A GENETIC AND DEVELOPMENTAL STUDY OF THE

NOTCH LOCUS OF DROSOPHILA MELANOGASTER

by

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ABSTRACT

The sex-linked Notch locus plays an important role in embryogenesis and determination of many adult structures of the fruit fly, Drosophila melanogaster. Mutation at this locus can cause lethality in embryonic or later stages, as well as morphological abnormalities of the adult eyes, wings, bristles and legs. Alleles of the Notch locus can be broadly grouped into three classes: 1) recessive lethal Notch (N) alleles, which may be deficiencies or point mutations, 2) Abruptex (Ax) alleles, which are probably point mutations and may be either lethal or viable, and 3) viable recessive alleles with visible phenotypes, which affect a variety of traits and are point mutations. The present investigation was initiated with a view to understanding the relationships between N and Ax alleles and the nature of their role in development, and has consisted mainly of the following approaches: 1) an examination of the phenotypes of certain unusual N alleles and the phenotypic responses to alteration of the dosage of these alleles in relation to wild-type (N^+), 2) an examination of the interaction of Ax alleles with N alleles and with one another, and 3) developmental studies of the conditional (temperature-sensitive) phenotypes associated with certain Notch-locus genotypes.

The results of the N-allele dosage study indicate that a single mutation in the Notch locus can affect different functions associated with this locus in fundamentally different ways. Depending on the genotype and phenotype examined, the responses of

various $\underline{N}$ alleles to dosage changes suggest that mutation at the
Notch locus may result in reduced, increased or novel activity
at the locus.

Four ethyl methanesulfonate-induced $\underline{Ax}$ alleles have been
examined, none of which is cytologically abnormal in salivary
gland chromosome preparations, and at least three of which map
within the Notch locus. Depending on culture conditions and
the alleles involved, $\underline{Ax}/\underline{N}$ heteroallelic combinations may be
viable or lethal. All $\underline{Ax}/\underline{N}$ combinations studied exhibited less
severe Abruptex phenotypes (bristle loss and wing vein gapping)
than the respective $\underline{Ax}/\underline{Ax}$ homozygotes. However, the $\underline{Ax}$ alleles
differed from one another in their effects on the wing nicking
of the $\underline{N}$ alleles, in that the viable allele $\underline{Ax^{9B2}}$ and the semi-
lethal allele $\underline{Ax^{E1}}$ both suppressed wing nicking, whereas the two
viable alleles $\underline{Ax^{E2}}$ and $\underline{Ax^{16172}}$ both enhanced wing nicking.
Furthermore, heteroallelic combinations of $\underline{Ax}$ alleles which
affected nicking in different direction, were lethal ($\underline{Ax^{E1}}/\underline{Ax^{E2}}$,
$\underline{Ax^{E1}}/\underline{Ax^{16172}}$, $\underline{Ax^{9B2}}/\underline{Ax^{16172}}$), whereas combinations of $\underline{Ax}$ alleles
with similar effects on nicking were viable ($\underline{Ax^{E1}}/\underline{Ax^{9B2}}$, $\underline{Ax^{E2}}/$
$\underline{Ax^{16172}}$).

The temperature-shift experiments have revealed an interest-
ing pattern of temperature-sensitive periods (TSPs) for lethality
or adult morphological abnormalities associated with various
Notch-locus genotypes. TSPs for lethality may be monophasic
occurring in the embryo ($N^{60g11}/N^{60g11};Dp^{51b7}$), or the second
larval instar ($\underline{Ax^{16172}/N^{264-40}}$), or they may be polyphasic, occur-
ring in embryo, larval and pupal stages ($N^{264-103}/\underline{fa^{no}}$). On the

other hand, the TSPs for all the adult morphological abnormalities examined occur during the third larval instar, including rough eyes and wing nicking (N^{60g11}/+, $N^{264-103}$/spl), leg segment fusion ($N^{264-103}$/+, $N^{264-103}$/spl), wing vein gapping (Ax^{16172}/+) and disturbance of bristle numbers ($N^{264-103}$/spl, Ax^{16172}/+).

Several molecular models are discussed in relation to the observations on N-allele dosage and interactions of the Ax and N alleles. The results are consistent with the hypothesis that the Notch locus is a regulator gene influencing many developmental processes, that mutations can affect the activity either of the entire gene or of various parts of the gene individually, and that N and Ax mutations usually affect this regulatory system in opposite ways from one another.

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INTRODUCTION

The sex-linked Notch locus (standard map position 3.0)
plays an important role in embryogenesis and in the determination
of many adult structures of Drosophila melanogaster. Mutation at
this locus can result in a wide array of defects, such as
lethality in embryonic or later stages, and morphological
abnormalities of the adult eyes, wings, bristles, and legs. In
spite of a great deal of information collected over a period of
many years concerning various alleles of this complex locus, no
unified theory has yet been advanced which satisfactorily
explains the nature of its role in development. With a view to
understanding this role and the nature of recently recovered
atypical mutant alleles, the present investigation was initiated.
As outlined in more detail below, the main approaches to this
problem have been: 1) examination of the phenotypes of certain
mutant alleles of the Notch locus, their phenotypic responses to
alterations in relative mutant:wild-type allele dosage, and
their interactions with one another; and 2) developmental studies
of various conditional (temperature-sensitive) phenotypes
associated with certain Notch-locus genotypes.

Owing to the incredible complexity of the Notch locus, it
will be useful at this point to include a brief review of the
findings of other investigators. Mutations in the Notch locus
can be broadly grouped into three classes - the Notches, the
Abruptexes, and the recessive visible mutations. Since
mutations within either of the first two classes generally

exhibit qualitatively similar phenotypes, these groupings
probably reflect, for the most part, similar functional defects.
However, the recessive visible mutations are much more hetero-
geneous with respect to phenotype, and thus cannot be regarded
as a functionally similar group. The relative genetic positions
of a number of alleles within the locus are summarized in
Figure 1.

Typical Notch (N) mutants, which define the locus, are
recessive lethals with a dominant phenotype consisting of serra-
tions at the tips and along the edges of the wings (Plate 1a)
and/or delta-like thickenings at the ends of the longitudinal
wing veins (Plate 1a,b), a generally increased number of thoracic
microchaetae, and other bristle disturbances (BRIDGES AND BREHME
1944). The lethality of flies homo- or hemizygous for N muta-
tions is associated with a gross hypertrophy of the embryonic
nervous system at the expense of ectodermally derived structures
and a failure of mesodermal tissues to differentiate (POULSON
1939a, b, 1940). N mutations may result from cytologically
visible deficiencies, position-effect inactivations of intact
(wild-type) Notch loci associated with chromosome rearrangements,
or cytologically invisible, genetically separable changes within
the Notch locus (LINDSLEY AND GRELL 1968). Cytological analysis
of deficiency Notches has provided convincing proof that the
Notch locus is located in the polytene chromosome band 3C7, and
that a deficiency for this band is sufficient to cause the Notch
phenotype (MOHR 1932; SLYZINSKA 1938; DEMEREC 1939). Duplica-
tions of genetic material which contain a wild-type copy of the

FIGURE 1   Genetic map of the Notch locus. Lethal alleles are

below the line, non-lethal alleles above. Solid

lines represent information obtained from the following refer-

ences: WELSHONS 1958a, b, 1965, 1971; WELSHONS & VON HALLE 1962;

WELSHONS, VON HALLE & SCANDLYN 1963. Broken lines represent

information obtained from data contained in the present report.

The shorter lines above and below the main map indicate the

approximate locations of mutations which have not been positioned

critically with respect to all the sites on the main map. Note,

however, that from the published data and information:

1) Ax^{E2}, Ax^{59b}, Ax^{59d}, N^{J14}, N^{f10}, and N^{h21} are to the right of

spl; 2) Ax^{E1} is to the right of fa^{no}; 3) Ax^{9B2} is to the right

of N^{40}; 4) Ax^{9B2} and Ax^{59d} are to the left of N^{Co}; 5) fa^{g} is

between N^{e11} and fa^{no}.

Ax^{9B2}

Ax^{E2}

nd nd^2

N^{co} Ng^{11}

fa^{noE}

N^{J24}

N^{103}

Ax^{59d}

Ax^{59b}

Ax^{E1}

l$_{(1)}$N^2

spl

N^{nic}

l$_{(1)}$N^B

l$_{(1)}$N^3

N^{J14}, N f$_{10}$, N h$_{21}$

no

fa

N^{40}

N

N

fa g

fa

l$_{(1)}$ N^1

N e^{11}

PLATE 1   Wings of a) N^8/+, b) N^{g11}/+, c) +/+;Dp, d) Ax^{E2}/Ax^{E2}, and e) OR females, raised at 22°C. Magnification 18x.

a
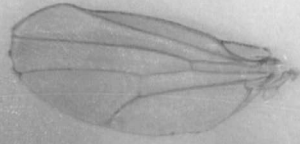
b
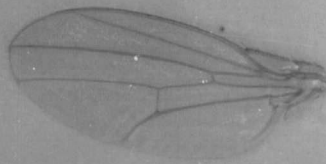
c

d

e

Notch locus (N^+) completely abolish the wing nicking phenotype
in deficiency heterozygotes, allow N homo- and hemizygotes to
live, and cause a new phenotype - Confluens, consisting of extra
wing veinlets (Plate 1c) - when their presence results in more
than the usual number of N^+ loci, in either males or females
(MORGAN, SCHULTZ AND CURRY 1941; LEFEVRE 1952; WELSHONS 1965).
These observations have led to the hypothesis that the dosage of
the N^+ gene product determines the expression of the mutant
phenotypes associated with the Notch locus (WELSHONS 1965).

Generally, the point N mutants behave as though they were
deficiencies and allow pseudodominant expression of the normally
recessive Notch-locus eye mutants facet (fa), facet-glossy
(fa^g), split (spl), and the wing mutant notchoid (nd), when
heterozygous with these mutants. In addition, in combination
with the wing mutant facet-notchoid (fa^{no}), they cause lethality.
Mutants of the N type can be classed as amorphs (WELSHONS 1965).
However, there have been several reports in the literature of
N mutants which do not behave entirely like deficiencies, in
that expression of the wing nicking phenotype is variable and
interactions with the intralocus recessive visible mutations are
in some cases reduced or absent (LINDSLEY AND GRELL 1968;
WELSHONS, personal communication). In all cases however, the N
mutations are lethal when homo- or hemizygous (WELSHONS 1965).
Additional atypical N mutants exist, which, besides having mild
expression of one or more of the typical Notch phenotypes, are
associated with abnormalities not seen in other N mutants
(WELSHONS 1956a; WELSHONS AND VON HALLE 1962). The mild

expression of the typical Notch phenotypes of these classes of mutations, suggests that they may not be completely amorphic, but instead have intermediate activity between $\underline{N}^+$ and $\underline{N}$ deficiencies (i.e. are hypomorphic to $\underline{N}^+$). However, the fact that certain of the $\underline{N}$ mutants have phenotypes not associated with deficiencies for band 3C7, suggest that they may be defective in some way other than by producing less gene product or a product having decreased function. In addition to the $\underline{N}$ mutations having mild or atypical phenotypes, there exists within the Notch locus a number of recessive lethals which lack dominant adult phenotypes, but since they allow pseudodominant expression of the recessive visibles (WELSHONS 1965), and have embryological defects similar to those of $\underline{N}$ homozygotes (POULSON 1968), these lethals can also be included in the $\underline{N}$ class of mutations. Clearly the $\underline{N}$ class of mutations is not entirely homogeneous, since it includes dominant $\underline{N}$ amorphs, non-visible recessive lethals, and atypical Notches.

The possibility that some non-amorphic $\underline{N}$ mutations may be hypomorphic or neomorphic, might be studied by investigation of the effects of gene dosage on the phenotypes of these mutants. Theoretically, these two mutant classes can be distinguished by their response to alteration of the relative dosage of mutant product with respect to wild-type product (cf MULLER 1932). A major difficulty with this approach is that so far the product of the Notch locus is unknown, so that the relative proportions of mutant and wild-type products can only be inferred by the genetic constitution and phenotypic expression of the genotype.

The insertion of duplications of the N^+ locus into autosomes permits manipulation of the numbers of mutant and wild-type alleles. In the present investigation, the effects of altering $N:N^+$ gene dosage have been observed for four different N alleles. The results indicate that this approach is valid, although there remain some reservations which have yet to be settled experimentally.

The Abruptex mutations characteristically cause a reduction in the numbers of certain bristles, and interruptions in wing venation (Plate ld). The original Abruptex allele, Ax^{28a}, is viable in the hemizygous and homozygous condition, and when heterozygous with deficiencies for the Notch locus, both the wing nicking phenotype of Notch and the bristle and wing vein phenotypes of Ax^{28a} are suppressed (MOHR 1932). Wing nicking does occur with a low incidence in Ax^{28a}/N individuals (LEFEVRE, RATTY AND HANKS 1953). The suppression of the Notch phenotype by Ax^{28a}, and the presence of an extra band adjacent to band 3C7 in salivary gland preparations of Ax^{28a} chromosomes led to the hypothesis that Ax^{28a} was a duplication of the Notch locus with the Abruptex phenotype resulting from a position effect associated with the proximity of the two loci (MORGAN, SCHULTZ AND CURRY 1941). However, on the basis of mutational data and the fact that other known duplications of the N^+ locus produce a Confluens phenotype, LEFEVRE and co-workers suggested that the extra band in the Ax^{28a} chromosome was not 3C7 (LEFEVRE et al. 1953). Recently, extrapolating from studies on two recessive lethal Ax mutations, WELSHONS (1971) has suggested that Ax^{28a} may indeed

consist of two 3C7 bands, one of which contains a dominant $\underline{Ax}$ mutation that would be lethal without the adjacent wild-type locus.

The present report describes the results of investigations on five $\underline{Ax}$ mutations, their interactions with several $\underline{N}$ mutants and their interactions with one another, with a view to resolving the nature of $\underline{Ax}$ alleles and their relationship to Notch. While no decision can be made from the results of the present study as to whether $\underline{Ax}^{28a}$ really is duplicated for salivary band 3C7, the data to be presented show that in $\underline{Ax}/\underline{N}$ heterozygotes: 1) $\underline{N}$ mutants suppress the phenotypes of $\underline{Ax}$ mutations, 2) not all $\underline{Ax}$ mutants suppress the wing nicking of $\underline{N}$ mutants, and 3) the suppression of wing nicking by certain $\underline{Ax}$ mutants does not appear to be associated with duplication of the Notch locus. Finally, and perhaps most significantly, these studies have revealed an unexpected system of lethal interactions among the Abruptex mutants.

As indicated earlier, the third group of Notch-locus mutations, the recessive visibles, are phenotypically and therefore probably also functionally very heterogeneous. Note also (Figure 1) that the two eye mutant sites $\underline{fa}$ (including $\underline{fa}^{g}$) and $\underline{spl}$, are separated genetically by the site of the wing mutant $\underline{fa}^{no}$, and that the wing mutant sites $\underline{fa}^{no}$ and $\underline{nd}$, are separated by the position of the eye mutant $\underline{spl}$. The eye mutations complement one another (i.e. the $\underline{fa}/\underline{spl}$ and $\underline{fa}^{g}/\underline{spl}$ heterozygotes are wild-type in appearance) as well as the two wing mutants. On the other hand, $\underline{fa}^{g}/\underline{fa}$, $\underline{nd}/\underline{nd}^{2}$ and $\underline{fa}^{no}/\underline{nd}$ have intermediate

phenotypes compared to the respective homozygotes (WELSHONS 1965). As mentioned in the discussion of $\underline{N}$ mutants, the recessive visible mutations are expressed pseudodominantly or result in lethality when heterozygous with $\underline{N}$ mutations. In addition to this, it has recently been reported that heterozygotes of $\underline{fa}^g$ with $\underline{Ax}^{59b}$ or $\underline{Ax}^{59d}$, also express the $\underline{fa}^g$ phenotype, although less so than in $\underline{fa}^g/\underline{fa}^g$ flies (WELSHONS 1971). The present investigation has only concerned certain combinations of recessive visibles with $\underline{N}$ and $\underline{Ax}$ mutations, and has not dealt systematically with recessive visible mutations as a group. Nevertheless, as will be discussed later, the data allow certain conclusions about the nature of $\underline{fa}^{no}$ and $\underline{spl}$.

In the course of investigating the interactions between various Notch-locus mutations, outlined above, several instances of temperature-sensitive (ts) expressions of lethal or morphological phenotypes, have been discovered. Mutations with phenotypes that are expressed conditionally as a function of temperature have been useful tools in the analysis of development in such diverse organisms as Drosophila (DRIVER 1931; TARASOFF AND SUZUKI 1970; SUZUKI 1970), bacteriophages (EPSTEIN, BOLLE, STEINBERG, KELLENBERGER, BOY DE LA TOUR, CHEVALLEY, EDGAR, SUSMAN, DENHARDT, AND LIELAUSIS 1963), and slime molds (LOOMIS 1969). The utility of ts mutants in developmental studies results from the ability to manipulate the temperature impinging on the organism, at specific time intervals during development. The present report describes experiments involving temperature shifts at different times during the development of selected

Notch-locus genotypes, which have been used to define tempera-
ture-sensitive periods (TSPs) for several of the conditional
lethal and morphological phenotypes. The results show that TSPs
for lethality may be found at several stages of development,
ranging from the egg to the pupa stage, whereas the TSPs for
adult morphological phenotypes (including eye facet pattern,
wing nicking, wing vein gaps, bristle loss, and fusion of leg
segments) all occur in the third larval instar.

The results of the present investigation are consistent
with the hypothesis that the Notch locus is a regulator gene
which controls many developmental processes. According to this
hypothesis, the phenotypes associated with most $\underline{N}$ mutations
reflect reduced repressor activity, whereas the phenotypes of
$\underline{Ax}$ mutations appear to reflect increased repressor activity.
Molecular models illustrating this concept are discussed in
relation to the data.

MATERIALS AND METHODS

I. Description of strains and mutant stocks.

The wild-type strain (hereafter designated OR) used in all experiments was derived from the highly inbred strain Oregon R^{369}, by the series of crosses outlined in Figure 2. The OR stock was established early in 1969, from a single brother-sister mating of the type shown in the last line in Figure 2, and has been kept in mass culture since that time. This stock is vigorous and fertile at both 20°C and 29°C. OR individuals frequently exhibit slight branching of the posterior crossvein, which is typical of Oregon R strains (LINDSLEY AND GRELL 1968), and also occasionally have gaps in the posterior crossveins. No gapping of the longitudinal veins has been seen in this stock, and variation in bristle numbers is slight. Sample bristle counts of OR males and females are contained in Appendix 8 (lines 10-12).

Brief descriptions of the mutations and chromosome rearrangements used are presented in Tables 1-3. Except where noted with an asterisk (*) more information can be obtained in LINDSLEY AND GRELL 1968.

FIGURE 2   Mating scheme used to synthesize isogenic wild-type

strain (OR).

$$\frac{FM6}{y} \;;\; \frac{SM1 \;;\; TM2}{T(2;3)e} \;;\; \frac{spa^{pol}}{spa^{pol}} \;\female \quad X \quad OREGON\ R^{369} \quad \male$$

$$\frac{FM6}{+} \;;\; \frac{SM1}{+} \;;\; \frac{TM2}{+} \;;\; \frac{spa^{pol}}{+} \;\female \quad X \quad \frac{FM6}{Y} \;;\; \frac{SM1 \;;\; TM2}{T(2;3)e} \;;\; \frac{spa^{pol}}{spa^{pol}} \;\male$$

$$\frac{FM6}{+} \;;\; \frac{SM1}{+} \;;\; \frac{TM2}{+} \;;\; \frac{spa^{pol}}{+} \;\female \quad X \quad \frac{+}{Y} \;;\; \frac{SM1}{+} \;;\; \frac{TM2}{+} \;;\; \frac{spa^{pol}}{+} \;\male$$

$$\frac{+}{+} \;;\; \frac{+}{+} \;;\; \frac{+}{+} \;;\; \frac{+}{+} \;\female \quad X \quad \frac{+}{Y} \;;\; \frac{+}{+} \;;\; \frac{+}{+} \;;\; \frac{+}{+} \;\male$$
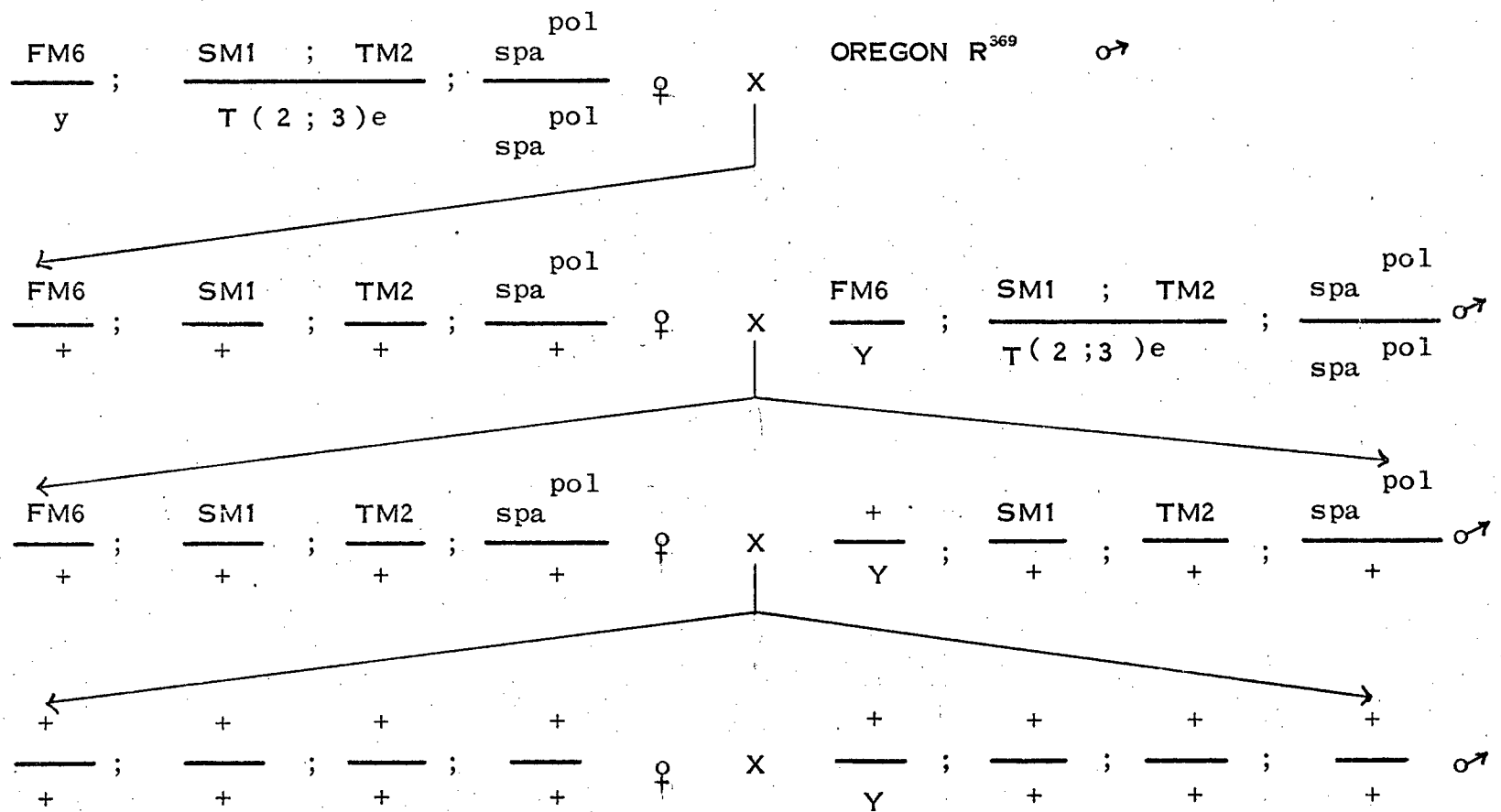
TABLE 1   Symbols and phenotypic descriptions of non-Notch-
           locus mutations used.

Name	Symbol	Location	Phenotype
Bar	$\underline{B}$	X-57.0	Narrow eye.
Bar of Stone	$\underline{B}^S$	X-57.0	Extremely narrow eye.
bobbed-lethal	$\underline{bb}^1$	X-66.0	Recessive lethal in XX females or XO males.
brown-Variegated	$\underline{bw}^V$	2-104.5	Dominant mottled brown eye colour.
carnation	$\underline{car}$	X-62.5	Eye colour dark ruby; orange in combination with $\underline{v}$.
crossveinless	$\underline{cv}$	X-13.7	Wing crossveins missing.
Curly	$\underline{Cy}$	2-6.1	Wings curved upwards; recessive lethal.
deep orange	$\underline{dor}$	X-0.3	Orange eye colour.
deep orange-lethal	$\underline{dor}^1$	X-0.3	Recessive lethal $\underline{dor}$ allele.
ebony	$\underline{e}$	3-70.7	Black body colour.
ebony-sooty	$\underline{e}^S$	3-70.7	Black body colour; allele of $\underline{e}$.
forked	$\underline{f}$	X-56.7	Bristles shortened and bent.
Hairy wing	$\underline{Hw}$	X-0.0	Dominant, extra bristles along wing veins and on head and thorax.
lethal(1)J1	$\underline{l(1)J1}$	X-0.0	Recessive lethal; covered by $\underline{y}^+$ duplications.
miniature-2	$\underline{m}^2$	X-36.1	Wing size reduced.
ruby	$\underline{rb}$	X-7.5	Eye colour ruby; white in combination with $\underline{w}^a$.
sparkling-poliert	$\underline{spa}^{pol}$	4-3.0	Eyes small, glazed.

Name	Symbol	Location	Phenotype
tinylike	$\underline{tyl}$	X-36	Small bristles.
Ultrabithorax-130	$\underline{Ubx}^{130}$	3-58.8	Large haltere size; recessive lethal.
vermilion	$\underline{v}$	X-33.0	Eye colour bright scarlet.
white	$\underline{w}$	X-1.5	White eye colour.
apricot	$\underline{w}^{a}$	X-1.5	Apricot eye colour; allele of $\underline{w}$.
eosin	$\underline{w}^{e}$	X-1.5	Yellowish-pink eye colour; allele of $\underline{w}$.
yellow	$\underline{y}$	X-0.0	Yellow body and bristle colour.
yellow-2	$\underline{y}^{2}$	X-0.0	Yellow body, darker bristles; allele of $\underline{y}$.

TABLE 2   Symbols and descriptions of Notch-locus alleles used.

Allele	Symbol Used	Description
*Abruptex-9B2	Ax^{9B2}	Bristle loss; wing vein gapping - see Results.
*Abruptex-59d5	Ax^{59d}	Recessive lethal; bristle loss and wing vein gapping.
*Abruptex-16172	Ax^{16172}	Extensive bristle loss and wing vein gapping - see Results.
*Abruptex-E1	Ax^{E1}	Recessive lethal; bristle loss and wing vein gapping - see Results.
*Abruptex-E2	Ax^{E2}	Bristle loss and wing vein gapping - see Results.
facet-glossy	fa^{g}	Irregular eye facet array and glazed eye surface.
facet-notchoid	fa^{no}	Nicked wings, thick wing veins; lethal when heterozygous with most N mutations.
*facet-notchoid-E	fa^{noE}	Like fa^{no}, but milder wing phenotype.
Notch-8	N^{8}	Cytologically deficient for several salivary chromosome bands including 3C7. Recessive lethal; nicked wings and thick wing veins.
Notch-264-40	N^{40}	Phenotypically like N^{8}, but not cytologically deficient.
Notch-264-103	N^{103}	Not cytologically deficient. Recessive lethal; temperature sensitive wing nicking, fusion of leg segments, and interaction with other alleles - see Results.
Notch-60g11	N^{g11}	Not cytologically deficient. Recessive lethal; temperature sensitive wing nicking and eye facet disarry - see Results.

Allele	Symbol Used	Description
*Notch-70k27	N^{70k27}	Recessive lethal; weak wing nicking, eyes normal; from origin, should also contain Ng^{11} mutant site – see Appendices 3, 4.
Notch-Confluens	N^{Co}	Not cytologically deficient. Recessive lethal; weak wing nicking; strong extra wing vein (Confluens) phenotype in presence of extra N^+ loci – see Results.
split	spl	Eyes reduced in size and with irregular facet array; missing or doubled bristles frequent.

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*Alleles not described in LINDSLEY AND GRELL (1968).

TABLE 3. Symbols and descriptions of chromosome rearrangements used.

Name	Symbol Used	Markers Carried	Description
Bar of Stone, white-plus Y	$B^S \; w^+ \cdot Y$	$w^+ - N^+; B^S$	Insertion of X chromosome markers into Y chromosome.
Bar of Stone, yellow-plus Y	$B^S \; y^+ \cdot Y$	$y^+ - l(1)Jl^+; B^S$	Insertion of X chromosome markers into Y chromosome.
delta-49	$dl49, y \; Hw \; m^2$	y, Hw, m^2	Inversion in central region of X.
delta-49	$dl49, tyl \; bb^1$	bb^1, tyl	Inversion in central region of X.
Duplication (1;2)51b7	Dp	w^+, N^+	Insertion of X chromosome markers into right arm of chromosome 2.
*Duplication (1;Y)59k9(4)	$Dp^{59k9(4)}$	y^2, $l(1)Jl^+$	Insertion of X chromosome markers into Y chromosome #.
*Duplication (1;Y)60d19(1)	$Dp^{60d19(1)}$	y^2, $l(1)Jl^+$	Insertion of X chromosome markers into Y chromosome #.
*Duplication (1;Y)67g24(1)	$Dp^{67g24(1)}$	$y^2 - dor^+$ (inclusive)	Insertion of X chromosome markers into Y chromosome #.
First Multiple-6	$FM6$	y, B	Multiply inverted X; female sterile.
*lethal First Multiple-6	$l(FM6)$	y, B, l	FM6 chromosome carrying EMS-induced lethal.
Muller-5	$M5$	w^a, B	Multiply inverted X.
Second Multiple-1	$SM1$	Cy	Multiply inverted second chromosome.

Name	Symbol Used	Markers Carried	Description
*Translocation (1;Y)2E	T(1;Y)2E	y, l(1)J1$^+$; dor$^+$	Insertion of X chromosome markers into Y chromosome (RAYLE & HOAR 1969).
*Translocation (2;3)e	T(2;3)e	e	Reciprocal translocation between Second and Third chromosomes. Recessive lethal.
Third Multiple-2	TM2	Ubx^{130}, e^s	Multiply inverted Third chromosome.
*Compound X	$\widehat{XX}$/Y	+	Compounded X chromosomes.
*yellow-white-forked Compound X	$\widehat{XX}$,y w f/Y	y, w, f	Compounded X chromosomes.

* Rearrangements not described in LINDSLEY AND GRELL (1968).

GREEN, personal communication.

II. Use of the symbol $\underline{Dp}$.

As noted in Table 3, the symbol $\underline{Dp}$ is used to denote the presence of a second chromosome containing an insertion of the $\underline{w}^+$-$\underline{N}^+$ region. To avoid confusion over the number of $\underline{N}^+$ alleles present, the symbols $\underline{Dp}^+$ or + are <u>not</u> used in the text to denote a normal (non-duplication-bearing) second chromosome. For example, the notation $\underline{N}$/+;$\underline{Dp}$ describes a female containing one mutant $\underline{N}$ allele and one $\underline{N}^+$ allele on the X chromosomes, and one $\underline{N}^+$ allele on the second chromosome.

III. Incubation temperatures.

Several different temperatures were used for incubation of developing cultures in various parts of this investigation. The cultures kept at 20°C, 25°C, 28°C, and 29°C were all grown in incubators, each of which held the respective temperatures to ± 0.5°C. The other temperatures of 20.5°C, 21.5°C, and 22°C, were found in different areas within temperature-regulated rooms in which the average temperature controls were set at 22°C. In these rooms, the actual temperature depended largely on position within the room and, to some extent, on seasonal weather changes. The temperatures stated are those observed in checks made at the time of the experiment in question. In a previous report, the actual 20.5°C temperature was rounded off to 21°C (FOSTER AND SUZUKI 1970).

IV.   Procedure for temperature-shift experiments.

The critical time during development of ts mutants when temperature induces mutant phenotypes (or temperature-sensitive period, abbreviated TSP), can be determined by shifting cultures from one temperature to another at different successive intervals.

A sufficient number of eggs for experiments involving temperature shifts were obtained by mating approximately 100 aged females (3 to 8 days after eclosion) with 30 to 40 males, two to three days prior to egg collection. For collection of eggs, the parents were kept at room temperature in empty half-pint bottles lying on their sides. The bottles were capped with fresh yeasted petri plates containing Drosophila medium which were changed daily. These conditions ensured good egglays.

For most shift experiments, eggs were collected within a two hour interval from approximately 100 fertilized females. In early experiments, the eggs were collected directly on medium in culture bottles, but in later experiments, they were collected on medium contained in petri dishes, then transferred to bottles containing more food. The use of plates facilitated immediate assessment of the success of an egglay, thereby conserving food in unsuccessful cases. It was found that the best egglays were obtained on plates containing fresh moist medium, whose surface had been scratched with a needle and sprinkled with live dried baker's yeast. Furthermore, the best results were obtained when egglays were performed in darkness with the surface of the medium held vertically.

The larval instars present in the cultures at the time of shifting were identified according to the morphology of their mouthparts. Samples of larvae were placed in a drop of water on a microscope slide, crushed under a coverslip, and examined microscopically. The larval instars could readily be determined by the number of teeth present on the mandibular hooks (BODENSTEIN 1950). No special techniques were needed to recognize non-larval stages.

In experiments involving genotypes with known or suspected embryonic TSPs, eggs were collected on petri plates within a one hour period. The food in these plates was not transferred to bottles containing more food until after the temperature shifts. It was reasoned that the smaller volume of food and air and the thinness of the walls of the plates would allow more rapid temperature equilibration of the medium, thereby allowing a more accurate determination of the TSP.

In one experiment, synchronization of developmental time in cultures was attempted through the isolation of first instar larvae immediately after they had hatched from the egg. However, by the third instar, larvae in such cultures were not particularly well-synchronized, so this method was not repeated.

Accurate staging of third instar larvae relative to pupation time was achieved by performing shift experiments in a manner different from that outlined above. In these experiments, several batches of eggs laid by a relatively small number of parents over several days, were collected on regularly changed petri plates, and shifted from one temperature to another at the

same time. White prepupae (a very transitory condition at the beginning of the prepupal stage (BODENSTEIN 1950)) were then isolated at defined times after the shift. Thus, if it can be assumed that development of third instar larvae takes about the same length of time for different larvae, the interval from a shift to pupation was determined very precisely.

Details of the scoring of the various shift experiments are presented along with the results of the particular experiment concerned.

V. Preparation of specimens for scanning electron microscopy.

The ability of the scanning electron microscope to maintain objects at different heights within focus makes it a useful tool in the illustration of fine morphological detail. In the present study, the scanning electron microscope has been used to prepare illustrations of mutant eye phenotypes and, in one temperature shift experiment, to provide a record of the eye facet data.

Eyes of adult flies were prepared for viewing with the scanning electron microscope in the following manner. The flies were decapitated and the heads placed in chloroform for three or more days. The heads were then removed from the chloroform and allowed to air-dry for at least three days. Omission of the chloroform treatment resulted in the collapse of many of the eyes upon dessication. The dried heads were mounted on aluminum discs with household cement, coated with gold-palladium alloy in a vacuum evaporator, and photographed with a Cambridge Steroscan scanning electron microscope.

VI. Procedure for wing nick counts.

In order to obtain quantitative data on $\underline{N}:\underline{N}^+$ dosage and $\underline{N}/\underline{Ax}$ interactions, counts were made of the number of individuals with 0, 1, or 2 nicked wingtips. Samples of flies for these counts were obtained by rearing the progeny of 3-10 pairs of parents (3-5 day egglays) in 1/4 pint bottles containing Drosophila medium. Except where noted otherwise, scoring was limited to the tips of the wings.

VII. Procedure for bristle and wing vein gap counts.

To obtain quantitative data on $\underline{Ax}$ phenotypes, and their interactions with $\underline{N}$ mutations and one another, counts were made of the number of individuals with 0, 1, 2, (etc.) of a given bristle, and of the number of individuals with gaps in the longitudinal wing veins. See Figure 3 for the names and positions of the bristles and wing veins scored. Culture conditions were the same as described for wing nicking counts.

VIII. Statistical procedures used on nicking, bristle and vein gap data.

The mean bristle (or wing nick or vein gap) frequency per fly ($\overline{x}$) was calculated by the formula

$$\overline{x} = \frac{0n_o + n_1 + 2n_2 + 3n_3 + \ldots}{n}$$

FIGURE 3   The anatomical positions of a) bristles and b) wing

veins discussed in the text (LINDSLEY & GRELL 1968).

a.

ANTERIOR ─┐

MEDIAN ─┐ } ORBITALS

POSTERIOR ─┘

OCELLARS

ANTERIOR ─┐ VERTICALS

POSTERIOR ─┘

POSTVERTICALS

THORACIC
MICROCHAETAE

ANTERIOR ─┐ } DORSOCENTRALS

POSTERIOR ─┘

ANTERIOR
POSTALARS

ANTERIOR ─┐ } SCUTELLARS

POSTERIOR ─┘

b.

L1 ─┐

L2 ─┤

L3 ─┤ } LONGITUDINAL VEINS

L4 ─┤

L5 ─┘

POSTERIOR ─┐ } CROSS VEINS

ANTERIOR ─┘
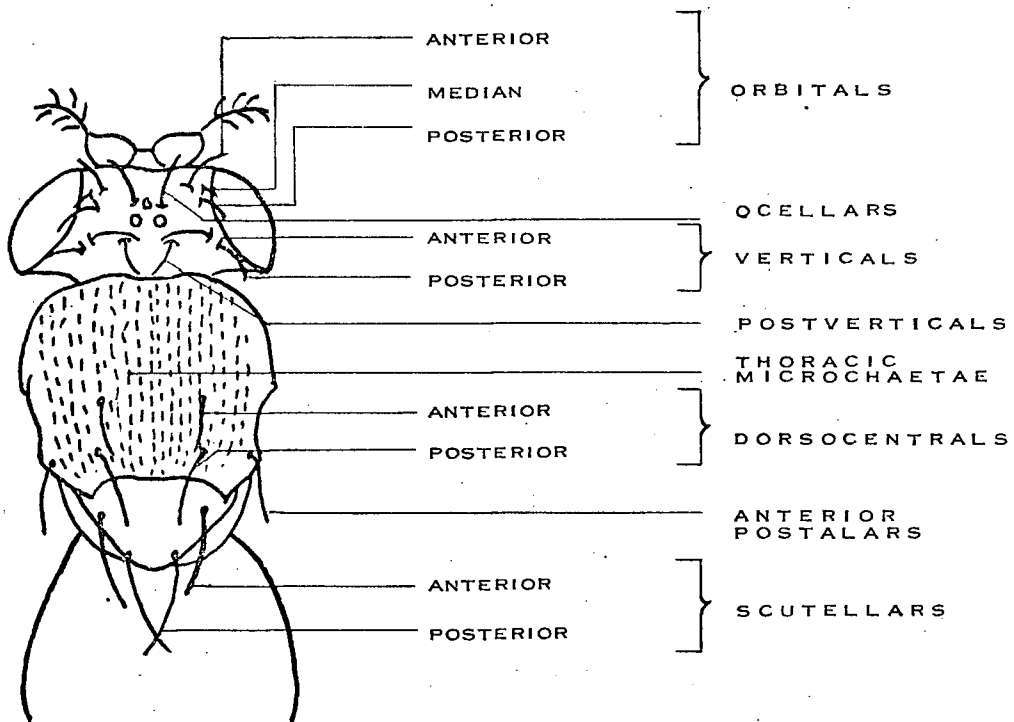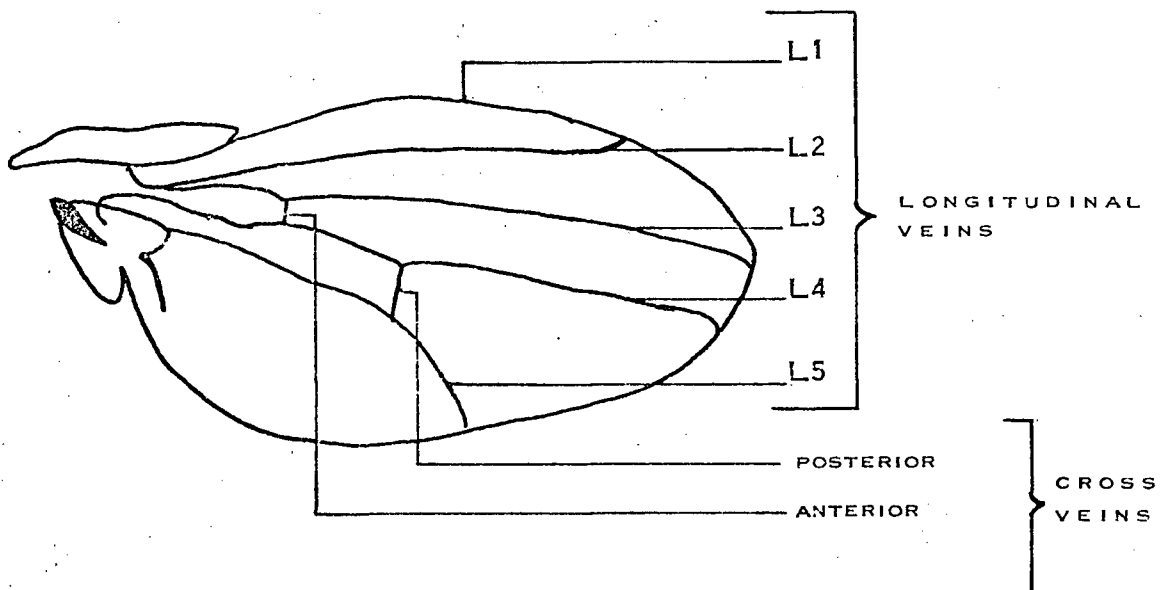
where n_0, n_1, n_2 are the number of flies with 0, 1, or 2 bristles (gaps, nicks), and n is the total number of flies.

Standard deviation (S.D.) was calculated by the formula

$$S.D. = \sqrt{\frac{1}{n-1} \sum_{i=0}^{k} n_i (x_i - \bar{x})^2}$$

where x_i = 0, 1, 2 (etc.) bristles (or nicks or gaps).

One sided 95% confidence intervals for $\bar{x}$ were calculated according to the formula

$$\text{confidence interval} = \frac{S.D.}{\sqrt{n-1}} \, t_{.95}$$

where $t_{.95}$ is the percentile value for Student's distribution with n-1 degrees of freedom (SPIEGEL 1961). Two values of $\bar{x}$ were said to be significantly different at the 95% level of confidence, if their confidence intervals did not overlap.

IX. <u>Calculation of index of phenotypic expression of bristle</u> <u>and wing vein gap phenotypes</u>.

The index of phenotypic expression, used to summarize bristle and wing vein gap phenotypes in Tables 18-20, 24-26, and 31, relates mutant bristle or wing vein gap frequencies to the respective wild-type frequencies. The wild-type index is defined as 1.00, and progressively lower indices indicate increasingly severe expression of the mutant phenotypes. Index of expression of bristle phenotypes is given by the formula

$$\text{index} = \frac{\text{mutant bristle frequency}}{\text{wild-type bristle frequency}} \, .$$

Index of expression of wing vein gap phenotypes is given by the formula

$$index = \frac{8-(\underline{mutant\ wing\ vein\ gap\ frequency})}{8}\ .$$

<u>RESULTS</u>

A. PHENOTYPES OF SELECTED $\underline{N}$ AND $\underline{N}^+$ COMBINATIONS

I. Effects of gene dosage on the phenotypes of $\underline{N}^8$ and $\underline{N}^{40}$.

One hundred six $\underline{N}^8$/+ progeny from the cross $\underline{N}^8$/dl49,
$\underline{y}$ $\underline{Hw}$ $\underline{m}^2$ ♀ x $\underline{OR}$ ♂, and 68 $\underline{N}^{40}$/+ progeny from the cross $\underline{w}^a$ $\underline{N}^{40}$
$\underline{rb}$/dl49,$\underline{y}$ $\underline{Hw}$ $\underline{m}^2$ ♀ x $\underline{OR}$ ♂, were raised at 20.5°C and examined for
wing nicking. Both wings of all individuals were nicked at the
tips and along the proximal part of the trailing edges. Examina-
tion of smaller samples of the same genotypes raised at 29°C
revealed no marked effect of temperature either on the degree of
expression or number affected. Since $\underline{N}^8$ is deleted for more loci
than are carried by $\underline{Dp}$, the genotypes $\underline{N}^8/N^8$;$\underline{Dp}$ and $\underline{N}^8/N^8$;$\underline{Dp}/\underline{Dp}$
were lethal. However, since $\underline{N}^{40}$ closely resembles $\underline{N}^8$ phenotypi-
cally, in both wing nicking and in its interactions with the
recessive visible Notch-locus alleles, it was assumed that the
dosage results obtained with $\underline{N}^{40}$ would resemble those of a
deficiency for the locus. This assumption is supported by the
observation that $\underline{N}^8/\underline{N}^{40}$;$\underline{Dp}$ females had a wing phenotype indis-
tinguishable from those of either $\underline{N}^{40}$/+ or $\underline{N}^8$/+, and by observa-
tions (reported later) on similar combinations of $\underline{N}^{40}$ and $\underline{N}^8$
with other $\underline{N}$ mutants.

In order to study the phenotypic effects of altering the
$\underline{N}^{40}$:$\underline{N}^+$ ratio, zygotes containing varying numbers of $\underline{N}^{40}$ and $\underline{N}^+$
loci were generated by the crosses outlined in the footnote to

Table 4. N^{40}/Y;Dp males and N^{40}/N^{40};Dp females are quite viable at both 20.5°C and 29°C (Table 4). The wings of the N^{40}/N^{40};Dp females had serrations both at the tips and along the trailing edges of the wings (although occasionally a female was nicked in only one wingtip), a phenotype very similar to that of $N^{40}/+$ heterozygotes. On the other hand, the N^{40}/N^{40};Dp/Dp and 1(FM6)/ N^{40};Dp females (both of which carried two N^+ loci) had wild-type wings. Thus, N^{40}-bearing females which have only one dose of N^+ express the typical Notch mutant phenotype, whereas females with two doses of N^+ are wild-type, in accord with the rules described by WELSHONS (1965). Furthermore, the N^{40}/Y;Dp/Dp males and 1(FM6)/N^{40};Dp/Dp females (cross 2, Table 4), each of which had an extra dose of N^+, had a Confluens wing phenotype, again predicted by WELSHONS (1965).

Both N^{40}/N^{40};Dp and N^{40}/N^{40};Dp/Dp females appeared to be fully fertile. Four of the latter were mated to w/Y males, and no w^a/w-eyed individuals were recovered among 135 female progeny, confirming that the parents were homozygous for Dp (note that N^{40} was linked to w^a in these experiments, and that Dp carries w^+). If only one dose of Dp were present, half of the females should have had the w^a/w eye phenotype. While N^{40}/Y;Dp males were fertile, their N^{40}/Y;Dp/Dp brothers were almost completely sterile. However, 2 out of 16 males tested did yield 3 and 7 w^+ progeny respectively, when mated to 3 w^a/w^a females each. The infertility of N^{40}/Y;Dp/Dp males is not surprising in view of the triplication of all the loci (except N^+) carried by the duplication.

TABLE 4   Viability of N^{40}/N^{40};Dp and N^{40}/N^{40};Dp/Dp females in relation to siblings at different temperatures.

		PROGENY					
		FEMALES				MALES	
CROSS*	TEMPERATURE	l(FM6)/N	l(FM6)/N;Dp**	N/N;Dp	N/N;Dp;Dp	N/Y;Dp	N/Y;Dp/Dp
1	20.5°C	26	26	20	-	47	-
	29°C	74	98	68	-	69	-
2	20.5°C	53	161	72	44	103	41
	29°C	65	157	94	62	168	42

* 1.   l(FM6)/w^a N^{40} rb ♀ x w^a N^{40} rb;Dp ♂

  2.   l(FM6)/w^a N^{40} rb;Dp ♀ x w^a N^{40} rb;Dp ♂

** includes l(FM6)/N;Dp/Dp females (cross 2 only).

The wing nicking phenotypes of N^8 and N^{40} combinations are summarized in Table 5. The genotypes $N^8/+$, $N^{40}/+$, $N^8/N^{40};Dp$, and $N^{40}/N^{40};Dp/Dp$ females were wild type in terms of wing nicking, as has been reported for $N/Y;Dp$ males and $N/+;Dp$ females (LEFEVRE 1952; WELSHONS 1965). Thus, in terms of the characteristic Notch wing nicking phenotype, N^{40} responded to changes in gene dosage in the manner expected of a complete amorph.

In passing, it should be noted that minor eye facet disruptions frequently occurred in the $N^{40}/Y;Dp$, $N^{40}/Y;Dp/Dp$, $N^{40}/N^{40};Dp$ and $N^{40}/N^{40};Dp/Dp$ progeny of cross 2 (Table 4), but seldom in the progeny of cross 1. The irregularities were more extensive and more frequent in $N/Y;Dp/Dp$ than in $N/Y;Dp$ males, but no such difference was detectable between $N/N;Dp/Dp$ and $N/N;Dp$ females. These observations suggest that homozygosis of some factor on the duplication-bearing chromosome may be responsible for the eye roughness, although it cannot be ruled out that some kind of maternal effect on Dp resulted in the facet disarray, since Dp came from the female parent in cross 2 but not in cross 1. Similar mild eye facet irregularities have also been seen in $+/Y;Dp$ males and $+/+;Dp$ females, although no careful studies were made of these genotypes. This point will be referred to when the N^{g11} gene-dosage results are described.

II. Effects of gene dosage on the phenotypes of N^{103}.

The frequency of wingtip nicking in $N^{103}/+$ females is much lower at 20°C and 22°C than at 25°C and 29°C (Table 6). The

TABLE 5   Summary of the effects of varying gene dosage on the wing phenotypes of N^8 and N^{40}.

GENOTYPE	NUMBER OF N^+ LOCI	WING PHENOTYPE*
FEMALES		
$N^8/+$	1	1.0 N
$N^{40}/+$	1	1.0 N
N^8/N^{40};Dp	1	1.0 N
N^{40}/N^{40};Dp	1	1.0 N
$N^{40}/+$;Dp	2	+
N^{40}/N^{40};Dp/Dp	2	+
$N^{40}/+$;Dp/Dp	3	Co
MALES		
N^{40}/Y;Dp	1	+
N^{40}/Y;Dp/Dp	2	Co
$+/Y$;Dp	2	Co

* 1.0 N = all individuals have nicked wings; + = wild-type wings; Co = Confluens wings

TABLE 6   Number of wings of $N^{103}/+$ females exhibiting nicking when raised at different temperatures.

TEMPERATURE	CROSS*	% NICKED INDIVIDUALS	MEAN NUMBER OF NICKED WINGTIPS PER FLY (+ 95% CONFIDENCE INTERVAL)	NUMBER OF FLIES EXAMINED
20°C	1	74	0.97 ± .12	103
	2	80	1.25 ± .09	213
22°C	1	71	1.03 ± .14	91
	2	82	1.34 ± .09	218
25°C	1	100	1.97 ± .05	143
	2	100	1.99 ± .02	337
29°C	1	100	2.00	90
	2	100	2.00	228

* 1.   OR ♀ x y w^a N^{103};Dp ♂

    2.   M5/y w^a N^{103} ♀ x OR ♂

difference in nicking observed between crosses 1 and 2 (Table 6)
at the two lower temperatures probably reflects a difference in
genetic background between the two crosses, since the N^{103} stock
was outcrossed to M5/M5 females to generate the female parents
for cross 2. Although the frequencies of wing nicking appear to
be similar at 25°C and 29°C, the wings of N^{103}/+ females were
more extensively nicked at 29°C than at 25°C, with serrations at
the tips and along both the leading and trailing edges (Plate 2).
The degree of nicking of these females at 29°C was definitely
more extreme than that of N^8/+ or N^{40}/+ flies, whereas the nick-
ing phenotype in N^{103}/+ females raised at 25°C was less pro-
nounced than in N^8/+ and N^{40}/+ females, the wings frequently
being only slightly incised at the tips. The enhanced nicking
seen in N^{103}/+ females at 29°C has a counterpart in the pheno-
type of N^{103}/Y;Dp males raised at this temperature. At the three
lower temperatures the wings of such males are wild-type, as is
the case with most other N alleles (including the deficiency
Df(w^{RJ3})/Y;Dp, which was recently examined). However, N^{103}/Y;Dp
males raised at 29°C frequently had thickened wing vein tips
(usually L5), reminiscent of the thickenings seen in typical
N/+ heterozygotes. From these observations, and the fact that
N^{103}/fa^{no} is viable at 20°C-25°C (Appendix 1), it is clear that
N^{103} does not behave like a deficiency at any of the temperatures
studied. At the lower temperatures the relatively mild expres-
sion of the wing nicking phenotype suggests that N^{103} is
hypomorphic rather than amorphic at these temperatures. On the
other hand, at 29°C the mutant wing expression of N^{103}/+ females

PLATE 2   Wings of N^{103}/+ females raised at a) 22°C, b) 25°C,

c) 29°C. Magnification 20x.
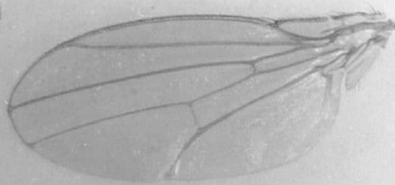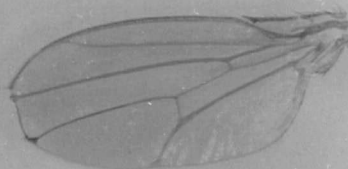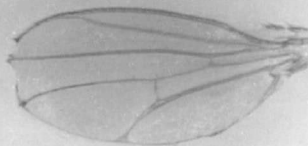
is even more pronounced than of heterozygotes for N deficiencies, the Notch wing phenotype even being expressed in males. This may indicate that at 29°C a defective product of N^{103} competes with or partially inactivates the N^+ allele product.

At 29°C only the $N^{103}/+$ females (Table 6) exhibited fusion of tarsal segments, whereas the legs of their $N^{103}/+;Dp$ sisters and $N^{103}/Y;Dp$ brothers had the normal number of distal segments (1 metatarsus and 4 tarsi). At 20°C-25°C all three genotypes had normal legs. Thus the mutant leg phenotype behaves like the wing phenotype, in that it is suppressed both in males and heterozygous females in the presence of a duplication for N^+. It is pertinent here to note that all N^{103}/fa^{no} females raised at 25°C, and some when raised at 20°C or 22°C, also had fused tarsi (Appendix 1). Furthermore, occasional heterozygotes of another Notch allele (N^{70k30}) with fa^{no} survive and at least some of these also exhibit tarsal fusion (Appendix 4). These results lead one to suspect that the leg phenotype may be associated with greatly reduced function at the Notch locus (i.e. intermediate between $N/+$ and N/N), although it must be pointed out that surviving N^{g11}/fa^{no} heterozygotes had normal legs (Appendix 1).

$N^{103}/N^{103};Dp$ females have good viability at 20°C-25°C, but at 29°C considerable mortality (Table 7) results from the sticking to the medium of newly eclosed females. Six $N^{103}/N^{103};Dp$ females recovered at 29°C exhibited much more severe wing nicking and tarsal fusion than their 29°C $N^{103}/+$ sibs. Wing nicking data for the $N^{103}/N^{103};Dp$ females raised at the three

TABLE 7   Viability of N^{103}/N^{103};Dp females* in relations to siblings, at different temperatures.

TEMPERATURE	FEMALES			MALES		
	M5/N;Dp	M5/N	N/N;Dp	M5/Y;Dp	M5/Y	N/Y;Dp
20°C	74	59	70	42	63	72
22°C	89	85	75	62	77	81
25°C	110	106	128	91	130	110
29°C	68	56	6	26	88	98

* Progeny of the cross M5/y w^a N^{103} ♀ x y w^a N^{103};Dp ♂

lower temperatures are presented in Table 8. At all three temper-
atures the incidence of wing nicking was significantly lower than
in N^{103}/+ females raised at the same temperatures (Table 6).
The reduction of nicking at 20°C and 22°C was much greater than
that at 25°C. Thus at temperatures at which the wing nicking of
N^{103}/+ is milder than that of N^8/+ or N^{40}/+, doubling the N:N^+
ratio of N^{103} significantly reduces the expression of the wing
nicking phenotype. On the other hand, at 29°C N^{103}/+ flies
have more extensive wing nicking than N^8/+ or N^{40}/+ females, and
the extent of wing nicking is further increased in N^{103}/N^{103};Dp
females.

III. Effects of gene dosage on the phenotypes of N^{g11}.

When raised at 25°C or 29°C, N^{g11}/+ females resemble typi-
cal N heterozygotes but have weaker wing nicking phenotypes than
N^8/+ or N^{40}/+ heterozygotes. At these temperatures the eyes
possess a wild-type facet pattern with only occasional minor ir-
regularities (Plate 3a). When raised at 20°C-22°C on the other
hand, all N^{g11}/+ females have a lower incidence of wing nicking
and exhibit a "rough" eye phenotype (consisting of disarrayed
ommatidia and duplicated interommatidial bristles) which extends
over the posterior three-quarters to four-fifths of the eye
(Plate 3b). In an earlier investigation of N^{g11}/+ females
obtained from the cross OR ♀ x w^a N^{g11} rb/B^S $w^+ \cdot Y$ ♂, 78.4%
(530/676) of the individuals raised at 29°C, and 3.5% (24/681)
of the individuals raised at 20.5°C, had one or both wingtips

TABLE 8   Number of wings of $N^{103}/N^{103};Dp$ females exhibiting
           nicking when raised at different temperatures.

TEMPERATURE	% NICKED INDIVIDUALS	MEAN NUMBER OF NICKED WINGTIPS PER FLY (± 95% CONFIDENCE INTERVAL)	NUMBER OF FLIES EXAMINED
20°C	10	0.10 ± .07	63
22°C	11	0.15 ± .10	72
25°C	90	1.64 ± .10	128

PLATE 3   Scanning electron micrographs of $N^{g11}/+$ females
raised at a) 20.5°C, and b) 29°C. Magnifications
a) 270x, b) 290x.

a

b
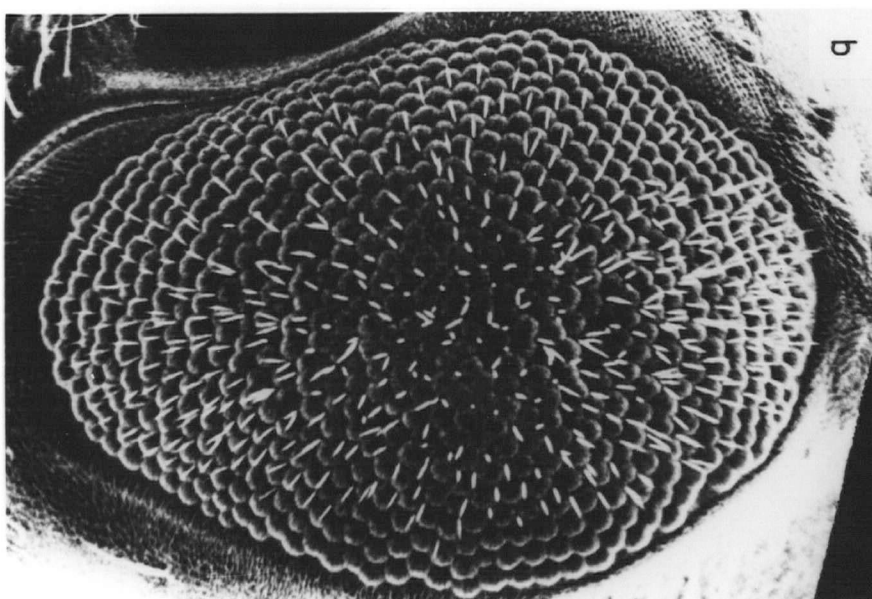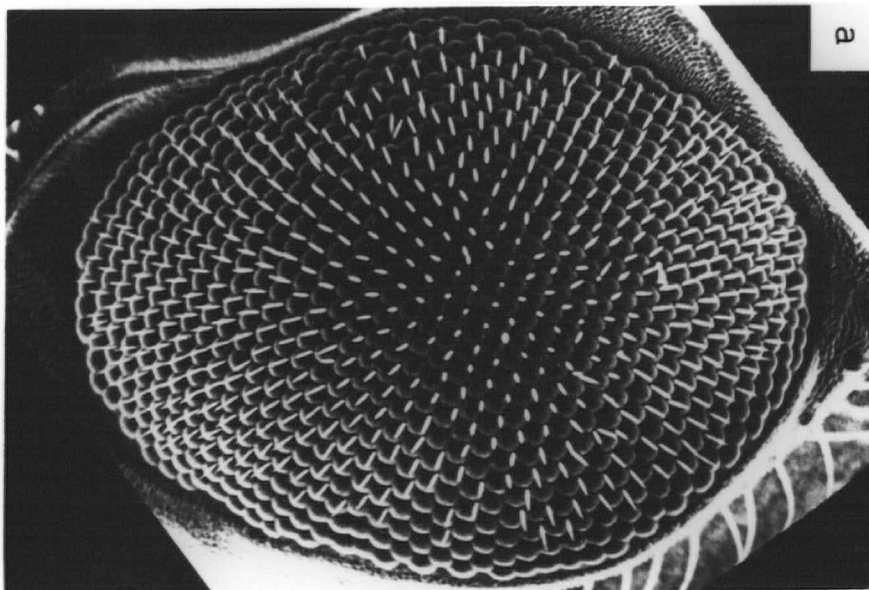
nicked (FOSTER AND SUZUKI 1970). More recent data obtained from flies raised at several temperatures confirm these findings (Table 9). No significant difference in wingtip nicking was apparent between 20°C and 22°C, but the frequency of nicking increased progressively at 25°C and 29°C. The differences between the progeny of crosses 1 and 2 at 25°C and 29°C, may reflect genetic background differences (see footnote to Table 9). Thus, the mutant eye phenotype of N^{g11}/+ females is only expressed at lower temperatures, while expression of the wing nicking phenotype is increased at higher temperatures.

The effect of an extra N^{+} locus on the expression of N^{g11} phenotypes was examined in the female progeny of the cross w/w ♀ x w^{a} N^{g11} rb/Y;Dp ♂ (Table 10). Since the duplication contains the w^{+} allele, duplication-bearing progeny could be distinguished unambiguously from their non-duplication-bearing sisters on the basis of their eye colours. All N^{g11}/+ female progeny raised at 20.5°C were mutant in both eyes and 6% of individuals exhibited wingtip nicking, while at 29°C all eyes were wild type and 95% of individuals had nicked wings (Table 10). In N^{g11}/+;Dp females no wing nicking was observed at either temperature, and at 29°C the eye facet pattern was wild type. On the other hand, considerable facet disarray was observed at 20.5°C in these females. The mutant phenotype in the N^{g11}/+;Dp flies classed as "R" (Table 10) was not as marked as in their N^{g11}/+ sisters, and 78 of the 108 "R" females were mutant in only one eye. Nevertheless, the eye roughness of N^{g11}/+;Dp females is much more extensive than that seen in the occasional +/+;Dp

TABLE 9   Number of wings of N^{g11}/+ females exhibiting
nicking when raised at different temperatures.

TEMPERATURE	CROSS*	% NICKED INDIVIDUALS	MEAN NUMBER OF NICKED WINGTIPS PER FLY (± 95% CONFIDENCE INTERVAL)	NUMBER OF FLIES EXAMINED
20°C	1	0	0.00	152
	2	1	0.01 ± .02	241
22°C	1	2	0.02 ± .02	161
	2	1	0.01 ± .01	273
25°C	1	10	0.11 ± .05	184
	2	24	0.28 ± .05	287
29°C	1	67	0.85 ± .12	103
	2	76	1.19 ± .06	257

* 1.   OR ♀ x w^a N^{g11} rb/B^S w^+.Y ♂

2.   M5/w^a N^{g11} rb ♀ x OR ♂

Note that the M5/N parents for cross 2 were obtained by
the cross M5/M5 ♀ x w^a N^{g11} rb;Dp ♂

TABLE 10   The dosage effect of N^+ on wing and eye phenotypes of N^{g11}-bearing females.

			PHENOTYPE			
			NUMBER IN EACH CLASS			
GENOTYPE	TEMPERATURE	EYE COLOUR	N^+R*	NR	N^+R^+	NR^+
N^{g11}/+	20.5°C	apricot	179	11	0	0
	29°C	apricot	0	0	7	155
N^{g11}/+;Dp	20.5°C	wild-type	108	0	115	0
	29°C	wild-type	0	0	177	0

*N = one or both wings nicked; N^+ = wings not nicked; R = one or both eyes with extensive facet disarray; R^+ = no extensive eye facet disarray.

female (or in N^{40}/N^{40};$\underline{Dp}$ and N^{40}/N^{40};$\underline{Dp}/\underline{Dp}$ females, Table 4), so it can be assumed that the eye phenotype of N^{g11} is not completely suppressed in the presence of an extra $\underline{N^+}$ locus, in contrast to the complete suppression of the Notch wing phenotype in such cases.

It is instructive to describe the appearance of N^{g11}/Y;$\underline{Dp}$ males. The wings of such males are wild type, with no wing nicking or thickened veins. However, males of this genotype have essentially the same eye phenotype and temperature-sensitivity as N^{g11}/+ females. Thus, in the presence of the duplication, $\underline{N^{g11}}$-bearing males exhibit no wing nicking or thickened wing veins but express the mutant eye phenotype. This is similar to the addition of an extra dose of $\underline{N^+}$ to $\underline{N^{g11}}$-bearing females (Table 10), except that in the females the mutant eye phenotype is considerably diminished.

Frequently, $\underline{N^{g11}}$/Y;$\underline{Dp}/\underline{Dp}$ males are found in stock cultures ($\widehat{XX}$,$\underline{y}$ $\underline{w}$ $\underline{f}$; $\underline{Dp}$ ♀ x $\underline{w^a}$ $\underline{N^{g11}}$ $\underline{rb}$/Y;$\underline{Dp}$ ♂, incubated at 20°C-22°C). Such males are relatively infertile, have Confluens wings, and exhibit a much more regular eye facet pattern than their $\underline{N^{g11}}$/Y; $\underline{Dp}$ brothers. The eyes of such males are not completely wild-type, but since this is also true of N^{40}/Y;$\underline{Dp}/\underline{Dp}$ males, it cannot be ascertained whether the eye facet disruption is due to the presence of $\underline{N^{g11}}$ or to homozygosis of $\underline{Dp}$. However, it is significant that in spite of any irregularities caused by homozygosis for $\underline{Dp}$, increasing the dose of $\underline{N^+}$ decreases the mutant eye phenotype of N^{g11} in males. This is similar to the effect seen in $\underline{N^{g11}}$/+;$\underline{Dp}$ females.

Further studies on the effects of gene dosage on the pheno-
types of N^{g11} have revealed that the addition of an extra dose
of N^{g11} affects the eye and wing phenotypes in opposite direc-
tions and, unexpectedly, that flies with the genotype N^{g11}/N^{g11};
Dp have a temperature-sensitive lethal phenotype (Table 11).
The lethality will be discussed in a later paragraph. Expression
of the mutant eye phenotype in the few N^{g11}/N^{g11};Dp females which
survived at 20.5°C and 22°C was much more extreme than in $N^{g11}/+$
females, in contrast to the reduction of the mutant eye phenotype
caused by the extra N^+ in $N^{g11}/+$;Dp females (Table 10). The eyes
of surviving N^{g11}/N^{g11};Dp females were reduced in size by about
two-thirds and showed an irregular facet pattern over the whole
eye with greatly multiplied numbers of interommatidial setae,
giving a brush-like appearance to the eye surface. Furthermore,
the enhancement of the mutant eye phenotype was apparent in
N^{g11}/N^{g11};Dp females raised at 25°C and 29°C, unlike $N^{g11}/+$
females, which are wild type at these temperatures. In contrast
to the eye phenotypes, expression of the Notch wing phenotype
was reduced in N^{g11}/N^{g11};Dp compared to $N^{g11}/+$, at all tempera-
tures. No thickening could be detected in the wing veins of the
N^{g11}/N^{g11};Dp females recovered at 20°C-22°C, whereas $N^{g11}/+$
females raised at the same temperature have detectable, though
often small thickenings at the tips of the veins (Plate 1b). At
25°C no wing nicking was seen in 43 scorable females, compared
to 10% and 24% of the $N^{g11}/+$ females (Table 9). At 29°C 22%
(10/42 from experiment 1, and 6/32 from experiment 2, Table 11)
of the N^{g11}/N^{g11};Dp females showed wingtip nicking, compared to

TABLE 11   Viability of N^{g11}/N^{g11};Dp and N^{g11}/N^{g11};Dp/Dp females in relation to their siblings, when raised at different temperatures.

| | | PROGENY | | | | | |
| | | FEMALE | | | MALE | | |
CROSS*	TEMPERATURE	M5/N;Dp	M5/N	N/N;Dp**	M5/Y;Dp	M5/Y	N/Y;Dp
1	20.5°C	102	91	1	90	87	92
	29°C	53	53	42	29	85	65
1	20°C	164	158	0	128	157	118
	22°C	168	159	2	173	191	195
	25°C	97	102	44	74	104	101
	29°C	43	35	32	10	40	48
2	20.5°C	131	42	24	83	35	113
	29°C	49	13	37	17	9	57

* 1.   M5/w^a N^{g11} rb ♀ x w^a N^{g11} rb/Y;Dp ♂

   2.   M5/w^a N^{g11} rb;Dp ♀ x w^a N^{g11} rb/Y;Dp ♂
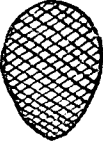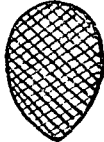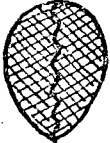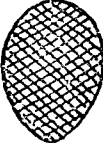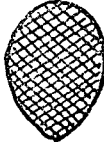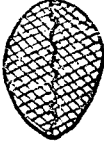
** Includes N/N;Dp/Dp females (cross 2 only).

67% and 76% in the N^{g11}/+ samples (Table 9). Thus it can be seen that increasing the dose of N^{g11} relative to N^+ causes reduction of the Notch wing phenotypes (as observed with N^{103} at 20°C-25°C), whereas expression of the mutant eye phenotype is enhanced.

Not surprisingly, the addition of a second dose of N^+ to flies already carrying 2 doses of N^{g11} and 1 of N^+, causes considerable reduction in the severity of the mutant eye phenotype seen in N^{g11}/N^{g11};Dp females. The eyes of all N^{g11}/N^{g11};Dp/Dp females (cross 3, Table 11) were much less mutant in appearance than those of N^{g11}/N^{g11};Dp females raised at the same temperature, being larger in size and having less severe facet and bristle effects. However, the eyes of N^{g11}/N^{g11};Dp/Dp females were not identical to those of N^{g11}/+, in that they generally had a more mutant facet array and were smaller at all temperatures. The effects of Dp on eye facet pattern, noted earlier, may partly account for the former observation, and inspection of certain other stocks (w^a rb, w^a spl, and w^a spl rb) has suggested that the reduction in eye size may be at least partly related to some factor carried in the rb-containing stocks. Nevertheless, the fact that the N^{g11}/N^{g11};Dp/Dp females have less severely affected eyes than N^{g11}/N^{g11};Dp females, is consistent with the other observations on gene dosage.

A summary of the main effects of gene dosage and temperature on the eye and wing phenotypes of N^{g11}, is presented in Figure 4. It can be seen that the mutant eye phenotype is enhanced both by lower temperatures and by increased N^{g11}:N^+ ratio, whereas the wing nicking phenotype is decreased by these

FIGURE 4   Summary of the effects of relative $\underline{N}:\underline{N}^+$ dosage and

of temperature on the eye and wing phenotypes of

$\underline{N}^{g11}$-bearing flies. The relative size and pattern of the eyes

indicates the relative degree of expression of the mutant eye

phenotypes: ⬚ = wild-type eye facet arrangement; ⬚ = exten-

sive facet disarray; ⬚ = occasional facet disarray. Wing

phenotypes: + = wild-type (no nicks, no thickened wing veins);

.2N, etc., indicates approximate frequency of Notch-winged in-

dividuals having a nick in one or both wing tips; Co = Confluens

wings.

GENOTYPE		EYES			WINGS		
		20 - 22°C	25°C	29°C	20-22°C	25°C	29°C
N^{g11}/N^{g11} ; Dp	♀				+	ON	.2 N
N^{g11}/N^{g11} ; Dp / Dp	♀				+	+	+
$N^{g11}/+$	♀				.02 N	.1-.2 N	.7-.8 N
N^{g11}/Y ; Dp	♂				+	+	+
$N^{g11}/+$; Dp	♀				+	+	+
N^{g11}/Y ; Dp / Dp	♂				Co	Co	Co

conditions. Thus, in terms of wing nicking the N^{g11} allele

behaves more like N^+ (i.e., becomes less hypomorphic) at progres-

sively lower temperatures. On the other hand, in terms of the

eye phenotype N^{g11} becomes progressively more mutant at lower

temperatures and increased N^{g11}:N^+ ratio, a result which is

opposite to the behaviour expected of a hypomorph.

Turning to the lethal phenotype, the data in Table 11

(crosses 1, 2) show that N^{g11}/N^{g11};Dp females usually die at

20°C-22°C but survive at higher temperatures. No significant

pupal mortality was observed in these crosses, indicating that

death of N^{g11}/N^{g11};Dp females occurs at some stage prior to

puparium formation. The possibility that the lethality might be

due to a temperature-sensitive bobbed-lethal (bb^l) allele on the

N^{g11}-bearing chromosome (which would be covered by bb^+ on the Y

chromosome in N^{g11}/Y;Dp males, and therefore would not cause

lethality), was excluded by the absence of lethality in the

cross $1(FM6)/w^e$ bb^l ♀ x w^a N^{g11} rb/Y;Dp ♂(165 w^e bb^l/Y ♂,

150 $1(FM6)/w^a$ N^{g11} rb ♀ , 178 w^e bb^l/w^a N^{g11} rb ♀ , disregarding

Dp). Moreover, the results of experiment 3 (Table 11) suggest

that N^{g11}/N^{g11};Dp/Dp females survive at 20.5°C, which in turn

suggests that the relative dosage of N^{g11} and N^+ determines the

viability in this case. Test matings of 11 fertile females

raised at 20.5°C (cross 3, Table 11) to w/Y males, yielded no

w^a/w female or w^a/Y male progeny (out of 248 female and 98 male

total progeny), confirming that these females were homozygous

for Dp. The lethality of N^{g11}/N^{g11};Dp females at low tempera-

tures contrasts strikingly with the observed viability of

N^{40}/N^{40};Dp (Table 4) and N^{103}/N^{103};Dp (Table 7) flies. Thus, lethality in the presence of N^{g11} is not due to some defect in the N^{+} allele carried by Dp, but to the properties of N^{g11} itself. The results of developmental studies on this lethality will be reported in a later section.

In addition to the phenotypes already described, it was noted that N^{g11}/N^{g11};Dp females raised at 20°C-22°C had certain abnormalities, such as sparse thoracic microchaetae and frequently missing ocellar bristles, generally characteristic of Abruptex mutations. Expression of this phenotype was reduced at 25°C and was absent at 29°C, which is the same response to temperature observed for the eye phenotype. This aspect of N^{g11} will be referred to again.

IV. Effects of gene dosage on the phenotypes of N^{Co}.

The N^{Co} stock used for the present study (w^{a} N^{Co}) was derived by recombination from a w^{a} N^{Co} rb chromosome, after it was discovered that the latter contained both a bb^{1} allele and an enhancer of wing nicking on the X chromosome (Appendix 2). The derived w^{a} N^{Co} stock lacks both the lethal and the modifier.

In a sample of 100 $N^{Co}/+$ females obtained from the cross OR ♀ x w^{a} N^{Co}/Y;Dp ♂ at 22°C, 42% had a nick in one or both wingtips. The non-nicked $N^{Co}/+$ females could be reliably distinguished from their non-nicked $N^{Co}/+$;Dp sisters (97 were examined), which exhibited a characteristic strong Confluens phenotype (compare $N^{Co}/+$ and $N^{Co}/+$;Dp wings in Plate 4), like

PLATE 4   Wings of a) $\underline{N^{Co}}/+$, b) $\underline{N^{Co}}/+;\underline{Dp}$, and c) $\underline{N^{Co}}/\underline{N^{Co}};\underline{Dp}$ females raised at 22°C. Magnification 18x.
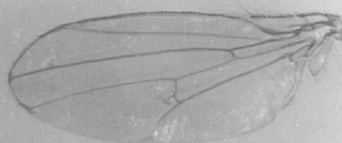
that of N^{Co}/Y;Dp males. The enhancement of the Confluens pheno-
type in the presence of an extra N^+ locus is similar to that
seen in $N^{Co}/Dp(1;1)Co$ flies (WELSHONS 1956a, b), and contrasts
with the reduction of the N^{g11} eye phenotype in the presence of
an extra N^+ locus.

The effect of increasing the N^{Co}:N^+ ratio was determined
from the progeny of the cross: $M5/w^a\ N^{Co}$ ♀ x $w^a\ N^{Co}/Y$;Dp ♂,
at 22°C. The number of N^{Co}/N^{Co};Dp females (36 out of 240 off-
spring, with 40 expected) indicates that N^{Co}/N^{Co};Dp is not a
lethal genotype at 22°C. However, these females were weak,
moving about very slowly and frequently becoming mired in the
food medium. Of 28 flies whose wings could be scored (not mired
in food), 5 (18%) exhibited wingtip nicking, compared to 42% in
the $N^{Co}/+$ females. The Confluens phenotype of these females
was even more extreme than that of $N^{Co}/+$;Dp females (Plate 4),
and the wings themselves were curved downwards and held at right-
angles to the body. Thus, the Confluens phenotype is enhanced
by increasing or decreasing the N^{Co}:N^+ ratio, whereas expression
of the wing nicking phenotype is reduced or abolished. In
addition, the N^{Co}/N^{Co};Dp females had a disrupted eye facet
phenotype not seen in the other N^{Co}-bearing combinations.

To summarize briefly, the observations on the response of
N^{Co} to changes in the N:N^+ ratio suggest that this allele is
hypomorphic in terms of the defect responsible for wing nicking,
but not so far as the Confluens phenotype is concerned. More-
over, the fact that Confluens is known to result from increased
N^+ dosage suggests that N^{Co} may be hypermorphic in this regard,

especially when it is remembered that $\underline{N}^{Co}/Y;\underline{Dp}$ males have a more

extreme phenotype than $\underline{N}^{+}/Y;\underline{Dp}$ males.

Thus, the properties of $\underline{N}^{Co}$ indicate a clear functional distinc-

tion within the Notch locus for the wing nicking and Confluens

phenotypes.

V. The phenotypes of $\underline{N}^{x}/\underline{N}^{y};\underline{Dp}$ combinations.

An examination of the phenotypes of combinations of $\underline{N}^{103}$,

$\underline{N}^{Co}$, and $\underline{N}^{g11}$ with $\underline{N}^{40}$ and $\underline{Dp}$, has yielded results consistent

with the findings of the gene dosage investigations and with the

assumption that $\underline{N}^{40}$ can be considered an amorphic allele. Com-

parison of the wingtip nicking data with those for the respective

$\underline{N}/+$ heterozygotes (compare Table 6 and cross 1, Table 12, and

compare Table 9 and crosses 2 and 3, Table 12) shows that the

frequencies and patterns of temperature sensitivity of wing

nicking in $\underline{N}^{x}/\underline{N}^{40};\underline{Dp}$ females are remarkably similar to those for

$\underline{N}^{x}/+$. The few statistically significant differences are likely

due to differences in genetic background. The frequency of

wingtip nicking of $\underline{N}^{g11}/\underline{N}^{40};\underline{Dp}$ and $\underline{N}^{Co}/\underline{N}^{40};\underline{Dp}$ females from

crosses 4 and 5 (Table 12) is also quite similar to the frequency

among the respective $\underline{N}^{x}/+$ females (note that 81% of 74 $\underline{N}^{Co}/+$

progeny of the cross $\underline{OR}$ ♀ x w^{a} $\underline{N}^{Co}$ rb/Y;$\underline{Dp}$ ♂, had nicked wing-

tips). Furthermore, the disordered eye facet phenotype seen in

$\underline{N}^{g11}/+$ females at low temperature was also expressed in the

$\underline{N}^{g11}/\underline{N}^{40};\underline{Dp}$ females at 20°C-22°C, and the strong wing nicking

and tarsal fusion seen in $\underline{N}^{103}/+$ females at 29°C was expressed

TABLE 12   Number of wings of N^x/N^{40};Dp females exhibiting
            wing nicking when raised at different temperatures.

GENOTYPE	CROSS*	TEMPER -ATURE	% NICKED INDIVID- UALS	MEAN NUMBER OF NICKED WINGTIPS PER FLY (± 95% CONFIDENCE INTERVAL)	NUMBER OF FLIES EXAMINED
N^{103}/N^{40};Dp	1	20°C	82	1.38 ± .14	104
		22°C	73	1.22 ± .18	67
		25°C	99	1.84 ± .07	88
		29°C	100	2.00	72
N^{g11}/N^{40};Dp	2	20°C	7	0.10 ± .09	59
		22°C	7	0.08 ± .08	59
		25°C	38	0.55 ± .18	55
		29°C	82	1.4 ± .3	22
N^{g11}/N^{40};Dp	3	20°C	4	0.04 ± .05	52
		22°C	5	0.05 ± .05	65
		25°C	33	0.40 ± .19	55
		29°C	61	0.8 ± .3	18
N^{g11}/N^{40};Dp	4	20.5°C	9	–	81
		29°C	83	–	86
N^{Co}/N^{40};Dp	5	20.5°C	73	–	45

* 1. $M5/w^a$ N^{40} rb ♀ x $\underline{y}$ w^a N^{103}/Y;Dp ♂

  2. $M5/w^a$ N^{40} rb ♀ x w^a N^{g11} rb/Y;Dp ♂

  3. $M5/w^a$ N^{40} rb;Dp ♀ x w^a N^{g11} rb/B^S w^+.Y ♂

  4. 1(FM6)/w^a N^{g11} rb ♀ x w^a N^{40} rb/Y;Dp ♂

  5. 1(FM6)/w^a N^{Co} rb E-N^{70k27} bb^1 ♀ x w^a N^{40} rb/Y;Dp ♂

in the N^{103}/N^{40};Dp females at this temperature. Also, the wings
of the N^{Co}/N^{40};Dp females were similar to those of N^{Co}/+ in
appearance. The recessive behaviour of N^{40} to the wing nicking,
eye, and leg phenotypes of the other N mutants, and the absence
of a strong Confluens phenotype in N^{Co}/N^{40};Dp females, strongly
supports the assumption that N^{40} can be regarded as an amorphic
allele. Observations made on limited numbers of N^{g11}/N^{8};Dp and
N^{Co}/N^{8};Dp females raised at 20.5°C (24 and 5 flies from the
crosses N^{8}/dl49,y Hw m^{2} ♀ x w^{a} N^{g11} rb/Y;Dp ♂, and N^{8}/dl49,y
Hw m^{2} ♀ x w^{a} N^{Co} rb/Y;Dp ♂respectively), confirmed the reces-
siveness of N^{8} to the N^{g11} eye phenotype, to the wing nicking
phenotypes of both N^{g11} and N^{Co}, and the abolition of the
Confluens phenotype.

Observations on the phenotypes of trans combinations of
the three N mutants with mild wing nicking, confirm and extend
the results obtained in the gene dosage investigations. As
would be predicted if the mild wing nicking of the N/+ hetero-
zygotes reflects hypomorphic activity, no wing nicking was
observed among 89 N^{103}/N^{g11};Dp (22°C), 33 N^{103}/N^{Co};Dp (22°C),
and 213 N^{g11}/N^{Co};Dp (20.5°C)* females, a striking reduction
compared to the respective N/+ heterozygotes. Moreover, the eye

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*Heterologous combinations obtained from the respective crosses:

M5/y w^{a} N^{103} ♀ x w^{a} N^{g11} rb/Y;Dp ♂

M5/y w^{a} N^{103} ♀ x w^{a} N^{Co}/Y;Dp ♂

l(FM6)/w^{a} N^{g11} rb ♀ x w^{a} N^{Co} rb/Y;Dp ♂

phenotype of N^{103}/N^{g11};Dp and the Confluens phenotype of N^{103}/N^{Co};Dp females were intermediate between those of the respective N/+ and N/+;Dp combinations, indicating that N^{103} is intermediate between N^+ and N^8 or N^{40} in these respects also. The eye and wing vein phenotypes of N^{g11}/N^{Co};Dp, on the other hand, were essentially identical to those of the respective N/+ heterozygotes, suggesting that N^{g11} is amorphic or at least very hypomorphic in terms of the Confluens function, and likewise N^{Co} in terms of the mutant eye function, even though both alleles have considerable activity in the function whose absence causes extensive wing nicking. Observations such as these, combined with the results of the gene dosage investigation, support the notion that mutations at the Notch locus may only affect certain of the functions of this locus as determined phenotypically. In fact, it appears that a single mutation can affect different Notch-locus functions in fundamentally different ways, as exemplified by the wing nicking and Confluens phenotypes of N^{Co}.

B. ORIGIN AND MAPPING OF THE ABRUPTEX MUTATIONS

Four of the five $\underline{Ax}$ alleles studied ($\underline{Ax^{E1}}$, $\underline{Ax^{E2}}$, $\underline{Ax^{16172}}$

and $\underline{Ax^{9B2}}$) have been examined in detail with respect to the

phenotypes of the wing veins and of certain bristles. The first

two of these mutants were recovered among the progeny of ethyl

methanesulfonate (EMS)-treated $\underline{OR}$ males in this laboratory

(Appendix 3), and $\underline{Ax^{16172}}$ and $\underline{Ax^{9B2}}$ were also EMS-induced

(LEFEVRE, WELSHONS, personal communications). $\underline{Ax^{E1}}$ is semilethal

as a hemi- or homozygote and in $\underline{trans}$ heterozygous combinations

with most $\underline{N}$ mutants. The few individuals which do manage to

eclose are very weak and usually become mired in the food medium

shortly after hatching. $\underline{Ax^{E2}}$ is not as extreme as $\underline{Ax^{E1}}$ and is

both viable and fertile as a hemi- and homozygote. The $\underline{Ax}$

alleles, $\underline{Ax^{16172}}$, $\underline{Ax^{9B2}}$ and $\underline{Ax^{59d}}$, were kindly supplied by

Dr. W. J. WELSHONS. $\underline{Ax^{16172}}$ and $\underline{Ax^{9B2}}$ are viable in the hemi-

zygous and homozygous condition, although homozygous $\underline{Ax^{9B2}}$

females are poorly fertile and this stock is kept by crossing

hemizygous $\underline{Ax}$/Y males to compound-X females. The fifth mutant,

$\underline{Ax^{59d}}$, which was not examined in great detail, was maintained

in $\underline{cis}$ combination with the recessive visible mutant $\underline{fa^g}$.

$\underline{Ax^{59d}}$ is almost a complete recessive lethal, has cytologically

normal salivary gland chromosomes, and has been mapped genetical-

ly within the Notch locus between $\underline{spl}$ and $\underline{N^{Co}}$ (WELSHONS 1971).

The salivary gland chromosomes of $\underline{Ax^{E1}}$, $\underline{Ax^{E2}}$, $\underline{Ax^{9B2}}$, and $\underline{Ax^{16172}}$

have also been examined cytologically, and all appear to be

normal in the Notch region of the X chromosome (KAUFMAN, person-

al communication). In addition, as will be described below, Ax^{E1}, Ax^{E2}, and Ax^{9B2} have been mapped genetically at sites within the Notch locus.

In the mapping of Ax^{E1}, advantage was taken of the facts that Ax^{E1} is viable and fertile when heterozygous with the non-Abruptex recessive visible mutations in the Notch locus, and that the Ax^{E1}/N and fa^{no}/N genotypes are lethal. Thus, in the cross $w^a\ fa^{no}\ spl\ rb/w^+\ Ax^{E1}\ rb^+$ ♀ x $w^a\ N^{40}\ rb/B^S\ w^+.Y$ ♂, the only surviving female progeny should be non-disjunctants, "break-through" Ax^{E1}/N and fa^{no}/N females, or $fa^{no+}\ Ax^+$ crossovers between fa^{no} and Ax^{E1}. The results of two such crosses are presented in Table 13. It can be seen that there were appreciable numbers of surviving Ax^{E1}/N and $fa^{no}\ spl/N$ females, which were very weak and sterile and could easily be recognized phenotypically. The B^S class of females results from non-disjunction of the maternal X chromosomes. The $w^+\ rb^+$ class of exceptions was unexpected, since these would at first appear to be recombinants within the Notch locus unaccompanied by recombination for the closely linked flanking eye-colour markers. However, some of these females differed from wild type in that they were Abruptex-like (wing vein gaps) or had nicked wings. Seven of the exceptional females survived long enough to be test-crossed to $w^a\ spl\ rb$ males, and five of these crosses yielded progeny. The results of these and subsequent crosses, which are recorded in Appendix 5, indicated that the $w^+\ rb^+$ exceptions were not the products of recombination, but were triploid females. The pertinent observations supporting this conclusion are that:

TABLE 13   Results of cross for the genetic localization of Ax^{El}.

SERIES*	NUMBER OF MALE PROGENY	GENOTYPE OF SURVIVING FEMALE PROGENY				
		$\underline{w^+ \; spl \; rb}$	$\underline{w^+ \; rb^+}$	$\underline{B^s}$	$\underline{N/Ax^{El}}$	$\underline{N/fa^{no} \; spl}$
1	46,986	9	6	31	160	17
2	11,542	5	2	25	184	10
TOTALS	58,528	14	8	56	344	27

* Series 1: 24 cultures in half-pint bottles, 25-30 females per culture; mostly 3 day broods but some were longer (5-6 days); total eglaying period 24-28 days.

  Series 2: 40 cultures in quarter-pint bottles, with 5 sets of 8 bottles each having 1, 2, 3, 4, or 5 parent females, respectively. Neither the total number of progeny nor the ratio of $rb:rb^+$ male progeny showed any effects of increasing numbers of females, so the data were pooled. Eggs were collected in 3 day broods for a total of 6 broods.

1) these females were poorly fertile compared to the w^+ spl rb

females, and they yielded male, female and sterile intersex

progeny; 2) some of the F_1 female progeny from the test crosses

were also semisterile and yielded intersexes, while others were

fully fertile and yielded only male and female progeny; 3) from

the phenotypes of the exceptional females and those of their

progeny and one subsequent generation, at least four of the

original fertile w^+ rb^+ females must have carried the chromosomes

w^a fa^{no} spl rb, w^a N^{40} rb, and w^+ Ax^{E1} rb^+. All the w^+ spl rb

female exceptions tested (see Appendix 5) behaved like normal

diploids and passed the w^+ spl rb chromosome to their progeny.

These represent true crossovers between fa^{no} and Ax^{E1}, and posi-

tion Ax^{E1} to the right of fa^{no}. From these data no decision can

be made as to the position of Ax^{E1} with respect to spl, although

it would seem that if Ax^{E1} is to the right of spl, the two

mutants must be extremely closely linked. This is based on the

fact that 14 crossovers between fa^{no} and Ax^{E1} were recovered,

but none between Ax^{E1} and spl. The frequency of recombination

between fa^{no} and Ax^{E1} (Table 13) is 0.05%, which is greater than

the map distance between fa^{no} and spl (0.03%) recorded by WEL-

SHONS (1958). Unfortunately, the present results and the data

of WELSHONS cannot be compared in order to position Ax^{E1} with

respect to spl, since genetic background, temperature and other

culture conditions were likely different in the two investiga-

tions. Nevertheless, it is probably safe to conclude that Ax^{E1}

maps within the Notch locus, close to or at spl.

In order to map Ax^{E2} with respect to other mutants at the

Notch locus, all of the male progeny of $\underline{w^a}$ $\underline{fa^{no}}$ $\underline{spl}$ $\underline{rb}/\underline{w^+}$ $\underline{Ax^{E2}}$ $\underline{rb^+}$ females were scored for their visible phenotypes. The results of two experiments are presented in Table 14. For positioning $\underline{Ax^{E2}}$, the relevant recombinants recovered were the $\underline{w^a}$ $\underline{fa^{no}}$ $\underline{Ax^{E2}}$, $\underline{spl}$ $\underline{rb}$, $\underline{w^a}$ $\underline{fa^{no}}$ $\underline{spl}$ $\underline{Ax^{E2}}$, and $\underline{rb}$ classes, which place $\underline{Ax^{E2}}$ to the right of $\underline{spl}$ (see Appendix 6 for progeny tests of these recombinants). When the double crossover classes $\underline{w^a}$ $\underline{fa^{no}}$ $\underline{rb}$ and $\underline{fa^{no}}$ $\underline{Ax^{E2}}$ are included, the genetic map distance between $\underline{fa^{no}}$ and $\underline{spl}$ is calculated to be 0.05 unit and between $\underline{spl}$ and $\underline{Ax^{E2}}$ 0.01 unit. This observation, combined with the fact that $\underline{spl}$ lies approximately equidistant between $\underline{fa^{no}}$ and $\underline{nd}$ in WELSHONS' map (1965) suggests strongly that $\underline{Ax^{E2}}$ lies within the presently defined limits of the Notch locus. It should be noted at this point that when linked in the $\underline{cis}$ position, $\underline{fa^{no}}$ completely suppresses the wing vein but not the bristle pheno-types of $\underline{Ax^{E2}}$. This and other interactions of $\underline{Ax^{E2}}$ with $\underline{fa^{no}}$ and $\underline{spl}$ are described briefly in Appendices 6, 7.

Although $\underline{Ax^{16172}}$ was not mapped extensively, the available data suggest that this mutant probably also maps within the Notch locus. Use was made of the discovery that both the $\underline{Ax^{E2}}/\underline{Ax^{9B2}}$ and $\underline{Ax^{16172}}/\underline{Ax^{9B2}}$ combinations are lethal, while $\underline{Ax^{E2}}/\underline{Ax^{16172}}$ females are both viable and fertile. Thus, in the cross $\underline{w^a}$ $\underline{Ax^{E2}}$ $\underline{rb}/\underline{w^+}$ $\underline{Ax^{16172}}$ $\underline{rb^+}$ ♀ x $\underline{w^a}$ $\underline{Ax^{9B2}}$ $\underline{rb}/Y$ ♂, the only surviving female progeny should be $\underline{Ax^+}$ recombinants or non-disjunctants, whereas all males should survive. In one experi-ment of this kind 12,873 males were recovered and no recombinant females were observed. Crossing over in the $\underline{w^a}$-$\underline{Ax}$ and $\underline{Ax}$-$\underline{rb}$

TABLE 14   Results of crosses for the genetic localization of Ax^{E2}.

MALE PROGENY GENOTYPES	CROSS*		PERCENT CROSSOVERS
	1	2	
w^a fa^{no} spl rb	8,185	8,295	
+ Ax^{E2} +	8,052	8,045	
w^a Ax^{E2} +	179	130)	
+ fa^{no} spl rb	164	150)	1.77
w^a fa^{no} Ax^{E2} +	3	3)	
+ spl rb	2	7)	0.05**
w^a fa^{no} spl Ax^{E2} +	0	2)	
+ + rb	1	0)	0.01***
w^a fa^{no} spl +	493	464)	
+ Ax^{E2} rb	609	463)	5.76
w^a fa^{no} rb	0	1	
+ fa^{no} Ax^{E2} +	1	0	
TOTALS	17,689	17,560	

* 1. w^a fa^{no} spl rb/Ax^{E2} ♀ x y w^a/Y ♂

2. w^a fa^{no} spl rb/Ax^{E2} ♀ x w^a fa^{no} spl rb/Y ♂

Cross 1 consisted of 12 cultures, cross 2 of 18, in half-pint bottles, 5 pairs of parents per bottle. Eggs were collected in 3-5 day broods over a total period of 27 days.

** Includes double crossovers w^a fa^{no} rb and + fa^{no} Ax^{E2} +

*** Includes double crossover w^a fa^{no} rb

regions as observed in the males occurred with normal frequencies. This indicates that Ax^{E2} and Ax^{16172} must be situated close to one another, probably within the Notch locus. The lethal inter-actions mentioned above will be described more fully in a later section.

The results of two crosses used to map Ax^{9B2} are reported in Table 15. The five Ax-N recombinants recovered place Ax^{9B2} between N^{40} and N^{Co}. The observed frequencies of crossing over within the Notch locus (0.09% between both N^{40} and Ax^{9B2}, and Ax^{9B2} and N^{Co}) were rather high when compared with the 0.03-0.04% between N^{40} and N^{Co} observed by WELSHONS (1958b), although recombination with the flanking markers w^{a} and rb occurred with near-normal frequencies (Table 15). This could reflect reduced viability of Ax^{9B2} males compared to Ax^{+}, especially when one considers the possible interaction of Ax^{9B2} with genetic modi-fiers, which undoubtedly exist in both N stocks and in the w^{a} fa^{no} spl Ax^{E2} rb stock. Nevertheless, the available crossover data and the origin of Ax^{9B2}, indicate that Ax^{9B2} is a point mutant which maps within the Notch locus. Thus the genetic data strongly suggest the inclusion of all four Ax alleles tested within the limits of the Notch locus.

TABLE 15   Results of crosses for the genetic localization of Ax^{9B2}.

PROGENY CROSSOVER CLASS	CROSS*					
	1			2		
	GENOTYPE	NUMBER	%	GENOTYPE	NUMBER	%
	+ Ax +	3017	–	+ Ax rb	2259	–
w^a–Ax	w^a Ax +	55	1.72	w^a Ax rb	28	1.24
Ax–rb	+ Ax rb	125	3.91	+ Ax +	91	4.02
Ax–N	+ + rb	3	0.09	w^a + rb	2	0.09

* Male progeny of crosses:

1. w^a N^{40} rb/+Ax^{9B2} + ♀ x w^a fa^{no} spl Ax^{E2} rb/Y ♂

2. w^a N^{Co} +/+Ax^{9B2} rb ♀ x w^a fa^{no} spl Ax^{E2} rb/Y ♂

Cultures were incubated at 22°C in quarter-pint bottles (11 bottles, cross 1; 20 bottles, cross 2), with 5-8 female parents per bottle. Since fertility was variable, especially in cross 2, brood length was adjusted (3-7 days) to increase the number of progeny in some cultures. Total egg collection period was 10-15 days.

C. PHENOTYPES OF ABRUPTEX MUTATIONS

I. Ax^{E1}.

The most conspicuous phenotypic features of Ax^{E1}/+ females
raised at 20.5°C, are the presence of terminal gaps in at least
one of the L5 wing veins in nearly all individuals and the high
incidence of missing ocellar and postvertical bristles, the post-
verticals generally being more strongly affected than the
ocellars (consult Figure 3 for the positions of wing veins and
bristles scored). Other bristles are not affected so strongly,
although the postorbital setae and thoracic microchaetae are
noticeably sparser and less regularly arrayed than in OR flies.
Sample wing vein gap and ocellar and postvertical bristle counts
of Ax^{E1}-bearing flies are presented in Table 16 (see Appendix 8
for sample bristle counts of OR flies). The females in lines 1
and 2 are genotypically similar with respect to Ax^{E1} but derived
from different crosses. Thus, the slight differences in values
probably reflect background genotypic variation. It can be seen
that in the Ax^{E1}/+ heterozygotes, the numbers of postvertical
and ocellar bristles are reduced and the incidence of wing vein
gaps is high. Addition of an N^+ locus (compare sibs in lines 2
and 3) strikingly increases ocellar bristle number and reduces
the number showing postvertical bristle or vein gap phenotypes.

By comparing lines 2 and 4 (remembering that the back-
ground genotypes are different), the effect of autosomal inser-
tion of the Notch locus can be measured since N^8 is a deletion

TABLE 16   Counts of ocellar and postvertical bristles and wing vein gaps in various combinations of Ax^{El} at 20.5°C.

GENOTYPE	CROSS*	NUMBER OF OCELLARS			NUMBER OF POSTVERTICALS			WING VEIN GAPS** 1 OR MORE		
		0	1	2	0	1	2	0	L5	L4 & L5
$Ax^{El}/+$ ♀	1	55	34	8	94	3	0	4	89	4
$Ax^{El}/+$ ♀	2	72	16	2	72	9	9	0	75	15
$Ax^{El}/+$;Dp ♀	2	1	5	88	32	29	33	46	48	0
Ax^{El}/N^8;Dp ♀	3	15	2	2	19	0	0	0	18	1
Ax^{El}/N^{40};Dp ♀	4	62	10	1	62	8	3	0	73	-
Ax^{El}/Y;Dp♂	4	60	7	1	68	0	0	11	57	-

* 1. OR ♀ x Ax^{El}/B^S w^+.Y ♂

2. w^a rb/w^a rb ♀ x w^a Ax^{El}/Y;Dp♂

3. N^8/dl-49,y Hw m^2 ♀ x w^a Ax^{El}/Y;Dp♂

4. l(FM6)/Ax^{El} ♀ x w^a N^{40} rb/Y;Dp♂

** Scoring of wing vein gaps: 1) progeny of crosses 1-3 were scored either for no gaps, or for gaps in L5 only (1 or both wings), or for gaps L4 and L5 (1 or both wings); 2) progeny of cross 4 were only scored for the absence or presence of wing vein gaps.

of the entire locus. In general, the two sets of results are similar in that vein gapping is increased and the bristle numbers decreased. The point mutant N^{40} (line 5) interacts similarly to N^8, thereby behaving as an amorph with respect to its phenotypic interaction with Ax^{El}. In terms of both allelic dosage and phenotype the Ax^{El}/Y;Dp males (line 6) are similar to the females in lines 1 and 2.

In contrast to the results with N^8 and N^{40}, 92 Ax^{El}/N^{Co};Dp females from the cross $1(FM6)/Ax^{El}$ ♀ x w^a N^{Co} rb/Y;Dp ♂ exhibited no wing vein gaps. Thus, N^{Co} behaves like a dose of N^+ in terms of its morphological interaction with Ax^{El}.

Death of flies hemizygous or homozygous for Ax^{El} occurs in the late pupa or partially-eclosed adult stages with the few "escapers" rapidly becoming mired in the food. By placing the culture bottles on their sides, emerging adults could be prevented from falling into the medium, and specimens thus be obtained for examination. Such flies have deformed wings and crippled legs and usually die within three days. Although the genital systems of Ax^{El}/Y males are normal and motile sperm are produced (KAUFMAN, personal communication), they made no attempt at copulation when placed with aged virgin wild-type females. The bristle data for Ax^{El}/Y hemizygotes presented in Table 17 show that none of the males examined possessed ocellars, postverticals, or anterior scutellars, and that the dorsocentrals were usually entirely missing. Orbitals and posterior scutellars were reduced in frequency by one-third to one-half, and about two-thirds, respectively. In contrast to the drastic reduction in

TABLE 17   Counts of bristles in eclosed Ax^{E1}/Y males*
raised at 20.5°C.

TYPE OF BRISTLE	NUMBER OF FLIES IN EACH PHENOTYPIC CLASS							MEAN NUMBER OF BRISTLES PER FLY (± 95% CONFIDENCE INTERVAL)
	0	1	2	3	4	5	6	
ORBITALS	0	0	9	32	36	10	1	3.57 ± .16
OCELLARS	88	0	0	–	–	–	–	0.00
POSTVERTICALS	88	0	0	–	–	–	–	0.00
VERTICALS	0	0	0	1	84	–	–	3.99 ± .02
DORSOCENTRALS	78	6	1	0	0	–	–	0.09 ± .06
ANTERIOR SCUTELLARS	85	0	0	–	–	–	–	0.00
POSTERIOR SCUTELLARS	37	35	13	–	–	–	–	0.72 ± .14

* Data are the pooled observations on Ax^{E1}/Y male progeny which
survived in 5 crosses performed for other purposes. The
general format of these crosses was l(FM6)/Ax^{E1} x "x", where
"x" was spl Ax^{E2}, fa^{no} Ax^{E2}, fa^{no} spl Ax^{E2}, two fa^{no} Ax^{E2}
recombinants derived independently, and Ax^{9B2}.

numbers of the other bristles examined, the verticals were pre-
sent in wild-type frequency. Homozygous $\underline{Ax^{E1}}$ females (which
occasionally eclosed in $\underline{l(FM6)/Ax^{E1}}$ ♀ x $\underline{Ax^{E1}/B^S}$ $\underline{w^+}$.Y ♂ stock
cultures) were not examined in detail, but their phenotype was
generally like that of the males. Wing vein gap frequencies
could not be obtained in these flies owing to the extreme deform-
ity of the wings, but little wing venation and no wing nicking
was observed in a few individuals whose wings were sufficiently
extended to permit examination.

II. $\underline{Ax^{E2}}$.

The most characteristic features of $\underline{Ax^{E2}}$ hemi- and homozy-
gotes are the nearly complete absence of anterior orbital
bristles and the presence of terminal gaps in the L5 (and often
L4) wing veins (see Figure 3). Other bristles are also affected,
but to a lesser extent. The results of sample bristle and wing
vein gap counts of hemizygous and homozygous $\underline{Ax^{E2}}$ individuals
raised at 22°C and 29°C are summarized in Table 18. It can be
seen that at 22°C the number of orbital bristles was reduced by
one-third, while the verticals, postverticals, dorsocentrals, and
scutellars were present in nearly wild-type frequencies. The
ocellar bristles were slightly reduced in numbers at the low
temperatures, and there was a small but significant sex-differ-
ence in ocellar frequency, females having a lower frequency than
males. At 22°C every $\underline{Ax^{E2}}$ hemi- or homozygote had gaps in from
two to four wing veins, with no significant differences between

TABLE 18 Summary of the bristle and wing vein gap phenotypes of Ax^{E2} * #

GENOTYPE	ORBITALS		OCELLARS		POSTVERTICALS		VERTICALS	
	22°C	29°C	22°C	29°C	22°C	29°C	22°C	29°C
Ax^{E2}/Y ♂	0.66	0.64^T	1.00	0.46^T	1.00	0.38^T	1.00	0.99^T
Ax^{E2}/Ax^{E2} ♀	0.67	0.62^T	0.97	0.15^{TS}	1.00	0.14^{TS}	1.00	0.99
$Ax^{E2}/+$ ♀	0.99^H	0.95^{TH}	1.00	0.95^{TH}	1.00	1.00^H	1.00	1.00

| GENOTYPE | DORSOCENTRALS | | SCUTELLARS | | | | WING VEINS | |
			ANTERIOR		POSTERIOR			
	22°C	29°C	22°C	29°C	22°C	29°C	22°C	29°C
Ax^{E2}/Y ♂	1.00	0.89^T	1.00	0.74^T	1.00	0.97^T	0.72	0.39^T
Ax^{E2}/Ax^{E2} ♀	1.00	0.91^T	1.00	0.86^{TS}	1.00	0.97^T	0.71	0.54^{TS}
$Ax^{E2}/+$ ♀	1.00	0.97^{TH}	1.00	1.00^H	1.00	1.00^H	0.88^H	0.81^{TH}

* The numbers presented represent the "index of phenotypic expression" of the bristle or wing vein phenotypes, with 1.00 equal to wild-type, and smaller numbers indicating progressively more severe expression of the mutant phenotypes. See Methods and Materials for the formulae which define "index of phenotypic expression" for both bristles and wing vein gaps.

See Appendix 8 for actual counts of bristles and wing vein gaps.

T Statistically significant differences between the 29°C and the 22°C frequency.

S Statistically significant difference between homozygous females and hemizygous males at this temperature.

H Statistically significant difference between heterozygous and homozygous females at this temperature.

the sexes. At 29°C the frequencies of all the bristles except
the verticals were reduced, the ocellar bristle sex-difference
was much more pronounced than at lower temperatures, and two
other bristles, (postverticals and anterior scutellars) showed
significant sex-differences which were not apparent at lower
temperatures. It is also noteworthy that while the ocellar and
postvertical frequencies were lower in females than in males,
the anterior scutellar frequency was higher in females. The
frequency of wing vein gaps increased significantly at 29°C,
and here also there was a sex-difference, the males having more
gaps than the females. Thus, females were more mutant than
males with respect to the ocellar and postvertical phenotypes,
but less mutant than males with respect to the anterior scutellar
and wing vein gap phenotypes.

The last line of Table 18 summarizes the wing vein and
bristle data obtained from Ax^{E2}/+ heterozygotes at 22°C and 29°C.
All mutant phenotypes were greatly reduced compared to those of
the homozygotes at each temperature, and temperature sensitivity
was only detectable for the orbitals, ocellars, dorsocentrals,
and wing vein gaps.

III. Ax^{16172}.

The characteristic appearance of Ax^{16172} hemi- and homo-
zygotes is one of markedly reduced head and thoracic bristle
frequencies, and extensive gaps in wing venation. These pheno-
types are much more extreme than those of Ax^{E2}. In addition,

the wings of Ax^{16172} often are curved or drooped downwards,
deformed at the bases, and frequently contain prominent bubbles,
and the eyes frequently contain regions of ommatidial disruption
or are irregular in outline.

The results of bristle and wing vein gap counts of Ax^{16172}
males and females raised at 22°C and 29°C are summarized in
Table 19. In the samples examined no postvertical and no or very
few ocellar bristles appeared in either sex at either temperature.
There appeared to be a slight increase in ocellar frequency at
29°C, the difference between the 22°C males and 29°C males being
significant at the 95% level. Other than this, the ocellar,
postvertical, and vertical bristle frequencies were not affected
by either sex or temperature. The frequencies of the other
bristles examined (orbitals, dorsocentrals, and scutellars), and
of wing vein gaps, were affected significantly by both tempera-
ture and sex in the presence of Ax^{16172}. However, it can be
seen that there did not appear to be a consistent pattern, either
of temperature sensitivity or of sexual dimorphism, except that
temperature sensitivity, if it occurred in both sexes, was
always in the same direction in males and females for a given
phene. The orbital frequency was increased in both sexes at
29°C compared to the lower temperatures, and in both cases the
frequency was significantly lower in females than in males. It
should be noted here than in an earlier sample of Ax^{16172} flies
raised at 20.5°C, the frequency of wing vein gapping and loss of
certain bristles differed from the present sample (Appendix 9).
Since the two sets of data were obtained at different times, the

TABLE 19   Summary of the bristle and wing vein gap phenotypes of Ax^{16172} * #

GENOTYPE	ORBITALS		OCELLARS		POSTVERTICALS		VERTICALS	
	22°C	29°C	22°C	29°C	22°C	29°C	22°C	29°C
Ax^{16172}/Y ♂	0.26	0.53^T	0.00	0.03	0.00	0.00	0.97	0.98
Ax^{16172}/Ax^{16172} ♀	0.16^S	0.42^{TS}	0.00	0.01	0.00	0.00	0.98	0.99
Ax^{16172}/+ ♀	0.68^H	0.70^H	0.80^H	0.39^{TH}	0.43^H	0.31^H	1.00^H	1.00^H

GENOTYPE	DORSOCENTRALS		SCUTELLARS				WING VEINS	
			ANTERIOR		POSTERIOR			
	22°C	29°C	22°C	29°C	22°C	29°C	22°C	29°C
Ax^{16172}/Y ♂	0.76	0.38^T	0.35	0.14^T	0.90	0.37^T	0.17	0.14
Ax^{16172}/Ax^{16172} ♀	0.68^S	0.20^{TS}	0.56^S	0.24^{TS}	0.96	0.49^{TS}	0.44^S	0.33^{TS}
Ax^{16172}/+ ♀	1.00^H	0.99^H	1.00^H	0.94^{TH}	1.00^H	1.00^H	0.71^H	0.58^{TH}

* See footnotes to Table 18.

See Appendix 9 for actual counts of bristles and wing vein gaps.

difference could result from modifying genes accumulated during the interval. The data for Ax^{16172}/+ heterozygotes (Table 19) show that the expression of the Abruptex phenotype was much milder in heterozygous than in homozygous females, and that where temperature differences existed in both homozygotes and heterozygotes, they were in the same direction. The data for Ax^{16172} can be summarized briefly as follows: 1) the higher temperature pushes the frequency of the orbital bristles closer to wild type, whereas the frequencies of dorsocentrals, scutellars, and wing vein gaps become more mutant at 29°C; 2) females are more mutant than males in terms of the orbital and dorsocentral frequencies, whereas males are more mutant than females with regard to the scutellar frequencies and wing vein gap frequencies; and 3) expression of all the mutant phenotypes of Ax^{16172} is reduced in Ax/+ heterozygotes compared to homozygotes.

IV. Ax^{9B2}.

The characteristic features of Ax^{9B2} hemi- and homozygotes are marked reductions in the frequencies of ocellar, postvertical, and dorsocentral bristles. Other bristles are usually present in near-wild-type frequencies, although the anterior scutellars are often missing. The extent of wing vein interruptions at 22°C range from mild to moderate, often overlapping wild-type. At higher temperatures occasional nicks appear in the wing tips.

The results of bristle, wing-vein gap, and wing nick counts

of Ax^{9B2} hemi- and homozygotes are summarized in Table 20. It

can be seen that at the lower temperature, homozygous females

had higher frequencies of dorsocentrals and posterior scutellars

than males. No significant sex differences were seen for the

other bristles or for the wing vein gaps at low temperatures. In

another sample of Ax^{9B2} males and females raised at 20.5°C, the

frequencies of certain bristles differed significantly from the

present 22°C sample and there was a sex-difference in the post-

vertical but not the posterior scutellar frequency (Appendix 10).

These differences likely result from genetic background differ-

ences, since the two sets of data were obtained from different

crosses (see Appendix 10). Comparing the 22°C and 29°C data, it

can be seen that at the higher temperature, the frequencies of

postvertical and dorsocentral bristles and of wing vein inter-

ruptions were significantly raised, whereas the frequency of

posterior scutellar bristles was lowered. Also note that at

29°C but not at 22°C, a few flies (which were included in the

"6" class, Appendix 10) possessed an extra median orbital

bristle. No sex-differences were observed at 29°C, but this may

only be due to the small number of individuals examined. Ap-

parently an autosomal temperature-sensitive modifier of Ax which

resulted in lethality of Ax^{9B2} hemi- and homozygotes at 29°C,

was present in the Ax^{9B2} stock. This is inferred from the re-

sults of certain crosses performed to investigate lethal inter-

actions among Abruptex mutants (see Table 30).

The data for $Ax^{9B2}/+$ females (Table 20) reveal an interest-

ing contrast between the orbitals and the other bristles. The

TABLE 20   Summary of the bristle and wing vein gap phenotypes of Ax^{9B2} * #

GENOTYPE	ORBITALS		OCELLARS		POSTVERTICALS		VERTICALS	
	22°C	29°C	22°C	29°C	22°C	29°C	22°C	29°C
Ax^{9B2}/Y ♂	0.99	0.99	0.00	0.04	0.00	0.22[T]	0.99	0.99
Ax^{9B2}/Ax^{9B2} ♀	0.99	0.99	0.01	0.00	0.00	0.20[T]	0.99	1.00
Ax^{9B2}/+ ♀	0.91[H]	–	0.06[H]	–	0.01	–	1.00	–

| GENOTYPE | DORSOCENTRALS | | SCUTELLARS | | | | WING VEINS | |
			ANTERIOR		POSTERIOR			
	22°C	29°C	22°C	29°C	22°C	29°C	22°C	29°C
Ax^{9B2}/Y ♂	0.27	0.48[T]	0.79	0.69	0.92	0.77[T]	0.78	0.51[T]
Ax^{9B2}/Ax^{9B2} ♀	0.35[S]	0.50[T]	0.84	0.74	0.99[S]	0.85[T]	0.79	0.45[T]
Ax^{9B2}/+ ♀	0.80[H]	–	1.00[H]	–	1.00	–	0.99[H]	–

* See footnotes to Table 18.

\# See Appendix 10 for actual counts of bristles and wing vein gaps.

orbital frequency in the Ax^{9B2}/+ females was significantly lower (more mutant) than in homozygous females, whereas the other bristle frequencies were significantly higher or unchanged in the heterozygotes. It is possible that this sort of observation might result from heterozygosis of a recessive modifier of Ax caused by outcrossing the stock, but such a modifier would have to be specific for the orbital effect of Ax^{9B2}.

A summary of the phenotypic differences between the three viable Ax mutant strains is presented in Table 21. Comparison reveals that besides the obvious quantitative differences, there appear to be qualitative differences between Ax^{9B2}, on the one hand, and Ax^{E2} and Ax^{16172}, on the other. This is particularly obvious with Ax^{E2} and Ax^{9B2}, since Ax^{E2} has a fully penetrant orbital phenotype, with only minor loss of other bristles, whereas Ax^{9B2} has fully penetrant expression of ocellar, post-vertical, and dorsocentral phenotypes, with only minor orbital loss. Furthermore, in both Ax^{E2} and Ax^{16172} there were instances of females being either more or less mutant than males for a given phene, whereas in Ax^{9B2} females were less mutant than males in every case where a significant sex-difference appeared (Table 22). As will become apparent in the results to follow, these observations are not the only manifestation of what must be basic differences between Ax^{9B2} and the other two Ax mutants.

TABLE 21   Comparison of the bristle and wing vein phenotypes
of the viable <u>Ax</u> alleles at 22°C.

	Ax^{E2}	Ax^{16172}	Ax^{9B2}
ORBITALS	XX	XX	O
OCELLARS	O	XX	XX
POSTVERTICALS	O	XX	XX
DORSOCENTRALS	O	X	XX
ANTERIOR SCUTELLARS	O	X	X
WING VEIN GAPS	XX	XX	X

O = no or only mild expression of mutant phenotype.

X = moderate mutant expression; penetrance not complete.

XX = complete penetrance of mutant phenotype (i.e., no
individuals possess the wild-type number of bristles).

TABLE 22   Summary of statistically significant sex differ-
            ences in bristle and wing vein gap frequencies
            observed in the viable Ax strains.

	Ax^{E2}	Ax^{16172}	Ax^{9B2}
ORBITALS		♀ > ♂ *	
OCELLARS	♀ > ♂		
POSTVERTICALS	♀ > ♂		♀ < ♂
DORSOCENTRALS		♀ > ♂	♀ < ♂
ANTERIOR SCUTELLARS	♀ < ♂	♀ < ♂	
POSTERIOR SCUTELLARS		♀ < ♂	♀ < ♂
WING VEIN GAPS	♀ < ♂	♀ < ♂	

*   ♀ > ♂ = females more strongly mutant than males.

    ♀ < ♂ = females less mutant than males.

D. INTERACTIONS OF NOTCH AND ABRUPTEX MUTATIONS

I. Viability of $\underline{N}/\underline{Ax}$ heterozygotes.

The viability data for the heterozygous $\underline{N}/\underline{Ax}$ combinations examined are summarized in Table 23. As WELSHONS (1971) reported, all of the combinations of $\underline{Ax}^{59d}$ with $\underline{N}$ alleles were lethal. However, $\underline{Ax}^{E1}$, which was originally detected on the basis of its lethality with $\underline{N}^{40}$, was lethal when heterozygous with $\underline{N}^{g11}$, $\underline{N}^{Co}$, and $\underline{N}^{8}$, but viable with $\underline{N}^{103}$ at 20°C-22°C. Death of $\underline{Ax}^{59d}/\underline{N}$ and $\underline{Ax}^{E1}/\underline{N}$ females occurs mainly in the pupal stages, as is the case with $\underline{Ax}^{59d}$ and $\underline{Ax}^{E1}$ hemizygotes and homozygotes. In contrast to these two mutants, $\underline{Ax}^{E2}$, $\underline{Ax}^{16172}$, and $\underline{Ax}^{9B2}$, which survive as hemi- and homozygotes, were viable as $\underline{Ax}/\underline{N}$ heterozygotes at 20.5°C-22°C, although there did appear to be some reduced survival of $\underline{Ax}^{E2}/\underline{N}^{40}$, $\underline{Ax}^{E2}/\underline{N}^{Co}$, and $\underline{Ax}^{16172}/\underline{N}^{103}$ flies. The mortality of the latter combinations did not appear to be correlated with a noticeable incidence of pupal lethality. At 29°C, on the other hand, most of the $\underline{Ax}/\underline{N}$ combinations, particularly those involving $\underline{Ax}^{16172}$, were poorly viable, the reduced survival being associated with a corresponding increase in pupal death. The relatively greater lethality of $\underline{Ax}^{16172}/\underline{N}$ at 29°C is not paralleled by lethality of $\underline{Ax}^{16172}$ hemizygotes or homozygotes at this temperature.

TABLE 23   Summary of the viability of heterozygous combinations of different Ax and N alleles.

ABRUPTEX ALLELE	TEMPERATURE	NOTCH ALLELE				
		N^8	N^{40}	N^{103}	N^{g11}	N^{Co}
Ax^{59d}	20-22°C	–	L*	L	L	–
	29°C	–	–	L	L	–
Ax^{E1}	20-22°C	L	L	V	L	L
	29°C	–	–	L	L	–
Ax^{E2}	20-22°C	V	V	V	V	V
	29°C	V	V^R	V	V	V^R
Ax^{16172}	20-22°C	V	V	V	V	V
	29°C	V^R	L	L	L	L
Ax^{9B2}	20-22°C	V	V	V	V	V
	29°C	V	V^R	V^R	V	V

* L = lethal or semilethal (0-5% survival in relation to siblings).

V^R = reduced viability (5-30% survival in relation to siblings).

V = viable (greater than 30% survival).

II. The effects of $\underline{N}$ mutants on the bristle and wing vein phenotypes of $\underline{Ax}$ mutations.

In order to study further the phenotypic interactions of $\underline{N}$ and $\underline{Ax}$ mutants, a detailed examination was made of the bristle and wing phenotypes of the viable $\underline{Ax}/\underline{N}$ combinations. The results show that all five $\underline{N}$ mutants act similarly on the expression of the Abruptex phenotypes, although it will be noted that there are differences that appear to be $\underline{N}$ allele-specific.

The bristle and wing vein data obtained from heterozygous combinations of $\underline{Ax}^{E2}$, $\underline{Ax}^{16172}$, and $\underline{Ax}^{9B2}$ with five different $\underline{N}$ mutants, are summarized in Tables 24, 25, and 26 respectively. Comparison with the data for the respective $\underline{Ax}$ homozygotes (Tables 18, 19, and 20) shows that where $\underline{Ax}$ alone caused marked bristle loss or wing vein gapping, most of the combinations with $\underline{N}$ had bristle and wing vein gap frequencies significantly closer to wild type. This resembles the suppression of Ax^{28a} phenotypes observed in $\underline{Ax}^{28a}/\underline{N}^{8}$ heterozygotes (MOHR 1932), and indicates that the suppression of Abruptex phenotypes by $\underline{N}$ mutants appears to be a general phenomenon. There do appear to be several exceptions, particularly with $\underline{N}^{103}$ and $\underline{N}^{g11}$ (Tables 24, 26); however, analysis of the $\underline{N}^{103}$ and $\underline{N}^{g11}$ exceptions tends to confirm the rule that $\underline{N}$ mutants suppress Abruptex phenotypes, and most of the other exceptions appear to reflect bristle loss caused by the $\underline{N}$ mutants themselves.

Dealing with the latter exceptions first, it can be seen that at 29°C, heterozygotes of $\underline{Ax}^{9B2}$ with $\underline{N}^{8}$, $\underline{N}^{40}$, and $\underline{N}^{Co}$

TABLE 24   Summary of the bristle and wing vein gap phenotypes of Ax^{E2}/N heterozygotes.*#

GENOTYPE	ORBITALS		OCELLARS		POSTVERTICALS		VERTICALS	
	22°C	29°C	22°C	29°C	22°C	29°C	22°C	29°C
Ax^{E2}/N^8	0.97[Su]	0.78[TSu]	1.00[Su]	0.99[Su]	1.00	1.00[Su]	1.00	0.99
Ax^{E2}/N^{40}	0.96[Su]	0.79[TSu]	1.00[Su]	0.95[Su]	0.99	1.00[Su]	1.00	0.99
Ax^{E2}/N^{Co}	0.98[Su]	0.86[TSu]	1.00[Su]	0.99[Su]	1.00	1.00[Su]	1.00	1.00
Ax^{E2}/N^{103}	0.68[L]	0.76[TSu]	1.00[Su]	0.99[Su]	1.00	1.00[Su]	1.00	1.00
Ax^{E2}/N^{g11}	0.95[Su]	0.98[Su]	0.99	1.00[Su]	1.00	1.00[Su]	1.00	1.00

GENOTYPE	DORSOCENTRALS		SCUTELLARS ANTERIOR		SCUTELLARS POSTERIOR		WING VEINS	
	22°C	29°C	22°C	29°C	22°C	29°C	22°C	29°C
Ax^{E2}/N^8	1.00	1.00[Su]	1.00	0.99[Su]	1.00	1.00	0.95[Su]	0.93[Su]
Ax^{E2}/N^{40}	1.00	1.00[Su]	1.00	1.00[Su]	0.99	1.00	0.98[Su]	0.98[Su]
Ax^{E2}/N^{Co}	1.00	1.00[Su]	1.00	0.97[Su]	1.00	1.00	0.99[Su]	0.98[Su]
Ax^{E2}/N^{103}	1.00	0.99[Su]	1.00	0.99[Su]	1.00	1.00	0.73[SuL]	0.88[TSu]
Ax^{E2}/N^{g11}	1.00	1.00[Su]	1.00	0.99[Su]	1.00	0.99	0.82[SuL]	0.93[TSu]

*   See footnote to Table 18.
\#   See Appendix 11 for actual counts of bristles and wing vein gaps.
T   Statistically significant difference between the 29°C and the 22°C frequency.
Su Statistically significant suppression of Ax phenotypes compared to Ax/Ax females.
L   Suppression of Ax phenotypes significantly less than with N^8, N^{40}.

TABLE 25 Summary of the bristle and wing vein gap phenotypes of Ax^{16172}/N heterozygotes. * #

GENOTYPE	ORBITALS 22°C	ORBITALS 29°C	OCELLARS 22°C	OCELLARS 29°C	POSTVERTICALS 22°C	POSTVERTICALS 29°C	VERTICALS 22°C	VERTICALS 29°C
Ax^{16172}/N^8	0.70^{Su}	0.68^{Su}	1.00^{Su}	1.00^{Su}	1.00^{Su}	0.97^{Su}	0.99	0.98
Ax^{16172}/N^{40}	0.73^{Su}	–	1.00^{Su}	–	1.00^{Su}	–	1.00^{Su}	–
Ax^{16172}/N^{Co}	0.82^{Su}	–	1.00^{Su}	–	0.99^{Su}	–	1.00^{Su}	–
Ax^{16172}/N^{103}	0.57^{SuL}	–	0.12^{SuL}	–	0.60^{SuL}	–	1.00^{Su}	–
Ax^{16172}/N^{g11}	0.63^{SuL}	–	0.93^{SuL}	–	0.38^{SuL}	–	1.00^{Su}	–

			SCUTELLARS					
GENOTYPE	DORSOCENTRALS 22°C	DORSOCENTRALS 29°C	ANTERIOR 22°C	ANTERIOR 29°C	POSTERIOR 22°C	POSTERIOR 29°C	WING VEINS 22°C	WING VEINS 29°C
Ax^{16172}/N^8	1.00^{Su}	0.99^{Su}	1.00^{Su}	0.98^{Su}	0.99^{Su}	1.00^{Su}	0.75^{Su}	0.75^{Su}
Ax^{16172}/N^{40}	1.00^{Su}	–	0.99^{Su}	–	0.99^{Su}	–	0.75^{Su}	–
Ax^{16172}/N^{Co}	1.00^{Su}	–	1.00^{Su}	–	1.00^{Su}	–	0.76^{Su}	–
Ax^{16172}/N^{103}	1.00^{Su}	–	0.97^{Su}	–	0.99	–	0.72^{SuL}	–
Ax^{16172}/N^{g11}	1.00^{Su}	–	0.80^{SuL}	–	1.00^{Su}	–	0.62^{SuL}	–

* See footnotes to Tables 18, 24

\# See Appendix 12 for actual counts of bristles and wing vein gaps.

TABLE 26   Summary of the bristle and wing vein gap phenotypes of Ax^{9B2}/N heterozygotes. * #

GENOTYPE	ORBITALS		OCELLARS		POSTVERTICALS		VERTICALS	
	22°C	29°C	22°C	29°C	22°C	29°C	22°C	29°C
Ax^{9B2}/N^8	0.99	0.91^{TE}	0.11^{Su}	0.17^{Su}	0.82^{Su}	0.34^{T}	1.00	0.90^{TE}
Ax^{9B2}/N^{40}	0.99	0.87^{TE}	0.08^{Su}	0.11^{Su}	0.81^{Su}	0.51^{TSu}	1.00	1.00
Ax^{9B2}/N^{Co}	0.99	0.94^{TE}	0.63^{Su}	0.06^{Su}	0.84^{Su}	0.64^{TSu}	1.00	1.00
Ax^{9B2}/N^{103}	0.74^{E}	0.95^{T}	0.63^{Su}	0.00^{T}	0.92^{Su}	0.80^{Su}	1.00	1.00
Ax^{9B2}/N^{g11}	0.85^{E}	0.97^{T}	0.00	0.02	0.01	0.92^{TSu}	1.00	1.00

| GENOTYPE | DORSOCENTRALS | | SCUTELLARS | | | | WING VEINS | |
			ANTERIOR		POSTERIOR			
	22°C	29°C	22°C	29°C	22°C	29°C	22°C	29°C
Ax^{9B2}/N^8	0.81^{Su}	0.58^{TSu}	1.00^{Su}	0.99^{Su}	1.00	1.00^{Su}	0.86^{Su}	0.63^{TSu}
Ax^{9B2}/N^{40}	0.82^{Su}	0.62^{TSu}	1.00^{Su}	1.00^{Su}	0.99	1.00^{Su}	0.93^{Su}	0.90^{Su}
Ax^{9B2}/N^{Co}	0.75^{Su}	0.51^{TL}	1.00^{Su}	0.99^{Su}	0.99	1.00^{Su}	0.89^{Su}	0.81^{TSu}
Ax^{9B2}/N^{103}	0.82^{Su}	1.00^{TSu}	1.00^{Su}	1.00^{Su}	0.98	1.00^{Su}	0.60^{E}	-
Ax^{9B2}/N^{g11}	0.51^{SuL}	0.54^{L}	0.32^{E}	1.00^{TSu}	0.99	0.96	0.68^{E}	0.92^{TSu}

* See footnotes to Tables 18, 24.
# See Appendix 13 for actual counts of bristles and wing vein gaps.
E Significantly greater bristle loss or wing vein gapping than in Ax^{9B2}/Ax^{9B2} homozygotes.

(Table 26), had lower (more mutant) orbital bristle frequencies than Ax^{9B2} homozygotes (Table 20). Similar orbital loss is observed in the respective N/+ heterozygotes raised at 29°C. Note also that in the heterozygotes of Ax^{E2} with these N mutants, orbital bristle frequencies were significantly reduced at 29°C compared to 22°C, although they were still less mutant than Ax^{E2} homozygotes grown at 29°C (Table 24). The loss of vertical bristles in heterozygotes of Ax^{9B2} with N^8 at 29°C, but not with other N alleles, may reflect an interaction between Ax^{9B2} and the verticals (vt) locus, located adjacent to N in bands 3C5-6 (GERSH 1965). This possibility arises from the fact that vt is hemizygous in N^8 heterozygotes, and that a homozygous deficiency for vt causes reduction of the number of vertical bristles (GERSH 1965). The other exception (non-suppression of the dorso-centrals phenotype in Ax^{9B2}/N^{Co}, Table 26) cannot be explained in terms of the phenotype of N^{Co}/+, and probably is specific for this Ax/N combination.

Inspection of Tables 24-26 shows that there are several instances where Ax combinations with N^{103} and N^{g11}, either do not suppress the Ax phenotypes at 22°C, or cause significantly less suppression than N^8 or N^{40}. It is significant that at this temperature, both N^{103} and N^{g11} express relatively mild Notch phenotypes, both in extent of wing nicking (Tables 6, 9), and in their interactions with the recessive Notch-locus mutations (WELSHONS, personal communication; and see Appendix 1). Further-more, in most (9 out of 12) of the cases for which sufficient data are available (Tables 24, 26), significantly greater sup-

pression of the $\underline{Ax}$ phenotypes occurs at 29°C, the temperature at which both $\underline{N}^{103}$ and $\underline{N}^{g11}$ exhibit stronger Notch phenotypes (Tables 6, 9). Furthermore, the few $\underline{Ax}^{16172}/\underline{N}^{g11}$ flies which survived at 29°C, also showed reduction of the Abruptex phenotypes when compared to 22°C flies (Appendix 12). The most glaring exception to this (the ocellars of $\underline{Ax}^{9B2}/\underline{N}^{103}$, Table 26), can be accounted for by the observation that $\underline{N}^{103}/+$ heterozygotes also show drastically reduced numbers of ocellar bristles at 29°C. Thus, it appears that the exceptional behaviour of $\underline{N}^{103}$ and $\underline{N}^{g11}$ occurs mainly at temperatures at which the Notch phenotypes of these mutants are only mildly expressed, with more typical Notch behaviour (in terms of suppression of $\underline{Ax}$ phenotypes) occurring at higher temperatures at which $\underline{N}^{103}$ and $\underline{N}^{g11}$ behave more like the amorphic $\underline{N}$ alleles.

Bristle counts made on $\underline{Ax}^{E1}/\underline{N}^{103}$ females raised at 22°C, (which were viable, in contrast to the lethality of the other $\underline{Ax}^{E1}/\underline{N}$ combinations), strengthen the rule that $\underline{N}$ mutants suppress Abruptex phenotypes. Comparison of the data on $\underline{Ax}^{E1}/\underline{N}^{103}$ females (Table 27) with those from $\underline{Ax}^{E1}/Y$ males (Table 17) reveals that each bristle frequency was closer to wild type in the $\underline{Ax}/\underline{N}$ females than in the $\underline{Ax}/Y$ males (with the exception of the verticals, which were essentially wild type in each case). It should be remembered, however, that $\underline{N}^{103}$ does not behave as a deficiency for the Notch locus at 22°C, and that the suppression of $\underline{Ax}^{E1}$ phenotypes by $\underline{N}^{103}$ may reflect the presence of significant levels of activity in the $\underline{N}^{103}$ gene product. Comparison of the $\underline{Ax}^{E1}/\underline{N}^{103}$ data (Table 27) with that for $\underline{Ax}^{E1}/+$ (Table 16), shows that

TABLE 27   Counts of bristles in Ax^{El}/N^{103} females* raised at 22°C.

TYPE OF BRISTLE	NUMBER OF FLIES IN EACH PHENOTYPIC CLASS							MEAN NUMBER OF BRISTLES PER FLY (± 95% CONFIDENCE INTERVALS)
	0	1	2	3	4	5	6	
ORBITALS	0	0	0	1	58	22	13	4.50 ± .13
OCELLARS	84	8	2	–	–	–	–	0.13 ± .07
POSTVERTICALS	25	26	43	–	–	–	–	1.19 ± .15
VERTICALS	0	0	0	0	94	–	–	4.00
DORSOCENTRALS	0	1	33	29	31	–	–	2.96 ± .15
ANTERIOR SCUTELLARS	0	1	93	–	–	–	–	1.99 ± .02
POSTERIOR SCUTELLARS	0	0	94	–	–	–	–	4.00

*Progeny of the cross $\underline{M5}/\underline{y}\ \underline{w}^a\ \underline{N}^{103}\ ♀$ x $Ax^{El}/\underline{B}^S\ \underline{w}^+·Y\ ♂$

the ocellars are quite comparable in the two genotypes, but the postverticals are more wild type in $\underline{Ax}/\underline{N}$ than in $\underline{Ax}/+$, and more comparable to $\underline{Ax}/+;\underline{Dp}$ (Table 16). It may be that both the partial inactivation and the partial function associated with $\underline{N}^{103}$, possibly acting by different mechanisms, are responsible for the suppression of the $\underline{Ax}^{E1}$ phenotypes.

From the foregoing observations, notwithstanding the exceptions noted, it can be stated in summary that: 1) when in trans heterozygous combination, $\underline{N}$ mutants generally reduce expression of the bristle and wing vein phenotypes of $\underline{Ax}$ mutations, and 2) high temperatures, which enhance the interactions of $\underline{N}^{103}$ and $\underline{N}^{g11}$ with the recessive visible mutants at the locus, also increase the suppression of the Abruptex mutant phenotypes by these two $\underline{N}$ alleles.

III. The effects of $\underline{Ax}$ mutants on the wing nicking phenotypes of $\underline{N}$ mutations.

The examination of the phenotypes of the different available $\underline{Ax}/\underline{N}$ heterozygotes has revealed that the $\underline{Ax}$ mutants studied fall into two classes with respect to their effects on the $\underline{N}$ mutants. Both $\underline{Ax}^{E2}$ and $\underline{Ax}^{16172}$ enhance wing nicking, whereas $\underline{Ax}^{9B2}$ and $\underline{Ax}^{E1}$ suppress nicking. The enhancement of nicking by $\underline{Ax}^{E2}$ and $\underline{Ax}^{16172}$ is evident in the frequencies of nicking with $\underline{N}^{103}$, $\underline{N}^{Co}$, and $\underline{N}^{g11}$ (compare the data in Table 28 with Table 6 for $\underline{N}^{103}/+$, and Table 9 for $\underline{N}^{g11}/+$; and note that 42% of $\underline{N}^{Co}/+$

TABLE 28   Nicking frequencies in wings of Ax/N heterozygotes.*

N ALLELE	Ax^{E2} 22°C	Ax^{E2} 29°C	Ax^{16172} 22°C	Ax^{16172} 29°C	Ax^{9B2} 22°C	Ax^{9B2} 29°C
N^8	2.00	2.00	2.00	2.00	$0.02 \pm .01^L$	$1.48 \pm .17^L$
N^{40}	2.00	2.00	2.00	-	$0.22 \pm .08^L$	2.00
N^{Co}	$1.99 \pm .02^H$	2.00^H	$1.99 \pm .02^H$	-	0.00^L	$0.94 \pm .24$
N^{103}	$1.60 \pm .11^H$	2.00	$1.63 \pm .19^H$	-	$0.07 \pm .04^L$	2.0
N^{g11}	$0.19 \pm .06^H$	$1.97 \pm .05^H$	$0.07 \pm .06$	-	0.00	$0.42 \pm .21$

* The figures presented are the mean number of nicked wingtips per fly, ± 95% confidence intervals. Absence of a confidence interval indicates no variability in the samples examined. See Appendices 11-13 for actual counts of nicked wings.

H Significantly higher frequency of wing nicking than in N/+ heterozygotes.

L Significantly lower frequency of wing nicking than in N/+ heterozygotes.

individuals had nicked wings, cf page 58). In the heterozygotes
of Ax^{E2} and Ax^{16172} with N^8 and N^{40}, the frequency of nicking
was the same as for the N/+ heterozygotes (100%), but the wing
serrations were much deeper in the presence of Ax. The enhance-
ment of the wing nicking phenotype of N mutants by Ax^{E2} and
Ax^{16172} is the opposite of what has been reported for Ax^{28a}
(MOHR 1932). On the other hand, Ax^{9B2} suppresses both the in-
tensity and frequency of nicking due to N (Table 28). The
enhancement of nicking in Ax^{9B2}/N heterozygotes at 29°C compared
to 22°C (Table 28) likely is related to the occasional nicking
seen in Ax^{9B2} hemi- and homozygotes at this temperature
(WELSHONS, personal communication).

Accurate data concerning the wing phenotype of Ax^{E1}/N^{103}
could not be collected, owing to the frequent deformity and
crumpling of the wings observed in flies of this genotype. How-
ever, in those flies whose wings were sufficiently extended to
permit observation, wing nicking occurred less frequently than
in N^{103}/+ females at this temperature. The suppression of wing
nicking has generally been observed when Ax^{E1}/N heterozygotes
have been available, including the Ax^{E1}/N^{40} breakthroughs re-
covered in the mapping of Ax^{E1} (Table 13). The facts that Ax^{E1}
and Ax^{9B2} possess normal salivary chromosome banding and map
within the Notch locus suggest that duplication of the Notch
locus is not a necessary condition for suppression of wing nick-
ing by an Abruptex mutant.

In summary, the foregoing observations indicate that there
are two classes of Abruptex mutation: 1) those which enhance

the wing nicking of Notch mutants (Ax^{E2} and Ax^{16172}), and 2)
those which suppress wing nicking (Ax^{E1} and Ax^{9B2}, as well as
Ax^{28a}). It has already been noted that Ax^{9B2} differs qualita-
tively from Ax^{E2} and Ax^{16172} (Tables 21, 22), and it will be
seen that the interactions among the different Ax mutants,
analyzed in the following section, provide further evidence that
these two classes of Abruptexes must differ in fundamental ways.

E. INTERACTIONS BETWEEN DIFFERENT ABRUPTEX MUTATIONS

Viability data for all the heterozygous Ax^x/Ax^y combinations tested, and for the Ax homozygotes, are summarized in Table 29. The time of death of the lethal genotypes is mainly pupal, with some also dying as newly eclosed adults. It can be seen that combinations of the lethals Ax^{E1} and Ax^{59d} with the viable mutations Ax^{E2} and Ax^{16172}, are lethal (as are Ax^{E1}/Ax^{59d} heterozygotes). This type of lethal interaction is reminiscent of the lethality of most N mutants with fa^{no}, and is hence perhaps not unexpected. Surprisingly, however, the viable mutation Ax^{9B2} is lethal when heterozygous with the other two viable Abruptexes (Ax^{E2} and Ax^{16172}), while both Ax^{E1}/Ax^{9B2} and Ax^{E2}/Ax^{16172} are viable. This type of lethality is most unusual, and can be called "negative complementation", as opposed to the usual type of complementation in which two lethal alleles (x,y) produce a viable heterozygote (x/y). The lethality of Ax^{9B2}/Ax^{E2} and Ax^{9B2}/Ax^{16172} has been confirmed at both 22°C and 29°C (Table 30). However, it can be seen in the control cross (M5/Ax^{9B2} ♀ x Ax^{9B2}/Y ♂) that Ax^{9B2} homo- and hemizygotes exhibit significant lethality (also pupal) at 29°C. This cross was started by crossing M5/M5 females to stock Ax^{9B2}/Y males, then backcrossing F_1 females to stock Ax^{9B2}/Y males. Since the Ax^{9B2}/Y male progeny are fully viable in the other two crosses, it can be concluded that there is at least one recessive autosomal temperature-sensitive modifier of Ax in the Ax^{9B2} stock, which kills Ax^{9B2} individuals at 29°C.

TABLE 29   Summary of viability of various heterozygous combinations of $\underline{Ax}$ alleles at 22°C.

	Ax^{E1}	Ax^{59d}	Ax^{9B2}	Ax^{E2}	Ax^{16172}
Ax^{E1}	L*	L	V	L	L
Ax^{59d}		L	-	L	L
Ax^{9B2}			V	L	L
Ax^{E2}				V	V
Ax^{16172}					V

* L = lethal

  V = viable

TABLE 30   Relative viability of Ax^{9B2}/Ax^{9B2}, Ax^{9B2}/Ax^{E2}, and Ax^{9B2}/Ax^{16172} at 22°C.

Ax^X	TEMPERATURE	PROGENY GENOTYPES*			
		FEMALES		MALES	
		$M5/Ax^X$	Ax^{9B2}/Ax^X	$M5/Y$	Ax^{9B2}/Y
Ax^{E2}	22°C	124	0	100	122
	29°C	131	0	101	121
Ax^{16172}	22°C	86	0	76	94
	29°C	136	0	76	98
Ax^{9B2}	22°C	101	96	95	128
	29°C	151	23	85	38

* Progeny of the cross $M5/Ax^{9B2}$ ♀ x Ax^X/Y ♂, where x = E2, 16172, or 9B2.

Correlation of the observed pattern of viability and lethality with the effects of $\underline{Ax}$ mutants on the wing nicking of $\underline{N}$ mutants, reveals the striking fact that only those $\underline{Ax}$ mutants which affect wing nicking $\underline{in\ the\ same\ way}$ give viable phenotypes when heterozygous with each other, whereas those $\underline{Ax}$ alleles which affect wing nicking in opposite directions from one another, are lethal in heterozygous combination. The effects of $\underline{Ax}^{59d}$ on wing nicking could not be determined, since none of the $\underline{Ax}^{59d}/\underline{N}$ combinations tested were viable. The viability of $\underline{Ax}^{9B2}/\underline{Ax}^{59d}$ has not yet been checked. It is also noteworthy that the viable combinations of the Notch-suppressing $\underline{Ax}$'s investigated in the present study ($\underline{Ax}^{9B2}/\underline{Ax}^{9B2}$, and $\underline{Ax}^{E1}/\underline{Ax}^{9B2}$) are essentially female-sterile. In other experiments, furthermore, it has been observed that $\underline{Ax}^{E1}/\underline{Ax}^{E1};\underline{Dp}$ females are sterile, although they lay many eggs. This is similar to the observation that the combination $\underline{Ax}^{59b}/\underline{Ax}^{59d};\underline{Dp}$ is also relatively infertile (WELSHONS 1971). Notwithstanding the possible effects of the modifier present in the $\underline{Ax}^{9B2}$ stocks, the observed correlation of $\underline{Ax}$ properties with respect to lethality and sterility, with the effects on Notch wing nicking, must reflect some fundamental difference between the two classes of Abruptex mutations.

Further information concerning the two types of Abruptexes was obtained from examination of the bristle and wing phenotypes of the viable $\underline{Ax}^{x}/\underline{Ax}^{y}$ combinations and of a few surviving "breakthrough" individuals from the lethal genotypes. The $\underline{Ax}^{9B2}/\underline{Ax}^{E2}$ heterozygotes examined were recovered among progeny of the mapping cross $\underline{w}^{a}\ \underline{Ax}^{E2}\ \underline{rb}/+\ \underline{Ax}^{16172}\ +\ \female\ \times\ \underline{w}^{a}\ \underline{Ax}^{9B2}\ \underline{rb}/Y\ \male$

performed at 22°C (all of the observed breakthroughs from this cross were considered to be Ax^{9B2}/Ax^{E2}, since no extreme $\underline{Ax}$ females with wild-type eye colour were recovered). The results (Table 31) show that the overall mutant appearance of surviving Ax^{E1}/Ax^{E2} individuals is generally more extremely Abruptex than is the case for $\underline{Ax}^{E1}$ males or $\underline{Ax}^{E2}$ males or females (compare Table 31 with Tables 17 and 18). Similarly, the Ax^{E2}/Ax^{9B2} females which managed to eclose were much more extremely mutant than either homozygote. Comparison of the Ax^{E2}/Ax^{9B2} bristle data (Table 31) with those for $\underline{Ax}^{E2}$ and Ax^{9B2} homozygotes (Tables 18, 20), shows that this is particularly obvious in the frequencies of the orbitals, verticals, dorsocentrals, and scutellars. Only two Ax^{E1}/Ax^{16172} females were recovered, and these possessed no bristles except the verticals (Appendix 14). Comparison with the data in Tables 17 and 19 shows that this genotype also was more mutant than the respective hemi- and homozygotes. In addition to the above observations, it was noted that the meso- and meta-thoracic legs of Ax^{E1}/Ax^{E2}, Ax^{E2}/Ax^{9B2}, and Ax^{E1}/Ax^{16172} females were often deformed, the thoracic microchaetae were much sparser than those of the homozygotes, and the wings of these females were so deformed that venation could not be examined. In contrast to the more extreme appearance of the above three genotypes, the phenotypes of the viable combinations Ax^{E2}/Ax^{16172} and Ax^{E1}/Ax^{9B2} were intermediate between the respective homozygous (or hemizygous) genotypes (compare the data for the heterozygous genotypes in Table 31, with the respective hemi- or homozygous genotypes in Tables 17-20). Detailed vein

TABLE 31   Summary of the bristle and wing vein phenotypes of heterozygous combinations of different $\underline{Ax}$ alleles at 20-22°C *#.

GENOTYPE	ORBITALS	OCELLARS	POSTVERTICALS	VERTICALS
Ax^{E1}/Ax^{E2}	0.01^{E}	0.00	0.00	1.00
Ax^{E1}/Ax^{9B2}	0.56	0.00	0.00	1.00
Ax^{E2}/Ax^{16172}	0.45^{I}	0.00	0.00	0.99
Ax^{E2}/Ax^{9B2}	0.00^{E}	0.00	0.00	0.55^{E}

GENOTYPE	DORSOCENTRALS	SCUTELLARS ANTERIOR	POSTERIOR	WING VEINS
Ax^{E1}/Ax^{E2}	0.08	0.00	0.98	–
Ax^{E1}/Ax^{9B2}	0.30^{I}	0.07^{I}	0.87^{I}	–
Ax^{E2}/Ax^{16172}	0.80^{I}	0.89^{I}	1.00	0.43
Ax^{E2}/Ax^{9B2}	0.00^{E}	0.43^{E}	0.79^{E}	–

* See footnote to Table 18.

See Appendix 14 for actual counts of bristles and wing vein gaps.

E Frequency significantly more mutant than in respective hemi- or homozygotes.

I Frequency intermediate between that of respective hemi- or homozygote.

gap counts were not made of the Ax^{El}/Ax^{9B2} females, but it was noted that the vein gapping was also intermediate between Ax^{El}/Y males and Ax^{9B2}/Ax^{9B2} females. From the foregoing account, it can be seen that the extent of expression of mutant phenotypes of the available heterozygous Abruptex combinations is correlated with the viability of those combinations. The lethal genotypes (Ax^{El}/Ax^{E2}, Ax^{El}/Ax^{16172}, Ax^{E2}/Ax^{9B2}) all exhibit more extreme mutant phenotypes than those of the individual homo- or hemizygotes, whereas the viable genotypes (Ax^{El}/Ax^{9B2} and Ax^{E2}/Ax^{16172}) exhibit intermediate phenotypes. Presumably the lethality of the three former genotypes actually results from the enhanced mutant phenotypes of these combinations.

Disregarding Ax^{59d} for the moment, the observation on Abruptex mutant interactions can be summarized as follows: 1) trans-heterozygotes for Ax mutations which have opposite effects on the wing nicking of N mutants, exhibit negative complementation, having more extreme mutant phenotypes than either Ax homozygote alone and resulting in lethality, and 2) heterozygous combinations of Ax mutations which have the same effect on wing nicking, are viable, and their phenotypes are intermediate between those of the respective Ax homozygotes. Ax^{59d} cannot as yet be placed in either of these classes of Abruptexes, since this Ax is completely lethal when heterozygous with Ax^{El}, Ax^{E2}, Ax^{16172}, and all N mutants tested.

F. DEVELOPMENTAL STUDIES OF SELECTED GENOTYPES

I. N^{g11}/+ - TSPs for wing nicking and eye facet disruption.

As reported above, the mutant eye phenotype of N^{g11}/+
females is only expressed at low temperatures, whereas the wing
nicking phenotype is expressed weakly at low temperatures and is
enhanced at higher temperatures (Figure 4). As will be discus-
sed, the results of several shift experiments show that both the
eye and wing phenotypes of N^{g11}/+ females have third larval in-
star TSPs. In addition, the data have revealed a temperature-
dependent polarized pattern of eye facet arrangement.

In the first experiment, cultures established from 2-hour
egglays (see footnote to Table 32) were shifted from 20.5°C to
29°C, and vice versa, at successive intervals after egg collec-
tion, and the developmental stage of the flies at the time of
each shift was noted. Adult females emerging in these cultures
were scored for wing nicking and the eye phenotype, eyes being
scored as mutant if any part of the eye contained disrupted
ommatidia. The results show that in shift-ups the number of
mutant eyes rose sharply between 120 and 144 hours, when only
third instar larvae were present, while at the same time the
proportion of nicked wings dropped drastically (Table 32). Simi-
larly, only third instar larvae were present when shift-downs
(between 72 and 84 hours of development) decreased the number of
mutant eyes and increased the frequency of wing nicking (Table
33). Thus the TSPs for both the eye and wing phenotypes of N^{g11}

TABLE 32   Eye and wing phenotypes of N^{g11}/+ adult females shifted from 20.5°C to 29°C at different successive intervals.

| CULTURE NUMBER | TIME OF SHIFT | | NUMBER OF FLIES IN EACH PHENOTYPIC CLASS | | | | | |
	CULTURE AGE (HR)	DEVELOPMENTAL STAGE	R N*	R+N	R N+	R+N+	%R	%N
1	24	I**	0	38	0	23	0	62
2	48	I, some II	0	28	0	27	0	51
3	72	II, some III	0	31	0	19	0	62
4	96	III	0	55	0	8	0	87
5	120	III	1	54	3	4	6	89
6	144	III, some P	9	0	54	0	100	14
7	168	P, some III	2	0	49	0	100	4
8	192	P	0	0	43	0	100	0
9	216	P	1	0	171	0	100	0.6
10	240	P	0	0	118	0	100	0
11	264	P	0	0	123	0	100	0
12	NOT SHIFTED		1	0	180	0	100	0.6

*R = rough eyes (disrupted facet arrangement); R+ = wild-type eyes; N = nicked wings; N+ = wild-type wings.

** I, II, III = 1st, 2nd, 3rd larval instar, respectively; P = prepupae & pupae.

TABLE 33   Eye and wing phenotypes of Ng^{11}/+ adult females shifted from 29°C to 20.5°C at different successive intervals.

CULTURE NUMBER	TIME OF SHIFT CULTURE AGE (HR)	DEVELOPMENTAL STAGE	NUMBER OF FLIES IN EACH PHENOTYPIC CLASS R N*	R+N	R N+	R+N+	%R	%N
1	24	I, some II*	0	0	53	0	100	0
2	36	II, some I	1	0	16	0	100	6
3	48	II	4	0	51	0	100	7
4	60	III	7	0	64	0	100	10
5	72	III	8	0	100	1	99	7
6	84	III	1	8	0	9	6	50
7	96	P, some III	0	13	0	15	0	46
8	108	P	0	28	0	12	0	70
9	132	P	0	9	0	8	0	52
10	144	P	0	20	0	8	0	71
11	NOT SHIFTED		0	9	0	9	0	50

* See Table 32 for explanation of symbols.

occur during the third larval instar, well before the final differentiation of the imaginal discs into the adult organs.

In the preceding experiment it was noted that some of the flies, in cultures shifted during the third larval instar, had only small patches of mutant eye tissue, and that the position and extent of this tissue was related to the time and direction of the shift. To investigate this pattern more closely, eggs collected on petri plates were allowed to develop for approximately 24 hours, at which time they were hatching in large numbers. After the plates had been cleared of all larvae by washing, newly-hatched first-instar larvae were picked from the plates and placed on food in shell vials (100 larvae per vial) which were incubated at 20.5°C or 29°C. Shifts from one temperature to the other were timed from the end of the larva-collection period (i.e. from within 20 minutes after hatching from the egg), and were carried out at shorter intervals during the third larval instar. The results show that in the shift-ups, the number of eyes with mutant tissue rose sharply, and the frequency of nicked wings dropped dramatically between 84 and 108 hours, when only third-instar larvae were present (Table 34). In the shift-downs, the number of mutant eyes dropped between 66 and 84 hours, and the frequency of nicked wings rose sharply between 60 and 72 hours, both intervals occurring during the third instar (Table 35). These times coincide fairly well with those shown in Tables 32 and 33, if allowance is made for the duration of the egg phase in the earlier experiment.

As many flies with mutant eyes as possible, from vials 5-9

TABLE 34 Eye and wing phenotypes of $N^{g11}/+$ adult females shifted from 20.5°C to 29°C at different successive intervals (Experiment 2).

CULTURE NUMBER	TIME OF SHIFT HOURS AFTER EGG HATCH	TIME OF SHIFT DEVELOPMENTAL STAGE	NUMBER OF FLIES IN EACH PHENOTYPIC CLASS R N*	R+N	R N+	R+N+	%R	%N
1	0	I*	0	8	0	7	0	53
2	24	I	0	24	0	11	0	69
3	48	II	0	21	0	26	0	45
4	72	II, III	0	21	0	4	0	84
5	84	III	2	19	0	5	8	81
6	96	III	15	8	9	0	75	72
7	108	III	9	0	16	0	100	36
8	120	III, some P	3	0	29	0	100	9
9	132	P, some III	1	0	31	0	100	3
10	144	P	0	0	33	0	100	0
11	168	P	1	0	30	0	100	3
12	192	P	0	0	37	0	100	0

* See Table 32 for explanation of symbols.

TABLE 35   Eye and wing phenotypes of Ng^{11}/+ adult females shifted from 29°C to 20.5°C at different successive intervals (Experiment 2).

CULTURE NUMBER	TIME OF SHIFT		NUMBER OF FLIES IN EACH PHENOTYPIC CLASS					
	HOURS AFTER EGG HATCH	DEVELOPMENTAL STAGE	R N*	R^+N	R N^+	R^+N^+	%R	%N
1	12	I*	0	0	21	0	100	0
2	24	II	0	0	28	0	100	0
3	36	III	0	0	32	0	100	0
4	43	III	1	0	25	0	100	4
5	48	III	0	0	25	0	100	0
6	60	III	3	0	25	0	100	11
7	66	III	7	0	23	0	100	23
8	72	III, some P	5	7	11	5	57	43
9	84	P, some III	0	18	0	14	0	56
10	96	P	0	13	0	10	0	57

* See Table 32 for explanation of symbols.

of the shift-ups (Table 34), and vials 6-8 of the shift-downs (Table 35), were examined with a high power dissecting microscope (100x), and the position of the mutant tissue in one eye of each fly was drawn on mimeographed eye diagrams of the type shown in Figure 5, along with a note on the wing phenotype of the fly. Each line of the diagram represents five rows of ommatidia, and the orientation of the lines reflects the general orientation of the rows of ommatidia. After being scored in this manner, eyes were then graded according to the following system. Starting from the posterior end, the eye was divided into six regions, each consisting of five vertical rows of ommatidia (see Figure 5). When the boundary between mutant and wild-type tissue lay predominantly in region 1, the fly was scored as 1, and so forth. The results show that the anterior boundary of the mutant tissue in the shift-ups (Table 36) and the posterior boundary in the shift-downs (Table 37), migrated anteriorly in a vertical strip across the eye, with increasing age at the time of shifting. (The absence of shift-down boundaries in region 1 is due to the accidental loss of a 54-hour shift-down culture before it could be scored.) The anterior boundaries in the shift-down series did not migrate, and lay predominantly in region 5 (Table 37). This is generally the anterior limit of mutant tissue seen in all N^{g11}/+ females raised at low temperature. In addition to the correlation of mutant eye tissue with time of shifting, the frequency of wing nicking can also be correlated with the position of the mutant eye tissue, as shown in Table 38. Here it can be seen that in the shift-ups, nicking decreased markedly as the

FIGURE 5   Diagram used for scoring position of mutant eye

tissue boundaries. Anterior end is to the right.
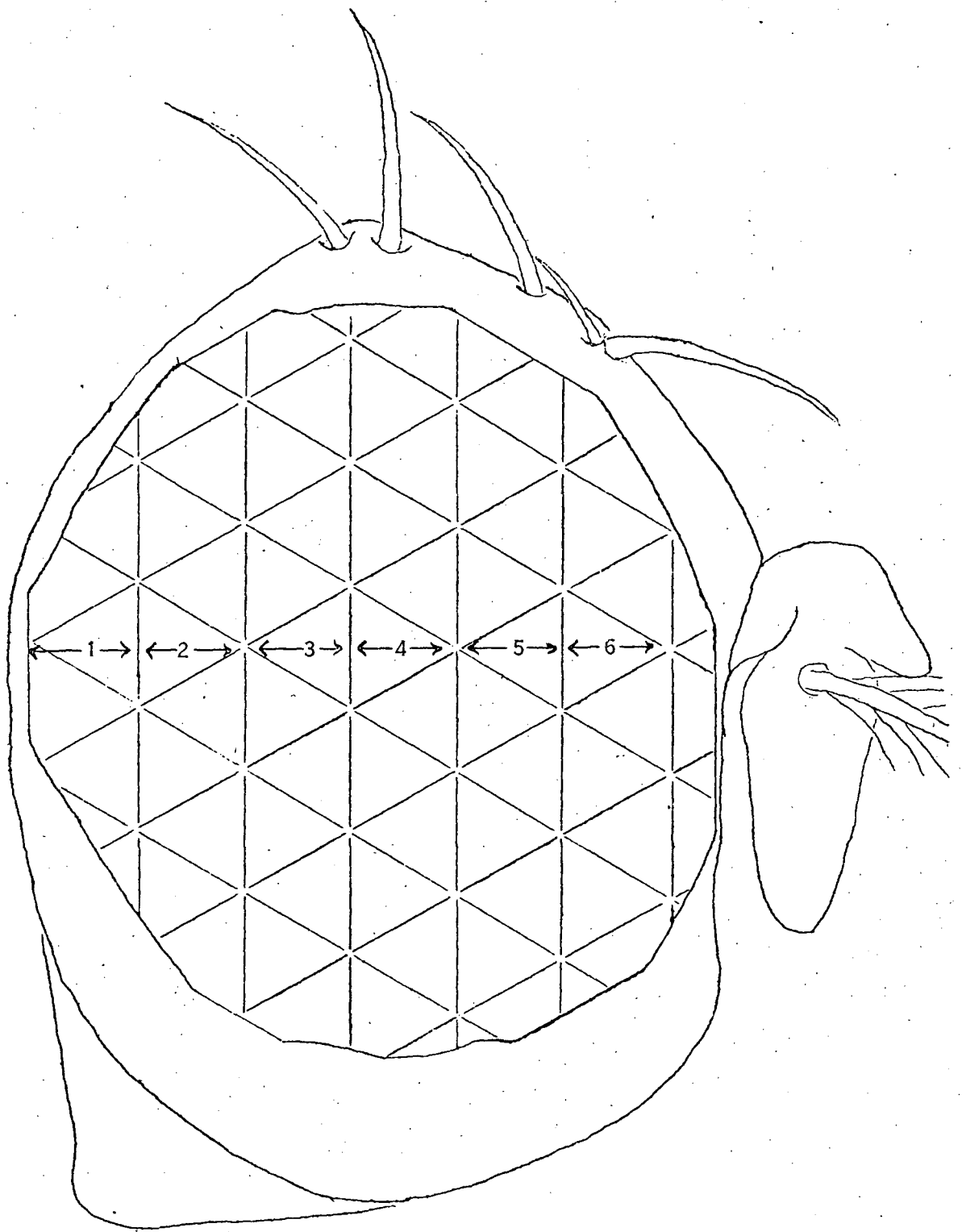
TABLE 36   Positions of anterior boundaries of mutant tissue
extending from the posterior rim of the eyes of
$N^{g11}/+$ females, shift-up experiment 2*.

VIAL NUMBER	NUMBER OF FLIES WITH BOUNDARY IN GIVEN REGION OF EYE					
	1**	2	3	4	5	6
5	2	0	0	0	0	0
6	4	15	4	0	0	0
7	0	12	12	0	0	0
8	0	2	15	11	1	0
9	0	0	0	6	16	2

* Data were obtained from flies recorded in Table 34.

** See Figure 5.

TABLE 37   Positions of anterior and posterior boundaries of mutant tissue in the centre of the eyes of $N^{g11}/+$ females, shift-down experiment 2*.

VIAL NUMBER	BOUNDARY	NUMBER OF FLIES WITH BOUNDARY IN GIVEN REGION OF EYE					
		1**	2	3	4	5	6
6	anterior	0	0	0	0	19	5
	posterior	0	4	15	4	1	0
7	anterior	0	0	0	1	17	5
	posterior	0	5	3	6	9	0
8	anterior	0	0	0	1	10	3
	posterior	0	0	0	8	5	1

* Data were obtained from flies recorded in Table 35.

** See Figure 5.

TABLE 38   Correlation of the occurrence of wingtip nicking
            with position of the boundary of mutant eye tissue.

| | | MUTANT EYE TISSUE BOUNDARY IN REGION | | | | |
EXPERIMENT	WING PHENOTYPE*	1	2	3	4	5,6
SHIFT-UP (anterior boundary)	N	4	17	4	1	0
	N^+	2	12	27	16	19
SHIFT-DOWN (posterior boundary)	N	0	0	1	4	4
	N^+	0	9	17	14	12

* N = nicked wingtip(s); N^+ = wild-type wingtips.

boundary of mutant eye tissue progressed anteriorly from region
2 to region 4, whereas in the shift-downs, wing nicking increas-
ed as the boundary shifted from region 3 to 4, although the rise
was not as dramatic as the drop in the shift-up series (Table 38).

Since developmental synchrony of the larvae was relatively
poor in the previous experiment, the method of white prepupa iso-
lation (see Materials and Methods) was used in order to time the
migration of mutant eye tissue across the eye more precisely.
Several batches of eggs were collected in successive 2- to 6-hour
intervals, on petri plates containing food, over a 48- and 72-
hour period. The 72-hour series was incubated at 20.5°C until
the first puparia were detected, at which time all of the plates
in this series were shifted to 29°C. Each plate in the 48-hour
series was placed at 29°C immediately after collection, and the
entire series was shifted to 20.5°C upon the appearance of the
first puparia. After the shifts at both temperatures, white
prepupae only were collected at defined times from the plates,
placed into vials, and allowed to develop into adults. Eyes and
wings of emergent adult females were examined with a dissecting
microscope, and the eyes were further examined and recorded
photographically with a scanning electron microscope. The
results, summarized in Figure 6, show that during the 23 hours
preceding puparium formation, the longer the interval between a
shift-up and puparium formation (i.e. the earlier the shift-up
during development), the less mutant eye tissue was found, the
mutant area always beginning at the posterior edge of the eye and
extending anteriorly (Figure 6a). On the other hand, the longer

FIGURE 6   The eye and wing phenotypes of N^{g11}/+ females

            shifted from a) 20.5°C to 29°C, and b) 29°C to

20.5°C at different times before puparium formation. Note that

the third larval instar lasts about 66 hr. at 20.5°C, and 40 hr.

at 29°C. = wild-type eye facet arrangement; = mutant eye

facet arrangement; ▢ = number of flies with wild-type wings;

 ▣ = number of flies with one or both wings nicked. Note that

the anterior rim of the eye is to the left.

a. SHIFT-UP

EYE PHENOTYPE

EYE TSP

NUMBER OF FLIES WITH NICKED WINGS

HOURS FROM SHIFT TO PUPATION

b. SHIFT-DOWN

EYE PHENOTYPE

EYE TSP

NUMBER OF FLIES WITH NICKED WINGS
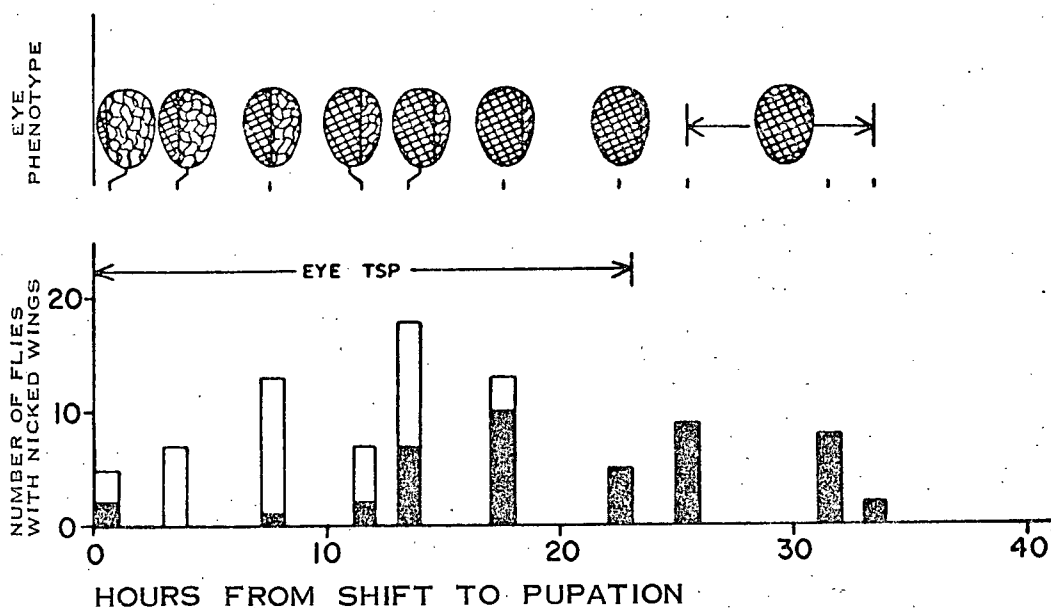
HOURS FROM SHIFT TO PUPATION
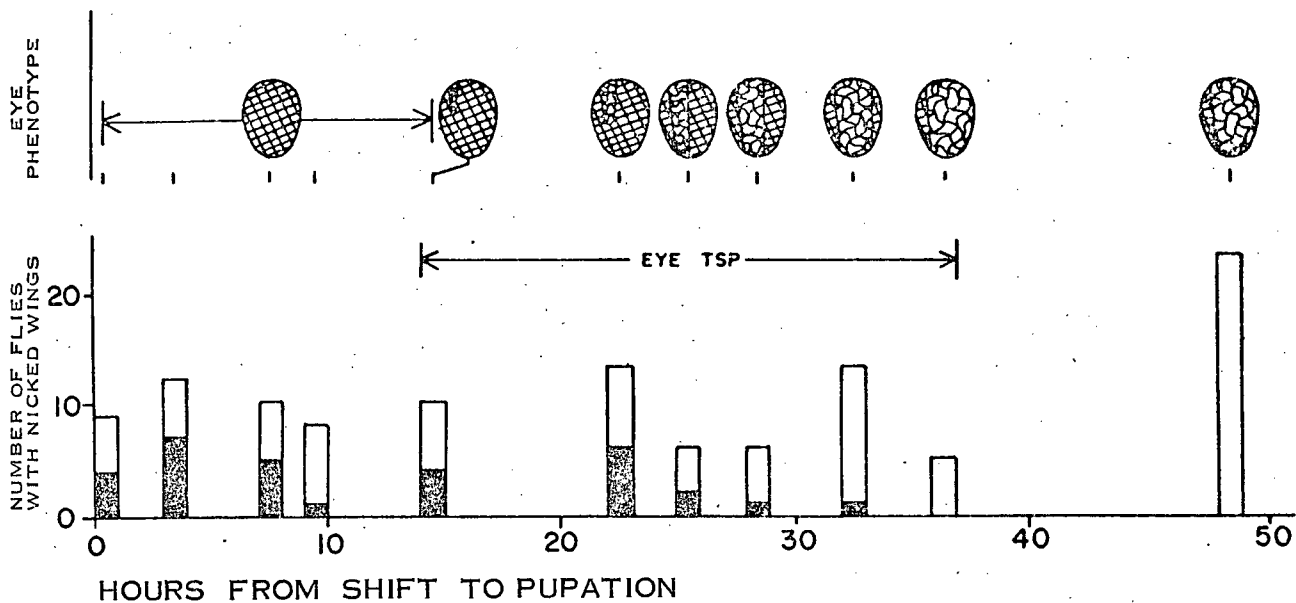
the interval between a shift-down and puparium formation, the more mutant eye tissue was found, beginning at a point near but not at the anterior edge of the eye and extending posteriorly (Figure 6b). These observations are entirely consistent with the previously observed posterior-to-anterior progression of the boundary between mutant and non-mutant eye tissue (Tables 36, 37). The synchrony obtained in this experiment was much better than in the previous ones, since the positions of the mutant eye tissue boundaries in the adult flies raised from prepupae isolated at any given time after a shift, generally differed by only 3-4 rows of facets. This in turn indicates that there was little difference in the developmental rates of different third-instar larvae in these cultures. The eye TSP can be defined as the time (which is about 23 hours at both 20.5°C and 29°C) during which the mutant tissue boundary progresses anteriorly across the eye (Figure 6). It is interesting to note that the eye TSP occurs earlier in the third instar at 29°C than at 20.5°C. From the wing nicking data presented in Figure 6, it can be seen that in the shift-up series the frequency of nicked wings drops about half way through the eye TSP, and conversely, in the shift-down series the frequency of nicked wings rises about half way through the eye TSP. These data, and those in Table 38, indicate that in the shift-ups the wing nicking TSP ends about half way through the eye TSP, and in the shift-downs the wing TSP begins during the eye TSP.

In summary, the results of the temperature-shift experiments with N^{g11}/+ females reported here, have shown that:

1) the TSPs for both the rough eye and wing nicking phenotypes occur during the third larval instar; 2) the eye facet pattern is affected by temperatures in a vertical wave that proceeds anteriorly from the posterior rim of the eye during the eye TSP; and 3) the wing nicking TSP appears to begin and end during the eye TSP.

II. N^{103}/spl - TSPs for wing nicking, eye facet disruption, tarsal fusion, and bristle disruptions.

Flies heterozygous for the ts mutant N^{103} and the recessive eye mutant spl exhibit a ts expression of wing nicking, eye facet array, and tarsal fusion phenotypes (WELSHONS, personal communication; and see Table 6). Both the eye and wing mutant phenotypes of N^{103}/spl females are enhanced at high temperatures and decreased at low temperatures, and tarsal fusion only occurs at high temperatures. At 28°-29°C, the eyes of N^{103}/spl flies resemble those of N^{g11}/+ females raised at 20°-22°C (Plate 3), except that facet disarray extends over the entire surface of the eye. At 20°-22°C, the eyes of N^{103}/spl approach wild type, but usually contain small, irregular areas of facet disarray.

Since preliminary shift experiments roughly localized the TSPs for eye, wing, and leg phenotypes to the third instar-pre-pupal period, and since increases or decreases in the numbers of certain bristles appeared to be affected by temperature shifts at these stages (data not presented here), the prepupa isolation

method already used with N^{g11}/+ was used to time these events
more precisely. Eggs from the cross $\underline{M5/y}$ $\underline{w^a}$ $\underline{N^{103}}$ ♀ x $\underline{spl/Y}$ ♂
were collected at 22°C on food in petri plates in successive 12-
hour intervals for several days. Half of these plates (shift-up
series) were incubated at 20°C and the other half (shift-down
series) were shifted to 28°C immediately after collection. Plates
were incubated at the respective temperatures until the appear-
ance of puparia. White puparia were isolated from the respective
cultures during a 12-hour period just before shifting, placed
into vials, and incubated further at the same temperatures. Then
the larvae and isolated puparia from the shift-up series were all
shifted from 20°C to 28°C; those from the shift-down series were
all shifted from 28°C to 20°C, and white puparia were collected
at defined times. Adult $\underline{N^{103}/spl}$ females which emerged from the
isolated prepupae were scored for eye, wing, leg, and bristle
phenotypes. The results (Figure 7) confirm that the TSPs for all
the phenotypes examined occur in the third larval instar. Note
that in the eyes, the boundary between strongly mutant tissue
and weakly mutant tissue, progresses anteriorly across the eye
with increasing larval age at the time of shifting, as was ob-
served in $\underline{N^{g11}}$/+ females. Furthermore, comparison with the
results for $\underline{N^{g11}}$/+ (Figure 6) reveals a striking similarity in
that the high-temperature eye TSPs of both genotypes end several
hours before the low-temperature eye TSPs, with respect to
puparium formation. It should be noted here that the partial
penetrance of $\underline{spl}$ in $\underline{N^{103}/spl}$ females at 20°C, precludes precise
determination of the limits of the eye TSPs (hence the "approxi-

FIGURE 7   The eye, wing, leg, and bristle phenotypes of

N^{103}/spl females shifted from a) 20°C to 28°C, and

b) 28°C to 20°C at different times prior to or after puparium

formation. ⬙⬙⬙ = eye facet arrangement approaches wild-type;

⬙⬙ = strongly mutant eye facet arrangement; ▪ = wingtip nick-

ing; ▫ = tarsal fusion; Ö = ocellar bristles; ● = dorsocentral

bristles; △ = anterior scutellar bristles; ▲ = vertical

bristles. Note that the anterior rim of the eye is to the left.

a. SHIFT-UP

APPROXIMATE EYE TSP

EYE PHENOTYPE

BRISTLE NO. IN RELATION TO WILD TYPE

+2.0

+1.0

0.0

-1.0

THIRD INSTAR

PREPUPA

-2.0

WING NICKING OR TARSAL FUSION

100

50

0

TIME OF SHIFT (HRS) BEFORE OR AFTER PUPARIUM FORMATION

30   20   10   0   10   20

BEFORE   AFTER

b. SHIFT-DOWN
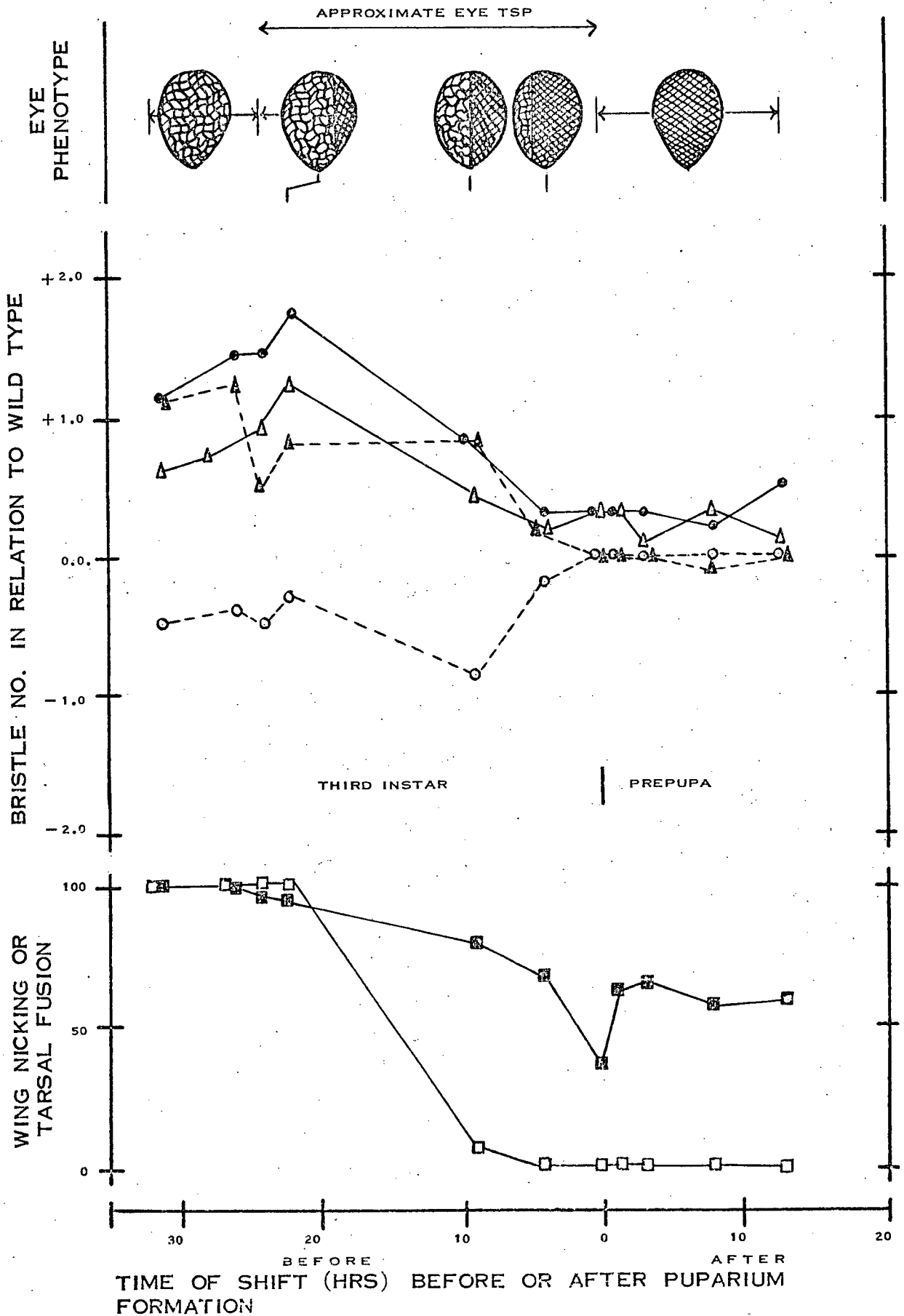
TIME OF SHIFT (HRS.) BEFORE OR AFTER
PUPARIUM FORMATION
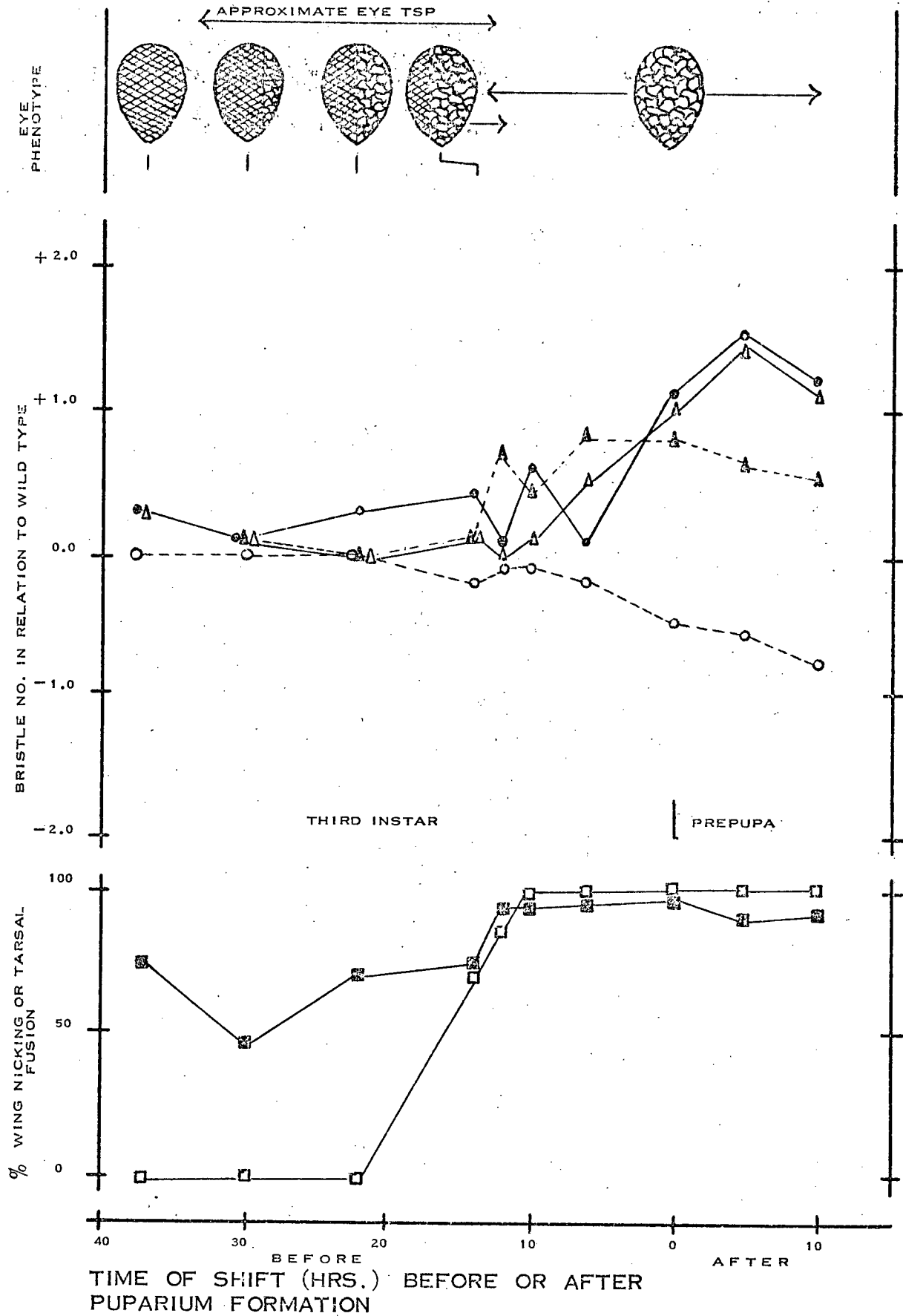
mate" eye TSPs depicted in Figure 7), but it can definitely be stated that at 28°C the eye TSP ended at least 10 hours before puparium formation. The bristle data show that shift-ups prior to 4 hours before puparium formation result in the appearance of extra vertical, dorsocentral, and anterior postalar bristles, and cause loss of ocellar bristles (Figure 7a). Conversely, shift-downs prior to 10-14 hours before puparium formation markedly restrict the increase or loss of these bristles (Figure 7b). All these phenotypes are seen in both N^{103}/+ and spl individuals, so the ts expression observed here cannot be definitely attributed to one or the other of these mutations.

To summarize briefly, the results of the temperature-shift experiments on N^{103}/spl females have shown that: 1) the eye facet and wing nicking phenotypes of N^{103}/spl have third-instar TSPs; 2) with respect to puparium formation, the eye TSP of N^{103}/spl ends several hours earlier at high temperatures than at low temperature, a pattern very similar to that observed in N^{g11}/+ females; and 3) the TSPs for fusion of tarsal segments, appearance of extra vertical, dorsocentral and anterior postalar bristles, and loss of ocellar bristles, all occur during the third larval instar.

III. OR - radiation-induced rough eye phenocopy.

The posterior-to-anterior progression of the boundary between mutant and non-mutant eye tissue in N^{g11}/+ and N^{103}/spl females shifted at successively later times during the eye TSP,

resembles the observations made by BECKER (1957). He found that a phenocopy of a "rough" eye could be induced by X-irradiation during the third instar-prepupa stages, and that with increasing age at the time of irradiation the irregular arrangement migrated anteriorly across the eye in a vertical band. Since the eye TSPs for both N^{g11}/+ and N^{103}/spl occur earlier at 29°C than at 20°-22°C (Figures 6, 7), it was asked whether the sensitivity of eye facet arrangement to radiation would also occur earlier at 29°C than at 20.5°C. The results of the experiment described below indicate that no difference exists in the radiation sensitive period (RSP) of OR flies irradiated during the third instar and prepupa stages, at 29°C or 20.5°C.

For this experiment, two groups of 15 OR females each (inseminated by OR males) were allowed to lay eggs at 20.5°C for several days on petri plates, which were changed every 24 hours (as for the prepupa-isolation temperature-shift experiments). All of the eggs laid by one group of females were transferred to 29°C immediately after collection. The other set of plates was kept at 20.5°C. Before irradiation, white prepupae were isolated from both series at defined times and kept at the respective temperatures until eclosion. At one defined time, all the plates in both series and the isolated pupae and prepupae were irradiated for 25 seconds from a Cobalt-60 source delivering approximately 4500 rads per minute. Immediately after radiation the cultures were placed back at their respective temperatures, and isolation of white prepupae at defined times was continued as before. The eyes of adult flies emerging from the isolated pre-

pupae were examined with a high power dissecting microscope (100x), and the position of irregular eye tissue was noted for each eye.

Penetrance of the rough eye phenocopy was 100% in recovered adult flies irradiated as prepupae or late third instar larvae and, as shown by the data in Table 39, synchrony was fairly good in flies irradiated up to 18 hours before puparium formation. These results, summarized in Figure 8, show that irradiation of OR flies during the larval or prepupal period causes roughly the same pattern of eye facet disturbance at both 29°C and 20.5°C. This indicates that unlike the TSPs for eye facet arrangement in N^{g11}/+ and N^{103}/spl, the end of the RSP for eye facet arrangement is not displaced at 29°C compared to 20.5°C.

IV. Ax^{16172}/N^{40} – TSP for lethality; Ax^{16172}/+ – TSPs for

wing vein gapping and loss of ocellar bristles.
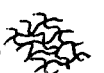
In the investigation of Ax mutant expression, it was found that expression of the ocellar bristle-loss and wing vein gap phenotypes in Ax^{16172}/+ females was temperature sensitive (Table 19) and that lethality of the combinations of Ax^{16172} with most N mutations was also ts (Table 23). Ax^{16172}/+ females raised at 29°C have significantly fewer ocellar bristles and more gaps in wing veins than females reared at 22°C (Appendix 9), and Ax^{16172} is lethal or semilethal in combination with N mutations at 29°C but viable at 22°C (Table 23). The temperature-shift experiments to be reported show that the TSP for lethality of Ax^{16172}/N^{40}

TABLE 39　　Number of rows of disrupted ommatidia in adult OR flies irradiated before or after puparium formation, at 20.5°C and 29°C.

TEMPERATURE	TIME OF RADIATION WITH RESPECT TO PUPARIUM FORMATION	NUMBER OF ROWS OF IRREGULAR OMMATIDIA AT ANTERIOR EDGE OF EYE		NUMBER OF EYES SCORED
		AVERAGE	RANGE	
20.5°C	17 hr. after (pupae)	(WILD TYPE)		14
	6.5 hr. after (prepupae)	3.5	3-4	10
	0.5 hr. after (prepupae)	7.3	6-9	10
	1.5 hr. before (3rd instar)	9.3	8-10	16
	6.5 hr. before (3rd instar)	11.8	10-13	22
	17.5 hr. before (3rd instar)	13.5	10-16	12
29°C	17 hr. after (pupae)	(WILD TYPE)		6
	0.5 hr. after (prepupae)	7.5	6-9	18
	1.5 hr. before (3rd instar)	9.5	7-11	26
	6.5 hr. before (3rd instar)	13.5	12-15	18
	18 hr. before (3rd instar)	*	9-16	4

* In the 29°C 18 hr. (before) sample, the 8-11 anterior rows of ommatidia were wild-type, the next 9-16 rows were rough in a vertical strip in the middle of the eye, and the 7-12 posterior rows were wild-type.

FIGURE 8   The eye phenotypes of <u>OR</u> flies irradiated at

different times before and after puparium formation,

at 20.5°C and 29°C. ▨ = wild-type facet arrangement; ✾ =

mutant eye facet arrangement. Note that the anterior rim of the

eye is to the left.

29°C

20.5°C

| | | | | |
|20|10|0|10|20|
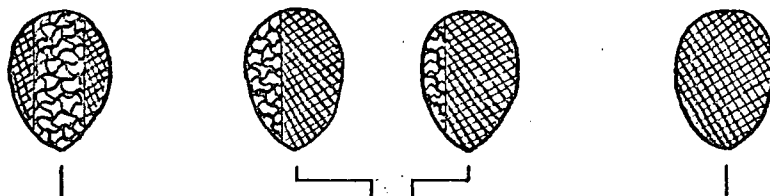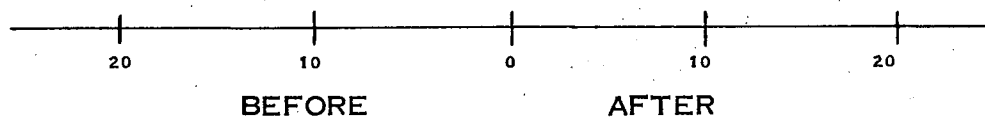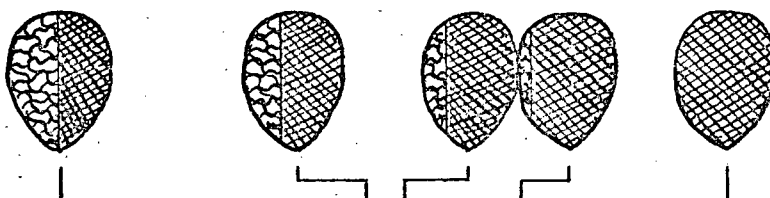
BEFORE   AFTER

TIME OF IRRADIATION BEFORE OR
AFTER PUPARIUM FORMATION (HOURS)

flies occurs during the second larval instar, whereas the TSPs for both the ocellar and wing vein phenotypes of $Ax^{16172}/+$ females occur in the third instar.

The cross $\underline{M5}/\underline{w^a}$ $\underline{N^{40}}$ $\underline{rb}$ ♀ x $\underline{Ax^{16172}/Y}$ ♂ was used to generate cultures for shifting which contained both $\underline{Ax}/+$ and $\underline{Ax}/\underline{N}$ females. Two experiments were performed with cultures established from 2-hour egglays by approximately 100 inseminated females each. In the first experiment, cultures incubated at 22°C (shift-up) or 29°C (shift-down) were shifted from 22°C to 29°C and vice versa at regular intervals. In the second experiment, cultures incubated at 22°C were shifted to 29°C at regular intervals and shifted back to 22°C 18 hours later (pulse-up); cultures incubated at 29°C were shifted to 22°C at regular intervals and shifted back to 29°C 24 hours later (pulse-down). This timing of pulse-shifts was adopted because 18 hours at 29°C is roughly the equivalent, in developmental terms, of 24 hours at 22°C.

The results of the shift-up and shift-down series show that shift-ups before or during the second larval instar (Table 40), and shift-downs after the second larval instar (Table 41), cause significant mortality of $\underline{Ax^{16172}}/\underline{N^{40}}$ females. This indicates that the TSP for $\underline{Ax}/\underline{N}$ lethality occurs in the second larval instar and possibly extends into the early third instar. The results of the pulse-shift experiments show that at no stage of the life cycle do 18-hour pulse-ups significantly reduce viability (Table 42), whereas in the pulse-down series, 24-hour incubations at 22°C during the second instar rescue most of the $\underline{Ax}/\underline{N}$ heterozygotes from pupal death (Table 43). The latter result

TABLE 40   Data indicating viability of Ax^{16172}/N^{40} females relative to their sibs when shifted from 22°C to 29°C at different successive intervals.

CULTURE NUMBER	TIME OF SHIFT		NUMBER OF PROGENY				% SURVIVAL OF Ax/N FEMALES*
	CULTURE AGE (HR)	DEVELOPMENTAL STAGE	VIABLE ADULTS			DEAD PUPAE	
			M5/Ax ♀	Ax/N ♀	M5/Y ♂	Ax/N ♀	
1	24	E**	85	1	75	86	2
2	48	I	87	5	43	75	6
3	72	II	33	18	57	33	35
4	96.5	III, some II	70	79	71	1	99
5	120	III	153	157	130	7	96
6	144	III	135	101	102	10	91
7	168	III, some P	79	81	63	3	97
8	192	P, some III	100	111	104	0	100
9	216	P	169	146	131	4	97
10	240	P	70	69	63	1	99
11	264	P	106	103	113	1	99
12	288	P	64	69	48	0	100
13	NOT SHIFTED UP		66	60	49	1	98

* % survival = viable Ax/N adults ÷ (viable Ax/N adults + dead Ax/N pupae) x 100%.
** E = egg stage. See Table 32 for other symbols.

TABLE 41   Data indicating viability of Ax^{16172}/N^{40} females relative to their sibs when shifted from 29°C to 22°C at different successive intervals.

CULTURE NUMBER	TIME OF SHIFT CULTURE AGE (HR)	TIME OF SHIFT DEVELOPMENTAL STAGE	NUMBER OF PROGENY VIABLE ADULTS M5/Ax ♀	NUMBER OF PROGENY VIABLE ADULTS Ax/N ♀	NUMBER OF PROGENY VIABLE ADULTS M5/Y ♂	DEAD PUPAE Ax/N ♀	% SURVIVAL OF Ax/N FEMALES*
1	18	I**	49	45	51	0	100
2	36	II	26	20	28	1	95
3	54	II, III	27	17	29	4	81
4	72	III	59	3	62	64	4
5	90	III, some P	54	1	36	47	2
6	108	P, some III	30	0	26	33	0
7	127	P	24	0	19	29	0
8	144	P	38	3	21	27	10
9	162	P	32	1	18	30	3
10	180	A, some P	29	0	20	21	0
11	NOT SHIFTED DOWN		102	3	83	109	3

* See Table 40.
** A = adult stage. See Table 32 for other symbols.

TABLE 42 Data indicating viability of Ax^{16172}/N^{40} females relative to their sibs when pulsed from 22°C to 29°C and back after 18 hours, at different successive intervals.

CULTURE NUMBER	TIME OF PULSE-UP CULTURE AGE (HR)	TIME OF PULSE-UP DEVELOPMENTAL STAGE (UP)	NUMBER OF PROGENY VIABLE ADULTS M5/Ax ♀	NUMBER OF PROGENY VIABLE ADULTS Ax/N ♀	NUMBER OF PROGENY VIABLE ADULTS M5/Y ♂	DEAD PUPAE Ax/N ♀	% SURVIVAL OF Ax/N FEMALES*
1	24–42	E**	49	50	52	2	96
2	48–66	I	43	60	37	1	98
3	72–90	II	60	68	43	2	97
4	96.5–114.5	III, some II	66	60	42	1	98
5	120–138	III	60	72	57	0	100
6	144–162	III	69	52	77	1	98
7	168–186	P, some III	57	55	32	1	98
8	192–210	P	62	86	60	0	100
9	216–234	P	93	84	68	3	97
10	240–258	P	42	63	57	1	98
11	264–282	P	80	77	60	5	94

* See Table 40.
** See Tables 32, 40 for explanation of symbols.

TABLE 43   Data indicating viability of Ax^{16172}/N^{40} females relative to their sibs when pulsed from 29°C to 22°C and back after 24 hours, at different successive intervals.

CULTURE NUMBER	TIME OF CULTURE AGE (HR)	PULSE-DOWN DEVELOPMENTAL STAGE (DOWN)	NUMBER OF PROGENY				% SURVIVAL OF Ax/N FEMALES*
			VIABLE ADULTS			DEAD PUPAE	
			M5/Ax ♀	Ax/N ♀	M5/Y ♂	Ax/N ♀	
1	18–42	I**	44	4	35	38	10
2	36–61	II	50	38	23	11	78
3	54–78	II, III	72	41	56	45	48
4	72–96	III	41	0	43	35	0
5	90–114	III, some P	54	2	37	58	3
6	108–132	P, some III	62	1	40	66	1
7	127–150	P	55	3	35	46	6
8	144–168	P	37	1	30	32	3
9	162–186	P	52	0	47	61	0

* See Table 40.
** See Table 32 for explanation of symbols.

confirms the second-instar TSP inferred from the reciprocal shift series (Tables 40, 41). Furthermore, the pulse-shift results suggest that only a relatively short incubation at 22°C during the second instar is sufficient to prevent death of most $\underline{Ax}/\underline{N}$ females, and that 18-hour pulses at 29°C are not sufficient to cause death (Tables 42, 43). The results of the shift-up, shift-down, and pulse-down experiments are summarized in Figure 9.

To determine the stage when loss of ocellar bristles and gapping of wing veins are affected by temperature, ocellar bristles and wing vein gaps were counted in the $\underline{M5}/\underline{Ax}^{16172}$ ($\underline{Ax}/+$) female progeny recorded in Tables 40-43. The results show clearly that in the shift-up series the frequency of wing vein gaps drops and the ocellar bristle frequency rises during the third larval instar (Figure 10a), and in the shift-down series the wing vein gap frequency rises and the ocellar frequency drops during the third instar (Figure 10b). Furthermore, the wing vein gap and ocellar frequencies become significantly (see footnote to Figure 10) more mutant when cultures are pulsed up during the third instar (Figure 10a), and likewise these phenotypes become significantly less mutant when cultures are pulsed down during the third instar (Figure 10b) (wild-type frequencies for wing vein gaps and ocellars are 0.0 and 2.0, respectively). These results indicate that the TSPs for both the wing vein gap and the ocellar bristle phenotypes of $\underline{Ax}^{16172}/+$ occur during the third larval instar.

The foregoing results can be summarized as follows: 1) the TSP for lethality of $\underline{Ax}^{16172}/\underline{N}^{40}$ females occurs during the second

FIGURE 9   Viability of Ax^{16172}/N^{40} shifted at different

stages of development. X = shift-up; O = shift-

down; Δ = pulse-down. The shift-ups are timed on the 22°C scale

(bottom), while shift-downs and pulse-downs are timed on the 29°C

scale (top).

DEVELOPMENTAL STAGES AT 29°C (APPROX.)

EGG 1ST 2ND 3RD PUPA ADULT

HOURS AT 29°C

0 50 100 150 200

% SURVIVAL OF A×$\frac{16172}{N}$ ^{40} FEMALES

100

80

60

40

20

0

HOURS AT 22°C

0 100 150 300

EGG 1ST 2ND 3RD PUPA
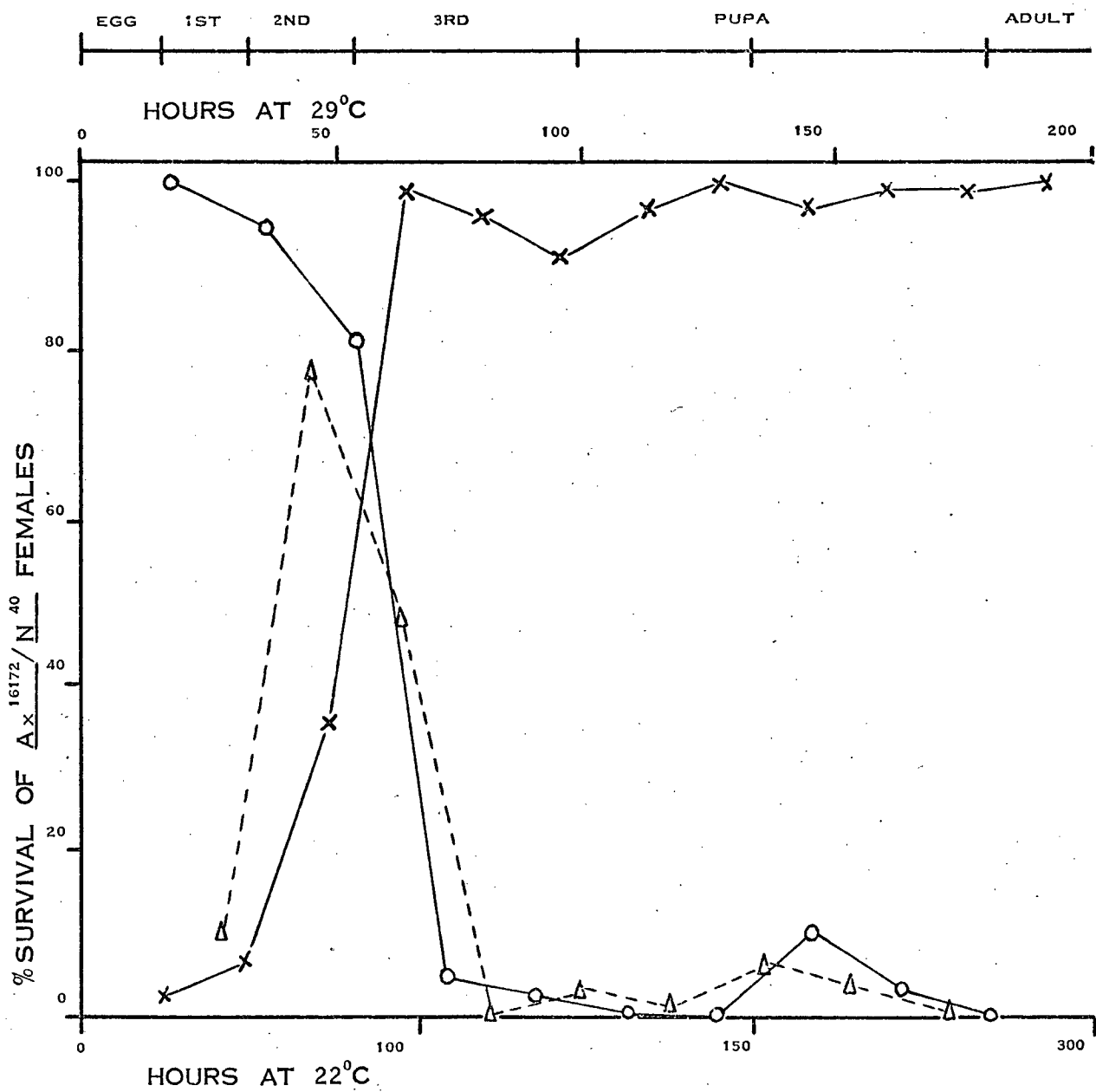
DEVELOPMENTAL STAGES AT 22°C (APPROX.)

FIGURE 10   The number of ocellar bristles and wing vein gaps

of $\underline{Ax}^{16172}$/+ females when a) shifted from 22°C to

29°C, or pulsed from 22°C to 29°C for 18 hr., and then back to

22°C, and b) shifted from 29°C to 22°C, or pulsed from 29°C to

22°C for 24 hr., and then back to 29°C. △ = wing vein gaps,

shift series; ▲ = wing vein gaps, pulse series; o = ocellar

bristles, shift series; ●. = ocellar bristles, pulse series. Note

that the points in the pulse series are plotted for the middle of

the pulse period. Note also that the drop in ocellar bristle

frequency at 155 hr. in the pulse-up series (a), is statistically

significant at the 95% level of confidence, and that the other

bristle and wing vein gap frequency changes occurring during the

third instar (shift-up, pulse-down and shift-down series), are

also statistically significant.

a. SHIFT- OR PULSE-UP

APPROXIMATE DEVELOPMENTAL STAGES AT 22°C
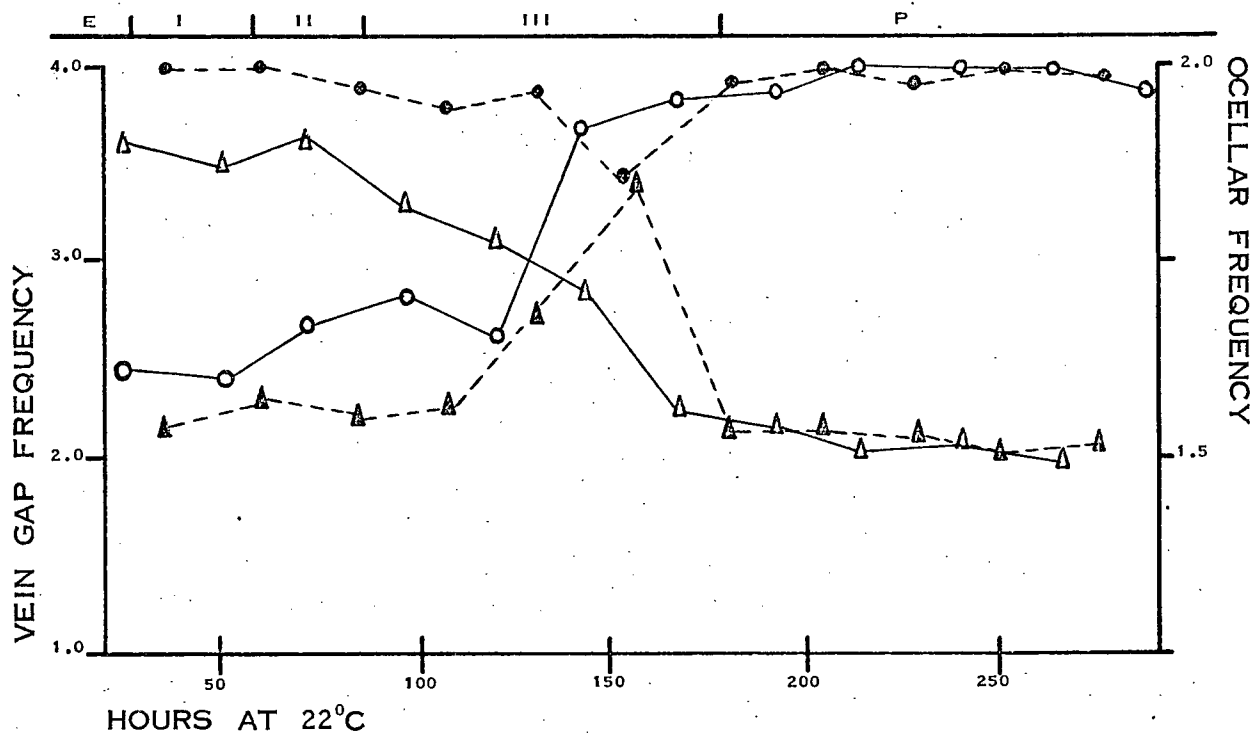
b. SHIFT- OR PULSE-DOWN

APPROXIMATE DEVELOPMENTAL STAGES AT 29°C
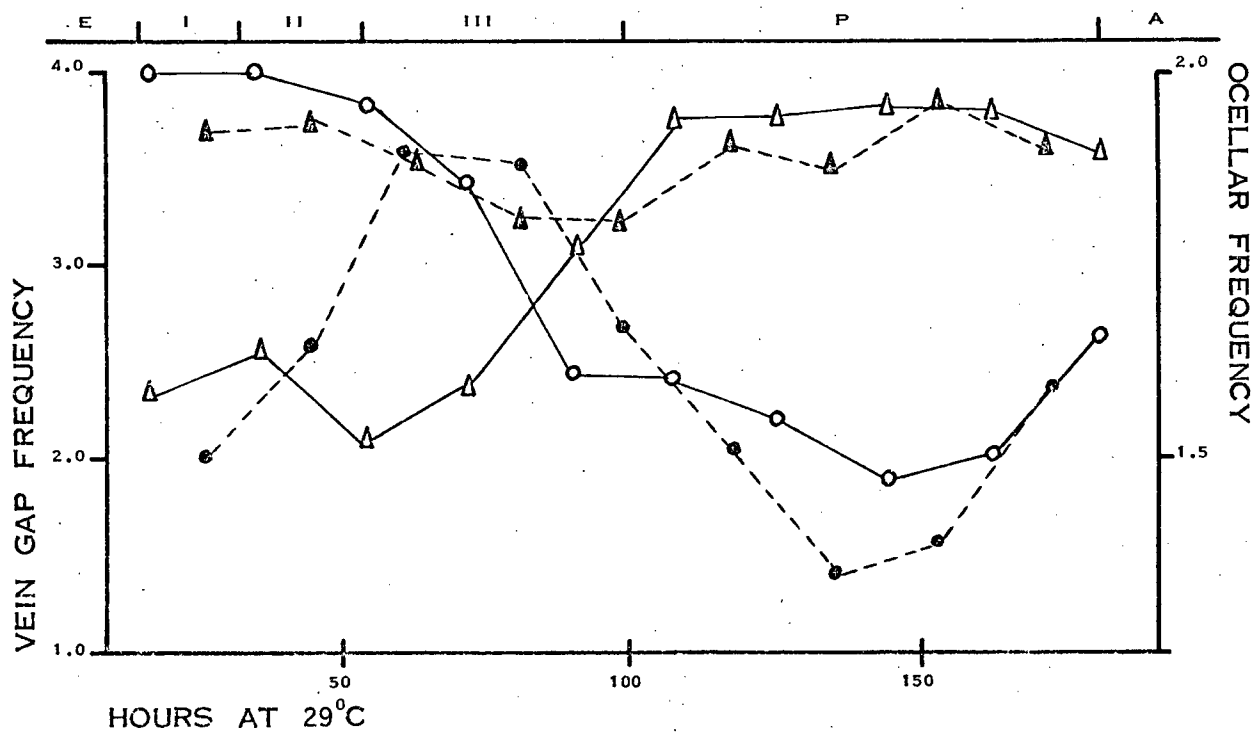
larval instar, although 18-hour pulses at 29°C during this stage are not sufficient to cause lethality; and 2) the TSPs for enhancement of wing vein gapping and ocellar bristle loss in $Ax^{16172}/+$ females occur during the third larval instar.

V. N^{103}/fa^{no} - TSPs for lethality.

Unlike most other N/fa^{no} combinations, which are lethal under all conditions, N^{103}/fa^{no} females die when raised at 29°C but survive at 22°C (Appendix 1). The results of the shift experiments to be reported here show that one or several TSPs for lethality extend from the egg through the larval stage into the prepupal or pupal stages, and that there are three distinct lethal phases in flies reared at 29°C. Moreover, additional observations suggest that at 22°C, whereas most N/Y hemizygotes die as embryos, N^{103}/Y embryos may survive into the third larval instar.

Eggs of the cross $M5/y\ w^a\ N^{103}\ ♀$ x $fa^{no}/Y\ ♂$ were collected for shift experiments to determine viability of N^{103}/fa^{no} females. Each culture was established from the 2-hour egglays of 100 inseminated females. As described previously for Ax^{16172}/N^{40} and $Ax^{16172}/+$, the first experiment consisted of 22°C-29°C (shift-up) and 29°C-22°C (shift-down) shifts, and the second experiment consisted of 18-hour pulses to 29°C at 24-hour intervals in 22°C cultures (pulse-ups), and 24-hour pulses to 22°C at 18-hour intervals in 29°C cultures (pulse-downs).

The results of the shift-up series show that shift-ups before the end of the third larval instar result in low survival (0-7%) of N^{103}/fa^{no} females, that after the third instar there is a jump to about 50% survival, and that successively later shift-ups after pupation allow correspondingly greater survival of N^{103}/fa^{no} flies (Table 44, Figure 11). Furthermore, when shifted up from the late embryo stage (24-hour egg) onward, death of N^{103}/fa^{no} females occurs from the prepupal or early pupal (eye phenotype and sex unscorable due to early death, see Table 41) to the late pupal stages (Figure 11). It is assumed that most of the unscorable dead pupae resulting from shift-ups before puparium formation were N^{103}/fa^{no} females. This is indicated by the absence of a temperature-effect on mortality of $M5/fa^{no}$ females and $M5/Y$ males and because the sum of the number of verifiable N^{103}/fa^{no} flies (surviving adults and dead pupae) and unscorable dead pupae is roughly equal to the number of $M5/fa^{no}$ females recovered (Table 44). Inspection of Figure 11 reveals an interesting pattern of lethality: 1) shift-ups from the late embryo to the end of the third larval instar cause death of about half of the N^{103}/fa^{no} females in the prepupa or early pupa stage, and about half in the late pupa stage; 2) shift-ups immediately after the third instar permit roughly half of the N^{103}/fa^{no} females to emerge as adults, the remainder dying as late pupae; and 3) progressively later shift-ups after pupation allow proportionately greater survival of N^{103}/fa^{no} females. On the other hand, the results of the shift-down series show that shift-downs as early as the first instar (18 hours) do

TABLE 44

Data indicating viability of N^{103}/fa^{no} females in relation of their sibs when shifted from 22°C to 29°C at different successive intervals.

| CULTURE NUMBER | TIME OF SHIFT | | NUMBER OF PROGENY | | | | | % SURVIVAL OF N^{103}/fa^{no} FEMALES* |
| | CULTURE AGE (HR) | DEVELOPMENTAL STAGE | VIABLE ADULTS | | | DEAD PUPAE | | |
			$M5/fa^{no}$ ♀	N^{103}/fa^{no} ♀	M5/Y ♂	N^{103}/fa^{no} ♀	UNSCOR-ABLE	
1	24	E**	32	0	28	20	12	0
2	48	I	116	1	88	65	46	1
3	72	II	118	0	115	62	85	0
4	96.5	II, III	96	5	64	45	43	5
5	120	III	112	4	78	55	63	3
6	144	III	62	6	55	39	35	7
7	168	P, some III	62	34	57	26	4	53
8	192	P, a few III	115	51	81	56	4	46
9	216	P	159	111	134	32	6	75
10	240	P	133	116	118	22	5	81
11	264	P	80	75	63	4	3	91
12	288	P	68	61	51	0	4	94
13	NOT SHIFTED UP		94	96	80	7	2	91

* % survival = viable N^{103}/fa^{no} adults ÷ (viable N^{103}/fa^{no} adults + dead N^{103}/fa^{no} pupae + unscorable dead pupae) x 100% (see text).

** See Tables 32, 40 for explanation of symbols.

FIGURE 11   Relative proportions of viable N^{103}/fa^{no} adult

females, late pupal N^{103}/fa^{no} female deaths, and

early pupal (unscorable) deaths, in cultures shifted from 22°C

to 29°C at different successive intervals. ▨ = viable

N^{103}/fa^{no} adults; ≡ = late pupal N^{103}/fa^{no} deaths; ▨ = unscor-

able early pupal deaths. This Figure is based on the data

presented in Table 44.

% IN EACH CLASS
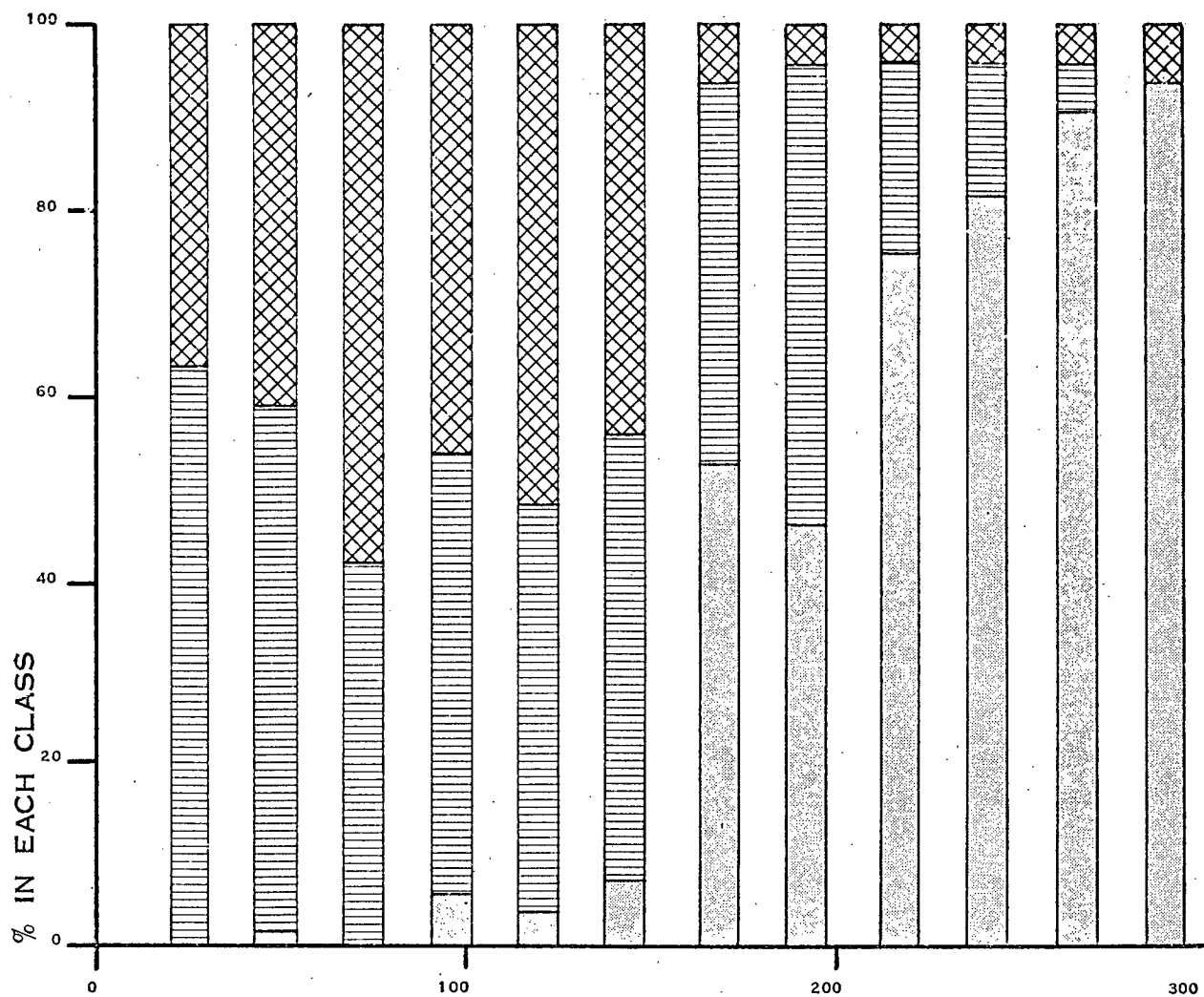
HOURS AT 22°C PRIOR TO SHIFT-UP

EGG 1ST 2ND 3RD PUPA
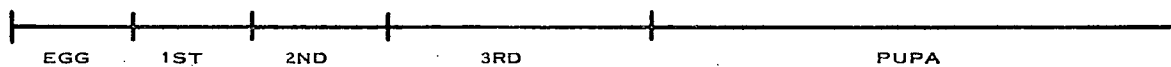
APPROXIMATE DEVELOPMENTAL STAGES AT 22°C

not permit significant survival of N^{103}/fa^{no} females, and that
furthermore very few die as pupae (Table 45) (the few which do
die as pupae may be attributed to asynchronous egglays). This
indicates that incubation of N^{103}/fa^{no} females at 29°C during the
embryonic stage induces death at some stage prior to puparium
formation (possibl even in the egg stage). The results describ-
ed above show that one TSP for lethality of N^{103}/fa^{no} occurs in
the early embryo stage, and another extends through one or more
of the larval instars and ends during the pupa stage. These
findings are summarized in Figure 12.

The results of the pulse-up series show that exposure to
29°C for 18-hour periods at any developmental stage after the
late embryo did not cause significant mortality of N^{103}/fa^{no}
females (Table 46). Similarly, pulse-downs after the embryo
stage did not result in survival of N^{103}/fa^{no} females (Table 47).
Survival in this case would not be expected, since each pulse-
down culture was incubated at 29°C during the embryo stage, and
it has already been shown that this results in death before
puparium formation (Table 45).

As was noted earlier, certain observations have provided
evidence that not all N^{103}/Y males die as embryos at 22°C.
During the experiments reported above, larvae were selected from
22°C and 29°C cultures at the times of shifts and their develop-
mental stages determined according to the morphology of their
mouthparts and anterior spiracles. In the 22°C, but not in the
29°C series, some of the larvae had yellow mouthparts (about
20%-25% of the 1st-2nd instar larvae sampled, fewer of the 3rd

TABLE 45   Data indicating viability of N^{103}/fa^{no} females in relation to their sibs when shifted from 29°C to 22°C at different successive intervals.

	TIME OF SHIFT		NUMBER OF PROGENY				
			VIABLE ADULTS			DEAD PUPAE	
CULTURE NUMBER	CULTURE AGE (HR)	DEVELOPMENTAL STAGE	$M5/fa^{no}$ ♀	N^{103}/fa^{no} ♀	$M5/Y$ ♂	N^{103}/fa^{no} ♀	UNSCORABLE
1	18	I*	31	0	18	3	0
2	36	I, II	63	2	52	2	0
3	54	II, some III	57	0	56	0	0
4	72	III	22	0	31	2	1
5	90	III, some P	47	1	44	0	4
6	108	P, some III	28	0	30	0	2
7	127	P	25	0	31	0	0
8	144	P	17	0	23	0	0
9	162	P	36	0	51	1	0
10	180	A, some P	59	0	47	0	1
11	NOT SHIFTED DOWN		57	0	54	1	0

* See Tables 32, 41 for explanation of symbols.

FIGURE 12   Time of death of N^{103}/fa^{no} females in relation to

time of shift from 22°C to 29°C. Note that the

"pupa" stage as used here includes the prepupa stage. Note also

that the actual time of death has not been critically ascertained

for early embryo shift-ups, but that death does occur before the

prepupa stage.
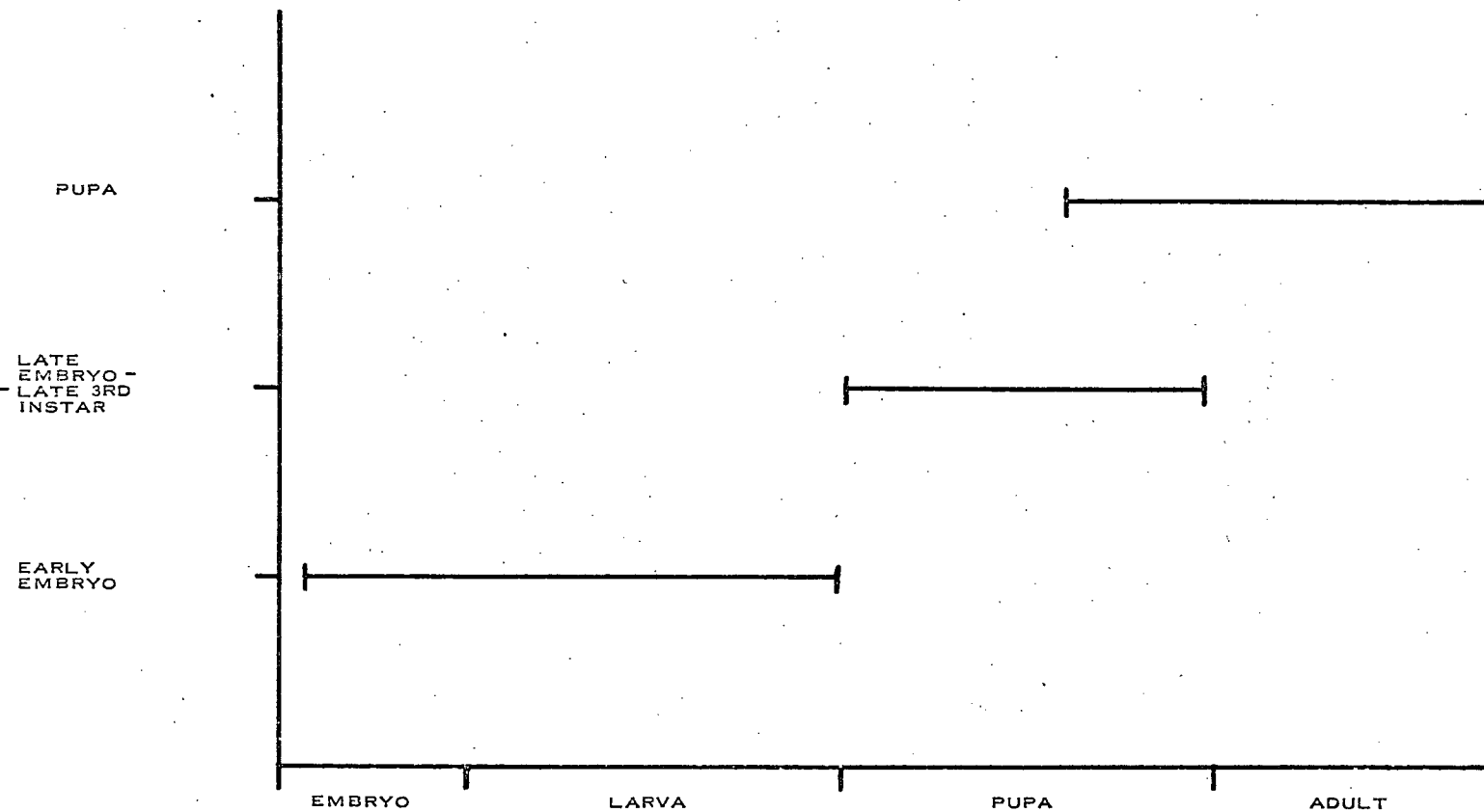
TABLE 46   Data indicating viability of N^{103}/fa^{no} females in relation to their sibs when pulsed from 22°C to 29°C and back after 18 hours, at different successive intervals.

| | TIME OF | PULSE-UP | NUMBER OF PROGENY | | | |
| | | | VIABLE ADULTS | | | TOTAL DEAD PUPAE (NOT SCORED) |
CULTURE NUMBER	CULTURE AGE (HR)	DEVELOPMENTAL STAGE (UP)	$M5/fa^{no}$ ♀	N^{103}/fa^{no} ♀	$M5/Y$ ♂	
1	24–42	E*	59	47	50	1
2	48–66	I	61	69	61	7
3	72–90	II	63	34	43	11
4	96.5–114.5	II, III	55	56	61	6
5	120–138	III	56	44	43	4
6	144–162	III	115	77	72	11
7	168–186	P, some III	69	66	53	13
8	192–210	P, (+2 III)	53	44	39	1
9	216–234	P	48	63	40	1
10	240–258	P	66	61	53	4
11	264–282	P	48	53	32	6

* See Tables 32, 40 for explanation of symbols.

TABLE 47   Data indicating viability of N^{103}/fa^{no} females in relation to their sibs when pulsed from 29°C to 22°C and back after 24 hours, at different successive intervals.

| CULTURE NUMBER | TIME OF PULSE-DOWN | | NUMBER OF PROGENY | | | | |
| | | | VIABLE ADULTS | | | DEAD PUPAE | |
	CULTURE AGE (HR)	DEVELOPMENTAL STAGE (DOWN)	$M5/fa^{no}$ ♀	N^{103}/fa^{no} ♀	$M5/Y$ ♂	N^{103}/fa^{no} ♀	UNSCORABLE
1	18-42	I*	39	0	35	1	0
2	36-61	I, II	80	0	52	0	1
3	54-78	II, some III	16	0	36	4	3
4	72-96	III	12	0	21	0	0
5	90-114	III, some P	42	0	56	2	4
6	108-132	P, some III	25	0	32	0	2
7	127-150	P	18	0	36	0	0
8	144-168	P	38	0	28	1	4
9	162-186	P	35	0	50	0	4
10	180	A, some P	42	0	32	1	0

* See Tables 32, 41 for explanation of symbols.

instars), indicating that they were mutant for the gene y. From the genotypes of the parents used to generate these cultures ($M5/y\ w^a\ N^{103}\ \female\ \times\ fa^{no}/Y\ \male$), these must have been $y\ w^a\ N^{103}/Y$ males. Recombination between N^{103} and y, giving $y\ N^+/Y$ males, is unlikely, since these mutations are only 3.0 map units apart (LINDSLEY AND GRELL 1968), and furthermore, $M5$ suppresses crossing over in the X chromosome. The y larvae had smaller mouthparts and developed more slowly than their y^+ (black mouthparts) sibs. Moreover, third instar y larvae did not have visible teeth on the mouthhooks, compared to 9-12 teeth in the y^+ larvae. No prepupae or pupae with y mouthparts were seen. These results indicate that unlike most other N/Y hemizygotes (POULSON 1939b, 1968), N^{103}/Y males incubated at 22°C survive the embryo stage and may reach the third larval instar before dying. The absence of y larvae at 29°C suggests that N^{103}/Y males do not survive the embryonic stage at this temperature.

The preceding results can be summarized as follows: 1) TSPs for lethality of N^{103}/fa^{no} females occur in the embryonic stage and also during the larval instars; 2) shift-ups during the early embryo stage cause lethality before puparium formation, whereas later shift-ups cause death after puparium formation; 3) pulse-ups (18-hour periods at 29°C) from the late embryo stage onward, do not cause significant mortality of N^{103}/fa^{no} females; and 4) N^{103}/Y hemizygotes die as embryos at 29°C, but at 22°C they may survive into the third instar, dying before puparium formation.

VI. $N^{g11}/N^{g11};Dp$ - TSP for lethality.

As described in the section dealing with gene dosage, the combination $N^{g11}/N^{g11};Dp$ has a lethal phenotype which is ts. Females of this genotype survive at 29°C, but at 20°C-22°C they die at some stage prior to puparium formation (Table 11). The temperature-shift experiments reported here show that the TSP for lethality is restricted to the embryo stage, unlike those previously described for Ax^{16172}/N^{40} and N^{103}/fa^{no}.

In the first experiment, 2-hour egglays from approximately 200 $M5/w^a N^{g11} rb$ females (inseminated by $w^a N^{g11} rb/Y;Dp$ males) were collected at 20.5°C (shift-up) and 29°C (shift-down), and shifted from one temperature to the other at regular intervals. The results of the shift-up series show that the shift-up during the early embryo stage (0 hour after egg collection) allows survival of $N^{g11}/N^{g11};Dp$ females, but that shift-ups during or after the late embryo stage (from 24 hours onward) cause death of this genotype (Table 48). Conversely, shift-downs during the early embryo stage (0 hour) cause death of $N^{g11}/N^{g11};Dp$ females, whereas shift-downs immediately following the embryo stage (18 hours) or later do not cause lethality (Table 49). These results indicate that the TSP for lethality of $N^{g11}/N^{g11};Dp$ females occurs during the embryo stage.

In the second experiment, designed to localize the lethal TSP more precisely within the embryo stage, 1-hour egglays from approximately 300 females (see previous experiment) were collected at 22°C and placed at either 20°C (shift-up) or 28°C (shift-

TABLE 48   Data indicating viability of N^{g11}/N^{g11};Dp females in relation to their sibs when shifted from 20.5°C to 29°C at different successive intervals.

CULTURE NUMBER	TIME OF SHIFT CULTURE AGE (HR)	TIME OF SHIFT DEVELOPMENTAL STAGE	MALES M5/Y	MALES M5/Y;Dp	MALES N/Y;Dp	FEMALES M5/N	FEMALES M5/N;Dp	FEMALES N/N;Dp	% SURVIVAL OF N/N;Dp FEMALES*
1	0	E**	18	7	50	35	37	46	156
2	24	E	46	24	37	34	38	0	0
3	48	I	37	40	32	41	49	0	0
4	72	II	31	37	34	50	60	0	0
5	96	III	37	32	51	56	41	0	0
6	120	III	37	22	26	20	27	0	0
7	144	III, some P	49	38	31	47	41	0	0
8	168	P, some III	37	44	43	54	54	0	0
9	194	P	41	44	39	39	38	0	0
10	216	P	38	64	50	58	55	1	2
11	NOT SHIFTED UP		162	107	149	138	147	5	3

* % survival = number observed ÷ number expected x 100%, where number expected = total progeny (except N^{g11}/N^{g11};Dp) ÷ 5.

** See Tables 32, 40 for explanations of symbols.

TABLE 49   Data indicating viability of $N\underline{g^{11}}/N\underline{g^{11}};\underline{Dp}$ females in relation to their sibs when shifted from 29°C to 20.5°C at different successive intervals.

CULTURE NUMBER	TIME OF SHIFT CULTURE AGE (HR)	TIME OF SHIFT DEVELOPMENTAL STAGE	NUMBER OF PROGENY MALES M5/Y	MALES M5/Y;Dp	MALES N/Y;Dp	FEMALES M5/N	FEMALES M5/N;Dp	FEMALES N/N;Dp	% SURVIVAL OF N/N;Dp FEMALES*
1	0	E**	14	9	10	2	10	0	0
2	18	I	24	15	24	14	20	20	103
3	36	II	5	5	7	3	5	3	60
4	54	III	23	8	20	16	12	11	63
5	72	III	17	4	16	14	12	16	127
6	90	III, some P	33	15	35	29	32	31	108
7	106	P, some III	24	12	29	22	31	19	81
8	126	P	21	10	22	16	18	24	138
9	144	P	32	12	31	31	29	18	67
10	187	P	24	11	40	31	24	27	86
11	NOT SHIFTED DOWN		47	21	61	43	66	40	84

* See Table 48.

** See Tables 32, 40 for explanation of symbols.

down). Shifts from one temperature to the other were performed
hourly during most of the embryo stage. The results of the
shift-ups show that shifting from 20°C-28°C before 12 hours does
not result in lethality, but that the viability drops markedly
in the 12- to 14-hour shift-ups (Table 50). In the shift-down
series, death of nearly all N^{g11}/N^{g11};Dp females occurs in shifts
from 28°C-20°C before 6 hours, but viability rises markedly in
the 6- to 9-hour shift-downs (Table 51). These results, sum-
marized in Figure 13, indicate that the TSP for lethality of
N^{g11}/N^{g11};Dp females occupies a relatively short interval, occur-
ring about the middle of the embryo stage.

VII. Summary of temperature-shift results.

The findings of the temperature-shift experiments are sum-
marized in Figure 14. It can be seen that the TSP for each adult
morphological phenotype occurs during the third larval instar,
whereas TSPs for lethality occur during several stages of develop-
ment, depending on the genotype involved.

Although the lethal TSPs appear to be markedly different
from one another (Figure 14), there are some similarities worth
mentioning. In both cases where the TSP is embryonic (N^{103}/fa^{no}
and N^{g11}/N^{g11};Dp), incubation at the non-permissive temperature
causes death at some stage prior to pupation. Furthermore, in
both of the larval TSP cases, death occurs after puparium forma-
tion, although Ax^{16172}/N^{40} females all die in late pupal stages,
and death of N^{103}/fa^{no} females is divided about evenly between

TABLE 50   Data indicating viability of Ng^{11}/Ng^{11};Dp females in relation to their sibs when shifted from 20°C to 28°C at different successive intervals during the embryo stage.

CULTURE NUMBER	TIME OF SHIFT-UP (HOURS)	NUMBER OF PROGENY						% SURVIVAL OF N/N;Dp FEMALES*
		MALES			FEMALES			
		M5/Y	M5/Y;Dp	N/Y;Dp	M5/N	M5/N;Dp	N/N;Dp	
1	3	26	18	45	38	27	27	88
2	4	15	14	27	16	16	19	108
3	5	14	8	22	21	24	10	56
4	6	32	25	29	35	32	35	114
5	7	26	15	29	19	19	24	111
6	8	9	11	23	14	10	12	90
7	9	39	31	41	38	41	30	79
8	10	20	12	32	32	26	27	111
9	11	17	14	16	22	12	15	93
10	12	50	24	40	33	41	13	35
11	13	15	18	21	21	18	2	11
12	14	14	8	16	13	18	1	7
13	15	40	39	31	34	37	0	0
14	16	33	19	27	20	21	0	0
15	17	25	15	23	21	28	0	0
16	NOT SHIFTED	40	53	51	43	54	0	0

* See Table 48.

TABLE 51   Data indicating viability of N^{g11}/N^{g11};Dp females in relation to their sibs when shifted from 28°C to 20°C at different successive intervals during the embryo stage.

CULTURE NUMBER	TIME OF SHIFT-DOWN (HOURS)	NUMBER OF PROGENY						% SURVIVAL OF N/N;Dp FEMALES*
		MALES			FEMALES			
		M5/Y	M5/Y;Dp	N/Y;Dp	M5/N	M5/N;Dp	N/N;Dp	
1	2	48	46	36	34	42	0	0
2	3	47	45	55	39	48	2	4
3	4	37	34	35	38	45	1	3
4	5	37	56	46	37	62	2	4
5	6	37	28	39	15	30	6	20
6	7	51	43	56	37	44	17	37
7	8	71	38	56	43	47	21	41
8	9	30	25	28	33	24	26	93
9	10	40	42	49	44	39	38	89
10	11	46	43	35	33	45	29	72
11	12	35	39	56	56	50	40	85
12	13	37	36	65	45	39	37	83
13	14	44	36	43	52	41	43	100
14	15	65	39	58	66	70	63	106
15	16	50	50	70	44	52	57	107
16	NOT SHIFTED	41	24	35	39	40	33	92

* See Table 48.

FIGURE 13   Viability of $N^{g11}/N^{g11};Dp$ females shifted at

different times during the embryo stage.

O———O shifted from 20°C to 28°C;

X————X shifted from 28°C to 20°C.

Note that the arrows indicate the approximate time of egg hatch.
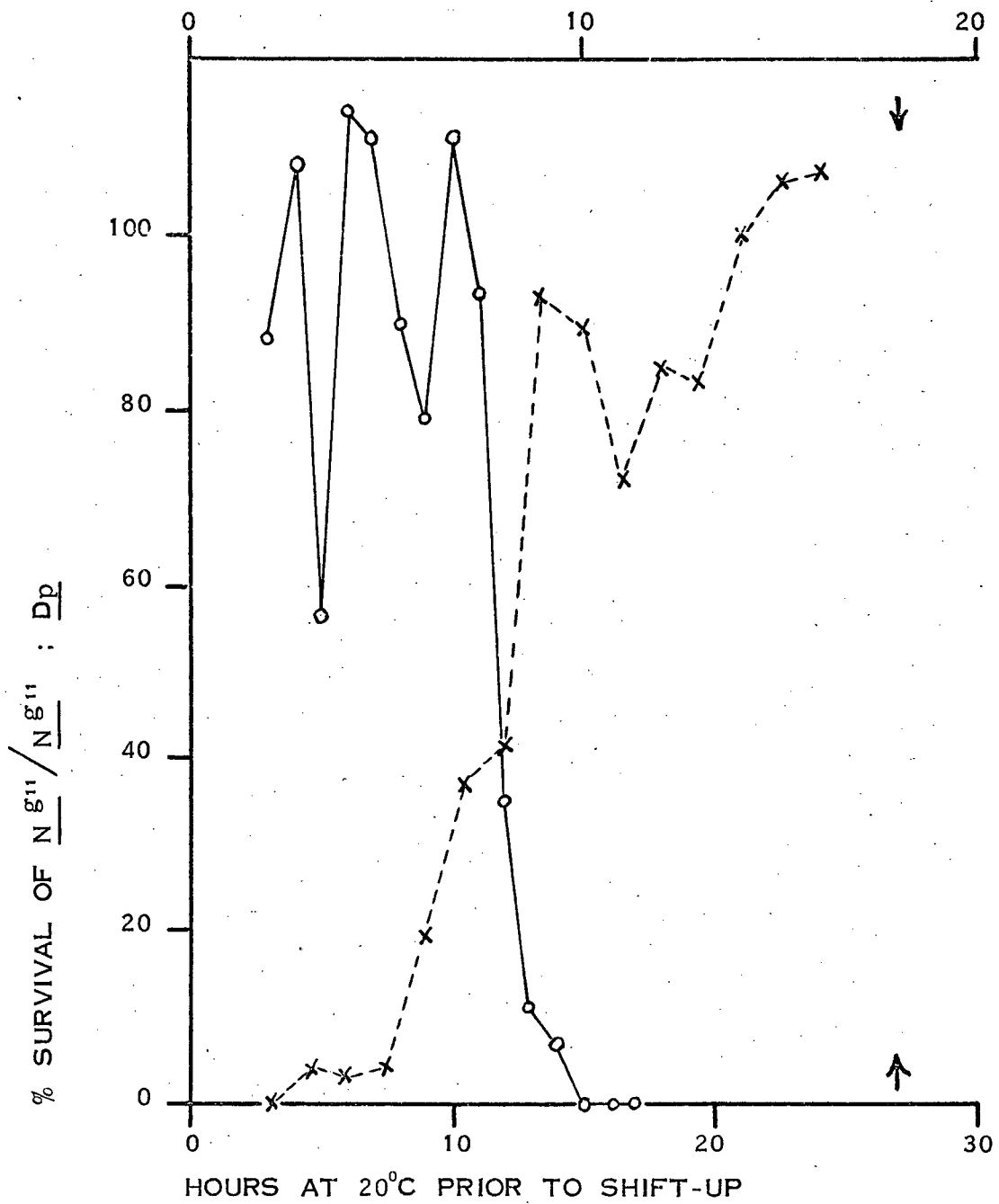
FIGURE 14   Temperature sensitive periods for lethality and

               adult morphological phenotypes of selected Notch-

locus mutant genotypes. Note that the TSPs for adult morphology

include TSPs for: 1) eye facet arrangement and wing nicking

(N^{g11}/+); 2) eye facet arrangement, wing nicking, tarsal

segment fusion, and bristle disturbances (N^{103}/spl); and 3) wing

vein gapping and ocellar bristle loss (Ax^{16172}/+). Note that

this figure does not indicate the lengths or relative positions

of the TSPs during the third instar.

DEVELOPMENTAL STAGE
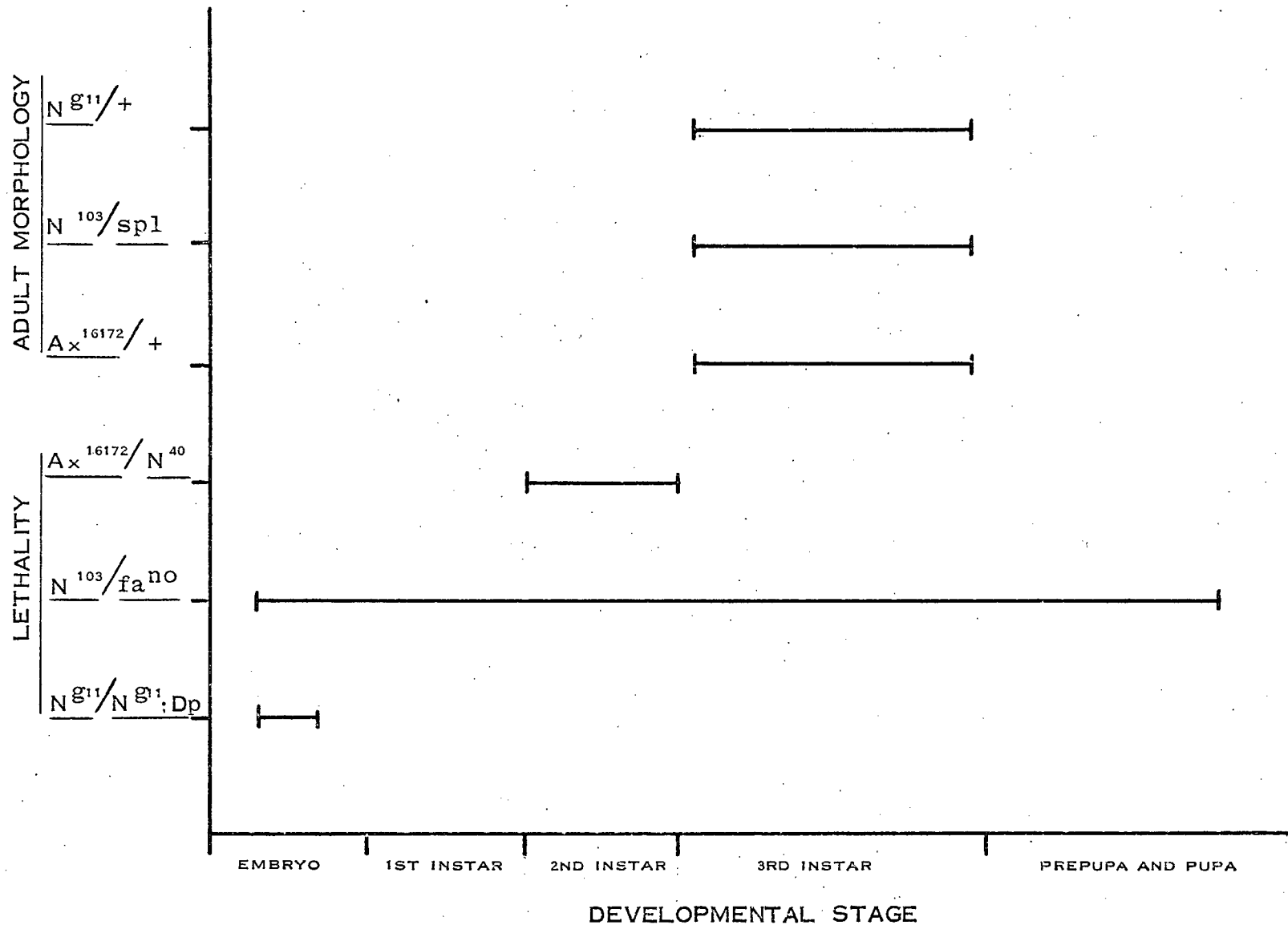
early and late pupal stages (Figure 11). It should be stressed that the data for N^{103}/fa^{no} do not rule out the possibility that there are several discrete TSPs for lethality, rather than the single one indicated in Figure 14.

DISCUSSION

The present investigation has consisted primarily of an examination of the properties of a variety of mutations within the complex Notch locus which exhibit unusual phenotypes. This includes mutations of the Notch type as well as Abruptex alleles, which do not resemble $\underline{N}$ mutations phenotypically but nevertheless are located within the Notch locus (Figure 1). In addition to the genetic complexities of the locus, the alleles within this region exhibit multiple phenes, which point to an important role in development. The following discussion will deal mainly with the phenotypic responses to alterations of the relative numbers of wild type ($\underline{N}^+$) and mutant ($\underline{N}$) alleles, the phenotypic inter-actions between $\underline{N}$ and $\underline{Ax}$ mutations and among different $\underline{Ax}$ alleles, and the developmental studies of certain conditional phenotypes associated with different Notch-locus genotypes. Since the strains used in this investigation were not co-isogenic, and (as noted in Results from time to time) certain minor differences could therefore have resulted from genetic background variability, the discussion of the $\underline{N}$ dosage and $\underline{Ax}$ results will be confined to those observations which appear to be generally repeatable in different genetic backgrounds. The present results do not elucidate a complete picture of the function of the Notch locus during development. Nevertheless, the hypothesis that its function is of a regulatory nature, rather than (in a morphological sense) of a structural nature, appears to be a plausible explanation of the data. Moreover, the data do appear

to require certain assumptions about the nature of the Notch-
locus product and several molecular models are discussed. It
should be emphasized that these models are primarily for the
purposes of illustration and discussion; the experiments report-
ed do not give precise information about the molecular nature of
the Notch-locus gene products(s). Ultimately, however, molecular
models of the Notch-locus product(s) will have to account for all
of the unusual and seemingly contradictory properties of the
mutant alleles of this complex locus.

As mentioned earlier, point $\underline{N}$ mutants exist which map as
discrete sites within the Notch locus, but are identical to $\underline{N}$
deficiencies both phenotypically and in their interactions with
other mutations within the Notch locus. These alleles can be
classed as amorphs (WELSHONS 1965), which by definition produce
either a biologically inactive gene product or no gene product
at all (MULLER 1932). Since the phenotypic effects of the point
mutation $\underline{N^{40}}$ are indistinguishable from the effects of $\underline{N^8}$, which
is known to be a deficiency, with respect to embryonic lethality
(POULSON 1939b, 1968), wing nicking frequency (Table 5), inter-
actions with Notch-locus recessive visibles (LINDSLEY AND GRELL
1968), suppression of $\underline{Ax}$ phenotypes (Tables 24-26), and reces-
siveness to other $\underline{N}$ alleles in $\underline{N^x/N^y};\underline{Dp}$ combinations (Table 12;
also see Table 16), it is reasonable to assume that $\underline{N^{40}}$ is indeed
an amorphic allele. By logical extension of this line of reason-
ing, $\underline{N}$ mutations which are not phenotypically like deficiencies,
such as $\underline{N^{g11}}$, $\underline{N^{Co}}$, and $\underline{N^{103}}$, cannot be amorphic. $\underline{N^{g11}}$ and $\underline{N^{Co}}$
not only exhibit a milder expression of certain Notch phenotypes

than deficiencies, but are also associated with additional phenotypic changes not caused by deficiencies. Depending on the temperature, the expression of N^{103} may be either milder or more extreme than deficiency of amorphic N mutations. As discussed below, analysis of these three exceptional Notches suggests that the non-amorphic N mutations may be interpreted as hypomorphic, neomorphic, hypermorphic or antimorphic depending upon the particular genotype and phenotype studied. Wing nicking and other typical Notch phenotypes exhibited by heterozygotes for deficiency or amorphic N mutations appear to be strictly regulated by N^+ gene dosage (WELSHONS 1965; and see Table 5). It follows that those N mutations showing a relatively mild wing nicking phenotype must be hypomorphic to N^+ (rather than amorphic) in terms of the product activity whose dosage determines wing nicking. This hypothesis is testable, since increasing the number of hypomorphic alleles (and presumably, therefore, the amount of product) should decrease expression of the mutant phenotype, as discussed by MULLER (1932). Thus, the reduced wing nicking (compared to N/+) observed in the N/N;Dp combinations of N^{g11}, N^{Co}, and N^{103} (at low temperatures), as shown in Tables 6, 8, and 12, is precisely the result expected if these three Notches are hypomorphic. It can be concluded that N^{g11} and N^{Co}, and N^{103} at low temperatures, are hypomorphic to N^+ with regard to wing nicking. Consistent with this interpretation is the observation that nicking of N^{40}, which is assumed to be amorphic, was not markedly reduced in N^{40}/N^{40};Dp females (Table 5). It should be pointed out that the term "hypomorph" does not necessarily

imply that the gene product has diminished activity; the term could equally well describe an allele which produces less product. However, the analysis to follow indicates that N^{g11} and N^{Co} cannot be hypomorphic in terms of all phenotypes, and that N^{103} is not hypomorphic at 29°C. This in turn suggests that at least in the case of N^{g11} and N^{Co}, "hypomorph" describes the product, rather than the amount of product.

Dealing with N^{103} first, it will be recalled that at 29°C, $N^{103}/+$ females show more intense wing nicking than $N^8/+$ or $N^{40}/+$ (compare Plates 1a, 2c), and also have fused leg segments. Furthermore, $N^{103}/Y;Dp$ males raised at 29°C have thickened wing veins, unlike males carrying other N mutations. As noted earlier, the existence of a tarsal fusion phenotype in at least some surviving N^{70k30}/fa^{no} heterozygotes (Appendix 4), and in N^{103}/fa^{no} at 25°C (Appendix 1), suggests that the leg phenotype may be associated with a level of N^+ product activity intermediate between that of $N/+$ and N/N. Recently, SHELLENBARGER (1971) has described a ts lethal in the Notch locus which also conditionally exhibits leg segment fusion and other adult morphological abnormalities. Reduced N^+ product activity, leading to extreme wing nicking and tarsal fusion, could result in 29°C $N^{103}/+$ heterozygotes if the N^{103} allele product either partially inactivates, or competes for receptor sites with the N^+ product. These alternatives are outlined diagrammatically in Figure 15. Note that the inactivation model (Figure 15a) requires the formation of di- or multimeric complexes of the Notch-locus product, whereas the competition model (Figure 15b) does not necessarily

FIGURE 15   Hypothetical molecular models to explain tarsal

fusion and enhanced wing nicking in N^{103}/+

females at 29°C.

a. INACTIVATION OF N^+ PRODUCT

N^{103} →

MUTANT
PRODUCT

MUTANT
COMPLEXES
WITH NO
FUNCTIONAL
ACTIVITY

N^+ →

WILD TYPE
PRODUCT

WILD TYPE
COMPLEX
(REDUCED
AMOUNT)
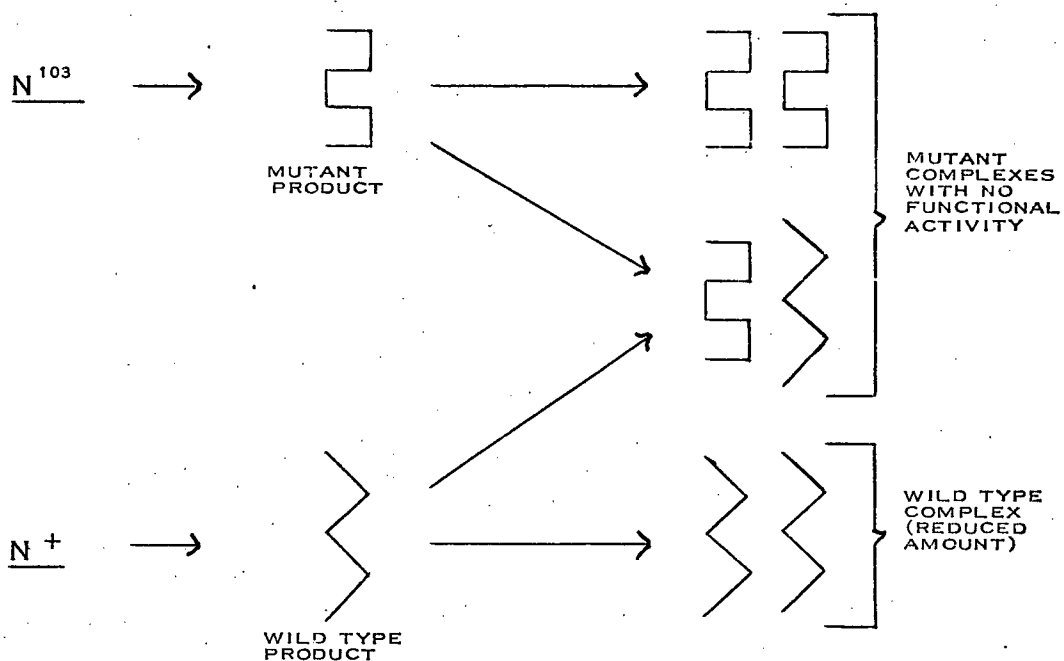
b. COMPETITION FOR RECEPTOR SITES

N^{103} →

MUTANT
PRODUCT

BLOCKED
RECEPTOR
SITE

RECEPTOR

N^+ →

WILD TYPE
PRODUCT

FUNCTIONAL
N^+ ·
RECEPTOR
COMPLEX
(REDUCED
AMOUNT)
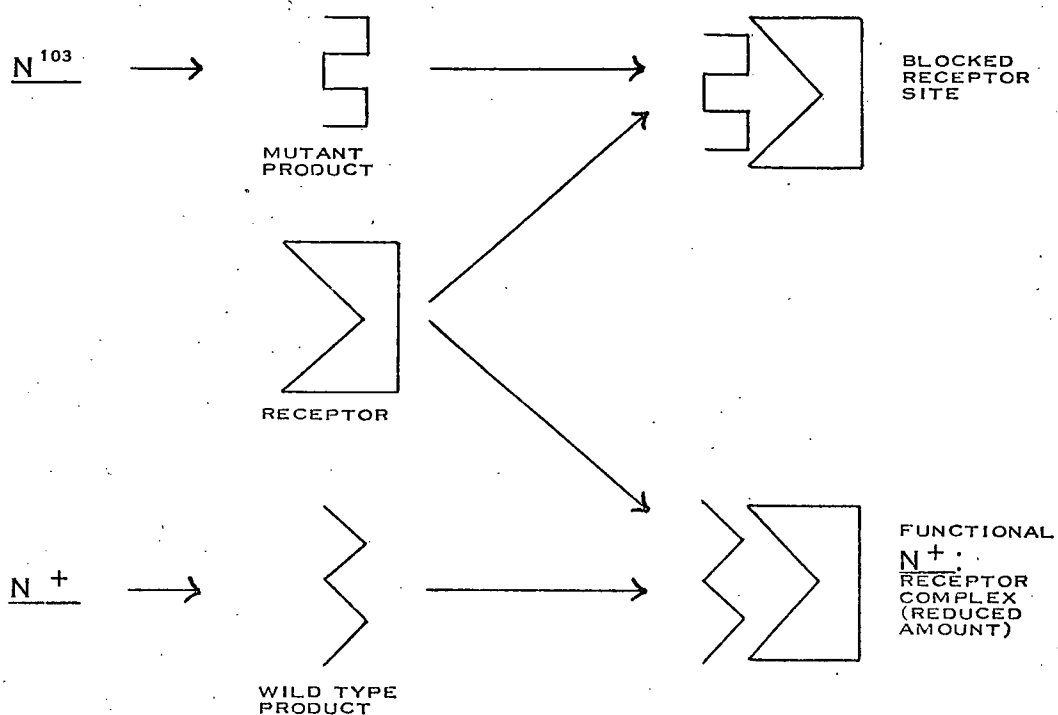
imply dimer formation, but does require a Notch-product:receptor complex. The formation of both types of complex has been proposed by WELSHONS (1965), to account for the array of pleiotropic effects associated with the Notch locus. Formally, both models (a) and (b) (Figure 15) can be classed as antimorphic interactions of N^{103} with N^+, in the sense that the N^{103} product has an "actively negative value" in terms of the amount of functioning N^+ product (MULLER 1932). A truly amorphic allele, such as the N^{40} mutation has been inferred to be, should not form a product which is able to complex with either the N^+ product or a receptor site. The observation that at 29°C N^{103}/N^{103};Dp females have even more exaggerated wing nicking and tarsal fusion phenotypes than $N^{103}/+$ females, is entirely consistent with the hypothesis that N^{103} behaves as an antimorph at 29°C.

The tarsal fusion associated with N^{103} at high temperatures is not always correlated with extreme wing nicking. This follows from the fact that N^{103}/spl females exhibited tarsal fusion but had less extensive wing nicking than $N^{103}/+$ when raised at 28°C (Figure 7) or 29°C. Without further experiments one cannot say whether this difference is due to extra-locus genetic modifiers in the spl stock or to the spl allele itself. The latter choice appears to be more likely, since fa^{no} spl/Y males have a milder wing nicking phenotype than fa^{no}/Y males (WELSHONS, personal communication). The possibility that non-Notch-locus modifiers may affect the phenotype of $N^{103}/+$ or N^{103}/spl, does not necessarily invalidate the hypothesis that

N^{103} is antimorphic at 29°C, since an altered "receptor" site (see model b, Figure 15) could formally be described as a genetic modifier.

The results of the dosage study of N^{g11} reveal that the eye facet and wing nicking phenotypes respond in opposite directions to both temperature and gene dosage (Figure 4). It has already been suggested that the decreased wing nicking in N^{g11}/N^{g11};Dp females at 22°C indicates that N^{g11} is hypomorphic in this regard. However, the increased expression of the mutant eye phenotype in such females is not the behaviour expected of a hypomorph. This, and the fact that deficiency N mutations and N^+ duplications do not express the eye phenotype seen in $N^{g11}/+$ females (although +/Y;Dp males do have occasional facet irregularities), suggests that the N^{g11} allele product is functionally altered (i.e. is neomorphic), as opposed to having reduced function, with respect to its role in eye development. Thus it can be assumed that at 20°C-22°C, the N^{g11} product plays an active role in eye development, either interacting or competing with the N^+ product (cf Figure 15) and thereby causing the mutant eye phenotype. A similar conclusion has been reached concerning the mutant spl (WELSHONS 1956b, 1971), whose eye phenotype is qualitatively identical to that of N^{g11}. The observation (Figure 4) that the mutant eye phenotype is expressed with increasing severity in the dosage series - 2 N^+ : 1 N^{g11}, 1 N^+ : 1 N^{g11}, 1 N^+ : 2 N^{g11} - is consistent with the hypothesis that the N^{g11} allele product is neomorphic at low temperature.

The opposite responses of the eye and wing phenotypes (i.e.

when the eye is mutant the wings are more wild type, and vice versa) of N^{g11} to temperature changes, may be explained if it is assumed that at 29°C, the N^{g11} product is less active, or less product is produced, than at 20°C-22°C. Thus it can be hypothesized 1) that at low temperatures, the N^{g11} product is able to participate in both eye and wing development and has sufficient wild-type acitivity to produce predominantly non-nicked wings, but is mutant in that function involved in eye facet arrangement; and 2) that at 29°C, the N^{g11} product is relatively inactive (or less N^{g11} product is produced), and is therefore unable to participate fully in wing development (causing a predominance of nicked individuals) and (in N^{g11}/+ females) unable to participate significantly in eye development, allowing the N^+ allele product to direct a normal eye facet arrangement. This hypothesis is illustrated diagrammatically in Figure 16. The assumption implied in this hypothesis, that normal eye facet arrangement is relatively independent of levels of gene product, whereas wing development is not, is supported by the facts that N^8/+ and +/+;Dp females have normal or near-normal eye facet arrangements, but have nicked (N^8/+) or Confluens (+/+;Dp) wing phenotypes. Since the eyes of N^{g11}/N^{g11};Dp females at 29°C are unmistakably mutant (Figure 4), the N^{g11} product must be produced at 29°C, and must compete or interact with the N^+ product. This, and the incomplete penetrance of wing nicking in N^{g11}/+ females at 29°C (Table 9), emphasizes the point that even at 29°C the activity of N^{g11} is not totally abolished.

It is also noteworthy that two cases of genetic suppression

FIGURE 16   Hypothetical molecular model to explain opposite

response to temperature of eye and wing phenotypes

of N^{g11}.

a. 22° C

N^+ → WILD TYPE PRODUCT

N^{g11} → ACTIVE MUTANT PRODUCT
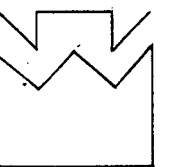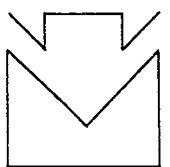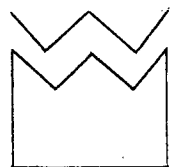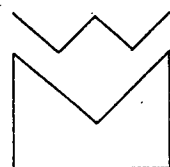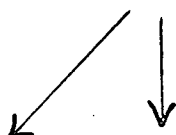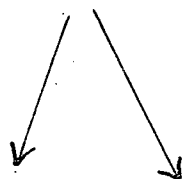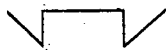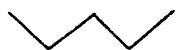
WING   EYE   WING   EYE

ACTIVE PARTICIPATION OF N^{g11} IN EYE AND WING DEVELOPMENT

b. 29°C

N^+ → WILD TYPE PRODUCT

N^{g11} → INACTIVE MUTANT PRODUCT
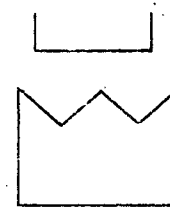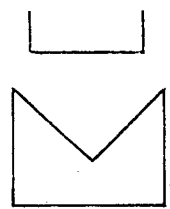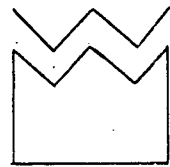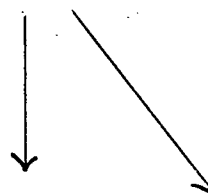
WING   EYE   WING   EYE

REDUCED PARTICIPATION OF N^{g11} IN EYE AND WING DEVELOPMENT

of the N^{g11} eye phenotype, one due to an extra-locus modifier (E-N^{70k27}), and the other to a mutation within the Notch locus (N^{70k30}), are both associated with increased wing nicking (Appendices 3, 4). Both cases are consistent with the interpretation that mutant N-allele product is removed from competitive activity, either by a reduction in the amount produced, or in the affinities which permit the competition or interaction.

The lethality of N^{g11}/N^{g11};Dp females at 20°C-22°C, but not at 25°C-29°C (Table 11), can also be explained in terms of the hypothesis outlined above. The fact that the flies of the genotypes N^{40}/N^{40};Dp (Table 4), N^{103}/N^{103};Dp (Table 7), N^{Co}/N^{Co}; Dp, and N^{x}/N^{y};Dp are all viable, rules out the possibility that the N^{+} activity of Dp itself is insufficient to allow survival. Furthermore, the viability of N^{g11}/N^{g11};Dp/Dp females (Table 11), N^{g11}/Y;Dp males, and N^{g11}/N^{8};Dp and N^{g11}/N^{40};Dp females, indicates that the 2 N^{g11} : 1 N^{+} allele ratio is the factor which determines the lethality of N^{g11}/N^{g11};Dp females at 20°C-22°C. If, in fact, at 22°C the N^{g11} product has sufficient activity to compete with the N^{+} product, then twice as much defective wild-type product could be sufficient to interfere with N^{+} activity, pushing it below the threshold necessary for viability. The TSP for lethality of N^{g11}/N^{g11};Dp females is embryonic (Figure 13) and the phenotype associated with the embryonic death of N hemizygotes has been well characterized (POULSON 1940). If the lethality in N^{g11}/N^{g11};Dp females is due to antimorphic (antagonistic) action by the N^{g11} product against N^{+} activity, embryos incubated at 20°C should exhibit disturbances similar to N hemi-

zygotes, whereas if the lethality results from neomorphic (competitive) action, such embryos should have some other phenotype. An examination of the phenotypes of N^{g11}/N^{g11};Dp embryos incubated at 22°C or 29°C, has not yet been completed, so a conclusion on this point must be postponed. The viability of N^{g11}/N^{g11};Dp females at 25°C-29°C, is entirely consistent with the hypothesis that the N^{g11} gene product has less antagonistic or competitive activity at higher temperatures (Figure 16).

The Abruptex-like phenotype of N^{g11}/N^{g11};Dp females which survive at 20°C-22°C (and those which are incubated at 29°C during the embryo stage and then shifted down, Table 48) will be referred to later, and will not be discussed here except to note that the neomorph hypothesis (Figure 16a) can account for this observation also.

Like N^{g11}, N^{Co} behaves like a hypomorph in terms of wing nicking but not its other phenotypes. The Confluens phenotype, which is mildly expressed in $N^{Co}/+$ females, and strongly expressed in $N^{Co}/+$;Dp females and N^{Co}/Y;Dp males (WELSHONS 1956a; Plate 4), is also known to result from increased N^{+} dosage (MORGAN et al. 1941; LEFEVRE 1952; WELSHONS 1965). This suggests that N^{Co} is probably hypermorphic compared to N^{+}, in terms of the function responsible for the Confluens phenotype. This hypothesis is supported by the observation that N^{Co}/N^{Co};Dp females have a much more extreme Confluens phenotype than $N^{Co}/+$;Dp females (Plate 4). The possibility that the strong Confluens phenotype results from some special interaction between N^{Co} and Dp is ruled out by the fact that N^{Co}/N^{8};Dp and N^{Co}/N^{40};Dp females have essentially

identical phenotypes to those of N^{Co}/+ (Table 12). In fact, the reduction of the Confluens phenotype in N^{Co}/N^X;Dp females is predicted by, and therefore lends support to, the hypothesis that N^{Co} is hypermorphic for the Confluens function.

It is interesting to speculate on how a molecule such as the N^{Co} product (assuming, for the moment, that the Notch locus does produce a single product) can be hypomorphic in one sense and hypermorphic in another. One possible explanation for this phenomenon is that the N^{Co} mutation causes a change in a single part of the Notch-locus product responsible for wing control, such that in one cellular milieu it causes wing nicking and in another it causes extra veins. Alternatively, N^{Co} may affect the tertiary structure (folding) of the Notch-locus product, such that a site normally responsible for the completion of wingtip development is altered thereby leading to the wing nicking phenotype. The same tertiary alterations could also affect a site regulating wing vein production and thereby produce extra veins. Implicit in the latter type of reasoning is the assumption that the Notch-locus product has several active sites, each of which is involved in different aspects of development. The validity of this assumption is supported by inspection of the genetic map of the Notch locus (Figure 1), which reveals that mutations with similar phenotypes generally map at similar sites within the locus (e.g., $\underline{fa}$ and $\underline{fa^g}$, $\underline{fa^{no}}$ and $\underline{fa^{noE}}$, $\underline{nd}$ and $\underline{nd^2}$, and the $\underline{Ax}$ and $\underline{Ax}$-like mutations). As discussed later, this clustering is expecially striking for the $\underline{Ax}$ mutations.

Before discussing the phenotypes of $\underline{Ax}$ mutations and their

interactions with $\underline{N}$ mutations and each other, the question of
whether $\underline{Ax}$ mutations represent duplications of the Notch locus,
as suggested for $\underline{Ax^{28a}}$ by MORGAN et al. (1941), should be dis-
cussed. The duplication hypothesis was based on the observations
that $\underline{Ax^{28a}}/\underline{N^8}$ females exhibited reduced wing nicking (MOHR 1932)
and that there was an extra band in the Notch-locus region of the
X in salivary chromosome preparations of $\underline{Ax^{28a}}$ (MORGAN et al.
1941). KAUFMAN (personal communication) has examined the banding
patterns of salivary chromosomes carrying $\underline{Ax^{E1}}$, $\underline{Ax^{E2}}$, $\underline{Ax^{16172}}$,
and $\underline{Ax^{9B2}}$, and none of these mutations are associated with visible
duplications or deficiencies. Furthermore, the mutagen EMS was
used to induce $\underline{Ax^{E1}}$ and $\underline{Ax^{E2}}$ (Appendix 3), $\underline{Ax^{16172}}$ (WELSHONS,
personal communication), and $\underline{Ax^{9B2}}$ (LEFEVRE, personal communica-
tion). If the fact that EMS induces missense mutations in T4
bacteriophage (KRIEG 1963) is also true in Drosophila, then these
four $\underline{Ax}$ alleles are not likely to be duplications. Finally,
genetic recombination tests show that in the presence of $\underline{Ax}$
alleles, crossing over is not reduced within the Notch locus or
in the immediately adjacent regions (Tables 13-15, and see com-
ment about $\underline{Ax^{16172}}$ mapping, p. 72), a criterion which has been
used by others to infer the absence of chromosome aberrations
(GREEN AND GREEN 1956; CARLSON 1958; WELSHONS AND VON HALLE 1962).
It can also be noted that $\underline{Ax^{E2}}$ and $\underline{Ax^{16172}}$ enhance the wing nick-
ing effect of $\underline{N}$ alleles (Table 28), and that $\underline{Ax^{E1}}$ is usually
lethal in combination with $\underline{N}$ mutations (Table 23). This also
tends to rule out the need to postulate that these alleles are
duplications. $\underline{Ax^{9B2}}$ does suppress wing nicking, but the cyto-

logical evidence and its mutagenic origin suggest that this allele is not a duplication either. However, the observed cross-over frequency (0.09%) between Ax^{9B2} and each of two flanking $\underline{N}$ alleles ($\underline{N}^{40}$ and $\underline{N}^{Co}$) (Table 15) is rather high compared to the total recombination frequencies (0.03% between the two $\underline{N}$'s measured directly, and a total of 0.04% between $\underline{N}^{40}$-spl and $\underline{spl}$-$\underline{N}^{Co}$) reported by WELSHONS (1958b). On the other hand, the cross-over frequency between $\underline{fa}^{no}$ and spl observed in the present study (Tables 13, 14) was also higher than that reported by WELSHONS (1958a), suggesting that culture and genotypic conditions may at least be partially responsible for the difference. Moreover, as noted in Results, the possibility that genetic modifiers (of $\underline{N}$, Ax^{9B2}, and $\underline{fa}^{no}$ spl Ax^{E2}) affected the relative viability of non-crossover ($\underline{Ax}^{9B2}$) and crossover ($\underline{Ax}^{+}$) progeny (Table 15), cannot be discounted. Thus it can be assumed that factors other than an intra-Notch-locus duplication, may account for the apparent increases in $\underline{N}$-$\underline{Ax}^{9B2}$ recombination. In summary, it appears likely that $\underline{Ax}^{E1}$, $\underline{Ax}^{E2}$, $\underline{Ax}^{16172}$, and $\underline{Ax}^{9B2}$ are all point mutations within the Notch locus. In the following discussion, therefore, it is assumed that these mutations are not duplications or parti-al duplications of the Notch locus.

Like the Notch class of mutations, the Abruptex class of mutations can be readily defined phenotypically. However, as was observed with the $\underline{N}$ mutations examined, within this group there appear to be allele-specific differences. In fact, on the basis of the patterns of bristle loss (Table 21), sexual dimorphism (Table 22), interaction with $\underline{N}$ mutations (Table 28), and inter-

actions among the Ax mutations themselves (Tables 29-31), there are at least two distinct sub-groups of Ax mutations. Nevertheless, the fact that in terms of the bristle phenotypes, all five N alleles tested interacted similarly (in Ax/N heterozygotes) with all Ax mutations tested (Tables 24-27), emphasizes that the Ax's should be treated as a single class of mutations.

Since the Ax mutations behave differently from known N deficiencies, it is obvious that Ax mutations cannot be amorphic. The data seem to indicate that each Ax mutant may affect the different functions controlled by the Notch locus in different ways, and furthermore that certain of these functions may be affected in more than one way. The suppression of both the Ax and N mutant phenotypes in Ax^{9B2}/N heterozygotes (Tables 26, 28) cannot be attributed to partial intra-cistronic complementation, whereby a hybrid polymer restores some wild-type activity to the gene product, since in the case of the deficiency, N^8, there is no N-allele product to participate in polymer formation. If, on the other hand, it is assumed that Ax^{9B2} is hypermorphic, the increased activity on the part of Ax^{9B2} could suppress the wing nicking of N, and reduced or complete lack of function on the part of the N allele could diminish the bristle loss and wing vein gapping caused by the hypermorphic Ax allele (compare with the suppression of Confluens in N^{Co}/N^x;Dp). This very explanation was advanced by MULLER (1932) to explain the reduced expression of N and Ax phenotypes in Ax^{28a}/N^8 heterozygotes. By the wing nicking criterion, Ax^{E2} and Ax^{16172} would be hypomorphic, since wing nicking is enhanced by these alleles (Table 28).

On the other hand, the suppression of Ax^{E2} and Ax^{16172} phenotypes by $\underline{N}$ mutations suggests that these two alleles are hypermorphic, like Ax^{9B2}. Unfortunately, this model cannot accommodate the observation that $\underline{Ax}$ phenotypes are suppressed in $\underline{Ax}/+$ females compared to $\underline{Ax}/\underline{Ax}$ females or $\underline{Ax}/Y$ males (Tables 16-20), since they should be enhanced if the $\underline{Ax}$ alleles were truly hypermorphic. Similarly, it can be deduced that the $\underline{Ax}$ mutations are not (entirely) neomorphic so far as their effect on bristle numbers is concerned, since in this case $\underline{N}$ deficiencies or point mutations should not suppress the $\underline{Ax}$ mutant phenotypes. From the foregoing discussion it is apparent that some rather special assumptions may be warranted in order to reconcile the seemingly conflicting observations.

A way out of the morass described above, which is consistent with the observations on the atypical Notches, may be found by combining recent suggestions (BRITTEN AND DAVIDSON 1969; WRIGHT 1970) that the Notch locus is a regulator gene, with the fact that some regulatory loci in bacteria comprise both repressor and activator elements (GAREN AND ECHOLS 1962a, b; ENGLESBERG, IRR, POWER AND LEE 1965). If we assume that the Notch locus is this type of regulator gene, or at least that different elements of the wild-type Notch-locus product tend to oppose or balance one another in the developmental processes they influence, then the suppression of $\underline{Ax}$ phenotypes by both $\underline{N}^+$ and $\underline{N}$ alleles could be accommodated. To borrow the regulator gene terminology, we may suppose that the bristle loss caused by $\underline{Ax}$ mutations is due to a repression of the bristle-forming mechanism. As diagrammed

in Figure 17, this could come about a) by mutation of an activator element to a non-functional form ($\underline{A}^-$), or b) by mutation of a repressor element to a hyper-functional form ($\underline{R}^H$). It is also possible that a single mutation could affect both types of elements. Thus, according to these models, $\underline{Ax}$ mutations may be hypo- or amorphic changes ($\underline{A}^-$) and/or hyper- or antimorphic changes ($\underline{R}^H$) in the Notch-locus product. Accordingly, an amorphic Notch mutation would be represented as $\underline{A}^-\underline{R}^-$. The hypothesis that mutation of $\underline{N}^+$ to $\underline{Ax}$ causes bristle loss due to increased repression of bristle-forming activity, is consistent with the observation that $\underline{N}$ deficiencies usually cause increased bristle numbers (MOHR 1932). Thus, mutation of $\underline{N}^+$ to $\underline{N}$ could result in reduced repression of bristle-forming activity, leading to the increased bristle numbers. As will be discussed later, the model outlined in Figure 17 can also account for the $\underline{Ax}^x/\underline{Ax}^y$ interactions. This model is not meant to imply that there are two discrete parts to the Notch locus, although, as will be shown later, the genetic evidence does suggest that the left half of the Notch locus is functionally matched or paired with the right half. It is possible, although difficult to prove, that a whole series of mutually antagonistic regulator elements make up the Notch-locus product. In other words, the Notch locus could be an "integrator" gene, as postulated by BRITTEN and DAVIDSON (1969), which controls the transcription of many "producer" (structural) genes.

By considering the expected gene products of $\underline{Ax}/\underline{Ax}$, $\underline{Ax}/+$, and $\underline{Ax}/\underline{N}$ (Table 52), we can see how the models presented in

FIGURE 17   Model of Notch locus comprising antagonistic

elements.

a.

A −   R +

↓

REPRESSOR

↓

BRISTLE LOSS DUE TO
LOWERED ACTIVATOR
FUNCTION

b.

A+ R^H

↓   ↓

ACTIVATOR   HYPER-ACTIVE
OR ANTAGONISTIC
REPRESSOR

↓   ↓   ↓

BRISTLE LOSS DUE TO EXCESS
REPRESSOR FUNCTION

c.

A +   R +

↓   ↓

ACTIVATOR   REPRESSOR

↓   ↓

NORMAL BRISTLE DEVELOPMENT
MEDIATED BY BALANCED NOTCH-
LOCUS PRODUCT(S).
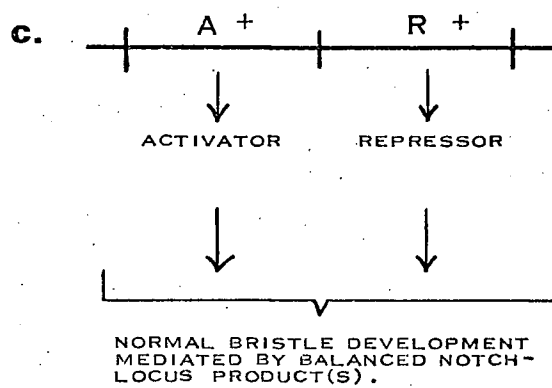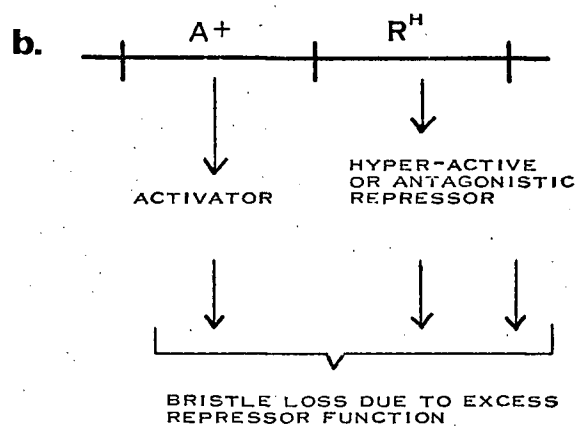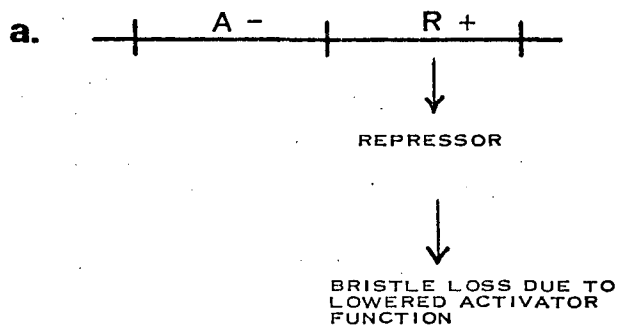
TABLE 52   Expected product proportions of $\underline{Ax}/\underline{Ax}$, $\underline{Ax}/+$, and
$\underline{Ax}/\underline{N}$, according to activator:repressor model
(Figure 17).

GENOTYPE	EXPECTED PRODUCT PROPORTIONS	
	CASE (a)	CASE (b)
$\underline{Ax}/\underline{Ax}$	2 R^+: 0 A^+	2 R^H: 2 A^+
$\underline{Ax}/+$	2 R^+: 1 A^+	1 R^H: 1 R^+: 2 A^+
$\underline{Ax}/\underline{N}$	1 R^+: 0 A^+	1 R^H: 1 A^+

Figure 17 can explain the phenotypes associated with these geno-
types. If we assume that R^H has twice the repressive activity
of R^+ then the quantitative equivalence of cases (a) and (b) is
more apparent (Figure 17, Table 52). It is easy to see from
Table 52 that Ax/+ should be phenotypically closer to wild-type
than Ax/Ax, since in both cases there is a smaller excess of
repressor over activator functions in Ax/+ than in Ax/Ax. There
is still an excess of repressor over activator function in the
Ax/N product (Table 52), but if we remember that in the model
there is less excess repressor in Ax/N than in Ax/Ax, in relation
to the rest of the genome, the relatively milder mutant phenotype
of Ax/N is not too surprising. Note that according to this model,
suppression of Ax phenotypes by N^+ occurs by a different mechan-
ism tha suppression by N mutants. Also, this model is not
inconsistent with the dimer- or complex-formation models based on
the N allele-dosage studies (Figures 15, 16).

Yet another feature of the models presented in Figure 17
is that they could also account for the phenotypes of N^{g11} and
spl, which have been inferred to be neomorphs on the basis of
their responses to alterations of gene dosage (Figure 4) or to
modifiers (WELSHONS 1956b, 1971), respectively. Accordingly,
so-called neomorphic mutations may actually be mutations which
cause intra-gene-product imbalances of the hypo- or hypermorphic
variety, as opposed to true hypo- and hypermorphs, which by
definition affect the synthesis or function of the whole gene
product.

As noted earlier, the Abruptexes tend to map in one region

of the Notch locus. Inspection of Figure 1 shows that those $\underline{Ax}$
alleles which have been mapped with any degree of precision are
situated in the right half of the genetic map. Furthermore, by
viture of its phenotype of sparse thoracic microchaetae (WELSHONS
1965) and its larval-pupal lethal phase (WRIGHT 1970), the lethal
mutation $\underline{l(1)N^B}$, which maps in this region (Figure 1), can also
be regarded as Abruptex-like. It has already been noted that
under certain conditions, $\underline{N^{g11}}$ (which maps near the right limit
of the Notch locus (Figure 1)) manifests Abruptex-like character-
istics. Moreover, $\underline{nd}^2$, which also maps near the right limit
(Figure 1), expresses both $\underline{N}$-like (wing-nicking) and $\underline{Ax}$-like
(vein gapping) phenotypes (WELSHONS, personal communication).
Thus it becomes increasingly apparent that non-amorphic mutations
which affect similar developmental processes are likely to be
positioned at similar sites within the Notch locus. The separa-
tion of $\underline{fa}^{no}$ and $\underline{nd}$, which have similar phenotypes, appears to
contradict this generalization. However, as the following dis-
cussion will show, this exception can be reconciled with the
generalization that different regions within the Notch locus are
specific in terms of their developmental function.

As stated earlier, the Notch locus appears to consist of
two parts which are functionally related to one another. This is
inferred from a correlation of the genetic positions of a number
of non-amorphic alleles within the locus, with their inter-allelic
complementation pattern. All heteroallelic combinations among
the recessive visible mutations, $\underline{fa}^g$, $\underline{spl}$, $\underline{fa}^{no}$, and $\underline{nd}$, except
$\underline{fa}^{no}/\underline{nd}$, exhibit complementation (i.e., are non mutant in appear-

ance) (WELSHONS 1965). Recently, it has been reported that Ax^{59b} and Ax^{59d} do not complement fa^g, in that the eyes of Ax/fa^g heterozygotes are rough, although not glossy (WELSHONS 1971). Moreover, the Ax-like allele, $l(1)N^B$, allows complete pseudodominant expression of fa^g, the $l(1)N^B/fa^g$ heterozygote having eyes which are both rough and glossy, unlike the other (N-like) $l(1)N$ alleles, which allow only partial pseudodominance of fa^g (WELSHONS 1965). If the genetic map of the Notch locus (Figure 1) is fitted to these patterns of complementation, such that noncomplementing alleles are situated opposite one another, a spiral genetic map is obtained (Figure 18). This pattern is strikingly reminiscent of the spiral genetic map generated by congruence of the linear genetic map with the circular complementation map of the ad-8 locus in Neurospora crassa (KAPULER AND BERNSTEIN 1963).

Assuming that the correlation of the genetic map with the complementation pattern (Figure 18) is not fortuitous, several interpretations of its significance are possible. One possibility is that during the evolution of Drosophila, an intra-band tandem repeat of genetic material occurred (BAUER 1943), and that while maintaining similar roles in development, the two halves evolved divergently. It is conceivable, for example, that specific functions originally common to both halves were selectively lost in one or the other region, thereby leading to the apparent division of functions now observed. Another possibility is that the tertiary structure of the Notch locus product has a spiral configuration. This interpretation has been given to the spiral correlation of the genetic and complementation maps of the ad-8

FIGURE 18   Correlation of the genetic map positions and

complementation pattern of certain Notch-locus

mutations. Alleles positioned opposite one another are non-

complementary. The position of $\underline{l(1)N^B}$ with respect to $\underline{Ax^{59d}}$

has not been determined.

nd

no
fa

fa^g

spl

$Ax^{59} d$

$l(1) N^B$
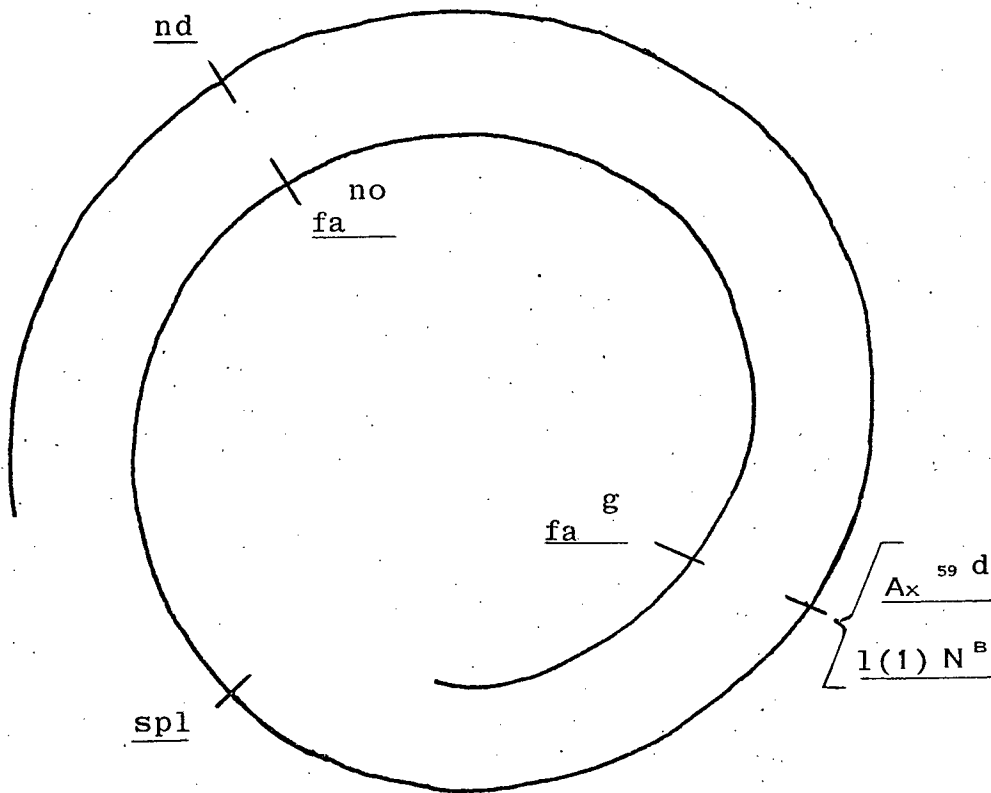
locus in N. crassa (KAPULER AND BERNSTEIN 1963), although CRICK

and ORGEL (1964) strongly attacked this interpretation.   There

have been several reported cases of circular complementation maps

in Drosophila (CARLSON 1961; SUZUKI AND PROCUNIER 1969) and other

organisms (FINCHAM AND DAY 1965), and it may be that as more com-

plex genetic systems are analyzed, many more examples will be

discovered.   In fact, SHELLENBARGER (1969) has found that the

complementation pattern of 17 EMS-induced lethals within the

Notch locus fits a circular map.   It would be very interesting

indeed to see whether the genetic positions of these lethals are

correlated with the complementation map, and whether this pattern

fits the spiral map presented in Figure 18.

Throughout the preceding discussion it has generally been

implied or assumed that the Notch locus produces a single gene

product.   This has also been the opinion of other investigators,

who based their conclusions on the non-complementation (lethality)

of all heteroallelic N mutant combinations tested (WELSHONS 1965)

and the similarity of the embryonic abnormalities in hemi-, homo-,

and heterozygotes of various lethal N alleles (POULSON 1968).

Further support for a single Notch-locus product comes from con-

sideration of the interactions of fa^{no}, spl and Ax^{E2} (Appendices

6, 7).   The genotype fa^{no} spl Ax^{E2}/+ + + expresses a rough eye

phenotype which is virtually indistinguishable from that of spl/

spl, whereas the genotypes fa^{no} spl +/+ + Ax^{E2}, + spl +/+ + Ax^{E2},

and fa^{no} + +/+ + Ax^{E2} do not have rough eyes.   The genotype fa^{no}

+ +/+ spl Ax^{E2} has a mild rough eye phenotype which overlaps wild

type, but this is no more extreme than the phenotype of + + +/+

spl Ax^{E2}. (Flies of the genotype fa^{no} + Ax^{E2}/+ spl + have not been examined yet.) The phenotypic differences between the cis and trans configurations indicates that the fully penetrant spl phenotype of fa^{no} spl Ax^{E2}/+ + + is not due to an additive effect of the three mutant alleles acting independently, but to the presence of the three mutant sites in the same product molecule.

In addition to the enhancement of the spl mutant eye phenotype by Ax^{E2}, the coupling of fa^{no} to Ax^{E2} completely suppresses the wing vein gap phenotype of Ax^{E2} and significantly suppresses an extreme bristle-loss phenotype associated with the coupling of spl to Ax^{E2} (Appendix 7). This enhancement of one phenotype and suppression of the others is rather unusual, especially since spl separates the fa^{no} and Ax^{E2} mutant sites (Table 14, Figure 1). However, examination of the spiral map (Figure 18) suggests the following explanation. If we assume that the enhancement of the spl eye phenotype is due to an extension of the effects of the fa^{no} lesion to the right of the mutant site (Figure 1), the suppression of the spl Ax^{E2} bristle phenotype (and the Ax^{E2} wing vein phenotype) may be due to a comparable effect to the left of fa^{no}, influencing the sites which are complementary to the spl-Ax region (i.e., the fa^{g} region, see Figure 18). Admittedly this is not the only possibility, since effects of the fa^{no} mutation on the tertiary folding of a Notch-locus product molecule containing the spl and Ax^{E2} mutant sites, could equally well account for the observations. Nevertheless, the interactions of these three mutant alleles suggest at least that the region of the Notch locus spanning the fa^{no}-Ax^{E2} mutant sites produces a single

molecular product. Furthermore, these observations indicate that fa^{no} is not entirely a hypomorphic allele, as was suggested by WELSHONS (1965) on the basis of N/fa^{no} lethality, since in this event we would not expect enhancement of spl. WELSHONS (1971) has recently reported that the coupling of an amorphic (N) allele to spl results in inactivation of the spl mutant function.

One of the phenomena underlying the differences between the two groups of Abruptexes is the unusual system of lethal inter- actions among the Ax alleles. It is not difficult to conceive of a lethal/non-lethal heterozygote causing lethality, such as Ax^{E1}/Ax^{E2}, Ax^{E1}/Ax^{16172}, Ax^{59d}/Ax^{E2}, and Ax^{59d}/Ax^{16172} (Table 29), in the same way that N/fa^{no} heterozygotes are usually lethal. HOUSE (1959a) has also reported a lethal interaction between Ax^{28a} and the lethal allele Ax^{A}. However, the observation that certain heteroallelic combinations of viable Ax alleles (Ax^{9B2}/Ax^{E2} and Ax^{9B2}/Ax^{16172}) result in lethality (Tables 29, 30) is an entirely different matter. At first sight, one might postulate that this kind of interaction, or negative complementation, indi- cates that the products of Ax^{9B2} and Ax^{E2} (or Ax^{16172}) are mutual- ly antagonistic and that the resulting inactivation of these gene products is responsible for the lethality. However, the observa- tion that the lethal Ax^{x}/Ax^{y} combinations exhibit severe bristle loss phenotypes (Table 31), and the fact that relatively severe hypomorphic situations, like the viable and semilethal N/fa^{no} genotypes, exhibit bristle disturbances in the opposite direction, tend to discount the Ax-product inactivation theory. One plaus- ible alternative is that the two groups of Ax's affect generally

different sets of functions (possibly overlapping one another to some extent). In the case of the viable alleles, these regulatory upsets would not be sufficient to cause homozygous lethality, but combinations of regulator mutations whose range of effects differ might affect enough functions to cause lethality. This type of model is illustrated in Figure 19, and is consistent with the different phenotypic pattern expressed by $\underline{Ax}^{9B2}$ compared to the other two alleles (Table 21). Another alternative is that the two groups of $\underline{Ax}$'s differ fundamentally in terms of the primary lesion in the Notch-locus product. For instance, if we refer to the models presented in Figure 17, the heterozygous combinations of an $\underline{A}^-$-type $\underline{Ax}$ (model a) and an $\underline{R}^H$-type $\underline{Ax}$ (model b) would result in the product ratio of 1 $\underline{R}^+$: 1 $\underline{R}^H$: 1 $\underline{A}^+$, which might easily exhibit a more extremely mutant phenotype than either homozygous $\underline{A}^-/\underline{A}^-$ or $\underline{R}^H/\underline{R}^H$ (see Table 52). Either of the above models could account for the observed lethal patterns and also accommodate the viability of $\underline{Ax}^{E1}/\underline{Ax}^{9B2}$ (Table 29). Whether or not $\underline{Ax}^{59d}/\underline{Ax}^{9B2}$ is lethal has little bearing on the validity of these models, since if lethality occurred it could be attributed either to effects on functions not affected by $\underline{Ax}^{9B2}$ (Figure 19 model), or simply to $\underline{Ax}^{59d}$ being more severely mutant than $\underline{Ax}^{E1}$ (either model). From the data presently available, therefore, it is not possible to distinguish between these types of models, if indeed either is the correct one, and a test of these ideas probably must await the development of methods to characterize the Notch-locus product biochemically.

Although lethality of the $\underline{Ax}^{9B2}/\underline{Ax}^{E2}$ type has not been re-

FIGURE 19   "Range of function" model to explain lethality

of $\underline{Ax}^{9B2}/\underline{Ax}^{E2}$ and $\underline{Ax}^{9B2}/\underline{Ax}^{16172}$. The presence

of an arrow in a space indicates that the given genotype is

mutant in the function represented by that space.

GENOTYPE HYPOTHETICAL FUNCTIONS AFFECTED DEVELOPMENTAL RESULT

HOMOALLELIC

A_x^{9B2} / A_x^{9B2}

A_x^{E2} / A_x^{E2}

$A_x^{16172} / A_x^{16172}$

2 FUNCTIONS AFFECTED;
GENOTYPE SURVIVES.

HETEROALLELIC

A_x^{9B2} / A_x^{E2}

A_x^{9B2} / A_x^{16172}

3 FUNCTIONS AFFECTED;
GENOTYPE DIES.

A_x^{E2} / A_x^{16172}

2 FUNCTIONS AFFECTED;
GENOTYPE SURVIVES

ported before, two cases of synthetic lethality involving an $\underline{Ax}$ allele have been reported. In one case, combining the recessive lethal allele $\underline{Ax}^A$ with the third-chromosome mutant Hairless ($\underline{H}$) results in lethality of $\underline{Ax}^{A}/+;\underline{H}/+$ females, while in the second case $\underline{Ax}^{28a}/Y;\underline{H}/+$ and $\underline{Ax}^{28a}/\underline{Ax}^{28a};\underline{H}/+$ are lethal above 26°C (HOUSE 1959a). This is most interesting, since $\underline{H}$, which is itself a recessive lethal, has a bristle loss and wing vein gapping phenotype similar to that of $\underline{Ax}$ (GOWEN 1933). Moreover, $\underline{H}$, which by itself has no effect on wing vein L2, enhances the gapping of L2 caused by $\underline{Ax}^{28a}/\underline{Ax}^{28a}$ (HOUSE 1955), $\underline{H}/+$ and $\underline{Ax}^{28a}/+$ enhance one another in double heterozygotes, and $\underline{N}^8/+;\underline{H}/+$ females have reduced expression of both the $\underline{N}$ and $\underline{H}$ phenotypes, an effect resembling that seen in $\underline{Ax}^{28a}/\underline{N}^8$ flies (HOUSE 1959a). The resemblance between $\underline{H}$ effects and $\underline{Ax}$ effects appears to be more than a chance similarity. The $\underline{Ax}^{28a}$-like interactions between $\underline{H}$ and $\underline{N}^8$ strengthen this contention, and show the dual involvement of both $\underline{N}^+$ and $\underline{H}^+$ activity in normal development. The observed interactions of $\underline{H}$, $\underline{Ax}$, and $\underline{N}$ support the hypothesis that (in contrast to the $\underline{Ax}/+$ situation, which must be a case of dilution of $\underline{Ax}$ mutant product by $\underline{N}^+$ product) the reduced expression of $\underline{Ax}$ in $\underline{Ax}/\underline{N}$ heterozygotes reflects lowered $\underline{Ax}$ activity in relation to the rest of the genome (Table 52).

The existence of Notch-locus modifiers at other loci is not surprising at all. In fact, the existence of such "modifiers" is predicted by the hypothesis that the Notch locus is a regulatory gene which influences several developmental systems. According to this hypothesis, modifiers of Notch-locus alleles might reflect

changes in the response potential of developmental systems under Notch-locus control, or they could be mutations in other regulator genes. Besides the interaction with $\underline{H}$, many cases of modifiers of $\underline{N}$ mutant alleles have been reported, a few of which are suppressors (MORGAN 1919) and enhancers (Appendices 2-4) of wing nicking, an enhancer of $\underline{spl}$ (WELSHONS 1956b; VON HALLE 1965), and modifiers which cause head deformities in the presence of $\underline{N}$ (HILLMAN 1961). Consideration of the results of the present investigation, therefore, must be tempered by the realization that genetic modifiers of the alleles studied could have, and very likely did, influence observations especially of the quantitative type, as was noted from time to time in Results. Nevertheless, the results of the investigations of $\underline{N}$ allele dosage effects and $\underline{Ax}$ interactions with $\underline{N}$ and one another, seem to be sufficiently uniform for the purposes of the present discussion. It can also be stated that preliminary results from $\underline{Ax^{E2}}$, $\underline{Ax^{16172}}$, and $\underline{Ax^{9B2}}$ strains made co-isogenic for $\underline{OR}$ autosomes* (Figure 20), confirm both the lethality of $\underline{Ax^{9B2}/Ax^{E2}}$ and $\underline{Ax^{9B2}/Ax^{16172}}$ and the morphological differences between $\underline{Ax^{9B2}}$ and $\underline{Ax^{E2}}$.

The sexual dimorphisms frequently encountered in the phenotypes of the $\underline{Ax}$ mutations (Table 22) cannot be so easily dismissed as being due to genetic background variation, since these differences appeared in sibling males and females reared under

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*Probably for part of the X chromosome also, since the autosomal inversions $\underline{SM1}$ and $\underline{TM2}$ would tend to increase recombination between the $\underline{Ax}$ and $\underline{+^o}$ X chromosomes.

FIGURE 20   Mating scheme used to replace autosomes of

recessive viable Ax stocks with OR autosomes.

+° = chromosome from OR stock.

$$\frac{FM6}{y} \; ; \; \frac{SM1 \; ; \; TM2 \; pol}{T(2;3)e \; ; \; pol} \; ; \; \frac{+}{+} \; ; \; \frac{+}{+} \; ; \; \frac{+}{+} \quad \male$$

$$\times$$

$$\frac{FM6}{Ax} \; ; \; \frac{SM1 \; ; \; TM2 \; pol}{+ \; ; \; + \; ; \; +} \; ; \; \frac{+}{Y} \; ; \; \frac{+}{+} \; ; \; \frac{+}{+} \quad \female$$

$$OR \quad \male$$

$$\times$$

$$\frac{Ax}{+^o} \; ; \; \frac{SM1 \; ; \; TM2 \; ; \; +^o}{+^o \; ; \; +^o \; ; \; ?} \quad \female$$

$$OR \quad \male$$

$$\times$$

$$\frac{Ax}{+^o} \; ; \; \frac{+^o \; ; \; +^o \; ; \; +^o}{+^o \; ; \; +^o \; ; \; ?} \; ; \; \frac{Ax}{Y} \; ; \; \frac{+^o}{+^o} \; ; \; \frac{+^o}{?} \quad \male$$
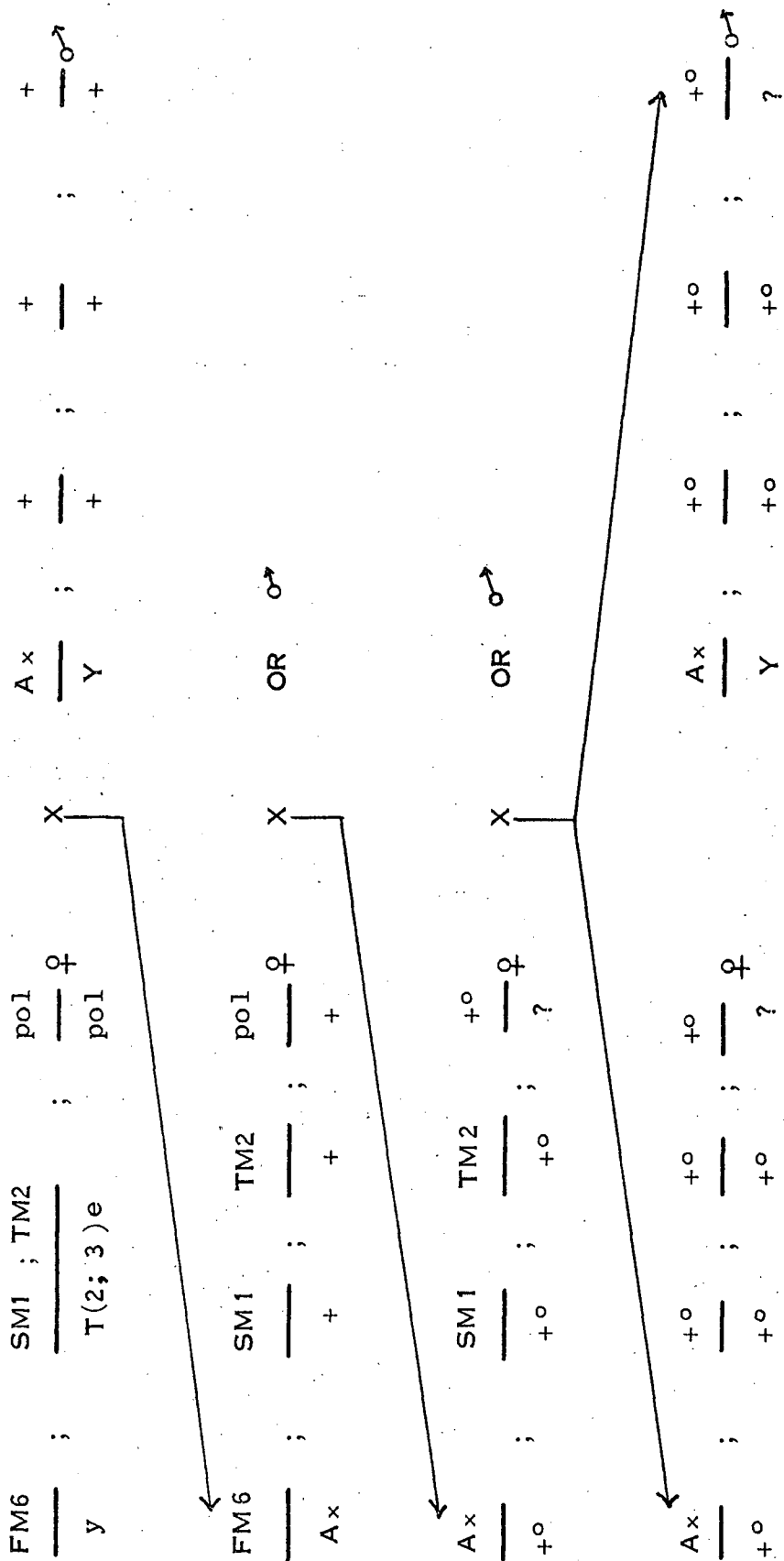
identical conditions. Since the Notch locus is sex-linked, in-
complete or aberrant dosage compensation of the $\underline{Ax}$ mutant pro-
ducts is one possible explanation. By definition $\underline{N}^+$ is dosage
compensated, since one dose of $\underline{N}^+$ in males produces the same
phenotype as two doses in females (STERN 1960). The fact that a
single dose of $\underline{N}^+$ in $\underline{N}/+$ females leads to a mutant phenotype does
not reflect defective dosage compensation of $\underline{N}^+$ (since dosage
compensation concerns male-female differences), but indicates
that $\underline{N}^+$ is "haplo-insufficient" in females (MOHR 1932). The in-
terpretation that defective dosage compensation of $\underline{Ax}$ accounts
for the sexual dimorphism of mutant phenotypes may be weakened by
the fact that certain autosomal mutants also exhibit sexual di-
morphism. For example, the fourth-chromosome mutant cubitus-
interruptus ($\underline{ci}$), which causes wing vein gaps, and its dominant
allele, $\underline{ci}^D$, are significantly less mutant in females than in
males (HOUSE AND EBERSOLE 1971). It is possible that sexual
physiological differences could explain dimorphisms such as these.
On the other hand, LUCCHESI (personal communication), on the basis
of autosomal and sex-linked enzyme specific activities in triploid
and intersex (2X;3A) females, has suggested that sex-linked dos-
age compensation is a special case of "diploid" regulation, which
normally operates in autosomes as well as the sex chromosomes.
Thus, the phenotypic sex differences of the $\underline{Ax}$ and $\underline{ci}$ alleles
could both result from defective dosage regulation of the mutant
gene products.

The results of the temperature-shift experiments with
several different Notch-locus genotypes, show that the presence

of the $\underline{N}^+$ gene product is necessary for normal development at
several stages of the Drosophila life cycle. The most striking
observation is that the TSPs for the adult morphological pheno-
types all occur during the third larval instar, whereas TSPs for
lethality occur during several stages of development, depending
on the genotype involved.

The TSPs for the eye facet, wing nicking, bristle disturb-
ance, wing vein gapping, and leg segment fusion phenotypes, all
occur prior to pupation (in the third instar), which is the stage
when the actual differentiation of the adult morphological struc-
tures takes place. This suggests that during the third larval
instar, activity of the Notch locus affects determination of the
pattern of differentiation of the cells in imaginal discs respon-
sible for the adult eyes, wings, legs, and epidermis. Recently,
SHELLENBARGER (1971) reported that heat shocks of larvae homo-
zygous for the ts Notch-locus lethal allele, $\underline{1(1)N}^{ts-1}$, induce
mutant adult phenotypes such as eye- and headlessness, rough eye,
small wing, notched wing, and leg segment fusion, although he
found that heat shocks during the pupa stage also cause some
adult morphological abnormalities. Recently, it has been report-
ed that a ts allele ($\underline{ss}^{a40a}$) at the spineless locus, which is
definitely a differentiation pattern-determining gene, also has
a third instar TSP (for conversion of antennal to leg structures)
(GRIGLIATTI AND SUZUKI 1971). Ultimately, in order to understand
the significance of pattern-determination TSPs, we must know the
molecular properties of the gene products of loci such as $\underline{N}^+$ and
$\underline{ss}^+$.

The restriction of TSPs for morphological traits to the third larval instar contrasts with the TSPs for lethality, which occur at several stages of development. This provides an interesting comparison with the pattern of TSPs for lethality and visible phenotypes observed for the sex-linked ts lethal mutation l(1)E6^{ts} (GRIGLIATTI AND SUZUKI 1970). In the case of l(1)E6^{ts}, the lethal TSP occurred at the end of the third instar, whereas TSPs for deposition of pteridine pigments were embryonic-second instar (Malphigian tubules) or late pupal (testis and eye). The biphasic nature of the pigment TSPs of l(1)E6^{ts} is not surprising, since the early TSP is for larval tissue and the late TSP for adult tissue, whereas the Notch-locus morphological TSPs were all for adult phenotypes. Another contrast is that the TSPs for the mutant pigment deposition of l(1)E6^{ts} occurred at about the same time as pigment deposition normally takes place, whereas the TSPs for Notch-locus-mediated morphological traits occur well before the differentiation of the structures affected. This suggests that these particular developmental functions of the N$^+$ locus occur before the actual differentiative processes, while l(1)E6^{ts} may more closely affect differentiation. This is consistent with the hypothesis that the Notch locus is a regulator gene.

The polarized progression of eye facet arrangement seen in both N^{g11}/+ (Figure 6) and N^{103}/spl (Figure 7) females shifted at progressively later stages during the third instar, suggests that a wave of determination of ommatidial organization originates in that portion of the eye disc destined to form the posterior edge of the adult eye, and then progresses anteriorly. These observa-

tions and conclusions are very similar to those of BECKER (1957) who found that a rough-eye phenocopy could be induced in wild-type flies by X-irradiation during the third instar-prepupal stages, and that the irregular arrangement migrated anteriorly across the eye in a vertical band with increasing larval age at the time of irradiation. BECKER's results differ from the present temperature-shift results, since X-ray sensitivity occurred during both larval and prepupal stages (BECKER 1957), while the TSPs for eye facet arrangement end before the prepupa stage (Figures 6, 7). Moreover, in both N^{g11}/+ and N^{103}/spl the 29°C eye TSPs end earlier with respect to puparium formation than at 20°C-22°C (Figures 6, 7). In order to determine whether the sensitivity of eye facet arrangement to radiation also occurs earlier at 29°C, I repeated BECKER's experiments with OR flies incubated at 20.5°C and 29°C. The results (Figure 8) confirm that the RSP for eye facet arrangement extends into the prepupa stage, and also show that there is no difference in the RSP at these two temperatures. The temporal differences between the stages of sensitivity to radiation and temperature shifts suggest that radiation may affect eye development at a different level of complexity than the effects of temperature on the Notch-locus primary gene product.

Further to the question of eye differentiation, KURODA (1970) has reported that a posterior-to-anterior gradient of ommatidial differentiation occurs in the eye discs of mature third-instar Oregon-R larvae, cultured in vitro. This is also a later developmental stage than the beginning of the TSPs reported here.

Furthermore, he observed that irradiation of mature third-instar discs immediately after isolation, preferentially inhibited the organization of ommatidium-forming cells in the anterior part of the eye disc, an observation consistent with the results of BECKER (1957) and those reported here (Figure 8). The observation (KURODA 1970) that visible ommatidial precursor-cell-cluster formation occurs later than the TSPs reported here, is further evidence that the Notch-locus role in the development of adult structures is pre-differentiative, or else occurs at a very early stage of differentiation.

In passing, it can be noted that posterior-to-anterior progression of eye facet differentiation has been observed in the mosquito <u>Aedes</u> <u>aegypti</u> as well as other insects (WHITE 1961), thereby suggesting that this pattern of development may be a general one so far as compound eyes are concerned. Indeed, polarized determination of other adult structures in insects may also be the rule, since a proximal-to-distal progression of conversion of aristal to leg, or leg to aristal segments, has been observed with $\underline{ss^{a40a}}$ (GRIGLIATTI AND SUZUKI 1971). It would be of interest in this regard to see whether tarsal segment fusion in $\underline{N^{103}}/+$ heterozygotes also proceeds in a proximal-to-distal direction. This was not examined for in the $\underline{N^{103}}/\underline{spl}$ temperature shift experiment (Figure 7).

The several different lethal TSPs observed in the present experiments (Figure 14) indicate that mutation at the Notch locus can affect vital functions at several discrete developmental stages. Moreover, the fact that the TSPs for lethality differ

from those for adult morphological phenotypes suggests that the vital processes affected may be only loosely related to the morphological processes (cf GRIGLIATTI AND SUZUKI 1970).

The monophasic lethal TSPs of Ax^{16172}/N^{40} (Figure 9) and $N^{g11}/N^{g11};Dp$ (Figure 13), may indicate that these genotypes are each defective in only one vital process, whereas the length of the TSP (or TSPs) and heterogeneity of kill-periods (Figure 12) of N^{103}/fa^{no} may be interpreted in several ways. Although the N^{103}/fa^{no} shift data (Tables 44-47) do not allow a critical decision as to whether the TSP is monophasic or polyphasic, results recently reported by SHELLENBARGER (1971) suggest that it may be polyphasic. He found that peak sensitivity of $1(1)N^{ts-1}$ homozygotes to temperature occurred during the embryo, first-second instar, and prepupal stages. TARASOFF and SUZUKI (1970) described sex-linked lethal mutations in D. melanogaster with both monophasic and polyphasic TSPs and proposed that polyphasic TSPs may reflect: 1) repetitive gene activation and inactivation; 2) tissue-specific activation and inactivation of a gene; or 3) repetitive use of a gene product which is synthesized only once during development. Any one or a combination of these hypotheses could explain the polyphasic TSP of $1(1)N^{ts-1}$ (and N^{103}/fa^{no}, if it is polyphasic), but suggestion (2) is particularly attractive, considering the wide array of pleiotropic effects associated with Notch-locus mutations and since position-effect variegation of certain Notch-locus recessive mutants (COHEN 1962) suggests that the Notch locus can function autonomously in different tissues.

The observation that death of N^{103}/fa^{no} females occurs at

three distinct developmental stages, depending on their stage
when shifted to 29°C (Figure 12), further indicates that the TSP
may be polyphasic, or at least that more than one developmental
event is vitally affected. This pattern of lethality is similar
to that observed in $\underline{1(1)N}^{ts-1}$, in which embryonic heat treatments
result in death before puparium formation and later treatments
cause death just before or at the emergence of the adult (SHELLEN-
BARGER 1971). It should be pointed out that the bimodal lethal
phase (early pupal or late pupal) of $\underline{N}^{103}/\underline{fa}^{no}$ females shifted up
during the larval stages (Figure 11) could result from genetic
background heterogeneity. Such modification of the lethal phase
has been reported for unconditional lethals (HADORN 1961), and
recently for a conditional lethal (SUZUKI 1970). Nevertheless,
this does not affect the conclusion that $\underline{N}^{103}/\underline{fa}^{no}$ probably has
more than one TSP for lethality.

As discussed earlier, the monophasic embryonic TSP for
lethality of $\underline{N}^{g11}/\underline{N}^{g11};\underline{Dp}$ females may or may not result from em-
bryonic disturbances similar to those of $\underline{N}$ homozygotes, depending
on whether $\underline{N}^{g11}$ is antimorphic or neomorphic in this instance.

The monophasic second instar TSP for lethality of $\underline{Ax}^{16172}/$
$\underline{N}^{40}$ is very interesting, since most monophasic ts lethals in
$\underline{Drosophila}$ have TSPs in either the embryo or late third instar-
pupal stages (SUZUKI 1970). Incubation of $\underline{Ax}^{16172}/\underline{N}^{40}$ at 29°C
during the TSP does not prevent the determination or differentia-
tion of imaginal discs, since death occurs in late pupae or
partially-eclosed adults. However, the fact that $\underline{Ax}^{16172}$ hemi-
and homozygotes are not ts lethal, and the assumption that $\underline{N}^{40}$ is

amorphic, suggest that $\underline{Ax^{16172}/N^{40}}$ larvae do not possess $\underline{suf}$-

$\underline{ficient}$ product activity when incubated at 29°C during the TSP.

Thus, ts lethality of this genotype may reflect a hypomorphic

gene-product activity. Other than this, the data do not suggest

the nature of the defect responsible for inviability, although we

can assume that the function affected is regulatory rather than

structural, since the TSP is so far removed from the differentia-

tive (pupal) stage.

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APPENDIX 1

Interactions of N^{g11} and N^{103} with fa^g and fa^{no}

Since N^{g11} was known to have a ts eye phenotype (WELSHONS AND VON HALLE 1962), and N^{103} to have ts interaction with spl, and ts wing and leg phenotypes (WELSHONS, personal communication), a check on the interaction of these N alleles with fa^g and fa^{no} was made.

To investigate the interactions with fa^g, the following crosses were performed at 20°C, 22°C, 25°C, and 29°C:

fa^g/fa^g ♀ x w^a N^{g11} rb/B^S $w^+ \cdot Y$ ♂, and

fa^g/fa^g ♀ x y w^a N^{103};Cy Dp Bw^V ♂

At all temperatures the N/fa^g progeny of both crosses had eyes with disarrayed ommatidia and the glazed appearance of fa^g hemi- or homozygotes. No difference between the roughness of N/fa^g and that of fa^g alone, could be detected in any of the heterozygotes excepting N^{g11}/fa^g at 20°C and 22°C. In the latter cases, the roughness was no greater than one would expect from the combination of the $N^{g11}/+$ and fa^g phenotypes. The pseudo-dominant expression of fa^g observed with N^{g11} and N^{103}, is consistent with that reported for fa with these two N alleles (LINDSLEY AND GRELL 1968).

Both N^{g11}/fa^{no} and N^{103}/fa^{no} were found to be relatively viable at low temperatures, but lethal at 29°C. The crosses used, and the results obtained, appear below.

Cross 1: $\underline{w^a}\ \underline{fa^{no}}/\underline{w^a}\ \underline{fa^{no}}$ ♀ x $\underline{w^a}\ \underline{N^{g11}}\ \underline{rb}/\underline{B^S}\ \underline{w^+}\cdot Y$ ♂

Results:

	PROGENY	
Temperature	B^+ ♀♀	B^S ♂♂
20°C	31	121
22°C	3	11
25°C	16	113
29°C	0	47

Cross 2: $\underline{M5}/\underline{w^a}\ \underline{N^{g11}}\ \underline{rb}$ ♀ x $\underline{w^a}\ \underline{fa^{no}}$ ♂

Results:

	PROGENY		
Temperature	B ♀♀	B^+ ♀♀	B ♂♂
20°C	266	40	239
22°C	109	38	62
25°C	133	35	121
29°C	80	0	55

Cross 3: $\underline{M5}/\underline{y}\ \underline{w^a}\ \underline{N^{103}}$ ♀ x $\underline{w^a}\ \underline{fa^{no}}$ ♂

Results:

	PROGENY		
Temperature	B ♀♀	B^+ ♀♀	B ♂♂
20°C	244	194	204
22°C	64	83	71
25°C	138	86	129
29°C	71	0	69

Significant pupal lethality was seen in the 25°C and 29°C cultures involving $\underline{N^{g11}}$, although this was not sufficient to account for all the expected female progeny. For example, in

cross 2, 28 dead pupae were counted in the 29°C culture. Of the 24 which had developed to sufficiently advanced stages, 23 were N^{g11}/fa^{no} females, many of which had drastically reduced head and eye sizes on one or both sides. Other morphological phenotypes were not examined. No significant pupal lethality was observed in the 20°C or 22°C N^{g11} cultures, nor in any of the N^{103} cultures. Furthermore, the data show that at the three lower temperatures, N^{103}/fa^{no} was much more viable than N^{g11}/fa^{no}. Temperature-shift studies of the N^{103}/fa^{no} lethality are reported in Results (part F, section V).

The wings of both N^{103}/fa^{no} and N^{g11}/fa^{no} adults were deeply serrated at the tips and along both edges (N^{103}/fa^{no} being more extreme in this regard), had extremely thickened wing veins, and frequently contained large bubbles. In addition, N^{103}/fa^{no} females raised at 25°C had fused tarsal segments, while those raised at lower temperatures only occasionally had fused tarsi (1 out of 24 examined at 22°C). The legs of the N^{g11}/fa^{no} females which survived, did not show any tarsal fusion.

APPENDIX 2

Removal of $E\text{-}N^{70j}$ and bb^l from the N^{Co} chromosomes, and mapping of $E\text{-}N^{70j}$

In the course of the first experiments designed to study the effects of gene dosage on N^{Co}, it became apparent that the initial w^a N^{Co} rb chromosome contained a bobbed-lethal (bb^l) allele, since all attempts to make this chromosome homozygous failed, and the cross $\underline{l(FM6)}/w^e$ bb^l ♀ x w^a N^{Co} rb/Y;Dp ♂ yielded no non-Bar-eyed female progeny. In order to remove the bb^l mutant from the w^a N^{Co} rb bb^l chromosome, recombinants between N^{Co} and the flanking eye colour mutations were obtained from w^a N^{Co} rb bb^l/+ + + + females, the wild-type X chromosome having come from the OR stock. Two strains were established, w^a N^{Co}, and N^{Co} rb, from which the bb^l had been removed, as indicated by the following results of the cross $\underline{l(FM6)}/x$ ♀ x $\underline{In(1)dl49,tyl}$ bb^l/Y ♂ (♂♂ were not counted).

FEMALE PROGENY PHENOTYPE	PARENTAL ♀ X-CHROMOSOME		
	w^a N^{Co} rb bb^l	N^{Co} rb	w^a N^{Co}
B eyes	143	65	129
B^+ eyes	0	74	133

In the course of routine checks it was discovered that the w^a N^{Co} chromosome was associated with a significantly lower frequency of wing nicking than the N^{Co} rb and w^a N^{Co} rb bb^l chromosomes, suggesting that an enhancer of notching present in the original stock is not present in the w^a N^{Co} strain. From the data

tabulated below, it can be seen that the N^{Co} $\underline{rb}$ strain is not significantly different from the original strain in terms of wing nicking, whereas the frequency of individuals with nicked wingtips is halved in the $\underline{w^a}$ N^{Co} stock.

GENOTYPE	CROSS	NO. OF FEMALES		% NICKED FEMALES
		NICKED	NOT NICKED	
$\underline{w^a}$ N^{Co} $\underline{rb}$ $\underline{bb^1}$ + + + +	$\underline{OR}$ ♀ x $\underline{w^a}$ N^{Co} $\underline{rb}$ $\underline{bb^1}$/Y;$\underline{Dp}$ ♂	60	14	81
N^{Co} $\underline{rb}$ + +	$\underline{OR}$ ♀ x N^{Co} $\underline{rb}$/Y;$\underline{Dp}$ ♂	95	17	85
$\underline{w^a}$ N^{Co} + +	$\underline{OR}$ ♀ x $\underline{w^a}$ N^{Co}/Y;$\underline{Dp}$ ♂	42	58	42

The enhancer of nicking, designated $\underline{E-N^{70j}}$, was localized genetically in the following manner.

N^{Co}-containing female progeny from the cross:

+ N^{Co} $\underline{rb}$ + + + +/$\underline{y}$ + + $\underline{cv}$ $\underline{v}$ $\underline{f}$ $\underline{car}$ ♀ x $\underline{y}$ + + $\underline{cv}$ $\underline{v}$ $\underline{f}$ $\underline{car}$ ♂,

were scored for wingtip nicking and for the markers $\underline{y}$, $\underline{cv}$, $\underline{v}$, $\underline{f}$, and $\underline{car}$. The results are tabulated below.

RECESSIVE MARKERS	0 WINGS N	1 WING N	2 WINGS N	% N INDIVIDUALS
+ + + + +	71	133	124	78%
+ + + + $\underline{car}$	4	14	19	89%
+ + + $\underline{f}$ $\underline{car}$	28	46	64	80%
+ + $\underline{v}$ $\underline{f}$ $\underline{car}$	18	54	81	88%
+ $\underline{cv}$ $\underline{v}$ $\underline{f}$ $\underline{car}$	21	21	10	60%
$\underline{y}$ + + + +	1	5	11	94%
+ + + $\underline{f}$ +	1	0	3	—

RECESSIVE MARKERS	0 WINGS N	1 WING N	2 WINGS N	% N INDIVIDUALS
+ + v f +	1	8	6	93%
+ cv v f +	3	2	1	50%
+ + v + +	5	14	10	83%
+ cv v + +	6	5	7	67%
+ cv + + +	3	4	0	57%
y + + f car	0	1	1	-
+ cv + + car	0	1	0	-
y + v f +	0	0	1	-
y + v + +	0	0	1	-

It can be seen that all the genotypes of which appreciable numbers were available, exhibited wingtip nicking in 78% or more of individuals, except each class resulting from a crossover between N^{Co} and cv, and containing cv, which had wingtip nicking frequencies ranging from 50% to 67%. This suggests that E-N^{70j} is situated to the left of cv.

Although the following cannot be regarded as conclusive, additional observations made on some of the progeny listed above, suggest that E-N^{70j} is located closer to rb than to cv. The evidence is as follows. During the course of scoring this cross, it was noticed that some of the N^{Co} cv v f car females had darker eyes than others of this genotype, and that these females had a lower incidence of wing nicking than the lighter-eyed females. Of 37 females examined in this regard, 13 had darker eyes and 24 had the lighter eyes. Only 4 of the darker-eyed flies exhibited nicking, whereas 18 of the lighter-eyed females were nicked. If

it is assumed that the darker-eyed females have the genotype
$+ \underline{N^{Co}} + \underline{cv} \ \underline{v} \ \underline{f} \ \underline{car}/\underline{y} + + \underline{cv} \ \underline{v} \ \underline{f} \ \underline{car}$, and the lighter-eyed females
$+ \underline{N^{Co}} \ \underline{rb} \ \underline{cv} \ \underline{v} \ \underline{f} \ \underline{car}/\underline{y} + + \underline{cv} \ \underline{v} \ \underline{f} \ \underline{car}$, these data suggest that
$\underline{E-N^{70j}}$ is situated close to $\underline{rb}$. This assumption is supported by
the observations that: 1) all $\underline{y}^+ \ \underline{N}^+ \ \underline{cv} \ \underline{v} \ \underline{f} \ \underline{car}$ females examined
(their genotype presumably being $+ + + \underline{cv} \ \underline{v} \ \underline{f} \ \underline{car}/\underline{y} + + \underline{cv} \ \underline{v} \ \underline{f}$
$\underline{car}$, since interference is essentially complete in the $\underline{y}$-$\underline{rb}$
region) had the darker eye colour, and 2) all $\underline{y}^+ \ \underline{N^{Co}} \ \underline{cv}^+ \ \underline{v} \ \underline{f} \ \underline{car}$
females examined (presumably $+ \underline{N^{Co}} \ \underline{rb} + \underline{v} \ \underline{f} \ \underline{car}/\underline{y} + + \underline{cv} \ \underline{v} \ \underline{f} \ \underline{car}$)
had the lighter eye colour.

APPENDIX 3

Screening procedures used to isolate new Notch-locus mutations

Procedure No. 1, diagrammed on the next page, was designed to screen for new mutations which were lethal when heterozygous with N. $\underline{OR}$ males which had been fed 0.0125 M EMS in 1% sucrose for 24 hours, were mated to compound-X ($\widehat{XX}/Y$) females of the geno-type shown. Individual male progeny of this cross, raised at room temperature (20°C-22°C), were each mated to 2 $\underline{1(FM6)/1(1)J1}$ and 2 $\widehat{XX},\underline{y}$ $\underline{w}$ $\underline{f};\underline{B}^S$ $\underline{y}^+\cdot Y$ females in the same vial. Those vials which produced both $\underline{y}$ and $\underline{y}^+$ female progeny, but no males, were saved as putative lethals covered by the $\underline{N}^+$ duplication $\underline{B}^S$ $\underline{w}^+\cdot Y$. The $\underline{1(FM6)/+}$* females from the vials scored as putative lethals, were mated individually to $\underline{w}^{\underline{a}}$ N^{40} $\underline{rb}/\underline{B}^S$ $\underline{w}^+\cdot Y$ males. Progeny of this cross were scored for the absence or presence of $\underline{B}^+$ females, indicating the respective presence or absence of a Notch-locus lethal failing to complement with $\underline{N}^{40}$. From approximately 2600 chromosomes tested in this manner, 32 putative Notch-locus lethals were recovered. Of these, 1 was not lethal, 19 were lethal but not covered by the $\underline{N}^+$ duplication, 11 were lethals covered by the $\underline{N}^+$ duplication, but were not lethal when heterozygous with $\underline{N}^{40}$, and 1 was a Notch-locus lethal. This lethal, originally designat-ed $\underline{1(1)N}^{\underline{E1}}$, was later renamed $\underline{Ax}^{\underline{E1}}$ when it was found to be an Abruptex allele (see Table 13 for mapping of $\underline{Ax}^{\underline{E1}}$).

EMS
↓

$\widehat{XX}$,$\underline{y}$ $\underline{w}$ $\underline{f}$;$\underline{B^S}$ $\underline{w^+}\cdot Y$ ♀ x +/Y ♂

$\underline{1(FM6)}/\underline{1(1)J1}$ ♀)
) x +*/$\underline{B^S}$ $\underline{w^+}\cdot Y$ ♂ (individual ♂)
$\widehat{XX}$,$\underline{y}$ $\underline{w}$ $\underline{f}$;$\underline{B^S}$ $\underline{y^+}\cdot Y$ ♀)

$\underline{1(FM6)}/+^*$	$\underline{1(1)J1}/+^*$	$\underline{1(FM6)}/\underline{B^S}$ $\underline{w^+}\cdot Y$	$\underline{1(1)J1}/\underline{B^S}$ $\underline{w^+}\cdot Y$	$\widehat{XX}$,$\underline{y}$ $\underline{w}$ $\underline{f}$;$\underline{B^S}$ $\underline{w^+}\cdot Y$	+*/$\underline{B^S}$ $\underline{w^+}\cdot Y$
($\underline{y^+}$ females)		(males die)		($\underline{y}$ females)	(males die if +* carries a lethal)

$\underline{1(FM6)}/+^*$ ♀ x $\underline{w^a}$ $\underline{N^{40}}$ $\underline{rb}$/$\underline{B^S}$ $\underline{w^+}\cdot Y$ ♂

$\underline{1(FM6)}/\underline{w^a}$ $\underline{N^{40}}$ $\underline{rb}$	+*/$\underline{w^a}$ $\underline{N^{40}}$ $\underline{rb}$	$\underline{1(FM6)}/\underline{B^S}$ $\underline{w^+}\cdot Y$	+*/$\underline{B^S}$ $\underline{w^+}\cdot Y$
($\underline{B}$ females live)	(B^+ females die if +* carries N-locus lethal)	(males die)	(males live if +* carries lethal covered by duplication)
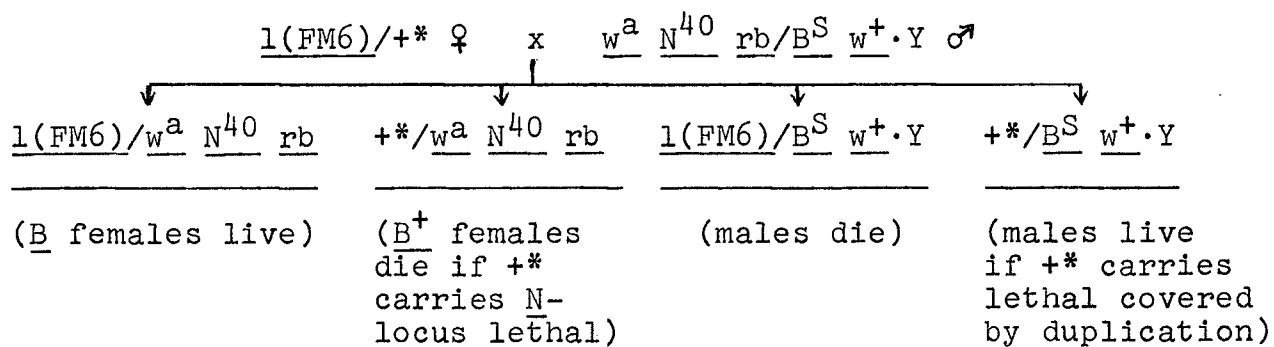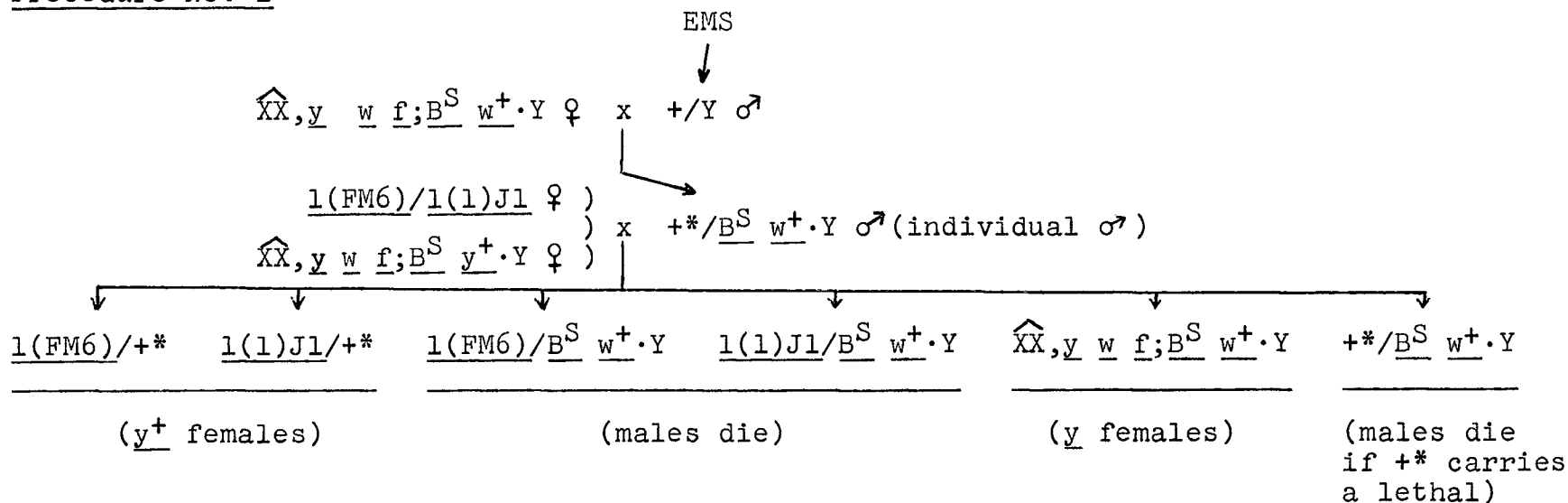
Procedure No. 2 (not diagrammed) was used to screen for visible, recessive viable mutations in the Notch locus. OR males which had been fed 0.005 M EMS in 1% sucrose, were mated to $\widehat{XX}$/Y females. Male progeny of this cross, raised at room temperature, were screened directly for visible phenotypes affecting wing nicking, wing venation, or eye facet arrangement. Of a total of 4686 male progeny examined 111 were saved for further testing. These were mated individually to virgin $\widehat{XX}$/Y females. Of these 19 were sterile, 67 did not yield mutant progeny, 3 were autosomal dominants, 7 were lethals, and 15 were X-linked visibles. Of the last group, two proved to be in the Notch locus. On the basis of subsequent tests, these were designated Ax^{E2} and fa^{noE}, respectively (see Appendix 4 for mapping of fa^{noE}, and Table 14 for mapping of Ax^{E2}).

Procedure No. 3, diagrammed on the next page, was designed to screen for 1) revertants of N^{g11} to N^+, and 2) forward mutations of N^{g11} to N^{amorph} (either as the result of mutation at the N^{g11} site, or at a site cis to N^{g11}). It can also be used to screen for extra-locus modifiers of Notch, as the results show. w^a N^{g11} rb/B^S $w^+ \cdot Y$ males which had been fed 0.0125 M EMS in 1% sucrose for 24 hours, were mated to d149/w^a N^{40} rb females (8 half-pint bottles, 20 ♀ ♀ x 4-5 ♂ ♂ each). The eyes of female progeny, raised at 20.5°C, were scored for white eye colour (putative N^+ revertants) or non-mutant eye facet pattern (N^+, N, or modifier mutations). Of 3008 female progeny examined, none were white-eyed, but 11 red-eyed flies with wild-type or nearly wild-type eye facet patterns, were saved for further testing. Of these 2 were sterile, 5 did not breed true, 1 was probably non-disjunctant for the maternal X chromosomes (there also appeared to be non-disjuction of the N^{g11}/B^S $w^+ \cdot Y$ chromosomes in the treated male parents) and was discarded, and 2 bred true and were saved. Stocks established from these 2 putative revertant females were provisionally named Re^{70k27} and Re^{70k30}, respectively. Subsequent tests indicated that the partial eye revertant Re^{70k27} was a sex-linked, recessive lethal, dominant enhancer of wing nicking (Appendix 4), so this mutation was re-named $E-N^{70k27}$. The full eye revertant Re^{70k30} is phenotypically a notch mutation, and I have so far been unable to separate it from the N^{g11} site (Appendix 4). Re^{70k30} has therefore been re-named N^{70k30}. Note that where N^{70k30} is referred to elsewhere in this report, it is taken to include the N^{g11} mutant site.

Procedure No. 3

EMS
↓

$\underline{dl49},\underline{y}$ $\underline{Hw}$ $\underline{m^2}/\underline{w^a}$ $\underline{N^{40}}$ $\underline{rb}$ ♀   x $\underline{w^a}$ $\underline{N^{g11}}$ $\underline{rb}/\underline{B^S}$ $\underline{w^+} \cdot Y$ ♂

20.5°C

$\underline{dl49},\underline{y}$ $\underline{Hw}$ $\underline{m^2}/\underline{w^a}$ $\underline{N^{g11}}$ $\underline{rb}$ ♀ $\underline{w^a}$ $\underline{N^{40}}$ $\underline{rb}/\underline{w^a}$ $\underline{N^{g11}}$ $\underline{rb}$ ♀
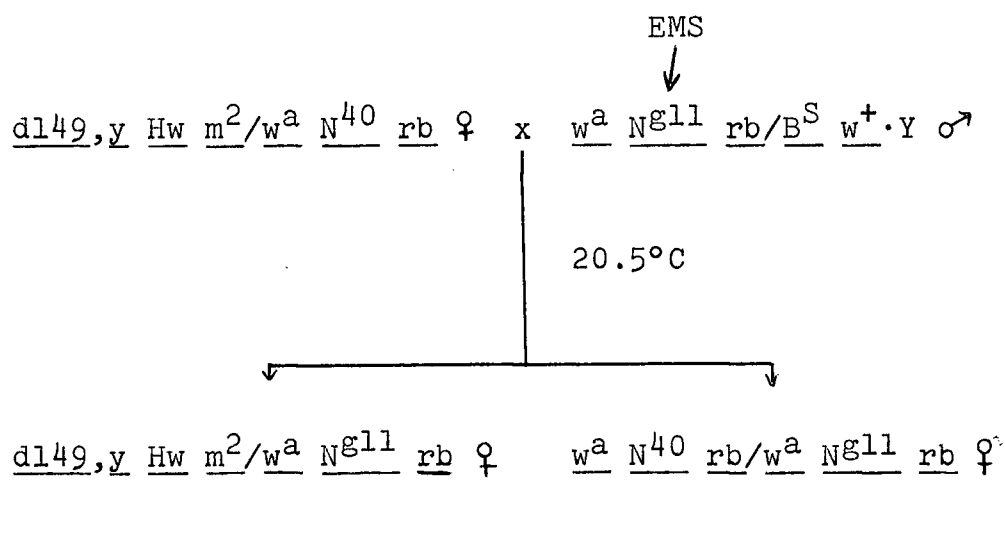
Score eyes of red-eyed females for facet pattern. Wild-type eyes indicate $\underline{N^{g11}} \to \underline{N^+}$, $\underline{N^{g11}} \to N$, or extra locus modifier.

Females die unless $\underline{N^{g11}} \to \underline{N^+}$ mutation has been induced (score for appearance of white-eyed females).

Re-test putative mutants further by mating to $\underline{dl49},\underline{y}$ $\underline{Hw}$ $\underline{m^2}$ males.

APPENDIX 4

Description and mapping of fa^{noE}, $E\text{-}N^{70k27}$, and N^{70k30}

I. fa^{noE}

The new mutation fa^{noE} was so named, on the basis of its phenotype and, as described below, the lack of recombination between fa^{no} and fa^{noE}. fa^{noE} has thickenings at the ends of the wing veins, and occasional nicks in the wingtips. Expression of both phenotypes is mild compared to fa^{no}, and heterozygotes of fa^{noE}/fa^{no} are intermediate in appearance. fa^{noE}/spl, fa^{noE}/fa, and fa^{noE}/fa^g have wild-type wings and eyes. N^8/fa^{noE} and N^{40}/fa^{noE} are lethal, and N^{g11}/fa^{noE} is semilethal at 20.5°C (148 male and 8 female progeny were recovered from the cross $\underline{y}\ fa^{noE}/\underline{y}\ fa^{noE}\ ♀ \times \underline{w}^a\ N^{g11}\ \underline{rb}/\underline{B}^S\ \underline{w}^+\cdot Y\ ♂$) and lethal at 29°C.

In order to map fa^{noE} with respect to fa^{no}, two series were run of the cross $fa^{noE}\ \underline{cv}/\underline{w}^a\ fa^{no}\ \underline{spl}\ \underline{rb}\ ♀ \times \underline{w}^a\ N^{40}\ \underline{rb}/\underline{B}^S\ \underline{w}^+\cdot Y\ ♂$. Series 1 consisted of 24 cultures, each with 25 pairs of parents per 1/2-pint bottle, transferred to fresh bottles for a total of 6 broods, each of which lasted 3 days except that in 8 cultures, one brood lasted 7-8 days, and in 14 cultures there were 1 or 2 5-6 day broods. Series 2 consisted of 9 cultures, each with 5 pairs of parents per 1/4-pint bottle, transferred every 3-4 days for a total of 5 broods. Male and female progeny of these crosses were counted. The results, tabulated below, show that no $fa^{no}\text{-}fa^{noE}$ crossovers ($N^{40}/+$ females) were recovered, although 22

N^{40}/fa^{no} spl and 6 N^{40}/fa^{noE} breakthroughs survived and 41,830

males were counted. This indicates that fa^{noE} is very closely

linked to fa^{no} in the Notch locus.

SERIES	TOTAL MALES	FEMALES			
		B^S	N/fa^{no} spl	N/fa^{noE}	$N/+$
1	38,119	35	20	5	0
2	3,711	8	2	1	0
TOTAL	41,830	43	22	6	0

II. $E-N^{70k27}$

$E-N^{70k27}$ (abbreviated $E-N^{k27}$) maps to the left of w^a (see

below), is a recessive lethal, and in cis or trans combination

with N^{g11} causes a marked reduction in the 20-22°C rough eye

phenotype of N^{g11} and an increased frequency of wing nicking.

With practice, $E-N^{k27}$ $N^{g11}/+$ + can readily be distinguished from

$N^{g11}/+$ and from wild-type, at 20-22°C. From the cross $E-N^{k27}$ w^a

$N^{g11}/dl49$ ♀ x OR ♂, 7% (10/146) of $E-N^{k27}$ w^a $N^{g11}/+$ + + females

raised at 20.5°C, and 27% (84/316) raised at 22°C, had nicked

wings. $E-N^{k27}/N^{Co}$ females also are more strongly nicked than

$N^{Co}/+$. Lethality of $E-N^{k27}$ occurs during the pupa stage, and is

preceded by the formation of prominent melanotic tumors, which

become visible during the third instar. Both the lethality and

tumor formation are absent in males carrying a duplication of the

dor^+ region, but $E-N^{k27}$ and dor may not be allelic, since $E-N^{k27}/$

dor has wild-type eyes and $E-N^{k27}/dor^1$ survive (see below).

E-N^{k27} was mapped to the left of w$^{\underline{a}}$ by the cross E-N^{k27} w$^{\underline{a}}$ N^{g11} rb/+ + + + ♀ x w$^{\underline{a}}$ rb ♂. Eggs were collected for 3 days in 10 half-pint bottles (5 pairs of parents per bottle), and progeny were raised at 22°C. The recombination frequencies, calculated from the female data tabulated below, are:

1.60% (E-N^{k27}-w$^{\underline{a}}$), 1.51% (w$^{\underline{a}}$-N^{g11}), and 4.17% (N^{g11}-rb).

	GENOTYPE	NO. OF PROGENY	
		FEMALES	MALES
Non-crossovers	E-N w$^{\underline{a}}$ N^{g11} rb	1039	0
	+ + + +	1067	1033
Single cross-overs E-N-w$^{\underline{a}}$	E-N + + +	(not distinguishable from non-crossovers)	
	+ w$^{\underline{a}}$ N^{g11} rb	18	0
Single cross-overs w$^{\underline{a}}$-N^{g11}	E-N w$^{\underline{a}}$ + +	19	0
	+ + N^{g11} rb	15	0
Single cross-overs N^{g11}-rb	E-N w$^{\underline{a}}$ N^{g11} +	45	0
	+ + + rb	49	48
TOTALS		2252	1081

The following results of pair-matings in vials (M5/E-N^{70k} w$^{\underline{a}}$ ♀ x Duplication ♂ - see below), indicate that E-N^{70k} is situated in the dor region. Note that p-DTS is a dor^1 allele (PRATT 1970).

X/Dp·Y MALE PARENTS	PROGENY				LARVAL MELA-NOTIC TUMORS
	M5/X ♀	X/E-N ♀	M5/Y ♂	E-N/Dp·Y ♂	
Df(1)sc^{8}w^a/Dp^{59k9}(4)	45	49	0	0	present
y^{59b} z/Dp^{60d19}(1)	14	13	12	0	present
y dor/T(1;Y)2E	32	43	23	26	absent
p-DTS rb/Dp^{67g24}(1)	11	18	12	17	absent
w^a N^{g11} rb/B^S w$^+$·Y	39	49	28	0	present

III. N^{70k30}

N^{70k30}/+ females have a wild-type eye facet arrangement at 22°C, and enhanced wing nicking compared to N^{g11}/+. Lethality of N^{70k18}/fa^{no} is variable, depending on the genetic background (see below). Those that die, do so in the late pupal stage (20-22°C). This is unlike N^{g11}/fa^{no}, some of which survive to adulthood, the rest apparently dying before pupation (Appendix 1). Controlled experiments have not been done, so it cannot be ascertained whether the difference between N^{g11}/fa^{no} and N^{70k30}/fa^{no} is due to genetic background differences, or to the N^{g11}→N^{70k30} mutational change.

Eighty-seven percent (134/154) of the N^{70k30}/+ females raised at 22°C (w^a N^{70k30} rb/dl49,y Hw m^2 ♀ x OR ♂), had one or both wingtips nicked.

Viability of N^{70k30} with fa^{no} was investigated in the following crosses and results.

Cross 1. w^a N^{70k30} rb/dl49,y Hw m^2 ♀ x w^a fa^{no} ♂

Progeny: 151 dl49/fa^{no} ♀ , 23 N^{70k30}/fa^{no} ♀ , 108 dl49/Y ♂

Cross 2. w^a fa^{no}/w^a fa^{no} ♀ x w^a N^{70k30}/B^S w^+·Y ♂

Progeny: 233 fa^{no}/B^S w^+·Y ♂, 1 N^{70k30}/fa^{no} ♀ .

Surviving N/fa^{no} females have deeply serrated wings and very thick veins. The lone female survivor of cross (2) had fused tarsal segments (other N/fa^{no} females not examined).

The results of the mapping cross w^a N^{70k30} rb/+ + + ♀ x w^a rb ♂, reported below, indicate that the mutation responsible for conversion of N^{g11} to N^{70k30}, is situated within the Notch locus, at or very close to the N^{g11} site. Eggs were collected in 3 day broods (6 broods) in 10 half-pint bottles (5 pairs per bottle), progeny were raised at 22°C, and females were scored for eye colour and facet pattern. Among 27,671 female progeny counted, w^a-N and N-rb recombination was standard (1.42% and 4.95%, respectively), but no confirmed crossovers between N^{70k30} and N^{g11} were recovered. One white-eyed (w^a rb) female was recovered with eyes similar to those of N^{g11}/+, but when backcrossed to w^a rb males, this exception only produced 3 female progeny, none of which were N^{g11}/+.

APPENDIX 5

Tests of exceptional female progeny from Ax^{E1} mapping crosses (see Table 13)

A. spl rb ♀ ♀

The results of individual matings of 11 of the w^+ spl rb females, all of which had notched wings, to w^a spl rb males, are reported below. Of the other females, two were sterile and one was not tested because she was not virgin.

	PROGENY					
	♀ ♀				♂ ♂	
♀ No.	spl rb	w^a spl rb	w^a N spl rb	N spl rb	spl rb	w^a spl rb
1	13	0	0	0	8	1
2	10	1	9	0	13	0
3	11	0	10	0	17	0
4	27	0	19	1	15	0
5	14	1	17	0	14	0
6	20	0	12	0	12	0
7	29	2	20	0	29	1
8	38	0	32	2	34	1
9	23	0	25	0	30	0
10	41	0	32	0	41	0
11	36	2	32	1	31	0

These results indicate that the spl rb exceptions were all true crossovers between fa^{no} and spl, rather than resulting from

phenocopy-like events. The absence of $\underline{N}$ females in the progeny of female No. 1 probably resulted from selective death of this class due to late scoring of this vial.

B. $\underline{w^+ \ rb^+}$ ♀ ♀

The results of crosses of the $\underline{w^+ \ rb^+}$ female exceptions (note that only No. 5 was definitely virgin when discovered), to $\underline{w^a \ spl \ rb}$ males, are reported below.

PARENTAL ♀ No.			1			2			3			4			5		
Parental wings			notched			notched			abruptex			notched			notched		
PROGENY Phenotypes		Sex	♀	♂	I	♀	♂	I	♀	♂	I	♀	♂	I	♀	♂	I
Eye colour	Eye facets	Wings															
+	+	$\underline{Ax}$, +	24	0	10	5	0	5	3	0	3	6	0	6	29	0	3
$\underline{w^a}$	+	$\underline{Ax}$, +	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
$\underline{rb}$	+	$\underline{Ax}$, +	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
$\underline{w^a}$ $\underline{rb}$	$\underline{spl}$	+	3	0	0	2	0	1	1	0	2	0	1	8	3	0	6
$\underline{w^a}$	$\underline{spl}$	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
$\underline{rb}$	$\underline{spl}$	+	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$\underline{w^a}$ $\underline{rb}$	$\underline{spl}$	$\underline{N}$	5	1	3	3	1	4	0	1	2	3	0	2	6	2	2
$\underline{w^a}$	$\underline{spl}$	$\underline{N}$	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
$\underline{rb}$	$\underline{spl}$	$\underline{N}$	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
+	+	$\underline{N/Ax}$	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0

The "I" progeny sex class comprised intersexes. These individuals possessed sexcombs and were either male-like or female-like with respect to their genitalia and body markings. All flies of this class were sterile, and the flies themselves were

weak and usually died earlier than the true males and females.
The presence of intersexes among their progeny suggests that the
w^+ rb^+ exceptional females were triploids, a conclusion which is
supported by the results of further tests, made on the F_1 female
progeny. The tests of the different classes of F_1 females will
be considered separately.

The results of progeny tests of F_1 virgin females with wild-
type eye facets and $\underline{Ax}$ or $\underline{Ax}^+$ wing veins (crossed to $\underline{w^a}$ $\underline{spl}$ $\underline{rb}$
$\sigma\sigma$), are presented in the following table. The data indicate
that F_1 females No. 1-1, 1-5, and 2-5 were triploids. Females
1-1 and 1-5 carried $\underline{Ax}^{El}$ and either $\underline{w^a}$ $\underline{spl}$ $\underline{rb}$ or $\underline{w^a}$ $\underline{fa}^{no}$ $\underline{spl}$ $\underline{rb}$,
and furthermore the paucity of male progeny suggests (although
not conclusively) that they also carried $\underline{N}^{40}$. The split notched,
and split non-notched daughters of female no. 2-5, and the fact
that 2-5 herself was apricot, and neither notched nor $\underline{Ax}$-like,
suggests that this female had the genotype $\underline{w^a}$ $\underline{N}^{40}$ $\underline{rb}/\underline{w^a}$ $\underline{Ax}^{El}/$
$\underline{w^a}$ $\underline{spl}$ $\underline{rb}$. Females number 1-3, 2-3, 3-1, 3-2, 4-2, 4-3, 4-5, 4-7,
5-1 and 5-2 were diploids with the genotype $\underline{w^a}$ $\underline{fa}^{no}$ $\underline{spl}$ $\underline{rb}/\underline{Ax}^{El}$,
and females number 2-1 and 2-7 were diploids with the genotype
$\underline{w^a}$ $\underline{spl}$ $\underline{rb}/\underline{Ax}^{El}$.

The results of crosses of F_1 virgin split-eyed normal-winged
females to $\underline{w^a}$ $\underline{spl}$ $\underline{rb}$ males are presented below (top of p. 247).

These data indicate that F_1 females 1-2, 1-7, 2-2 and 2-6
were diploids with the genotype $\underline{w^a}$ $\underline{fa}^{no}$ $\underline{spl}$ $\underline{rb}/\underline{w^a}$ $\underline{spl}$ $\underline{rb}$. The
small number of "white split notched" males from female 1-7
likely resulted from overcrowding and late scoring of the vial.

Progeny tests of F$_1$ ♀♀ with + eye facets and $\underline{Ax}$ or $\underline{Ax}^+$ wing veins.

F$_1$ ♀ No.	F$_1$ ♀ Pheno.	F$_2$ Sex	White split	White split notched	Apricot split	Apricot split notched	Ruby split	Ruby split notched	$\underline{Ax}$ $\underline{Ax}^+$	Apricot $\underline{Ax};\underline{Ax}^+$	Ruby $\underline{Ax};\underline{Ax}^+$
1-1	$\underline{w^+}\ \underline{rb^+}$ / $\underline{Ax^+}\ \underline{N^+}$	(♀	9	0	0	0	0	0	8	1	0
		(♂	1	0	0	0	0	0	0	0	0
		(I	2	0	0	0	0	0	2	0	0
1-3	$\underline{w^+}\ \underline{rb^+}$ / $\underline{Ax^+}\ \underline{N^+}$	(♀	17	0	2	0	0	0	17	2	1
		(♂	0	21	0	0	0	0	0	0	0
1-5	$\underline{w^+}\ \underline{Ax^+}$ / $\underline{N^+}\ \underline{rb^+}$	(♀	6	0	0	0	0	0	6	0	0
		(♂	0	0	0	0	0	0	0	0	0
2-1	$\underline{w^+}\ \underline{Ax}$ / $\underline{rb^+}$	(♀	42	0	0	0	0	0	39	0	0
		(♂	34	0	2	0	0	0	0	0	0
2-3	$\underline{w^+}\ \underline{Ax}$ / $\underline{rb^+}$	(♀	31	0	2	0	1	0	53	2	0
		(♂	2	26	0	3	1	2	0	0	0
2-5	$\underline{w^a}\ \underline{N^+}$ / $\underline{rb^+}\ \underline{Ax^+}$	(♀	7	1	0	0	1	0	0	6	0
		(♂	0	0	0	0	0	0	0	0	0
		(I	1	0	0	0	0	0	2	0	0

F_1 ♀ No.	F_1 ♀ Pheno.	F_2 Sex	White split	White split notched	Apricot split	Apricot split notched	Ruby split	Ruby split notched	Ax Ax^+	Apricot $Ax;Ax^+$	Ruby $Ax;Ax^+$
2-7	$\frac{w^+}{rb^+}$ Ax	♀	25	0	1	0	0	0	20	0	0
		♂	28	0	0	0	0	0	0	0	0
3-1	$\frac{w^+}{rb^+}$ Ax	♀	23	0	1	0	0	0	28	2	0
		♂	1	25	0	0	0	1	0	0	0
3-2	$\frac{w^+}{rb^+}$ Ax	♀	23	0	2	0	0	0	33	1	0
		♂	2	26	0	1	0	0	0	0	0
4-2	$\frac{w^+}{Ax^+}$ $\frac{N^+}{rb^+}$	♀	24	0	3	0	2	0	42	0	0
		♂	1	21	0	1	0	1	0	0	0
4-3	Ax	♀	41	0	1	0	0	0	49	0	1
		♂	3	26	0	2	0	0	0	0	0
4-5	Ax	♀	41	0	0	0	1	0	33	1	0
		♂	3	18	0	0	0	1	0	1	0
4-7	Ax	♀	17	0	2	0	1	0	29	1	1
		♂	0	18	0	1	0	0	0	0	0

F_2 PHENOTYPE

F_1 ♀ No.	F_1 ♀ Pheno.	F_2 Sex	White split	White split notched	Apricot split	Apricot split notched	Ruby split	Ruby split notched	$\dfrac{Ax}{Ax^+}$	Apricot $Ax;Ax^+$	Ruby $Ax;Ax^+$
5-1	Ax	(♀	12	0	2	0	1	0	15	3	0
		(♂	2	20	0	1	0	0	0	0	0
5-2	Ax	(♀	16	0	1	0	0	0	23	1	1
		(♂	1	19	0	0	0	0	0	0	0

F$_2$ PROGENY

F$_1$ ♀ No.	♀♀		♂♂	
	white split	white split notched	white split	white split notched
1-2	49	0	16	18
1-7	56	0	23	3
2-2	17	0	14	12
2-6	54	0	27	10

Results of progeny tests of white split notched females to w^a spl rb males are presented below.

F$_2$ PROGENY

F$_1$ ♀ No.	white split			white split notched		
	♀	♂	I	♀	♂	I
1-4	1	0	0	0	0	0
1-6	2	0	0	0	3	0
2-4	65	0	0	0	65	0
2-8	54	2	0	1	57	0
4-1	7	4	1	5	1	1
4-6	20	6	5	4	1	0

These data indicate that F$_1$ females 2-4, 2-8 and 1-6 were diploids with genotype w^a fa^{no} spl rb/w^a fa^{no} spl rb. This indicates that parental females 1 and 2 were non-virgin, both having been inseminated by their w^a fa^{no} spl rb brothers. Females 4-1,

and 4-6, on the other hand, were triploids, which, judging from
their origin and the phenotypes of their progeny, must have had
the genotype w^a N^{40} rb/w^a fa^{no} spl rb/w^a spl rb.

It has already been inferred that the presence of intersexes
among their progeny suggests that the original w^+ rb^+ female
exceptions were triploids. Careful consideration of the pheno-
types of the original females, and those of the succeeding two
test generations, confirms this hypothesis for at least four of
the exceptions. Notwithstanding the known non-virginity of
females 1 and 2, the data indicate that females 1, 2, 4, and 5
all contained the chromosomes w^a fa^{no} spl rb, w^a N^{40} rb, and w^+
Ax^{E1} rb^+, and that female 3 had at least w^a fa^{no} spl rb and
w^+ Ax^{E1} rb^+.

APPENDIX 6

Tests of exceptional progeny from Ax^{E2} mapping crosses

Series 1

Male progeny whose phenotypes suggested they were recombinants for fa^{no}, spl, or Ax^{E2} were mated to $\widehat{XX}/Y$ females to establish stocks for further testing. The phenotypes of the original males are described below (see footnote to Table 14 for the crosses used).

Recombinant No. (Culture-Brood)	PHENOTYPE	
	EYES	WINGS
1-3	apricot	notched
2-3	apricot	notched
5-7	ruby, split	+
7-4	+	notched
8-2	ruby, split	+
8-4	ruby	+
8-8	apricot	notched

Recombinant 8-8 was sterile. The fertile recombinants all bred true, the progeny male phenotypes being the same as those of the respective parental males. Because certain of the bristle phenotypes of the apricot notched stocks resembled those of Ax^{E2}, several individuals from each of the fertile notched stocks, and from the ruby split stock 8-2 were mated to Ax^{E2}/Ax^{E2} virgin females, along with a control cross of Ax^{E2}/Ax^{E2} ♀ x fa^{no} spl ♂.

The presence or absence of wing vein gaps in the progeny of these crosses was scored, and the results are tabulated below. The figures presented are the pooled data from progeny of up to 24 fertile individual matings of males from a given stock to Ax^{E2}/Ax^{E2} females.

Recombinant No.	PROGENY			
	♀ ♀		♂ ♂	
	No gaps	1 or more gaps	No gaps	1 or more gaps
1-3	73	977	0	1079
2-3	65	1041	0	1023
7-4	23	631	0	607
8-2	554	231	0	663
CONTROL	561	184	0	755

These data suggest that 1-3, 2-3, and 7-4 all contained the mutant Ax^{E2}, whereas 8-2 did not, and that therefore their genotypes were: w^a fa^{no} Ax^{E2}, w^a fa^{no} Ax^{E2}, fa^{no} Ax^{E2}, and spl rb, respectively, although the nature of the control cross does not eliminate the possibility that spl suppresses the Ax^{E2} wing vein phenotype. It will later be shown that this is not the case. These data indicated that Ax^{E2} was to the right of fa^{no}, and from the single rb recombinant, probably to the right of spl. Unfortunately, these stocks were all accidentally lost before further tests could be made, but the data from the next series confirm the inferred map position of Ax^{E2}.

Series 2

Male progeny whose phenotypes indicated that they were recombinant for $\underline{fa^{no}}$, $\underline{spl}$, or $\underline{Ax^{E2}}$, were mated to XX/Y females to establish a stock of the recombinant chromosome. The phenotypes of these original males are recorded below.

Recombinant No.	PHENOTYPE	
	EYES	WINGS
1-3	apricot	notched
1-4	apricot	notched
2-4	ruby, split	+
3-4	ruby, split	+
4-3	apricot	notched
5-3	ruby, split	+
9-4	ruby, split	+
10-4	apricot, extreme split	notched
12-1	apricot, extreme split	notched
12-5	ruby, split	+
13-3	white	notched
15-4	ruby, split	+
17-3	ruby, split	+

The "extreme split" recombinants 10-4 and 12-1 had very narrow eyes with only a few facets present in an amorphous, glazed matrix of eye tissue. In each case except for male 1-4, which was sterile, the phenotypes of the respective male progeny (when mated to XX/Y females) were identical to those of the original recombinants.

In addition to the male recombinants, the following female recombinants from experiment 2 were recovered.

	PHENOTYPE	
Recombinant No.	EYES	WINGS
1-2	apricot	notched
5-5	ruby, split	+
7-2	ruby, split	+
7-3	apricot	notched
14-3	ruby, split	+
18-2	apricot	notched

The phenotypes of the male progeny of the recombinant females are tabulated below.

	PHENOTYPES OF PROGENY					
Recombinant No.	white split notched	apricot split notched	white notched	apricot notched	white split	ruby split
1-2	21	1	3	17	0	0
5-5	21	0	0	0	0	28
7-2	23	0	0	0	0	28
7-3	14	1	0	25	0	0
14-3	23	0	0	0	1	13
18-2	11	0	0	22	0	0

As was observed with each original recombinant (both sexes), the Abruptex wing vein gap phenotype was not present in any of their progeny. However, the bristle phenotypes of the "apricot notched", and "apricot split notched" males were similar to those of Ax^{E2}/Y males, while the "white notched", and "split ruby" males were more like wild-type, although even these had bristle defects. Consequently, in order to determine whether Ax^{E2} was present in any of these recombinants, males from most of the recombinant cultures were mated to virgin Ax^{E2}/Ax^{E2} females, and

the female progeny were inspected for wing vein gaps and for the number of anterior orbital bristles present. The results of these tests are reported below.

PARENTAL ♂ PHENOTYPE	REC. #	♀ PROGENY PHENOTYPES					
		No. of flies with		No. of flies with given No. of anterior orbitals		
		0 gaps	1 or more gaps	0	1	2
apricot notched	1-2	1	51	52	0	0
apricot notched	1-3	0	40	40	0	0
apricot notched	4-3	0	53	53	0	0
apricot notched	7-3	0	34	34	0	0
apricot notched	18-2	1	40	41	0	0
white notched	13-3	45	2	15	21	11
apricot split notched	10-4	3	74	77	0	0
apricot split notched	12-1	22	154	176	0	0
ruby split	3-4	33	7	35	4	1
ruby split	5-3	18	24	26	15	1
ruby split	5-5	27	17	27	14	3
ruby split	7-2	15	36	23	19	9
ruby split	9-4	31	13	37	6	1
ruby split	12-4	28	16	26	16	2
ruby split	14-3	20	20	32	6	2
ruby split	15-4	29	12	25	12	4
ruby split	17-3	25	39	46	14	4

The high frequency of occurrence of wing vein gaps, and the absence of orbitals in the apricot notched, and apricot split notched heterozygotes with Ax^{E2}, indicate that both recombinant classes contain the Ax^{E2} mutant site, and have the genotypes w^a fa^{no} Ax^{E2} and w^a fa^{no} spl Ax^{E2}, respectively. Conversely, the lower frequencies of wing vein gaps, and presence of some flies possessing anterior orbitals, in the heterozygotes of white notched, and ruby split, with Ax^{E2}, indicates that Ax^{E2} is not present in these recombinants, which must have the genotypes w^a fa^{no} rb and spl rb, respectively. These data place Ax^{E2} to the right of spl. This conclusion was subsequently confirmed when the recombinant genotype spl Ax^{E2} was recovered as a single male in the progeny of the cross w^a fa^{no} spl $Ax^{E2}/+ + + + ♀$ x OR ♂, the female parents of which were the progeny of a cross between OR females and recombinant stock 12-1. All spl Ax^{E2} individuals established from this recombinant, have the characteristic Ax^{E2} wing phenotype, have a rough eye phenotype intermediate between that of fa^{no} spl Ax^{E2} and spl, and have markedly reduced bristle frequencies compared to spl or Ax^{E2} alone.

APPENDIX 7

Interactions of fa^{no}, spl, and Ax^{E2}

The phenotypes of various combinations of fa^{no}, spl, and Ax^{E2} indicate that: 1) coupling of spl and Ax^{E2} results in extreme expression of the spl eye phenotype, extreme bristle loss, and near-normal expression of the Ax^{E2} wing vein gap phenotype; 2) coupling of fa^{no} to spl Ax^{E2} results in further enhancement of the spl eye phenotype, suppression of bristle loss compared to spl Ax^{E2}, and complete suppression of wing vein gapping; 3) coupling of fa^{no} to Ax^{E2} does not cause a mutant eye phenotype, possibly slight enhancement of bristle loss compared to Ax^{E2}, and complete suppression of wing vein gapping. The eye and wing phenotypes of various combinations of alleles at 20°C-22°C are summarized below in tabular form. The rough eye and wing vein gap phenotypes (where expressed) are enhanced at 29°C. Note that the spl eye phenotype is normally completely recessive in spl/+.

GENOTYPE	EYES	WINGS
fa^{no} spl Ax^{E2}/Y ♂	very narrow, glazed, few or no discrete ommatidia	nicked, thick veins, no vein gaps
spl Ax^{E2}/Y ♂	intermediate between fa^{no} spl Ax^{E2} and spl in size; discrete ommatidia but strongly spl	vein gaps like Ax^{E2}
fa^{no} Ax^{E2}/Y ♂	wild type	nicked, thick veins, no vein gaps
fa^{no} spl Ax^{E2}/+ + + ♀	about same size and roughness as spl/spl	wild type

spl Ax^{E2}/+ + ♀	occasional roughness, overlapping wild type; slightly smaller than +.	occasional vein gaps
spl Ax^{E2}/fa^{no} ♀	like spl Ax^{E2}/+ +	
fa^{no} spl/Ax^{E2} ♀	wild type	occasional vein gaps

Bristle counts of various combinations of fa^{no}, spl, Ax^{E2}, and +, are tabulated on the following pages. The following crosses were used to generate the genotypes listed in the table.

LINE	CROSS
1	XX/Y ♀ x fa^{no} spl Ax^{E2}/Y ♂
2	XX/Y ♀ x spl Ax^{E2}/Y ♂
3	XX/Y ♀ x w^a fa^{no} Ax^{E2}/Y ♂
4,5	OR ♀ x fa^{no} spl Ax^{E2}/Y ♂
6,7	OR ♀ x spl Ax^{E2}/Y ♂
8,9	Ax^{E2}/Ax^{E2} ♀ x fa^{no} spl rb/Y ♂
10,11	OR ♀ x fa^{no} spl rb/Y ♂
12,13	OR ♀ x w^a fa^{no} Ax^{E2}/Y ♂

LINE	GENOTYPE	TEMP.	ORBITALS							OCELLARS			POSTVERTICALS		
			0	1	2	3	4	5	6	0	1	2	0	1	2
1	fa^{no} spl Ax^{E2}/Y	20.5°C	0	15	43	1	0	0	0	48	10	1	10	25	24
2	spl Ax^{E2}/Y	20.5°C	39	6	0	0	0	0	0	45	0	0	43	2	0
3	fa^{no} Ax^{E2}/Y	20.5°C	0	0	2	11	39	0	0	3	8	41	0	0	52
4	) fa^{no} spl Ax^{E2}/+ + +	20.5°C	0	0	29	57	141	36	10	7	78	178	1	10	262
5	)	29°C	0	1	7	30	58	1	0	97	0	0	10	44	43
6	) spl Ax^{E2}/+ +	20.5°C	0	1	50	77	38	9	0	73	68	34	5	42	128
7	)	29°C	3	5	56	15	7	0	0	86	0	0	52	29	5
8	) fa^{no} spl +/+ + Ax^{E2}	20.5°C	0	0	0	0	74	12	2	0	0	88	0	0	88
9	)	29°C	0	0	0	3	55	4	0	43	16	3	0	11	51
10	) fa^{no} spl/+ +	20.5°C	0	0	0	0	0	3	118	0	3	118	0	0	121
11	)	29°C	0	0	0	0	19	44	40	63	34	6	0	9	94
12	) fa^{no} Ax^{E2}/+ +	20.5°C	0	0	0	0	0	14	104	0	0	118	0	0	118
13	)	29°C	0	0	0	0	6	34	78	0	5	113	0	0	118

	DORSOCENTRALS					SCUTELLARS											WING VEIN GAPS								
LINE	0	1	2	3	4	TOTAL (lines 1,2 not scored separately)											0	1	2	3	4	5	6	7	8
						0	1	2	3	4															
1	6	16	21	13	3	8	19	16	15	1							59	0	0	0					
2	28	13	3	0	0	27	14	4	0	0							(gaps in L5 (100%); many in L4 also)								
						ANTERIOR			POSTERIOR																
						0	1	2	0	1	2														
3																	52	0	0	0					
4	0	0	0	5	268	9	88	176	0	2	271						272	0	0	0					
5	0	3	19	33	41	89	7	0	2	9	85						97	0	0	0					
6	0	0	0	2	173	19	48	108	0	1	174						156	(+18 with 1 or more gaps)							
7	0	6	32	40	8	77	9	0	20	39	27						28	(+57 with 1 or more gaps)							
8	0	0	0	0	88	0	0	88	0	0	88						86	1	1	0					
9	0	0	2	20	40	6	28	28	0	0	62						32	14	16	0					
10	0	0	0	0	121	0	0	121	0	0	121						119	0	0	0					
11	0	0	0	2	101	1	18	84	0	0	103						102	0	0	0					
12	0	0	0	1	117	0	1	117	0	0	118						115	0	0	0					
13	0	0	0	7	111	0	0	118	0	1	117						118	0	0	0					

APPENDIX 8

Counts of bristles and wing vein gaps in Ax^{E2} and OR flies

Data in lines 1-7 were taken from progeny of stock cultures (Ax^{E2}/Ax^{E2} ♀ x Ax^{E2}/Y ♂), raised in uncrowded conditions. Lines 8,9 were obtained from progeny of OR ♀ x Ax^{E2}/Y ♂ . Lines 10-12 were obtained from progeny of stock OR cultures, reared in un-crowded conditions (females raised at 29°C were not examined in detail, but there were no obvious differences compared to males). Note that males and females in lines 2,3 are sibs; males and females in lines 4-7 are sibs; females in lines 8,9 are sibs, males and females in lines 10,11 are sibs.

The data presented in this and the following appendices are the number of flies with a given number of bristles of a particu-lar type (or wing vein gaps, or nicked wings), followed by $\bar{x}$ ± one-sided 95% confidence intervals for the mean. See Methods and Materials for explanation of $\bar{x}$ and confidence intervals.

LINE	GENOTYPE		TEMP.	ORBITALS							
				0	1	2	3	4	5	6	$\bar{x}$
1	Ax^{E2}/Y	♂	20.5°C	-	-	-	-	-	-	-	-
2	Ax^{E2}/Y	♂	20.5°C	0	0	0	1	46	1	0	4.00 ± .06
3	Ax^{E2}/Ax^{E2}	♀	20.5°C	0	0	0	2	67	0	0	3.97 ± .04
4	Ax^{E2}/Y	♂	22°C	0	0	0	2	163	0	0	3.99 ± .02
5	Ax^{E2}/Ax^{E2}	♀	22°C	0	0	0	1	165	2	0	4.01 ± .02
6	Ax^{E2}/Y	♂	29°C	0	0	2	25	138	0	0	3.82 ± .05
7	Ax^{E2}/Ax^{E2}	♀	29°C	0	0	3	30	86	0	0	3.70 ± .08
8	$Ax^{E2}/$ +	♀	20.5°C	0	0	0	0	1	5	144	5.95 ± .04
9	$Ax^{E2}/$ +	♀	29°C	0	0	0	0	4	27	75	5.67 ± .09
10	OR	♂	22°C	0	0	0	0	0	0	109	6.00
11	OR	♀	22°C	0	0	0	0	0	0	139	6.00
12	OR	♂	29°C	0	0	0	0	0	4	138	5.97 ± .03

LINE	OCELLARS				POSTVERTICALS				VERTICALS					
	0	1	2	$\overline{x}$	0	1	2	$\overline{x}$	0	1	2	3	4	$\overline{x}$
1	0	1	73	1.99 ± .03	0	2	72	1.97 ± .04	-	-	-	-	-	-
2	0	1	47	1.98 ± .04	0	0	44	2.00	-	-	-	-	-	-
3	1	4	64	1.91 ± .07	0	5	64	1.93 ± .06	-	-	-	-	-	-
4	0	2	163	1.99 ± .02	0	2	163	1.99 ± .02	0	0	0	0	165	4.00
5	0	12	156	1.93 ± .04	0	1	167	1.99 ± .01	0	0	0	1	167	3.99 ± .01
6	52	74	39	0.92 ± .10	77	50	38	0.76 ± .10	0	0	0	6	159	3.96 ± .03
7	87	29	3	0.29 ± .08	90	25	4	0.28 ± .08	0	0	0	4	115	3.97 ± .03
8	0	0	150	2.00	0	0	150	2.00	0	0	0	0	150	4.00
9	0	12	93	1.89 ± .06	0	1	105	1.99 ± .02	0	0	0	1	105	3.99 ± .02
10	0	3	106	1.97 ± .03	0	0	109	2.00	0	0	0	0	109	4.00
11	0	3	136	1.98 ± .02	0	2	137	1.99 ± .02	0	0	0	0	139	4.00
12	0	1	141	1.99 ± .01	0	0	142	2.00	0	0	0	0	142	4.00

	DORSOCENTRALS						ANTERIOR SCUTELLARS				POSTERIOR SCUTELLARS			
LINE	0	1	2	3	4	$\overline{x}$	0	1	2	$\overline{x}$	0	1	2	$\overline{x}$
1	0	0	0	1	73	3.99 ± .03	0	0	74	2.00	0	0	74	2.00
2	0	0	0	0	47	4.00	–	–	–	–	–	–	–	–
3	0	0	0	1	68	3.99 ± .03	–	–	–	–	–	–	–	–
4	0	0	0	2	163	3.98 ± .02	0	0	165	2.00	0	0	165	2.00
5	0	0	0	0	168	4.00	0	0	168	2.00	0	0	168	2.00
6	0	3	12	43	107	3.54 ± .09	10	62	87	1.48 ± .08	0	10	149	1.94 ± .03
7	0	0	5	32	82	3.65 ± .09	6	23	90	1.71 ± .09	0	7	112	1.94 ± .04
8	0	0	0	0	150	4.00	0	0	150	2.00	0	0	150	2.00
9	0	0	2	10	94	3.87 ± .07	0	0	106	2.00	0	0	106	2.00
10	0	0	0	0	109	4.00	0	0	109	2.00	0	0	109	2.00
11	0	0	0	0	139	4.00	0	0	139	2.00	0	0	139	2.00
12	0	0	0	0	142	4.00	0	0	142	2.00	0	1	141	1.99 ± .01

	WING VEIN GAPS									
LINE	0	1	2	3	4	5	6	7	8	$\bar{x}$
1	–	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–	–
4	0	0	133	18	12	0	0	0	0	2.26 ± .08
5	0	0	130	23	13	0	0	0	0	2.30 ± .08
6	0	0	9	16	40	36	42	6	7	4.85 ± .19
7	0	0	15	15	73	6	1	1	0	3.69 ± .14
8	63	38	49	0	0	0	0	0	0	0.91 ± .12
9	17	21	62	3	2	0	0	0	0	1.54 ± .15
10	109	0	0	0	0	0	0	0	0	0.00
11	139	0	0	0	0	0	0	0	0	0.00
12	142	0	0	0	0	0	0	0	0	0.00

APPENDIX 9

Counts of bristles and wing vein gaps in Ax^{16172} flies

Data in lines 1-6 were taken from progeny of stock cultures (Ax^{16172}/Ax^{16172} ♀ x Ax^{16172}/Y ♂), raised in uncrowded conditions. Data in lines 7,8 were obtained from the cross: OR ♀ x Ax^{16172}/Y ♂ . Note that males and females in lines 1,2 are sibs; males and females in lines 3-6 are sibs; females in lines 7,8 are sibs. See Methods and Materials for explanation of $\bar{x}$ and confidence intervals, and see Appendix 8.

LINE	GENOTYPE	TEMP.	ORBITALS							
			0	1	2	3	4	5	6	$\bar{x}$
1	Ax^{16172}/Y ♂	20.5°C	16	33	50	10	0	0	0	1.50 ± .14
2	Ax^{16172}/Ax^{16172} ♀	20.5°C	26	35	22	5	0	0	0	1.07 ± .16
3	Ax^{16172}/Y ♂	22°C	23	61	77	21	2	0	0	1.55 ± .11
4	Ax^{16172}/Ax^{16172} ♀	22°C	54	59	34	5	1	0	0	0.95 ± .12
5	Ax^{16172}/Y ♂	29°C	0	1	21	33	42	0	0	3.20 ± .10
6	Ax^{16172}/Ax^{16172} ♀	29°C	0	2	32	22	7	0	0	2.54 ± .10
7	$Ax^{16172}/+$ ♀	22°C	0	0	0	9	95	15	3	4.10 ± .09
8	$Ax^{16172}/+$ ♀	29°C	0	0	0	0	73	13	1	4.17 ± .08

	OCELLARS				POSTVERTICALS				VERTICALS					
LINE	0	1	2	$\bar{x}$	0	1	2	$\bar{x}$	0	1	2	3	4	$\bar{x}$
1	109	0	0	0.00	109	0	0	0.00	0	0	1	20	88	3.80 ± .07
2	88	0	0	0.00	88	0	0	0.00	0	0	0	9	79	3.90 ± .06
3	183	1	0	0.01 ± .01	184	0	0	0.00	0	0	2	21	161	3.86 ± .05
4	153	0	0	0.00	153	0	0	0.00	0	0	1	12	140	3.91 ± .05
5	92	5	0	0.052 ±.026	97	0	0	0.00	0	0	1	6	90	3.92 ± .04
6	62	1	0	0.016 ±.017	63	0	0	0.00	0	0	0	4	59	3.94 ± .03
7	5	40	77	1.59 ± .09	43	54	25	0.85 ± .11	0	0	0	0	122	4.00
8	35	37	15	0.77 ± .14	44	33	10	0.61 ± .13	0	0	0	0	87	4.00

LINE	DORSOCENTRALS						ANTERIOR SCUTELLARS				POSTERIOR SCUTELLARS			
	0	1	2	3	4	$\bar{x}$	0	1	2	$\bar{x}$	0	1	2	$\bar{x}$
1	0	5	47	38	19	2.65 ± .14	44	48	12	0.75 ± .12	1	11	97	1.88 ± .06
2	2	9	44	25	8	2.32 ± .16	22	38	28	1.07 ± .14	0	10	78	1.89 ± .06
3	0	2	45	83	54	3.03 ± .10	79	81	24	0.70 ± .09	4	29	151	1.80 ± .06
4	1	9	51	53	39	2.78 ± .13	35	65	52	1.11 ± .10	2	10	141	1.91 ± .05
5	15	31	39	9	3	1.53 ± .12	73	22	2	0.27 ± .06	39	45	13	0.73 ± .08
6	25	30	7	1	0	0.79 ± .09	34	27	2	0.49 ± .07	19	27	17	0.97 ± .10
7	0	0	0	0	122	4.00	0	0	122	2.00	0	0	122	2.00
8	0	0	0	5	82	3.94 ± .05	2	6	79	1.89 ± .07	0	0	87	2.00

	WING VEIN GAPS								
LINE	0	1	2	3	4	5	6	7	8	$\bar{x}$
1	0	0	0	0	0	2	18	18	64	7.41 ± .15
2	0	0	0	0	5	3	6	4	4	5.95 ± .54
3	0	0	0	0	11	23	36	32	63	6.68 ± .17
4	0	0	0	2	82	22	17	1	0	4.46 ± .12
5	0	0	0	0	2	4	26	34	27	6.86 ± .17
6	0	0	0	0	9	17	29	0	0	5.36 ± .18
7	0	0	91	24	6	0	0	0	0	2.30 ± .09
8	0	0	12	30	45	0	0	0	0	3.38 ± .13

APPENDIX 10

Counts of bristles, wing vein gaps and wing nicking in $\underline{Ax^{9B2}}$

$\underline{flies}$

Data were obtained from progeny of the following crosses.

LINE	CROSS
1,2	$\underline{1(FM6)/Ax^{9B2}}$ ♀ x $\underline{Ax^{9B2}/Y}$ ♂
3-6	$\underline{M5/Ax^{9B2}}$ ♀ x $\underline{Ax^{9B2}/Y}$ ♂ (see Table 30)
7	$\underline{OR}$ ♀ x $\underline{Ax^{9B2}/Y}$ ♂

Note that $\underline{Ax^{9B2}/Y}$ males were obtained from stock cultures (XX,$\underline{y}$ $\underline{w}$ $\underline{f}/Y$ ♀ x $\underline{Ax^{9B2}/Y}$ ♂). Female parents (lines 1,2) were obtained from the cross $\underline{1(FM6)/Ax^{E1}}$ ♀ ($\underline{Ax^{E1}}$ stock) x stock $\underline{Ax^{9B2}/Y}$ ♂ . Female parents (lines 3-6) were obtained from the cross $\underline{M5/M5}$ ♀ x stock $\underline{Ax^{9B2}/Y}$ ♂ . Males and females in lines 1,2 are sibs; males and females in lines 3-6 are sibs. See Methods and Materials for explanation of $\bar{x}$ and confidence intervals, and see Appendix 8.

| | | | ORBITALS | | | | | | | |
LINE	GENOTYPE	TEMP.	0	1	2	3	4	5	6	$\bar{x}$
1	Ax^{9B2}/Y	♂ 20.5°C	0	0	0	0	0	2	87	5.98 ± .03
2	Ax^{9B2}/Ax^{9B2}	♀ 20.5°C	0	0	0	0	0	3	75	5.96 ± .04
3	Ax^{9B2}/Y	♂ 22°C	0	0	0	0	0	11	117	5.91 ± .05
4	Ax^{9B2}/Ax^{9B2}	♀ 22°C	0	0	0	0	0	3	93	5.97 ± .03
5	Ax^{9B2}/Y	♂ 29°C	0	0	0	0	0	3	36	5.92 ± .08
6	Ax^{9B2}/Ax^{9B2}	♀ 29°C	0	0	0	0	0	1	22	5.96 ± .08
7	Ax^{9B2}/+	♀ 22°C	0	0	0	1	12	31	63	5.46 ± .12

LINE	OCELLARS				POSTVERTICALS				VERTICALS					
	0	1	2	$\overline{x}$	0	1	2	$\overline{x}$	0	1	2	3	4	$\overline{x}$
1	88	1	0	0.01 ± .02	89	0	0	0.00	0	0	0	1	88	3.99 ± .02
2	75	2	1	0.05 ± .06	70	3	5	0.17 ± .10	0	0	0	2	76	3.97 ± .03
3	128	0	0	0.00	128	0	0	0.00	0	0	0	9	119	3.93 ± .04
4	95	1	0	0.01 ± .02	96	0	0	0.00	0	0	0	4	92	3.96 ± .04
5	36	3	0	0.08 ± .08	26	9	4	0.44 ± .19	0	0	0	1	38	3.97 ± .05
6	23	0	0	0.0	17	3	3	0.39 ± .27	0	0	0	0	23	4.0
7	95	12	0	0.11 ± .06	105	2	0	0.02 ± .03	0	0	0	0	107	4.00

LINE	DORSOCENTRALS						ANTERIOR SCUTELLARS				POSTERIOR SCUTELLARS			
	0	1	2	3	4	$\overline{x}$	0	1	2	$\overline{x}$	0	1	2	$\overline{x}$
1	8	30	50	1	0	1.49 ± .13	0	13	76	1.85 ± .07	0	2	87	1.98 ± .03
2	2	2	72	2	0	1.95 ± .08	0	7	71	1.91 ± .06	1	3	74	1.94 ± .06
3	28	60	40	0	0	1.09 ± .11	7	40	81	1.58 ± .09	1	19	108	1.84 ± .06
4	13	33	50	0	0	1.39 ± .13	4	24	68	1.67 ± .10	0	2	94	1.98 ± .03
5	0	10	21	7	0	1.92 ± .19	5	14	19	1.37 ± .20	3	12	24	1.54 ± .18
6	0	2	19	2	0	2.00 ± .16	2	8	13	1.48 ± .25	0	7	16	1.70 ± .18
7	0	0	25	37	45	3.19 ± .13	0	0	107	2.00	0	0	107	2.00

LINE	WING VEIN GAPS										WING NICKING			
	0	1	2	3	4	5	6	7	8	$\bar{x}$	0	1	2	$\bar{x}$
1														
2														
3	16	26	62	20	3	0	0	0	0	1.75 ± .14	127	0	0	0.00
4	9	20	58	6	0	0	0	0	0	1.66 ± .13	93	0	0	0.00
5	0	2	4	7	11	8	3	1	0	3.89 ± .41	35	1	0	0.03 ± .05
6	0	0	2	3	4	5	3	0	1	4.44 ± .65	19	1	0	0.05 ± .09
7	102	5	0	0	0	0	0	0	0	0.05 ± .04	107	0	0	0.00

APPENDIX 11

Counts of bristles, wing vein gaps, and wing nicking in
Ax^{E2}/N heterozygotes

The heterozygotes examined were generated by the following
crosses.

LINE	CROSS
1,2	$M5/N^8$ ♀ x Ax^{E2}/Y ♂
3,4	$M5/w^a$ N^{40} rb ♀ x Ax^{E2}/Y ♂
5,6	$M5/y$ w^a N^{103} ♀ x Ax^{E2}/Y ♂
7,8	$1(FM6)/w^a$ N^{g11} rb ♀ x Ax^{E2}/Y ♂
9,10	$M5/w^a$ N^{g11} rb ♀ x Ax^{E2}/Y ♂
11,12	Ax^{E2}/Ax^{E2} ♀ x w^a N^{g11} rb/B^S $w^+ \cdot Y$ ♂
13,14	$M5/w^a$ N^{Co} ♀ x Ax^{E2}/Y ♂

Note that the figures in Table 24 were calculated from
results of the crosses of M5/N females to Ax^{E2}/Y males. See
Methods and Materials for explanation of $\bar{x}$ and confidence inter-
vals, and see Appendix 8.

LINE	GENOTYPE	TEMP.	ORBITALS							
			0	1	2	3	4	5	6	$\bar{x}$
1	Ax^{E2}/N^8	22°C	0	0	0	0	3	25	158	5.83 ± .05
2		29°C	0	0	0	4	24	23	11	4.66 ± .18
3	Ax^{E2}/N^{40}	22°C	0	0	0	0	3	18	88	5.78 ± .08
4		29°C	0	0	0	1	15	3	9	4.71 ± .32
5	Ax^{E2}/N^{103}	22°C	0	0	0	5	171	12	2	4.06 ± .05
6		29°C	0	0	0	3	18	16	5	4.55 ± .21
7		20.5°C	0	0	0	0	4	13	107	5.83 ± .07
8		29°C	0	0	0	0	1	8	70	5.87 ± .07
9	Ax^{E2}/N^{g11}	22°C	0	0	0	0	7	33	117	5.70 ± .08
10		29°C	0	0	0	0	0	5	36	5.88 ± .09
11		20.5°	0	0	0	0	1	23	138	5.85 ± .05
12		29°C	0	0	0	0	0	5	28	5.85 ± .11
13	Ax^{E2}/N^{Co}	22°C	0	0	0	0	3	10	103	5.86 ± .07
14		29°C	0	0	0	1	4	14	10	5.14 ± .26

LINE	OCELLARS				POSTVERTICALS				VERTICALS					
	0	1	2	$\bar{x}$	0	1	2	$\bar{x}$	0	1	2	3	4	$\bar{x}$
1	0	0	186	2.00	0	2	184	1.99 ± .02	0	0	0	0	186	4.00
2	0	1	63	1.98 ± .03	0	0	64	2.00	0	0	0	2	62	3.97 ± .04
3	0	0	109	2.00	0	2	107	1.98 ± .02	0	0	0	0	109	4.00
4	0	3	25	1.89 ± .11	0	0	28	2.00	0	0	0	1	27	3.96 ± .06
5	0	0	190	2.00	0	0	190	2.00	0	0	0	0	190	4.00
6	0	1	41	1.98 ± .04	0	0	42	2.00	0	0	0	0	42	4.00
7	4	8	112	1.87 ± .07	0	0	124	2.00	-	-	-	-	-	-
8	0	2	77	1.97 ± .03	0	1	78	1.99 ± .02	-	-	-	-	-	-
9	0	5	152	1.97 ± .03	0	2	155	1.99 ± .02	0	0	0	0	157	4.00
10	0	0	41	2.00	0	0	41	2.00	0	0	0	0	41	4.00
11	3	14	145	1.88 ± .05	0	0	162	2.00	-	-	-	-	-	-
12	0	0	33	2.00	0	0	33	2.00	-	-	-	-	-	-
13	0	0	116	2.00	0	0	116	2.00	0	0	0	0	116	4.00
14	0	1	28	1.97 ± .06	0	0	29	2.00	0	0	0	0	29	4.00

	DORSOCENTRALS						ANTERIOR SCUTELLARS				POSTERIOR SCUTELLARS			
LINE	0	1	2	3	4	$\bar{x}$	0	1	2	$\bar{x}$	0	1	2	$\bar{x}$
1	0	0	0	0	186	4.00	0	1	185	1.99 ± .01	0	1	185	1.99 ± .01
2	0	0	0	1	63	3.98 ± .03	0	1	63	1.98 ± .03	0	0	64	2.00
3	0	0	0	0	109	4.00	0	1	108	1.99 ± .02	0	3	106	1.97 ± .03
4	0	0	0	0	28	4.00	0	0	28	2.00	0	0	28	2.00
5	0	0	0	0	190	4.00	0	0	190	2.00	0	0	190	2.00
6	0	0	0	2	40	3.95 ± .06	0	1	41	1.98 ± .04	0	0	42	2.00
7	0	0	0	0	124	4.00	0	3	121	1.98 ± .03	1	2	121	1.97 ± .04
8	0	0	0	22	57	3.72 ± .09	0	3	76	1.96 ± .04	1	4	74	1.92 ± .06
9	0	0	0	0	157	4.00	0	1	156	1.99 ± .01	0	1	156	1.99 ± .01
10	0	0	0	1	40	3.98 ± .05	0	1	40	1.98 ± .05	0	1	40	1.98 ± .05
11	0	0	0	2	160	3.99 ± .02	1	0	161	1.99 ± .02	0	1	161	1.99 ± .01
12	0	0	0	6	27	3.82 ± .12	0	0	33	2.00	0	0	33	2.00
13	0	0	0	0	116	4.00	0	1	115	1.99 ± .02	0	0	116	2.00
14	0	0	0	0	29	4.00	0	2	27	1.93 ± .09	0	0	29	2.00

	WING VEIN GAPS										WING NICKING			
LINE	0	1	2	3	4	5	6	7	8	$\bar{x}$	0	1	2	$\bar{x}$
1	125	18	22	0	0	0	0	0	0	0.38 ± .10	0	0	178	2.00
2	36	9	12	0	0	0	0	0	0	0.58 ± .19	0	0	60	2.00
3	75	6	4	0	0	0	0	0	0	0.16 ± .09	0	0	95	2.00
4	22	1	1	0	0	0	0	0	0	0.13 ± .16	0	0	25	2.00
5	0	0	96	6	1	0	0	0	0	2.08 ± .05	12	22	82	1.60 ± .11
6	9	6	6	0	1	0	0	0	0	1.00 ± .41	0	0	28	2.00
7	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9	26	23	86	0	0	0	0	0	0	1.44 ± .12	112	21	2	0.19 ± .06
10	23	3	5	2	0	0	0	0	0	0.58 ± .30	0	1	34	1.97 ± .05
11	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13	93	8	0	0	0	0	0	0	0	0.08 ± .05	0	1	106	1.99 ± .02
14	23	1	2	0	0	0	0	0	0	0.19 ± .20	0	0	26	2.00

APPENDIX 12

Counts of bristles, wing vein gaps, and wing nicking in Ax^{16172}/N heterozygotes

The heterozygotes examined were generated by the following crosses.

LINE	CROSS
1,2	M5/N[8] ♀ x Ax^{16172}/Y ♂
3,4	M5/w^a N[40] rb ♀ x Ax^{16172}/Y ♂
5	d149,y Hw m^2/w^a N[40] rb ♀ x Ax^{16172}/Y ♂
6	M5/y w^a N[103] ♀ x Ax^{16172}/Y ♂
7,8	l(FM6)/w^a Ng[11] rb ♀ x Ax^{16172}/Y ♂
9	M5/w^a Ng[11] rb ♀ x Ax^{16172}/Y ♂
10,11	M5/w^a N[Co] ♀ x Ax^{16172}/Y ♂

Note that the figures in Table 25 were calculated from results of the crosses of M5/N females to Ax^{E2}/Y males. See Methods and Materials for explanation of $\bar{x}$ and confidence intervals, and see Appendix 8.

| | | | ORBITALS | | | | | | | |
LINE	GENOTYPE	TEMP.	0	1	2	3	4	5	6	$\overline{x}$
1	Ax^{16172}/N^8	22°C	0	0	0	1	158	28	6	4.20 ± .06
2		29°C	0	0	0	6	15	9	0	4.10 ± .23
3	Ax^{16172}/N^{40}	22°C	0	0	0	2	112	37	13	4.37 ± .09
4		29°C	0	0	0	0	0	1	0	–
5		20.5°C	0	0	0	1	134	21	1	4.14 ± .05
6	Ax^{16172}/N^{103}	22°C	1	1	10	9	41	0	0	3.42 ± .20
7	Ax^{16172}/N^{g11}	20.5°C	0	0	0	9	130	9	0	4.00 ± .05
8		29°C	0	0	0	0	1	2	0	4.7
9		22°C	0	0	2	21	83	0	0	3.76 ± .08
10	Ax^{16172}/N^{Co}	22°C	0	0	0	2	40	49	34	4.92 ± .12
11		29°C	0	0	0	0	1	0	0	–

| | OCELLARS | | | | POSTVERTICALS | | | | VERTICALS | | | | | |
LINE	0	1	2	$\bar{x}$	0	1	2	$\bar{x}$	0	1	2	3	4	$\bar{x}$
1	0	1	192	1.99 ± .01	0	0	193	2.00	0	0	0	3	190	3.98 ± .02
2	0	0	30	2.00	0	2	28	1.93 ± .08	0	0	0	3	27	3.90 ± .10
3	0	0	164	2.00	0	2	162	1.99 ± .02	0	0	0	1	163	3.99 ± .01
4	0	0	1	–	0	0	1	–	0	0	0	0	1	–
5	0	2	161	1.99 ± .02	0	4	159	1.98 ± .02	0	0	0	0	163	4.00
6	50	10	2	0.23 ± .11	15	20	27	1.19 ± .18	0	0	0	0	62	4.00
7	5	19	124	1.80 ± .07	62	59	25	0.75 ± .10	0	0	0	1	147	3.99 ± .01
8	0	2	1	1.3	0	0	3	2.0	0	0	0	0	3	4.0
9	3	9	94	1.86 ± .07	47	39	20	0.75 ± .13	0	0	0	0	106	4.00
10	0	1	124	1.99 ± .02	0	2	123	1.98 ± .02	0	0	0	0	125	4.00
11	0	0	1	–	0	0	1	–	0	0	0	0	1	–

LINE	DORSOCENTRALS						ANTERIOR SCUTELLARS				POSTERIOR SCUTELLARS			
	0	1	2	3	4	$\bar{x}$	0	1	2	$\bar{x}$	0	1	2	$\bar{x}$
1	0	0	0	0	193	4.00	0	0	193	2.00	0	2	191	1.99 ± .02
2	0	0	0	1	29	3.97 ± .06	0	1	29	1.97 ± .06	0	0	30	2.00
3	0	0	0	1	163	3.99 ± .01	0	2	162	1.99 ± .02	0	2	162	1.99 ± .02
4	0	0	0	0	1	-	0	0	1	-	0	0	1	-
5	0	0	0	0	163	4.00	0	1	162	1.99 ± .01	0	0	163	2.00
6	2	3	20	18	19	2.79 ± .23	0	4	58	1.94 ± .06	0	1	61	1.98 ± .03
7	0	0	4	32	112	3.73 ± .07	4	26	118	1.77 ± .07	0	0	148	2.00
8	0	0	0	1	2	3.7	0	0	3	2.0	0	0	3	2.0
9	0	0	10	21	75	3.61 ± .11	8	26	72	1.60 ± .11	0	0	106	2.00
10	0	0	0	2	123	3.98 ± .02	0	0	125	2.00	0	0	125	2.00
11	0	0	0	0	1	-	0	0	1	-	0	0	1	-

	WING VEIN GAPS										WING NICKING			
LINE	0	1	2	3	4	5	6	7	8	$\bar{x}$	0	1	2	$\bar{x}$
1	0	0	167	5	0	0	0	0	0	2.03 ± .03	0	0	184	2.00
2	0	1	27	1	0	0	0	0	0	2.00 ± .09	0	0	30	2.00
3	2	2	108	4	0	0	0	0	0	1.98 ± .06	0	0	133	2.00
4	-	-	-	-	-	-	-	-	-	-	0	0	1	-
5	-	-	-	-	-	-	-	-	-	-	0	0	163	2.00
6	0	0	30	7	1	0	0	0	0	2.24 ± .14	4	6	28	1.63 ± .19
7	0	0	0	8	28	39	59	0	0	5.11 ± .14	129	3	2	0.05 ± .04
8	1	2	0	0	0	0	0	0	0	0.7	0	0	3	2.0
9	0	0	25	9	23	2	1	0	0	3.08 ± .23	56	4	0	0.07 ± .06
10	0	9	93	0	0	0	0	0	0	1.91 ± .05	0	1	115	1.99 ± .02
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-

APPENDIX 13

Counts of bristles, wing vein gaps, and wing nicking in Ax^{9B2}/N heterozygotes

The heterozygotes examined were generated by the following crosses.

LINE	CROSS
1,2	$M5/N$ ♀ x Ax^{9B2}/Y ♂
3	$dl49,y$ Hw m^2/w^a N^{40} rb ♀ x Ax^{9B2}/Y ♂
4,5	$M5/w^a$ N^{40} rb ♀ x Ax^{9B2}/Y ♂
6,7	$M5/y$ w^a N^{103} ♀ x Ax^{9B2}/Y ♂
8,9	$1(FM6)/w^a$ N^{g11} rb ♀ x Ax^{9B2}/Y ♂
10,11	$M5/w^a$ N^{g11} rb ♀ x Ax^{9B2}/Y ♂
12,13	$M5/w^a$ N^{Co} ♀ x Ax^{9B2}/Y ♂

Note that the figures in Table 26 were calculated from results of the crosses of $M5/N$ females to Ax^{9B2}/Y males. See Methods and Materials for explanation of $\bar{x}$ and confidence intervals, and see Appendix 8.

LINE	GENOTYPE	TEMP.	ORBITALS							
			0	1	2	3	4	5	6	$\bar{x}$
1	Ax^{9B2}/N^{8}	22°C	0	0	0	0	0	3	112	5.97 ± .03
2		29°C	0	0	0	1	5	23	37	5.45 ± .15
3	Ax^{9B2}/N^{40}	20.5°C	0	0	0	0	1	3	207	5.98 ± .02
4		22°C	0	0	0	0	0	6	95	5.94 ± .04
5		29°C	0	0	0	0	12	20	25	5.23 ± .18
6	Ax^{9B2}/N^{103}	22°C	0	0	0	0	93	28	18	4.46 ± .10
7		29°C	0	0	0	0	0	2	5	5.7 ± .4
8	Ax^{9B2}/N^{g11}	20.5°C	0	0	0	0	5	34	83	5.64 ± .09
9		29°C	0	0	0	0	1	4	15	5.70 ± .23
10		22°C	0	0	0	4	27	36	49	5.12 ± .14
11		29°C	0	0	0	0	0	4	21	5.84 ± .13
12	Ax^{9B2}/N^{Co}	22°C	0	0	0	0	0	3	78	5.96 ± .04
13		29°C	0	0	0	1	0	11	24	5.61 ± .19

LINE	OCELLARS				POSTVERTICALS				VERTICALS					
	0	1	2	$\bar{x}$	0	1	2	$\bar{x}$	0	1	2	3	4	$\bar{x}$
1	91	23	1	0.22 ± .07	7	27	81	1.64 ± .10	0	0	0	2	113	3.98 ± .02
2	47	16	3	0.33 ± .12	31	25	10	0.68 ± .16	0	0	2	22	42	3.61 ± .12
3	160	41	10	0.29 ± .07	5	24	182	1.84 ± .05	0	0	0	0	211	4.00
4	86	14	1	0.16 ± .07	8	22	71	1.62 ± .11	0	0	0	0	101	4.00
5	46	10	1	0.21 ± .11	16	24	17	1.02 ± .18	0	0	0	0	57	4.00
6	25	53	61	1.26 ± .11	5	12	122	1.84 ± .07	0	0	0	0	139	4.00
7	7	0	0	0.0	0	3	4	1.6 ± .5	0	0	0	0	7	4.00
8	121	1	0	0.01 ± .02	119	3	0	0.02 ± .03	0	0	0	0	122	4.00
9	19	1	0	0.05 ± .09	0	3	17	1.85 ± .15	0	0	0	0	20	4.00
10	116	0	0	0.00	115	0	1	0.02 ± .03	0	0	0	1	115	3.99 ± .02
11	24	1	0	0.04 ± .07	0	4	21	1.84 ± .13	0	0	0	0	25	4.00
12	15	31	35	1.25 ± .14	4	19	58	1.67 ± .11	0	0	0	0	81	4.00
13	31	4	0	0.11 ± .10	4	18	14	1.28 ± .19	0	0	0	0	36	4.00

LINE	DORSOCENTRALS						ANTERIOR SCUTELLARS				POSTERIOR SCUTELLARS			
	0	1	2	3	4	$\bar{x}$	0	1	2	$\bar{x}$	0	1	2	$\bar{x}$
1	0	0	26	35	54	3.24 ± .13	0	0	115	2.00	0	0	115	2.00
2	0	0	47	16	3	2.33 ± .12	0	1	65	1.98 ± .03	0	0	66	2.00
3	0	2	30	84	95	3.29 ± .09	0	1	210	2.00 ± .01	1	1	209	1.99 ± .02
4	0	0	18	37	46	3.28 ± .13	0	1	100	1.99 ± .02	0	2	99	1.98 ± .03
5	0	0	36	16	5	2.46 ± .15	0	0	57	2.00	0	0	57	2.00
6	0	0	34	33	72	3.27 ± .12	0	0	139	2.00	1	5	133	1.95 ± .04
7	0	0	0	0	7	4.0	0	0	7	2.0	0	0	7	2.0
8	1	13	105	3	0	1.90 ± .06	90	25	7	0.32 ± .09	0	3	119	1.98 ± .03
9	0	1	15	2	2	2.25 ± .29	0	0	20	2.00	0	0	20	2.00
10	0	5	104	7	0	2.02 ± .05	63	32	21	0.64 ± .12	0	3	113	1.97 ± .03
11	0	2	19	3	1	2.12 ± .21	0	0	25	2.00	0	2	23	1.92 ± .10
12	0	0	27	26	28	3.01 ± .16	0	0	81	2.00	0	2	79	1.98 ± .03
13	0	0	35	1	0	2.03 ± .05	0	1	35	1.97 ± .05	0	0	36	2.00

	WING VEIN GAPS										WING NICKING			
LINE	0	1	2	3	4	5	6	7	8	$\bar{x}$	0	1	2	$\bar{x}$
1	40	23	51	1	0	0	0	0	0	1.11 ± .15	113	2	0	0.02 ± .01
2	3	12	13	10	12	7	6	0	0	2.97 ± .37	11	11	42	1.48 ± .17
3	62	68	80	0	0	0	0	0	0	1.09 ± .10	177	23	10	0.20 ± .06
4	62	19	16	0	0	0	0	0	0	0.53 ± .13	78	18	2	0.22 ± .08
5	24	18	12	1	0	0	0	0	0	0.82 ± .20	0	0	57	2.00
6	0	1	38	36	61	2	0	0	0	3.18 ± .13	129	8	1	0.072 ±.041
7	2	0	0	0	0	0	0	0	0	–	0	0	7	2.0
8	0	0	32	19	11	2	2	0	0	2.83 ± .16	66	0	0	0.00
9	6	3	5	4	0	1	0	0	0	1.58 ± .59	11	5	3	0.58 ± .34
10	0	2	35	10	9	0	0	0	0	2.46 ± .19	56	0	0	0.00
11	13	7	4	0	0	0	0	0	0	0.63 ± .28	15	8	1	0.42 ± .21
12	33	20	24	0	0	0	0	0	0	0.88 ± .17	80	0	0	0.00
13	2	13	20	0	0	0	0	0	0	1.51 ± .18	13	12	11	0.94 ± .24

APPENDIX 14

Counts of bristles and wing vein gaps in heteroallelic

Ax^x/Ax^y combinations

The data on the following pages were obtained from the following crosses.

LINE	CROSS
1,3	$\underline{1(FM6)/Ax^{E1}}$ ♀ x $\underline{Ax^{E2}/Y}$ ♂
2	$\underline{Ax^{E2}/Ax^{E2}}$ ♀ x $\underline{Ax^{E1}/B^S}$ $\underline{w^+ \cdot Y}$ ♂
4	$\underline{1(FM6)/Ax^{E1}}$ ♀ x $\underline{Ax^{16172}/Y}$ ♂
5,6	$\underline{1(FM6)/Ax^{E1}}$ ♀ x $\underline{Ax^{9B2}/Y}$ ♂
7	$\underline{Ax^{16172}/Ax^{16172}}$ ♀ x $\underline{Ax^{E2}/Y}$ ♂
8	$\underline{Ax^{E2}/Ax^{E2}}$ ♀ x $\underline{Ax^{16172}/Y}$ ♂
9	$\underline{w^a}$ $\underline{Ax^{E2}}$ $\underline{rb}/+$ $\underline{Ax^{16172}}$ $+$ ♀ x $\underline{w^a}$ $\underline{Ax^{9B2}}$ $\underline{rb}/Y$ ♂

The wings of all the genotypes except $\underline{Ax^{E2}/Ax^{16172}}$ (lines 7,8) were too deformed to score accurately for wing vein gaps. The data for $\underline{Ax^{E2}/Ax^{16172}}$ females are tabulated below.

LINE	NUMBER OF FLIES WITH GIVEN NUMBER OF WING VEIN GAPS									
	0	1	2	3	4	5	6	7	8	$\bar{x}$
7	0	0	0	0	41	11	7	2	1	4.56 ± .20
8	0	0	1	3	35	15	8	3	1	4.59 ± .23

See Methods and Materials for explanation of $\bar{x}$ and confidence intervals, and see Appendix 8.

| | | | ORBITALS | | | | | | | |
LINE	GENOTYPE	TEMP.	0	1	2	3	4	5	6	$\bar{x}$
1		20.5°C	13	0	0	0	0	0	0)	
2	Ax^{E1}/Ax^{E2}	20.5°C	10	0	0	0	0	0	0)	0.04 ± .07
3		20.5°C	2	1	0	0	0	0	0)	
4	Ax^{E1}/Ax^{16172}	20.5°C	2	0	0	0	0	0	0	–
5	Ax^{E1}/Ax^{9B2}	20.5°C	0	0	17	43	50	6	0	3.39 ± .10
6		20.5°C	0	0	20	35	33	10	0	3.34 ± .16
7	Ax^{E2}/Ax^{16172}	20.5°C	0	4	24	26	12	0	0	2.70 ± .18
8		20.5°C	0	6	21	30	11	0	0	2.68 ± .18
9	Ax^{E2}/Ax^{9B2}	22°C	28	0	0	0	0	0	0	0.0

LINE	OCELLARS				POSTVERTICALS				VERTICALS					
	0	1	2	$\bar{x}$	0	1	2	$\bar{x}$	0	1	2	3	4	$\bar{x}$
1	13	0	0		13	0	0		0	0	0	0	13)	
2	10	0	0	0.0	10	0	0	0.0	0	0	0	0	10)	4.0
3	3	0	0		3	0	0		0	0	0	0	3)	
4	2	0	0	–	2	0	0	–	0	0	0	1	1	–
5	116	0	0	0.00	116	0	0	0.00	0	0	0	0	116	4.00
6	98	0	0	0.00	98	0	0	0.00	0	0	0	0	98	4.00
7	66	0	0	0.00	66	0	0	0.00	–	–	–	–	–	–
8	68	0	0	0.00	68	0	0	0.00	0	0	1	1	66	3.96 ± .06
9	28	0	0	0.0	28	0	0	0.0	1	6	10	8	3	2.21 ± .34

	DORSOCENTRALS						ANTERIOR SCUTELLARS				POSTERIOR SCUTELLARS			
LINE	0	1	2	3	4	$\bar{x}$	0	1	2	$\bar{x}$	0	1	2	$\bar{x}$
1	11	2	0	0	0		13	0	0		0	0	13)	
2	6	3	1	0	0	0.31 ± .19	10	0	0	0.0	0	1	9)	1.96 ± .07
3	2	1	0	0	0		3	0	0		0	0	3)	
4	2	0	0	0	0	-	2	0	0	-	2	0	0	-
5	30	37	49	0	0	1.16 ± .13	97	16	3	0.19 ± .07	4	28	84	1.69 ± .09
6	21	35	42	0	0	1.21 ± .14	90	8	0	0.08 ± .05	1	21	76	1.77 ± .08
7	0	0	26	26	14	2.82 ± .16	1	16	49	1.73 ± .10	0	1	65	1.98 ± .03
8	0	1	6	15	46	3.56 ± .15	0	11	57	1.84 ± .09	0	0	68	2.00
9	28	0	0	0	0	0.0	16	11	1	0.46 ± .19	2	8	18	1.57 ± .21