NEGATIVE COMPLEMENTATION AT THE NOTCH LOCUS OF *DROSOPHILA MELANOGASTER*[1,2]

GEOFFREY G. FOSTER[3]

Department of Zoology, The University of British Columbia, Vancouver, B.C., Canada

Manuscript received October 18, 1974
Revised copy received April 10, 1975

ABSTRACT

Four Abruptex alleles (Ax^{E1}, Ax^{E2}, $Ax^{s^{E2}}$, and Ax^{16172}) have been mapped within the Notch locus. Based on their visible phenotypes and their interactions with one another and with *N* mutations, the *Ax* alleles can be divided into two groups. Heterozygous combinations of members of the same group are intermediate in phenotype compared to the respective homozygotes, whereas heterozygotes of *Ax* alleles from different groups exhibit negative heterosis, being much less viable and more extremely mutant than either homozygote. It is suggested that the Notch locus is a multi-functional regulator ("integrator") gene, whose product possesses both "repressor" and "activator" functions for the processes it regulates.

FEW genes have remained as enigmatic in the face of intensive and prolonged study as the Notch locus of *Drosophila melanogaster*. The importance of this locus in development has inspired much research (POULSON 1939a, b, 1940, 1945, 1968; WELSHONS 1958a, b, 1965, 1971; WELSHONS and VON HALLE 1962; FOSTER and SUZUKI 1970; FOSTER 1973a) and speculation (WELSHONS 1965; WRIGHT 1970; FOSTER 1973a, b) about its mode of action and function, but all this activity has not yet produced a satisfying picture of what the Notch locus actually does.

The reason the Notch locus is so poorly understood may be because the most common type of mutation at this locus probably tells us the least about the locus. The key may lie, not with the *Notch* (*N*) mutations, which define the locus, but with a phenotypically dissimilar group of mutant alleles, the Abruptex (*Ax*) mutations. The relationship between *N* and *Ax* mutations has remained obscure over the years. Although a close relationship was suspected more than three decades ago (SLYZINSKA 1938), until recently there was room for doubt as to whether *N* and *Ax* mutations were really different variants of the same gene, or represented sites in adjacent loci (MOHR 1932; SCHULTZ, in MORGAN, SCHULTZ and CURRY 1941; LEFEVRE, RATTY and HANKS 1953).

[1] This research was supported by grants to D. T. SUZUKI by the National Research Council of Canada (A-1764) and the National Cancer Institute of Canada (6051).

[2] Taken in part from material submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Genetics at The University of British Columbia.

[3] Recipient of a Killam Predoctoral Fellowship. Present address: Division of Entomology, CSIRO, Canberra, Australia, 2601.

The characteristic Abruptex phenotype consists of interrupted wing venation and a reduction in the number of bristles, unlike the Notch mutations, which have serrated wing tips and a generally increased number of bristles. The original Abruptex allele, Ax^{28a}, is viable in hemizygous and homozygous condition, and when heterozygous with deficiencies of the Notch locus, both the Notch phenotype and the bristle and wing-vein phenotypes of Ax^{28a} are suppressed (MOHR 1932; LEFEVRE, RATTY and HANKS 1953). The suppression of the Notch phenotype, and the appearance of an extra band in the Notch-locus region in Ax^{28a} polytene chromosome preparations, led to the hypothesis that Ax^{28a} was a duplication of the Notch locus, the Abruptex phenotype resulting from a position effect (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 (extra wing vein) and not an Abruptex phenotype, LEFEVRE, RATTY and HANKS (1953) suggested that Ax^{28a} was not a duplication. The mapping of two lethal Ax alleles within the Notch locus finally showed that Notch and Abruptex were really the same gene, but did not resolve the question of the nature of Ax^{28a}, since this mutation could still have been a duplication of the Notch locus, with one of the elements occupied by an otherwise lethal Ax allele (WELSHONS 1971). Data to be presented in the present paper lessen the need to describe Ax^{28a} as a duplication, since they show that three other nonlethal Ax alleles map within the Notch locus.

Even with the controversy as to their identity set aside, however, the Abruptex mutants promise to provide entertainment for geneticists for some time to come. Independent discoveries in two laboratories (FOSTER 1971, 1972; PORTIN and RUOHONEN 1972) have suggested that the Abruptex alleles are a most unusual group of mutations. While heterozygous combinations of some homozygous viable Ax alleles are viable and of intermediate phenotype compared to the homozygotes, certain of the heterozygotes exhibit marked negative heterosis and are either completely inviable or are much more extremely mutant than either parent. The present report describes the results of invstigations on four Ax mutations, and their interactions with several N mutations and with one another. In addition to describing the system of negative heterosis the data show that: (1) N mutations generally suppress the phenotypes of Ax mutations, and (2) not all Ax mutations suppress the wing nicking of N mutations.

MATERIALS AND METHODS

1. Genetic strains and mutations

Consult LINDSLEY and GRELL (1968) for descriptions of non-Notch locus mutations, and for further information on mutants (below) marked with an asterisk.

A. Notch locus mutations

Ax^{E1}—Ethylmethane sulfonate (EMS)-induced (FOSTER 1971); inviable as hemi- and homozygote; polytene chromosomes normal; see RESULTS.

Ax^{E2}—EMS-induced (FOSTER 1971); viable and fertile as hemi- and homozygote; polytene chromosomes normal; see RESULTS.

Ax^{16172}—EMS-induced in the laboratory of E. LEWIS (WELSHONS, personal communication); viable and fertile in hemi- and homozygote; polytene chromosomes normal; see RESULTS.

Ax^{9B2}—EMS-induced in the laboratory of G. LEFEVRE, JR. (personal communication); hemi- and homozygous viable; homozygous females poorly fertile; polytene chromosomes normal; see RESULTS.

fa^{no}—(facet-notchoid)—recessive viable; notched-wing phenotype; lethal when heterozygous with most *N* alleles.*

N^{Co}—weak Notch phenotype; gives Confluens phenotype when in combination with duplications of N^+.*

N^8—strong Notch phenotype; cytological deficiency for Notch and adjacent loci.*

$N^{264\text{-}40}$—(N^{40})—strong Notch phenotype.*

$N^{264\text{-}10^{3}}$—(N^{10^3})—temperature-sensitive (ts) Notch phenotype (FOSTER 1973a).*

N^{60g11}—(N^{g11})—ts Notch and rough-eye phenotype (FOSTER and SUZUKI 1970).*

spl—(split)—disrupted eye facet phenotype.*

B. Balancer chromosome

*l(FM6)—FM6** balancer chromosome carrying EMS-induced lethal mutation(s).

C. Genetic background of strains

Ax^{E1} and Ax^{E2} were originally induced in Oregon-R *X* chromosomes. Ax^{16172} and Ax^{9B2} are of uncertain lineage. Ax^{E2} and Ax^{16172} were maintained as homozygous stocks, while Ax^{9B2} was maintained by crossing males to compound-*X* females. Ax^{E1} was maintained as the following stock: $l(FM6)/Ax^{E1} \times Ax^{E1}/B^s w^+Y$. Except where noted in RESULTS, no attempt was made to make the genetic backgrounds of the different *Ax* stocks co-isogenic,

The N^{Co} strain was derived from a $w^a N^{Co} rb$ strain obtained from W. J. WELSHONS. The original stock was found to carry a bb^l allele and an enhancer of Notch which mapped between *rb* and *cv* (FOSTER 1971). The offending portions of *X* chromosome were replaced with Oregon-R material.

N^{g11}, N^{40}, and N^{10^3} were maintained in the following stocks: $\widehat{XX}.y\ w\ f$ ♀♀ × $w^a N^{40}$-$rb;Dp^{51b7}$ ♂♂; $\widehat{XX}.y\ w\ f$ ♀♀ × $y\ w^a N^{10^3};Dp^{51b7}$ ♂♂; $l(FM6)/w^a N^{g11} rb$ ♀♀ × w^a-$N^{g11} rb/B^s w^+Y$ ♂♂.

The autosomes of these stocks were derived from Oregon-R_{369}, but the *X* chromosomes were not made co-isogenic.

N^8 was maintained in the stock:

$N^8/In(1)d149,y\ Hw\ m^2$ ♀♀ × $In(1)d149,y\ Hw\ m^2$ ♂♂, and was not made coisogenic with the other *N* stocks.

II. Procedure for bristle, wing-vein gap and wing-nick counts

Quantitative data on the phenotypic expression of *Ax* homozygotes and various heterozygous *Ax/Ax* and *Ax/N* combinations were obtained through counts of individuals with 0, 1, 2 (etc.) of a given bristle or gaps in the longitudinal veins. Likewise *N*/+ and *N/Ax* heterozygotes were scored for wingtip nicking. Samples of flies for these counts were obtained by rearing the progeny of 3-10 pairs of parents (3–5 day oviposition period) in quarter-pint bottles containing Drosophila medium, at various temperatures as specified in RESULTS.

RESULTS

Abruptex mutant phenotypes

Ax^{E2}, Ax^{9B2}, and Ax^{16172} are viable alleles, whereas Ax^{E1} is poorly viable as a hemi- and homozygote. Similarly, Ax^{E2}, Ax^{9B2}, and Ax^{16172} are generally viable when heterozygous with *N* mutations, although Ax^{16172} is ts in this regard (FOSTER 1973a). Ax^{E1}, on the other hand, is inviable when heterozygous with most *N* mutations, except for the ts N^{10^3} (FOSTER 1973a), with which it is viable at 22° but not at 29°.

Although a direct comparison between the *Ax* mutations must be viewed with caution, because the strains were not co-isogenic, their phenotypes suggest that

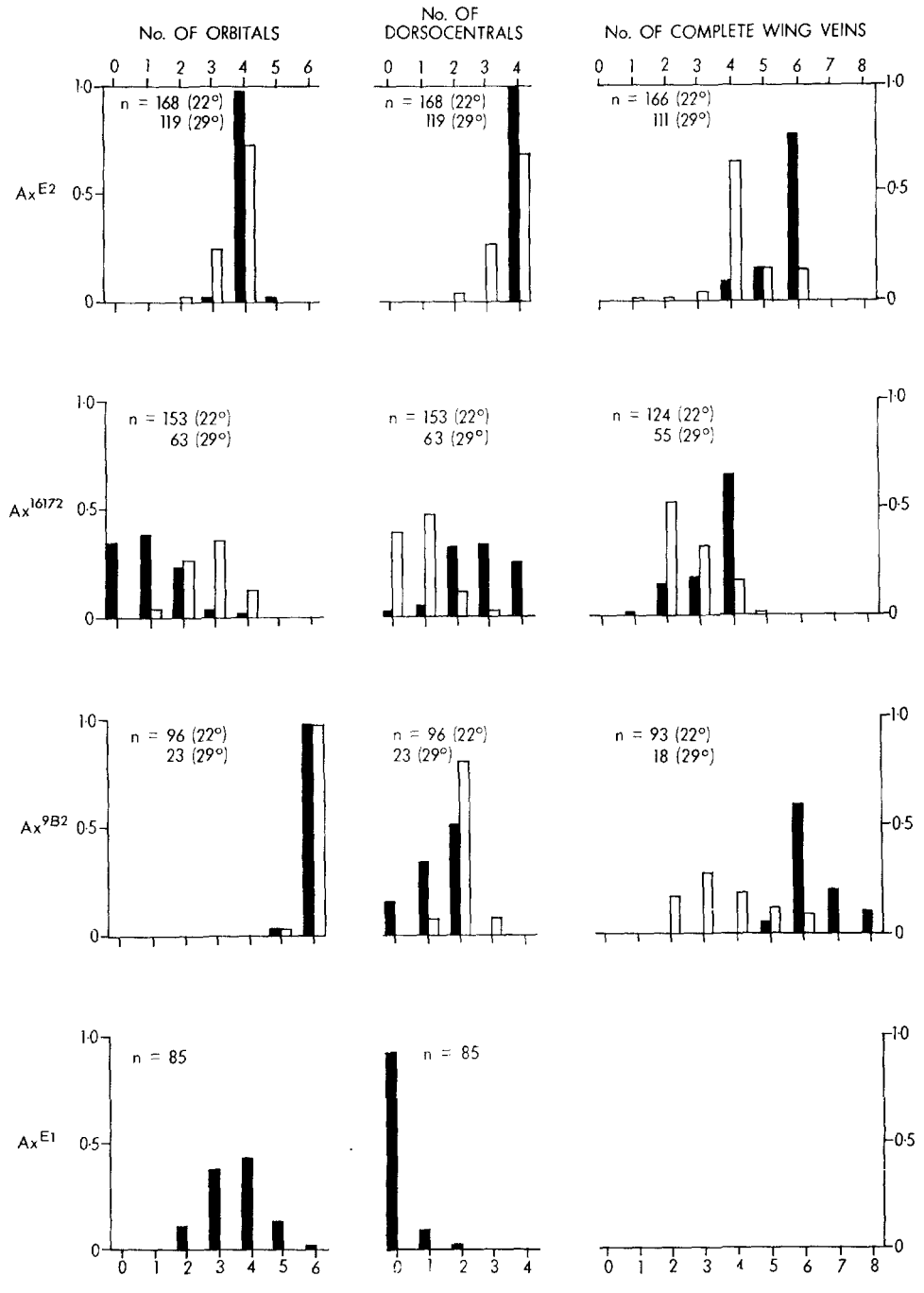

FIGURE 1.—Abruptex bristle and wing vein phenotypes. Data are for homozygous Ax^{E2}, Ax^{16172}, and Ax^{9B2} females raised at 22° or 29°, and Ax^{E1} males raised at 20.5°. ■ 22° or 20.5°, □ 29°.

qualitative as well as quantitative differences exist between the different alleles. Bristle and wing-vein-gap counts made on homozygous females (hemizygous males in the case of Ax^{E1}) are summarized in Figure 1. Comparing the viable alleles at 22° (1) both Ax^{E2} and Ax^{16172} have fully penetrant orbital bristle and wing-vein-gap phenotypes, whereas Ax^{9B2} does not; (2) Ax^{9B2} is fully penetrant for a dorsocentral-bristle phenotype, unlike Ax^{E2} and Ax^{16172}. At 29°, the counts differ quantitatively in several cases compared to the 22° values, but the overall pattern remains the same. These observations suggest that Ax^{9B2} differs qualitatively from Ax^{E2} and Ax^{16172}, whereas there is no indication from these data or from counts of ocellar, postvertical, or scutellar bristles (FOSTER 1971), that Ax^{E2} and Ax^{16172} differ from one another qualitatively, except perhaps in their pattern of temperature sensitivity.

Examination of the viable Ax strains raised at 22° or 29° (Figure 1), reveals that each is ts. However, the three strains differ in their patterns of temperature sensitivity. Only Ax^{E2} is internally consistent, all the characters being more mutant at one temperature (29°) than at the other (22°). Ax^{16172} and Ax^{9B2}, on the other hand, are "schizophenic", since in some respects they are more mutant at 29° than at 22°, while in others they are less mutant. Thus the relationship between a given Ax allele and its various forms of phenotypic expression does not appear to be a simple one.

Because Ax^{E1} is inviable, this allele cannot be compared easily with the recessive alleles. At 20.5°, most $Ax^{E1}/+$ females possess gaps in the L5 wing veins, and some in the L4 veins, the number of ocellar and postvertical bristles are reduced compared to wild type, while the orbitals and dorsocentrals are only silhgtly affected (FOSTER 1971). The few Ax^{E1} hemi- and homozygotes which do manage to eclose usually become mired in the food medium. By laying the bottles on their sides, a few Ax^{E1} males were obtained (Figure 1). Data are the pooled observations on Ax^{E1}/Y progeny which survived in five crosses performed for other purposes (FOSTER 1971). In addition to the phenotypes depicted, these males entirely lacked ocellar, postvertical, and anterior scutellar bristles, and they had deformed legs and wings with the latter showing little evidence of venation. Such males usually died within three days of emergence, and made no attempt at copulation with virgin females, although they produced motile sperm.

Interactions among the Abruptex mutations

Viability data for all the Ax combinations examined are summarized in Table 1. The time of death of the inviable genotypes is mainly pupal, with some individuals dying as newly eclosed adults. Note that heterozygotes of the semilethal allele Ax^{E1} with Ax^{9B2} are viable, whereas those with Ax^{E2} and Ax^{16172} are inviable. Thus among these four alleles there appear to be two complementary groups, within which heterozygotes are viable, and between which they are inviable.

Further information concerning the two types of Abruptexes was obtained from phenotypic examination of the viable Ax^{x}/Ax^{y} combinations and a few

TABLE 1

Summary of viability of various heterozygous and homozygous combinations of Ax *alleles.*

	Ax^{E1}	Ax^{9B2}	Ax^{E2}	Ax^{16172}
Ax^{E1}	I*	V	I	I
Ax^{9B2}		V	I	I
Ax^{E2}			V	V
Ax^{16172}				V

* I=inviable or semilethal.
V=viable.

surviving "break-through" individuals from the inviable genotypes (Figure 2). The appearance of surviving Ax^{E1}/Ax^{E2} individuals is generally more mutant than in Ax^{E1} or Ax^{E2} hemi- and homozygotes, as is shown in the orbital bristle (Figure 2) and wing-vein phenotypes (see below), although there were exceptions to this: (1) the dorsocentral bristles of Ax^{E1}/Ax^{E2} females (Figure 2) did not differ significantly from those of Ax^{E1} males (Figure 1), and (2) the posterior scutellar bristles of Ax^{E1}/Ax^{E2} females were wild-type like Ax^{E2}, whereas Ax^{E1} males had considerably reduced numbers (FOSTER 1971). (It should be noted that males and females from a single stock frequently differ significantly —females sometimes more mutant, sometimes less—in specific Ax phenotypes (FOSTER 1971), so that the above exceptions may be spurious). Like Ax^{E1}/Ax^{E2}, the Ax^{E2}/Ax^{9B2} females which managed to eclose were much more extremely mutant than either homozygote. In contrast to the above genotypes, the viable combinations Ax^{E2}/Ax^{16172} and Ax^{E1}/Ax^{9B2} were phenotypically intermediate between the respective homozygous (or hemizygous) genotypes. Detailed vein-gap counts were not made of the Ax^{E1}/Ax^{9B2} females, but it was noted that wing structure and venation were also intermediate between Ax^{E1} males and Ax^{9B2} females. Only two Ax^{E1}/Ax^{16172} females were ever observed, and these possessed no bristles except the verticals (usually not affected by Ax mutations), i.e., this genotype was also more mutant than the respective hemi- and homozygotes (Figure 1). In addition, the meso- and metathoracic legs of the inviable genotypes are often deformed, thoracic microchaetae are much sparser than in homozygotes, and the wings are so deformed that venation cannot be easily examined.

The above data show that the expression of visible mutant phenotypes of the heterozygous Abruptex combinations is correlated with the viability of those combinations. The inviable genotypes all exhibit more extremely mutant phenotypes than those of the individual homo- or hemizygotes, whereas the viable genotypes exhibit intermediate phenotypes. Thus the negative heterosis manifested most obviously at the level of viability is also apparent in terms of visible phenotypes.

Although the above data were obtained with non-coisogenic strains, a later check using Ax^{E2}, Ax^{16172}, and Ax^{9B2} strains made coisogenic for Oregon-R auto-

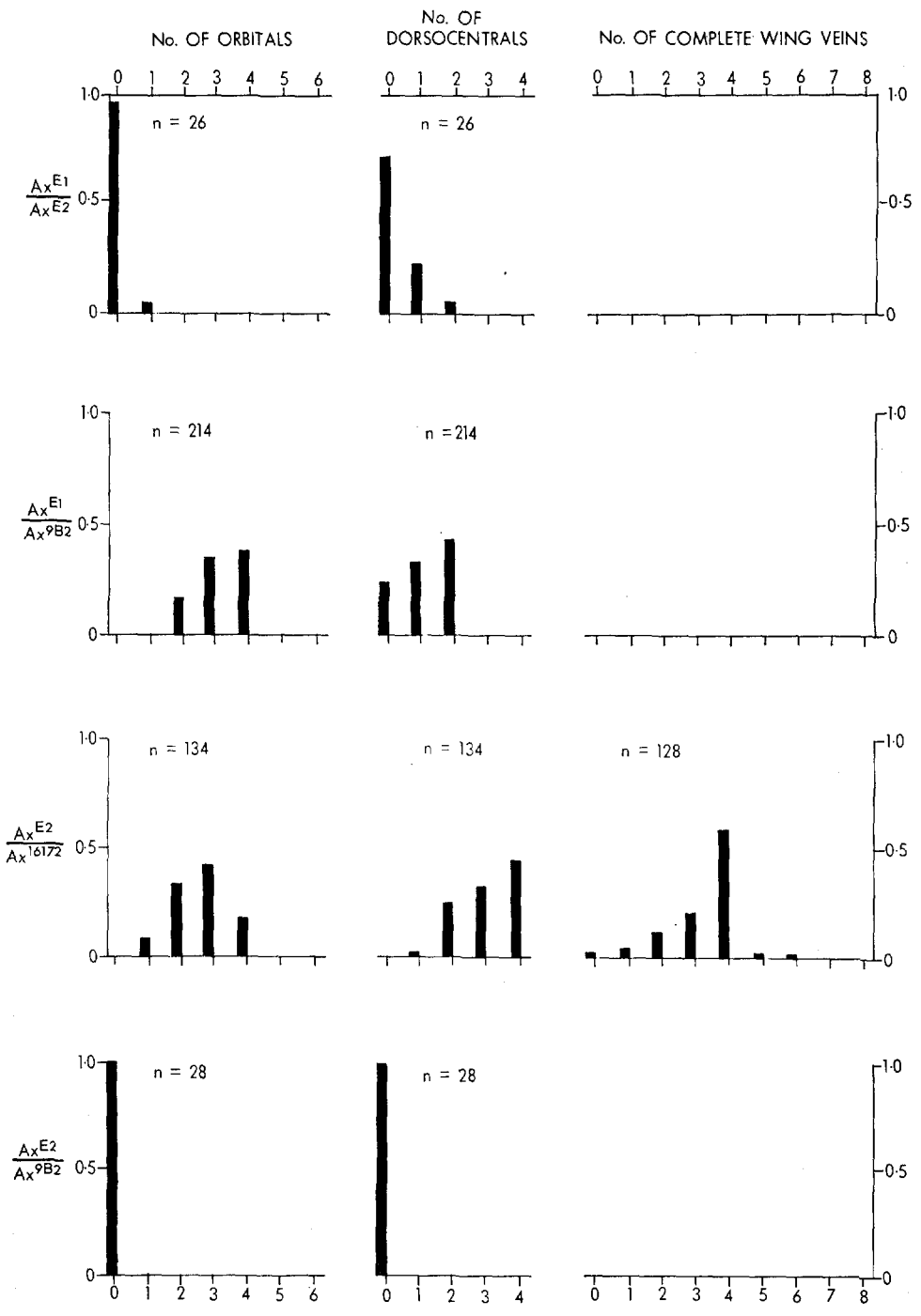

FIGURE 2.—Bristle and wing-vein phenotypes of heteroalleic *Ax* combinations. All flies were raised at 20.5° except Ax^{E2}/Ax^{9B2} (22°).

somes and probably part of the *X* chromosome also (FOSTER 1971) confirmed the lethality of Ax^{E2}/Ax^{9B2} and Ax^{16172}/Ax^{9B2}, the viability of Ax^{E2}/Ax^{16172}, and the different phenotypic patterns of Ax^{E2} and Ax^{9B2}. The results of this exercise also suggested that the original Ax^{16172} stock contained one or more codifiers of *Ax* expression, since the new line had a much more mutant appearance than that described in Figure 1. Nevertheless, this does not detract from the overall pattern of the results.

Interactions between Notch and Apruptex mutations

In order to determine whether mutual suppression of phenotypic expression, as in N^{8}/Ax^{28a} (MOHR 1932), is a general property of *Ax/N* heterozygotes, the phenotypes of the viable *Ax/N* combinations were examined. The data obtained from *trans* heterozygous combinations of Ax^{E2}, Ax^{16172}, and Ax^{9B2} with five different *N* mutations are summarized in Figures 3-5. Comparison with Figure 1 shows that where homozygous *Ax* caused marked bristle loss or wing-vein gapping, most of the heterozygous combinations with *N* had bristle and wing-vein-gap frequencies closer to wild type. There do appear to be several exceptions, particularly with N^{103} and N^{g11}; however, analysis of the N^{103} and N^{g11} exceptions tends to confirm the rule that *N* mutations suppress Abruptex phenotypes, and the others appear to be trival. Dealing with the latter exceptions first, at 29° heterozygotes of Ax^{9B2} with N^{8}, N^{40}, and N^{Co}, had fewer orbital bristles (Figure 5) than Ax^{9B2} *homozygotes* (Figure 1), but similar orbital bristle loss is observed in the respective *N*/+ heterozygotes raised at 29°. Turning to N^{103} and N^{g11}, there are several instances at 22° where these alleles either do not suppress Abruptex phenotypes, or the suppression is markedly less than that of N^{8} or N^{40} (Figures 3, 5). Significantly, both N^{103} and N^{g11} are ts, with relatively mild or atypical expression of the Notch phenotype at 22° (FOSTER and SUZUKI 1970; FOSTER 1973a). Usually, N^{103} and N^{g11} suppress *Ax* phenotypes at 29°, the temperature at which both exhibit Notch phenotypes (Figures 3, 5). Thus it appears to be a general rule that *N* mutations suppress Abruptex phenotypes. In passing it can be noted that Ax^{E1}/N^{103} heterozygotes raised at 22° also expressed much milder Apruptex phenotypes than Ax^{E1} hemizygotes (FOSTER 1971), but since N^{103} does not behave as a deficiency at 22° (FOSTER 1973a), it is questionable whether this suppression is due to the action of the *N* mutation, as such, or to significant levels of wild-type activity in the N^{103} gene product.

In contrast to the general suppression of Abruptex phenotypes by *N* mutations, the action of *Ax* mutations on Notch phenotypes is not uniform. Like Ax^{28a} (MOHR 1932), Ax^{9B2} suppresses the wing nicking caused by *N* mutations, although it appears to be ts in this regard (Table 2). On the other hand, both Ax^{E2} and Ax^{16172} increase the wing-nicking frequencies of the weaker *N* alleles (Table 2), and markedly enhance the extent of nicking caused by the stronger alleles, N^{8} and N^{40}. Although Ax^{E1}/N^{103} females reared at 22° usually have deformed wings, some were sufficiently extended to permit examination, and showed less nicking than in $N^{103}/+$ females at this temperature. In addition, the Ax^{E1}/N^{40} breakthroughs recovered in the mapping of Ax^{E1} (Table 3) had less wing nicking than $N^{40}/+$. The facts that Ax^{E1} and Ax^{9B2} possess normal salivary

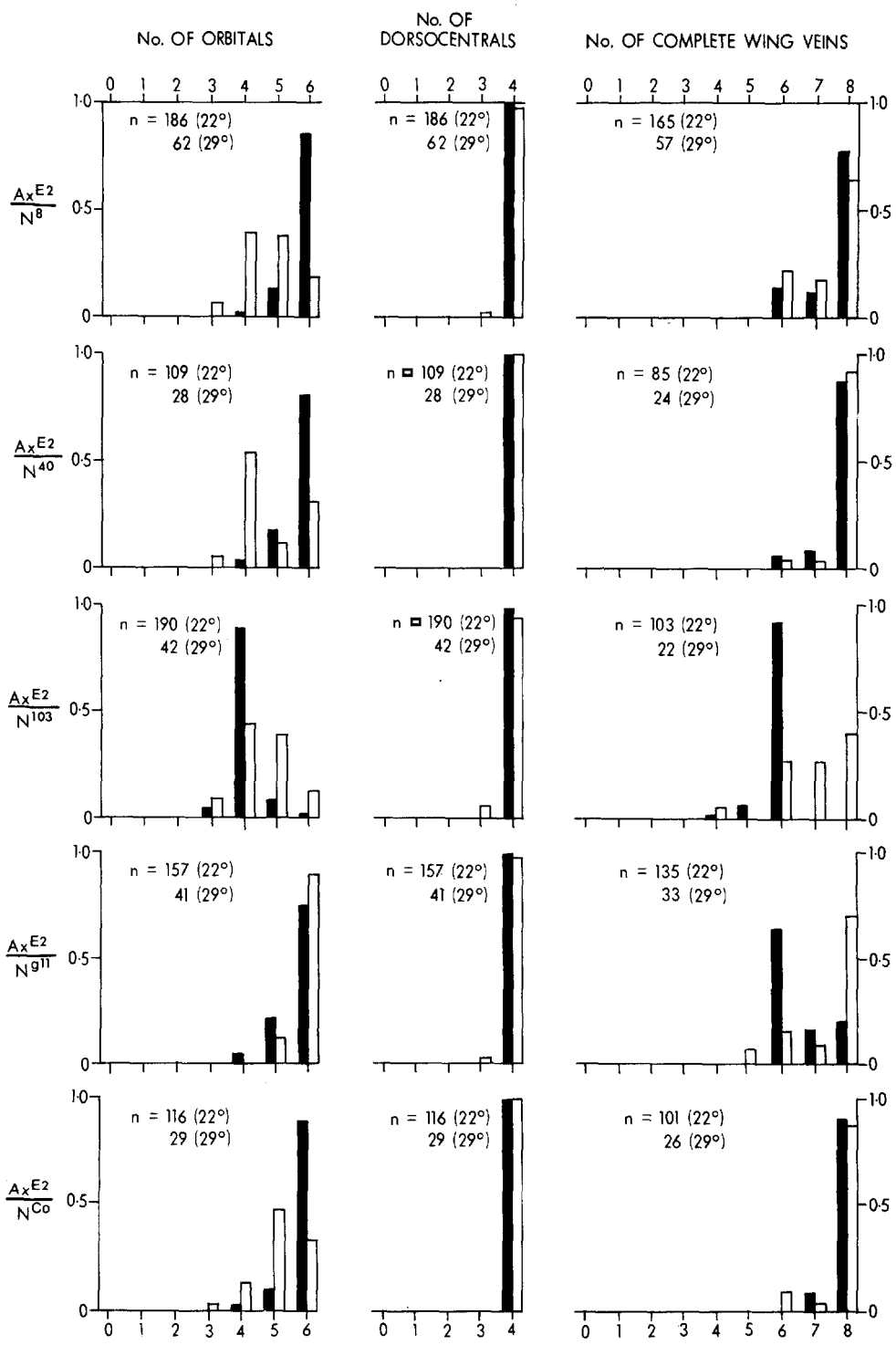

FIGURE 3.—Bristle and wing-vein phenotypes of Ax^{E2}/N heterozygotes. ■ 22°, □ 29°.

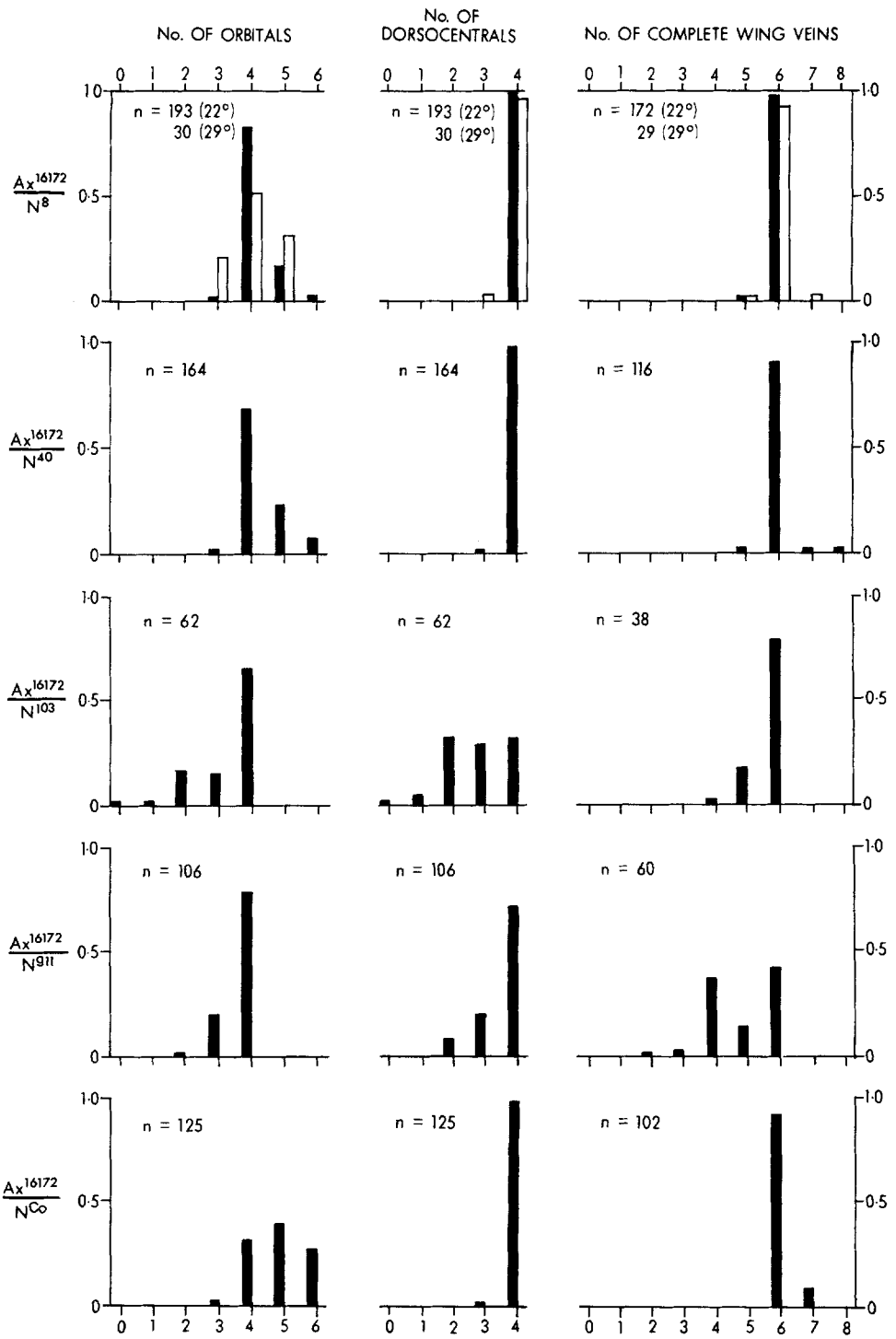

FIGURE 4.—Bristle and wing-vein phenotypes of Ax^{16172}/N heterozygotes. ■ 22° □ 29°.

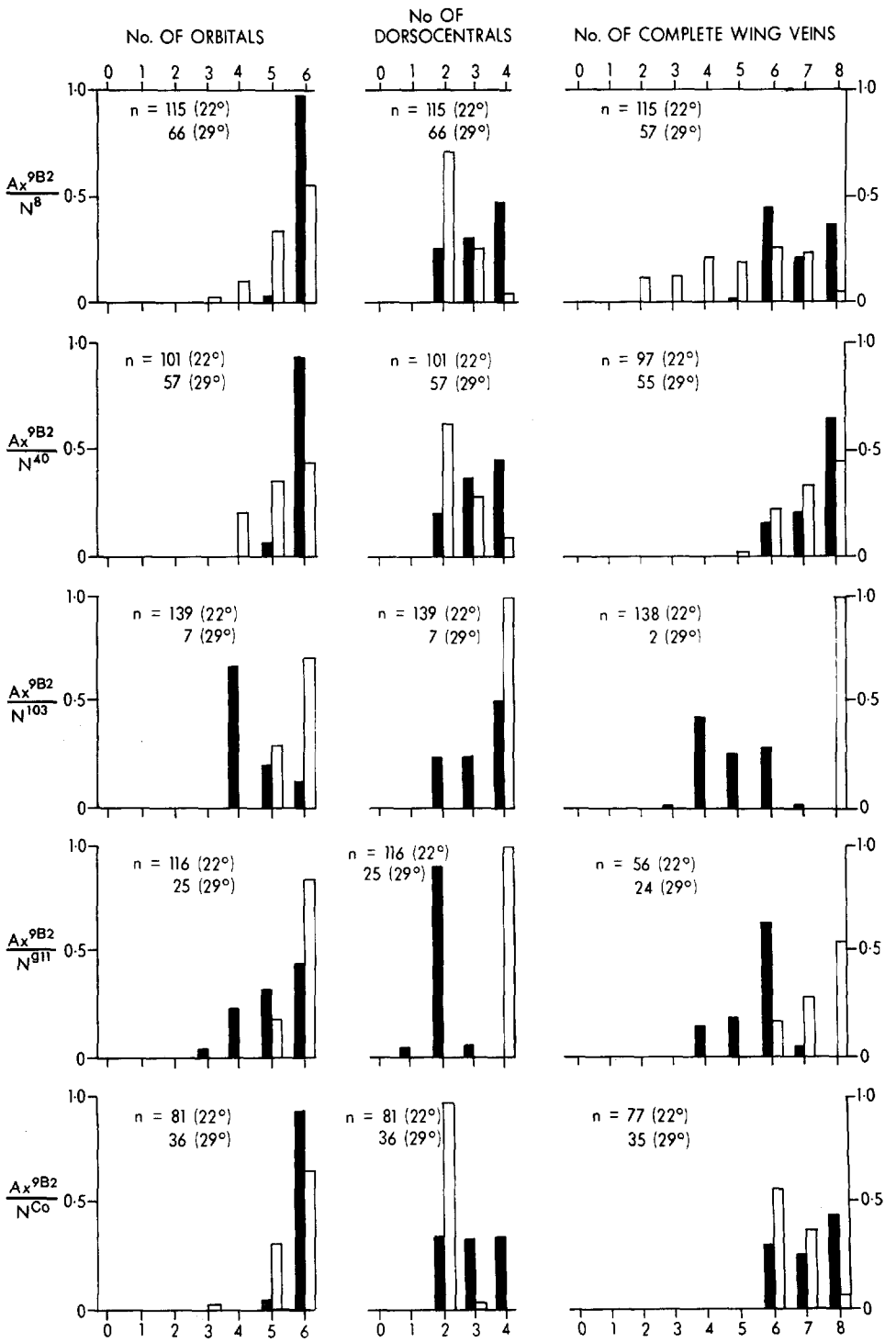

FIGURE 5.—Bristle and wing-vein phenotypes of Ax^{9B2}/N heterozygotes. ■ 22°, □ 29°.

TABLE 2

Wing nicking in N/Ax *heterozygotes.**

N alleles		*Ax* alleles			
		Ax^{+}	Ax^{E2}	Ax^{16172}	Ax^{9B2}
N^{8}	22°	100	100	100	2
	29°	—	100	100	83
N^{40}	22°	100	100	100	20
	29°	—	100	—	100
N^{103}	22°	82	90	90	7
	29°	100	100	—	100
N^{g11}	22°	1	17	7	0
	29°	76	100	—	37
N^{Co}	22°	42	100	100	0
	29°	—	100	—	64

* Percent of total individuals with nicks in one or both wingtips.

chromosome banding and map within the Notch locus (see below) suggest that duplication of the locus is not a necessary condition for suppression of wing nicking by an *Ax* mutant.

If the action of *Ax* alleles on Notch phenotypes is correlated with their interactions with one another (Table 2, Figure 2), the interesting fact emerges that heterozygotes within either the suppressor or the enhancer group (i.e., Ax^{E1}/Ax^{9B2}, and Ax^{E2}/Ax^{16172}) are viable and have intermediate phenotypes, while all heterozygotes *between* the two groups exhibited extreme negative heterosis resulting in inviability in all the cases examined. Thus the evidence from direct phenotypic comparisons, complementation tests, and interactions with *N* alleles, indicates that there are two functionally distinct groups of *Ax* mutations.

Genetic mapping of the Ax mutations

Ax^{E1}—In the mapping of Ax^{E1}, the inviability of Ax^{E1}/N and fa^{no}/N was used

TABLE 3

Results of cross for the genetic localization of Ax^{E1}

Series*	Number of male progeny	Phenotype of surviving female progeny				
		w^{+} *spl rb*	w^{+} rb^{+}	B^{s}	N/Ax^{E1}	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; 3–6-day broods, total oviposition period 24–28 days.

Series 2: 40 cultures in quarter-pint bottles, 1–5 females per bottle, 3-day broods for 6 broods.

to set up a selective system for recombinants. In the cross w^a fa^{no} *spl* *rb*/+ Ax^{E1} + ♀♀ × w^a N^{40} *rb*/B^s w^+ *Y* ♂♂, the only surviving female progeny should be nondisjunctants (B^s) or fa^{no+} Ax^+ recombinants. The results of two such crosses are presented in Table 3. There were appreciable numbers of surviving Ax^{E1}/*N* and fa^{no} *spl*/*N* females, both of which were sterile and very weak and could be recognized easily. The w^+ rb^+ class of progeny proved not to be recombinants, but were in fact w^a fa^{no} *spl* *rb*/w^a N^{40} *rb*/w^+ Ax^{E1} rb^+ triploids (FOSTER 1971), indicating that fa^{no} and Ax^{E1} had enough complementary activity between them to rescue the genotype from death. The w^+ *spl* *rb* progeny represent true crossovers between fa^{no} and Ax^{E1}, and position 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*; however, if Ax^{E1} is to the right of *spl*; the two mutants must be very closely linked, since 14 crossovers between fa^{no} and Ax^{E1} were recovered (0.05%), but none between Ax^{E1} and *spl*. This frequency of recombination between fa^{no} and Ax^{E1} is greater than the distance between fa^{no} and *spl* (0.03%) recorded by WELSHONS (1958b), but this cannot be used to order Ax^{E1} and *spl*, since genetic background and other conditions were likely different in the two investigations.

Ax^{E2}—In order to map Ax^{E2}, all of the male progeny of w^a fa^{no} *spl* *rb*/w^+ Ax^{E2} rb^+ females were scored for their visible phenotypes. The results (Table 4) place Ax^{E2} 0.01 unit to the right of *spl*, while the fa^{no}–*spl* distance was 0.5 unit. This observation, combined with the fact that *spl* lies approximately equidistant between fa^{no} and *nd* in WELSHONS' (1965) map, suggests strongly that Ax^{E2} lies within the limits of the Notch locus.

Ax^{16172}—In the attempt to map Ax^{16172} with respect to Ax^{E2}, use was made of the inviability of Ax^{E2}/Ax^{9B2} and Ax^{16172}/Ax^{9B2}. In the cross w^a Ax^{E2} *rb*/+ Ax^{16172} + ♀♀ × w^a Ax^{9B2} *rb* ♂♂, the only surviving female progeny should be Ax^+ recombinants or nondisjunctants, whereas all males should survive. In a single experiment of this kind, 12,873 males and no recombinant females were observed. Crossing over in the adjacent w^a–*Ax* and *Ax*–*rb* regions as observed in

TABLE 4

Results of crosses for the genetic mapping of Ax^{E2}

Genotype	Cross* 1	Cross* 2
w^a fa^{no} + Ax^{E2} +	3	3
+ + *spl* + *rb*	2	7
w^a fa^{no} *spl* Ax^{E2} +	0	2
+ + + + *rb*	1	0
w^a fa^{no} + + *rb*	0	1
+ fa^{no} + Ax^{E2} +	1	0
TOTAL ♂ PROGENY	17,689	17,560

* 1. w^a fa^{no} *spl* + *rb*/+ + + Ax^{E2} + ♀♀ × *y* w^a ♂♂
2. w^a fa^{no} *spl* + *rb*/+ + + Ax^{E2} + ♀♀ × w^a fa^{no} *spl* *rb* ♂♂

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.

TABLE 5

Results of crosses for the genetic mapping of Ax^{9B2}

	Cross*	
	1	2
Total ♂ progeny	3200	2259
$Ax - N$ crossovers	3 (+ + rb)	2 (w^a + rb)

* Progeny of crosses:
1. $w^a\ N^{40}\ rb/+\ Ax^{9B2} + ♀♀ \times w^a fa^{no}\ spl\ Ax^{E2}\ rb\ ♂♂$
2. $w^a\ N^{Co} +/+\ Ax^{9B2}\ rb\ ♀♀ \times w^a\ fa^{no}\ spl\ Ax^{E2}\ rb\ ♂♂$

Cultures were incubated at 22° 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 periods varied from 10–15 days.

the males occurred with normal frequencies. This indicates that Ax^{E2} and Ax^{16172} must be situated close to one another, and that Ax^{16172} is probably within the Notch locus.

Ax^{9B2}—Two crosses were used to map Ax^{9B2}, both of the format N/Ax^{9B2} ♀♀ × fa^{no} *spl rb* ♂♂ (plus appropriate flanking markers). The only expected survivors are Ax^{9B2} males, nondisjunctant females, and N^+ Ax^+ recombinants of both sexes. The results (Table 5) place Ax^{9B2} between N^{40} and N^{Co}, i.e., within the Notch locus. The observed frequencies of crossing over (0.05% between N^{40} and Ax^{9B2}, and 0.04% between N^{Co} and Ax^{9B2}, and not 0.09% for each region as erroneously stated in FOSTER 1971) were rather high compared with the total N^{40}–N^{Co} distance of 0.03%–0.04% reported by WELSHONS (1958b), although recombination with flanking markers occurred in normal frequencies. The high values in the present data could reflect reduced viability of Ax^{9B2} males compared to Ax^+, since some cultures were overcrowded; in addition, the possible action on Ax^{9B2} of genetic modifiers in both of the N stocks and in the w^a fa^{no} *spl* Ax^{E2} *rb* stock, cannot be ignored. Regardless of these frequencies, the available crossover data and cytological information, 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 Notch locus.

DISCUSSION

The present investigation has consisted primarily of an examination of the properties of a variety of homozygous and heterozygous combinations of Notch-locus mutations. At the outset, therefore, it should be emphasized that the mode of action and function of the Notch locus may be several steps removed from the phenotypic manifestation of its genotypic state. Having said this, it will be recognized that much of what will be said on the following pages is at best speculative. Because of the genetic complexity of the Notch locus (e.g., WELSHONS 1965), the pleiotropic nature of its mutant alleles, and its involvement at many stages of development (SHELLENBARGER 1971; FOSTER 1973a), it

is tempting to assume that its function is of a regulatory nature. For the purposes of illustration this has been assumed in the following discussion, although other possible roles of the Notch locus cannot be ruled out.

Since the strains used in the investigation were generally not coisogenic, and (as noted in RESULTS from time to time) certain minor differences could therefore have resulted from genetic background variability, the discussion will be confined to those observations which appear to be repeatable in different genetic backgrounds.

As a group, the Abruptex mutations can be readily defined phenotypically. Within this group, allele-specific differences in the patterns of bristle loss, their effects on N mutations and interactions among the *Ax* mutations themselves, indicate that there are at least two distinct subgroups of *Ax* mutations. Nevertheless, the fact that all five *N* alleles tested tended to suppress the phenotypes of both kinds of *Ax* allele (Figure 3-5) emphasizes that the *Ax*'s are essentially a single class of mutations.

Since the *Ax* mutations behave differently from *N* deficiencies, obviously they are not amorphic alleles. The data suggest 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^{8} heterozygotes cannot be attributed to intracistronic complementation, whereby a hybrid multimer restored some wild-type activity to the gene product, since in the case of N^{8} there is no *N*-allele product to participate in multimer 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 of the *N* allele could diminish the bristle loss and wing-vein gapping caused by the hypermorphic *Ax* allele. This explanation was advanced by MULLER (1932) to explain the reduced expression of *N* and *Ax* phenotypes in Ax^{2Sa}/N^{8} heterozygotes. Similarly, from the suppression of Ax^{E2} and Ax^{16172} phenotypes by *N* mutations, these two alleles could be said to be hypermorphic. By the wing-nicking criterion, however, Ax^{E2} and Ax^{16172} are *hypo*morphic, since nicking is enhanced by these alleles. Moreover, this model cannot accommodate the observation that *Ax* phenotypes are suppressed in *Ax*/+ females compared to homozygotes, since they should be *enhanced* if the *Ax* alleles were truly hypermorphic. Similarly, it can be inferred that the *Ax* mutations are not neomorphic so far as their effect on bristle numbers is concerned, since in this case *N* deficiencies or point mutations should not suppress the *Ax* mutant phenotypes. It seems apparent that some rather special assumptions may be warranted in order to reconcile these seemingly conflicting observations.

One way out of the morass described above 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 systems in bacteria contain elements which possess both repressor and activator functions (GAREN and ECHOLS 1962a,b; ENGLESBERG *et al.* 1965). If we can stretch the analogy as far

as the Notch locus of *D. melanogaster* (without making any assumptions as to whether the regulation is transcriptional or translational), i.e., that different forms of the wild-type Notch-locus product tend to oppose or balance one another in the developmental processes they influence, then the suppression of *Ax* alleles by both N^+ and *N* alleles could be accommodated. To borrow the regulator-gene terminology, we may suppose that the bristle loss caused by *Ax* mutations is due to a "repression" of some part of the bristle-forming mechanism. As diagrammed in Figure 6, this could come about (a) by a mutation causing an activator function to become non- or hypo-functional (A^-), or (b) by a mutation causing a repressor function to become hyper-functional (R^H). It is also possible that other changes, such as hyper-activator or hyper-repressor, could occur, and that a single mutation could affect both R and A functions, either in the same or in different ways. For example, *Ax* mutations may be hypo- or amorphic (A^-) and/or hyper- or antimorphic (R^H) changes in part of the Notch-locus product. An amorphic *N* mutation could be represented as A^-R^-. The hypothesis that mutation of N^+ to *Ax* increases the net repression of bristle-forming activity is not inconsistent with the observation that *N* deficiencies usually cause increased bristle numbers. Mutation to *N* could result in relaxed regulation of bristle-forming activity, leading directly or indirectly to increased bristle numbers. The model presented in Figure 6 can also account for the phenotypes of mutations such as *spl*, which has been inferred to be a neomorph on the basis of its response to modifiers (WELSHONS 1956, 1971). Accordingly, so-called neomorphic mutations may merely 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 discussed later, the "antagonistic function" model may also partly account for the Ax^x/Ax^y interactions. This model is not meant to imply that there are two discrete parts to the Notch locus, although the left half of the locus does appear to be functionally matched or paired with the right half (FOSTER 1973b). It is possible, although difficult to prove on present evidence, that whole series of mutually antagonistic regulator elements make up the Notch-locus product. In other words, the Notch locus could be an "integrator" gene along the general lines postulated by BRITTEN and DAVIDSON (1969) which controls the output of many "producer" (structural) genes.

Although this model is somewhat simplistic and may not satisfy all the data, by considering the expected gene products of *Ax/Ax*, *Ax/+*, and *Ax/N* (Table

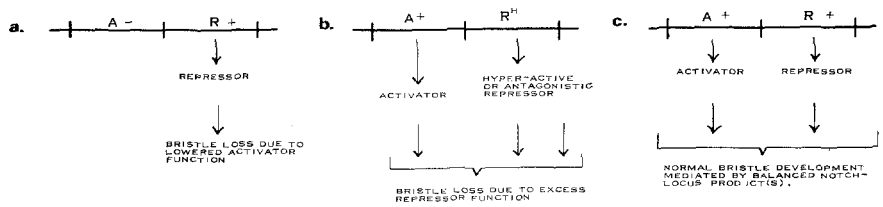

FIGURE 6.—"Antagonistic function" model of Abruptex mutations.

6), we can see how it can explain the phenotypes associated with these genotypes. If we assume that R^H has twice the repressive activity of R^+, then the quantitative similarity of cases (a) and (b) is more apparent (Figure 6, Table 6). It is easy to see that $Ax/+$ should be phenotypically closer to wild type than Ax/Ax, since in both cases (a) and (b) there is a smaller excess of repressor over activator function than in Ax/Ax. There is still an excess of repressor-over-activator function in the Ax/N product (Table 6); 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 phenotype of Ax/N is not too surprising. Note that suppression of Ax phenotypes by N^+ is postulated to occur by a different mechanism than suppression by N mutations.

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 noncomplementation (inviability) of all heteroallelic N mutant combinations (WELSHONS 1965) and the similarity of the embryonic abnormalities in hemi-, homo-, and heteroallelic combinations of various N alleles (POULSON 1968). Further support for a single Notch-locus product comes from the *cis/trans* differences observed in heterozygotes of fa^g or *spl* with Ax^{59b} or Ax^{59d} (WELSHONS 1971) and from the interactions of fa^{no}, *spl* and Ax^{E2} (FOSTER 1971). The genotype fa^{no} *spl* Ax^{E2}/ + + + expresses a rough-eye phenotype which is virtually indistinguishable from that of homozygous *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 that of + *spl* Ax^{E2}/+ + +. The difference between the *cis* and *trans* configurations indicates that the *spl* phenotype of fa^{no} *spl* Ax^{E2}/+ + + is not due to additive effects of the three-mutant alleles acting independently, but to the presence of the three mutant sites in the same product. In contrast to the enhancement of the *spl* phenotype, the coupling of fa^{no} to *spl* Ax^{E2} or Ax^{E2} alone completely suppresses the wing-vein gap (but not the bristle) phenotype of Ax^{E2}. This enhancement of the *spl* phenotype and suppression of the Ax phenotype is unusual, especially since *spl* separates the fa^{no} and Ax^{E2} mutant sites. These observations indicate that fa^{no} may not be entirely a hypomorphic allele, as was inferred by WELSHONS (1965) on the basis of N/fa^{no} inviability, since in this event we would not expect enhancement

TABLE 6

Expected product proportions of Ax/Ax, Ax/+, *and* Ax/N, *according to antagonistic functions model*

Genotype	Expected product proportions	
	Case (a)	Case (b)
Ax/Ax	2 R^+ : 0 A^+	2 R^H : 2 A^+
Ax/+	2 R^+ : 1 A^+	1 R^H : 1 R^+ : 2 A^+
Ax/N	1 R^+ : 0 A^+	1 R^H : 1 A^+

of *spl*. (WELSHONS (1971) has reported that the coupling of an amorphic (*N*) allele to *spl* results in suppression of the *spl* mutant phenotype.) Two possible explanations are: (1) that the effects of fa^{no}, *spl*, and Ax^{E2} happen through changes in the tertiary folding of the Notch-locus product; and (2) that the fa^{no}, *spl*, and Ax^{E2} lesions each directly affect several of the functions of the Notch-locus product, but in different, internally inconsistent ways.

One of the manifestations of the underlying differences between the two groups of *Ax* mutations is the system of lethal interactions among the *Ax* alleles. It is not difficult to conceive of a lethal/non-lethal heterozygote causing inviability, such as Ax^{E1}/Ax^{E2} and Ax^{E1}/Ax^{16172} (Table 1), and Ax^{59d}/Ax^{E2} and Ax^{59d}/Ax^{16172} (FOSTER 1971), in the same way that N/fa^{no} heterozygotes are usually inviable. HOUSE (1959) has also reported a lethal interaction between Ax^{28a} and the inviable allele Ax^{A}. On the other hand, the inviability of certain combinations of viable alleles is somewhat different. It could be postulated that the products of Ax^{9B2} and Ax^{E2} (or Ax^{16172}) are antagonistic and that inactivation of these gene products is responsible for the inviability. However this seems unlikely, since the inviable Ax^{x}/Ax^{y} genotypes exhibit severe bristle-loss phenotypes, and relatively severe "hypomorphic" situations like N/fa^{no} (however, see above) exhibit bristle disturbances in the opposite direction. One plausible alternative is that the two groups of *Ax* mutations affect 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 inviability, but combinations of mutations whose range of effects differ might affect enough functions to cause inviability. This type of model is illustrated in Figure 7, and is consistent with the different phenotypic pattern expressed by Ax^{9B2} compared to the other two alleles (Figure 1). This model cannot explain the interactions with *N* mutations, however, and we are forced to consider more elaborate models, such as that in Figure 6 (without necessarily excluding that in Figure 7). With the "antagonistic function" model of the Notch locus (Figure 6), we could suppose that the two groups of *Ax* mutations differ fundamentally in terms of the primary lesion in the Notch-locus product. Unfortunately, it is not obvious that the heterozygous combination of an A^{-}-type *Ax* and an R^{H}-type *Ax*, would lead to a more extremely mutant phenotype, since the product ratio $1\ R^{+} : 1\ R^{H} : 1\ A^{+}$ (cf. Table 6) does not increase the net "repressor" activity compared to that of the respective homozygous combinations, according to the model. Thus a new dimension may have to be added to the model of the Notch-locus product, involving interaction between the actual product molecules of two different alleles.

The type of interaction alluded to above, interallelic complementation, presumably acts by a similar mechanism whether the net phenotypic result is an enhanced or a reduced mutant phenotype; the case where allelic interaction results in a more mutant phenotype, as in certain *Ax* combinations, is termed "negative complementation" (FINCHAM 1966). Complementation at the intracistronic level is now generally held to be indicative of direct interaction between the molecular products of two different alleles (CRICK and ORGEL 1964; FINCHAM

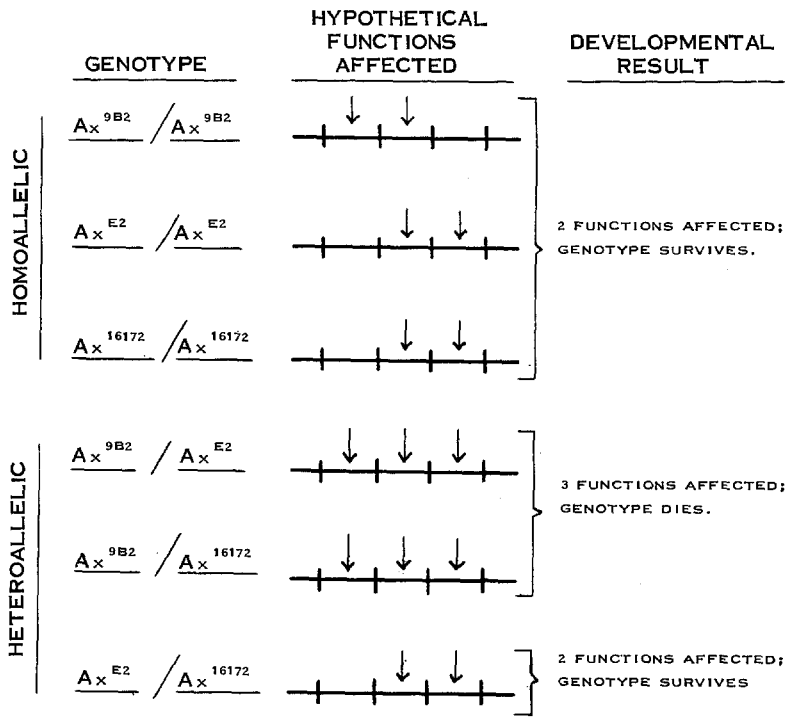

FIGURE 7.—"Range of function" model of Abruptex mutations. The presence of an arrow in a space indicates that the given genotype is defective in the function represented by that space.

1966). However, in the case of multi-functional molecules, as the Notch-locus product is postulated to be, there appears to be no need to invoke this type of interaction in the case of complementation among certain types of mutation (e.g., the Notch-locus recessive visibles *fa*^{g}, *fa*^{no} and *spl*, which are phenotypically distinct from one another and are essentially wild type in *trans*- heteroallelic combinations). The negative complementation between certain *Ax* alleles, however, does appear to require the existence of subunit interaction, at least according to presently accepted assumptions about gene action.

Several workers have proposed, or commented upon, models of gene-product structure based on patterns of interallelic complementation (e.g., KAPULER and BERNSTEIN 1963; CRICK and ORGEL 1964; FINCHAM 1966; FOSTER 1973b). Although models of this sort may be of questionable value so far as their actual relation to molecular structure is concerned, they do serve to point out that multimeric interaction need not be a simple lining-up of homologous segments of gene products (cf. CRICK and ORGEL 1964; FINCHAM 1966), although this must also be viewed in the light of possible evolutionary gene duplication (FOSTER 1973b). The fact that there are two groups of *Ax* alleles, with no complementation within groups, but with negative complementation between groups, does not itself suggest a structure for the Notch-locus product. However, as a starting point, it may be worthwhile considering a modification of the scheme (FOSTER

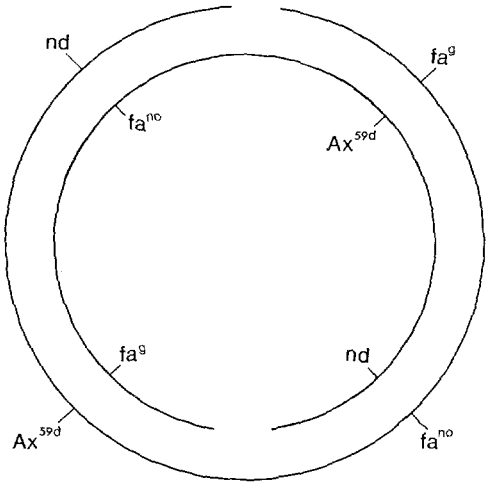

FIGURE 8.—Model of subunit interaction at the Notch locus, based on correlation of genetic map and complementation pattern (see FOSTER 1973b).

1973b) in which the Notch-locus product was depicted as a possible spiral. Using the same assumptions as in FOSTER (1973b), a similar model of the Notch-locus product can be derived, but involving two (or more) product molecules (Figure 8).

In spite of the foregoing, we should finish on a cautionary note. It is already obvious, from N^{g}/Ax, that suppression of one mutation's phenotype by an allele need not involve a multimeric type of interaction. Thus, while present considerations appear to require subunit interaction, it is not inconceivable that even the strong negative heterosis exhibited between the Ax groups could result through some mechanism other than multimeric interaction. At this stage it is not possible to resolve this point satisfactorily, and indeed it is questionable whether any amount of purely genetic investigation will do so. This is more apparent when we consider that: (1) we have no idea how many levels of organization separate the actual functioning of the Notch-locus product and what can be measured phenotypically; and (2) relatively large differences as measured phenotypically (either visible phenotypes or viability) may correspond to relatively minor changes in actual gene products as they might be measured by chemical or physical means, or *vice versa.*

I am deeply grateful to DR. DAVID SUZUKI for his support, encouragement and friendship during this investigation. I thank DR. W. J. WELSHONS for supplying me with mutant strains and information about them, and DR. T. C. KAUFMAN for examining the polytene chromosomes of the Ax strains.

LITERATURE CITED

BRITTEN, R. J. and E. H. DAVIDSON, 1969 Gene regulation for higher cells: a theory. Science **165:** 349–357.

CRICK, F. H. C. and L. E. ORGEL, 1964 The theory of inter-allelic complementation. J. Mol. Biol. **8:** 161–165.

ENGLESBERG, E., J. IRR, J. POWER and N. LEE, 1965 Positive control of enzyme synthesis by gene C in the L-arabinose system. J. Bacteriol. **90**: 946–957.

FINCHAM, J. R. S., 1966 *Genetic Complementation* (Benjamin, New York), 143 pp.

FOSTER, G. G., 1971 *A Genetic and Developmental Study of the Notch Locus of Drosophila melanogaster*. Ph.D. Thesis, The University of British Columbia, Vancouver. ——, 1972 Genetic studies of the Notch locus of *Drosophila melanogaster*. Genetics **71**: Suppl. s18. ——, 1973a Temperature-sensitive mutations in *Drosophila melanogaster* XIII. Temperature-sensitive periods of the lethal and morphological phenotypes of selected combinations of Notch-locus mutations. Develop. Biol. **32**: 282–296. ——, 1973b Is the Notch locus of *Drosophila melanogaster* a tandem repeat? Correlation of the genetic map and complementation pattern of selected mutations. Genetics **73**: 435–438.

FOSTER, G. G. and D. T. SUZUKI, 1970 Temperature-sensitive mutations in *Drosophila melanogaster*, IV. A mutation affecting eye facet arrangement in a polarized manner. Proc. Natl. Acad. Sci, U.S. **67**: 738–745.

GAREN, A., and H. ECHOLS, 1962a Genetic control of induction of alkaline phosphatase synthesis in *E. coli*. Proc. Natl. Acad. Sci. U.S. **48**: 1398–1402. ——, 1962b Properties of two regulating genes for alkaline phosphatase. J. Bacteriol. **83**: 297–300.

HOUSE, V. L., 1959 A comparison of gene expression at the Hairless and Abruptex loci in *Drosophila melanogaster* Anat. Rec. **134**: 581–582.

KAPULER, A. M. and H. BERNSTEIN, 1963 A molecular model for an enzyme based on a correlation between the genetic and complementation maps of the locus specifying the enzyme. J. Mol. Biol. **6**: 443–451.

LEFEVRE, G., JR., F. J. RATTY, JR. and G. D. HANKS, 1953 Frequency of Notch mutations induced in normal, duplicated and inverted X-chromosomes of *Drosophila melanogaster*. Genetics **38**: 345–359

LINDSLEY, D. L. and E. H. GRELL, 1968 *Genetic Variations of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. No. 627.

MOHR, O. L., 1932 On the potency of mutant genes and wild-type allelomorphs Proc. Sixth Int. Cong. Genetics, Ithaca **1**: 190–212.

MORGAN, T. H., J. SCHULTZ and V. CURRY, 1941 Investigations on the constitution of the germinal material in relation to heredity. Yearbook, Carnegie Inst. Wash. **40**: 282–287.

MULLER, H. J., 1932 Further studies on the nature and causes of gene mutations. Proc. Sixth Int. Cong. Genetics, Ithaca **1**: 213–255.

PORTIN, P., 1974 Allelic contracomplementation possible mechanism for initiating sympatic speciation. Nature **247**: 216–217.

PORTIN, P. and M. RUOHONEN, 1972 A new type of allelic interaction at the *Ax* locus of *Drosophila melanogaster*. Drosoph. Inf. Serv. **49**: 70.

POULSON, D. F., 1939a The developmental effects of a series of Notch deficiencies in the X-chromosome of *Drosophila melanogaster*. Proc. Seventh Int. Genetical Cong. (Edinburgh), 240–241. ——, 1939b Effects of Notch deficiencies. *Drosophila* Info. Serv. **12**: 64–64. ——, 1940 The effects of certain X-chromosome deficiencies on the embryonic development of *Drosophila melanogaster*. J. Exp. Zool. **83**: 271–325. ——, 1945 Chromosomal control of embryogenesis in *Drosophila*. Amer. Natur. **79**: 340–363. ——, 1968 The embryogenetic function of the Notch locus in *Drosophila melanogaster*. Proc. Twelfth Int. Cong. Genetics, Tokyo **1**: 143.

SHELLENBARGER, D. L., 1971 A temperature-sensitive Notch mutant of *Drosophila melanogaster*. Genetics **68**: 561–562.

SLYZINSKA, H., 1938 Salivary chromosome analysis of the white-facet region of *Drosophila melanogaster*. Genetics **23**: 291–299.

WELSHONS, W. J., 1956 Dosage experiments with split mutants in the presence of an enhancer of split. *Drosophila* Info. Serv. **30:** 157–158. ——, 1958a A preliminary investigation of pseudoallelism at the Notch locus of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **44:** 254–258. ——, 1958b The analysis of a pseudoallelic recessive lethal system at the Notch locus of *Drosophila melanogaster*. Cold Spring Harbor Symp. Quant. Biol. **23:** 171–176. ——, 1965 Analysis of a gene in *Drosophila*. Science **150:** 1122–1129. ——, 1971 Genetic basis for two types of recessive lethality at the Notch locus of *Drosophila*. Genetics **68:** 259–268.

WELSHONS, W. J. and E. S. VON HALLE, 1962 Pseudoallelism at the Notch locus in *Drosophila*. Genetics **47:** 743–759.

WRIGHT, T. R. F., 1970 The gentics of embryogenesis in *Drosophila*. Advances in Genetics **15:** 261–395.

Corresponding editor: B. H. JUDD