# THE *NOTCH* LOCUS OF *DROSOPHILA HYDEI*: ALLELES, PHENOTYPES AND FUNCTIONAL ORGANIZATION[1]

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ABSTRACT

The Notch (*N*) locus of *Drosophila hydei* and a series of its alleles and phenotypes are described. Some models are discussed to explain the opposite effects of some alleles on the structure of the wing, the neomorphic action of $N^{Ax}$ over typical *N* alleles and the interaction with the mutation Costal-nick (*Cnk*).

IN *D. melanogaster* typical Notch (*N*, DEXTER 1914) mutations are sex-linked hemizygous lethals causing excessive neurogenesis during embryogenesis (POULSON 1939, discussed by WRIGHT 1970). Heterozygous ($N/N^+$) females survive, have reasonable viability and fertility, but show a spectrum of morphological anomalies (MOHR 1919, 1924; MORGAN, BRIDGES and STURTEVANT 1925; LINDSLEY and GRELL 1968). Among them are thickened wing veins and nicks ("notches") along the margin. It has been suggested that in the heterozygotes the *N* phenotype is provoked by a reduced amount of gene product ("haplo-insufficient," LIFSCHYTZ and GREEN 1979) since heterozygotes of $N^+$ with *N* deficiencies show the same characteristic phenotype (MOHR 1924; POULSON 1945, 1967). Duplications generally are able to suppress the *N* genotype when they include the $N^+$ locus (BRIDGES and BREHME 1944; RATTY 1954). Furthermore, an excessive vein tissue, "Confluens," phenotype develops with an overdose of the $N^+$ locus (SCHULTZ 1941; MORGAN, SCHULTZ and CURRY 1941; LEFEVRE, RATTY and HANKS 1953; WELSHONS 1965).

The genetic complexity of the *D. melanogaster* locus has been studied by WELSHONS (1958a,b, 1965, 1974). METZ and BRIDGES (1917), however, already noticed recessive visibles that mapped close to the *N* locus and showed their phenotype in heterozygous combination with *N*. Among them are facet (*fa*), split (*spl*) and notchoid (*nd*). Later, other alleles were found that behave as recessive lethals but do not exhibit the dominant adult *N* phenotype in heterozygotes. They can be considered as hypomorphs rather than amorphs (WELSHONS 1965; WRIGHT 1970), apparently having sufficient activity to assure normal morphogenesis. A morphologically distinct type of mutants, Abruptex (*Ax*), that do not produce notches in the wing blade nor thickening of wing veins but suppress distal parts of veins instead and tend to suppress certain

[1] Dedicated to HANS GLOOR (Geneva, Switzerland) on the occasion of his 65th birthday.

bristles (mainly anterior dorso-centralis) are now recognized as Notch alleles, *i.e.*, they map in the same locus (WELSHONS 1971; FOSTER 1972, 1975). They will be designated $N^{Ax}$ henceforth. WELSHONS (1965) and PORTIN (1975) studied recombination and complementation and concluded that N is a monocistronic locus.

In contrast to the overwhelming amount of data on *D. melanogaster* that will be discussed, almost nothing is known about alleles, phenotypes and functional organization of the *N* gene in other Drosophilids, although presumed homologous mutations have been recorded for at least seven other species (SPENCER 1949). In this paper attention is given to a series of alleles of N (1-22.3, SPENCER 1949) of *D. hydei*. Mainly from a study of phenotypes and on the working hypothesis of a single structural cistron, we discuss some possible models for the organization of the Notch locus in this species.

## MATERIALS AND METHODS

### *Symbols*

After having established their allelism with described and still available *N* mutants ($N^{68}$, $N^{69l}$, $T(X;3)N^{70b3}$), the mutants were given a symbol $N^{-}$ or $N^{Ax-}$ depending on whether their phenotype at 25°, being the standard temperature of culture, was "typical" *N* or "Abruptex"-like, $N^{Ax}$ (Figure 1). Temperature-sensitive alleles are given the symbol $N^{ts-}$. They show a typical N phenotype at high temperature and an $N^{Ax}$ phenotype at low temperature.

### *Mutants and special chromosomes*

$w^{m}Co$ = Dp(1)16 $B_2$-17 $B_1$ (VAN BREUGEL 1970, 1971a): This duplication in fact consists of a small section of the X chromosome. It includes the white (*w*) locus, which is submitted to position-effect variegation by adhering heterochromatin, and an unaffected $N^{+}$ locus. The whole duplication occasionally slips as a kind of transposon from the Y to the X chromosome (VAN BREUGEL 1971a) and possibly also to autosomes (J. HACKSTEIN, NIJMEGEN, personal communication). The $N^{+}$ locus on the duplication is able to suppress haplo-insufficient hemizygous mutant *N* alleles, making it possible to study phenotype and lethality of compound genotypes. In the tables we replaced the symbol $w^{m}Co$ by the notation ($N^{+}$). In general, it makes little difference whether $N^{+}$ was introduced on the X or on the Y chromosome.

The presence of the duplication could be followed by means of the variegating *w* gene. Heterozygotes of two presumed *N* alleles were made from crosses $N^1/N^{+} \times N^2/w^{m}CoY$, and lethality of the compound $N^1/N^2$ was considered an important criterion for allelism. In the same manner homozygotes for a given allele were made and tested for lethality. Using the $w^{m}$ locus as an indicator, we also found genotypes (arisen by X-Y nondisjunction or X-Y exchanges) having, respectively, a supernumerary $w^{m}CoY$ chromosome or an extra $w^{m}Co$ duplication on their X chromosome.

In(1)$w^{m2H}$ *and* In(1)$w^{m2G}$ (HESS and GREEN 1965; VAN BREUGEL 1970): Both chromosomes were used to keep *N* alleles in stocks. (1) $w^{m2H}/N \times w^{m2H}/Y$: the $w^{m2H}$ homozygotes emerge later and do not easily come to maturity thus favoring the *N* progeny. (2) $w^{m2G}/N \times N/w^{m}CoY$: This is balanced because $w^{m2G}$ is lethal. This system can be used for most *N* alleles. Some spontaneous nondisjunction progeny ($N/../w^{m}CoY$) must be selected out.

T(1;2)$v^{t3}$ (KOBEL and GLOOR 1971): This translocation is homozygous lethal, but the hemizygous male survives. It can be used like the former chromosomes to balance *N* alleles: $T(1;2)v^{t3}/N \times T(1;2)v^{t3}/Y$. The *v* break point is slightly distal from the *N* locus giving rise to $N^{+}$ recombinant chromosomes (with low frequency) that must be selected out.

Costal-nick (Cnk, FREI and VAN BREUGEL 1983): *Cnk* is a dominant sex-linked mutant producing wing nicks and vein interruptions. Recombination frequency is 16% with *m* (miniature), 28% with *y* (yellow) and 36% with *sn* (singed). *Cnk*, thus, is located proximal to *m*.

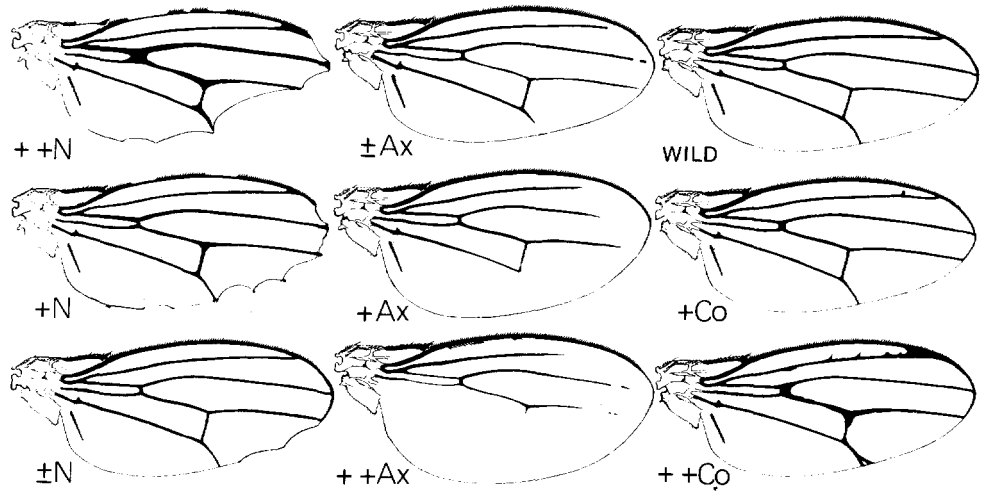


FIGURE 1.—Wing typology used to characterize the phenotypes of various Notch genotypes in Tables 3 and 4. *N* = typical Notch patterns, *Ax* = Abruptex-like patterns, wild = wild-type wing pattern, Co = Confluens patterns by $N^+$ overdoses. Expression: ± = weak, + = medium, ++ = strong.

*Outcrosses*

For outcrosses, either wild-type strain (Madeira or Leiden) or *w*-marked stocks were used. The background genotype did not dramatically influence *N* expression if compared with the $w^{m2}$ balanced stocks.

*Wing phenotypes*

Phenotypes were studied microscopically on wings embedded in Euparal. The average degree of expression of phenotypes was judged from at least ten wings for each genotype or other variable, using the typology presented in Figure 1. In most cases all wings could be classified neatly in the same class, indicating a small individual spread and limited influence of the residual genotype.

*Culture conditions*

Flies were reared at 25° unless otherwise indicated. In temperature experiments batches of eggs were collected during 4–8 hours at 25° and transferred to the new temperature.

## RESULTS

### *General aspects*

The Notch mutations presented arose either spontaneously or were X-ray induced. They were incidentally collected over a period of 15 years by several people and were selected on grounds of their well-defined phenotypes, either *N* or $N^{Ax}$ (Figure 1). They were identified as alleles on a combination of criteria (1) sex-linked, (2) phenotype, (3) homo- and hemizygous lethals either embryonic (*N*) or pupal ($N^{Ax}$), (4) lethal or extreme *N* in combination with known *N* alleles, (5) full compensation (*N* alleles) or partial compensation ($N^{Ax}$ alleles) of the developmental defects by the duplication $w^mCo(N^+)$, (6) reasonably balanced by $T(1;2)v^{t3}$ or $w^{m2}$.

Typical *N* (1-22.3, SPENCER 1949) of *D. hydei* is a sex-linked recessive embry-

onic lethal with, in the heterozygous ($N/N^+$) condition, the following possible phenes: thickening of the wing veins with delta endings (a rather constant feature), notches in the anterior and posterior wing margin ($N^{69l}$, a variable character probably connected with cell death, see VAN BREUGEL *et al.* 1981), eye reduction (mostly under bad culture conditions), fusion of tarsal segments (some alleles at higher temperature) and, incidentally, split-like doubling of thoracic macrochaetae. Like in *D. melanogaster*, females with a single dose of $N^+$ are phenotypically *N*, with $2N^+$ they are wild type, but with $3N^+$ or $4N^+$ they reach progressive Confluens (Figure 1) wing phenotypes (VAN BREUGEL 1971b) with net- or plexus-like structure of the $L_2$, $L_4$, $L_5$ longitudinal veins and both crossveins. In the male we never observed incised wings. This proves a dosage compensation mechanism: a single $N^+$ allele functions in the male as two $N^+$ do in females, moreover $2N^+$ in the male show a Confluens phenotype much the same as $4N^+$ in the female.

A second group of alleles is temperature sensitive, $N^{ts}$, showing *N* phenes at higher and $N^{Ax}$ phenes at lower temperatures. Although some of the phenotypic traits of the stable alleles (*N*, $N^{Ax}$) are also somewhat influenced by temperature, their overall phenotype remains always clearly either *N* or $N^{Ax}$.

$N^{Ax}$ alleles differ in many respects from typical *N* alleles. They show homo- and hemizygous survival up to the pupal stage, but flies usually do not emerge. In the pupal stage animals reach, however, an almost complete degree of metamorphosis before they die. In the heterozygous ($N^{Ax}/N^+$) condition, flies show distal vein interruptions and often lack anterior dorsocentral bristles.

Table 1 compiles the alleles under consideration, *viz.*, nine typical *N* alleles (group A), two $N^{ts}$ alleles (group B) and three $N^{Ax}$ alleles (group C).

Among various wing-nick mutants tested we found few that were not allelic with *N*; a number were lost before they could be tested. One particular independent mutation showed a strongly aberrant wing phenotype when combined with *N* alleles of group A (Figure 2a and b). This mutation called costal-nick (*Cnk*) shows at least 20% recombination with *N* and shows normal-sized wings with nicks along the margin, particularly in the costal region and at low temperatures. The veins are interrupted as in $N^{Ax}$ mutants. At 28° the phenotype can be wild type (Figure 3d). The peculiar interactions between *Cnk* and *N* will be considered in the following sections.

### *Cytological localization of the locus*

Localization was possible on the basis of three chromosomal aberrations involved with *N* mutations, namely, $In(1)N^{68}$, $T(X;3)N^{70b3}$ and $Df(1)N^{79}$. MUKHERJEE (1965) found that a white deficiency ($w^{Df\text{-}686}$) which lacks the bands 16 D3 to 17 A1.2 also showed a Notch phenotype and was male lethal. Thus, *N* must be included in this deletion. Figure 4 shows the results of a cytological analysis of the aberrations that narrowly delimit the *N* locus to the bands 16 D3 (4). The cytological map is the one used earlier (VAN BREUGEL, RAY and GLOOR 1968) to localize the white locus, whereas the numbering of bands is retained from the original BERENDES (1963) map.

TABLE 1

*Alleles of the Notch locus of* D. hydei

| Short symbol | Origin and references | Short description |
|---|---|---|
| Group A | | |
| $N^{68}$ | X-ray induced, VAN BREUGEL, VAN BREUGEL 1970, VAN BREUGEL *et al.* 1975 | Inversion(1)Notch-68. Recessive lethal dominant nicked wing phenotype, veins thickened. |
| $N^{69l}$ | X-ray induced, FREI, KOBEL and GLOOR 1971, VAN BREUGEL *et al.* 1981 | Notch-69l. Presumed point mutation. Phenotype as $N^{68}$. |
| $N^{70b3}$ | X-ray induced, FREI, FREI and VAN BREUGEL 1982 | Translocation(1:3)Notch-70b3. Break in X between $16D_2$ and $16D_4$. Phenotype as $N^{68}$. |
| $N^{79}$ | X-ray induced | Deficiency(1)Notch-79. Polytene bands $16D_{1,2}$ (3.4) missing (map: VAN BREUGEL *et al.* 1968). Phenotype as $N^{68}$. |
| $N^{80}$ | Spontaneous | Notch-80. Chromosomes normal. Phenotype as $N^{68}$. |
| $N^{80.6}$ | X-ray induced | Notch-80.6. Chromosomes normal. Phenotype as $N^{68}$. |
| $N^{81}$ | X-ray induced | Notch-81. Chromosomes normal. Phenotype as $N^{68}$. |
| $N^{125}$ | Spontaneous | Notch-125. Chromosomes normal. Phenotype as $N^{68}$. |
| $N^{78.9}$ | Spontaneous $w^+N$ revertant from $w^{iv}N^{Ax\text{-}fv}(w^mCo)/+$, VAN BREUGEL | Notch-78.9. Phenotype as $N^{68}$. Lost. |
| Group B | | |
| $N^{ts\text{-}K}$ | Unknown, KOBEL | Notch-*ts*-KOBEL. Chromosomes normal. Temperature-sensitive. Phenotype from *N* (25°) to *Ax* (17°). |
| $N^{ts\text{-}83}$ | Spontaneous from homozygous $N^K(w^mCo)$ | Notch-*ts*-83. Chromosomes normal. Temperature-sensitive. Phenotype as $N^K$ but more in direction of *Ax*. |
| Group C | | |
| $N^{Ax\text{-}66h}$ | X-ray induced, KOBEL (=$Ax^{66h}$), KOBEL and GLOOR 1971 | Notch-Abruptex 66h. Chromosomes normal. Phenotype weak *Ax*, distal vein interruptions, anterior dorsocentrals often absent. |
| $n^{Ax\text{-}81.5}$ | Spontaneous | Notch-Abruptex-81.5. Chromosomes normal. Phenotype moderate *Ax*. |
| $N^{Ax\text{-}fv}$ | Spontaneous, VAN BREUGEL (=$Ax^{fv}$), FREI and VAN BREUGEL 1982 | Notch-Abruptex-faintvein. Chromosomes normal. Phenotype strong *Ax*. Anterior wing margin with groups of extra medial triple row bristles. |

Entries without reference refer to unpublished new mutants.

### *Viability of* N *alleles and compounds*

By means of the $w^mCo$ ($N^+$) duplication, *N* alleles could be brought together in various combinations and compounds. Table 2 gives a compilation of the viability of such genotypes. It appears that homo- and hemizygotes of $N^{ts}$ alleles

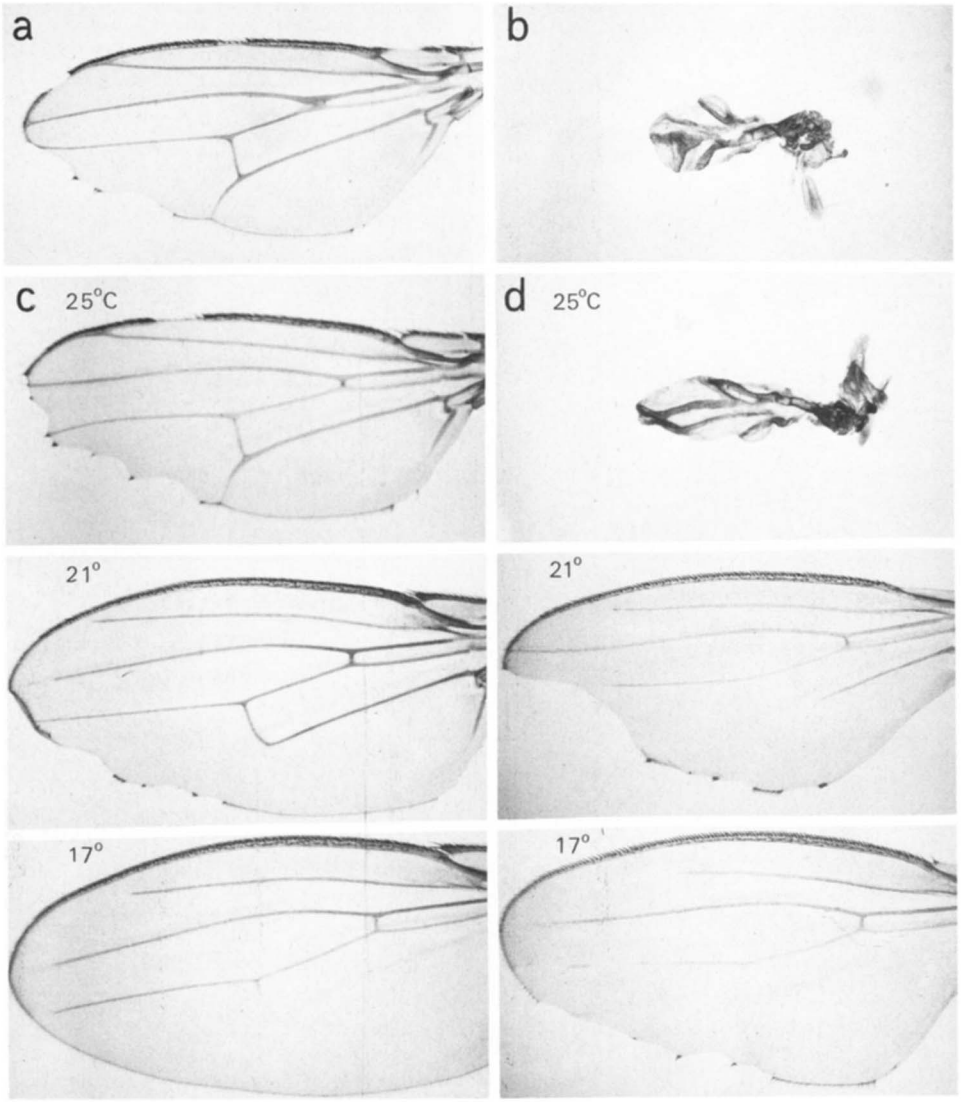


FIGURE 2.—Wing phenotypes produced by stable (a, b) and temperature-sensitive (c, d) Notch alleles in heterozygotes (left column) and double heterozygotes with dominant *Cnk* (right column). a, $N^{79}/T(1;2)v^{t3}$, thickening of proximal crossvein enhanced by $T(1;2)v^{t3}$; b, $N^{79}/Cnk$, reduction of the wing to a stump or vestigial-like phenotype as characteristic for group A alleles combined with *Cnk*; c, $N^{ts\text{-}83}/w^{m2H}$, temperature-dependent phenotype at three different temperatures; d, $N^{ts\text{-}83/Cnk}$, temperature-dependent reduction of the wing by interaction with *Cnk*. At low temperature a sum of *Ax* and *Cnk* effects is observed but no special interaction.

at low temperature have $N^{Ax}$ phenotype, lethal in the pupal stage, whereas at higher temperature the *N* type of embryonic lethality results (column 1).

All heterozygotes over $N^{+}$ are viable (column 2), whereas two different *N* alleles (within group A), or a combination of *N* and $N^{Ax}$ (group A and C), or combinations of $N^{ts}$ alleles (group B) with any other mutant allele, proved to be lethal (column 3).

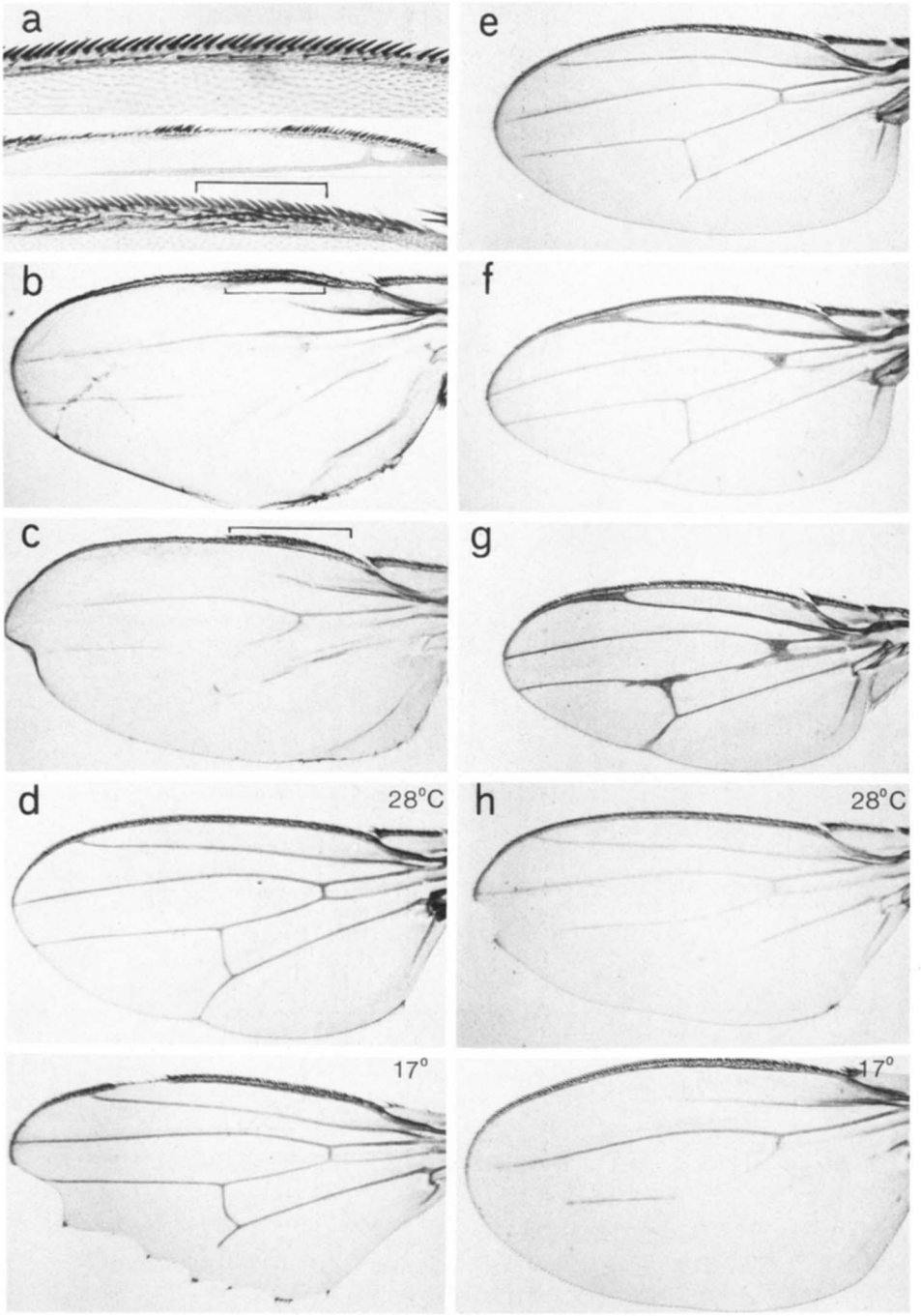


FIGURE 3.—Wing phenotypes of some Notch and/or Costal nick mutants. a, respectively: wild-type wing margin; typical Notch with gaps in the MR bristle pattern; $N^{Ax\text{-}fv}/(N^+)Y$ ♂: with an indicated stretch of supernumerary MR bristles (see also b, c). b, $N^{Ax\text{-}fv}/w^{m2H}$ ♀ strong $N^{Ax}$ expression. c, $N^{Ax\text{-}fv}/N^{ts\text{-}K}(N^+)$ ♀ 25°, $(N^+)$ compensates $N^{ts\text{-}K}$, but $N^{Ax\text{-}fv}$ is fully expressed as in heterozygotes. d, $Cnk/w^{m2H}$ ♀ at two temperatures. e, $N^{Ax\text{-}fv}(N^+)/N^{ts\text{-}K}(N^+)$ ♀ 25°, two doses of $(N^+)$ reduce the $N^{Ax}$ expression in comparison with b and c. f, $N^{Ax\text{-}fv}/(N^+)Y/(N^+)Y$ ♂, two $(N^+)$ doses suppress $N^{Ax}$, whereas a fairly strong Confluens pattern shows up. The latter indicates some $N^+$ activity of $N^{Ax\text{-}fv}$. g, $N^+/(N^+)Y/(N^+)Y$ ♂, strong Confluens phenotype. h, $N^{Ax\text{-}fv}/Cnk$ ♀ at two temperatures. Notice (1) the restored normal bristle pattern along the $L_1$ margin, (2) the lack of costal bristles is typical of *Cnk* at 17°, (3) the large wing surface as compared with group A *N*/*Cnk* heterozygotes.

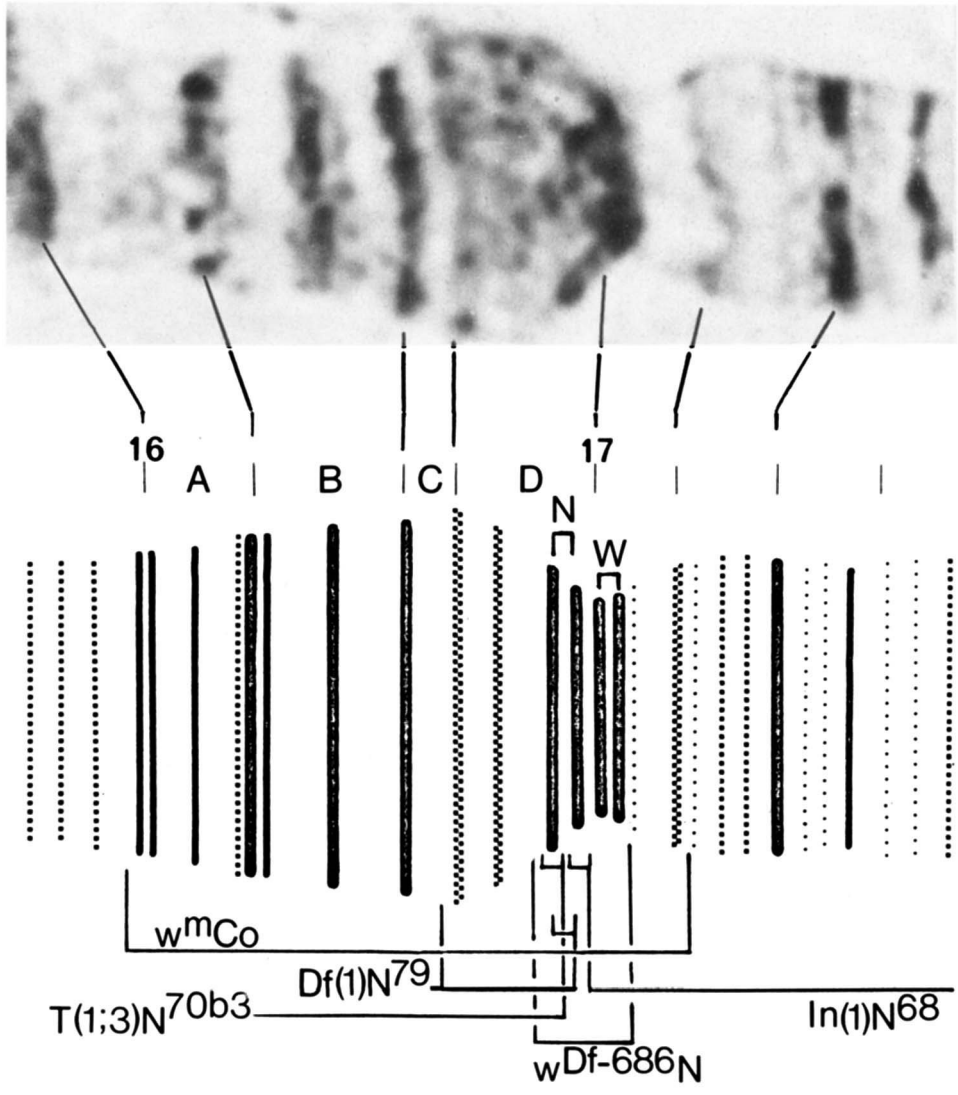


FIGURE 4.—Cytological localization of breakage points in five chromosomal aberrations restricting the *N* locus to bands 16D3(4).

When in any such genotype an extra $N^+$ locus was introduced (column 4), all combinations were viable. In hemizygous males the expression of all alleles is much the same as in homozygous females (column 5). With one extra dose of $N^+$ all alleles yield viable and fertile males, even though some male flies of $N^{Ax}$ type are too weak to emerge from the puparium (column 6).

We conclude that the duplication compensates fully for *N* embryonic lethality but only partially for $N^{Ax}$ pupal death. As yet we have not examined at which stage $N/N^{Ax}$ compounds are lethal.

TABLE 2

*Viability of Notch homozygotes, heterozygotes or heteroallelic compounds and the effect of the* $w^m Co(N^+)$ *duplication on survival of the flies*

| Allele $N^-$ | $N^-/N^-$ 1 | $N^-/N^+$ 2 | $N^-/N^{69l}$ 3 | $N^-/N^{69l}$ $(N^+)$ 4 | $N^-/Y$ 5 | $N^-/(N^+)Y$ 6 |
|---|---|---|---|---|---|---|
| Group A (17, 21, 25°) | | | | | | |
| $N^{68}$, $N^{69l}$, etc.[a] | − | ++ | − | ++ | − | ++ |
| Group B | | | | | | |
| $N^{ts\text{-}K}$ 17°C | + | ++ | − | ++ | + | +, ++ |
| 21 | ... | ++ | − | ++ | ... | ++ |
| 25 | − | ++ | − | ++ | − | ++ |
| $N^{ts\text{-}83}$ 17 | + | ++ | − | ++ | + | +, ++ |
| 21 | ... | ++ | − | ++ | ... | ++ |
| 25 | − | ++ | − | ++ | − | ++ |
| Group C (17, 21, 25°) | | | | | | |
| $N^{Ax\text{-}66h}$ | + | ++ | − | ++ | + | +, ++ |
| $N^{Ax\text{-}81.5}$ | + | ++ | − | ... | + | ... |
| $N^{Ax\text{-}fv}$ | + | ++ | − | ++ | + | +, ++ |

Lethal = −, observed death in pupa = +, fertile flies emerge = ++, not established = ...

[a] Many other compounds have been tried with other alleles instead of $N^{69l}$. Their phenotypes follow the same rules.

### *Wing phenotypes and the influence of other mutations*

N *alleles of group A* show a rather constant wing pattern in heterozygotes (Table 3). There is no difference between the balanced genotype (column 2) or an outcrossed control (column 1), but heterozygotes with $T(1;2)v^{t3}$ (column 3) have in general an enhanced *N* wing expression (Figure 2a). Combinations of group A alleles with *Cnk* all show strongly reduced wings (column 4 vg, Figure 2b) with no difference between the *trans* or *cis* genotype. When an extra $N^+$ locus is introduced, the characteristic *Cnk* phenotype is expressed (column 5). These results invariably are the same at three tested temperatures.

$N^{ts}$ *alleles of group B* produce phenotypes from extreme *N* to extreme $N^{Ax}$ depending on temperature (Figure 2c). The type-transition was already evident from the viability (Table 2) but can be followed step by step in the morphology of the heterozygous flies bred at various temperatures (Table 3, columns 1–3). At intermediate temperatures either a combination of weak *N* and $N^{Ax}$ expression is obtained or, less frequently, wild-type phenotype. Depending on temperature, interactions with $N^{ts}$ alleles follow exactly the mode as indicated for *N* alleles, on the one hand, or for $N^{Ax}$ alleles, on the other. Thus, for example, $T(1;2)v^{t3}$ enhances the *N* expression of $N^{ts}$ alleles at high temperature; interaction with *Cnk* is strong in the *N* range and weak in the $N^{Ax}$ range of temperature-dependent expression (Table 3, column 4; Figure 2d). Also, the effect of an extra dose of $N^+$, *i.e.*, the degree of normalization (suppression) in the presence of $N^{ts}$, follows the same logical pattern (Table 3, column 5).

$N^{Ax}$ *alleles of group C* show different degrees of expression (column 1). The

TABLE 3

*Wing phenotypes produced by Notch alleles heterozygous with some other mutations in comparison with control*

| Allele $N^-$ | $N^-/N^+$ (control) 1 | $N^-/w^{m2G}$ 2 | $N^-/T(1;2)v^{f3}$ 3 | $N^-/Cnk$ or $N^-Cnk/+$ 4 | $N^-(N^+)/Cnk$ 5 |
|---|---|---|---|---|---|
| Group A (17, 21, 25°) | | | | | |
| $N^{68}$, $N^{69l}$, etc. | +*N* | +*N* | ++*N* | vg | +*Cnk* |
| Group B | | | | | |
| $N^{ts\text{-}K}$ 17°C | +,++*Ax* | +,++*Ax*[a] | +,++*Ax*[a] | +*Cnk*; ++*Ax* | +*Cnk*; +,++*Ax* |
| 19 | ... | ... | ±*Ax* | ... | ... |
| 21 | ±*N*[b]; ±*Ax* | ±*N*[b]; ±*Ax* | ±*N*[b]; ±*Ax* | ±vg | ... |
| 23 | ... | ... | +*N* | ... | ... |
| 25 | +*N* | +*N* | ++*N* | +vg | +*Cnk* |
| 27–28 | +*N* | ++*N* | ++*N* | ++vg | ±*Cnk*[c] |
| $N^{ts\text{-}83}$ 17 | ++*Ax* | ++*Ax* | ... | +*Cnk*; ++*Ax* | ... |
| 21 | +*Ax* | +*Ax* | ... | +*Cnk*; ++*Ax* | ... |
| 25 | ±*N* | +*N* | +*N* | +vg | ... |
| 28 | +,++*N* | +,++*N* | ... | ++vg | ... |
| Group C (17, 21, 25°) | | | | | |
| $N^{Ax\text{-}66h}$ | ±*Ax* | ±*Ax* | ±*Ax* | +*Cnk*; ++*Ax* | +*Ax*[d]; ±*Cnk* |
| $N^{Ax\text{-}81.5}$ | +*Ax* | +*Ax* | +*Ax* | ... | ... |
| $N^{Ax\text{-}fv}$ | ++*Ax* | ++*Ax* | ++*Ax* | +*Cnk*; ++*Ax*[d] | ++*Ax*[d]; ±*Cnk* |
| Wild-type $N^+$ (17, 21, 25°C) | Wild | Wild | Wild | +*Cnk* | ±*Cnk* |

$N^+$ = exceptional segregants from cross $N/N^+ \times N/w^mCoY$ carrying the $w^mCo$ duplication either on the X or on a free Y chromosome. Expression: ± = weak, + = typical medium, ++ = strong (see Figure 1), vg = wing reduced (vestigial-like), ... = not established.

[a] No extra marginal bristles.

[b] Few gaps only.

[c] Costa nearly normal.

[d] As [c] but $L_4$, $L_5$ shortened, often blisters. Front margin along $L_1$ wild type.

$N^{Ax\text{-}fv}$ allele shows the strongest vein interruptions but develops also (in contrast to $N^{Ax\text{-}66h}$ and $N^{Ax\text{-}81.5}$) large amounts of supernumerary bristles along the proximal half of the front margin of the wing. These bristles look like normal medial row bristles (MR, VAN BREUGEL and GROND 1980) and occur often as short parallel rows along the margin (Figure 3a and b). The regional grouping resembles the clonal patterning of bristles in typical *N* flies (VAN BREUGEL *et al.* 1981), but instead of a lack of bristles there is in $N^{Ax\text{-}fv}$ an abundance of MR.

The most extreme expression of this phenomenon can be seen in $N^{Ax}$ homo- and hemizygote lethals when the imaginal wings could be recovered from pupae. The unemerged flies showed a wing pouch lacking all veins but with an enormous number of bristles in a zone that corresponds to the costa and the first longitudinal vein along the front margin of the wing (Figure 5d). Heterozygotes ($N^{Ax\text{-}66h}/N^+$) have no extra MR, but in homo- and hemizygotes a wing pouch develops much the same as in $N^{Ax\text{-}fv}$. These aspects of the phenotype

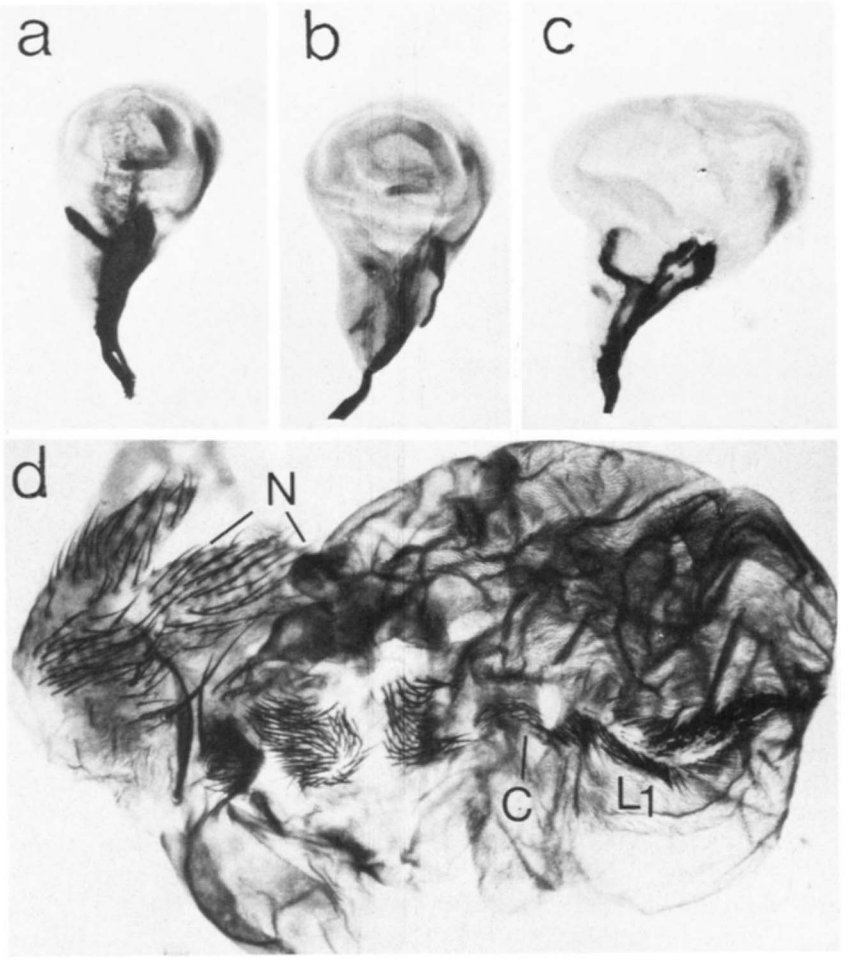


FIGURE 5.—Some $N^{Ax\text{-}fv}$ effects. a, Wild-type imaginal mesothoracic disc (stained for aldehyde-oxidase). b, $N^{Ax\text{-}fv}/+$, prospective wing area somewhat enlarged. c, $N^{Ax\text{-}fv}$ hemizygous male disc with double-sized wing area. d, $N^{Ax\text{-}fv}$ differentiated wing pouch, at higher magnification, dissected out from a pupa. No wing veins but large amounts of marginal bristles are seen in the C-$L_1$ region. N = notum, C = costa area, $L_1$ = area of first longitudinal vein.

may be bound to critical (threshold) values connected with the output (type of allele and dose) of the gene.

Heterozygous $N/N^+$ group A alleles (only some tested) show normal-sized imaginal discs. $N^{Ax\text{-}fv}/N^+$ larvae have slightly enlarged wing imaginal discs in third instar larvae (Figure 5b). In homo- and hemizygous $N^{Ax\text{-}fv}$, however, the wing part of the mesothoracic disc is considerably enlarged (Figure 5c). Since $N^{Ax\text{-}fv}$ wing parts are larger but do not develop wing veins, we conclude that $N^{Ax\text{-}fv}$ (and the locus as a whole) has something to do with the transition of wing blade tissue (trichome cells) into vein tissue, whereby a vein-forming substance might be involved.

$N^{Ax}$ alleles in combination with *Cnk* have nearly normal-sized wings with

some marginal incisions (Figure 3h), often with bristles in the area of the distal crossvein, and with shortened wing veins. The bristle pattern, however, along the $L_1$ vein, is restored to wild type. The overall structure of the wing is less abnormal than that observed in group A *N* alleles in combination with *Cnk*, where the wing is often reduced to some vestiges of the wing base area. Any attempt at a functional interpretation of the Notch locus will have to take into account that the independent mutant, *Cnk*, interacts very strongly with group A alleles but much less with group C alleles.

*The effect of an* $N^+$ *duplication on* N *alleles and* Cnk: *"allelic dominance" and neomorphic activity*

One extra dose of $N^+$ introduced in a heterozygous $N/N^+$ female fly gives wild-type wing structure (Table 4, column 1, group A alleles). It does not fully suppress, however, $N^{Ax}$ (group C) types of alleles, leaving a weak *Ax* effect. In males (column 5), too, one dose of $N^+$ makes the wing wild type with *N* but only weakens the expression of $N^{Ax}$. An $N^{Ax}$ residual expression was also present in $N^{ts\text{-}K}$ at 17°, whereas occasionally even a slight Confluens phenotype could be observed. It is clear, however, that at 17° the male phenotype is improved from a pupal lethal (with a veinless wing pouch) to a viable fly with a nearly normal wing upon receiving the $w^mCo$ duplication.

Our observations suggest that $N^{Ax}$ alleles, in contrast to *N*-type alleles or particularly *N*-deficiencies, do not represent amorphs or null-alleles, but rather have some residual $N^+$ activity combined with a neomorphic *Ax* activity. $N^{Ax\text{-}fv}$ males with two doses of $N^+$ show a stronger Confluens phenotype than do *N* $(N^+)$ $(N^+)$ or $N^+$ $(N^+)$ males. With respect to realization of Confluens, which represents an $N^+$ overdose phene, this indicates that $N^{Ax\text{-}fv}$ is not "silent" but contributes to the phenotype. On the other hand, two $N^+$ doses appear to suppress completely the proper *Ax* effect in $N^{Ax\text{-}fv}$ males since no vein interruptions are observed. In contrast to $N/N(N^+)$ $(N^+)$ and $N^+/N^+$ females, which have wild-type wings, homozygous $N^{Ax\text{-}fv}$ females with two $N^+$ doses show a combination of fairly strong *Ax* and medium Confluens expression. Apparently the two $N^{Ax\text{-}fv}$ genes act together with the two $N^+$ genes and promote the expression of the Confluens phene. On the other hand, the two extra $N^+$ doses are obviously not sufficient to suppress the *Ax* effect on wing veins. Considering the situation in males, in which two doses of $N^+$ are required to suppress the *Ax* effect of one $N^{Ax\text{-}fv}$ gene, we surmise by analogy that at least four $N^+$ duplications are necessary in females to suppress the *Ax* manifestation of two $N^{Ax\text{-}fv}$ genes.

The observation that $N^+/N^+$, $N/N^+(N^+)$ and $N/N(N^+)(N^+)$ females, as well as $N^+$ and $N/(N^+)$ males, all show wild-type wings is in agreement with the contention that typical *N*-type alleles are amorphs or null alleles, as are *N* deficiencies. In line with this, we found that $N^{Ax\text{-}fv}/N^+$ and $N/N^{Ax\text{-}fv}(N^+)$ females show the same strong *Ax* phenotype, which indicates that there is no interaction between *N* and $N^{Ax}$. Likewise, $N^{Ax\text{-}fv}/N^+(N^+)$ females have the same *Ax* phenotype as $N/N^{Ax\text{-}fv}(N^+)$ $(N^+)$ females, although basically the latter differ from the former by one additional *N* gene.

To explain that $N^+$ genes in $w^mCo$ duplications show weaker *Ax* than *N* phene suppression, one could hypothesize that the *N* locus in $w^mCo$ is subject

TABLE 4

*Wing phenotypes of Notch alleles compounds carrying the* $w^m$Co *duplication* ($N^+$), *showing* N *compensation and* Ax *neomorphic residual activity.*

| Allele $N^-$ | $N^-/N^+$ ($N^+$) 1 | $N^-/N^{69l}$($N^+$) 2 | $N^-/N^{ts\text{-}K}$($N^+$) 3[a] | $N^-/N^{Ax\text{-}fu}$($N^+$) 4 | $N^-$/($N^+$)Y 5 |
|---|---|---|---|---|---|
| Group A (17, 21, 25°) | | | | | |
| $N^{68}$, $N^{69l}$, etc. | Wild | +*N* | +*N* | ++*Ax* | Wild |
| Group B | | | | | |
| $N^{ts\text{-}K}$ 17°C | ... | ... | ... | ... | ±*Ax* |
| 21 | ... | ... | ... | ... | Wild |
| 25 | Wild | – | +*N* | ++*Ax* | Wild |
| $N^{ts\text{-}83}$ 17 | ... | ... | ++*Ax* | ... | ... |
| 21 | ... | ... | ±*N*; ±*Ax* | ... | ... |
| 25 | Wild | +*N* | +*N* | ++*Ax* | Wild |
| Group C (17, 21, 25°) | | | | | |
| $N^{Ax\text{-}66h}$ | ±*Ax*[b] | ... | ±*Ax* | ++*Ax* | ±*Ax* |
| $N^{Ax\text{-}81.5}$ | ... | ... | ... | ... | ±*Ax* |
| $N^{Ax\text{-}fu}$ | +*Ax* | – | – | ++*Ax* | +,++*Ax* |
| Wildtype | | | | | |
| $N^+$ 17 | ±Co[d] | Wild | ±*Ax*[b] | +*Ax*[c] | +Co |
| 21 | ... | ... | ±*Ax*[b] | ... | +Co |
| 25 | Wild; ±Co | Wild | Wild | +,++*Ax* | +Co |
| $N^+$ duplication | | | | | |
| $N^{Ax\text{-}fu}$($N^+$) 25°C | ±Co | +*Ax* | ±*Ax* | ++*Ax*; +Co | +,++Co |
| $N^{ts\text{-}K}$($N^+$) 17 | ... | ... | +,++*Ax* | ... | ... |
| 21 | ... | ... | ±*Ax* | ... | +,++Co |
| 25 | ... | ... | Wild | ±*Ax* | +,++Co |

Expression: ± = weak, + = medium, ++ = strong (see Figure 1), – = repeated combination, ... = not established.

[a] Phenotypes in this column all depend on temperature because of $N^{ts\text{-}K}$.

[b] Mainly $L_5$ is affected.

[c] Wild-type margin along $L_1$.

[d] Co, Confluens phenotype (see Figure 1).

to position-effect variegation in consequence of its vicinity to heterochromatin. The locus could comprise an *N* part and an $N^{Ax}$ part, whereby only the latter would be variegating. So, the duplication would compensate *N*-type defects normally but would have a reduced capacity to suppress *Ax*-type defects. Since it is known that in *D. hydei* (GLOOR, VAN BREUGEL and VOLKERS 1967) supernumerary Y chromosomes in this species suppress variegation, we could test the possibility of *Ax* variegation by comparing vein interruption measurements from $N^{Ax\text{-}fu}/w^mCoY$ males with those from $N^{Ax\text{-}fu}/w^mCoY/Y$ males. As we found no difference in vein interruptions, we consider the $N^+$ gene in the $w^mCo$ duplication as nonvariegating. Moreover, we are not compelled to question the single-structural-cistron-hypothesis for the Notch locus.

The $w^mCo$ duplication (see Table 3, column 5) also has a considerable effect on the expression of *Cnk*. The typical costal nick of *Cnk* disappears, and the

incisions along the remaining wing margin also become less frequent when two $N^+$ loci are present in the genome.

*Pleiotropic and antagonistic phenotypical effects*

The *N* (group A) and $N^{Ax}$ (group C) alleles not only affect the wing structures in opposite ways (Table 5) but also other organs. Lack of bristles, mainly anterior dorsocentrals, often occurs in $N^{Ax}$ and $N^{ts}$ genotypes provided the wing has an *Ax* type of expression (Table 6), whereas some *N* and $N^{ts}$ alleles may occasionally provoke doubling of thoracic bristles, particularly at 28°. Fusion of the tarsal elements in the leg is a typical *N* type of trait not seen in $N^{Ax}$ flies. *Ax* expression in the wing is a necessary condition for lack of anterior dorsocentrals, but strong *Ax* wing type does not imply a likewise pronounced bristle effect as we may conclude from $N^{Ax\text{-}fv}$ (Table 6). The temperature effect in $N^{Ax\text{-}66h}$ moreover seems opposite to that of $N^{ts}$ alleles, for which we have no real explanation.

In comments on the wing we have already mentioned the strong allele-specific interaction of *N* mutations with the wing nick mutation *Cnk*. Like the mesothoracic wing the metathoracic haltere structure is considerably reduced in *N/Cnk* heterozygotes when the *N* allele exerts an *N* type of action. Furthermore we remark that *Ax* halteres seem somewhat enlarged in comparison with wild type. Thus, the primary effects of the *N* gene on the wing and haltere are probably comparable. A pleiotropic effect of *N* on the metathoracic segment has never been noticed in *D. melanogaster*.

## CONCLUDING REMARKS AND DISCUSSION

*A comparison of species*

*D. hydei* and *D. melanogaster* differ considerably from each other. We mention here differences in the patterns in the polytene chromosomes (VAN BREUGEL, RAY and GLOOR 1968), the recombination frequency between and within genes (FREI 1974), the structure of the *w* locus (FREI 1975), the fertility factors and lampbrush structures on the Y chromosome (MEYER 1963), the bristle pattern on the first leg (*D. hydei* has no sexcomb) and the front margin of the wing (VAN BREUGEL and GROND 1980; VAN BREUGEL *et al.* 1981).

Although we have now three types of alleles of the *N* locus in *D. hydei* ($N^-$, $N^{ts}$ and $N^{Ax}$) we did not find recessive visibles comparable to facet, split or *notchoid* of *D. melanogaster*. We register neither hypomorphic mutations nor hypermorphic *Ax*'s able to suppress *N* in $N/N^{Ax}$ compounds (see e.g., LINDSLEY and GRELL 1968) nor signs of negative complementation as found in the latter species (FOSTER 1972, 1975; PORTIN and RUOHONEN 1972; PORTIN 1975, 1977a). In spite of some research in the literature we were not able to find in *D. melanogaster* a good homolog of the mutation *Cnk* interfering with *N*.

Taking into account the complicated temperature response of the $N^{ts}$ alleles of *D. hydei*, thus far unknown in *D. melanogaster*, we suggest that the construction of the *D. hydei N* locus may basically differ from that of *D. melanogaster*. THÖRIG, HEINSTRA and SCHARLOO (1981a, b) have recently questioned the

TABLE 5

*Antagonistic effects of* N- *and* $N^{Ax}$*-type alleles on wing morphology*

| N type | $N^{Ax}$ type |
|---|---|
| Smaller wing | Larger wing |
| Thicker veins | Thinner veins |
| Veins distally delta | Veins distally lacking |
| Incised margin | Smooth margin |
| Gaps of MR bristles along margin | Extra MR along margin |

TABLE 6

*Presence (+) or absence (−) of anterior dorsocentral bristles in relation to genotype and temperature*

| Genotype | Temperature | ♀ $--/w^{m2H}$ | ♀ --/Cnk | ♂ $--/w^{m}$CoY |
|---|---|---|---|---|
| Group A | | | | |
| ($N^{68}$, $N^{691}$, etc.) | 17 | + | + | + |
| | 21 | + | + | + |
| | 25 | + | + | + |
| | 28 | + | + | + |
| Group B | | | | |
| ($N^{ts\text{-}K}$, $N^{ts\text{-}83}$) | 17 | − | − | − |
| | 21 | − | − | − |
| | 25 | + | + | + |
| | 28 | + | + | + |
| Group C | | | | |
| $N^{Ax\text{-}fv}$ | 17 | ± | + | − |
| | 25 | + | + | − |
| | 28 | + | + | ± |
| $N^{Ax\text{-}66h}$ | 17 | + | + | ... |
| | 25 | − | + | ... |
| | 28 | − | − | ... |
| Cnk | 17 | + | Lethal | ... |
| | 25 | + | Lethal | ... |
| | 28 | + | Lethal | ... |

... = not established.

validity of the existing *D. melanogaster N* models [e.g., WRIGHT 1970; FOSTER 1975; SHELLENBARGER and MOHLER 1975 (two models) and PORTIN 1975, 1977a] and also the applicability of the very general BRITTEN and DAVIDSON (1969) model to the *N* locus. This prompts us to reconsider some current interpretations (for instance, that *Axs* are missense mutations) and to devise a new model for the *D. hydei* locus.

*N locus models*

*The possibility of missense mutations:* Although the *N* and Confluens phenotypes and their mutual exclusion apparently reflect quantitative aspects of function and gene-dose dependence of expression, the $N^{Ax}$-type mutants are rather indicative of inadequate function or activity. It is conceivable that the

typical *N* alleles represent various types of mutations, *viz*, locus deficiencies, nonsense mutations in the structural gene and mutations in a controlling part rendering the gene inoperative. Accordingly, the gene product in *N* homo- and hemizygotes would be absent or nonfunctional. *Ax*-type alleles may be viewed as missense mutations located in the structural region (for *D. melanogaster*, see PORTIN 1977b, 1981). In homo- and hemizygotes a product would be formed that may be sufficiently functional to overcome the developmentally critical period at embryogenesis. It may also be sufficiently operative to promote or reinforce the expression of the Confluens phene in genotypes with $N^+$ duplications. Realization of the *Ax* phene, however, would be a consequence of the defectiveness of the gene product. Different *Ax*-type mutations may give rise to gene products that differ in structure from the normal product and also among themselves according to the allele-specific change in the amino acid sequence. Depending on the type of structural change in the produced molecules, *Ax* alleles may exhibit at the level of phenotypic expression a variety of allele differences. For a given *Ax* allele, moreover, the strength of *Ax* phene expression in euploids and carriers of $N^+$ duplications may essentially depend on the ratio of normal *vs.* mutant genes, rather than on the number of gene doses present. Ratio dependence may be taken to mean that the activity of *Ax* mutant genes is controlled in much the same way as that of normal $N^+$ genes.

Although a number of *Ax* phenotypes may be explained on the basis of missense mutations, we feel that the opposed characteristics of wing pattern aberrations in $N^{Ax}$- and *N*-type heterozygotes (Table 5) reflect rather alternative (antagonistic) states in Notch locus mutants. As a further complication the temperature-sensitive ($N^{ts}$) alleles not only may achieve the full *N* and $N^{Ax}$ type of expression but also overlapping phenotypes or even wild type depending on temperature. A temperature-sensitive protein as the result of a missense mutation generally is believed to have function or no function depending on temperature. In the $N^{ts}$ alleles of *D. hydei* there seems to be an array of abnormal function, normal function or no function. These indications prompt us to envisage a formal explanation of gene function in which the $N^{Ax}$ mutations, $N^{ts}$ and possibly also some $N^-$ alleles, are viewed as genetic defects impairing control of gene activity rather than affecting the structural product itself.

*The possibility of impaired control:* The newly proposed *N* locus model (Figure 6), fashioned in analogy to the derepression type of the operon model, assumes a single *cis*-regulated structural part that is transcribed when the repressor is not bound to the controlling element of the gene whereby derepression would depend on the presence of repressor-bound inducer molecules, and repression on their absence. Induction during normal development would take place at given time points, *e.g.*, $t_1$ and $t_2$, leaving intervals during which the gene(s) are repressed (for arguments of active periods see FOSTER and SUZUKI 1970; FOSTER 1973; VAN BREUGEL, VERMET-ROZEBOOM and GLOOR 1975; SUZUKI *et al.* 1976; SHELLENBARGER and MOHLER 1978). *N*-type mutants interfering with control of gene activity would be noninducible because of an undissociable binding of the repressor to the mutant controlling element. $N^{Ax}$ types, on the other hand, would impair the binding of the repressor to the mutant-controlling

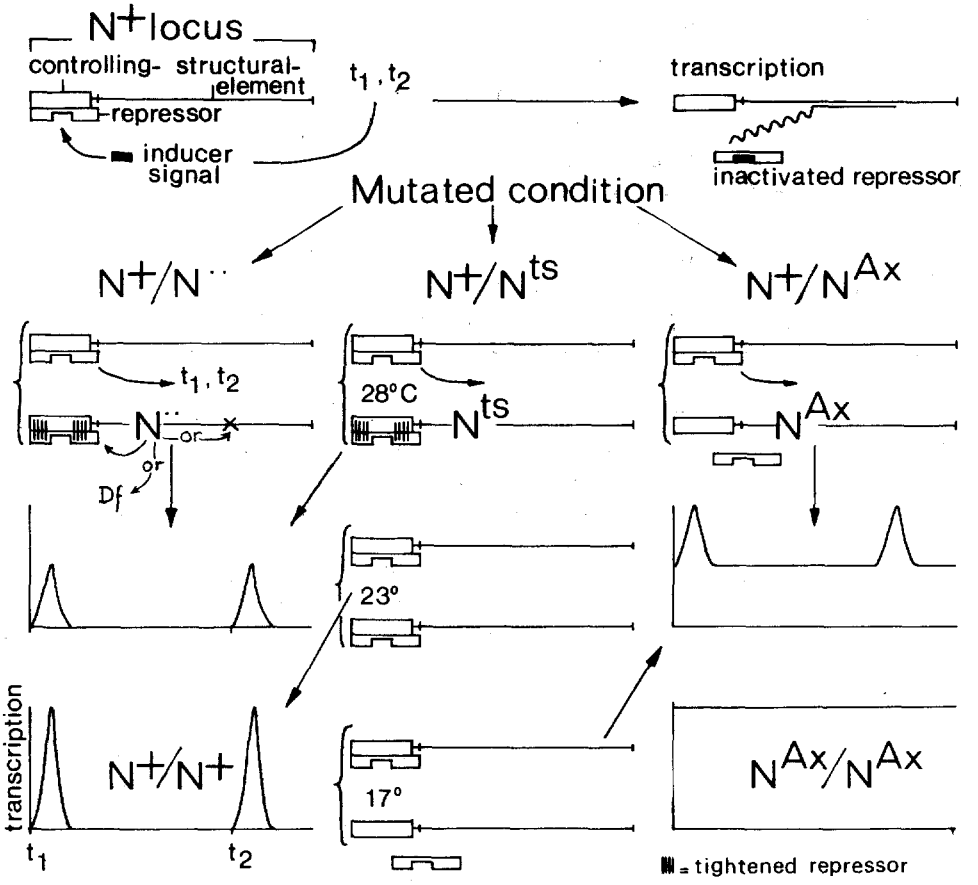


FIGURE 6.—A functional model of the Notch locus of *D. hydei*.

element. The gene(s) would thus be transcribed constitutively, or nearly so. We surmise that the neomorphic *Ax* manifestations of $N^{Ax}$ alleles might indeed be due to gene activity during those intervals in which wild-type $N^+$ genes are normally repressed (e.g., between $t_1$ and $t_2$). Different expression of $N^{Ax}$ alleles may be explained by unequal remnant affinities between repressor and mutant-controlling elements resulting in different levels of near-constitutive transcription. In $N^{ts}$ mutants at high temperature, binding of the repressor would be tight and transcription noninducible. Binding would be looser at intermediate temperatures, allowing induction similar to wild type. Very loose binding at low temperature would lead to constitutive activity and, thus, to the manifestation of an *Ax* phenotype. Constitutive activity of $N^{Ax}$ alleles or of $N^{ts}$ alleles at low temperature during the normal induction phases ($t_1$ and $t_2$) may allow survival at embryogenesis of homo- and hemizygotes, on one hand, and, on the other hand, may permit the expression of Confluens in carriers of inducible $N^+$ duplications.

*Suppression and interaction*

We must assume that, judged from the wing phenotype, either the translocation break point in the X chromosome (position-effect) or a separate enhancer on the $T(1;2)v^{t3}$ translocation is able to suppress the $N^+$ locus on this chromosome. There is also some reason to believe that the output of the $N^+$ gene in heterozygous $N/N^+$ flies can be lowered experimentally and quite specifically on the transcriptional level since injection of actinomycin D in third instar larvae is able to enhance some of the *N* phenes [like fusion of the tarsal elements and bristle doubling (VAN BREUGEL, VERMET-ROZEBOOM and GLOOR 1975; VAN BREUGEL and VAN DER AART 1979)]. It would be interesting to study the action of the drug on the $N^+$-overdose Confluens types and the newly found *Ax* and *ts* alleles.

The *Cnk* mutation is also haplo-insufficient. The heterozygote shows an aberrant wing phenotype, whereas the homozygote is lethal. The apparent joint action between *Cnk* and *N* leading to reduced wings and halteres suggests important functions of both genes in the morphogenesis of these structures. Their interaction can be explained in various ways: (1) accepting two serial enzymes in the same biosynthetic pathway. Both haplo-insufficient mutants might lower the output of the chain with 50% each. This would reduce the output of the total chain finally to about 25%; (2) two enzymes in separate chains whose products have to cooperate to obtain normal morphogenesis; (3) on the grounds of common traits with *N* (incisions and vein interruptions) it could well be that $Cnk^+$ is something like an evolutionary duplication of (part of?) the $N^+$ locus. Both genes could then be involved in the *same* enzymatic reaction. It is obvious that the double mutant *N/Cnk* then will be more extreme than either of the heterozygotes. Alternatively, one could assume that mutated *Cnk* (like $N^{Ax}$) cannot bind a repressor that it shares with the *N* locus. In *N/Cnk*, more of this repressor will then be available to suppress the $N^+$ locus. In our model (Figure 6) this would heavily affect $N/N^+$-insufficient types (group A alleles) but less so the $N^{Ax}$ alleles, as indeed our results have shown with respect to the size of the wing. Concerning the structure of the wing we remark that the double mutant $N^{Ax\text{-}fu}/Cnk$ shows a slightly suppressed *Cnk* phenotype, particularly in the costal region; on the other hand there is a small increase in vein interruptions indicative of a slight *Ax* enhancement. This proves that *Ax* and *Cnk* are not totally indifferent to each other. A general functional relationship between *N* alleles and *Cnk* as suggested in point 3 is sustained by the observation that $w^m Co(N^+)$ not only suppresses *N* but also to some extent *Cnk*.

Since recently a series of flavin enzymes have been stipulated to be the Notch structural product (THÖRIG, HEINSTRA and SCHARLOO 1981a, b) we presently are studying these enzymes in *D. hydei*. When we know more about the *D. hydei* flavin enzymes, in the near future, we may be able to test the various models directly on the enzymatic level.

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