THE INTERACTION OF SOME WING MUTANTS OF DROSOPHILA MELANOGASTER

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CAROLE A. SACK
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This is to certify that the

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THE INTERACTION OF SOME WING MUTANTS

OF DROSOPHILA MELANOGASTER

presented by

Carole A. Sack

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Herman M. Slatis
Major professor

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ABSTRACT

THE INTERACTION OF SOME WING MUTANTS OF DROSOPHILA MELANOGASTER

by

Carole A. Sack

A large number of different mutant genes affect the wings of *Drosophila melanogaster* by causing notching or greater degrees of excision of their surface area. In this study, the interaction of some of these wing excision mutants was examined by determining the extent of excision produced by a wide variety of genotypes. Attention was centered on the effects produced on +/+, *vg*/+, and *vg*/vg genotypes by the alleles in the Notch pseudoallelic system (facet, facet-notched, facet-notchoid, notchoid, Notch-8, Notch-69, and split). Other loci also were tested for evidence of interaction and bifid and scalloped were found to interact with both the vestigial and Notch loci.

The phenotypes that have been seen range from the fully normal wing to excision patterns so great that only a small stub of a wing is produced. The phenotypes that have been observed for most of these genotypes can be placed into a single continuous series that is characterized by an ever-increasing amount of excision. Any given genotype produces flies with wings that fall into a short continuous portion of the entire series. Some of the genotypes that include bifid can only be described by the construction of a portion of a

second series of phenotypes, which interconnects with the main series at one end.

An analysis of the amount of wing excision produced by genotypes of the Notch and vestigial loci leads to the development of a theory that these two loci contribute in consistent manners toward the physiological changes that result in wing excision. The observations can be interpreted as showing that certain genetic differences can be given quantitative values of "excision units," and that the variability that is caused by the environment and the uncontrolled portion of the genotype can also be described in terms of "excision units." Thus, the effects of heredity and of environment in the production of a phenotype can be related in terms of the same units.

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OF DROSOPHILA MELANOGASTER

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Carole A.nn Sack

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Introduction

The study of the action of a mutant gene may be useful as a method of suggesting a mode of action for the wild type allele. For example, Waddington's (1940) developmental study of the wing mutant, miniature, of Drosophila melanogaster, revealed that the abnormally small cell size of the wings results from the absence of wing cell expansion in the prepupae or pupae. The gene action of the wild type allele at the miniature locus, therefore, appears to be necessary for normal wing cell expansion.

Any given phenotype is dependent not on a single gene but rather on the entire complex of genes making up the genome. Thus, the phenotype of the wing mutant, miniature, may be altered if other wing mutants are also present in the same genome. Manifestation of a character, particularly if it is dependent on more than one gene, must be the result of gene interaction. It is not feasible to study the numerous and undoubtedly complex interactions of the entire genome at one time, but some insight may be obtained through the observation of less complex situations.

In the past, various pigmentation systems were found to be of value for this type of investigation. One such system was the eye pigment system of Drosophila melanogaster. The interaction of certain eye color mutants studied both through the use of conventional genetic combinations and through the special transplantation techniques of Beadle and Ephrussi (1937) helped elucidate the gene action of some of the loci affecting eye pigmentation.

The problem of gene interaction is an important one in view of the fact that interactions are involved in the development of all of the characteristics of an organism. For most traits or structures, however, there is not even a partial answer to the question of what interactions have occurred during their formation. The purpose of this study was to examine the formation of the wings of *Drosophila melanogaster* through the observation of the interactions of some of the mutant genes which affect them. The effects of these interactions could supply information concerning not only the developmental mechanisms of the wing but also the nature of the gene actions for the loci studied.

The wings of *Drosophila melanogaster* are suitable for such a study because of their relatively simple morphology. Each wing is a thin membrane supported by a system of tubular veins. The membrane is formed by the apposition of two layers of the integument; where the veins exist the two layers separate forming a cavity which is continuous with the haemocoel. The wing morphology of wild type flies shows little variability, the shape and form of the wing as well as the venation pattern being constant. Numerous mutants are known, however, which alter the wild type pattern to varying degrees and in different ways.

This study was primarily concerned with only one of the ways in which the wing morphology can differ from wild type: the excision of marginal portions of the wing. Excision can range the entire spectrum from loss of one or two marginal hairs from the distal wing tip to the loss of all but a basal stub of the wing. The excision effect results from the interaction of certain genotypes with environmental factors. The present study was largely directed toward the analysis of the amount of excision produced by a variety of genotypes within a normal environment.

Literature Review

Possible Developmental Mechanisms of Wing Excision Mutants

The development of the normal wing and of some of the wing excision mutants has been described by several authors. Auerbach (1936) gave a detailed description of the development of the wing during the larval stages, while the development during the pupal stage was the primary emphasis of Waddington (1940). Goldschmidt (1937a and 1938) also conducted developmental studies and then attempted to describe a developmental mechanism for the processes leading to wings of reduced size. He claimed, without giving historical evidence, that the wing anlagen of mutants causing wing excision develop normally up to a certain stage after which marginal portions of the developing wing degenerate and are resorbed; according to him, the larger the amount of excision, the earlier the degenerative process begins. Thus, mutants with a slight notching effect would not show degeneration until the pupal stage, but in mutants which have extensive excision of the wing area, the degenerative process would begin early in larval life. Goldschmidt suggested that some necessary developmental factor might be entering the wing and spreading throughout it in a gradient fashion from base to tip. Areas in which this factor would not penetrate or would not penetrate in sufficient quantities would degenerate.

Waddington (1940), however, found no evidence of degeneration in either whole mounts or cross-sections of developing wings and claimed that the hypothesis of degeneration was not necessary to explain the events seen during the development of wing excision mutants. Furthermore, if degeneration of tissue were to occur at a very early stage in the more severely affected mutants, the degeneration would have to occur before the invagination which forms the wing bud. Before invagination the material which will become marginal tissue lies in the center of the wing-forming area; any degeneration would leave holes in this area and holes have never been observed.

Waddington then presented his own hypothesis of the action of wing excision mutants. Observing that, in spite of the early manifestation of some of these mutants, there are no major readjustments of the longitudinal veins to the new wing form, he concluded that excision must take place after the determination of the veins. Since the first visible effects of some of these mutants is at the time of invagination when an alteration in the size and position of the wing invagination may be observed, Waddington suggested that the veins must be determined before the invagination to form the wing occurs. In this manner, some portion of the wing-forming area is determined to form veins. If the position of the wing invagination is altered in relation to the wing-forming area, this might cause a disruption of the venation system with half-veins possibly being formed. Since this is not observed, Waddington concluded that only one surface of the wing is determined for vein formation and that the vein-determined surface induces vein formation on the other surface.

He suggested that if the invagination to form the wing fold were to occur in the wrong relation to the vein-determined surface, portions of that surface might pass around the apex of the wing and come to lie on the wrong side of the wing. When on the wrong side of the wing, the vein-determined surface would no longer form veins unless appropriately induced. Waddington thus hypothesized that the wing excision genes have little or no effect on vein determination but instead alter the relation between processes leading to the venation system and those which determine the position and shape of the invagination to form the wing bud.

This hypothesis, with its assumption that the area in which vein determination occurs does not also shift its position, does not lend itself well to experimentation and has never been directly tested.

There have been no recent hypotheses for a wing excision mechanism, but a number of observations on possible mechanisms have been made from studies of the mutant vestigial.

The Vestigial Locus

The present study, like many studies in the past, used the second chromosome mutant vestigial (vg) as its focal point. The wings of this mutant are reduced to such an extent that only the basal portions remain. The vg allele thus represents one of the strongest of the wing excision mutants. The mutant allelomorphs of the vestigial locus, however, form a series ranging from the wild type isoallele, vestigial-nicked (vg^{ni}), which differs from wild type in

the $\underline{vg}^{ni}/\underline{vg}$ combination, to an extreme reduction that produces the tiny stub of vestigial-no wing ($\underline{vg}^{nw}$).

The research of the earlier investigators can be classified roughly into four categories: the interaction of the vestigial locus with temperature, the vestigial locus and developmental time, the interaction of vestigial and mutants at other loci, and vestigial and the developmental pattern of the wing. From these various types of studies have emerged further attempts to define the general mechanism of wing excision as well as the specific action of the vestigial gene. None of the other wing excision mutants have received the attention given thus far to the vestigial locus.

The Vestigial Locus and Interaction with Temperature: One of the more interesting aspects of the vestigial locus is its interaction with temperature to produce a variation of phenotype within any one genotype (Blanc, 1945; Friedland and Harnly, 1945; Harnly, 1929, 1930, 1933, 1936, 1940, and 1942; Harnly and Harnly, 1935; Li and Tsui, 1936; Roberts, 1918; Stanley, 1931 and 1935; and Tanaka, 1960).

The large amount of work completed by the authors listed above included the interaction of the vg gene with temperature and also the interaction of various vestigial alleles and allelic combinations with temperature. These experiments culminated with the elucidation of two theories: one concerning the gene action of the vestigial locus (Harnly, 1951b) and the other concerning developmental mechanisms of wing formation (Harnly, 1951a).

The theory of gene action of the vestigial mutants was developed by the analysis of the heat reaction curves of the alleles and allelic combinations at the vestigial locus. The theory assumed that the mutant vestigial alleles are performing in much the same way as the wild type allele, but are doing it less successfully, a concept that was first suggested by Stanley (1935). It was postulated that the optimum temperature for the vestigial gene reaction or its product reaction is shifted to a higher temperature, which for some alleles, appears to be above the lethal temperature.

The second theory emerging from the temperature experiments concerned the size and number of parts present in the wing. Analysis of the data led to the conclusion that size and number are different characteristics and that they are controlled by two independently acting gene complexes. One complex controls the proportions of the parts to the whole and the other determines the absence or presence of a part.

Vestigial and Developmental Time: Braun (1939) discovered that prolongation of the time of development could increase the wing size produced by some of the alleles of the vestigial locus. Child (1939) then increased developmental time by adding ethyl p-hydroxybenzoate to his culture medium. He found that vestigial flies that developed on this medium had larger wings than the controls. He interpreted his results as an interaction between a destructive factor in the wing and the rate of development, an interpretation which supports

Goldschmidt's hypothesis of a degenerative process but is contrary to his suggestion that a developmental factor is necessary for complete wing form.

Braun (1942) increased developmental time by starving the larvae and found that the wings of vestigial-notched (vg^{no}), a less extreme vestigial allele, showed a stronger degree of excision or smaller wings with increased time of development. On the contrary, wings of the slow-growing *vg* flies showed less excision and an increase in size over *vg* controls. Braun concluded that his results, although somewhat ambiguous, substantiated the interpretation of Child. Both Child and Braun failed to compare their results with the results of the temperature studies with *vg*. In these studies it was found that higher temperatures, which shorten developmental time, produce an increase in wing size.

Green and Oliver (1940) used a class of genetic factors known as Minutes, which prolong developmental time. They combined Minutes with heterozygous *vg* and found an association between the increased frequency and degree of excision and the prolongation of the developmental period. They suggested that such results could be explained by assuming that the *vg* allele produces something which either inhibits or destroys a wing substance already present, thereby acting against normal wing development. The delay in the developmental period permitted either a greater production of this substance or a longer time interval in which it could act. This hypothesis of gene action for *vg* was not presented as a refutation of the Goldschmidt hypothesis of degeneration, but it did suggest an alternative mechanism for wing excision. Additional similar evidence for this mechanism of gene action for vg was later provided by Green (1946).

Vestigial and Interaction with Other Loci: The interaction of the vestigial locus with other genetic factors has not been well studied. In a series of papers, Goldschmidt and his coworkers presented the results of investigations of dominance modifiers of vg and its alleles (Gardner, 1942; Goldschmidt, 1935 and 1937b; Goldschmidt and Gardner, 1942; and Goldschmidt and Hoener, 1937). The term 'dominigene' was applied to those genes which altered the dominance relations of vg such that flies heterozygous for vg and carrying the dominigenes exhibited notching of the wing tips. Through selection, Goldschmidt developed a dominigene stock and claimed to identify some of its genes which were acting as dominance modifiers. A number of known mutants were also combined with this stock and some were shown to have a dominigene effect. Many of the results of the experiments with the dominigene stock were criticized by Blanc (1946) who, at Goldschmidt's suggestion, reanalyzed that work and performed further experiments. Blanc concluded, in part, that some of the dominigene identifications and effects reported by the Goldschmidt group were questionable and that "cominance modifiers of vestigial are ubiquitous." Blanc found that the cominigene stock represented a highly complex collection of genes interacting to produce wing notching and that such a complex system could not easily be interpreted.

Blanc concluded his study by tentatively suggesting, in what is probably an oversimplification, that the mode of action of the dominigenes involves the alteration of time relationships of the developmental processes which ultimately lead to wing formation.

Outside of the work done with Goldschmidt's dominigene stock and several small studies by Waletzky (1940a and 1940b) and Silverman (1954), very little other work has been done on the interaction between *vg* and other loci.

Vestigial and Patterns of Wing Development: From the temperature interaction studies with the vestigial locus, several observations were made on the patterns of the resulting wing phenotypes. The wings of flies grown at higher temperatures have more of the wing present, that is, they are more normal, than the wings of flies grown at lower temperatures. Harnly and Harnly (1935) noted that there was a pattern of increase in wing material or replacement of regions of the wing with increasing temperatures. The first replacement took place along the second and third longitudinal veins, the region along the fourth vein and the third posterior cell was enlarged next, followed by lateral replacement, and finally by the replacement of the distal margin of the wing. McGovern *et al.* (1946) concurred with the Harnlys in their description of the increasing normality of the *vg* wing with increasing temperature.

Materials and Methods

Drosophila Stocks Used

The stocks used in this study are listed in Table 1. The stocks were used as received; it was assumed that major modifying genes were absent from them. Unlike most of the genes used, both facet-notched (fa^{n}) and facet-notchoid (fa^{no}) were linked with mutants at other loci. These mutants, yellow (*y*) and white (*w*), apparently do not affect the wing morphology. They also showed no interaction effects when tested by Harnly (1942) in combination with *vg*. For these reasons, yellow and white were not removed from the stocks in which they were present. The mutant fa^{n} was X-ray induced in a chromosome bearing the inversion *delta-49* and, according to Lindsley and Grell (1967), should be separable from it, but this has apparently never been done. The inversion has no known phenotypic effect.

The mutants cut-notch (ct^{n}) and notchy (*ny*) were also linked with other mutants, but neither forked (*f*) nor ocelliless (*oc*) are known to affect wing morphology. Because these two stocks were only used in a preliminary aspect of this work, the wing mutants ct^{n} and *ny* were not isolated.

Only one previously undescribed mutant has been used. Early in this study, a stock was developed, through the method of selection, that segregated for heterozygous *vg* flies that had a high proportion of notching of the wing tips. There arose in this stock, presumably through spontaneous mutation, a mutant which was first distinguished

Table 1. The stocks used and their sources.

Stock	Symbol	Location	Source
bifid	bi	1-6.9	D
clipped	cp	3-45.3	B
Confluens	Co	1-3.0	A
cut-notch in ct^{n} oc	ct^{n}	1-20.0	B
cut-six	ct^{6}	1-20.0	E
facet	fa	1-3.0	B
facet-notched in In(1)dl-49 y fa^{n}	fa^{n}	1-3.0	D
facet-notchoid in y w fa^{no}	fa^{no}	1-3.0	D
miniature	m	1-36.1	C
Notch-8	N^{8}	1-3.0	A
Notch-69f29	N^{69f29}	1-2.3	*
notchoid	nd	1-3.0	A
notchy in ny f	ny	1-32	B
Oregon-R	wild type stock		C
scalloped	sd	1-51.5	B
short wing	sw	1-64.0	B
split from w^{a} spl	spl	1-3.0	A
vestigial	vg	2-67.0	C
vestigial-nicked	vg^{ni}	2-67.0	B
vestigial-no wing	vg^{nw}	2-67.0	B
vestigial-Ultra	vg^{U}	2-67.0	B

Table 1. (continued)

Sources: A - Bowling Green State University
B - California Institute of Technology
C - Michigan State University
D - University of Chicago
E - Yale

from other flies of the selected stock by the considerable degree of excision that it produced. Subsequently this new mutant was identified as an allele of the Notch locus and was named N^{69f29}. The superscript 69f29 represents the year, month, and day of the identification of the mutant. In this paper N^{69f29} will be abbreviated to N^{69}.

The Notch locus is located near the left end of the first (*X*) chromosome. Welshons (1958) and Welshons and von Halle (1962) have described Notch as a complex locus. The locus appears to contain two different groups of mutants. One group is a series composed of the dominant Notch (*N*) mutants which cause notching of the wing tip and which are hemizygous and homozygous lethal. The other group is compomes of the recessive mutants facet (*fa*), facet-glossy (fa^{g}), facet-notched (fa^{n}), facet-notchoid (fa^{no}), notchoid (*nd*), and split (*spl*). These mutants affect either eye size and the appearance of the facets of the eye (fa^{g} and *spl*), the margin of the wing (fa^{n}, fa^{no}, and *nd*), or both (*fa*). All of these recessives were used in this study with the exception of fa^{g}. When combined with a dominant Notch mutant, the recessives which affect the eyes tend to have a pseudo-dominant effect such that the Notch pehnotype and the eye phenotype of the recessive are both expressed. The combination of a dominant Notch mutant with a recessive which affects the wings results in an abnormal wing phenotype and, in some cases, and abnormal eye phenotype (Glass, 1933). Welshons and von Halle demonstrated the occurrence of crossing over between various mutants of this complex. Since the term pseudoallele is applied to those genes which behave as alleles but are separable by crossing over, the mutants of the Notch locus constitute a pseudoallelic series.

Confluens (Co) is a tandem duplication for the Notch locus and causes thickening and irregularities of the veins of the wing. Combination of Co with a dominant Notch mutant produces a wild type wing except for a slight thickening of longitudinal vein III, which is a characteristic of both Co and N.

Techniques and Materials

All of the matings made for this study were single pair matings using virgin females aged three to five days. Each pair was placed in a shell vial containing approximately one inch of medium at the bottom. The parental flies were removed after five to six days and discarded. All of the experiments were conducted at 25° C.

The medium used throughout this study was a modification of the semi-synthetic medium of Carpenter (1950). Both 5 ml. of propionic acid and 0.5 g. methyl p-hydroxybenzoate were added to each liter of medium to act as mold inhibitors. A small drop of fresh yeast solution was placed on the surface of the medium in each vial prior to its use.

For those crosses resulting in flies with affected wings, it was necessary to remove and retain the wings for more detailed study than was possible with the wings attached to the body. When possible, the right wings of at least thirty randomly selected flies from each such cross were removed with a pair of fine forceps. The wings were placed on slides and, as suggested by Delcour and Lints (1966), were covered with a drop of chloralphenol (1 g. phenol and 1 g. chloral hydrate per 25 ml. distilled water was found to be suitable). After three to five minutes the chloralphenol was blotted up and the wings mounted in a drop of Permount.

Methods of Observation

The living progeny from each type of cross were examined under a binocular microscope. Unusual phenotypes such as thoracic bristle alterations, haltere modifications, and eye size and facet changes were noted but not described in any detail. Description of the wing phenotype from living flies was usually limited to the position in which the wing is held and the general nature of any marginal excision. Some genotypes so reduced the wing that its removal was difficult or impossible, and description of these wings was made directly from the living flies. The mounted wings were observed under a compound microscope at a magnification of 31.25 (12.5 ocular and 2.5 objective).

Terminology

In this paper, the descriptions of the affected wings contain the words notching and excision. Notching is used in reference to absence of portions of the wing apex. Excision is used to refer to the absence of wing material from any area of the wing. The use of the word excision is not meant either to suggest or imply any mechanism leading to the absence of portions of the wing.

The terminology used in this paper in the descriptions of the wings is presented in Figure 1.

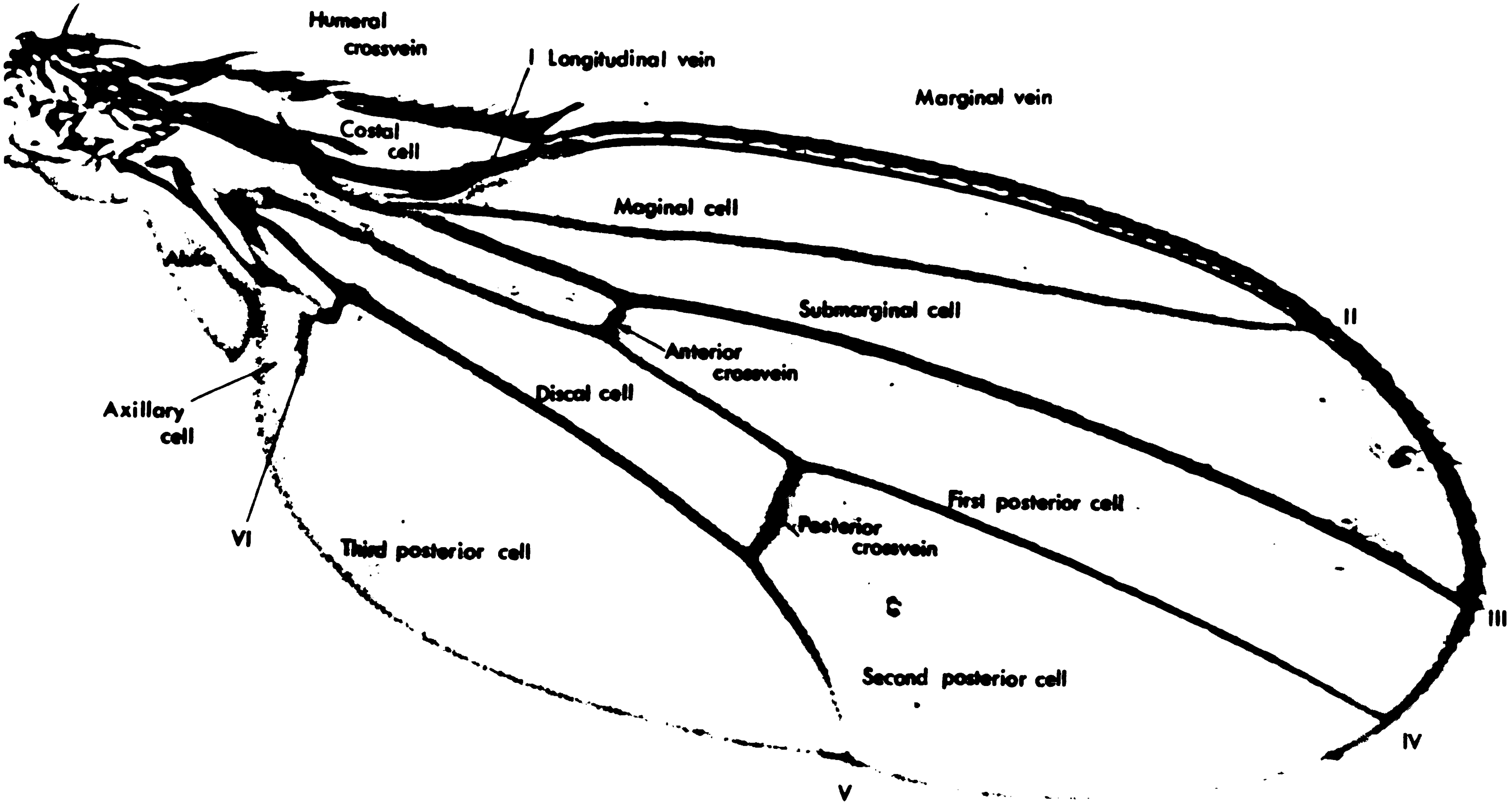

Figure 1. Wing terminology

Results

The goal of this study was to determine the extent of wing excision produced by a variety of genotypes and to identify the role of each gene in the production of excision. Extensive reports had indicated that the dominance relationships of the vestigial locus are dependent on the remainder of the genotype. Therefore, the investigation centered upon the changes produced by other genotypes in their interactions with +/+, *vg*/+ and *vg*/*vg*.

Identification of N^{69}

In a stock which was undergoing selection for the expression of apical notching of the wing in heterozygous *vg*, a new mutant arose and was identified as a member of the Notch (*N*) locus. This mutant formed a basis for many of these experiments. The manner of its identification is described below.

The new mutant, which showed an unusually large amount of wing excision, was first found in a female fly heterozygous for *vg*. A stock carrying the mutant and homozygous for *vg* was then established. Analysis of the segregation ratios for this stock (Table 2) and for the testcross of the progeny of an outcross (Table 3) reveals that the new mutant is dominant for notching, sex-linked, and lethal in males. It also produces distinctly different phenotypes with homozygous *vg* and with heterozygous *vg*. The mutant gene was isolated from *vg* and established in a balanced stock using the balancer chromosome *FM1*, *In(1)sc*^{8} *+ dl-49*, *y*^{31d} *sc*^{8} *w*^{a} *lz*^{s} *B*.

Table 2. Segregation ratio for the N^{69}; vg stock. The phenotypic cross was N^{69}; vg x vg .

vg		N^{69}; vg	
Males	Females	Males	Females
27	26	0	22

Expected phenotypic ratio, one class lethal - 1:1:0:1

Chi square = 0.56, d.f. = 2 0.70 P 0.90

Table 3. Testcross assumed to be *N^{69}*/+; *vg*/+ x +/*Y*; *vg*/*vg* . Parental females were from the phenotypic cross *N^{69}*; *vg* x *vg* .

Wild type		vg		N^{69}/+; vg/+		N^{69}/+; vg/vg	
Males	Females	Males	Females	Males	Females	Males	Females
46	54	42	46	0	47	0	40

Expected phenotypic ratio, two classes lethal - 1:1:1:1:0:1:0:1

Chi square = 2.56, d.f. = 5 0.70 P 0.90

Expected sex ratio, two classes of males lethal - 2 :1 .

Chi square = 0.22, d.f. = 1 0.50 P 0.70

After it was established that the new mutant is sex-linked, a crossover experiment was performed to locate its map position. Females carrying the mutant were individually mated to $\underline{w}^a$ spl males. The resulting females were backcrossed to $\underline{w}^a$ spl males and the progeny examined for recombinants. The crossover data, which is summarized in Table 4, indicate that the new mutant is 0.84 crossover units to the right of the white (w) locus, which is commonly assigned a map position of 1.5. The data also indicate that the new mutant is very closely linked to split (spl) with only one recombinant found in 1428 chromosomes tested. Split is a pseudoallelic member of the Notch locus and commonly assigned a map position of 3.0. The single recombinant between the new mutant and spl is most readily interpreted on the assumption that the new mutant lies to the left of split (spl).

Because the new mutant is closely linked to spl, it would be expected that its crossover map position would be very close to 3.0, but on the assumption that white (w) is at 1.5, the value obtained in this experiment is 2.34. Redfield (1955) conducted experiments which indicated that the standard map values in this region of the X chromosome may not always represent accurate crossover distances. Although the standard distance between white and split is given as 1.5, she found that the crossover distance between these two loci was between 0.5 and 0.8 in the absence of any inversion on other chromosomes and increased to as high as 6.3 in the presence of several inversions on other chromosomes. In addition, the new mutant appears

Table 4. Linkage data for N^{69}. Parental females were w^{a} *spl*/+ N^{69}. Parental males were w^{a} *spl*/*Y*.

Parental type females:	w^{a}	spl	+	- 767
	+	+	N	- 649
Redombinant females:	w^{a}	+	N	- 4
	+	spl	+	- 7
	w^{a}	+	+	- 0
	+	spl	N	- 0
	w^{a}	spl	N	- 0
	+	+	+	- 1
Total:				1428

Percent recombination for w^{a} - *N* = 12/1428 = 0.84

cytologically to be associated with a small deletion, which often reduces crossing over in adjacent regions. In view of this information and keeping in mind the very close linkage of the new mutant and _spl_, it is probable that the corssover distance of 0.84 between the white locus and the new mutant represents the distance between white and split under the conditions of this experiment. It was concluded, therefore, that the crossover data indicate that the new mutant could be at or near the Notch locus.

Phenotypically the new mutant is a typical Notch (Figure 2, A and B). The wings, which are slightly outheld, are almost always notched at the margin between longitudinal veins III and IV. The notched area often extends somewhat anterior to III and/or posterior to IV. There is usually a slight marginal excision along the proximal border of the third posterior cell and the axillary cell. Occasionally there is a small excision of the marginal vein in the area where it is joined by longitudinal vein I. Veins III, IV, and VI are thickened and all the longitudinal veins terminate in deltas at the wing margin. Rarely, flies may have one but not both wings unaffected by notching, but all the wings show the vein effects. The bristles may be slightly affected with the doubling of an anterior scutellar bristle in approximately 25 percent of the flies; other slight thoracic bristle abnormalities, such as extra bristles, may be noted.

It was concluded from the segregation ratios, the crossover study, and the phenotype that the new mutant is one of the dominant

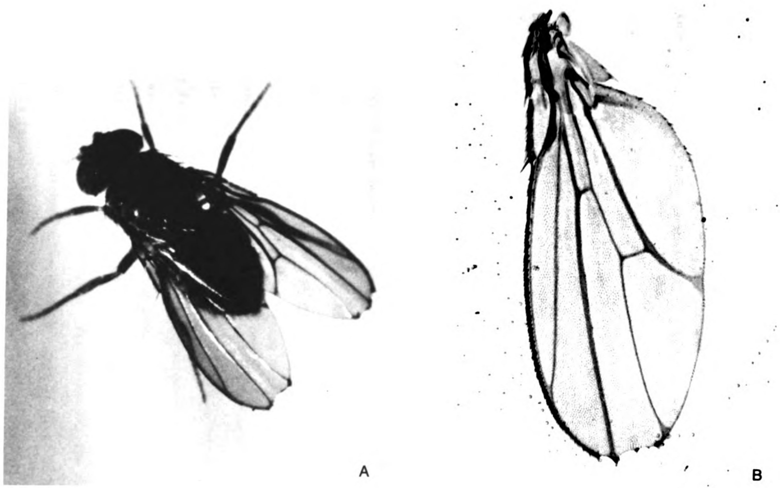

Figure 2. $N^{69}/+;+/+$

Notch alleles of the Notch locus. Further support for this identification was obtained when the progeny of a cross of the new mutant to Confluens (_Co_), a tandem duplication for the Notch locus, had wild type wings (vein III was not thickened in $\underline{N}^{69}$/_Co_ as had been reported for other _N_/_Co_ compounds).

The Notch Pseudoalleles and $\underline{N}^{69}$

In addition to the dominant Notch alleles, many of the recessive pseudoalleles also produce some phenotypic effect upon the wing. The degree of penetrance of wing notching was determined for each of the recessive pseudoalleles and for $\underline{N}^{69}$ by outcrossing each mutant to the wild type stock, Oregon-R. The results of these crosses, which are summarized in Table 5, provide penetrance values for the heterozygous females and hemizygous males resulting from the outcrosses. Penetrance values for homozygous mutant females were determined from matings of flies of the stock cultures.

As shown in Table 5, all of the $\underline{N}^{69}$ females have notched wings, but the penetrance of notching among the pseudoalleles is quite variable. For the _fa_ and $\underline{fa}^{n}$ pseudoalleles there is a considerable difference in the incidence of notching between the homozygous females and hemizygous males. The absence of notching in the heterozygous females resulting from outcrosses of the pseudoalleles indicates that all of the pseudoalleles are completely recessive for a mutant wing phenotype. The reason for the appearance of small numbers of notched males among the non-_fa_ males in the outcross of _fa_ is not clear. The notching possibly results from

Table 5. Penetrance of notching in N^{69} and the Notch pseudoalleles.

	x wild type (Ore-R)			
	Males		Females	Females
	al*/Y	+/Y	al/+	al/al
fa	42.98	1.07	0.00	11.90
fa^{n}	97.07	0.00	0.00	0.00
fa^{no}	7.34	0.00	0.00	10.91
nd	100.00	0.00	0.00	100.00
spl	0.00	0.00	0.00	0.00
N^{69}	lethal	0.00	100.00	lethal

* al symbolizes the pseudoallele

All values based on a minimum of 200 progeny from ten parental pairs.

the presence of Y-linked factors which cause some wing notching. The fa stock may also contain minor autosomal notching genes which, for some reason, lead to wing notching in some males but not in any of the females. Although not included in Table 5, it might be noted here that the wild type stock used showed no incidence of wing notching.

It has long been known that the combination of a dominant Notch allele with one of the recessive pseudoalleles may result in an exaggerated mutant wing phenotype, but detailed descriptions of the resulting wing phenotypes have not been given in the literature. In order to better understand the interallelic interactions of this locus and to serve as a basis for comparison with later studies using vg, the wing phenotypes resulting from the combination of N^{69} with each of the recessive Notch pseudoalleles were studied.

Facet: The eyes of fa flies are roughened in appearance. The wings may appear normal or may be notched between longitudinal veins III and IV (Figure 3). In the combination N^{69}/fa, the eye phenotype of fa is expressed. The wings of flies of this genotype show more excision than either N^{69}/+ or fa/fa (Figure 4). The marginal vein is usually excised on both sides of the juncture with vein I. Vein II is full length, reaching the normal wing margin, but the distal end of vein III may be excised. There is always some marginal excision on both sides of vein III and a deep excision near vein IV. Excision continues along the distal portion of vein VI such that about one-half of the axillary cell is removed. The alula is present but reduced in size. Both veins III and V are thickened, and veins II and IV may appear slightly irregular. In contrast to the doubling of the anterior scutellar bristles of some N^{69}/+ flies, some of the N^{69}/fa flies have doubling of a postscutellar bristle.

Facet-notch: The eyes of fa^{n} appear normal. In the stock used here, the males usually have wings notched between veins III and IV (Figure 5). The females show no notching of the distal wing margin, but both males and females may show some loss of marginal hairs from the alula.

The genotype N^{69}/fa^{n} (Figure 6) results in anterior marginal excisions and vein thickenings similar to those observed in the interaction of *fa* and N^{69}. However, in N^{69}/fa^{n}, heavy excision begins at or slightly proximal to vein V and continues along the posterior margin of the wing, resulting in the removal of about one-half of the third posterior cell and complete removal of the axillary cell. The alula may be completely absent or only a small vestige may remain. The eyes of this genotype may appear very slightly roughened and slightly reduced in size.

Facet-notchoid: The eyes of fa^{no} are wild type. Both males and females of the stock used have a small tendency toward notching between veins III and IV. Veins III and V are thickened and all the longitudinal veins terminate in deltas at the marginal vein (Figure 7).

The combination of fa^{no} and N^{69} is nearly lethal. Only a few

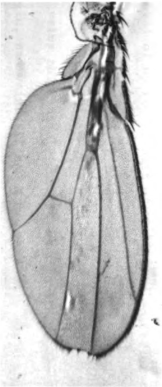

Figure 3. fa/Y

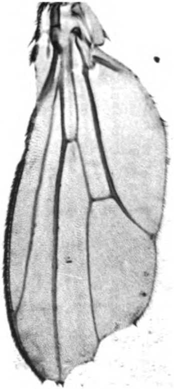

Figure 4. N^{69}/fa

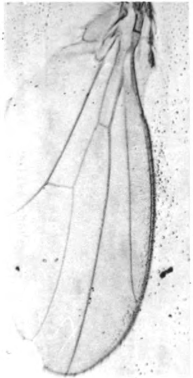

Figure 5. fa^n/Y

Figure 6.
N^{69}/fa^n

progeny successfully emerge from the pupae and these are poorly viable. In those progeny which do eclose, the veins are all very thickened and end in large deltas (Figure 8). Vein II is full length and veins III and V are very nearly full length. Heavy excisions are present along the anterior, apical, and posterior margins of the wing. The eyes of this genotype are small and like facet (fa) in appearance.

Notchoid: The eyes of nd are sometimes reduced in size and roughened somewhat. Unlike the three fa alleles, the wings of both sexes of nd are strongly affected (Figure 9). The longitudinal veins appear to lie closer together than normally. Veins III and IV are thickened and deltas are present at the marginal terminations of the longitudinal veins. Veins II and III reach the normal wing margin. Marginal excision, although variable, is extensive. From about one-half to three-fourths of the proximal marginal vein between veins I and II is removed or affected. Portions of the costal cell and its marginal vein are also removed. Effects on the posterior margin can vary from a narrow marginal excision up to virtually the complete removal of the third posterior cell and removal of up to one-half of the second posterior cell. The alula may also be reduced in size.

The flies of the genotype N^{69}/nd have narrow wings with all the veins thickened (Figure 10). Vein II reaches the normal wing margin, and veins III and V may have only small amounts of their terminal ends excised. The pattern of excision is similar, though

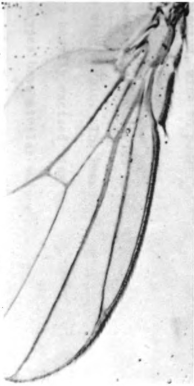

Figure 7. fa^{no}/Y

Figure 8. N^{69}/fa^{no}

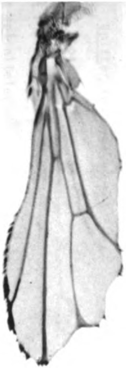

Figure 9. nd/Y

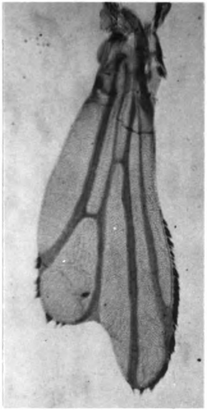

Figure 10. N^{69}/nd

slightly greater than either *nd*/*nd* or N^{69}/fa^{n}.

The N^{69}/*nd* flies are not very viable; almost all of those that emerge from the pupae become stuck in the medium shortly afterwards. Many of the flies are unable to eclose successfully. Some apparently never break open the pupal case while others only partially emerge. The eyes of this compound (N^{69}/*nd*) are small and roughened in appearance.

Split: The eyes of *spl* flies are small and roughened; the wings appear normal. In the combination N^{69}/*spl* the eyes have the *spl* phenotype, but the wings appear unaffected by the presence of the *spl* allele.

Summary of the Wing Effect of the Notch Locus Alleles: The strongest of the Notch locus mutants studied was N^{69}. A single dose of this allele always produces an abnormal wing phenotype. Because the homozygote is lethal, the wing phenotype which would result from a double dose of N^{69} is unknown. In contrast, the other five pseudoalleles of the Notch locus that were studied are completely recessive, with no penetrance in the heterozygous state.

In terms of a mutant wing phenotype, the strongest of the recessive pseudoalleles is *nd*, which affects both the veins and the wing margin. The homozygote of this pseudoallele produces a more heavily excised wing than the N^{69}/+ heterozygote, but the two genotypes have similar vein phenotypes.

As outlined in Figure 11, the three facet alleles, *fa*, fa^{n},

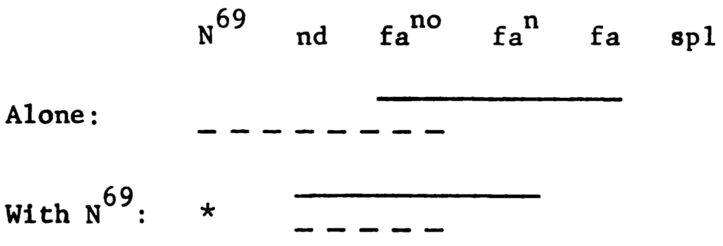

Figure 11. Comparison of the similar excision effects with the similar vein effects among alleles of the Notch locus and their compounds with N^{69}. The Notch locus alleles have been arranged in decreasing order of penetrance of a mutant wing effect (both excision and vein effects).

_____ = similar excision effect

_ _ _ = similar vein effect

* = lethal

and fa^{no}, all have roughly similar excision effects. In this respect, the three differ from one another only in the degree of penetrance of the excision effect in each sex. The fa^{no} allele also differs from the other facet alleles by the presence of vein thickenings and delta formations, in which it resembles N^{69} and *nd*. Thus, these three alleles do not fall into any orderly sequence of excision effects. The *spl* allele differs from the other Notch locus alleles studied by the absence of any phenotypic effect on the wing.

Because all of the recessive Notch pseudoalleles show no penetrance in heterozygotes with wild type, when N^{69} is combined with one of these recessives, any increase in excision over that of the dominant Notch wing phenotype is assumed to result from the interaction of the Notch mutant with its pseudoallele. All of the pseudoalleles tested, except *spl*, showed greater excision when heterozygous with N^{69} than that of N^{69}/+, the allele when heterozygous with wild type, and the allele when homozygous. Despite the differing degrees of penetrance and excision of the fa^{n}, fa^{no}, and *nd* pseudoalleles when homozygous and hemizygous, the wing phenotypes produced by each of these pseudoalleles when combined with N^{69} have similar degrees of excision. The pseudoalleles *nd* and fa^{no} differ from the other pseudoalleles because of their interaction with N^{69} to produce strong vein effects.

The Notch Locus and Vestigial

It was known from the time of its discovery that N^{69} interacts with heterozygous vg to produce a moderately reduced wing. A stock homozygous for vg and carrying N^{69} definitely confirmed that there is a strong interaction between the two genes.

The wings of homozygous vg flies, which are held at right angles to the body, are reduced to the basal portion (Figure 12). The excision is almost always extensive enough to remove both the anterior and posterior crossveins and the alula. Only the bases of the longitudinal veins are present. The halteres are reduced to either one or both of the basal segments; the distal segment, the capitellum, is never present. The postscutellar bristles are raised to a vertical position.

The results of an outcross of vg to wild type (Table 6) indicate that vg is almost, if not completely, recessive. The small percentage of notching among heterozygous females may result from other factors, such as environmental factors, rather than the penetrance of vg.

N^{69} and vg: When N^{69} is combined with heterozygous vg, the wings are always excised more than those of vg/+, but excision is far less than that of vg/vg flies (Figure 13). Marginal excision on the anterior border of the wings of N^{69}/+; vg/+ is variable. It begins just distal to the humeral vein and may continue along the margin to near the junction of vein II with the marginal vein; any excision in this area tends to remove primarily the marginal vein and only small portions of the marginal cell. At the apex of

Table 6. Penetrance of notching in crosses of Notch pseudoalleles to vg and wild type.

	x wild type (Ore-R)				x vestigial			
	al^{*}/Y	+/Y	al/+	+/+	al/Y; vg/+	+/Y; vg/+	al/+; vg/+	+/+; vg/+
fa	42.98	1.07	0.00	----	100.00	6.74	38.64	----
fa^{n}	97.07	0.00	0.00	----	100.00	0.29	0.51	----
fa^{no}	7.34	0.00	0.00	----	100.00	0.86	9.95	----
nd	100.00	0.00	0.00	----	100.00	2.31	86.82	----
spl	0.00	0.00	0.00	----	0.30	2.31	0.35	----
Ore-R	----	0.00	----	0.00	----	0.00	----	0.55

* - al symbolizes a pseudoallele

All values based on a minimum of 200 progeny from ten parental pairs, except for fa^{n} and fa^{no} where there were eight parental pairs.

the wing, vein II is full length or nearly full length, reaching the normal wing margin. Slight notching occurs between veins II and III, and a deep notch extends into the area of vein IV. Excision along the posterior wing margin is variable. A large marginal portion of the second posterior cell is usually present, but the third posterior cell may vary from having only a slightly excised border to almost complete removal of the entire cell. The axillary cell is usually not present and most or all of the alula is absent. As with $\underline{N}^{69}$/+ alone, the wings are slightly outheld and veins III and V are thickened. The halteres appear normal.

When the compound $\underline{N}^{69}$/+; *vg*/*vg* is formed, the effect upon the wing is striking (Figure 14). The wings are considerably reduced from even the small *vg* wing; usually they are small straight or bent projections, but, at the extreme, they may be represented by only a small knob on the side of the thorax. The halteres are also reduced, most often to the basal segment, but they may be entirely missing. There are not reports of interactions of other loci and *vg* producing this strong an effect upon wing reduction.

The phenotype of homozygous *vg* combined with a dominant Notch allele has not been described in the literature, but there are two references to the combination of Notch and heterozygous *vg*. Goldschmidt and Gardner (1942) briefly mention an interaction of *N* and of *fa* with heterozygous *vg* and the dominigene stock. McGovern *et al*. (1946) do not mention an interaction but must have been aware of it because they used the genotype $\underline{N}^{8}$/+; *vg*/+ in one of their

Figure 12. +/Y;vg/vg

Figure 13. N^{69}/+;vg/+

Figure 14. N^{69}/+;vg/vg

temperature studies. These references indicate that the reduced wing resulting from its combination with *vg*/+ is not unique to the N^{69} allele. As added confirmation and for comparison with N^{69}, N^{8} was combined with heterozygous *vg*. Approximately the same wing phenotype results from N^{8}/+; *vg*/+ as from N^{69}/+; *vg*/+ indicating that there may be a general interaction between Notch alleles and *vg*.

Another possibility concerning the results of the combination of dominant Notch alleles with *vg* is that the interaction may be specific for the *vg* allele and not a general one with other mutants at the vestigial locus. To test this possibility, N^{69} was combined with heterozygotes of several other vestigial alleles, vg^{ni}, vg^{nw}, and vg^{U}. The weak vestigial allele, vg^{ni}, is wild type when homozygous (a wild isoallele) and is only distinguished from the normal allele at the vestigial locus by the presence of notching in 27 percent of vg^{ni}/*vg* heterozygotes (Lindsley and Grell, 1967). When the genotype N^{69}/+; vg^{ni}/+ is formed, the wings strongly resemble N^{69}/+ except for the presence of notching between veins I and II, which is not seen in N^{69}/+ flies. The wings of vg^{nw} are smaller than homozygous *vg* and the wings of heterozygous vg^{U} (vg^{U}/+) are greatly reduced from wild type and resemble *vg* wings. When either of these strong alleles of vestigial is combined heterozygously with N^{69}, the phenotypes of the progeny reflect an interaction of the genes. The wings of N^{69}/+; vg^{nw}/+ flies resemble those of N^{69}/+; *vg*/+ except for additional excision along the anterior margin of the wing. The wings of N^{69}/+; vg^{U}/+ are small projections, as small or smaller than those of vg^{U}/+. The occasional doubling of scutellar bristles by N^{69} is enhanced in this combination; the scutellar bristles may be doubled or additional bristles may be present up to a total of eight instead of the normal four. Thus, it seems clear from the phenotypes resulting from the combinations of several different vestigial alleles with N^{69} that a general interaction takes place between the dominant Notch alleles and the vestigial locus.

Notch pseudodoalleles and vg: Table 6, which includes the control values from Table 5, summarizes the results of crosses between *vg* and the recessive Notch pseudoalleles. Because the degrees of penetrance alone do not always give an accurate indication of the results obtained from these crosses, each genotype is described below.

Facet-notched: The heterozygous females fa^{n}/+; *vg*/+ show the same small percentage of wing notching found in the control cross.

The hemizygous males fa^{n}/*Y*; *vg*/+ all have notched wings (Figure 15), but so do almost all of the fa^{n} control males. However, the wings of fa^{n}/*Y*; +/+ males are usually notched only between veins III and IV; fa^{n}/*Y*; *vg*/+ results in an extension of the marginal excision in about three-fourths of the wings to areas between veins II and III and between veins IV and V. About 15 percent of the wings also have slight excisions along the posterior margin.

Facet-notchoid: The fa^{no}/*Y*; *vg*/+ males (Figure 16) have a greatly increased incidence of notching over fa^{no}/*Y* males. The extent of the notching is not very great; it occurs between veins III and IV, sometimes extending to each side of this area.

About 10 percent of the fa^{no}/+; *vg*/+ show wing notching, as compared with no notching in fa^{no}/+ and only rare instances in *vg*/+ females. The pattern of excision among affected females resembles that of the fa^{no}/*Y*; *vg*/+ males.

Crosses between the *vg* stock and the fa^{no} stock and between the *vg* stock and the fa^{n} stock above often proved to be infertile. The reason for this lack of fertility is unknown. Attempts were made to develop the doubly homozygous stocks, fa^{n}; *vg* and fa^{no}; *vg*. Although a few doubly homozygous flies (which resembled *vg*/*vg*) were obtained for each genotype, crosses to develop stock cultures were unsuccessful.

Split: All of the genotypes resulting from crosses between *vg* and *spl* have small frequencies of wing notching, but none of these frequencies differ sufficiently from values for *vg*/+ to indicate any interaction effects.

Facet: The single *fa* allele in *fa*/+; *vg*/+ results in a greatly increased incidence of wing notching over both *fa*/+ and *vg*/+. The affected wings from flies of this genotype tend to have a slight marginal notch between veins III and IV with the excision rarely extending to either side of this area (Figure 17). In comparison,

Figure 15. fa^{n}/Y;vg/+

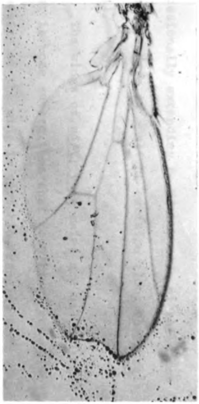

Figure 16. fa^{no}/Y;vg /+

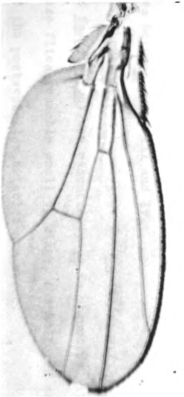

Figure 17. fa/+;vg/+

Figure 18. fa/fa;vg/+

all fa/Y; vg/+ males and fa/fa; vg/+ females (Figure 18) have notched wings. The marginal notch seen in these flies is more extensive than that of fa/+; vg/+ females; the major excised region lies between veins III and IV and very often extends to either side of this area. The wing notch also cuts more deeply into the first posterior cell. The fa/fa; vg/+ females show less tendency than fa/Y; vg/+ males for the notched area to extend beyond the marginal area between veins III and IV.

The genotypes homozygous for vg (fa/+; vg/vg, fa/fa; vg/vg, and fa/Y; vg/vg) are essentially vg in phenotype. The wings of these flies resemble small vg wings, the halteres are rediced, and the postscutellar bristles are erect.

Notchoid: Most of the females of the genotype nd/+; vg/+ have some notching of the wings between veins III and IV with the notching occasionally extending to either side of this area (Figure 19). Rarely, there is a slight excision along the margin of the axillary cell.

The wings of nd/Y; vg/+ males are all affected by excision that is more severe than that resulting from nd/Y alone (Figure 20). The size of the wing is variable, ranging from wings that extend only a short distance beyond the anterior crossvein to wings that are the full length of vein II. Vein I is present in part; veins II, III, and IV are usually present and appear to be lysing closer to one another than normal. The veins are thickened and irregular in form. The wings usually are long and are narrow due to the extensive reduction of the marginal, second posterior, and third posterior cells. The alula is absent.

Adding one more dose of *nd* to produce *nd*/*nd*; *vg*/+ females results in even more extensive reduction of the wings (Figure 21). Superficially these wings resemble large *vg* wings, but are large enough to include the anterior crossvein, completely or in part, and vein III is thickened. A second anterior crossvein is occasionally present (Figure 22). Despite the strong effect on the wings, the halteres of this genotype are normal in appearance.

The *nd*/+; *vg*/*vg* females have wings of the *vg* phenotype. Both the male *nd*/*Y*; *vg*/*vg* and the female *nd*/*nd*; *vg*/*vg* flies have extreme reduction of the wings (Figure 23). Only a basal stub of the wing remains, and the halteres are reduced to their basal segment or less.

Compounds of the Notch locus and vg: Once the wing phenotypes produced by the combinations of N^{69} with *vg* and the combinations of the recessive pseudoalleles with *vg* were known, it was decided to form some triple compounds composed of N^{69}, one of the pseudoalleles, and heterozygous vg in order to determine whether or not the interaction effects of these genes is cumulative.

The wings of N^{69}/*fa*; *vg*/+ flies were found to be unlike those of the combinations of any two of these mutants (Figure 24). Excision of the wings is more extensive and, at the extreme, may produce a wing composed primarily of veins III and IV and portions of the wing cells to either side. More commonly, vein II is present and extends to the normal margin, but the entire margin between

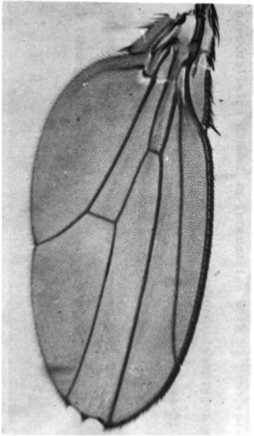

Figure 19. nd/+; vg/+

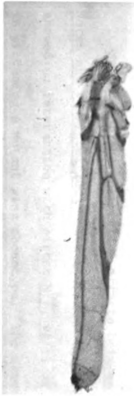

Figure 20. nd/Y; vg/+

Figure 21. nd/nd; vg/+

Figure 22: nd/nd; vg/+

Figure 23. nd/nd; vg/vg

veins I and II is excised, a deep notch extends into the area of veins III and IV, and the third posterior cell is completely removed.

The wings of the compound *N*^{69}/*fa*^{n}; *vg*/+ are also more reduced than those of the combination of any two of these mutants (Figure 25). The wing size is variable but is usually more reduced than that of *N*^{69}/*fa*; *vg*/+. The entire second and third posterior cells are removed along with vein V, the posterior crossvein, and portions of the discal cell.

The compound *N*^{69}/*nd*; *vg*/+ produces wings that resemble those of *vg*/*vg* (Figure 26). The compound *N*^{69}/*fa*^{no}; *vg*/+ is apparently lethal as attempts to obtain it were unsuccessful.

The wings of *N*^{69}/*spl*; *vg*/+ flies have the morphology typical of *N*^{69}/+; *vg*/+, confirming the failure of *spl* to show any effect on wing excision.

Summary of the interaction effects between alleles of the Notch and vestigial loci: The recessive Notch pseudoallele *spl* showed no interaction with either *N*^{69}, *vg*/+, or both *N*^{69} and *vg*/+ in producing a mutant wing phenotype. The other recessive Notch pseudoalleles showed an interaction with *vg* genotypes. An arbitrary scale of phenotypic classes was devised. Genotypes with no notching of the wing were assigned to the phenotypic class 0, and a value of 6 was given to the most strongly affected wings. The degrees of excision between these two extremes were assigned to classes 1 through 5. Although the phenotypes resulting from any one genotype

Figure 24.
$N^{69}/fa;vg/+$

Figure 25.
$N^{69}/fa^{n};vg/+$

Figure 26. $N^{69}/nd;vg/+$

varies, the typical phenotype for every genotype clearly fell within one of the phenotypic classes, except that genotypes with penetrance incomplete, but greater than one percent, were automatically assigned to class 1.

Wing excision for any Notch locus genotype increases as the vestigial locus genotype is changed from +/+ to _vg_/+ to _vg_/_vg_. It has been found that if the phenotypic classes are plotted along an arbitrary scale, the difference between the point described by the +/+ genotype, the _vg_/+ genotype, and the _vg_/_vg_ genotype can be shifted by a constant amount for every Notch locus genotype. This is illustrated in Figure 27, in which the distance between the +/+ and the _vg_/+ genotype is 3/13 as long as the distance between +/+ and _vg_/_vg_. With this arbitrary scale, all genotypes accurately indicate the effect of the interaction of the _vg_ and Notch loci.

The widths of the columns representing the phenotypic classes were dictated by the class values assigned to each genotype that was observed. The arbitrary nature of the assignment of widths is justifiable because there is no physiological or morphological basis for the distinctions between the phenotypic classes: they were chosen because of the ease of defining the degree of change from one class to the next. There was no intention of implying that the underlying basis for the difference between adjacent classes is directly related to a simple increment in the physiological conditions that determine the extent of wing excision.

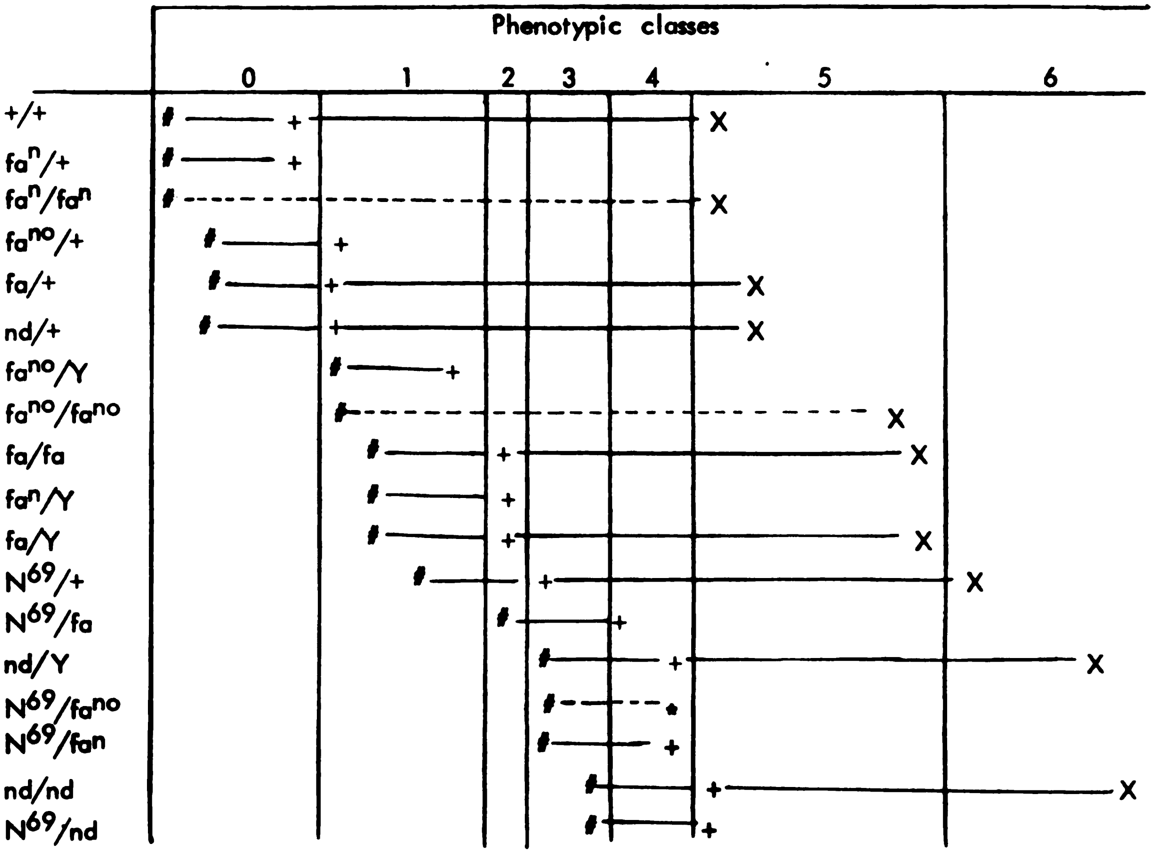

Figure 27: Graphic summary of the interaction observed between <u>vg</u> genotypes and genotypes of the Notch locus.

#	Notch locus genotype with +/+
+	Notch locus genotype with <u>vg</u>/+
X	Notch locus genotype with <u>vg</u>/<u>vg</u>
•	Apparently lethal genotype
-----	Phenotype with <u>vg</u>/+ unknown

Figure 27 leads to an important interpretation. The fact that the distance along the scale from the +/+ genotype to the *vg*/+ genotype to the *vg*/*vg* genotype is the same for all Notch locus genotypes suggests that the interaction between the Notch and vestigial loci is constant. Thus, one could describe the interaction of these two loci in terms of the effect of the Notch locus as providing a basic contribution to the excision value of the genotype, to which the *vg* locus contributes *additively* to the excision effect.

Six alleles at the Notch locus have been studied in detail: +, *fa*, fa^{n}, fa^{no}, *nd*, and N^{69}. The various genotypes that can be constructed from these alleles give a varriety of wing excision phenotypes when combined with +/+ and *vg*/+. An analysis was performed to determine whether these phenotypes indicate consistent interaction effects, or whether certain genotypes have phenotypes that would not have been expected from the study of the other available genotypes.

The order of the genotypes along the side of Figure 28 is determined largely by the increasing phenotypic effects in combination with +/+ and *vg*/+. The alleles along the top start with +, which is the allele contributing toward normal wing size, and end with *nd* and N^{69}, which are the alleles that contribute the most toward wing excision. In between, fa^{n}, *fa*, and fa^{no} have been placed in that sequence because this results in the top part of the figure showing a pattern that suggests orderability of effects of the genotypes on the amount of wing excision. If the combining qualities

	Notch locus alleles						Phenotypic values	
Genotype	+	fa^n	fa	fa^{no}	nd	N^{69}	+/+	vg/+
+/+	X						0	0
fa^n/+	X	X					0	0
fa/+	X		X				0	1
fa^{no}/+	X			X			0	1
nd/+	X				X		0	1
fa^{no}/Y				X			1	1
fa^n/Y		X					1	2
fa/fa			X				1	2
fa/Y			X				1	2
N^{69}/+	X					X	1	3
N^{69}/fa			X			X	2	4
N^{69}/fa^n		X				X	3	4
N^{69}/fa^{no}				X		X	3	*
nd/Y					X		3	4
nd/nd					X		3	5
N^{69}/nd					X	X	3	5

Figure 28. The failure of the Notch locus alleles to have clear orderability in the production of wing excision.

The top portion of the figure contains genotypes that have no excision in +/+. The middle portion includes genotypes with moderate excision with +/+ and vg/+, and the bottom portion contains genotypes with strong excision with vg/+. The figure has been arranged to show orderability in the top portion. It cannot be rearranged to show order in all three portions simultaneously.

* = Genotype apparently lethal.

of these three alleles were constant, the middle and bottom sections of the figure would also show this orderability. Instead, there are two separate exceptions to orderability: fa^{no}/Y and N^{69}/fa^{n}. The problem of ordering arises because fa^{n}/+; vg/+ has less excision than the comparable genotype with fa or fa^{no}, fa^{no}/Y; vg/+ has less excision than fa or fa^{n} in comparable genotypes, and N^{69}/fa; +/+ has less excision than the comparable genotypes with fa^{n} or fa^{no}. Thus, each of these three alleles is the least effective contributor to wing excision in one of its possible genotypes. This means that there must be at least two exceptions to any system for ordering these three alleles according to their effects on wing excision: their effects are not consistent in all combinations.

Other Loci and Vestigial

A search was conducted for clear interactions between vg and mutants other than at the Notch locus. The mutants bifid (bi), clipped (cp), cut-notch (ct^{n}), cut-six (ct^{6}), notchy (ny), miniature (m), scalloped (sd), and short wing (sw) were selected for investigation. Table 7 summarizes the results of crosses of these mutants to wild type and to the vg stock. The results indicate that all of the mutants are completely recessive in the heterozygous state, except cp, like vg, has a slight penetrance of notching in heterozygous females. The results of crosses of these mutants to the vg stock suggest that vg/+ may interact with many other wing size mutants by increasing the frequency of notching, although most of these interaction effects are of a small order.

Table 7. Penetrance of notching in crosses of various wing mutants to wild type and vg.

	x Ore-R		x vg		
Mutant	*/Y; +/+	*/+; +/+	*/Y; vg/+	*/+; vg/+	N^{69}
None	0.0	0.0	0.0	0.5	+
bi	44.6	0.0	54.5	0.0	+
cp	0.0	0.6	0.0	3.3	-
ct^{n}	100.0	0.0	100.0	2.5	+
ct^{6}	100.0	0.0	100.0	5.0	-
m	0.0	0.0	0.0	2.9	-
ny	18.6	0.0	23.1	2.7	-
sd	100.0	0.0	100.0	0.4	+
sw	0.0	0.0	1.6	0.0	-

* represents the mutant gene listed in the first column. Because cp is on the third chromosome, */Y and */+ indicate +/Y; cp/+ and +/+; cp/+ respectively.

All values are based on a minimum of 100 flies.

In addition to the genotypes listed in Table 7, the other possible genotypes for each mutant with *vg* were also examined. The only major interactions that were observed were with *sd* and *bi*. Each of the mutants listed in Table 7 was also crossed with the N^{69} stock to look for possible interaction. An interaction was found with *sd* and with *bi*. The interactions of *sd* and of *bi* with *vg* and N^{69} are described below. A possible interaction may also occur with ct^{n}. The wings of the genotype N^{69}+/+ ct^{n} have apical notching that is similar to that seen in the more extreme examples of N^{69}/+, but do not exceed its range of excision effects. This slight interaction will not be discussed further.

Scalloped: The margins of the wings of *sd* males and females are scalloped by excisions (Figure 29). The extent of the excisions is variable, but all of the wings are affected in the marginal areas between veins II and IV. About 50 percent of the wings are also affected by excisions in the marginal areas between veins I and II and veins IV and V. Less than half the wings have excisions along the border of the third posterior cell.

The genotype *sd*/+; *vg*/+ is usually wild type in phenotype. Close examination reveals, however, that about 5 percent of the wings have a partially-formed second anterior crossvein. The small incidence of notching, which occurs between veins III and IV, may indicate the presence of minor autosomal notching genes in the stock because the non-*sd* males, +/*Y*; *vg*/+, from the same cross and +/*Y*; +/+ males from an outcross to wild type also show a small incidence of notching (0.56 and 0.57 percent respectively).

As would be expected, the *sd*/*Y*; *vg*/+ males all have scalloped wings (Figure 30), but close examination of these wings reveals that the degree of excision is greater than in *sd*/*Y*; +/+ males. The marginal areas between veins I and II and veins IV and V, which were affected only about one-half the time in *sd* males, are always affected in *sd*/*Y*; *vg*/+ males. The excision along the anterior border between veins I and II is particularly greater in degree than in *sd* males. The margin along the third posterior cell is also more frequently affected with about two-thirds of the wings having excisions here as compared to less than one-half for *sd* males. Females of the genotype *sd*/*sd*; *vg*/+ show the same excision pattern as the *sd*/*Y*; *vg*/+ males.

The homozygous vg genotypes (*sd*/*sd*; *vg*/*vg*, *sd*/+; *vg*/*vg*, and *sd*/*Y*; *vg*/*vg*) all have wings that look like small *vg* wings. The halteres and postscutellar bristles resemble those of *vg*.

The compounds N^{69}+/+ *sd* and N^{8} +/+ *sd* were formed. The wings of the compound with N^{69} show slight interaction effects. The margin between veins I and II, which is unaffected in N^{69}/+ flies, shows excision in about 36 percent of the wings (Figure 31). On the other hand, the wings of the compound with N^{8} appear typically Notch with no excisions between veins I and II. The wings of the compound N^{69}+/+ sd; *vg*/+ largely resemble those of N^{69}/+; *vg*/+ (Figure 32).

Bifid: The *bi* mutant causes the longitudinal veins to be fused at their bases into a bifid stalk (Figure 33). In addition,

Figure 29.
sd/Y; +/+

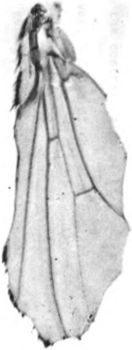

Figure 30.
sd/Y; vg/+

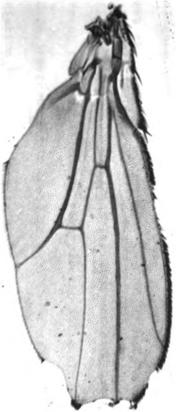

Figure 31.
N^{69} +/+ sd; +/+

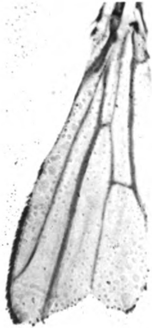

Figure 32.
N^{69} +/+ sd; vg/+

veins III ends in a delta at the wing margin and notching of the wing tip frequently occurs in males. The wings are also held out somewhat from their normal position.

The vein effect has complete penetrance in bi flies, but the notching effect shows considerable variation. The bi/bi females show no notching at all. The bi/Y males had a variable notching incidence depending on the origin of their father. In the bi stock the incidence of notching was 10 percent, but outcrosses of bi/bi females produced bi/Y males with notching incidences of 22.7 percent for a cross to sd, 44.6 percent for a cross to wild type, and 54.5 percent for a cross to vg. The reason for such a wide variation in these values is unknown.

There is not a clear interaction of bi and vg in genotypes with heterozygous vg. Although many of the bi/Y; vg/+ males have notched wings, their incidence of notching does not differ greatly from the control value of bi/Y males from an outcross to wild type. However, because of the wide variation in the values for wing notching in bi/Y control males, the possibility of interaction in this genotype to produce an increase in wing notching cannot be eliminated. The bi/bi; vg/+ females show slightly greater notching than vg/+ alone. Flies of bi/+; vg/+ are indistinguishable from wild type.

Combinations of bi with homozygous vg produce wings that depart strikingly from the vg phenotype. Wings of bi/+; vg/vg females (Figure 34) are usually long, narrow, and much larger than those of

Figure 33. bi/bi; +/+.

Figure 34. bi/+; vg/vg.

vg females. Unlike vg wings, vein II is complete, reaching the normal wing margin, which is present for short distances on each side of the vein. The anterior crossvein is usually present along with portions of the submarginal cell, the first posterior cell, and the discal cell. Also, about one-fourth of the wings have part or all of an additional anterior crossvein present. The halteres are also affected by the presence of the bi gene; they have all three segments present, although the distal segment, the capitellum, is reduced considerably in size from normal.

The addition of one more dose of bi to form bi/bi; vg/vg females produces further changes in the morphology of the flies away from the vg phenotype (Figures 35 and 36). The wings may be the long, narrow wing of bi/+; vg/vg females, but more often they have a 'lobster-claw' shape or some gradation between it and the long, narrow wing. The 'lobster-claw' wing extends to the normal wing margin in the area of vein II, but then is deeply incised at vein IV with vein V and the adjacent wing areas extending out in a lobe. Like bi/+; vg/vg, part or all of an extra anterior crossvein may be present. Vein II may be interrupted and not reach the marginal vein. The halteres are close to normal in appearance, but the capitellum, although larger than in bi/+; vg/vg flies, is still somewhat reduced from wild type.

The bi/Y; vg/vg males usually have the 'lobster-claw' type of wing (Figure 37). This wing may be larger, vein V somewhat longer, and more of vein IV may be present in the males than in the

Figure 35. bi/bi; vg/vg

Figure 36 .
bi/bi; vg/vg

Figure 37. bi/Y; vg/vg

females. The halteres appear to be completely normal in size and form in these males.

In all combinations of *bi* with homozygous *vg* the postscutellar bristles are erect and apparently unaffected by the presence of *bi*.

In order to look for the possible interaction of bi with dominant Notch alleles, the compounds $\underline{N}^{69}$+/+ *bi* and $\underline{N}^{8}$ +/+ *bi* were made. About 5 percent of the $\underline{N}^{69}$ wings show no notching of the wing tip, but about 40 percent of the wings of $\underline{N}^{69}$+/+ *bi* have no notching of the wing tip (Figure 38). The presence of *bi* in $\underline{N}^{69}$+/+; *bi*; *vg*/+ apparently has no effect upon the phenotype (Figure 39), but $\underline{N}^{69}$+/+ *bi*; *vg*/*vg* wings (Figure 40) are *vg* in appearance rather than the very small wing typical of $\underline{N}^{69}$/+; *vg*/*vg*. The reduction of the notching effect of the dominant Notch mutants when combined with bi is even more evident in the genotype $\underline{N}^{8}$ +/+ *bi* where about 85 percent of the wings have no notching as compared to 11 percent without *bi*.

Summary of the interaction effects of sd and bi with vg and N: The *sd* mutant, like the mutants of the Notch locus, also tends to act with heterozygous *vg* to produce a wing reduced from wild type. For wing excision, this interaction is recessive for the *sd* locus as *sd*/+; *vg*/+ is similar to +/+; *vg*/+. The interaction is intermediate for the *vg* locus as *sd*/*Y* or *sd*/*sd* with *vg*/+ is much more affected than the same sd genotypes with +/+ and less than with *vg*/*vg*.

As well as interacting with *vg*, *sd* also shows small interaction effects with $\underline{N}^{69}$. In its interaction with both *vg* and $\underline{N}^{69}$, *sd*

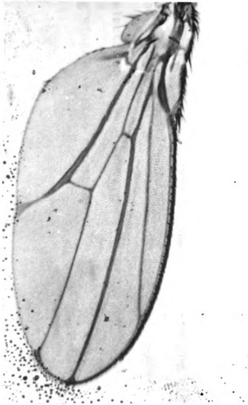

Figure 38. N^{69}+/+ bi;+/+

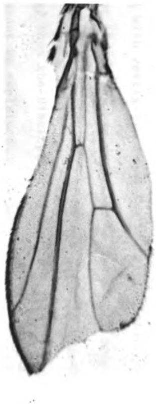

Figure 39.
N^{69}+/+ bi; vg/+

Figure 40. N^{69}+/+ bi; vg/vg

tends to have its strongest excision effect on the anterior margin of the wing.

The mutant *bi* may increase the incidence of notching in combinations with *vg*/+. However, in contrast with all other studied mutants that produced interactions, with *vg*/*vg* *bi* causes the wings and halteres to be increased in size toward normality. Like *vg*, the *bi* gene has an intermediately dominant effect, with *bi*/+ females having more wing area than +/+ and less than *bi*/*bi* when combined with *vg*/*vg*.

Contrary to these observations, the combination of *bi* with *vg* was examined by Waletzky (1939) who reported that there was no detectable effect from the combination. He also reported that disproportionately small wings were formed in the combination of *bi* with vestigial-nipped (vg^{np}), a weak vestigial allele. It is not clear from his report if he formed the same genotypes that were formed in this study. If he did form the genotypes with homozygous *vg*, then the discrepancy between his results and those found here cannot be explained.

Unidentified Modifiers and *vg*

It has been found that certain mutant genes combined with *vg*/*vg* can push the *vg* phenotype further away from the wild type phenotype, and that another mutant gene can push the *vg* phenotype toward normal. Selection within a homozygous *vg* stock for 'long' *vg* wings and 'short' *vg* wings was also attempted.

Because the *vg* stock used in this study was found to contain little variability upon which selection could be carried out, *vg* males were outcrossed to wild type, Ore-R, females. Pairs of the resulting heterozygotes were mated and their progeny observed for particularly long and short *vg* wings. From these crosses four stocks were developed with selection maintained each generation. Two of these stocks were selected for short *vg* wings as judged by their appearance under high power of a binocular microscope. The other two stocks were selected for long *vg* wings as judged by the presence of the anterior crossvein or an extension of vein II beyond the normal *vg* length. After twenty generations of selection, the short *vg* stocks did not clearly differ from the long *vg* stocks.

It was thought that the increase in wing size resulting from the interaction of *vg*/*vg* with higher temperatures might emphasize any differences between the long and short *vg* stocks, if differences actually were present. For this reason, flies from each stock were grown at 31° C from the egg to the adult stage. The wings of the resulting flies showed the expected increase in wing size toward normal. The wings of flies from the different stocks, however, showed a similar reaction to the temperature at which they were raised. Apparently selection within the *vg* lines was unsuccessful in definitely modifying the *vg* phenotype either toward or away from normal.

There are reports in the literature (Goldschmidt, for example) indicating that selection for genes which will modify the *vg*/+ effect to include wing notching can be successful. Such an attempt was made for this work. Outcrosses to wild type were made with each of the 'long' *vg* and 'short' *vg* stocks and the heterozygous *vg*/+ progeny were observed for wing tip notching. No notching was found among any of the progeny. Two non-selected *vg* stocks were also outcrossed to wild type and again no notching was found among the progeny (although other crosses of *vg* to Ore-R wild type that resulted in larger numbers of progeny did yield affected females).

Outcrosses of *vg* flies to three different stocks, *y*, *cn bw*, and *e*, were then made. The cross of a *vg* male to a *y* female yielded an affected female among the progeny. This female was mated to an affected male found among the progeny of a cross of a *vg* female to an *e* male. Only one affected female and no affected males were obtained from this cross. The affected female was mated to a *y* male resulting from the same cross. Several daughters of this cross had notching of the wing tips. These progeny were mated to *vg* males resulting from the same cross. Further selection on the progeny of one of these matings led to the establishment of a stock in which an affected female was mated to a *vg* male resulting from the same cross in each generation. Selection was continued for ten generations. At this time an affected female from the stock was mated to an affected male. The resulting progeny which were not *vg*/*vg* were 61 percent notched. The extent of the notching never exceeded excision between veins II and IV. The *vg*/*vg* flies resulting from this same cross appeared typically *vg*. Since these flies were assumed to be homozygous for the same modifying genes which led to notching of the _vg_/+ flies, it appears that these modifying genes were ineffective in modifying the _vg_/_vg_ genotype, although they were effective with _vg_/+.

Summary of unidentified modifiers of vg: Selection within _vg_/_vg_ stocks for genes which will modify the _vg_ phenotype toward or away from the normal _vg_ phenotype was not successful. Selection for genes which will modify the _vg_/+ phenotype away from normal can be successful, but these genes apparently do not have a strong enough effect to be readily identifiable in the _vg_/_vg_ phenotype.

Discussion

Phenotypic Series

Compounds formed of various combinations of alleles at the bifid, Notch, scalloped, and vestigial loci produce a wide spectrum of wing phenotypes. The genotypes formed from combinations of alleles at the Notch, scalloped, and vestigial loci can be arranged in a series of gradually increasing degrees of excision ranging from the wild type pattern of no excision (Pattern 0 in Figure 41) to almost complete absence of the wing (Pattern 6). Genotypes containing the *bi* gene produce a phenotypic series which interconnects with this series. An alternate set of phenotypes (Patterns 2a, 3a, and 4a) represents the observed departures from the series of patterns 0 to 6.

Series formed by combinations of alleles at the Notch, scalloped, and vestigial loci.

Patterns 0 to 6 have arbitrarily been chosen as representatives of the different degrees of excision. The excision patterns intermediate between each pair of representative patterns were also observed during the course of this study. No phenotypic pattern was found to be unique to any one genotype, but resulted from at least two different genotypes.

The least affected wing pattern always shows excision occurring first in the apical region of the wing. For the alleles of the Notch locus and for some of their compounds with *vg*/+, this excision typically occurs along the margin between veins III and IV. Occasionally, a wing of sd/sd or sd/Y flies may have only a single small excision in any part of the marginal area between veins II and IV.

Lateral excision appears to begin in the more proximal areas (Pattern 2) and then, with increasing degrees of excision, it extends toward the apex of the wing where the lateral and apical excisions appear to meet (Pattern 3). Further excision reduces the posterior area of the wing until a long, narrow wing composed primarily of vein II, part of vein III, and the immediately adjacent areas remains (Pattern 4). Further reduction of the wing occurs mostly in length, although slight lateral reducation also occur (Patterns 5 and 6). Complete absence of the wing did not occur on any fly of the genotypes observed, although flies of pattern 6 often had only a small stub of the wing remaining.

Harnly and Harnly (1935) and Harnly (1936) obtained a variety of wing shapes resulting from mutant allelic combinations at the vestigial locus and from the interaction of allelic combinations at various temperatures. They suggested that a series could be formed that would range from a vg phenotype to an almost normal wing. It appears from the Harnlys' descriptions that such a series of excisions resulting from combinations at the vestigial locus would differ only slightly from the wing pattern series described from the present study using alleles of the Notch, scalloped, and vestigial loci.

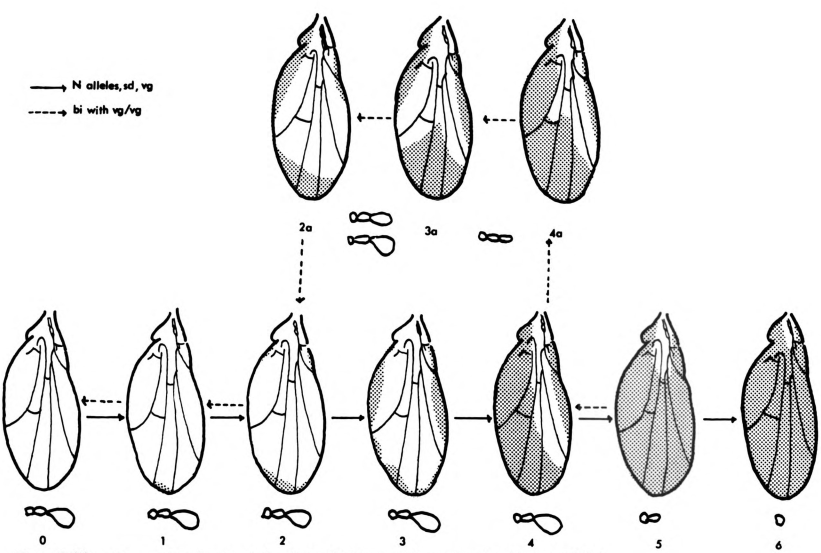

Figure 41. Wing patterns resulting from genotypes with the Notch locus alleles, scalloped, vestigial, and bifid.

Series formed by combinations with the bi gene.

Because the presence of the bi gene results in a definite reducation of excision in vg/vg flies, it seems natural to describe this series in terms of decreasing, rather than increasing, excision.

The smallest wing phenotype observed in the bi series resulted from the genotype N^{69}+/+ bi; vg/vg and resembled pattern 5 of Figure 41. None of the genotypes containing bi produced phenotypic patterns intermediate between patterns 5 and 4, making the bi series discontinuous at this point. The bi genotypes (bi/+, bi/bi, bi/Y) with vg/vg produce a series of phenotypes with gradually decreasing amounts of excision. This series of phenotypic patterns is represented by patterns 4, 4a, 3a, and 2a.

In this series, the long, narrow wing of pattern 4 appears to be increased in size by an addition of wing material along the area of vein V. Because excision still appears to cut deeply into the areas of veins III and IV, the wing has a strongly difurcated appearance (Pattern 3a). The most complete wing that occurs among the vg/vg genotypes with bi is one in which only the apex of the wing and small proximal lateral areas are absent. This wing is thus somewhat triangular in appearance (Pattern 2a).

None of the bi genotypes observed produced phenotypes resembling pattern 2, forming another discontinuity in this series. Patterns 1 and 0 result from bi genotypes combined with +/+ or vg/+.

Harnly and Harnly (1935) and Harnly (1936) have suggested that the wing patterns they observed among genotypes of the vestigial locus represent the sequence of patterns followed during the development of the normal wing. Thus, the excision patterns result because mutant gene action has caused development of the wing to be arrested at some stage of its formation. The present studies of the phenotypes resulting from the interactions between alleles of several loci indicate that at least one alternate pattern series can be described. This raises doubts concerning the assumption by the Harnlys that wing formation is related to a sequence of developmental patterns.

Phenotypic series of the halteres.

The halteres of Drosophila are modified wings. Their development from the dorsal metathoracic buds is similar to the development of the wings from the dorsal mesothoracic buds. It is not unexpected, therefore, when reduction of the wing also results in reduction of the size of the halteres.

The results of this study indicate, however, that the size of the haltere is not necessarily related to the size of the wing. Although the genotype *vg*/*vg* has both reduced wings and halteres, the genotype *nd*/*nd*; *vg*/+, which has *vg*-like wings, has halteres that do not appear to differ from normal.

Alteration of the haltere phenotype appeared to occur only in the presence of *vg*/*vg* in this study. Genotypes which increased the size of the haltere (Figure 41), and genotypes which further reduced the *vg*/*vg* wing also further reduced the haltere. This suggests that the size of the haltere is related to the size of the vestigial wing in vg/vg genotypes. Stanley (1935) also observed this by finding that when the wings of vg flies are increased in size due to development at high temperatures, the size of the halteres is also increased.

Excision Effects

Representation of the data.

Figure 27 represents a preliminary analysis of the data on the amount of wing excision produced by each of the observed genotypes. The most important conclusion derivable from that analysis is that for a given Notch locus genotype, the difference between +/+ and vg/+ is constant in terms of the variable scale of excision effects that was employed. Similarly, vg/vg consistently produces about four times as great a change in the excision effect as that produced by vg/+.

Figure 42 represents an attempt at analysis of all of the data that have been collected on the amount of wing excision for each genotype. The range of phenotypes produced by each genotype is represented by the length given to each of the bars. A phenotype that is only partially penetrant bisects the line between classes 0 and 1 in such a manner that the proportion of the bar in class 1 is equal to its penetrance. Similarly, the proportion of each bar in classes 1 and 2; 2 and 3; 2, 3, and 4; etc. indicates the proportion of expressivity of the genotype that is found in each of these

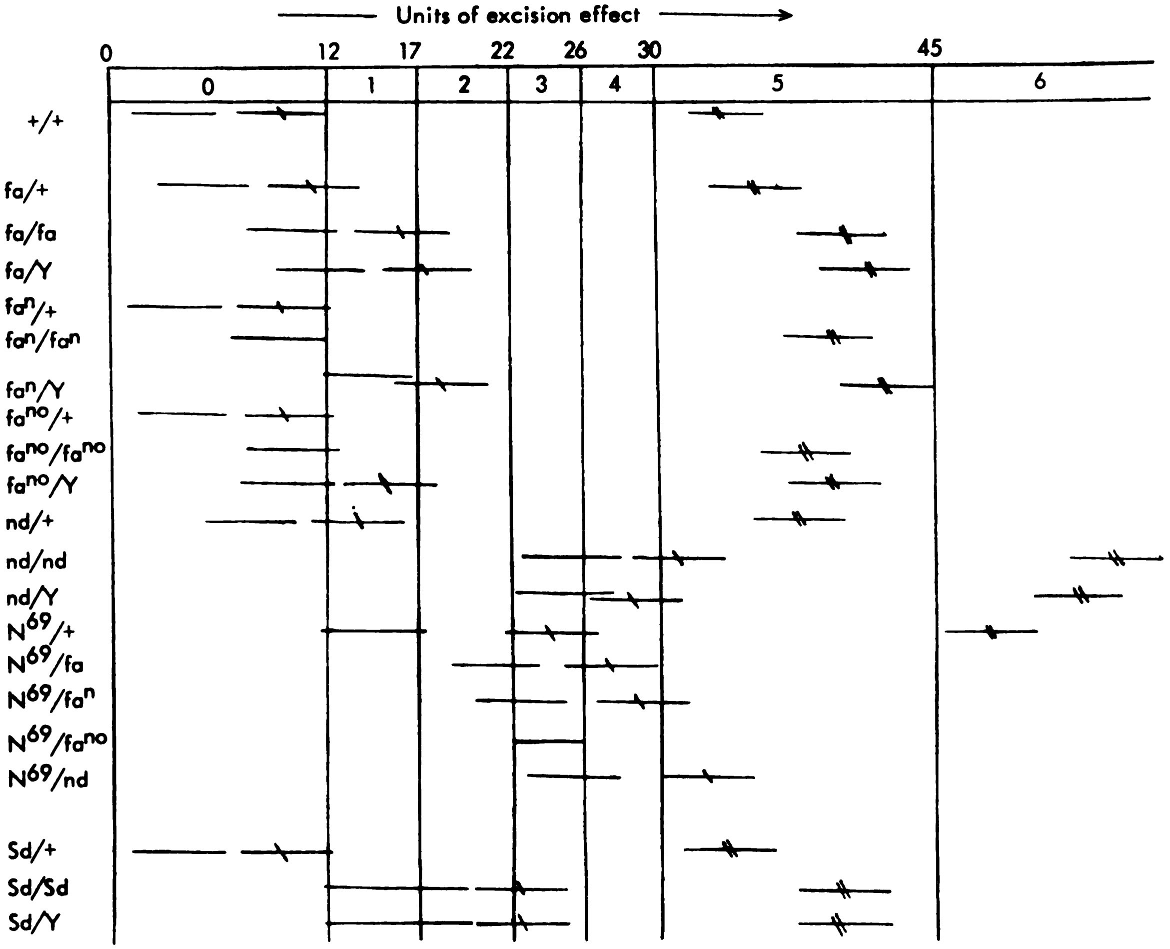

Figure 42 : Representation of the genetic interactions between the Notch, Scalloped, and Vestigial loci with an estimate of phenotypic variability.

—— +/+ at the vestigial locus

—+— vg/+

—++— vg/vg

phenotypic classes. No genotype has a discontinuous distribution of phenotypes that is discontinuous on this scale. Almost all of the bars have been given the same length, and the widths of the various classes have been adjusted to make this possible. A further constraint was placed on the class widths because of the attempt in Figure 42, as in Figure 27, to have a constant distance between +/+ and *vg*/+ and between +/+ and *vg*/*vg* for their combination with all Notch locus and scalloped locus genotypes.

These constraints interact to determine the width of each of the classes. For example: (1) N^{69}/*fa*; +/+ has a mean phenotype of 2 and N^{69}/*fa*; *vg*/+ has a mean phenotype of 4, and (2) N^{69}/+; *vg*/+ generally produces wings in phenotypic class 3, but some wings are in classes 2 and 4.

Exceptions

All of the data are not consistent with the scale used in Figure 42. An exception will occur if it is necessary to represent the phenotypic range of a given genotype by a bar that is either longer or shorter than the standard bar, or if the distance along the scale between +/+ and *vg*/+ or between +/+ and *vg*/*vg* is more or less than the standard distance that has been used in the figure. The obvious exceptions in Figure 42 are:

(1) N^{69}/fa^{no}; +/+ produces flies with wings only of class 3, and its phenotypic range is shorter than standard,

(2) N^{69}/+; +/+ produces wings that range from class 0 to class 2, so that its phenotypic range is greater than standard,

(3) the change in excision effect is greater between N^{69}/+; +/+ and N^{69}/+; *vg*/+ than for any of the other Notch locus genotypes,

(4) the change in excision effect is less between *nd*/*Y*; +/+ and *nd*/*Y*; *vg*/+ and between fa^{n}/*Y*; +/+ and fa^{n}/*Y*; *vg*/+ than for any other Notch locus genotypes,

(5) wings of *sd*/*sd*; +/+ and *sd*/*Y*; +/+ have a larger range of phenotypes than any of the other genotypes, and

(6) *sd*/*sd*; +/+ and *sd*/*Y*; *vg*/*vg* are predicted to be in class 6 if it is assumed that the change in excision effect is constant between +/+ and *vg*/*vg* for all loci, but these wings are in class 5.

If all the bars in Figure 42 were of the same length, it would indicate that the environmental and residual genetic variability for all genotypes is of the same order of magnitude. The one short bar (exception 1) may have been due to the small number of wings observed for that semi-lethal genotype. Of the bars that are longer than standard (exceptions 2 and 5), N^{69}/+; +/+ wings largely fall into a narrow phenotypic range near the border between class 1 and class 2, but a very few wings show no excision and so are of class 0. This range of phenotypes is clearly greater than the standard, and, because both wings of a fly are never of class 0, is apparently due largely to environmental factors that vary within a single individual, rather than to residual genetic variability. The great phenotypic range of *sd* stock flies suggests that the factors governing variability, which are relatively constant for all Notch locus genotypes, do not affect the *sd* locus in a comparable manner.

All of the other data represented in Figure 42 are consistent with the assumption that the phenotypic range is constant for all genotypes. From this, it would appear reasonable to conclude that for almost all of the genotypes observed, the phenotypic variability due to the environment and the uncontrolled portion of the genotype is of the same order of magnitude when measured on the scale of excision effects used in Figure 42.

If the change in amount of excision effect was constant for a given Notch locus or scalloped locus genotype when comparing compounds of +/+ with _vg_/+ or +/+ with _vg_/_vg_, it would be possible to interpret the interactions of these loci in a simple manner. Exceptions 3, 4, and 6 are in conflict with a simple interpretation. No obvious explanation can be given to account for exceptions 3 and 4. None of these exceptions is a major departure from the change in phenotype generally seen between +/+ and _vg_/+. Exception 6, like exception 5, suggests that the general rules observed for the Notch locus do not apply to the _sd_ locus.

All of the other genotypes represented in Figure 42 are consistent with the assumption that the change in excision effects for a given Notch locus genotype in combination with +/+ or _vg_/+ is constant, and that this is also true for the change between +/+ and _vg_/_vg_. From this, it would appear to be reasonable to conclude that the scale of excision effects used in Figure 42 generally measures a factor that is changed in a consistent manner by a change of genotype from +/+

to vg/+ to vg/vg.

Analysis

The goal of the analysis of these data is the development of a consistent theory of the role of various factors in the production of wing excision. The analysis has been dependent on the appropriate choice of a scale of "units of excision effect." The scale, as shown in Figure 42, appears to be appropriate, because, through its use, consistencies have been observed. For the purposes of the following discussion, it will be assumed that the exceptions that involve Notch locus alleles are accidental or trivial, and that the conclusions pertaining to the Notch and vestigial loci are correct. These conclusions are (1) for all genotypes the phenotypic variability due to the environment and the uncontrolled portion of the genotype is of the same order of magnitude in units of excision effect, and (2) for any Notch locus genotype, genotypic substitutions from +/+ to vg/+ or to vg/vg are accompanied by shifts of constant amounts in units of excision effect. In addition, it appears that these conclusions do not pertain directly to genotypes involving the sd locus.

"Units of excision effect" probably represent some definite quantitative change in the physiology of an individual fly. The scale of "units of excision effect" has changed the arbitrary identification of the phenotype in terms of classes 0 to 6 into a scale that apparently measures the underlying functional factors causing wing excision.

If this analysis is correct, then the effects of genetic interactions and of the residual genetic and environmental variability have been described in a single system of "units" of activity leading to wing excision. Thus, the range of phenotypic variability that has been ascribed to a single genotype is five units of excision effect, while for a given Notch locus genotype, the midpoint of its phenotypic range is increased by six units in going from +/+ to vg/+ and by 30 units in going from +/+ to vg/vg.

According to this interpretation, there must be at least 12 units of excision effect before the combination of genotype and environment will produce some wing excision, making the phenotype penetrant. Twelve units is, therefore, the lower threshold for wing excision. Below this, the wing is canalized into a normal phenotype. The widths of phenotypic classes 1 and 2 are each 5 units and classes 3 and 4 are each 4 units. In contrast with this, class 5 has a width of 15 units, and class 6 has no known upper limit, because these observations have not included any flies that are completely without wings. The great width of classes 5 and 6 in comparison with classes 1 through 4 suggests that there is a strong "developmental pressure" on the organism to produce at least some portion of the wing. This apparent damping effect on variability at the extremes of a phenotypic range has been described for other characteristics. Because the choice of the limits of the phenotypic classes was arbitrary, it is also possible that class 5 should have been subdivided, but the class designations appeared to fall into a natural series.

Three lines of evidence indicate that the "excision effect" is a complex characteristic. (1) The scalloped genotypes do not fall along the scale of excision units in the pattern determined by the interaction of Notch locus and vestigial genotypes (exceptions 5 and 6). This suggests that the apparently simple additivity of Notch and vestigial effects is of a different quality than the excision effects produced by the _sd_ locus genotypes. (2) The genotypes produced by combinations that include bifid do not fall along the same system of phenotypic patterns as the phenotypes produced by the other loci. Furthermore, the range of phenotypic classes produced by some genotypes that include _bi_ appears to be greater than the range for Notch locus genotypes. In addition, the _bi_ mutant apparently contributes to wing excision in some genotypes but to a greater amount of wing surface in _vg_/_vg_ genotypes. (3) The various pseudoalleles at the Notch locus cannot be assigned constant values that express their contribution to wing excision. As previously noted (see Figure 28), the amount of excision produced by facet, facet-notched, and facet-notchoid varies according to the allelic combination in which it occurs.

Summary

The purpose of this study was to examine the phenotypes produced by the interactions of various wing excision mutants and to utilize these phenotypes in the analysis of some factors that lead to wing excision.

Clear interactions that resulted in increased degrees of wing excision were found between some of the alleles of the Notch locus and vestigial (*vg*), between scalloped (*sd*) and vestigial, and between Notch-69 (N^{69}) and scalloped. In contrast, bifid (*bi*) was found to act as a partial suppressor of the wing excision effects of both N^{69} and *vg*/*vg*.

Compounds formed from these various genes result in a wide spectrum of wing phenotypes ranging from the wild type to a basal stub of the wing. It was found that these various phenotypes can be arranged into two interconnecting series of phenotypic patterns. Compounds of alleles of the Notch, scalloped, and vestigial loci form a continuous series of increasing degrees of excision. Compounds formed between *bi*/+, *bi*/*bi*, and *bi*/*Y* with homozygous *vg* result in phenotypic patterns which depart from this series forming an alternate but somewhat parallel series of phenotypes.

For further analysis of these phenotypes, an arbitrary scale ranging from 0 for the wild type wing to 6 for the most reduced wing was devised, and each genotype formed from alleles of the Notch and vestigial loci was assigned an average phenotypic value from this scale. It was found that the scale could be varied in

such a way as to suggest constant additivity for excision effects for all Notch locus genotypes when comparing compounds of +/+ with _vg_/+ and +/+ with _vg_/_vg_.

A more complete analysis of all of the data collected led to the construction of a scale of units of excision effect. This scale was constructed in such a manner that the constant nature of the additivity of excision effects of +/+, _vg_/+, and _vg_/_vg_ with the Notch locus genotypes was maintained. A further restriction was imposed on the construction of the scale so that the phenotypic range of each genotype would be constant.

The scale thus constructed led to some exceptions, but it was concluded that for any Notch locus genotype the change in excision effect from +/+ to _vg_/+ is a constant number of "excision units," and that the change in effect is also constant between +/+ and _vg_/_vg_. A second conclusion drawn is that, for almost all of the Notch locus genotypes, the phenotypic range is of the same order of magnitude when measured against the scale. It was further concluded that the _sd_ locus genotypes cannot be described by this same scale.

The scale of units of excision effect, which apparently measures some developmental change which leads to wing excision, can be used to describe certain genotypic differences as well as the phenotypic variability that results from the environment and uncontrolled portion of the genotype. The effects of the genotype and of the environment that lead to the production of a phenotype can, therefore, be expressed in terms of the same units.

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