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Genetic assimilation of part of a mutant phenotype

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SUMMARY

When the mutant *veinlet* was removed from a *Drosophila melanogaster* line selected for shortened vein length it was found that the selected polygenic background produced vein gaps in the absence of the major mutant. This example of genetic assimilation was unusual, however, in that the phenotype of the assimilated line was not exactly the same as the phenotype of the original selection line. It differed in two respects: only one of the longitudinal veins was affected in the assimilation line whereas all veins are shortened in the selected *ve* line, and vein gaps were subterminal in the assimilated line whereas they are terminal in *veinlet*. Modifiers of L 4 vein length were found to be located on both chromosomes II and III, though the chromosome II factor had a larger effect and was required for gap formation. The chromosome II gene was mapped to the same position as a similar L 4 vein length polygene (*PL(2)L4a*) found in another *veinlet* selection line and reported elsewhere. The pattern of expression of the *ve* mutant and the assimilated line genotype were compared using profiles of vein frequency at given points. The vein pattern profiles are discussed in relation to an hypothesis to explain the observed selection response and the effectiveness which these polygene combinations showed in modifying the much more complex *veinlet* phenotype.

1. INTRODUCTION

Environmental stimuli, such as temperature shocks, often permit discrimination among genotypes which otherwise give similar phenotypes. After a number of generations of selecting extremes, the phenotypic change is sometimes expressed in the absence of the stimulus. This has been called genetic assimilation (Waddington, 1953) or selection for subthreshold differences (Stern, 1958) and is probably best known through the work of Waddington (1953, reviewed 1961), Milkman (reviewed 1970) and others on crossveinless phenocopies, and the work of Waddington (1956) on bithorax phenocopies in *Drosophila melanogaster*.

Major mutants may also permit identification of combinations of polygenes which affect expression in particular ways. This is formally equivalent to the use of environmental stimuli to discriminate among genotypes, and has led to assimilation of mutant-related phenotypes in experiments on vibrissae number in mice

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(Dun & Fraser, 1958, 1959), scutellar bristle number in *Drosophila* (Rendel, 1959, 1968), and L 4 vein interruptions in *Hairless* mutant *Drosophila* (Scharloo, 1964).

The phenotype of an assimilated line is normally similar to the phenotype produced by the environmental stimulus or mutant gene. In a recent study of selected vein mutant lines of *Drosophila melanogaster*, however, we have found an example in which the selected mutant phenotype and the assimilated phenotype differed in an important respect. The third chromosome recessive *veinlet* (*ve*) is characterized by terminal gaps in each of the longitudinal veins (Plate 1). In crosses between a wild type stock and a *ve* line that had been selected for short veins, it was found that a high proportion of the F_1 *ve*/+ heterozygotes had subterminal gaps or thin areas in the fourth longitudinal vein (L 4). There were also a few terminal gaps in vein L 5. After seven generations of backcrossing such heterozygotes to the *ve* Short selection line, a derivative +/+ line was established. Progeny-testing showed that no *ve* alleles remained. In this derived line, however, a large proportion of flies had subterminal gaps in the L 4 veins. All other veins were wild type.

The occurrence of vein gaps in this +/+ line is an example of genetic assimilation. However, the assimilated phenotype differs from the mutant (*ve*/*ve*) phenotype in two respects. First, the *veinlet* mutation affects all of the longitudinal veins, whereas in the assimilated line only the L 4 is affected. Secondly, in mutant homozygotes at low expression the gaps in the veins are always terminal, whereas the L 4 vein gaps in the assimilated line are not terminal.

With respect to the fact that only vein L 4 is affected in the assimilated line, it is only part of the mutant phenotype that is assimilated, and there is a need to understand how the background selected to enhance *veinlet* expression should have such specific effects. With respect to the position of the gaps in vein L 4 there is a need to understand how selection to increase the length of terminal gaps should produce polygene combinations that induce gaps elsewhere, but not terminally. Complex interaction may be involved, and attempts to understand these relationships are the subject of this paper.

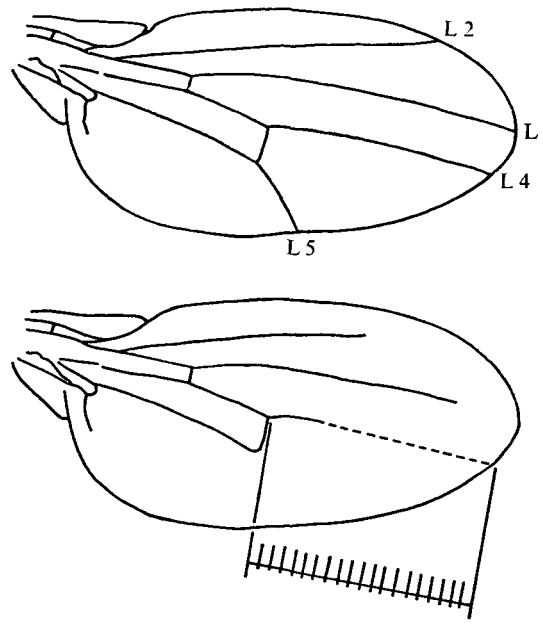
2. MATERIALS AND METHODS

The origin of the *veinlet* selection line has been described by Thompson (1974). Briefly, *ve* was outcrossed to a newly captured wild stock and resegregated in the F_2 to provide a base population for selection. Four replicate lines were then selected for reduction in the lengths of the L 2, L 3 and L 4 veins. All lines responded readily to selection as described by Thompson (1974). One of these four lines was used for the assimilation studies reported here. Vein nomenclature is shown in Text-fig. 1.

In addition to the *ve* Short selection line, the following stocks have been used: Oregon wild type, Eversden wild type (the newly captured stock to which *ve* was outcrossed before selection), and inbred marker stocks carrying the recessive mutants *yellow* (*y*, 1–0·0), *dumpy* (*dp*, 2–13·0), *black* (*b*, 2–48·5), *cinnabar* (*cn*, 2–

57·5), *curved* (*c*, 2–75·5), *brown* (*bw*, 2–104·5), and *scarlet* (*st*, 3–44·0). See Lindsley & Grell (1967) for descriptions.

Selection lines and crosses were maintained at 25 ± 1 °C on an agar-base oatmeal and molasses medium seeded with a suspension of live yeast. Vein lengths were measured on mounted wings as described by Thompson (1974). As in previous reports, vein lengths are expressed as the sine transformed ratio of vein length to total wing length unless otherwise stated.



Text-fig. 1. Wings of *Drosophila melanogaster*. Top, nomenclature for longitudinal veins. Bottom, *veinlet* wing showing region for which vein expression profiles were calculated. The L 4 vein from the tip of the wing to the intersection of the posterior crossvein was divided into 20 equal segments. See text for further discussion.

Vein profiles were made with a camera lucida. Mounted wings were projected on to graph paper ruled into 20 equal divisions. The graph was then arranged to divide the L 4 vein (from the distal tip to the intersection of the L 4 and posterior crossvein, see Text-fig. 1) into 20 equal units. This automatically corrects for variations in fly size. The presence or absence of vein material in each of these units was then recorded and summed for the whole sample (cf. House, 1953).

3. BACKCROSS OF *ve* SHORT HETEROZYGOTES AND ASSIMILATION OF A GAP PHENOTYPE

The *veinlet* selection line responded readily to selection, and by generation 12 the L 4 length had decreased significantly and the response had begun to plateau. A characteristic phenotype is shown in Plate 1. Only a very small piece of vein remains distal to the posterior crossvein.

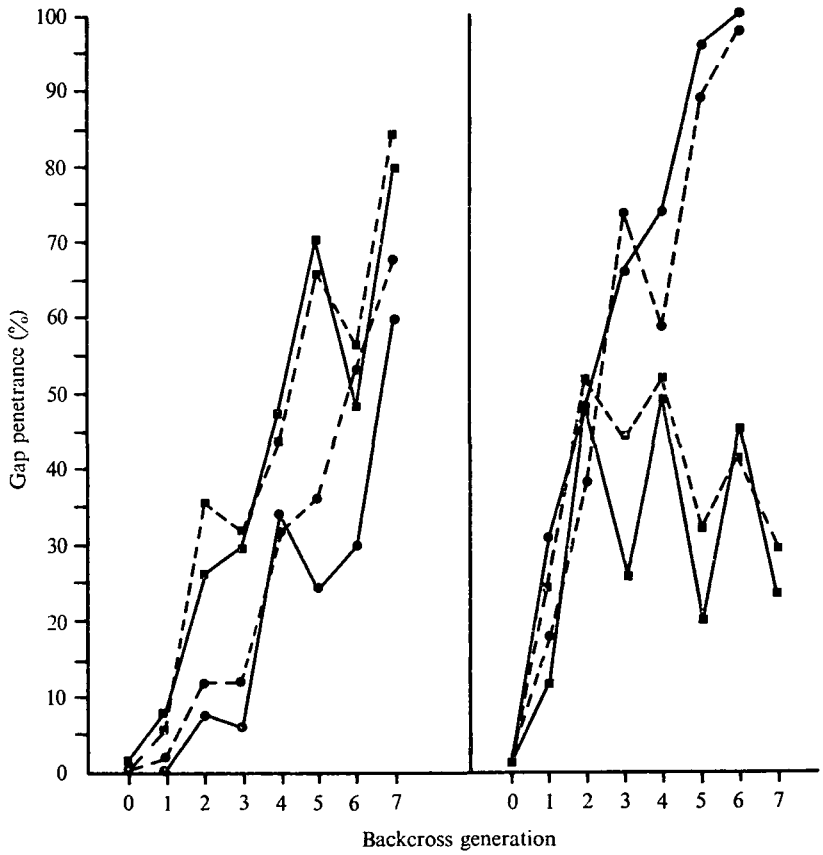
As part of another study (Thompson & Thoday, 1972; Thompson, 1974) *ve* Short selection lines had been crossed to wild-type stocks. In these crosses it was found that a significant proportion of the F_1 heterozygotes had gaps in the L 4 vein and in the tip of the L 5 vein. In one such cross, for example, 25 out of 300 females and 39 out of 300 males had gaps in the L 4, L 5 or both veins. No gaps were found in progeny from control crosses made with the unselected *ve* stock or *ve* Long selection lines. Thus, it is clear that selection had accumulated modifiers which allowed part of the *veinlet* phenotype to be penetrant in heterozygotes.

It should be pointed out before we go further that the word 'part', describing the mutant phenotype, will be used in two different ways in this and later discussions, for, as pointed out in the introduction, there are two phenomena to be considered. We use the word 'part' to refer either to the fact that only one particular vein out of the five longitudinal veins is affected, or to the fact that a particular region of the L 4 vein is affected more readily than other regions of the same vein. In this first section, however, we are only concerned with the frequency of gaps in one of the veins, and not with the position of the gaps.

The crosses between selected lines and wild type stocks only allowed us to measure gap penetrance in F_1 individuals which were heterozygous for the selected background and for *veinlet*. In order to study the effect which the homozygous *ve* Short background had upon the heterozygous expression of *veinlet*, the wild-type chromosomes were replaced with selection line chromosomes by backcrossing. The procedure also involved some selection of heterozygotes.

Heterozygotes from crosses of *ve* Short to Oregon wild type and to Eversden wild type were backcrossed to *ve* Short homozygotes for seven generations. Four backcross lines were maintained: two lines were originally heterozygous for the *ve* Short and Oregon backgrounds and two were heterozygous for the *ve* Short and Eversden backgrounds. In each set one line consisted of heterozygous females repeatedly backcrossed to *ve* Short males and one consisted of heterozygous males backcrossed to *ve* Short females. In the female backcross, recombination potentially allows all of the genetic background to become homozygous for the Short modifiers, except for the region very near *ve* itself. In the male backcross, on the other hand, the absence of recombination in most *Drosophila melanogaster* males restricts changes in the genotype to whole chromosome substitutions of the *X*, second and fourth chromosomes. The wild-type third chromosome must remain intact. Penetrance of this vein phenotype was scored in 50 +/*ve* flies of each sex in each line. Five pairs of heterozygous flies taken at random from those having L 4 vein gaps were selected as parents in each generation. The increase in gap frequency is shown in Text-fig. 2.

Although the original frequency of F_1 heterozygotes having L 4 vein gaps was very much lower than that observed in previous similar crosses, in all four lines backcrossing and selection resulted in a very high proportion of flies having gaps in one or, more often, in both wings.



Text-fig. 2. Backcrosses of *veinlet* Short: left, *ve* Short/Oregon backcrossed to *ve* Short; right, *ve* Short/Eversden backcrossed to *ve* Short. Solid lines are female responses, dotted lines are male responses (N = 50 for each sex). Solid squares (■) are lines in which heterozygous females were crossed to *ve* males; solid circles (●) are lines in which heterozygous males are crossed to *ve* females.

(i) *Backcrosses of Oregon/*ve *Short heterozygotes*

In the backcross of Oregon/*ve* Short heterozygotes, the male and female backcross lines parallel each other in having two or three stages of response (Text-fig. 2). Lowered viability caused the lines to be stopped at generation 7. These responses suggest that the inbred Oregon wild-type genome and the *ve* Short selected line differ in a number of factors which contribute to the formation of vein gaps in *ve* heterozygotes. As these factors become homozygous, gap frequency increases.

(ii) *Backcrosses of Eversden/*ve *Short heterozygotes*

The *ve* Short selection line had originated from an outcross to Eversden wild type, and the selected modifier complexes probably involve alleles which are also present in Eversden. It is not surprising therefore to find that the increase in gap frequency was more rapid in this set of lines. In the male backcross the increase

in gap frequency was rapid and almost linear. By the sixth generation almost 100% of the adults had large gaps in the L 4 veins of one or both wings. The female line responded very differently however. Gap frequency increased to only about 50%, then gradually decreased, fluctuating each generation with male offspring generally having a higher gap penetrance than female offspring.

The response of the male line is consistent with the hypothesis that the variation in L 4 vein length in this line is affected by a relatively small number of modifiers and that many of these are present in both *ve* Short and Eversden. Vein length modifiers on the second and fourth chromosomes rapidly become homozygous in the male backcross line. It should be noted that if the third chromosome contributes to gap production, the factors on that chromosome are heterozygous throughout the entire male backcross programme.

An explanation of the female backcross response is more difficult. Among the possibilities are the loss of modifiers from the *+ve* chromosome III by recombination, the separation of closely linked factors, or simply random fixation of some factors as an artifact of the breeding programme.

(iii) *The assimilated line*

The assimilated line was derived from the Eversden/*ve* Short male backcross line which had almost 100% gap penetrance by generation 6. A large number of heterozygotes from this line were pair-mated and, by progeny-testing, the cultures

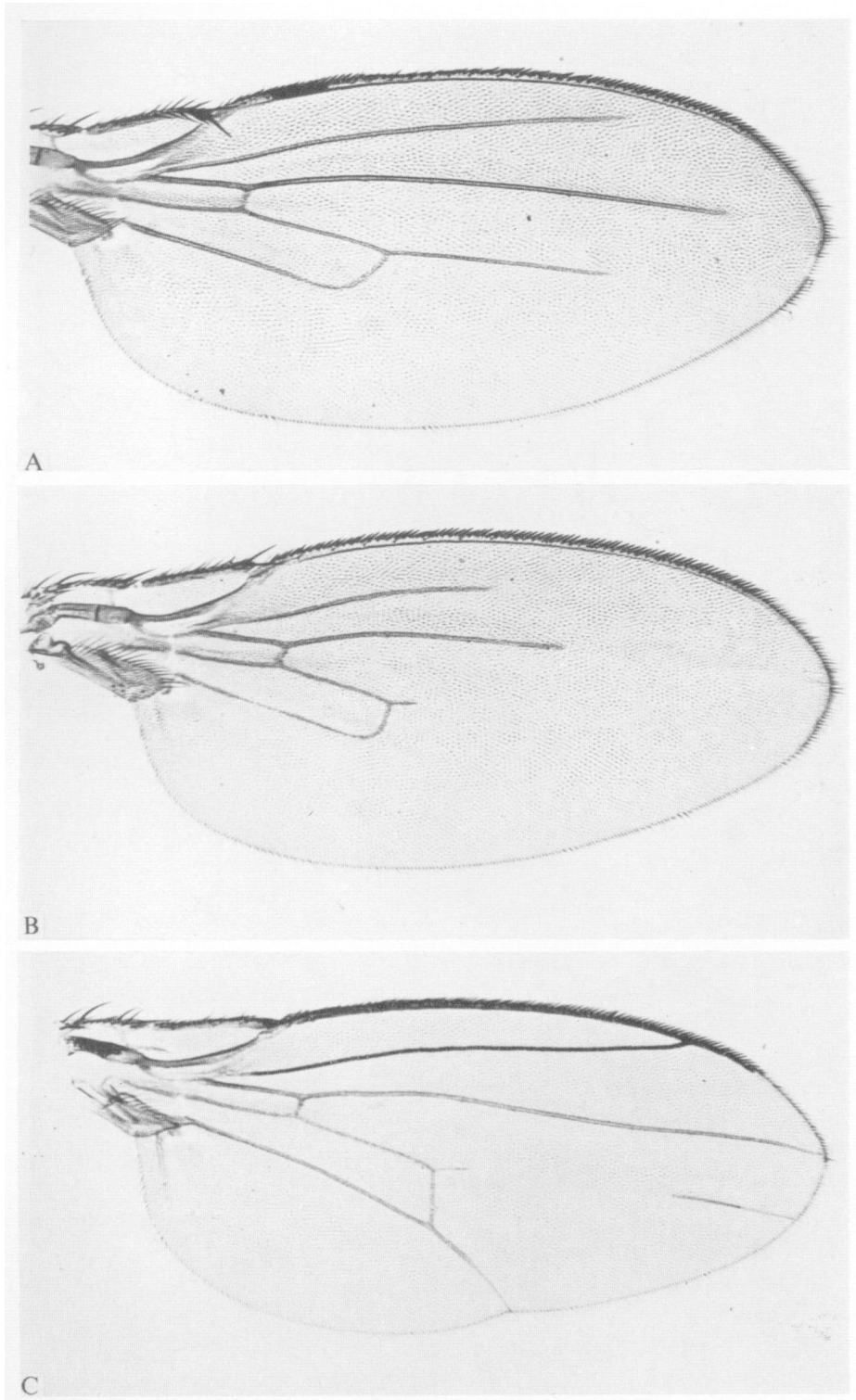
Table 1. *Relative L 4 vein length measurements for selection lines*

(Mean and standard deviation (N = 15) are given for the transformed ratio of vein length to total wing length ($\sin^{-1}\sqrt{(x)}/100$). For comparison, the Oregon wild-type measurements indicate the ratios expected for complete veins. The re-transformed mean is in parentheses.)

Selection line	Relative vein length	
	Females	Males
Oregon	0·807 ± 0·015 (0.974)	0·797 ± 0·018 (0.968)
ve Short	0·385 ± 0·016 (0.387)	0·378 ± 0·017 (0·375)
Assimilated line 1 (generation 10)	0·607 ± 0·016 (0.760)	0·648 ± 0·015 (0·819)
Assimilated line 2 (generation 10)	0·650 ± 0·008 (0.821)	0·652 ± 0·016 (0·834)

not segregating *ve* were identified. These were pooled and the frequency of gaps was scored. In replicate cultures the frequency of L 4 gaps was between 75 and 85%, demonstrating that genetic assimilation of part of the *ve* phenotype had occurred through selection for shorter veins in *veinlet* and the subsequent elimination of the *ve* mutant allele, by backcrossing and selection.

Two replicate cultures from the assimilation line were then selected further for increased expressivity (i.e. larger gaps). L 4 vein length decreased from about 0·750 to about 0·550 in one of these and from about 0·750 to about 0·600 in the other line. The vein phenotype of the assimilation line can be compared with *ve* in Plate 1, and the vein lengths are compared with *ve* Short in Table 1.



Wings of *Drosophila melanogaster*. A, *Veinlet* on an unselected genetic background. B, *Veinlet* Short. C, Wing from the assimilated line showing the subterminal L 4 gap.

4. GENETIC ANALYSIS OF THE ASSIMILATED LINE

(i) *Whole chromosome assays*

The analysis of heterozygous whole chromosome effects in the *ve* Short line is summarized in Table 2. Inbred *y*; *bw*; *ve st* females were mated to *ve* Short males, and the F_1 males were then backcrossed to *y*; *bw*; *ve st* females. Offspring carrying all combinations of heterozygous selection line chromosomes (II^s and III^s in Table 2) can be identified by eye colour (see Thompson, 1973). The second chromosome carries modifiers which have a highly significant effect upon L 4 vein length, and even though the third chromosome alone has no effect, there is a significant II × III chromosome interaction which further decreases L 4 vein length.

Table 2. *Relative L 4 vein length measurements in an assay of the effects of heterozygous whole autosomes in the* ve *Short selection line*

(Comparison is made with the inbred *y*; *bw*; *ve st* stock. Mean relative vein lengths ($N = 15$ in pooled replicates) for flies with various combinations of control (c) and heterozygous selected (s) chromosomes. Significance levels are derived from an analysis of variance.)

	Relative vein lengths				Significance levels		
	II^c; III^c	II^s; III^c	II^c; III^s	II^s; III^s	II	III	II × III
Females	0·523	0·461	0·561	0·444			
					< 0·001	ns	< 0·001
Males	0·506	0·447	0·523	0·433			

Table 3. *Gap penetrance* ($N = 100$) *in females* (*above the line*) *and males* (*below the line*) *having various combinations of second and third chromosomes from the assimilation line*

(GA = chromosome from the genetic assimilation line; Or = Oregon wild type chromosome. These combinations were obtained by using dominantly marked balancer chromosomes in a programme similar to that used for measuring heterozygous *ve* Short effects (Table 2).)

Chromosome III	Chromosome II		
	Or/Or	GA/Or	GA/GA
Or/Or	—	—	3/0
GA/Or	—	0/0	42/17
GA/GA	0/0	0/0	100/100

The whole chromosome effects in the assimilated line (Table 3) are similar to those in *ve* Short (Table 2). When the second chromosome from the assimilated line is homozygous, there is a low frequency of gaps, but this is increased dramatically when the third chromosome is also heterozygous. In the homozygous selection line, the frequency is further increased to 100%. The homozygous second chromosome is, therefore, required for gap formation, but the third chromosome carries one or more factors which, either when heterozygous or homozygous, modify the expression of the second chromosome factors.

(ii) *Location of the second chromosome factor*

It is clear from the whole chromosome assays that, although the third chromosome affects L 4 gap penetrance through an interaction with the second chromosome, the primary factor or factors are located on chromosome II. The location of these factors was estimated using percentage penetrance in individual cultures derived from single male recombinants as the phenotype assayed.

Table 4. *Location of a modifier region on chromosome II from the assimilation line*

(Numbers of recombinant males are shown in each of the two phenotypic classes, those with and those without gaps. See text for analysis of map position.)

Recombinant class				Phenotype	
				Gaps	No gaps
dp	b	cn	bw	0	32
+	+	+	+	35	0
dp	+	+	+	31	0
+	b	cn	bw	0	35
dp	b	+	+	6	0
+	+	cn	bw	0	10
dp	b	cn	+	11	22
+	+	+	bw	21	10
dp	+	+	bw	24	11
+	b	cn	+	10	21

In the first assay, females from the assimilated line were mated to an inbred marker stock carrying *dumpy*, *black*, *cinnabar*, and *brown*. Virgin F_1 females were backcrossed to *dp b cn bw* males, and the recombinants were picked up as males in the next generation. Fifty single males of each recombinant class were mated individually to several virgin females from the assimilation line. Recombinant chromosomes carrying factors contributing to L 4 vein gap formation would, therefore, be homozygous and these recombinants could be identified by the presence or absence of L 4 gaps (effectively an all or none classification) in the wings of their offspring. The results are shown in Table 4.

Recombinants between *cinnabar* (57·5 cM) and *brown* (104·5 cM) are of two types: those which carry the factor(s) affecting venation and those which do not. Among a total of 281 males tested, 42 recombinants occur between *cn* and the venation modifier and 88 between the modifier and *bw*. This places the modifier region at about 72 cM (42/281 = 0·149 = 14·9 cM; 88/281 = 31·3 cM; 57·5 (*cn*) + 14·9 = 72·4 cM).

To locate the modifier region more precisely and see whether it could easily be resolved into more than one factor, new markers were chosen which were nearer the modifier region. A cross similar to the one outlined above was made using an inbred stock marked with *black*, *cinnabar* and *curved* (75·5 cM). The *cn* to *c* distance (flanking the 'gap factor') is now only 18 cM. One critical assumption which

is made when changing marker stocks during a polygene location programme is that the two sets of 'standard' chromosomes carry identical 'wild type' alleles for the polygenic loci being studied on the test chromosome. The phenotypes of recombinants suggests that this is true in this case (Thompson and Thoday, 1974).

Table 5. *Second assay of the position of a modifier region on chromosome II, using markers nearer the region indicated in the first assay (Table 4)*

Recombinant class			Phenotype	
			Gaps	No gaps
b	cn	c	0	5
+	+	+	5	0
b	+	+	15	0
+	cn	c	0	15
b	cn	+	12	1
+	+	c	2	10

The results of the second recombination assay are shown in Table 5. The modifier region is clearly closer to *c* than to *cn*. There are 22 recombinants between the modifier and *cn*, which places the factor at about 73 cM ($22/25 \times 18$ cM = 15·8 cM from *cn*; $15{\cdot}8 + 57{\cdot}5 = 73{\cdot}3$ cM). This is, of course, only an approximate location and the polygenic locus may be a complex factor. But its map position agrees closely with the estimated position of a second chromosome factor which was located by measuring the effects of modifiers of L 4 vein length in homozygous *veinlet* selection lines (Thompson, 1975). In this latter estimate (which involved progeny-testing recombinants as described by Thoday, 1961), the factor appeared to be a single factor located at about $72{\cdot}5 \pm 2{\cdot}3$ cM. It was designated *PL(2)L4a* following the standard nomenclature outlined by Thompson & Thoday (1974).

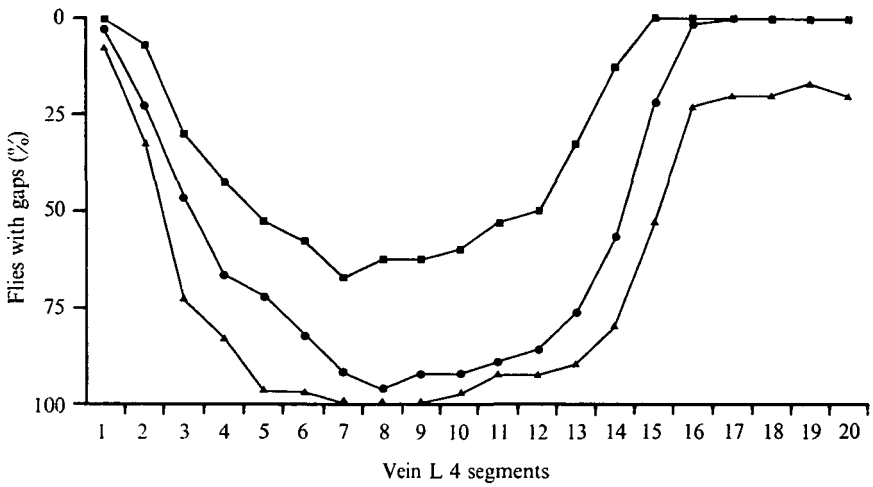
Since the third chromosome effects were only detectable through a chromosomal interaction, locations could not be attempted with the available stocks or markers.

5. PHENOTYPIC EFFECTS OF THE MUTANT AND THE ASSIMILATED LINE

In order to compare the phenotypic effects of the mutant and the assimilated line (which has many elements in common with the *ve* Short selected genome) profiles were drawn to show the regions of the L 4 vein affected by each. This allows us to observe phenotype frequencies in a way not possible by simple measurements of mean and variance. The L 4 vein from the posterior crossvein to the tip of the wing has been divided into 20 equal segments, and these are represented by the divisions on the horizontal axis of the profile. The number of flies lacking vein in each particular segment is plotted along the vertical axis.

The profiles for the assimilated line are shown in Text-fig. 3. Each sample has been cultured at a different temperature (20, 25 and 28 °C; only female wing profiles are illustrated). Two things can readily be seen from this graph. First, the focus for the assimilated line phenotype is different from that for the mutant. The gaps

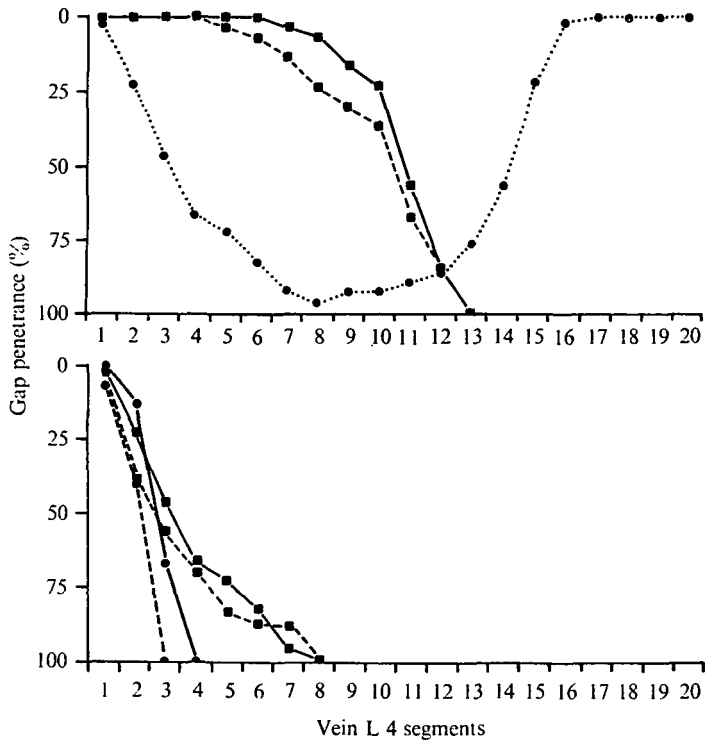
in the wings from all flies in the assimilated line are subterminal, rather than terminal as they are in *veinlet*. All flies raised at 25 °C or above have vein at the distal tip and base of the L 4. Secondly, it is clear that this focus does not change as temperature decreases and the phenotype becomes more extreme. The amount of vein simply decreases in a regular way by the enlargement of the subterminal gap distally and proximally. This implies that the genetic factors and temperature are influencing vein formation in different ways.



Text-fig. 3. Vein expression profiles of the assimilated line at different temperatures (female wings only): ■, 28 °C; ●, 25 °C; ▲, 20 °C. A gap penetrance of 0% for segment 1, for example, means that no flies lacked vein in that segment. A gap penetrance of about 55% in segment 6 (28° line) means that 55% of the flies lacked vein in the sixth 5% segment of the vein, as measured on camera lucida projections.

The phenotypic pattern of an unselected *ve* stock is compared with the assimilation line in Text-fig. 4(*a*). It can be seen that no *ve* flies have vein material in the distal third of the L 4 vein. The frequency of flies with vein increases in each segment approaching the junction of the posterior crossvein, however, and all flies have vein material in the first six divisions.

The phenotypic effects of *veinlet* and of the assimilated line have been summed and compared with the *ve* Short phenotype in Text-fig. 4(*b*). Since *veinlet* eliminated vein from the tip, and the assimilated line eliminated vein from the middle, their compound effects approximate the observed phenotype of the selected line. The representation of 'summed effects' predicts a few flies having slightly longer veins than were actually observed in the selected line itself. It must be remembered, however, that the assimilated line is not quite identical to the selected *ve* Short background. The absence of these longer vein flies could be explained by either a small interaction between *ve* and the selected background or by the loss of some minor modifiers from one of the chromosomes during the selection or backcross programme.



Text-fig. 4. (*a*). Vein expression profiles of *veinlet* (unselected) (■) and of the assimilated line at 25 °C (●). In the *veinlet* profile, female means are shown by solid lines and male means with dotted lines. The profiles show the frequency with which vein is found in each of 20 segments of the L 4 vein. (*b*). Profiles of *veinlet* Short (●) and the predicted phenotype of *veinlet* plus the assimilated background modifiers on a simple additive model (■).

These regional effects confirm previous reports (e.g. House, 1953) that mutants and some genotype-environment interactions affect parts of the L 4 vein in different ways.

6. DISCUSSION

When *veinlet* was removed from lines selected for shortened veins, it was found that the selected vein length polygenes produced gaps in the absence of the major mutant. But only part of the original mutant phenotype had been assimilated, in that only one of the five longitudinal veins was affected. This example of genetic assimilation provided a clear situation in which we could look at the relationship between the phenotypes of the selected genetic background and of the mutant. The study was, therefore, designed to answer two questions: what is the genetic basis of the assimilated phenotype and how could the relevant genetic variation be effective in modifying the much more complex *veinlet* phenotype?

Although *veinlet* has terminal gaps in all of the main longitudinal veins, genetic variation affecting vein expression has been shown to include polygenes having vein-specific effects. Thompson (1975) was able to select for changes in L 2, L 3,

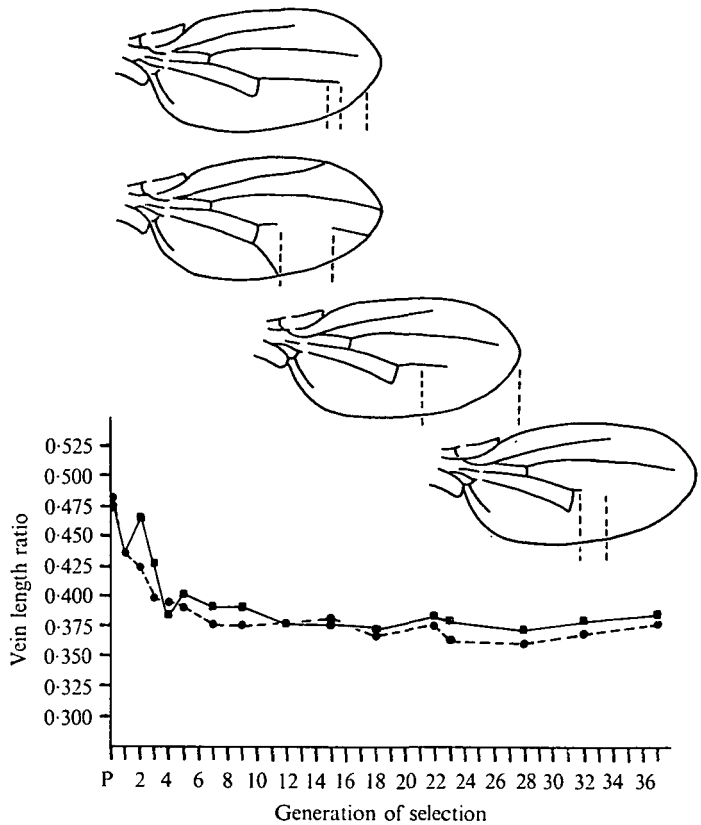
and L 4 vein lengths independently of each other. Indeed, whole chromosome assays showed that the genes responsible for these responses were often on different chromosomes, and this was confirmed by whole chromosome assays of the original *ve* Short selection line. Vein-specific modifier gene effects were also evident in the assimilated line. Only the L 4 vein had gaps. All other veins were wild type in appearance. Thus, assimilation of *part* of the mutant phenotype could occur because the genetic variation affecting vein length included loci which had effects limited to individual veins in the more complex venation pattern.

The second chromosome carried a single factor (designated *PL(2)L4a*) which was required for gap production, but which in turn was modified by one or more factors on the third chromosome. Waddington (1961) also discussed several examples in which one factor of comparatively large effect was necessary for the expression of an assimilated phenotype, while other less effective factors determined the degree of expression. Clearly, the segregation of one factor having comparatively large effects will make assimilation easier. Less-effective factors will be selected more efficiently after the primary factor has been fixed in the line, and there is then less chance of losing such minor factors by drift or random fixation. In this context, it would be instructive to know how often genetic assimilation occurs in which one factor does *not* account for the majority of the phenotypic response.

Assimilation should probably occur most readily in those characters which are least well buffered against genetic and environmental variation. Nevertheless, the fact that the L 4 vein was the only vein to show assimilation was probably due to the effectiveness of the factors segregating in the base population, rather than to any inherent sensitivity of the L 4 vein to assimilation. Some L 4 vein mutants may, however, lead to assimilation more readily than others. For example, during selection for decreased L 4 vein lengths in *cubitus interruptus Dominant* (ci^D) and *Hairless* (*H*), Scharloo (1962, 1964) produced non-*Hairless* flies having L 4 vein gaps but observed no assimilation in the ci^D line. He suggested that this might be due to the different canalisation profiles of these mutants.

The present analysis is not able to determine the precise developmental action of either *veinlet* or of the selected genetic background. It is possible, however, to compare the phenotypic effects of these two components. As mentioned previously, 'part' of the mutant phenotype was assimilated in two different senses: (1) only one of the five longitudinal veins was affected and (2) the region of the L 4 vein affected by the selected background was limited and different from that affected by the mutant. The modifier factors in the selected background produce gaps in the centre of the L 4 vein, while *veinlet* produces gaps only at the tip. The two phenotypes appear to be approximately additive. Thus, one might perhaps expect selection to have proceeded as follows (Text-fig. 5). First, flies were selected in which one or more factors increased the size of the distal gap, and in which *PL(2)-L4a* was segregating. The factors affecting the distal area of the vein quickly increased in frequency and *PL(2)L4a* became homozygous. Then the speed of the response slowed as less effective factors were accumulated to shorten the

remaining vein in the area of the posterior crossvein. Neither the selected background nor the mutant itself appear to affect the region immediately around the posterior crossvein, and a small fragment, therefore, remains.



Text-fig. 5. Selection response of *veinlet* Short and the proposed interaction of mutant and selected background which produced it. Symbols (■, females; ●, males) show the generations at which vein length samples were taken. The *veinlet* mutant (top wing) removes vein from the tip of the L 4 while the *PL(2)L4a* factor and associated modifiers removes vein primarily from the centre of the L 4 (second wing). During the first four generations the *PL(2)L4a* factor and polygenes acting on the distal region of the L4 vein (top wing) were selected to produce a wing having a relatively short L4 vein (third wing). During the remainder of the selection programme, modifiers acting upon the base of the L4 (fourth wing) were accumulated, causing a further small decrease in L 4 length between generations 4 and 23.

Studies of polygenic variation in a variety of mutant selection lines have shown that at least a proportion of the polygenes affecting vein mutant expression act independently of the mutant which they modify (Thompson, 1973, 1974). They apparently produce small, additive changes in the same or closely related developmental steps. The approximate additivity of the mutant and selected polygenic components of the *ve* Short and assimilation line phenotypes is consistent with this interpretation. Indeed, *PL(2)L4a* produces similar phenotypic effects when sub-

stituted into the genomes of several different mutants causing L 4 vein gaps (Thompson, 1975) and it is clearly not a mutant-specific modifier. Mutant-specific polygenic modifiers are known however (Scharloo, 1964; Fraser, 1967; and others).

On the basis of this limited study we may, therefore, suggest that an effective polygenic modifier is an allele which produces a small change of some type in the target character, whether this allele interacts with the mutant locus or not.

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