

## General and specific effects of modifiers of mutant expression

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### SUMMARY

Strains homozygous for mutants affecting the length of the L4 vein in *Drosophila melanogaster* were selected for increased or decreased vein length. Substitution of chromosomes selected for their effect on one mutant into the genome of a non-homologous mutant has shown that many modifiers of mutant expression must be considered as acting generally on the character rather than affecting only a specific mutant.

### 1. INTRODUCTION

All populations of outbreeding species show wide genetic variation, much of which is polygenic. To appreciate fully the significance of this variation, it is important to understand how it is related to the development of an organism. Though we still have little idea of its relevance to development or to fitness, it seems probable that a large amount of genetic variation is maintained by selection (Powell, 1971; Clegg & Allard, 1972; de Jong *et al.* 1972) and is, therefore, advantageous to the individual. The hypothesis that polygenic variation plays some fundamental role in development is further supported by the observation that polygenes are often found to be involved in the determination or the modification of dominance relationships, canalization, penetrance, and expressivity, all of which are characteristics of the genotype–phenotype relationship in complex organisms (Rendel, 1967; Ford, 1971; Thompson & Thoday, 1972; Belt & Burnet, 1972).

Polygenic modifiers may be defined as that part of quantitative variation which can be traced and measured by its effect upon the phenotype of mutant individuals. One way to approach the problem of variation and development is to ask how polygenic modifiers are related to the characters they affect. This is easily done for modifiers of the expression of wing vein length in mutants of *Drosophila melanogaster*. The specific question is, then, 'are modifiers of L4 vein length in the mutants *cubitus interruptus* (*ci*), *short vein* (*shv*), and *veinlet* (*ve*) specific to the single mutant in which their effects are first observed and measured, or are they simply modifiers of the formation of the L4 wing vein, which is shortened by all three mutants?'

### 2. STOCKS

The following recessive mutants were used in the course of this work. For detailed descriptions of mutants, see Lindsley & Grell (1967): (1) *cubitus interruptus* (*ci*, IV–0), L4 vein shortened; (2) *short vein* (*shv*, II–3.8), L2, L4, and in extreme cases L3 and L5 veins shortened; (3) *veinlet* (*ve*, III–0.2), all veins are shortened; (4) stocks marked with the recessive mutants *yellow* (*y*, chromosome I), *brown* (*bw*, chromosome II), and *scarlet* (*st*, chromosome III). The *y*; *bw*; *st* markers were used to derive inbred, unselected lines containing the three vein mutants: (a) *y*; *bw*; *st*; *ci*, (b) *y*; *shv* *bw*; *st*, and (c) *y*; *bw*; *ve* *st*.

Wings were mounted on microscope slides using DePeX mountant. Vein lengths and total wing length (measured from the fork of the L2 and L3 veins to the tip of the wing) were measured using an eyepiece graticule, and the analyses of variance were carried out on the sine transformed ratio of vein length to total wing length as in Thompson & Thoday (1972).

### 3. RESULTS AND DISCUSSION

To provide material with which to test the specificity of vein length modifiers of wing vein mutants, three mutants which shorten the L4 vein were outcrossed to a single newly captured wild-type stock, Eversden-14, and resegregated to provide mutant stocks with a large amount of genetic variation. Each was then divided into a number of sub-cultures which were selected either for longer or for shorter veins for over 35 generations, by which time the pairs of lines differed significantly in the expression of the L4 vein

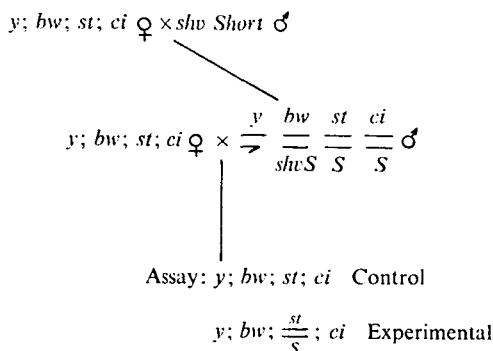


Fig. 1. The mating scheme used to substitute a selection line chromosome (S) into an unselected stock (in this example, *shv Short* chromosome III into *y; bw; st; ci*) is derived from the classical whole chromosome assay technique which capitalizes upon the fact that there is no crossing over in male *Drosophila*, so that selected and unselected chromosomes are inherited intact from the  $F_1$  male. The  $F_2$  classes can be identified by eye colour: white = control; red = selected II and selected III; scarlet = selected II only; brown = selected III only.

Table 1. *Relative L4 vein length measurements for selected lines at generation S-37*

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

Selection line	Relative vein length	
	♀	♂
Oregon	0.807 ± 0.015 (0.974)	0.797 ± 0.018 (0.968)
<i>ci</i> Long	0.782 ± 0.074 (0.958)	0.798 ± 0.020 (0.968)
<i>ci</i> Short	0.323 ± 0.035 (0.285)	0.333 ± 0.033 (0.301)
<i>shv</i> Long	0.658 ± 0.010 (0.832)	0.653 ± 0.015 (0.825)
<i>shv</i> Short	0.457 ± 0.014 (0.512)	0.436 ± 0.014 (0.475)
<i>ve</i> Long	0.798 ± 0.042 (0.968)	0.758 ± 0.070 (0.940)
<i>ve</i> Short	0.385 ± 0.016 (0.387)	0.378 ± 0.017 (0.375)

(Table 1). In all assays the effects of selected chromosomes are measured when they are heterozygous in a standard, inbred *y; bw; st* stock containing one of the vein mutants (stocks 4a, 4b and 4c above).

Table 2. *Effects of chromosomes II and III from the ve Short selection line (after ve allele removed by a 7-generation backcross programme) upon the expression of unselected ci.*

(Transformed mean vein lengths ( $N = 15$  of each sex and genotype) of flies with and without the selected chromosomes are given: II<sup>c</sup> and III<sup>c</sup>, control; II<sup>s</sup> and III<sup>s</sup>, selected chromosomes.)

Replicate	II <sup>c</sup> ; III <sup>c</sup>		II <sup>s</sup> ; III <sup>c</sup>		II <sup>c</sup> ; III <sup>s</sup>		II <sup>s</sup> ; III <sup>s</sup>	
	♀	♂	♀	♂	♀	♂	♀	♂
1	0.631	0.447	0.361	0.360	0.516	0.404	0.365	0.359
2	0.618	0.582	0.415	0.371	0.511	0.428	0.363	0.327
Total	0.624	0.514	0.388	0.366	0.514	0.416	0.364	0.343

Table 3. *Analysis of variance of the data in Table 2.*

Factor	d.f.	M.S.	F	P
Chromosome II	1	1.3832	162.7	< 0.001
Chromosome III	1	0.2459	28.9	< 0.001
Sex	1	0.2369	27.9	< 0.001
Replicate	1	0.0275	3.2	NS
II × III	1	0.0986	11.6	< 0.001
II × Sex	1	0.1004	11.8	< 0.001
III × Sex	1	0.0007	12.1 <sub>1</sub> <sup>224</sup>	NS
Other interactions	8	0.0181	2.1	NS
Residual (error)	224	0.0085	—	—

Table 4. *Effects of chromosome III, from shv Short, upon the expression of unselected ci.*

(Transformed mean vein lengths ( $N = 15$ ) with and without the selected third chromosome are given.)

Replicate	II <sup>c</sup> ; III <sup>c</sup>		II <sup>c</sup> ; III <sup>s</sup>	
	♀	♂	♀	♂
1	0.496	0.506	0.434	0.401
2	0.480	0.449	0.460	0.379
Total	0.488	0.478	0.447	0.390

Using appropriate crosses, single chromosomes, which had previously been shown by whole chromosome analysis to have a significant effect upon the L4 vein length in the selected line, were substituted into an unselected stock of a non-homologous mutant (Fig. 1). For example, chromosome II has a considerable effect upon the shortening of the L4 vein in the *ve Short* selection line (J. N. Thompson, unpublished data: effect of chrom. II,  $P < 0.001$ ). Chromosome II was then substituted into an unselected stock of *ci*, and its effect upon L4 length in the new mutant was measured. If the modifiers

accumulated on chromosome II were specific to the mutant in the original selection line, one would expect the chromosome to have a random effect when substituted into *ci*, depending upon the *ci*-specific modifiers it happened to carry. If, however, the modifiers were specific to the character, i.e. to L4 vein formation rather than to the mutant, the substituted chromosome should have an effect upon the expression of the second mutant comparable to its effect in the original selection line.

Table 5. *Analysis of variance of the data in Table 4.*

Factor	D.F.	M.S.	F	P
Chromosome (C)	1	0.0826	11.6	< 0.01
Replicate (R)	1	0.0060	1.2	NS
Sex (S)	1	0.0228	3.2	NS
C × R	1	0.0074	1.0	NS
C × S	1	0.0107	1.5	NS
R × S	1	0.0098	1.4	NS
C × R × S	1	0.0000*	—	—
Residual (error)	72	0.0071	—	—

\* The C × R × S factor is positive (0.00003) and not significant.

When chromosome II from *ve Short* is substituted into unselected *ci*, it also causes a considerable decrease in the length of the *ci* L4 vein ( $P < 0.001$ ; Tables 2 and 3). Thus, this second chromosome contains modifiers that shorten the L4 vein irrespective of the mutant present. Chromosome III, which contributes to the penetrance of the heterozygous *ve* allele, also has a significant effect upon the penetrance of heterozygous *shv* (J. N. Thompson, unpublished data) and a significant effect upon the vein length in *ci* ( $P < 0.001$ ; Tables 2 and 3).

Similarly, chromosome III from *shv Short* causes the L4 vein in both *shv* and *ci* to be shortened considerably (for effect in *ci*,  $P < 0.01$ ; Tables 4 and 5). These and similar substitutions demonstrate that within certain chromosomes removed from vein mutant selection lines, many of the polygenic modifiers are directly involved in some aspect of vein formation, and, thus, have qualitatively similar effects upon the expression of some phenotypically-similar mutants. This may be in contrast to the report by Scharloo (1964) that modifiers of *cubitus interruptus Dominant* have only a small effect upon the L4 expression of the dominant mutant *Hairless*, and vice versa, though no data are given.

Other examples, in which selected genetic backgrounds have qualitatively similar effects upon the expressions of related mutants, have been reported by Thompson & Thoday (1972), though Fraser (1968) reported that the modifier systems of the bristle mutants *scute* and *extravert* were independent. In the experiments of Thompson & Thoday, similarity in the effects of modifier backgrounds was shown by the high frequency of vein gaps in the  $F_1$  double heterozygotes from certain crosses among selected lines. When two mutants which affect the same vein (such as *radius incompletus* and *veinlet* which both cause gaps in the L2 vein) are selected for shorter veins, i.e. for less vein material, vein gaps are common in the  $F_1$  of crosses between the lines. When both lines have been selected for longer veins, i.e. for more vein material, extra-vein fragments are often observed in the  $F_1$  progeny. Crosses between Long and Short selected lines of different mutants are generally wild-type in appearance. These results imply that the various modifier backgrounds are at least functionally similar in increasing or decreasing the amount of vein material in the wing and support the present hypothesis that polygenic modifiers act independently of the major mutant.

Although the same modifiers may be involved in the responses of the various selection lines, the results so far only show that heterozygous whole chromosomes, carrying

modifiers of the expression of a wing vein mutant, act in a general way by affecting the development of the vein. The polygene location technique of Thoday (1961) will be used to see whether the same is true of much smaller regions around modifier loci within a selected chromosome. It would be surprising, however, if the numerous examples of similar effects of independently-obtained modifier backgrounds could all be traced to linkage of mutant-specific modifier factors.

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