GENETICS OF AN L_2 VENATION MUTANT IN *DROSOPHILA MELANOGASTER* I. MODE OF INHERITANCE AND EXPRESSION[1, 2]

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ABSTRACT

The results indicate the mutant phenotype is recessive; high penetrance being due to the homozygosity of factors found on both the second and third chromosomes. Selection for high and low lines of expression was initiated and after nine generations of selection for a high line and six generations for a low line, a significant difference between the two was obtained. Analysis of crosses between high and low lines, F_1 crosses, and backcrosses to both parental lines gives evidence for additive action of the polygenes controlling expression of the second longitudinal vein (L_2).

Some thirty mutant loci are known in *Drosophila melanogaster* (Braver, 1956) which produce an interruption of the longitudinal veins of the wing (Bridges and Brehme, 1944). Twelve of these have an effect on the second longitudinal vein (L_2). Five of these twelve confine their effect solely to L_2. It seemed of some interest therefore, to investigate the mode of inheritance and expression of an L_2 mutant phenotype arising in a wild-type stock maintained in mass culture at the Virginia Polytechnic Institute. The interest in a study of the genetic and environmental variables involved in the realization of L_2 interruption is further heightened by the apparent differences in the developmental events involved in the establishment of the second vein from those associated with the other longitudinal veins as revealed by the descriptive (Waddington, 1939) and experimental (Lees, 1941) studies of venation development in *Drosophila melanogaster.*

MATERIALS AND METHODS

The standard culturing medium of corn meal, agar, dextrose, and yeast, including the addition of the mold preventitive, tegosept M, was used. All crosses, except when otherwise noted, involve single pair replications, the parents of a given mating being placed initially in shell vials and transferred one day later to half pint bottles. All cultures were incubated at 26°C ± 1°C.

The following list comprises the stocks used in the investigation.

1. Oregon-R, a highly inbred wild type stock maintained in mass culture at The Ohio State University.
2. *Cy/Pm; H/Sb*, a balanced lethal stock with dominant markers for the second and third chromosomes.
3. The mutant stock being studied.

The Oregon-R stock was used in the determination of the mode of inheritance of the mutant. The *Cy/Pm; H/Sb* stock was used in the chromosome localization tests. The marker genes in the latter stock show the following characteristics: Curly (*Cy*)—a mutant associated with a second chromosome inversion, lethal as a homozygote and producing a strong upward curling of the wings in the heterozygous state. Plum (*Pm*)—a mutant associated with a second chromosome inversion, lethal as a homozygote and producing a reddish brown eye flecked with darker spots as a heterozygote. Hairless (*H*)—a homozygous lethal mutant associated

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with a third chromosome inversion, causing an absence of certain bristles and often producing interruption of the fourth and fifth (L_4, L_5) longitudinal veins and occasionally causing a break in L_2. Stubble (*Sb*)—a third chromosome mutant causing a shortening and thickening of the bristles of the fly as a heterozygote and lethality as a homozygote.

The degree of interruption of the L_2 vein was determined for the various progeny according to the following scheme, the score for a given individual being determined as the mean of the interruption of the left and right wings. The flies were classified under a binocular microscope (27 ×) into one of six classes (fig. 1) based on the percentage of L_2 missing, relative to the length of the vein extending from the junction of the second and third longitudinal veins to the junction of the

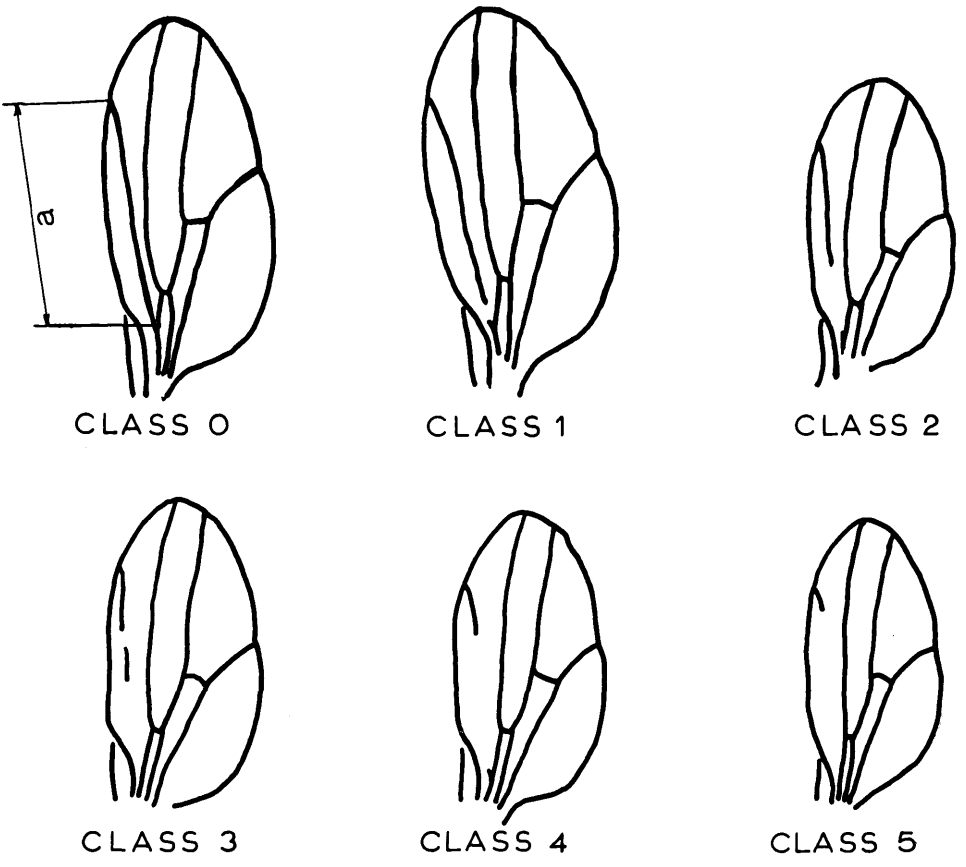

FIGURE 1. Classification of the degree of interruption of the second longitudinal vein.

second vein with the wing margin (distance "a" in fig. 1). The specifications of the six classes are as follows:

Class 0 Normal, wild type L_2 vein.
Class 1 0 to 20% of L_2 absent.
Class 2 20 to 40% of L_2 absent.
Class 3 40 to 60% of L_2 absent.
Class 4 60 to 80% of L_2 absent.
Class 5 80 to 100% of L_2 absent.

RESULTS AND DISCUSSION

Penetrance and Expression of the Mutant Stock

The percentage of individuals of the mutant stock having interrupted veins (the penetrance) at 26°C was very high, being 99.86 per cent. The distribution by sex into the various classes of phenotypic expression described under the section on methods (fig. 1) is summarized in table 1. Also included in this table is the percentage distribution relative to the various classes for the combined total of males and females.

A very low percentage of the flies at this temperature (26° C) fell into the extreme classes (0 and 5). The majority of the flies fell in class 2. The mean L_2 interruption for combined males and females was 33.24 per cent, calculated by the following method. Each class was assigned its midpoint value on the interruption scale and this value was then multiplied by the total number of flies in that class. Summation of these numbers for all classes, divided by the grand total of flies, yielded the average amount of interruption.

A chi-square test of the significance of the differences between the male and female distributions of table 1 yielded a p value less than 0.01 that this difference could be due to chance alone. Classes 0 and 1 were combined as were classes 3, 4, and 5 due to the small numbers.

TABLE 1

Distribution of L_2 interruption at 26° C in an unselected mutant stock

Class	0	1	2	3	4	5	Total
Males	0	51	214	89	1	2	357
Females	1	26	263	101	1	0	392
Percentage (Males and females combined)	0.13	10.24	63.44	25.27	0.27	0.27	100

Mode of Inheritance Tests With Oregon-R

The results of matings between the mutant stock and the Oregon-R stock are summarized in table 2. These results might be explained on the basis of a simple autosomal recessive mutant exhibiting greatly reduced penetrance in a new modifier background. Under this hypothesis, the F_2 segregating background would have to reduce the penetrance from 99.86 per cent of the mutant stock to 10.96 per cent. The genetic background of the backcross, which should be nearer the mutant stock than the average effect of the F_2 background, would have to reduce the penetrance to 26.34 per cent. This represents a rather extreme reduction of penetrance due to a change in the genetic background.

TABLE 2

Various matings of the mutant stock with Oregon-R at 26° C

Mating	Phenotype	Females	Males	Total	Penetrance (%)
Mutant x Oregon-R	Normal	293	293	586	
	Mutant	0	0	0	0.0
F_1 x F_1	Normal	843	790	1633	
	Mutant	20	26	46	2.74
F_1 x mutant	Normal	310	290	600	
	Mutant	46	45	91	13.17

These same results may also be explained by an alternate hypothesis. Assuming that the mutant condition may be due to the homozygosity of two independent recessive factors, the expected frequency of the mutant phenotype in the F_2 should be one-sixteenth or 6.25 per cent, assuming complete penetrance of the double recessive. The difference between observed and expected values might be due to differential mortality of the mutant in competition with the other genotypes. Assuming no differential mortality of genotypes other than the double recessive, it is possible to calculate the degree of differential elimination (s) of the latter genotype that would be necessary to explain the observed F_2 results. This calculated "s" value is 0.58.

The question is now raised as to whether the alternative hypothesis invoking a double recessive genotype showing 58 per cent differential elimination is capable of explaining the backcross data. A chi-square analysis of the backcross results shows no significant deviation (5-per cent level) between observed and expected values on the basis of this alternate hypothesis.

Chromosome Localization Tests

The standard technique of using a balanced *Cy/Pm; H/Sb* stock was employed. This stock was crossed to the mutant stock and Plum, Stubble males were selected from the progeny. These males were then back-crossed to the mutant females. The results of this cross are summarized in table 3, where m_2 and m_3 refer to the second and third chromosomes, respectively, of the mutant stock.

TABLE 3

Results of the m_2/m_2; m_3/m_3 x Pm/m_2; Sb/m_3 *matings*

Genotype	Normal	Interrupted	Total
Pm/m_2; Sb/m_3	175	0	175
Pm/m_2; m_3/m_3	173	9	182
m_2/m_2; Sb/m_3	137	0	137
m_2/m_2; m_3/m_3	1	122	123

If one invokes a single autosomal recessive gene on the third chromosome, a very strong inhibitory effect of the Plum chromosome would also have to be postulated. This would involve a penetrance reduction from 99.86 per cent to 4.94 per cent, which is not too likely. These results seem to be more compatible with the alternate hypothesis (two independent factors and differential mortality). Under this assumption, high penetrance is found when homozygosity of the second and third chromosomes exists. Very low penetrance is observed when homozygosity exists for the third chromosome only (m_3/m_3) or occasionally the second chromosome (m_2/m_2), (see table 4).

When the *Pm/m_2; m_3/m_3* × *m_2/m_2; m_3/m_3* and the *m_2/m_2; Sb/m_3* × *m_2/m_2; m_3/m_3* crosses were made, the combined data (table 4) again indicate that homozy-

TABLE 4

Combined results of the m_2/m_2; m_3/m_3 x Pm/m_2; m_3/m_3 *and* m_2/m_2; m_3/m_3 x m_2/m_2; Sb/m_3 *matings*

Genotype	Normal	Interrupted	Total	Penetrance (%)
m_2/m_2; Sb/m_3	369	2	371	0.54
Pm/m_2; m_3/m_3	387	13	400	3.25
m_2/m_2; m_3/m_3	10	457	467	98.00

gosity of the second and third chromosomes is necessary for high penetrance of the mutant phenotype. Of particular interest is the occasional penetrant fly found when homozygosity of the second chromosome is coupled with heterozygosity of the third. The penetrance is very high (98 per cent) when homozygosity of the second and third chromosomes exists. The penetrance is reduced sharply (to 3.25 per cent) when homozygosity of the third chromosome is coupled with heterozygosity of the second. It is almost zero (0.54 per cent) when the second chromosome is homozygous for the mutant factor, the third being heterozygous.

Effect of Selection on Expression of the Mutant Stock

In view of the general sensitiveness of the venation pattern in *Drosophila* to changes in genetic background, whether produced by environmental shocks (Waddington, 1953; Bateman, 1959; Milkman, 1960) or by gene substitutions (House and Yeatts, 1962), it seemed desirable to attempt, through selection, the separation of the mutant stock into lines showing increased (high) and decreased (low) degrees of expressivity. Selection was therefore carried out for high and low lines of expression at 26°C by means of single pair matings within the mutant stock.

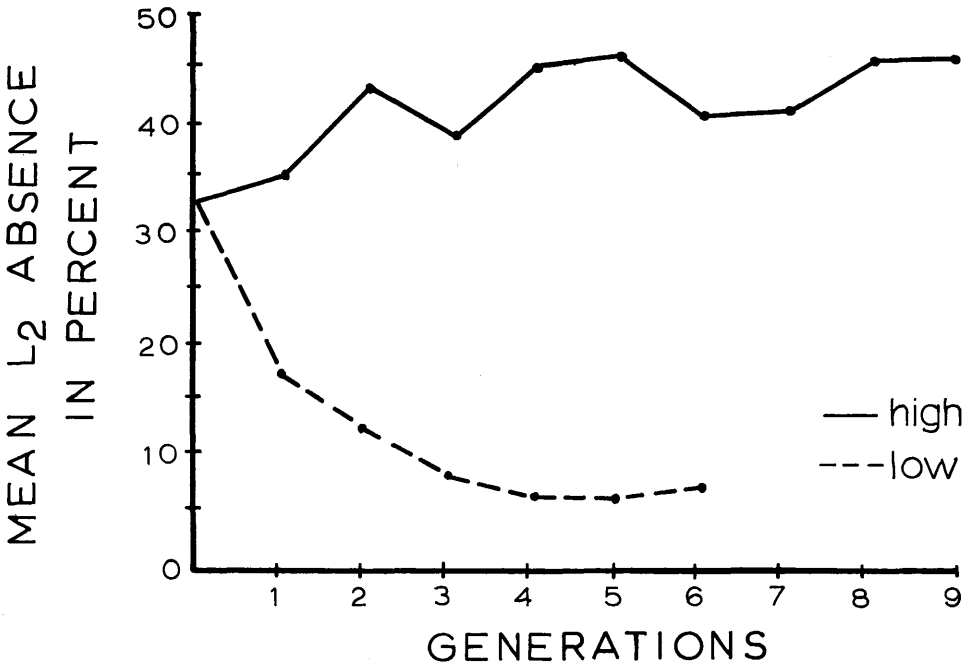

FIGURE 2. Mean expressivity in percent of L_2 absence for unselected stock and for each generation in the establishment of high and low lines.

The results of selection for nine generations for the high line and for six generations of selection for the low line are given in figure 2 for combined males and females. The initial mean expression of L_2 interruption before selection was 33.24 per cent.

The first generation of selection for a high line was characterized by a mean L_2 absence of 35.53 per cent. At the end of the second generation, the average amount of interruption had increased to 43.82 per cent. The third generation dropped back to a mean of 39.35 per cent, but rose again in the fourth generation

to 45.87 per cent, and reached its maximal value of 46.91 per cent at the end of five generations of selection. The ninth and final generation yielded an average L_2 interruption of 46.76 per cent.

Selection for the low line was more rapid than that in the high line. After one generation, the mean interruption had dropped to 18.14 per cent. The second generation was characterized by a mean value of 13.11 per cent and, by the end of the third generation, the average expression had been reduced to 9.1 per cent. The minimal value of 7.19 per cent L_2 interruption was recorded at the end of the fifth generation, and the sixth and final generation had a mean value of 8.27 per cent L_2 absence.

Starting with the unselected stock which showed approximately one-third of the L_2 missing, it was possible, by selection, to produce a high line characterized by approximately one-half of the vein absent, and a low line in which the average interruption was less than one-tenth of the length of the vein.

Although selection for high and low lines was initiated at the same time, selection for high expression extended over nine generations, while the period for selection for low expression was six generations. This difference in selection periods for the two lines resulted from decreased viability of the low line, leading to its loss. The selection for a low line was then reinitiated from the unselected mutant stock and continued for six generations.

Analysis of Crosses Between High and Low Lines

For the purpose of evaluating the genetical basis of the difference between the high and low lines produced by selection, reciprocal single pair matings were made between the two lines, followed by matings between F_1 individuals, and between the F_1 and the parental lines. No differences were observed between reciprocal matings; therefore, one can assume the absence of sex chromosome differences between the two lines The mean L_2 interruption and total flies scored for the unselected, high, low, F_1, F_2, and backcross populations are given in table 5.

TABLE 5

Mean L_2 interruption and number of flies scored for the unselected, high, low, F_1, F_2, and backcross populations at 26° C (males and females combined)

Population	Mean	Total
Unselected	33.24	349
High Line	46.76	185
Low Line	8.27	81
F_1	24.41	1239
F_2	29.40	860
Backcross to High line	36.73	1147
Backcross to Low line	17.01	315

These data are consistent with the assumption of additive effects of the modifier differences characterizing the two mutant lines. The F_1 population had a mean approximately half way between the parental means, the mean of the F_2 not deviating too greatly from the F_1 mean. The backcross data afford a further test for the hypothesis of additive action of the modifier differences characterizing the two lines (Mather, 1949). Under this assumption, the two backcross means would be expected to fall at the midpoint between the F_1 mean and the parental mean in question. For the backcross to the high line, a mean expression of 36.73 per cent L_2 interruption was obtained, in close approximation to 35.58 per cent expected under the additive hypothesis. Similarly, the mean of the backcross to the low line was 17.01 per cent, as opposed to an expected value of 16.34 per cent.

SUMMARY

A study has been made of the mode of inheritance and expression of a mutant causing an interruption of the second longitudinal vein in *Drosophila melanogaster*.

The results indicate the mutant phenotype is recessive, high penetrance being due to the homozygosity of factors found on both the second and third chromosomes. A very low penetrance of mutant expression is realized with homozygosity of either the second or third chromosome of the mutant stock, coupled with heterozygosity of the other chromosome.

Selection for high and low lines of expression was initiated and, after nine generations of selection for a high line and six for a low line, a significant difference between the two was obtained. These selection experiments suggest the presence of a polygenic system of modifiers which change the expression. Analysis of crosses between high and low lines, F_1 crosses, and backcrosses to both parental lines gives evidence for additive action of the polygenes controlling L_2 expression.

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