Genetics of an L2 Venation Mutant in Drosophila Melanogaster: II. Pattern Effects

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DROSOPHILA MELANOGASTER
II. PATTERN EFFECTS

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ABSTRACT

Pattern effects of the mutant $L_2$ vein in Drosophila melanogaster were analyzed at 18°, 26° and 30°C. Pattern profiles were made by dividing the vein into 20 equal intervals. These intervals were classified as to the presence or absence of vein material. The most frequent interruptions were found in the basal region of the vein. The vein interval 3, lying between 10 to 15 percent of the distance from the proximal end, was found to be the most sensitive region. This interval showed the highest frequency of interruption (in more than 50 percent of the flies), regardless of the temperature variable. The distal region was much more stable.

Differential pattern responses were found associated with changes in temperature and in sex. A general pattern characteristic of the mutant stock was noted at all three experimental temperatures.

INTRODUCTION

The venation system of Drosophila melanogaster lends itself quite well to genetic studies. Accurate quantitative data can be obtained by measuring the amount of vein or crossvein associated with the many venation mutants in this organism that both decrease and increase the amount of venation present, relative to the wild type. Also, the embryology of Drosophila concerned with wing development has been extensively studied by various workers (Auerbach, 1936; Bodenstein, 1950; Chen, 1929; and Waddington, 1939, 1940). The general results of these embryological investigations can be summarized as follows. The prepupal venation system is not identical with that found in the adult. At the prepupal stage, there is a vein at both the anterior and posterior margins of the wing, and also one in the middle of the wing that divides distally into branches which will later become the third and fourth longitudinal veins. The precursor of the fifth longitudinal vein is also present. The wing then swells up, due to an accumulation of liquid, and resembles an inflated balloon. Elongated processes are formed at the bases of cells on the upper and lower surfaces of the wing and these are joined together. The veins appear at this time, the developmental sequence of which has been described by Waddington (1940).

In a previous paper (Carlson, 1966), the classification of the degree of interruption of the second longitudinal vein ($L_2$) in the mutant stock being evaluated was given by arbitrarily setting up six classes of interruption. These six classes were as follows:

- Class 0—Normal $L_2$
- Class 1—0–20% of $L_2$ absent
- Class 2—20–40% of $L_2$ absent
- Class 3—60–80% of $L_2$ absent
- Class 4—80–100% of $L_2$ absent

This system takes into account the total amount of absence of vein and is not designed to shed light upon the pattern of this absence (i.e. a fly with 15% interruption in the basal segment of $L_2$, a fly with 15% interruption in the distal segment, a fly with 15% interruption in the central portion, and a fly with 5% basal, 5% central, and 5% terminal $L_2$ interruption are all classified alike in Class 1).

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From previous studies (Carlson, 1960), it has been shown that temperature changes the expression and penetrance of this mutant. This paper reports on an attempt to analyze the effects of temperature on the pattern of $L_2$ interruption of this mutant system.

MATERIALS AND METHODS

The standard culturing medium of corn meal, agar, dextrose, yeast, and mold inhibitor was used to culture the flies. Cultures were incubated at $26 \pm 1^\circ C$, $30 \pm 1^\circ C$, and $18^\circ C$. At the latter temperature, variation was as high as at $-4^\circ C$, this greater variation being due to a malfunctioning in the incubator detected, by means of a Taylor recording thermograph near the end of the experiment.

Matings at $26^\circ C$ and $30^\circ C$ involved single-pair replications, the parents of a given mating being placed initially in shell vials for 24 hours and then being transferred to half-pint bottles. Previous experience had shown that viability of the mutant stock was reduced at $18^\circ C$. Because of this reduced viability, four males and four females were used for matings at this temperature.

For analysis of pattern of mutant expression, the presence and absence of interruption was determined for the progeny of a given mating at each of twenty equally spaced intervals along the course of the second longitudinal vein. Wings from forty flies of each sex at each of the three experimental temperatures ($18^\circ C$, $26^\circ C$, and $30^\circ C$) were dissected off and mounted on glass slides. The wings were projected from a Tri-Simplex projector. Entries of less than 80 in Table 1 are due to unusable wings (i.e. torn or distorted). A grid ruled into twenty-one equally spaced lines was superimposed upon the wing image so that the $L_2$ vein was divided into twenty equal segments. The presence or absence of vein in each 5% segment was scored. By changing the angle of the ruled grid so that the points of intersection of $L_2$ with $L_3$, and of $L_2$ with the wing margin coincided with lines one and twenty-one of the grid, it was possible to compensate for variations in wing size.

Chi-square tests were performed by means of $k \times 2$ contingency tables, where $2 =$ interrupted vs. non-interrupted intervals, and $k =$ males vs. females or temperature variables, depending upon the data used.

RESULTS

The most frequently found pattern of expression was an interruption of the basal segment of the $L_2$ vein. A more graphic picture of the pattern of expression found in the mutant stock can be obtained by determining the presence or absence of venation for chosen intervals along the length of the $L_2$ vein from the points of junction with $L_3$ and with the anterior margin of the wing. The values obtained from this method are given in Table 1.

TABLE 1
Results of interruption for wings of males and females raised at $18^\circ$, $26^\circ$ and $30^\circ C.$ for each of 20 equal $L_2$ intervals. The numbers under intervals denote how many veins out of the total exhibited presence of $L_2$

| Sex | Temperature | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|-----|-------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|
| ♀   | $18^\circ$  | 9 | 18 | 71 | 42 | 36 | 37 | 48 | 63 | 67 | 69 | 72 | 70 | 72 | 71 | 70 | 73 | 71 | 71 | 66 | 64 | 54 | 73 |
| ♂   | $18^\circ$  | 9 | 74 | 49 | 35 | 40 | 51 | 63 | 71 | 75 | 75 | 74 | 73 | 72 | 70 | 72 | 72 | 71 | 71 | 71 | 68 | 54 | 75 |
| ♀   | $26^\circ$  | 9 | 58 | 6 | 2 | 2 | 4 | 13 | 20 | 40 | 64 | 86 | 72 | 72 | 73 | 74 | 74 | 74 | 74 | 74 | 68 | 74 |
| ♂   | $26^\circ$  | 9 | 73 | 22 | 6 | 6 | 13 | 24 | 39 | 48 | 60 | 67 | 71 | 72 | 72 | 74 | 76 | 76 | 76 | 76 | 76 | 76 |
| ♀   | $30^\circ$  | 9 | 52 | 11 | 5 | 5 | 6 | 16 | 22 | 38 | 49 | 57 | 61 | 67 | 71 | 75 | 78 | 78 | 78 | 78 | 78 | 71 | 76 |
| ♂   | $30^\circ$  | 9 | 56 | 17 | 5 | 7 | 9 | 18 | 25 | 35 | 47 | 50 | 58 | 63 | 64 | 66 | 69 | 71 | 71 | 71 | 70 | 61 | 72 |
The population of left and right wings within a given sex and a single temperature show no significant differences for any given $L_2$ interval. For this reason, the values for left and right wings were combined in Figures 1, 2, 3, 4, and 5, and in Table 1. The plotting of these intervals yields a profile of the regional pattern of interruption characterizing the mutant stock (fig. 1, 2, 3, 4, 5).

If the vein-making ability of the mutant stock is dependent, in any given region of $L_2$, upon a variable concentration of some vein-forming substance ($P$) and a threshold ($T$) for the venation response, the frequency scale would not be an appropriate one on which to compare the effects of genetic and environmental variables affecting the mutant phenotype (Wright, 1926; House, 1953). Assuming normal distributions with equal variances of a substance $P$ for each plotted interval of $L_2$, and a constant threshold for the venation effect, venation frequency for that interval can be thought of as that portion of the curve of variation exceeding the threshold. The relationship between the relative location of the mean and the threshold corresponding to any given frequency can also be thought of as that portion of the curve of variation exceeding the threshold. The relationship between the relative location of the mean and the threshold corresponding to any given frequency can be expressed as a normal deviate, $\frac{P-T}{\sigma}$. With a frequency of 50 percent interruption, the mean and the threshold would be the same and $\frac{P-T}{\sigma}=0$. For venation frequencies greater than 50 percent, the normal deviates will be positive. Venation frequencies less than 50 percent will produce negative values. This change from the frequency scale to one measuring the concentration of $P$ in units of standard deviation has been called the inverse probability transformation (Wright, 1926). The addition of five to the normal deviate value constitutes a probit and avoids the use of negative values.

![Figure 1. Pattern profiles of the mutant stock for males and females at 26°C.](image)

The pattern profiles of males and females for 20 equal intervals of $L_2$ from base (interval 1) to tip (interval 20) are plotted in Figure 1. In this figure both probit (left) and frequency-of-vein-present (right) scales are given. The profiles indicate quite clearly the greater frequency of interruption of the proximal segments of $L_2$, as opposed to that in the more distal regions. Chi-square tests of the differences in frequency of venation between regions 3 vs 10, 3 vs. 15, and 3 vs. 20 all yield probability values less than 0.01.

An interesting feature of the difference in expression between males and females
is revealed by a comparison of the male and female profiles of Figure 1. Females show a significantly lower tendency for venation formation in intervals 1 and 2 (chi-square test, p < .01) than do males, yet in the regions 10-19 there is no significant difference in frequency of interruption between the sexes. In general, females are more likely to exhibit a slightly greater degree of interruption at the three temperatures used in this study.

For the purpose of evaluating the effects of temperature on pattern, profiles for males and females at 18°C and 30°C have been plotted in Figures 2 and 3 for comparison with those obtained at 26°C (fig. 1). In addition, the pattern profiles for males only and for females only at all three temperatures have been plotted in Figures 4 and 5, respectively.

Sex differences found at 26°C for intervals 1 and 2 were not found for these same intervals at 18°C or 30°C (Table 1; fig. 1, 2, and 3). A common feature found at all three temperatures is that the profile curves for males and females
intersect in at least one point. At 30°C (fig. 3), the curves intersect at interval 9, and thereafter the frequency of \( L_2 \) vein absence in males is always greater than, or equal to, the frequency of \( L_2 \) vein absence in females, indicating a differential response for the various regions of the vein in the different sexes. There is, similarly, a reversal of temperature effect in the proximal region versus the distal regions of \( L_2 \), as seen in Figure 4 for 18° versus 26°C, and in Figure 5 for 18° versus 30°C. Another common feature of pattern expression for all temperatures is the characteristic dip in number of wings with venation of the basal region, followed by a steady climb to a plateau of increased number of wings having venation in the central region, with a slight dip in number of wings showing venation in the two terminal intervals.

A chi-square analysis for differences in the frequency of venation in females at 18°C, 26°C, and 30°C for intervals 1, 2, 9, 10, and 20 yields significant differences
for these intervals at the 1% level. The same analysis for males yields significant
differences (1% level) for intervals 1, 2, 9, and 10, but not for interval 20.

DISCUSSION

On the basis of Waddington's observations, House (1953) has postulated that
the presence or absence of venation at any given point along the vein is a function
of the presence or absence of basal processes sent out by the epithelial cells. In
the absence of these processes, or following their disappearance after formation
(apparently the case for L₂), a lacuna is formed which represents the vein channel.
In this framework, the theoretical vein-forming substance P would be thought of
as an inhibitory substance preventing the outgrowth of basal processes and there-
fore making possible the formation of the lacuna or vein. When P for a given
region of a vein drops below a critical threshold, T, basal process outgrowth takes
place and the vein lacunae are destroyed. Thus, to quote House, (1953, p. 323)
"The vein-forming process . . . would appear to be an all or none reaction, the
outcome of which is contingent on the conditions controlling the presence or
absence of outgrowth of cytoplasmic processes in the cells over-and-under-lying
the particular region in question."

This theoretical substance, P, then becomes identified as a material responsible
for inhibiting basal-process outgrowth. Its supposed regional variation relative
to a fixed threshold for inhibition forms the basis for the transformed frequency
profiles of Figures 1, 2, 3, 4, and 5.

In connection with these profiles, note that changes in sex, genotype, and
temperature do not affect the proximal and distal regions of L₂ in the same manner,
even when the changes are measured on the probit scale. This differential response
of proximal and distal regions of a longitudinal vein to changes in temperature
and sex has also been demonstrated by House (1952; 1953) for the fourth longi-
tudinal vein (L₄). In addition, changes in dosage of the cubitus interruptus (ci)
allele show the same differential response of proximal and distal regions of L₄
as do temperature changes.

Perhaps a more interesting comparison is that involving the reversal of the
effect of change in sex (fig. 1 and 3) and temperature (fig. 4 and 5) on the proximal
versus distal region of L₂ found in the current study, and the reversal of the response
of these regions of L₄ to changes in dosage of the ci⁺ allele in the presence of the
engrailed (en) substitution found by House (1953). Whether this difference in
behavior of the proximal and distal regions of the longitudinal veins in question
results from some underlying basic difference in the development of venation in
these regions or is peculiar to the mutants in question is unknown.

SUMMARY

General conclusions regarding pattern of L₂ interruption are as follows:
1. The most frequent interruption occurs at the proximal region of the vein.
Interval 3 showed the highest frequency of interruption, being absent in more than
50 percent of the wings examined at all three temperatures.
2. The distal region of the vein was stable. At all temperatures, intervals
12 through 17 showed less than 10 percent average interruption.
3. Differential responses to temperature changes were found between sexes
and between certain intervals.
4. A general pattern, characteristic of the mutant stock at all temperatures,
was present.

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REFERENCES


