STUDIES ON THE INTERACTION OF MUTATIONS AFFECTING THE CHAETAE OF DROSOPHILA MELANOGASTER. II. THE RELATION OF CHARACTER EXPRESSION TO SIZE IN FLIES HOMOZYGOUS FOR POLYCHAETOID, HAIRY, HAIRY WING, AND THE COMBINATIONS OF THESE FACTORS

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THE study of the results of combining various genetic factors affecting a particular embryonic system permits certain inferences concerning the nature of gene action. Favorable material for such a study is supplied by three Drosophila melanogaster mutations whose chief detectable effects are to increase the number of chaetae present on the fly. In the first paper of this series (Neel 1941) it was reported that the phenotype of a combination of any two or all three of these mutations generally represented more than the sum of the effects of these mutations when acting separately. A barrier to an analysis of the developmental reactions revealed by these data was the following circumstance: the eight different genotypes included in the study were in some cases characterized by significantly different mean fly sizes. Since previous work (Macdowell 1915, Neel 1940a) had shown that chaetae number is correlated with fly size, any attempt to analyze the data without first correcting for the size differences between genotypes would be to introduce an initial bias; the data then on hand were not considered adequate to make this correction.

In the present paper the equations for the regression on fly size of each of the five chaetal characteristics studied in the different genotypes are derived where possible. These equations are then utilized in the analysis of two related questions: (1) What do the interaction data, corrected for size differences, reveal concerning the embryonic reactions involved in the formation of supernumerary chaetae? (2) How are the regression coefficients in a genotype ab related to the coefficients observed in either a or b? This latter question is one on which we have at present little information, important though it is to the problem of related increases in the magnitudes of two measurements.

MATERIALS AND METHODS

The factors utilized in this study were Hairy wing (Hw, 1-o-o+), hairy (h, 3-26.5), polychaetoid (pyd, 3-39±2), yellow (y, 1-o-o), and sepia (se, 3-26.0). The first three factors increase the number of chaetae present. The y and se genes have no known effects on the chaetae but were used in the crosses as markers of Hw and h, respectively. After y Hw, se h, and pyd strains had

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been rendered highly isogenic with a long inbred Oregon wild type line through repeated crosses to, and extractions from, this line, the possible combinations of the three factors affecting the chaetae were synthesized. The study thus includes a total of eight genotypes: \( +^{O}r, y Hw, se h, pyd, y Hw; se h, y Hw; pyd, se h pyd, \) and \( y Hw; se h pyd. \) The considerations dictating the choice of these particular mutations and details concerning the synthesis of the stocks are given in the earlier paper.

Five characteristics pertaining to the chaetae were investigated: number of dorsocentral bristles, number of scutellar bristles, number of scutellar hairs, number of hairs on the right second longitudinal wing vein, and number of teeth in the right sex-comb. For each genotype the relation of these characteristics to mean fly size, as measured by femur length, was derived in the following manner: Flies of the desired genotype were allowed to oviposit on small food chips for eight to 12 hours. At the end of this time the eggs were divided into lots of either 100 or 200, and each lot placed in a \( 23 \times 95 \) mm shell vial which contained 10 cc of a yeast suspension absorbed on one sheet of Kleenex, an absorbent paper preparation. The concentration of the yeast suspension in different vials varied between 2.5 and 25 percent. By regulating the number of eggs introduced into the vial and the concentration of the yeast suspension, it was possible to get groups of flies of very different mean sizes. All experiments were conducted at \( 25.0 \pm 0.3 \)°C. To establish the nature of the regression obtaining in any one genotype, groups of 50 male flies raised at a particular food level were investigated as to mean femur length and the mean condition of each of the five chaetal characteristics. The data on each fly were kept separate. The examination of eight or nine such groups raised at different food levels gave a sufficient spread in the mean values of the characteristics to permit a derivation of the regression coefficients, using the method of least squares. To be certain of a considerable distribution in the values of the means, it was customary to select from the group raised at the lowest food level (initial condition: 200 eggs on 10 cc of a 2.5 percent yeast suspension) the 50 smallest males and derive the mean values in this special group.

The duration of the egg-larval-pupal stage is longer when flies are raised at a low food level than when food conditions are more favorable. In an earlier experiment, no relation between the length of egg-larval life and the number of dorsocentral bristles could be detected in \( pyd \) flies nor in flies containing another bristle mutation, Dichaete (NEEL 194oa). It is assumed, in the absence of any evidence to the contrary, that the same lack of correlation between character expression and the duration of development is true for all chaetae, both in \( pyd \) and in \( Hw \) and \( h \) flies. The differences in the length of the life cycle in different groups of flies, resulting from the technique employed, are thus regarded as having no effect upon the regressions observed.

RESULTS

The regressions in the different genotypes

The complete results of this procedure are too bulky for publication, but are on file with GENETICS. The exact nature of the regressions of the various chaetal
characteristics on femur length is difficult to determine when one of the variables, femur length, shows such relatively small variations. Thus, in any one case the regression shows good conformity to a straight line no matter whether the data are plotted arithmetically, logarithmically, or semi-logarithmically. Accordingly, the regressions were derived on the assumption of a linear relationship between the expression of the chaetal characteristic and femur length. The regressions, derived by the method of least squares, were of the form \( E = ax + b \), where \( x \) is the femur length, \( a \) the slope of the regression line, \( b \) the intercept on the Y-axis, and \( E \) consequently the estimated value of the characteristic at the given femur length. Values of \( a \) and \( b \) are given in table I.

Where a character showed no, or very slight, variation, regression constants could, of course, not be obtained. In a very few instances where the departure from wild type shown by a characteristic in a particular genotype was small, at lower body sizes this characteristic appeared essentially normal. In such cases the regression was derived only over that size range in which appreciable variation occurred. The range in femur length over which any one characteristic was observed to vary sufficiently from wild type to be studied is indicated in table I. The average number of paired determinations used in the derivation of each of the 33 regressions was 451.5 (9.03 groups). The standard error of estimate of \( E \) is also given in the table, as an indication of the dispersal of the points about the line of best fit. The regression equations are plotted in figure I. The projection of the regression line on the abscissa indicates the range in mean femur length studied, while its projection on the ordinate indicates the approximate range in the chaetal characteristic.

The effect of modifiers on the expression of the three factors

The differences between these eight genotypes which are described in table I and figure I are undoubtedly for the most part referable to the known genetic differences between the strains. However, an unknown portion of these differences may be ascribed to an initial failure to render these lines completely isogenic for all factors except the recognizable mutations under investigation, and to the appearance of different modifying factors in the strains during the eight to ten months that elapsed between the synthesis of the strains and their use in this study.

The \( se \ h \) data are pertinent in this connection. When the data on this genotype were first collected, they led to the following regressions (each regression based on 500 paired determinations (10 groups)): scutellar hairs: \( E = 0.0631x - 7.389 \); scutellar bristles: \( E = 0.00289x + 2.673 \); wing hairs: \( E = 0.0377x - 8.726 \); sex-comb teeth: \( E = 0.0116x + 4.102 \). When the values predicted on the basis of these equations were compared with the values reported in the earlier paper (Neel 1941), a discrepancy was apparent. Accordingly, the \( se \ h \) strain was crossed to, and the factors extracted from, the +\( Or \) line three additional times. The regressions were again derived and found to be in better agreement with previous results. It is this second set of \( se \ h \) data which is summarized in table I and figure I. At a femur length of 525\( \mu \) the following differences exist between the values predicted on the basis of the two sets of
Table 1

The regression of the five chaetal characteristics on femur length, in the eight different genotypes. Where the range of variation is small, or where there is only sporadic variation, no regression constants have been calculated. The constant \( a \) represents slope, and \( b \) the Y-intercept, in an equation of the form \( E = ax + b \), where \( x \) is mean femur length and \( E \) the estimated value of the characteristic at this femur length. The error appended to the \( a \) values is the standard error of estimate of \( a \). The constant \( s_{y-x} \) is the standard error of estimate of \( E \). The figure in parentheses after the "range of \( x \)" indicates the number of groups of 50 flies each included in that range.

<table>
<thead>
<tr>
<th>Character</th>
<th>Range of ( x )</th>
<th>( y ) ( \mu )</th>
<th>se ( y )</th>
<th>( y ) ( \mu ); se ( y )</th>
<th>se ( h ) ( \mu )</th>
<th>se ( h ); se ( h ) ( \mu )</th>
<th>se ( h ) ( \mu ); se ( h ) ( \mu )</th>
<th>se ( h ) ( \mu ); se ( h ) ( \mu )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>-</td>
<td>420.5-549.4</td>
<td>0.2</td>
<td>444.0-536.2</td>
<td>415.0-543.5</td>
<td>444.0-536.2</td>
<td>415.0-543.5</td>
<td>444.0-536.2</td>
</tr>
<tr>
<td>of ( x )</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
</tr>
<tr>
<td>Dorsocentral</td>
<td>a</td>
<td>0.016±0.00144</td>
<td>0.015±0.00206</td>
<td>0.024±0.0068</td>
<td>0.016±0.00198</td>
<td>0.0148±0.00663</td>
<td>0.0234±0.00250</td>
<td>0.0286</td>
</tr>
<tr>
<td>bristle</td>
<td>b</td>
<td>-0.603</td>
<td>-2.275</td>
<td>-3.364</td>
<td>-0.251</td>
<td>-2.314</td>
<td>-1.417</td>
<td>-1.417</td>
</tr>
<tr>
<td>Range</td>
<td>-</td>
<td>420.5-549.4</td>
<td>492.3-558.6</td>
<td>513.0-553.1</td>
<td>474.9-550.2</td>
<td>414.9-543.5</td>
<td>406.1-529.6</td>
<td>418.9-558.4</td>
</tr>
<tr>
<td>of ( x )</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
</tr>
<tr>
<td>Scutellar</td>
<td>a</td>
<td>0.00555±0.00512</td>
<td>0.00587±0.00513</td>
<td>0.00564±0.00222</td>
<td>0.00679±0.0040</td>
<td>0.0037±0.00645</td>
<td>0.0149±0.00000</td>
<td>0.0013</td>
</tr>
<tr>
<td>bristle</td>
<td>b</td>
<td>0.0483</td>
<td>3.580</td>
<td>2.684</td>
<td>2.605</td>
<td>-0.129</td>
<td>1.3.15</td>
<td>-1.476</td>
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<tr>
<td>Range</td>
<td>-</td>
<td>456.8-558.6</td>
<td>418.7-553.1</td>
<td>444.9-556.2</td>
<td>415.0-543.5</td>
<td>406.1-529.6</td>
<td>418.9-558.4</td>
<td>418.9-558.4</td>
</tr>
<tr>
<td>of ( x )</td>
<td>(g)</td>
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<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
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<tr>
<td>Scutellar</td>
<td>a</td>
<td>0.0054±0.00530</td>
<td>0.00172±0.00396</td>
<td>0.0145±0.00287</td>
<td>0.0087±0.0109</td>
<td>0.0049±0.0239</td>
<td>0.0852±0.00102</td>
<td>0.0852±0.00102</td>
</tr>
<tr>
<td>hairs</td>
<td>b</td>
<td>-1.519</td>
<td>-14.854</td>
<td>-8.219</td>
<td>-5.357</td>
<td>-14.775</td>
<td>5.615</td>
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<tr>
<td>Range</td>
<td>-</td>
<td>456.8-558.6</td>
<td>418.7-553.1</td>
<td>444.9-556.2</td>
<td>415.0-543.5</td>
<td>406.1-529.6</td>
<td>418.9-558.4</td>
<td>418.9-558.4</td>
</tr>
<tr>
<td>of ( x )</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
</tr>
<tr>
<td>Wing hairs</td>
<td>a</td>
<td>0.0147±0.00576</td>
<td>0.023±0.00713</td>
<td>0.0247±0.00297</td>
<td>0.0640±0.0129</td>
<td>0.0125±0.0070</td>
<td>0.0926±0.0050</td>
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<tr>
<td>b</td>
<td>-</td>
<td>-1.467</td>
<td>-7.533</td>
<td>-2.615</td>
<td>0.385</td>
<td>1.028</td>
<td>5.842</td>
<td></td>
</tr>
<tr>
<td>Range</td>
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<td>420.5-549.4</td>
<td>492.3-558.6</td>
<td>474.9-550.2</td>
<td>414.9-543.5</td>
<td>406.1-529.6</td>
<td>418.9-558.4</td>
<td>418.9-558.4</td>
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<tr>
<td>of ( x )</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
</tr>
<tr>
<td>Sex-comb teeth</td>
<td>a</td>
<td>0.0080±0.000828</td>
<td>0.0118±0.00113</td>
<td>0.0111±0.00160</td>
<td>0.0090±0.00138</td>
<td>0.0154±0.00640</td>
<td>0.0122±0.00075</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>5.606</td>
<td>3.640</td>
<td>4.656</td>
<td>5.633</td>
<td>2.106</td>
<td>2.009</td>
<td>4.832</td>
</tr>
<tr>
<td>Range</td>
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<td>420.5-549.4</td>
<td>456.8-558.6</td>
<td>418.7-553.1</td>
<td>444.9-556.2</td>
<td>415.0-543.5</td>
<td>406.1-529.6</td>
<td>418.9-558.4</td>
</tr>
<tr>
<td>of ( x )</td>
<td>(g)</td>
<td>(g)</td>
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<td>(g)</td>
<td>(g)</td>
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</tr>
</tbody>
</table>

* These values considerably influenced by one aberrant point.
regressions: scutellar hairs, $3.01 \pm 0.86$; scutellar bristles, $0.11 \pm 0.12$; wing hairs, $5.31 \pm 1.27$; and sex-comb teeth, $0.18 \pm 0.33$. In both cases where the differences are significant, the strain which had been crossed the three additional times showed the lower values. These results are most easily explained by assuming the accumulation in the strain of one or more positive modifiers of some of the chaetal characteristics under consideration, with the loss of these modifiers on outcrossing. The modifiers appear to be rather specific, in that they affect those two of the four characteristics under observation which are phenotypically most similar.

In the $se\ h$ case, the accumulation of modifiers was first detected because of the relatively large effect on the wing hairs. Comparison of the present data with the earlier results suggests that other less striking modifiers may have

**Figure 1.**—The relation between mean femur length and the mean number of chaetae present in five different regions, in $yHw, se\ h, pyd, yHw; se\ h, yHw; pyd, se\ h, pyd, yHw; se\ h, pyd$, and wild type *Drosophila melanogaster* males. Details in text.
accumulated in several strains. Hersh (1930) has recorded a similar rapid appearance of modifiers of a quantitatively variable characteristic in Drosophila melanogaster, Bar eye. Moreover, there can be little doubt that various wild type strains, in spite of their anatomical similarities, differ greatly with respect to their complement of minor modifying factors (cf. Pippin 1942). The interaction of these three factors might therefore appear significantly different if they were studied on another genetic background. It is obvious that under such circumstances one must be cautious in the development of theoretical treatments of the data.

**ANALYSIS OF THE DATA**

It is apparent from figure 1 that in the cases of two of the five characteristics studied (number of scutellar hairs, number of hairs on the right second longitudinal wing vein) the relations of the various regressions on femur length are essentially the same. In each case there is evident a family of straight lines in which, within the range of chance variation, at any given value of x over the range in x studied the slope of any single line tends to be directly proportional to the magnitude of its y value. In each case this series of lines can be transformed to a family of lines whose slopes are not significantly different by plotting the ordinate logarithmically. Moreover, the slopes of the wing hair regressions are quite similar to those of the scutellar hair regressions. Such a treatment of a third characteristic, the scutellar bristles, although it lessens the differences between lines with respect to their slopes, still leaves a situation where at a given x value the equations yielding the largest y values have the greatest slopes. But if in this latter case one plots as ordinate the logarithm of the bristle number minus four, one secures a family of parallel lines. The theoretical justification in the subtraction of four lies in the fact that normally three are four scutellar bristles present; the bristle number minus four is thus the number of extra bristles present. There are in the wild type only very sporadic hairs present on the scutellum, and none on the second longitudinal wing vein, so that the values shown for these characteristics refer without correction to the number of extra chaetae. In these three regions, then, the number of extra chaetae is apparently related to the size of the fly in a similar manner.

The situation with respect to the dorsocentral bristles appears to be different. Thus, when the ordinate is taken as the logarithm of the number of extra bristles, the smallest regression coefficient \((y_{HW}; se h)\) is 0.00183 ± 0.000216, and the largest \((se h pyd)\), 0.00877 ± 0.000480. But when the logarithm of the total number of dorsocentrals is plotted against femur length, there is a reasonable approximation to parallelism, with the greatest difference between any two slopes \((y_{HW}; se h—se h pyd)\) being 0.000423 ± 0.000484. While it is impossible to say to what extent this situation is due to the operation of chance and accumulated modifiers, the fact, to be discussed below, that the type of interaction seen here differs from that in the preceding three systems suggests that this difference is real and characteristic of the region.

The sex-comb data present a unique picture. The variation in the mean number of teeth in the right sex-comb is so small that the relations of the data
are not materially altered by logarithmic plotting. The seriation with respect to
the steepness of the regressions on an arithmetic plot is as follows: +Or, se h,
y Hw, pyd, se h pyd, y Hw; se h, y Hw; pyd, and y Hw; se h pyd, with the latter
two separated from the former by quite a gap. Unlike the other systems, this
seriation does not appear to be related to the magnitude of the departure from
wild type, but rather to the number of mutations present in the strain. For
instance, although the regression coefficient for y Hw; se h is significantly
greater than for +Or, flies of the former genotype have significantly fewer
teeth in the sex-comb at all sizes studied. The relative steepness of the regres-
sion in the two genotypes containing y Hw and pyd may indicate a type of
specific interaction.

As pointed out above, the present data, because of the relatively small range
of the variables, can equally well be treated as an expression of a linear relation
(y = ax + b) or of an exponential function (y = bx^k); for simplicity’s sake the
former treatment was chosen. Since the variations in mean femur length are
relatively small (although corresponding to an approximately six- to eightfold
variation in body weight), conversion of the femur lengths to their logarithmic
equivalents would not alter the relative femur lengths to an important extent.
The conversion of the considerably more variable numbers of hairs and bristles,
as described above, therefore gave essentially the same picture as would have
resulted from treating the data as an expression of the second relation, which
in its logarithmic form becomes log y = log b + k log x. In view of the large
amount of work done on biological relationships of this nature, it is of interest
to consider the data from this angle.

Although the equation y = bx^k has been found to describe a variety of cases
of correlated increase in two measurements, the exact biological significance
of the constants b and k remains unclear. In the present case, k may be re-
garded simply as the ratio of the percentage change in the number of chaetae
to the corresponding change in femur length. A more exact definition depends
on knowledge of the time course of determination of chaetae and femur length
—exponential, parabolic, or sigmoid—and such is lacking at present. The sig-
ificance of b is even more ambiguous. As the value of y at x = 1 in the origi-
nal system of measurement, it is in these cases a meaningless extrapolation.
However, as Hersch (1941) has pointed out, since our units of measurement
along the X-axis are arbitrary, we can select any point along this axis as the
origin of a new axis parallel to and coinciding with the old one, and if in so doing
we select an origin such that x = 1 has biological significance, then the values
of b acquire greater meaning. In the present case we can set x = 1 at the smallest
femur length held in common by all the genotypes studied with respect to a
given system. The values of y at x = 1 as read from the figure may then be
regarded simply as indices of the bristle-forming abilities of these genotypes in
the neighborhood of the smallest size at which all show extra bristles.

It is clear that in the present case, the effects of a given set of genes on b and
k vary according to the region under observation. When the scutellar and wing
hairs and the dorsocentral bristles are considered, k remains relatively con-
stant while b varies according to the genotype. The modifiers of the wing and
scutellar hairs studied in connection with se h also altered b without significantly affecting the value of k. In the scutellar bristles there is a direct relationship between the magnitudes of b and k; this is probably the first time such a relationship has been described. If we render the bristle data comparable with the hair data, by subtracting four from all means, the dorsocentral data approximate an inverse relationship between the magnitudes of b and k, a type of relationship first described by Hersh (1931), while for the scutellar bristles, k remains constant with increasing magnitudes of b, as in the scutellar and wing hairs. The latter situation is an interesting example of how in a group of related cases fundamental similarities in the manner of increase of two measurements may be obscured by the initial value of one of the measurements. Finally, the sex-comb teeth fail to show any consistent relationship between slope and intercept values. The possible significance of these findings in relation to correlated growth problems will be discussed later.

The foregoing considerations have been directed primarily at the second of the two questions raised in the introduction—namely, the manner of inheritance and interaction of regression coefficients. The first question concerned the types of embryonic reactions revealed within the various chaetae-forming regions, after all the genotypes have been equated to a common size. In the analysis of such a question one is primarily concerned with bringing out such fundamental similarities and differences as may exist between the various systems. The following attempt at analysis will be based on the assumption that the effects of the various factors in combination would be fully predictable from full knowledge of their effects when acting separately. Danforth and De Aberle (1927) and Wright (1941) have drawn attention to an alternate possibility, “the gene substitutions may bring about not only quantitative differences along a chain of reaction but also transmit differences in specificity. It is possible to dismiss any interaction effect as merely an inexplicable manifestation of specificity, an emergent in the sense of Lloyd Morgan” (Wright 1941, p. 321). While such specificities should be postulated only as a last resort, the possibility of their existence, which in the present imperfect state of our techniques can be neither confirmed nor denied, must be borne in mind. Another conceivable cause of apparent irregularities in interaction, applying particularly to a form like Drosophila with highly mosaic development, is that just as there exist demonstrable differences from region to region in the type of interaction observed, so there are intra-regional differences; the evaluation of interaction effects within, for example, the dorsocentral area is thus the average of several different types of interaction and as such of course does not fall into any simple scheme. These two possibilities will be touched on later.

In consequence of the type of regression observed, it makes no great difference to the picture of interaction effects in the wing hairs, scutellar hairs, or scutellar bristles what particular size is used as a reference point, so long as it is within the range in which all genotypes show extra chaetae. This is not true of the dorsocentral bristles and sex-comb teeth. Table 2 shows the calculated values for wing hairs, scutellar hairs, and scutellar bristles in the various
genotypes at an appropriate femur length. Differences between these values and the corresponding values given in the earlier paper are due to the reduction to a common size, to differences in newly arisen modifying factors, to the fact that these experiments were carried out at 25°C rather than at 24°C as in the preceding work, and to chance.

**Table 2**

Calculated values for the number of extra wing and scutellar hairs and scutellar bristles at the femur length indicated.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Femur Length (µ)</th>
<th>pyd</th>
<th>se h</th>
<th>y Hw</th>
<th>y Hw;se h</th>
<th>y Hw;pyd</th>
<th>se h pyd</th>
<th>y Hw;se h pyd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wing hairs</td>
<td>500</td>
<td>5.12</td>
<td>5.88</td>
<td>32.39</td>
<td>9.74</td>
<td>8.18</td>
<td>40.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>±0.95</td>
<td>±0.38</td>
<td>±1.48</td>
<td>±0.42</td>
<td>±0.37</td>
<td>±0.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scutellar hairs</td>
<td>525</td>
<td>22.79</td>
<td>3.93</td>
<td>41.21</td>
<td>4.78</td>
<td>35.55</td>
<td>50.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>±0.53</td>
<td>±0.23</td>
<td>±1.25</td>
<td>±0.26</td>
<td>±0.39</td>
<td>±1.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scutellar bristles</td>
<td>525</td>
<td>0.191</td>
<td>0.070</td>
<td>0.098</td>
<td>1.605</td>
<td>0.564</td>
<td>0.399</td>
<td>2.347</td>
</tr>
<tr>
<td></td>
<td>±0.066</td>
<td>±0.086</td>
<td>±0.090</td>
<td>±0.160</td>
<td>±0.095</td>
<td>±0.077</td>
<td>±0.103</td>
<td></td>
</tr>
</tbody>
</table>

The wing hair data, equated to an average femur length of 500µ, may be considered first. Although pyd acting separately has no effect on the wing hairs, in combination with either h or Hw it is a strong modifier of hair number, and in each case to about the same extent. The effects of pyd when acting separately are therefore either sub-threshold or else can only be expressed in the presence of h or Hw. Hw and h in combination interact very strongly. If some arbitrary value be assigned to pyd, it is obvious that the effects of Hw, h, and pyd are more multiplicative when the genes are acting in combinations of two than when all three are together. Probably the simplest hypothesis of factor interaction which brings regularity into these data is an adaptation of the inverse probability transformation which has been described by Wright (1920) and Wright and Chase (1936). We may assume that at this intermediate fly size the mean number of hairs which can appear on the second longitudinal wing vein has an upper limit in the neighborhood of 45—that is, not much above the number present in y Hw;se h pyd. It is an observable fact that when such a number is present, hairs are more or less regularly spaced along the entire length of the vein, and that when hair number rises much above this there is a tendency for hairs to be located on small diverticula off the main vein, or on thickenings of the vein. This fact introduced difficulties into the determination of wing hair number. In the adaptation of Wright’s scheme it is assumed that the addition to the genotype of a given factor tending to increase the number of wing hairs does not have the same phenotypic effect in all combinations of such factors, but has a relatively small effect when acting near the ends of the range, and a greater phenotypic effect near the center, with the exact relation between the “genetic determination” as abscissa and
the phenotypic effect of a vanishingly small increase in “genetic determination” as ordinate approximating in appearance a normal frequency curve. By “genetic determination” is meant the sum total of those genetic effects which tend to evoke hairs on the wing vein. It follows that the relation between “genetic determination” and phenotype, derived from summing the area under the curve to the left of any “genetic determination,” is given by the ogive of the normal frequency curve, with the upper asymptote at 45 hairs. “Genetic determination” is measured in terms of \( \sigma \) units, with practically the entire range of effective dosage being included in a spread of \( 6\sigma \). To establish the “genetic determination” corresponding to a particular phenotype, one first calculates the fraction of the maximum possible phenotype which this particular phenotype represents, and then, using a table of normal curve areas, determines at what abscissal value in terms of \( \sigma \) units from the left end of the normal frequency curve such a fraction of the total area is reached. Conversely, the phenotype resulting from a given “genetic determination” is measured in terms of the area included under the curve between \( 0\sigma \) and that particular \( \sigma \) value from the left end. Genetic effects are assumed to be additive along the abscissa. This hypothesis is illustrated in figure 2.

Since \( pyd \) has no visible effect when acting separately, it is necessary to assign an arbitrary \( \sigma \) value to it. Making this value equal to \( .3\sigma \) units, and deriving from the data a value of \( 1.88\sigma \) for \( Hw \) and \( 1.79\sigma \) for \( h \), we get the following results for the four combinations:

\[
\begin{array}{cccc}
\text{Observed } \sigma \text{ value} & 3.58 & 2.21 & 2.09 & 4.27 \\
\text{Expected } \sigma \text{ value} & 3.67 & 2.18 & 2.09 & 3.97 \\
\text{Expected phenotype} & 33.71 & 9.27 & 8.18 & 37.53
\end{array}
\]

Figure 2.—The assumed relation between “genetic determination” (as defined in text) and the number of hairs on the second longitudinal wing vein. It is apparent that a given increase in “genetic determination” (for example, one \( \sigma \) unit) has a much larger phenotypic effect in the middle of the scale than toward either end.
Comparison of the expected phenotypes with those given in table 2 reveals only one significant difference, in \( y \ Hw; \ se \ h \) \( pyd \). Even closer agreement could be obtained by allowing for the probable departures of the primary phenotypes from the "true" phenotypic values, as the result of chance and modifying factors. Moreover, there is no \textit{a priori} reason for assuming the relation between the strength of the "genetic determination" and the effect of a small increase in this determination to be exactly symmetrical; a slight positive skewness could help account for the \( y \ Hw; se \ h \) \( pyd \) discrepancy.

The phenotypic interactions seen in connection with the scutellar hairs are somewhat similar to those met in the wing hairs. Thus, \( pyd \) has no effect in the absence of \( Hw \) or \( h \), but combined with either acts as a positive modifier. Moreover, the relative effect of \( pyd \) is greatest in combination with \( h \)—that is, at an intermediate phenotype. However, if some reasonable value be assigned to the maximum mean number of scutellar hairs, and an attempt be made to treat the data in the fashion employed for the wing hairs, significant departures from expectation are observed. Polychaetoid interacts more strongly with \( se \ h \) than with \( y \ Hw \). Now, it is well known that mutations affecting chaetae number characteristically have a region or regions of maximum effect, with the departure from wild type decreasing as distance from that center increases (cf. \textsc{Neel} 1940b for discussion and references). The scutellar hairs in \( y \ Hw \) flies tend to be on the anterior part of this region, while in \( se \ h \) they are usually more posteriorly situated (\textsc{Neel}, 1941). We may therefore surmise, following the discussion of p. 59, that the "invisible field" of \( pyd \), whose existence we deduce from the interaction of \( pyd \) with the other factors, more nearly coincides with that of one of these factors than the other, and so differences in the phenotypic interactions result. The general type of interaction would still be as in the former case.

The scutellar bristles, equated to a femur length of 525\( \mu \), give a quite different picture of factor interaction than do the wing or scutellar hairs. Extra bristles are present in all three primary genotypes, with the seriation \( pyd > y Hw > se h \). However, the combination of \( Hw \) and \( h \) exhibits a very significantly greater departure from wild type than either of the other two combinations of two factors, and this marked interaction is maintained in the combination of all three factors. \textsc{Wright} (1935) has described on theoretical grounds a type of factor interaction where the phenotypic departure from an optimum (in this case, wild type) varies as the square of an additive primary genetic effect. The effects of \( pyd \) in combination with \( Hw, h \) and \( Hw; h \) show a fair approximation to such a scheme. If we accept the values observed in \( pyd, se h, y Hw, \) and \( y Hw; se h \) as a good approximation to the "true" values, then the comparison between observed and expected results is as follows.

\[
\begin{array}{cccccccc}
\text{Genotype:} & \text{pyd} & se h & y Hw & y Hw; se h & se h pyd & y Hw; pyd & y Hw; se h pyd \\
\text{\textit{Xobserved:}} & .44 & .26 & .31 & 1.27 & .63 & .75 & 1.53 \\
\text{\textit{Xexpected:}} & .70 & .75 & 1.71 \\
\end{array}
\]

There is a good correspondence between observation and expectation in two of the three cases. In view of the roles of chance and modifiers in determining...
bristle numbers, it seems entirely possible that, for example, the observed values for \textit{pyd} and \textit{y Hw; se h} are too high by an amount equal to one times the standard error of their determinations. Assuming, then, a value for \textit{pyd} of 0.126 and for \textit{y Hw; se h} of 1.462, the comparison between “observed” and expected becomes as follows:

| Genotype: \textit{pyd} \textit{se h} \textit{y Hw} \textit{y Hw; se h} \textit{se h \textit{pyd}} \textit{y Hw; pyd} \textit{y Hw; se h \textit{pyd}} |
|:---:|:---:|:---:|:---:|:---:|:---:|:---:|
| \sqrt{V_{\text{observed}}}: | -0.35 | 0.26 | 0.31 | 1.21 | 0.63 | 0.75 | 1.53 |
| \sqrt{V_{\text{expected}}}: | 0.61 | 0.66 | 1.56 |
| \text{x}_{\text{expected}}: | 0.372 | 0.436 | 2.434 |

This is a somewhat better fit. No other simple scheme has been found which results in so good a comparison between observation and expectation. We may therefore tentatively regard the scutellar bristle results as produced by a multiplicative interaction between two factors, with a third factor acting additively, the phenotype increasing as the square of these additive primary effects. \textit{Charles} (1938) has reported a comparable interaction of genes affecting the spotting pattern in the mouse: on a suitably transformed scale three of the four factors investigated appeared to act additively, while the fourth had a multiplicative effect.

In both \textit{y Hw} and \textit{se h} the frequency of extra scutellars was so low at intermediate and small femur lengths that the regressions could be derived over only a relatively limited range in fly size. This picture of factor interaction is therefore to be derived from the data only at relatively large fly sizes.

In view of the proximity of scutellar hairs and bristles, both were equated to the same size in the above treatment, a size in this case dictated by the scutellar bristle data, in order to facilitate the establishment of any possible interrelationships. None was found.

In the case of the dorsocentral bristles and teeth in the sex-comb, the exact picture of gene effects which one obtains depends to a much larger extent than in the preceding on the size at which one evaluates these effects. This is obvious from figure 1. However, the general features of the interactions observed in the dorsocentrales are the same at all sizes, in spite of important differences in detail. Although extra dorsocentrales were present in all three primary genotypes, in \textit{se h} they were so sporadic that a regression could not be derived. The supernumerary dorsocentrales evoked by \textit{pyd}, and, more rarely, by \textit{se h}, are quite restricted in distribution, usually falling in line with the normal dorsocentrales, while those associated with \textit{y Hw} have a more general distribution. In spite of the wide differences in the \textit{pyd} and \textit{se h} phenotype, the interaction of either with \textit{y Hw} results in a very similar, phenotypically superadditive end effect. On the other hand, the interaction of \textit{se h} with \textit{pyd} results in a fly with fewer dorsocentrales than \textit{pyd}. The fact that a similar, although non-significant, interaction between \textit{se h} and \textit{pyd} was noted in the earlier investigation makes it probable that this peculiar effect is not due to modifiers in the \textit{se h \textit{pyd}}, \textit{pyd}, or \textit{se h} strains. While this suggests competition effects within the relatively limited area in which these factors induce departures from wild type, the application of such a concept is difficult in view of the
strongly positive interaction seen in \( y Hw;se h \) \( pyd \). No consistent scheme relating all these effects has yet been developed.

Similar but even more complicated relations are apparent in the sex-comb teeth. Thus, \( y Hw \) and \( se h \) increase slightly the number of teeth in the sex-comb, but the combination of these two has significantly fewer teeth than normal. Polychaetoid flies also have fewer teeth than normal. However, the combination of \( y Hw;se h \) with \( pyd \) results in a fly with significantly more teeth than wild type.

It is obvious from the foregoing that, as nearly as can be judged from the phenotypic picture, the dynamic relations of these three genes differ significantly according to the part of the body under observation, even though the characteristic studied is more or less comparable in all regions. The question of "specific interactions," mentioned above, emerges most clearly in connection with such apparently irregular results as those pertaining to the sex-comb teeth. A related question of great importance to the ultimate interpretation of the apparent regional differences in factor interaction is this: do the factors involved have the same primary effects in all regions of the body, with the different end effects imposed by local circumstances, or does one factor have several different and independent roles to play? In the latter case there is of course no reason to expect the same type of interaction in all systems in which the factor plays a role, nor to attempt to interpret the phenotypic interactions seen here so that at one stage they fit into a common pattern (for a fuller discussion of this question see Sillow 1939 and Neel in press). Evidence as to the physiological versatility of a factor may be derived in various ways, and particularly from a study of multiple allelic series of factors with pleiotropic effects. Unfortunately for the present problem, mutations resulting in \( Hw \) and \( pyd \) have been reported but once. However, the \( +^h \) locus mutates relatively frequently. Most of the alleles which have been reported are similar to the one studied here, but one \( (h^{42+19}) \) is characterized by a restriction of supernumerary chaetae to the wing (Neel 1942b) and another \( (h^2) \) by a similar restriction of extra hairs to the wing and in addition by cylindrical bristles with javelin heads (Drosophila Information Service 9). (The location of the "javelin" mutation at 3-19.0 raises a question as to whether \( h^2 \) may be the result of a small chromosomal aberration involving the javelin locus.) The fact that the \( +^h \) gene may mutate in what appear to be qualitatively different directions may be taken as evidence of some biochemical independence of the various effects of the factor, although obviously decisive evidence is lacking. For the present the question of the relationship between the effects of these factors must be left open. Wright (1927, 1941) and Russell (1939) have described a somewhat similar case in the guinea pig, where the interaction of factors affecting pigmentation appears to be different in the epidermis, eye, and hair, and have interpreted it in terms of regional threshold differences. This explanation has been challenged by Sillow (1939). It is difficult to see how a similar explanation could be applied here.
THE RAPID ACCUMULATION OF MODIFIERS OF FLY SIZE IN THE STRAINS

It was suggested above that in these strains minor modifiers of bristle number had arisen with a relatively high frequency. The same appears to be true of modifiers of fly size. The relation between the concentration of the yeast suspension on which the flies were grown and mean femur length, when 100 eggs were introduced into a vial, is shown in figure 3. As yeast concentration increased above 10 percent there was no significant increase in femur length,

![Figure 3](image-url)

**Figure 3.**—The relation between food level and fly size in eight different strains of Drosophila. Details in text.

and for this reason the data on concentrations down to and including 10 percent have been averaged together. The "10+ percent" size seriation so obtained may be compared with the seriation observed at a yeast concentration of 15 percent in the earlier work. The differences between the two series are so great that either the determinations were subject to large errors or else genetic differences had appeared in the strains in the eight to ten months (14–18 generations) elapsing since the earlier work. Culture conditions were so carefully standardized that the first explanation appears insufficient. We have then to fall back on the second explanation and recognize a rapid appear-
ance of modifiers of size. These modifiers may be either positive (for example, the \( y \) \( Hw;se \) \( h \) stock) or negative (the wild type strain); there is no evidence as to whether or not they are the same ones concerned with chaetae number. Some four months after the constants had been determined in the wild type strain, it was found to contain a "mutability stimulating" factor (Neel 1942a). The majority of minor mutations produced over and above the normal rate under the influence of this factor were undoubtedly deleterious. There is no evidence as to how long the factor had been present in the strain prior to its discovery. If it had already been present in the strain for some time previous to the determination of the constants, this could account for the very considerable decrease in the size of the \( +^{Or} \) flies. However, the \( y \) \( Hw \) strain was also found to carry the factor, it having been introduced in crosses of this strain to the Oregon line, and yet the \( y \) \( Hw \) flies showed no size decrease. There was no evidence for the presence of the factor in other strains.

The significance of the size differences between strains was discussed in the earlier paper.

**THE RELATION BETWEEN NUTRITION AND SIZE IN THE VARIOUS GENOTYPES**

It is obvious from figure 3 that the seriation of genotypes with respect to mean femur length at 10+ percent yeast concentration is quite different from that at 2.5 percent. Under poor food conditions the four strains with the longest mean femur lengths all contain \( y \) \( Hw \), while under more favorable conditions the observed seriation is significantly different. A similar tendency was noted when 200 eggs were introduced into a vial, although the data are not so extensive.

All \( y \) \( Hw \) males were the offspring of \( y \) \( Hw/++ \) females, since, due to the poor viability and fertility of the homozygous \( y \) \( Hw \) females, it was necessary to propagate the various strains containing \( y \) \( Hw \) by heterozygous females. In any strain containing \( y \) \( Hw \) there was thus a segregation in each generation for \( y \) \( Hw \) and non-\( y \) \( Hw \) males. In such cases at low food levels the larger size of the \( y \) \( Hw \) males was readily discernible under the microscope. The size disparity was particularly marked in the offspring of \( y \) \( Hw/++ \); \( se \) \( h \) \( pyd \) females, the \( y \) \( Hw;se \) \( h \) \( pyd \) males having an easily detected size advantage over the ++; \( se \) \( h \) \( pyd \) segregants. These intra-strain differences are conclusive evidence that the larger size of the \( y \) \( Hw \) males at low food levels is not due to the chance accumulation of similar modifiers in the \( y \) \( Hw \) strains but is a function of \( y, Hw \), or some closely linked factor.

Among the thousands of mutant types that have been studied in various species of plants and animals in the past 40 years, only a very few have been reported as superior to wild type with respect to viability, fertility, or any other characteristic which could be of adaptive significance. These "favorable mutations" have recently been reviewed by Gustafsson (1941) and will not be further discussed here. However, it should be emphasized that in most of the previously discussed cases, like the present, the mutant type is superior to the wild type only under certain special conditions, which are yet not so spe-
cial but that they could conceivably arise in nature. Such differential responses of mutants are of particular interest from the standpoint of the theory of "preadaptation."

Bridges (1939) has very briefly mentioned the greater-than-normal viability of a yellow allele ($y^{34c}$). Nilsson (1931) described a case of increased resistance to high temperature in a $yw$ strain. Burkart and Stern (1933) drew attention to the high viability of the Blond translocation, characterized by a reciprocal interchange between the tips of the first and second chromosomes. This may be related to an involvement of the $y$ locus. If the present case is due to the yellow allele, this would constitute another possible instance of mutation at the $+y$ locus resulting in a phenotype which under certain conditions had a selective advantage over wild type. The suggested relatively high frequency of "favorable mutations" at this locus deserves further study. On the other hand, it would be equally significant if the factor responsible was $Hw$, since Demerec and Hoover (1939) have shown that this is a short duplication. Nilsson (1932) has reported that the Bar mutant, also due to a small duplication, is characterized by high viability under poor food conditions. These may therefore be cases where under certain conditions new chromatin has a selective advantage; such events provide a mechanism for intrachromosomal evolution. Unfortunately, in the present case it does not appear possible to reach a decision between these two alternatives, since the $Hw$ duplication first arose in combination with this particular $y$ and is so closely linked that it has not been observed to separate through crossing over.

Discussion

Needham and Lerner (1940) have pointed out the need for distinguishing between the actual relative growth of parts in relation to the whole or other parts (heterauxesis) and relative growth relationships between groups or individuals compared at maturity or any other given stage of development (allometry). A complication in many of the existing attempts to compare growth relationships in related organisms, or to study the inheritance of growth constants, has been the failure to distinguish between these two phases of relative growth. A further complication has been an almost complete lack of knowledge of, or control over, the gene differences contributing to the growth differences obtaining in the forms under analysis (e.g., Lerner 1937, 1941; Green 1933). It is not immediately clear whether the present data are entirely comparable to the usual phenomena included in studies of correlated growth. Certainly there is a superficial difference between increase in the numbers of such specialized structures as chaetae and the growth of an organ. However, earlier studies (Neel 1940b) have shown that in $pyd$ flies there is a close correlation between the number of dorsocentral bristles present and their total length—that is, each bristle number is distinguished by a more or less characteristic range in total bristle lengths. If this relationship is general for the various groups of chaetae studied, then correlating femur length with
gene interaction

chaetae number amounts to correlating size with the amount of chitin, etc., incorporated into certain special structures in various regions of the body. Among the various growth studies the present data are thus most nearly comparable to the type of investigation reviewed by Needham (1934), concerning the relation of chemical changes to increase in body size, with the difference that the substance under investigation here is distributed in the form of discrete units. Considered in this light, the data are quite pertinent to the problem of relative growth, since they constitute a unique study of the effects of single gene substitutions on the constants of allometry. The salient feature of the findings is the fact that the picture obtained of the genetic control of growth constants depends largely upon the particular structure studied. However, in the appearance of extra structures, on the basis of limited data, one relationship does appear to stand out: in some systems gene substitutions alter the magnitude of the departure from wild type without changing the ratio of the percentage increase of character and body size.

The differences in the various body regions in the relation between the magnitudes of slope and intercept in the different strains appear to be related to the nature of the phenotypic interactions. In those three cases agreeing in the relation between size and the number of extra chaetae, the picture of interaction effects is somewhat similar: primary genetic effects are often additive, although the exact relation between phenotype and this additive effect probably differs in the three cases. Such additive effects imply a similarity of gene action, although we are still in the dark as to what this similarity entails. Conversely, the non-additive interactions noted imply a regulation of the production of a given characteristic by different means. Such non-additive interactions are particularly prominent where the dorsocentral bristles and sex-comb teeth are concerned, and these same structures also showed an irregular relation between slope and intercept in the regressions. The question of a "pleiotropic" as compared with a "single" action of the factors studied has already been discussed.

A great deal of work has been performed on the inheritance of quantitative characters, and criteria have been developed for attempting to distinguish between an arithmetic and geometric action of the factors involved (cf. Charles and Smith 1939). Most of these studies have utilized plant material and have dealt with the interactions of large blocks of chromatin rather than single or a relatively few factors. The length of the life cycle and the paucity of genetic markers in most plants render a more precise approach difficult. Nevertheless, if the present data are indicative of the types of phenotypic interactions involved in the production of quantitative characters, then it seems to the author that in more complex cases relatively little light is shed on the nature of such inheritance by determining whether an unknown number of factors adhere more closely to a geometric than to an additive type of interaction. Rather, the admittedly much more difficult analysis of the effects of single gene substitutions is in the end the approach which must be used. Castle (1941) has recently summarized the relatively few attempts to do this.
for mammalian material. The results are fully as complicated as those reported here for Drosophila.

Harland and Atteck (1933) and Muller and Pontecorvo (1942) have attempted to explain the appearance of aberrant characteristics in crosses of similarly appearing organisms with the suggestion that in related forms similar phenotypes may be the result of somewhat different genetic systems. The present data furnish several examples of the control of a similar phenotype by different factors, and departures from either parental form in derived types. Thus, at large sizes $y Hw$ and $se h$ are similar as regards the number of hairs on the second longitudinal wing vein and the number of teeth in the sex-comb. Yet the compound $y Hw; se h$ has approximately six times as many wing hairs as either $y Hw$ or $se h$, but significantly fewer sex-comb teeth. At this rate, fairly large phenotypic aberrations could be obtained in a cross where the parental forms differed by a relatively few factors.

While it appears probable that "concealed genetic variability" of the type recently stressed by Dobzhansky et al. (1942) is the major cause of the phenomena of heterosis, a possible secondary role of factor interactions such as have been described above cannot be disregarded entirely.

SUMMARY

A study was made of the relation between body size and the number of five different kinds of chaetae present in Drosophila melanogaster males of the following genotypes: wild type, pyd, se h, $y Hw$, $y Hw; pyd$, $y Hw; se h, se h pyd$, and $y Hw; se h pyd$. In each case the regression of number of chaetae on femur length is adequately represented on an arithmetic plot by a straight line. The five families of straight lines, each representing the relation of a particular kind of chaetae to size in the eight strains, show different relations of slope and intercept. In each of three families the various strains differ in respect to the magnitude of the intercepts of their regression lines, but the ratio of percentage change in number of extra chaetae to percentage change in femur length remains constant within the limits of chance variation. In the other two families of lines much more irregular effects were noted.

The apparent nature of the phenotypic interactions of the factors depends to a considerable extent on the fly size at which these effects are evaluated. In the same three cases mentioned above, the data can be interpreted as indicating that primary genetic effects are mainly additive, but that the subsequent relation of effect to phenotype is different in each case. In the other two cases no satisfactory scheme of factor interaction has yet emerged.

Under poor food conditions male flies containing $y Hw$ showed a significantly smaller reduction in mean fly size than did flies not containing this combination of factors.

The relation of these findings to the problem of correlated growth is discussed.
GENE INTERACTION

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