STUDIES ON THE INTERACTION OF MUTATIONS AFFECTING
THE CHAETAE OF *DROSOPHILA MELANOGASTER*. I.
THE INTERACTION OF HAIRY, POLYCHAETOID,
AND HAIRY WING

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INTRODUCTION

ONE attack frequently used upon the problem of gene action consists in observing and attempting to interpret the results which follow when two or more mutations are brought together in a given strain of plants or animals. The present paper reports the results of such a study involving three *Drosophila melanogaster* mutations whose chief visible effects are upon the chaetae of the fly.

MATERIALS

The choice of mutations to be used in this investigation was dictated by four major considerations. The first of these was the desirability of employing factors whose effects alone and in combination with one another could be precisely evaluated. The second consideration was the assumption that an interpretation of factor interaction as seen in the adult can most profitably be attempted when the factors involved first bring about detectable departures from normal ontogeny at a relatively late stage of development (*cf.* BRINK 1927). Thirdly, any interaction of two or more genes can be studied more advantageously in case some data are at hand concerning the effects and modes of action of these genes when acting separately. Finally, it seemed probable that significant interaction effects were most likely to be found in cases where the factors studied affect the same characteristic.

For these reasons the following three *Drosophila melanogaster* mutations were selected for study: Hairy wing (*Hw*, 1-0.0), hairy (*h*, 3-26.5), and polychaetoid (*pyd*, 3-39 ± 2). The chief visible effects of all three of these factors are upon the same characteristic, the chaetae of the fly. These effects may be accurately measured. *Hw* has been particularly studied by ROKIZKY (1930), DEMEREC and HOOVER (1939), and NEEL (1940b). They found that *Hw* flies show extra hairs and bristles chiefly on the top and back of the head, on the dorsum and pleura of the thorax, and along the wing veins. The chief phenotypic effect of the *pyd* factor is upon the macrochaetae; NEEL (1940b) found extra bristles at 16 out of 20 loci investigated in *pyd* flies. NEEL concluded that the extra macrochaetae which are associated with the presence of either *Hw* or *pyd* in the genome are in some

cases the result of hypertrophy of hair primordia and in other cases are not to be associated with any element of the normal chaetal pattern. Extra bristles which do not correspond in position to a hair of the wild type pattern may either occur close to a normally located bristle or they may be found in a region which neither by the presence of hairs or bristles normally gives evidence of chaetal potencies. The *h* mutant, originally described by MOHR (1922), is characterized by the presence of supernumerary microchaetae along the wing veins and on the scutellum, the thoracic pleura, and the top of the head. Inasmuch as the primordia of the chaetae do not appear until about the twenty-seventh hour of pupal life at 25°C (ROBERTSON 1936), these mutations may from the phenotypic standpoint be classified as relatively "late-acting." However, we have little information as to how long the determinative processes which result in the eventual formation of a bristle precede the visible embryonic differentiation of that bristle. The fact (see below) that flies homozygous for any of these mutations are smaller than wild type points to some effect of these factors during larval life, since it is largely then that size is determined.

METHODS

The starting points for the synthesis of the desired combinations of factors were the following four stocks: *y Hw*, *se h*, *pyd*, and an inbred Oregon wild type. The yellow gene (*y*, 1-0.0) is so closely linked to *Hw* that no crossing over between these factors has been recorded. Similarly, sepia (*se*, 3-26.0) is closely linked to *h*. It was therefore possible in crosses to use *y* and *se* as marker genes for the presence of *Hw* and *h*, respectively.

Preliminary to the synthesis of stocks combining two or more of these bristle mutations, *y Hw*, *se h*, and *pyd* were introduced into a uniform genetic background by repeatedly crossing the mutant flies with flies of the inbred Oregon wild type strain and recovering the mutants in the F₂. This procedure of outcross and extraction was repeated 14 times in the cases of *se h* and *pyd*, and ten times for *y Hw*. BARTLETT and HALDANE (1935) have developed formulae for estimating the effectiveness of such a mating procedure in the production of a common genetic background in two strains. From their formulae it can be estimated that the average length of non-Oregon chromosome on either side of the closely linked *y* and *Hw* factors after ten outcrosses and extractions would be 17 genetic units, while after 14 outcrosses and extractions there would on the average be 13 non-Oregon units on either side of the closely linked *se* and *h*, or *pyd*. Since *Hw* is located at the extreme left end of the X, the estimate of 17 genetic units to either side must be modified to 17 genetic units to the right, and none, in the crossover sense, to the left. In what follows, reference will frequently be made to the deviation from normal which is associated with

the presence of a certain mutation in the genome. It is, of course, recognized that some portion of this deviation may be due to other genes introduced from the original strain which are closely linked to the particular mutation in question and which have not been replaced by their Oregon alleles. In other words, the study is not so much of the effects of single gene substitutions as of substitutions of small segments of chromosomes containing one or two readily detectable mutations.

A further source of undesirable genetic differentiation between the three "Oregonized" mutant strains would be residual heterogeneity in the Oregon strain and the occurrence of slight and phenotypically undetectable mutations during the progress of the out-crossing and extraction.

These prepared strains of *y Hw*, *se h*, and *pyd* were crossed so as to give the following four stocks: (1) *se h pyd*, (2) *y Hw; pyd*, (3) *y Hw; se h*, and (4) *y Hw; se h pyd*. The study which follows is based on these four strains, the three from which they were synthesized, and the standard Oregon strain, a total of eight different genotypes.

Males of these eight stocks were examined with respect to six characteristics: right fore-femur length, number of dorsocentral bristles, number of scutellar hairs, number of scutellar bristles, number of hairs on the right second longitudinal wing vein, and number of teeth in the right sex-comb. For the first and last two characteristics, the left measurement was used if for any reason (loss, injury) the right measurement was not available. Five of the characteristics are chaetal. The sixth, femur length, was included in the study since it seemed desirable to have a check on the mean size of flies of the various strains.

In any precise evaluation of gene effects it is necessary to raise flies in a standardized environment which is uniform for all flies studied. After considerable preliminary experimentation, the following culture technique was adopted. Well fed flies of the desired genotype were allowed to lay eggs on small food chips for eight to twelve hours. These eggs were divided into lots of 100, and each lot placed in a 23×95 mm shell vial which contained ten cc of a 15 percent yeast solution absorbed on one sheet of Kleenex. The vials were stoppered with cotton plugs and incubated at $24.0 \pm 0.3^{\circ}\text{C}$. Throughout the course of the experiments, yeast was procured in one pound cakes from the National Yeast Company. It is possible that there are culture methods yielding larger flies than the procedure described. This method, however, has the advantage of being one which can be accurately duplicated from time to time.

RESULTS

The six characteristics in the eight genotypes, under standard conditions

The condition of the various characteristics in the different genotypes is

shown in the columns of table 1 headed by an A. The B columns of the table are designed to furnish convenient frames of reference in evaluating the magnitude of the effects observed in combinations. These columns show what the expression of the characteristics would be in combinations of two or three mutations, if the effects of these mutations when combined were equal to the sum of their effects when acting separately—that is, if

TABLE 1

The results of combining mutations affecting the chaetae of Drosophila melanogaster. A columns, observed expression of the six characteristics investigated. B columns, expression expected in combinations of two or three mutations, on the basis of additive effects. The errors given are standard errors of the mean. Details in text.

GENOTYPE	FEMUR LENGTH (μ)		MEAN NUMBER OF DORSOCENTRAL BRISTLES		MEAN NUMBER OF SCUTELLAR HAIRS	
	A	B	A	B	A	B
+	595.9 $\pm$ 0.8		4.040 $\pm$ 0.016		0.0 $\pm$ 0.0	
<i>pyd</i>	559.2 $\pm$ 0.7		5.755 $\pm$ 0.072		0.0 $\pm$ 0.0	
<i>se h</i>	542.9 $\pm$ 0.8		4.030 $\pm$ 0.014		24.06 $\pm$ 0.23	
<i>y Hw</i>	556.5 $\pm$ 1.0		7.415 $\pm$ 0.120		4.50 $\pm$ 0.11	
<i>se h pyd</i>	537.6 $\pm$ 1.3	506.2 $\pm$ 1.3	5.680 $\pm$ 0.069	5.745 $\pm$ 0.074	34.25 $\pm$ 0.24	24.06 $\pm$ 0.23
<i>y Hw; pyd</i>	541.9 $\pm$ 0.9	519.8 $\pm$ 1.5	9.810 $\pm$ 0.159	9.130 $\pm$ 0.140	5.61 $\pm$ 0.11	4.50 $\pm$ 0.11
<i>y Hw; se h</i>	523.2 $\pm$ 1.1	503.5 $\pm$ 1.5	8.370 $\pm$ 0.074	7.405 $\pm$ 0.122	40.51 $\pm$ 0.24	28.56 $\pm$ 0.25
<i>y Hw; se h pyd</i>	535.4 $\pm$ 1.1	466.8 $\pm$ 1.7	12.680 $\pm$ 0.119	9.120 $\pm$ 0.141	54.92 $\pm$ 0.33	28.56 $\pm$ 0.25

GENOTYPE	MEAN NUMBER OF SCUTELLAR BRISTLES		MEAN NUMBER OF HAIRS ON II LONGITUDINAL WING VEIN		MEAN NUMBER OF TEETH IN SEX-COMB	
	A	B	A	B	A	B
+	4.000 $\pm$ 0.007		0.0 $\pm$ 0.0		10.165 $\pm$ 0.072	
<i>pyd</i>	4.375 $\pm$ 0.041		0.0 $\pm$ 0.0		9.965 $\pm$ 0.064	
<i>se h</i>	4.225 $\pm$ 0.033		8.46 $\pm$ 0.24		10.070 $\pm$ 0.070	
<i>y Hw</i>	4.140 $\pm$ 0.027		11.05 $\pm$ 0.28		10.915 $\pm$ 0.085	
<i>se h pyd</i>	5.190 $\pm$ 0.057	4.600 $\pm$ 0.053	10.82 $\pm$ 0.29	8.46 $\pm$ 0.24	11.080 $\pm$ 0.071	9.870 $\pm$ 0.119
<i>y Hw; pyd</i>	4.870 $\pm$ 0.059	4.515 $\pm$ 0.050	11.97 $\pm$ 0.30	11.05 $\pm$ 0.28	10.805 $\pm$ 0.069	10.715 $\pm$ 0.128
<i>y Hw; se h</i>	5.535 $\pm$ 0.048	4.365 $\pm$ 0.043	33.19 $\pm$ 0.37	19.51 $\pm$ 0.36	9.710 $\pm$ 0.064	10.820 $\pm$ 0.131
<i>y Hw; se h pyd</i>	7.015 $\pm$ 0.087	4.740 $\pm$ 0.060	38.35 $\pm$ 0.45	19.51 $\pm$ 0.36	12.025 $\pm$ 0.107	10.620 $\pm$ 0.146

their phenotypic effects were simply additive. The phenotype expected in a combination of mutations *b* and *c* on the basis of additive effects was calculated according to the formula

$$\text{Expected phenotype} = A + (B - A) + (C - A) = B + C - A,$$

where A is the numerical value of the characteristic in wild type flies and B and C its value in flies which carry the *b* and *c* mutations separately. The standard errors of the estimates in column B were calculated according to the formula

$$\sigma_E = \sqrt{\sigma_A^2 + \sigma_B^2 + \sigma_C^2},$$

where σ_A , σ_B , and σ_C are the standard errors of the appropriate values of

column A. The occurrence of additive effects in a compound would suggest, although it would not necessarily demonstrate, a developmental independence of the reactions conditioned by the bristle factors involved. Conversely, significant differences between corresponding entries in columns A and B may be taken as indications of factor interaction. If the value given in column A is greater than the corresponding figure in B, we may speak of super-additive effects; if it is less, of sub-additive effects.

The fact that the B columns are given for purposes of orientation in considering the data should be emphasized. From the standpoint of developmental physiology, there is no a priori reason for expecting agreement between comparable entries in the two columns.

The various mean values of the A columns are based upon measurements of 200 flies. All errors given in the table are standard errors. Since the standard error of the mean, σ_m , is related to the standard deviation, σ , by the formula $\sigma_m = \sigma/\sqrt{N}$ (where N represents the number of individuals), and since N is constant for all these measurements, the relative variability of a given characteristic in the different genotypes may be judged directly from the magnitude of σ_m . The distribution of most of the variates studied about their mean was essentially normal. However, in genotypes where the amount of deviation from wild type shown by such a trait as the dorso-central or scutellar bristles was small, the distribution curve was positively skewed, as might be expected.

Throughout this study the condition of the six characteristics was recorded separately for each fly. It was therefore possible to calculate for each genotype the correlation between any one characteristic and each of the others. Since six characteristics are involved, a total of 15 correlations was possible for each of the eight genotypes. In some cases the amount of variation shown by a characteristic was so small that no correlations which involved this measurement could be calculated. For this reason, only 83 out of a possible total of 120 correlations were derived. These are given in table 2.

The data contained in tables 1 and 2 can most readily be considered if the various characteristics are taken up one at a time.

Femur length. Femur length was used as a measure of fly size. NEEL (1940a) has reported that in the case of small groups of *pyd* males raised under diverse culture conditions, the relation between mean femur length and mean body weight appears to be linear (over a range in mean femur length from 516.0 ± 4.6 to $582.0 \pm 3.2\mu$, and in mean body weight from 0.600 ± 0.011 to 0.953 ± 0.004 mg). From the data collected at the time of that investigation, the equation for the regression of body weight on femur length can be calculated to be

$$Y = 0.0047X - 1.828$$

TABLE 2

The correlations between the various characteristics in the different genotypes. Possible correlations indicated by a (—) have not been calculated because of the limited variability displayed by one or both of the characteristics. The errors given with the correlations are standard errors.

CORRELATION	GENOTYPE							
	+	<i>pyd</i>	<i>se h</i>	<i>y Hw</i>	<i>se h pyd</i>	<i>y Hw; pyd</i>	<i>y Hw; se h</i>	<i>y Hw; se h pyd</i>
1. Femur length—teeth in sex-comb	0.165±0.069	0.210±0.068	0.258±0.066	0.060±0.071	0.138±0.069	0.162±0.069	0.235±0.067	0.014±0.071
2. Dorsocentral bristles—teeth in sex-comb	—	0.057±0.071	—	0.075±0.070	0.071±0.070	—0.019±0.071	0.139±0.069	0.179±0.068
3. Scutellar hairs—teeth in sex-comb	—	—	—0.056±0.071	0.028±0.071	0.200±0.068	0.068±0.070	0.135±0.069	0.047±0.071
4. Scutellar bristles—teeth in sex-comb	—	—0.044±0.071	—	—	—0.049±0.071	0.159±0.069	0.048±0.071	0.054±0.071
5. Wing hairs—teeth in sex-comb	—	—	—0.054±0.071	—0.030±0.071	—0.168±0.069	0.077±0.070	—0.059±0.071	0.014±0.071
6. Femur length—wing hairs	—	—	0.105±0.070	0.031±0.071	0.225±0.067	—0.007±0.071	0.061±0.071	0.014±0.071
7. Dorsocentral bristles—wing hairs	—	—	—	0.412±0.059	0.209±0.068	—0.167±0.069	—0.027±0.071	—0.079±0.070
8. Scutellar hairs—wing hairs	—	—	0.192±0.068	0.245±0.066	0.167±0.069	0.239±0.067	0.070±0.070	—0.004±0.071
9. Scutellar bristles—wing hairs	—	—	—	—	0.190±0.068	—0.149±0.069	—0.036±0.071	—0.050±0.071
10. Femur length—scutellar bristles	—	0.062±0.071	—	—	0.080±0.070	0.039±0.071	0.233±0.067	0.089±0.070
11. Dorsocentral bristles—scutellar bristles	—	0.014±0.071	—	—	0.166±0.069	0.091±0.070	0.094±0.070	0.222±0.067
12. Scutellar hairs—scutellar bristles	—	—	—	—	—0.105±0.070	—0.040±0.071	—0.202±0.068	—0.308±0.064
13. Femur length—scutellar hairs	—	—	0.007±0.071	—0.049±0.071	0.227±0.067	0.162±0.069	0.144±0.069	0.099±0.070
14. Dorsocentral bristles—scutellar hairs	—	—	—	0.310±0.064	0.210±0.068	0.049±0.071	0.041±0.071	—0.034±0.071
15. Femur length—dorsocentral bristles	—	0.109±0.070	—	—0.104±0.070	0.220±0.067	0.072±0.070	0.074±0.070	0.019±0.071

GENE INTERACTION

where Y equals mean body weight and X, mean femur length. This equation is strictly applicable only to the *pyd* flies used in its derivation, but it may be employed in connection with the present work to obtain an estimate of the weight of flies of the different genotypes, if the weights so calculated are regarded only as approximations. These weights are as follows: +—0.97 mg, *pyd*—0.80 mg, *se h*—0.72 mg, *y Hw*—0.79 mg, *se h pyd*—0.70 mg, *y Hw*; *pyd*—0.72 mg, *y Hw*; *se h*—0.63 mg, and *y Hw*; *se h pyd*—0.69 mg. All the strains containing only one bristle mutation are smaller than wild type. The *se h* flies are particularly small. This is paralleled by a higher larval and pupal mortality for *se h* than for +, *pyd*, or *y Hw*. In combinations the different factors do not reduce size as much as when acting separately, although the order of effectiveness remains the same.

The strain combining all three bristle factors is larger than *y Hw*; *se h*, although smaller than *y Hw*; *pyd* or *se h pyd*. This rather surprising fact may be due to the operation of some uncontrolled environmental variable. As an alternative, we may assume that the addition of *pyd* and the closely associated genes to the *y Hw*; *se h* complex somehow offsets the size-reducing tendency of the latter. In this connection, GONZALEZ (1923) noted that purple-arc flies lived longer than either purple or arc individuals, although both purple and arc acting separately tend to reduce viability. More recently, BRIERLY (1938) has recorded somewhat similar findings.

The correlations between the femur length of any one genotype and its various other characteristics are generally small and positive. However, seven out of the 31 correlations of table 2 which involve femur length exceed three times the value of their standard errors. This indicates that in the different genotypes there is a slight tendency, even under these conditions of limited size variability, for character expression to be positively linked with size or factors common to size and the character. Such a dependence upon a common variable would tend to establish correlations between the different chaetal traits of a genotype. It has been shown by a number of investigators that in instances where greater size variations are encountered, the expression of various characteristics is much more significantly related to fly size. This is certainly true in the case of *pyd* (NEEL 1940a), and observations made during the course of these experiments suggest that it is also true for *h* and *Hw*. The fact that there are such wide size differences between the various genotypes of this study thus raises a question which is fundamental to an investigation of this nature. Are we justified in drawing conclusions as to the relative effects of these three mutations, either alone or in combination, from groups of flies differing so widely in size? This problem will be returned to later.

These bristle mutations were originally selected for study, among other

reasons, because they were believed to be "late-acting." But the considerable differences in mean fly size between the strains probably indicate an action of the mutations during larval life, although no evidence is available as to the nature of this action.

Mean number of dorsocentral bristles. Both *pyd* and particularly *y Hw* flies are characterized by numerous extra dorsocentrals; *se h* individuals are not significantly different from wild type in this respect. In combination with *pyd*, the *se h* genes have no effect upon the dorsocentrals, yet in combination with *y Hw* the same factors have a positive influence, and in combination with *y Hw*; *pyd* a still stronger positive effect. The *se h pyd* flies do not deviate significantly from expectation on the basis of additive effects; the phenotypes of the other three compounds are super-additive.

KARP (1936) reported that *h* when not combined with any other bristle mutant had an insignificant tendency to reduce the number of sternopleural bristles, whereas in *Hw/+*; *h/h* females *h* had a positive influence on bristle number. However, *Hw/Hw*; *h/h* females had the same number of sternopleurals as *Hw/Hw*; *+^h/+^h* flies.

Mean number of scutellar hairs. Normally there are no hairs upon the scutellum. This condition obtains in *pyd* flies, but *y Hw* individuals have a few and *se h* flies have many hairs in this region. The distribution of the hairs differs in the latter two genotypes. In *y Hw* the hairs are usually on the anterior part of the scutellum, near and in the scutellar groove. In *se h* the hairs are more scattered over the whole scutellum, being particularly concentrated near its lateral and posterior margins. In this investigation, only those scutellar hairs were counted whose bases could be seen from a full dorsal view.

The *y Hw* and *se h* complexes interact strongly. In combination with either *se h* or *y Hw*, *pyd* acts as a fairly strong positive modifier; in combination with both of them it has an even stronger positive tendency. It appears that the effects on the scutellar region of *pyd* uncombined with any other bristle mutation are below the threshold which must be exceeded to give a visible effect; when that threshold is altered, as by the introduction of *y Hw* or *se h* into the genotype, the *pyd* effect is now detectable.

These results are in contrast to those of KARP (1936), who found the combination of *Hw* and *h* to have a sub-additive effect upon the number of sternopleural hairs.

Mean number of scutellar bristles. All three of the strains containing one bristle mutation show significantly more scutellar bristles than the wild type. Combinations of the various mutations uniformly show marked super-additive effects, which are most pronounced where the *se h* complex is involved.

It has been suggested that at least a portion of the action of *pyd* and

Hw consists in causing hair primordia to hypertrophy into bristles (NEEL 1940b). As we have seen, *se h* flies have many extra hairs on the scutellum. The interaction of *pyd* and *y Hw* with *se h* might accordingly be attributed to a tendency for hair primordia dependent upon *se h* to hypertrophy in the presence of *y Hw* or *pyd*. The same tendency might be true to a lesser extent of the interaction of *y Hw* and *pyd*.

This hypothesis finds confirmation in the correlations between scutellar hairs and scutellar bristles. These correlations are uniformly negative, although in only one out of the four cases are they clearly significant (table 2). As the number of bristles increases, the number of hairs tends to decrease. Inasmuch as both scutellar hairs and bristles show a slight tendency to be correlated positively with femur length, the influence of this association on the scutellar hair-scutellar bristle relationship was partialled out by the multiple correlation technique. The correlations between the scutellar hairs and bristles in the four genotypes then became: *se h pyd*, -0.127 ± 0.070 ; *y Hw; pyd*, -0.047 ± 0.071 ; *y Hw; se h*, -0.245 ± 0.067 ; and *y Hw; se h pyd*, -0.323 ± 0.062 . For the two significant correlations, the regression equations of hair number on bristle number were established as follows:

$$y Hw; se h: \quad Y = 47.2 - 1.2X$$

$$y Hw; se h pyd: \quad Y = 63.5 - 1.2X$$

The symbol Y represents hair number, and X, bristle number. In these genotypes, the addition of one bristle tends to be paralleled by the disappearance of about 1.2 hairs. This fits in well with the hypothesis of hypertrophy of hair primordia as a source of extra bristles, although it does not rule out the possibility that bristles appear independently of hairs and tend to suppress them. In the cases of *y Hw; pyd* and *se h pyd* it would have to be postulated that hair hypertrophy is rarer than in the other two genotypes.

Mean number of hairs on the right second longitudinal wing vein. The situation here is comparable to the findings regarding the scutellar hairs. In *se h* and *y Hw* flies, numerous hairs are located on the second longitudinal wing vein. Polychaetoid flies show the normal, hairless condition of this vein—yet in combination with the other two mutants, *pyd* has a positive effect upon wing hair number. Here again it is perhaps permissible to speak of a lowering of the threshold of visible effect by *se h* or *y Hw* and a consequent detectable influence of *pyd* when in combination with them. The interaction of *y Hw* and *se h* is characterized by very marked effects upon the wing hairs. This is illustrated in figure 1, which is a photomicrograph of typical wings from *y Hw*, *se h*, and *y Hw; se h* males. Moreover, wing venation is abnormal in the *y Hw; se h* compound. The irregular,

FIGURE 1.—The effects upon the wing hairs of *se h*, *y Hw*, and the combination of these two complexes, *y Hw; se h*. Left, *se h*; center, *y Hw*; and right, *y Hw; se h*. The wings pictured were chosen as typical of the genotypes which they represent. (Photographed by Ruby Gay Stewartson, Carnegie Institution, Cold Spring Harbor, N. Y.)

branching, and plexate veins of the $y\ Hw; se\ h$ wing seen in the figure are quite characteristic of flies of this genotype. This condition is even more pronounced in $y\ Hw; se\ h\ pyd$ individuals. When counts were made of the wing hairs of $y\ Hw; se\ h$ or $y\ Hw; se\ h\ pyd$ males, hairs were counted as being on the second longitudinal vein if they were located on a branch off this vein but were still closer to this vein than any other of the wing veins. This procedure may contribute in part to the apparent magnitude of the super-additive effects.

The figure illustrates the fact that the wing surface tends to be uneven in $y\ Hw$ and its compounds.

Mean number of teeth in the right sex-comb. The sex-comb normally consists of a single row of modified microchaetae known as teeth, located on the first tarsal segment. Both pyd and $se\ h$ flies have slightly although insignificantly fewer teeth in the sex-comb than wild type; $y\ Hw$ individuals possess significantly more. However, the combination of pyd and $se\ h$ results in a fly possessing significantly more teeth than wild type. Two factors which when acting separately appear to have if anything a negative influence on a structure have in combination a strong positive effect. The combination of either pyd or $se\ h$ with $y\ Hw$ gives flies having fewer teeth in the sex-comb than is the case in $y\ Hw$. However, when pyd and $se\ h$ are brought together again, this time in combination with $y\ Hw$, the same positive interaction is again observed:

In all genotypes containing $y\ Hw$ there is a tendency towards doubling of the sex-comb—that is, arrangement of the teeth in two or more rarely three closely parallel rows. Usually one row is large and the other row or rows small, consisting of one to four teeth. In $y\ Hw$, $y\ Hw; pyd$, and $y\ Hw; se\ h$, doubling is found in only three to six percent of the flies, but 49 percent of the $y\ Hw; se\ h\ pyd$ flies had doubled sex-combs. In this latter genotype there is a high correlation between the total number of sex-comb teeth present and the occurrence of doubling. When number of teeth is correlated with the alternative conditions of doubled and non-doubled, the value of biserial r is 0.794 ± 0.026 .

*The manifestation of the factors under
varied culture conditions*

Before any conclusions are drawn from these findings, the question of the general validity of the results should be considered. Would the relative magnitudes of the effects be the same under all culture conditions? The following experiment was undertaken to give partial answer to this question. Wild type, $se\ h$, $y\ Hw$, and $y\ Hw; se\ h$ flies were kept in the presence of abundant food for several days. Then cornmeal-molasses-agar cultures were made up according to the BRIDGES (1932) formula, seeded with

yeast, and a day later two pairs of flies of the appropriate genotype were introduced into each culture. During the development of the flies the cultures were kept at 24°C. Those offspring which appeared during the first three days of emergence were collected, and the mean number of dorso-central and scutellar bristles determined. The results are given in table 3. There is a tendency for bristle numbers to be slightly higher under these conditions than with the previously employed technique. However, the nature and extent of the effects remain very similar to those reported above.

TABLE 3

Mean number of dorsocentral and scutellar bristles present in +, y Hw, se h, and y Hw; se h male flies raised at 25°C on standard cornmeal-molasses-agar. The errors are standard errors of the mean. The letters A and B have the same significance as in table 1.

GENOTYPE	NUMBER OF FLIES	MEAN NUMBER OF DORSOCENTRAL BRISTLES		MEAN NUMBER OF SCUTELLAR BRISTLES	
		A	B	A	B
+	180	4.017±0.012		4.000±0.000	
y Hw	137	7.241±0.120		4.234±0.043	
se h	203	4.118±0.026		4.316±0.041	
y Hw; se h	94	8.830±0.111	7.342±0.123	5.851±0.075	4.550±0.059

Heterozygous pyd and h as dominance modifiers

Additional data on factor interaction were gathered during the synthesis of the various stocks used. Although both *pyd* and *se h* when acting separately behaved as recessives, flies simultaneously heterozygous for both these complexes showed significant departures from wild type bristle numbers. In table 4 is shown the mean number of bristles present at or near four different loci, in *se h*/+ +, *pyd*/+, and *se h* +/+ + *pyd* flies raised at room temperature (21±2°C) on standard cornmeal-molasses-agar. All strains had been made isogenic with the Oregon stock as reported above. Only those offspring which emerged within three days after the appearance of the first flies were used in the counts. Males and females were recorded separately, since females tend to have slightly more bristles than males.

The mean number per side per fly of anterior and posterior dorsocentrals, and of posterior scutellars, is the same for all genotypes, with one exception. There are significantly more bristles at or near the anterior dorso-central locus of *pyd*/+ males than at the same locus in *se h*/+ + or *se h* +/+ + *pyd* males. This may be a random occurrence, inasmuch as it was not duplicated in females of the same genotype. The frequent occurrence of supernumerary anterior dorsocentrals observed in all three strains is also characteristic of the Oregon wild type line under these conditions.

The situation with respect to the anterior scutellar locus is different. There are significantly more bristles present at this position in *se h* +/+ + *pyd* males and females than in either sex of *pyd*/+ or *se h*/+ +. Although non-allelic, these two recessive complexes when combined in the heterozygous condition can so reinforce each other's action as to result in a

TABLE 4

The interaction of heterozygous *pyd* and *se h* with each other and with *y Hw*. The symbols for the bristles used in the table have the following meanings: *M a dc*, mean number of anterior dorsocentral bristles per side per fly; *M p dc*, mean number of posterior dorsocentrals per side per fly; *M a sc*, mean number of anterior scutellar bristles present per side per fly; *M p sc*, mean number of posterior scutellars per side per fly.

BRISTLE	GENOTYPE					
	<i>se h</i> +/+		<i>pyd</i> /+		<i>se h</i> +/+ + <i>pyd</i>	
	♂ ♂	♀ ♀	♂ ♂	♀ ♀	♂ ♂	♀ ♀
<i>M a dc</i>	1.119 ± 0.015	1.194 ± 0.018	1.200 ± 0.018	1.220 ± 0.017	1.084 ± 0.013	1.208 ± 0.018
<i>M p dc</i>	1.005 ± 0.003	1.017 ± 0.006	1.008 ± 0.004	1.020 ± 0.006	1.005 ± 0.003	1.002 ± 0.002
<i>M a sc</i>	1.007 ± 0.004	1.033 ± 0.008	1.028 ± 0.007	1.046 ± 0.009	1.142 ± 0.017	1.272 ± 0.020
<i>M p sc</i>	1.000	1.000	1.004 ± 0.003	1.002 ± 0.002	1.002 ± 0.002	1.000
Number of half thoraces	436	458	494	603	430	515

BRISTLE	GENOTYPE					
	<i>y Hw</i>		<i>y Hw</i> ; <i>se h</i> +/+		<i>y Hw</i> ; <i>pyd</i> /+	
	<i>y Hw</i> (♂ ♂)	<i>y Hw</i> /++ (♀ ♀)	<i>y Hw</i> ; <i>se h</i> /++ (♂ ♂)	<i>y Hw</i> /++; <i>se h</i> /++ (♀ ♀)	<i>y Hw</i> ; <i>pyd</i> /+ (♂ ♂)	<i>y Hw</i> /++; <i>pyd</i> /+ (♀ ♀)
<i>M a sc</i>	1.339 ± 0.028	1.236 ± 0.021	1.641 ± 0.046	1.513 ± 0.034	1.826 ± 0.042	1.624 ± 0.038
<i>M p sc</i>	1.094 ± 0.017	1.105 ± 0.015	1.488 ± 0.043	1.522 ± 0.038	1.665 ± 0.043	1.662 ± 0.038
Number of half thoraces	298	390	170	226	230	283

significant deviation from wild type. GOLDSCHMIDT (1937) and GOLDSCHMIDT and HÖNER (1937) have reported somewhat similar phenomena.

By way of explanation of the probable restriction of dominance modification to the anterior scutellar locus, it might be pointed out that this locus is the only one of the four considered to be frequently marked by an extra bristle in *se h* flies. It is also often characterized by an extra bristle in *pyd* individuals, and is thus the place at which, phenotypically, *se h* and *pyd* exert a maximum of common effect. We could therefore conclude that although in heterozygous *pyd*/+ and *se h*/+ + there are no visible effects at this locus, there are nevertheless sub-threshold effects, which combine in the double heterozygote to reach the level of visible expression in some flies.

When *y Hw* males and *y Hw*/+ + females are made heterozygous for *se h* or *pyd*, there is likewise a significant effect upon bristle number. This is shown in table 4, using scutellar bristles as an example.

DISCUSSION

The combination of two and three factors affecting chaetal characteristics in a positive direction has resulted in flies deviating widely from an additive phenotype. This would imply that the relatively simple embryological systems assumed in the calculation of additive effects do not exist in these flies. Rather, some marked factor interaction, resulting in a strong mutual reinforcement of phenotypic effects, is indicated.

CSIK (1934), in a study of factor interaction among mutations affecting wing shape, observed "proportional" effects in many cases. Calculations show that the present data come slightly closer to showing proportional than additive effects. But the differences between expectation on the basis of proportional effects and the observed results are so large that the concept of proportional effects is obviously of no use in this case.

It would perhaps be possible to elaborate from the present data an hypothesis of factor interaction capable of bringing into line all or most of the observed data pertaining to the chaetae. Any attempt to do this at present, however, seems premature, since it overlooks the possible significance to a study of this nature of the size differences between the various genotypes. Does the comparison of character expression in groups of flies of very different average sizes give an accurate picture of gene effects, if character expression is correlated with size? It might be argued that inasmuch as size is genetically controlled, differences in size among genotypes are aspects of gene action which are a part of the total picture of gene effects. But small size may be due to structural defects in some part of the alimentary tract, inability to utilize satisfactorily some element of the diet, etc. Comparison of flies which are small due to causes of this nature with large flies is akin to comparing groups of flies raised under different nutritional conditions. Work aiming to evaluate the significance of these size differences between the eight genotypes is under way. It seems best to defer further speculation concerning the nature of the factor interaction seen here until these data are available.

A factor which in one genotype visibly affects only one or two chaetal characteristics may in another genotype prove to be a modifier of a third characteristic. For example, *pyd* in the Oregon background has no visible effect upon wing hairs, while *se h* has marked effects. However, the combination of *pyd* with *se h* has more wing hairs than simple *se h*. We are faced with two alternatives: (1) this modifying effect of *pyd* is a physiologically "new" effect, appearing only in *se h pyd* flies, or (2) the modifying effect is the expression of a physiological deviation from normal already present in *pyd* flies, but not reaching the threshold of visible expression. In this latter case we would recognize that a gene may have a rather general effect, which reaches the threshold of phenotypic deviation from

wild type only in certain limited areas. A factor which has only a slight visible effect might on this view induce sub-threshold variations in many characteristics.

Facts similar to the above can be found in the work of several investigators. PLUNKETT (1926) and STURTEVANT and SCHULTZ (1931) found, to quote PLUNKETT, that "in many cases a gene which alone has apparently no effect upon a particular bristle greatly intensifies the effectiveness of another gene in removing that bristle" (p. 192). HERSH (1929) marked different sections of the X chromosome with mutant genes and found that some sections acted as positive modifiers of eye-facet number in Bar-eyed flies, some as negative modifiers. HARNLY and HARNLY (1935) observed that although the only detectable effect of the dimorphos mutation when it is introduced into a wild type strain is a slight roughening of the eyes at high temperatures, in combination with vestigial, which strongly reduces wing size, dimorphos modifies both the size of the wing and the relation of wing area to the temperature prevailing during development. WADDINGTON (1939) postulates that in a case where a factor had no visible effect on the segmentation of the tarsus, nevertheless a "sub-threshold effect on the segmentation must have been present, since in compounds with other genes affecting segmentation, the most surprising degree of enhancement was seen" (p. 42).

The existence and determination of pattern in bristle mutants has aroused considerable discussion. In 1926 PLUNKETT pointed out that Dichaete flies, which are characterized by many missing bristles and abnormally held wings, deviate most widely from wild type in the vicinity of the presutural bristle and tend to become more normal as distance from this spot increases in any direction over the thorax. He postulated that during early embryonic development a precursor of a catalyst of a bristle-destroying reaction diffuses out from two bilaterally symmetrical centers in the egg. These centers correspond to the region of greatest effect of Dichaete in the adult. STURTEVANT and SCHULTZ (1931) and GOLDSCHMIDT (1931) have advanced similar hypotheses to explain the pattern of missing bristles seen in scute flies. However, MULLER (1932), CHILD (1935) and IVES (1939) have questioned the validity of the application of a diffusion hypothesis to the scute case.

Recently, SPARROW and REED (1940) studied a strain of *D. melanogaster*, the members of which possess many extra bristles. The trait seems to have a multigenic basis. They assume that the condition of the bristles in this strain can best be explained by the diffusion of a substance from a center sometime during development and cite as evidence for the assumption the following facts: (1) There is a gradient in bristle frequencies, extra bristles occurring most frequently in the anterior scutellar region, and usually

decreasing in frequency as distance from that point increases. (2) There is a significant positive association between affected bristle loci on the same side of the fly. (3) There is a gradient in left-right correlations between homologous bristles on the two sides of the fly, these being highest where extra bristle frequency is greatest. Point one, as clearly shown by CHILD (1935) in connection with scute, is scarcely proof for a diffusion hypothesis, since the appearance of a pattern may be a statistical phenomenon not corresponding to the condition in individual flies. As for points two and three, PLUNKETT (1926) and others since then have pointed out that evidence of this nature is valid only when the flies are genetically homozygous and have been raised under very uniform environmental conditions. Although the flies studied by SPARROW and REED were long inbred, there is room for some doubt as to the adequacy of the environmental control. The only statement by the authors with respect to this is: "the usual *Drosophila* culture methods were employed and cultures were reared at room temperatures which varied a few degrees from 21°C" (p. 412). The associations and correlations upon which their diffusion theory rests, although significant, are low. It seems possible that more carefully controlled temperature and culture conditions would reduce these statistics below the level of significance and so destroy the basis of a diffusion hypothesis. A simultaneous study of scute-8 by these authors revealed no evidence for diffusion phenomena.

NEEL (1940b) found that in *pyd* flies the presence of an extra bristle at one locus was entirely independent of the occurrence of an extra bristle at another locus. The *pyd* mutant is morphologically rather similar to the extra-bristled type studied by SPARROW and REED.

The data reported in this paper offer little suggestion of the existence of a developmental process coordinating the manifestation of the various chaetal characteristics of a fly. Correlations between the various chaetal traits of a genotype are usually not significant (table 2). The importance of size heterogeneity as a contributing factor to such significant correlations as do exist has already been mentioned. Nevertheless, it should be pointed out that in a few cases, mostly in γ *Hw* flies, there are some rather high correlations (γ *Hw* dorsocentral bristles-wing hairs, γ *Hw* dorsocentral bristles-scutellar hairs), which are as yet unexplained.

SUMMARY

Hw, *pyd*, and *h*, three *D. melanogaster* mutations characterized by an increase in the number of micro- and/or macrochaetae, were selected for a study of factor interaction. Femur length, number of dorsocentral bristles, number of scutellar hairs, number of scutellar bristles, number of hairs on the second longitudinal wing vein, and number of teeth in the sex-comb

were compared in +, *pyd*, *se h*, *y Hw*, *se h pyd*, *y Hw*; *pyd*, *y Hw*; *se h*, and *y Hw*; *se h pyd* males. By appropriate breeding techniques the strains had been rendered comparable with respect to almost all genes except the immediate regions on both sides of those detectable mutations which served to distinguish the strains.

All the mutant strains were considerably smaller than wild type.

The effects upon the chaetal characteristics of the mutations in combinations were generally super-additive—that is, greater than the sum of their effects when acting separately. The condition of the teeth in the sex-comb was an outstanding exception to this.

The absence, with some few exceptions, of significant correlations between the expression of the various chaetal traits indicates a developmental independence of these traits.

When culture conditions were varied, the relative effects of the mutations remained the same, although the absolute magnitudes of their effects changed.

Polychaetoid and *se h* when not combined with each other behaved as recessives. However, animals simultaneously heterozygous for both *se h* and *pyd* showed significant deviations from the wild type condition of the chaetae. Likewise, both heterozygous *se h* and *pyd* modify the expression of *y Hw*.

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