AN ANALYSIS OF THE EXPRESSION OF THE MUTANT "ENGRAILED" IN *DROSOPHILA MELANOGASTER*¹

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INTRODUCTION

THE fields of embryology and genetics find a common meeting ground in the study of the manner in which the genes control the development of an organism. Through analyses of the deviations from normality brought about by any specific mutant gene it is possible to obtain some idea of the processes of development in which it is involved and, therefore, probably also of those in which its wild type allele has had a part.

The mutant "engrailed" in *Drosophila melanogaster* seemed well suited for such an analysis for the following reasons. (1) The variability associated with each of the principal phenotypic effects of engrailed could be investigated experimentally since each is sensitive to environmental changes. (2) One of the effects of engrailed is rather unusual in that it produces a specific structure, a sex-comb, in a region of the tarsus where normally no such organ exists. Accordingly two sex-combs are found on one leg, the normal one and the mutant supernumerary. Thus the behavior of the two under various environmental conditions could be compared. (3) Variations in the sex-limited nature of the extra sex-comb could be observed under conditions which affect the normal sex-comb or sex itself.

There are several striking effects of engrailed, all of which are variable in their expression. (1) As just mentioned, there is an extra sex-comb in the male which is smaller than the wild type sex-comb and which is placed in a mirror image position to it on the outside of the first tarsal joint of the foreleg. Its size, as measured by the number of teeth, has been observed to vary from 0 to 11 teeth. (2) There is a median cleft in the scutellum which shifts from a flattening of the normally pointed posterior border to a complete separation of the two halves. (3) The wings are shorter, broader, and thinner than in the wild type. The venation is disturbed, with large or small gaps in the fourth and fifth longitudinal veins and an irregular branching plexus of extra veins. (4) Since this work was completed, a further effect of engrailed has been demonstrated by MRS. V. S. CURRY at the CALIFORNIA INSTITUTE OF TECHNOLOGY (unpublished). This is a rotation of the genitalia in males. Males with extremely rotated gen-

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italia are sterile, and hence in homozygous engrailed stocks there is selection for modifiers suppressing this effect. By making a balanced, that is heterozygous, stock MRS. CURRY is able to obtain homozygous engrailed males all of which show rotation.

Engrailed was first described by EKER (1929). It is an autosomal recessive gene, located in the second chromosome at locus 62.0. EKER describes the scutellar and wing modifications but makes no mention of the extra sex-comb. In his text figure of an engrailed male there seems to be such a sex-comb depicted on the left foreleg but not on the right. Its presence was discovered during a routine transfer of stocks (C. STERN, September 1937). This finding was confirmed by examination of the engrailed stock at the CALIFORNIA INSTITUTE OF TECHNOLOGY by the late DR. C. B. BRIDGES, and also by examination of a similar stock from the Department of Genetics of the CARNEGIE INSTITUTION OF WASHINGTON at Cold Spring Har-

FIGURE 1.—First tarsal joint of engrailed male showing primary (above) and secondary (below) sex-combs. Camera lucida drawing, 223X.

bor, N. Y. In the experimental work reported in this paper the latter stock was used. It was homozygous for the second chromosome recessive "purple" (*pr*, 2-54.5) as well as engrailed (*en*).

EXPERIMENTAL WORK

Sex-Combs

In *Drosophila* the sex-comb is a secondary sex character limited to the male. It consists of a row of modified bristles in the shape of a comb with heavy black teeth and is located on the inside (mesial side) of the first tarsal joint of the foreleg at its distal end.

The sex-combs of engrailed will henceforth be designated as primary and secondary. Primary refers to the sex-comb normally present on the mesial side of the foreleg; secondary to the extra one on the lateral side. They are shown in figure 1. The arrangement of the teeth in the primary sex-combs is almost invariably a straight row, but is much less regular in the secondary. If the number of teeth in the latter is small—from 1 to 5 or 6,—they are usually regularly placed, but as the number increases the arrangement becomes less uniform. Some representative types of sex-combs are shown in figure 2.

STURTEVANT (1929) has described a similar mutant in *D. simulans*, called "bipectinate." It is characterized by an extra sex-comb on the second tarsal segment, resembling the normal condition in *D. pseudoobscura* (see below). This mutant, like engrailed, is sex-limited in the expression of the extra sex-comb. Also like engrailed, it has other phenotypic effects which are not sex-limited—absence of one tarsal segment on every leg and changes in the shape of the wing.

It is interesting to note that features pertaining to sex-combs are of taxonomic importance. They are found in many, but not all, species of Dro-

FIGURE 2.—Various types of sex-combs. A, right primary, B, left secondary; C and D, left and right secondaries, respectively (19°), showing irregular arrangement of teeth. Camera lucida drawing, 223X.

sophila, and if present, they are characteristic in location, size, and form for the particular species. In species which possess them there is always a sex-comb on the first tarsal segment. A number of species normally have a second sex-comb on the inside of the second tarsal joint. The occurrence of this distal sex-comb is recorded below (STURTEVANT 1921, STURTEVANT and DOBZHANSKY 1936, KIKKAWA and PENG 1938).

SPECIES GROUP	DISTAL SEX-COMB
<i>melanogaster</i>	Present, except in <i>melanogaster</i> , <i>simulans</i> , and <i>bipectinata</i> *
<i>affinis</i>	Only 1 tooth
<i>obscura</i>	Present

* *D. bipectinata* has two sex-combs, one immediately behind the other, on the inside of the first tarsal joint.

MATHER and DOBZHANSKY (1939) made a statistical analysis of the number of teeth in the sex-combs in the two races of *D. pseudoobscura*. They found that the distal sex-comb had fewer teeth than the proximal and demonstrated a significant racial difference in the mean number of teeth in corresponding sex-combs, as well as a significant difference between some strains of the same race. They showed that if suitable statistical techniques are used, the number of teeth in the sex-combs may be employed as a taxonomic character to differentiate the two races with 90 percent of accuracy.

Effect of temperature upon the sex-combs

Methods.—In order to provide optimal developmental conditions, the flies used in the temperature experiments were bred in the following manner. Parent flies were placed, without etherization, in a fresh bottle of standard cornmeal-molasses-agar culture medium which had been well seeded with yeast. After 24–48 hours they were transferred to bottles containing small dishes of yeasted cornmeal-agar and allowed to oviposit until about 125 eggs were present. Then the dishes were removed and new ones put in their place. The number of eggs on each dish was counted and the entire contents of the dish transferred to a 10.5 cm fingerbowl containing a layer 1–1.5 cm thick of freshly yeasted cornmeal-agar. The number of eggs in each fingerbowl ranged from 110 to 135, averaging 125. A larger number was not used because of the possibility of overcrowding the developing larvae.

The fingerbowls containing eggs were placed in incubators at temperatures of 14°, 19°, 25°, and 29.5°C. The incubators were of the general type

TABLE I
*Mean number of teeth in sex-combs of engrailed.**

TEMP.	N	PRIMARY		SECONDARY	
		MEAN	σ	MEAN	σ
14°	180	10.42 ± 0.10	0.96 ± 0.07	6.26 ± 0.16	1.50 ± 0.11
19°	648	10.51 ± 0.05	0.92 ± 0.04	5.71 ± 0.11	1.88 ± 0.08
25°	622	11.04 ± 0.06	1.01 ± 0.04	3.57 ± 0.11	1.86 ± 0.08
29.5°	566	10.92 ± 0.06	1.01 ± 0.04	0.13 ± 0.02	0.34 ± 0.02

* These data approximate a normal distribution curve; therefore the mean and standard deviation are a legitimate form of analysis of them.

described by BRIDGES (1932) and maintained a constant temperature with no greater variation than $\pm 0.2^\circ$. When the larvae pupated, they were placed in half-pint culture bottles and allowed to hatch at the same temperature at which they developed.

As a quantitative evaluation of the size of the sex-combs the number of teeth present was determined in the following way. The forelegs were removed under a binocular dissecting microscope and placed on a dry slide. The teeth were counted under the high power of a compound microscope. Right and left sex-combs were recorded separately, but since analysis showed no significant difference in their mean sizes, the data were combined in computing the average number of teeth at each temperature. The mean and standard deviation together with their standard errors were calculated for both primary and secondary sex-combs. Differences between means were tested by the formula

$$\epsilon_d = \sqrt{\frac{\sigma_1^2}{N_2} + \frac{\sigma_2^2}{N_1}}$$

in which ϵ_d is the standard error of the difference, σ_1 and σ_2 are the standard deviations of the two means, and N_1 and N_2 are the two populations. If the observed difference was more than three times this value, it was considered significant.

Results.—The experimental results obtained by this procedure are presented in table 1. The primary sex-combs do not show a great deal of variation. Below are given the differences between their means and the standard errors of these differences. It may be seen that there is a significant difference between the high and low temperature groups but no such difference between the two members of each group.

TEMPERATURES	d	ϵ_d
14°-19°	0.09	0.11
* 14°-25°	0.62	0.11
* 14°-29.5°	0.50	0.12
* 19°-25°	0.53	0.08
* 19°-29.5°	0.41	0.08
25°-29.5°	0.12	0.08

* Significant.

These results fall in line with those of several investigators who have worked with both wild and mutant stocks in which only the normal, or primary, sex-comb was present. They found that the number of teeth is rather constant. There is a small amount of variability, but its range seems to be about the same under different environmental conditions. HOGE (1915) was able after seven generations of selection to produce a race which had a higher average number of teeth than the unselected controls. EIGENBRODT (1930) observed the effects of five different temperatures upon the number of teeth in the sex-combs of a homozygous forked Bar stock. He found no consistent or significant alteration in the number of teeth. COMBS (1937) made a further study of the effect of temperature on the sex-combs, using several mutant stocks and two temperatures. For all the stocks he found a slightly but significantly higher mean number of teeth at 27° than at 17°. The stability of the primary sex-comb is thus maintained rather well in a variety of conditions. Neither temperature nor selection can very effectively alter its size. However, the fact that there are significant differences between the number of teeth in the sex-combs of different homozygous stocks shows that genetic factors whose more obvious effect is not on the sex-comb nevertheless do influence the size of this structure.

In contrast to the results obtained for the primary sex-combs is the decided response to temperature exhibited by the secondary sex-combs. A decrease in tooth number is associated with an increase in temperature. At the three lower temperatures a large amount of variability is found—from 2 to 9 teeth at 14°, from 0 to 11 at 19°, and from 0 to 10 at 25°. At 29.5° there is a striking loss of secondary sex-combs—in 81.3 percent of the individuals studied they are completely absent. Only one fly was found with as many as three teeth in one of its secondary sex-combs. Below are given the differences, with their standard errors, between the mean tooth number at the various temperatures. Obviously all are significant.

TEMPERATURES	d	ed
14°-19°	0.55	0.15
14°-25°	2.69	0.15
14°-29.5°	6.13	0.07
19°-25°	2.14	0.11
19°-29.5°	5.58	0.08
25°-29.5°	3.44	0.08

In order to determine whether or not the variability in the number of teeth in the sex-comb on one foreleg was in any way connected with the variability on the other foreleg, correlation coefficients (*r*) were calculated separately for primary and secondary sex-combs on the right and left sides (table 2). None of the coefficients is high, and some are not significant, being less than three times the standard error of *r*. Even the highest of

TABLE 2

Coefficients of right-left correlation between the number of teeth in the primary and secondary sex-combs.

SEX-COMB	14°	19°	25°	29.5°
Primary	0.01 ± 0.11	0.38 ± 0.05	0.04 ± 0.01	0.15 ± 0.06
Secondary	0.23 ± 0.10	0.41 ± 0.03	0.24 ± 0.05	0.19 ± 0.06

these—namely, 0.41 ± 0.03 for the secondary sex-comb at 19°—indicates that no more than 16 percent of the variance between the two sides is due to factors common to both sides. The remaining 84 percent is random. Thus on the whole the two sides of the fly seem to vary independently with regard to the number of teeth in the sex-comb. Similar low positive correlations between the right and left sex-combs in races of wild type *D. melanogaster* were found by CARPENTER and BARROWS (CASTLE et al. 1906).

In his study of the bristle pattern of the mutant *Dichaete* in *D. melanogaster*, PLUNKETT (1926) points out that a low value of *r* is obtained when

there are uniform environmental conditions and genetic homogeneity. High correlations, on the other hand, will be given by stocks with numerous genetic differences or by homogeneous stocks when environmental influences are diverse. The low correlation coefficients in table 2 show that in those cases there must have been genetic and environmental uniformity; where the coefficients are higher it may be assumed that the external environment has played some role in effecting that correlation, although some genetic variability cannot be entirely excluded.

Of particular interest from a developmental point of view is the question of the correlation between primary and secondary sex-combs on the same foreleg (table 3). Again the values are all low or insignificant. They indicate that there is no association between the sizes of the two sex-combs on same leg, except possibly at 19°. PLUNKETT (1926) found a clear degree of association in *Dichaete* between the bristles on one side of the fly. However, in other mutants the situation is similar to engrailed where the association

TABLE 3
Coefficients of correlation between the number of teeth in the primary and secondary sex-combs on the same side.

SIDE	14°	19°	25°	29.5°
Right	0.15 ± 0.10	0.14 ± 0.05	-0.06 ± 0.06	-0.06 ± 0.06
Left	0.07 ± 0.10	0.17 ± 0.05	-0.06 ± 0.06	-0.02 ± 0.06

is lacking or doubtful. This has been demonstrated for scute-1 by CHILD (1935) and IVES (1939) and for polychaetoid by NEEL (1940b). These investigators concluded that each bristle locus behaves independently of the others.

The most important point brought out by these studies of the correlations between sex-combs is the high degree of independence of each of the four sex-combs on one engrailed fly. This also agrees with the different effects which changes in temperature had upon them—the primary varying little and increasing in size with increase in temperature, the secondary varying a great deal and decreasing in size with increase in temperature. This point will be considered in more detail later when the manner of action of the engrailed gene is discussed.

Relation of body weight to sex-combs

There is one general effect of temperature which is not often taken into account in investigations of temperature effects on various phenotypic characters—its effect on the body size of the fly. It is a common observation that flies reared at high temperatures are in general smaller than those reared in a cooler environment. NEEL (1940a) attacked this problem

of the relation of body size to character expression in the mutants *Dichaete* and *polychaetoid* of *D. melanogaster*. To determine whether the expression of engrailed was bound up with body size as it was with temperature, an analysis of these relations was undertaken. CASTLE et al. (1906) had already reported an increase in the number of teeth in the wildtype sex-comb with increase in size, using tibia length as an index of the latter.

Methods.—Flies of various sizes reared at the same temperature were obtained, using the eggdish-fingerbowl technique with different population densities (125, 300, 600, or 900 eggs in a fingerbowl). Another method employed was the rearing of 100 eggs on the amount of food contained in an eggdish (about 15 cc versus 125 cc in a finger bowl). Flies reared under crowded conditions on a limited amount of food were at a lower nutritional level than those less crowded and hence were smaller. The temperatures used were again 14°, 19°, 25°, and 29.5°.

When the larvae had pupated, they were placed in vials which contained a few cc of food. The adults usually hatched over a period of several days, especially from the more crowded cultures. Each day those which had emerged were collected and put into freshly yeasted culture bottles. There they were allowed to feed freely for 36–48 hours. The males were then etherized and weighed in groups of 50–200 on an analytical balance and the average weight for each group computed. Sex-comb counts were made for each size group.

Since body weight is closely and positively correlated with tibia length (NEEL 1940a), it may be used as an index of fly size, even though its use may be attended by a fairly large error. The chief cause of this error is the amount of food present in the gut, which in turn is determined by the inclination of the fly to feed, the availability of food, etc. Every effort was made to obtain as uniform and optimum conditions as possible.

Linear plots were made of the average weight and mean sex-comb tooth number of each group of flies, and a straight line was fitted to the points for each temperature by a modification of the least squares method which permitted a minimizing of the squared perpendicular distances from the points to the line and thus afforded the most correct fit. Each point represents flies grown under the same conditions. If successive hatches from the same population unit differed in average weight, the mean for the whole group was computed. Each point (with two exceptions) was calculated from more than 100 flies, hence 200 sex-combs, and many groups were still larger.

Results.—Primary sex-combs.—The data for the primary sex-combs are plotted in figure 3. A considerable range of body weights was obtained and, as might be expected, the smaller flies were found at the higher temperatures. Actually the group with the highest body weight was obtained at

19° instead of 14°, and the smallest at 25°, not 29.5°. In the latter case the technical difficulties in rearing flies at 29.5° reduced the population to less than half the size of that at 25°. Consequently the sampling error of mean weights may be large. At any rate the difference in weight between the smallest flies at 25° and the smallest at 29.5° is only 6 percent. In the former case it has been pointed out (EIGENBRODT 1930) that as incubation temperature decreases weight increases down to about 17°. Below this

FIGURE 3.—(left) Relation of mean body weight to mean number of teeth in primary sex-combs at four temperatures. The bars extending from each point show the extent of three times the standard error of mean tooth number. Equations are for the regression of mean tooth number (y) on mean body weight (x).

FIGURE 4.—(right) Relation of mean body weight to mean number of teeth in secondary sex-combs at three temperatures.

there is very little increase in weight. Similarly RIEDEL (1934) has shown that the tibia length of wild type flies increases with a temperature decrease until about 15–16°. Further reduction in temperature causes a decrease in tibia length. NEEL (1940a) obtained larger flies of the *Dichaete* mutant at 19° than at 14°.

At each temperature there is a range of sex-comb sizes corresponding to the body weights. The fit of the straight lines which have been plotted on the graph is an indication that the relationship of the size of the primary sex-combs to that of the whole organism approximates a rectilinear one. The vertical bars extending from each point show the extent of three times the standard error of the mean sex-comb tooth number. In all cases these

bars touch the fitted line. The equations on the graph are of the general form $y = ax + b$ and are specifically for the regression of the mean number of teeth in the sex-comb (y) on average body weight (x) at each temperature. It may be seen that the slope (a) of the lines is steepest at the higher temperatures, while the intercepts (b) are all rather near together.

An evaluation of the relationship of body size to the temperature effect must now be made. It is obvious from figure 3 that while there is a definite correlation between body weight and sex-combs within each temperature, this relationship does not hold for the same size of fly at different temperatures. For example, let us examine flies which weighed on the average 0.60 mg each. At 14° they have an average of 9.46 teeth in their primary sex-combs, at 19° , 9.68 teeth, and at 29.5° , 11.02. Between 14° and 19° there is not a great deal of evidence for a temperature effect distinct from size, but the difference between these two temperature groups and that at 29.5° is considerable and significant ($\epsilon_d = 0.08$ for $19-29.5^{\circ}$). Other similar average weights at different temperatures give the same picture. A comparison may also be made by calculating from the regression equations for each temperature what the tooth number would be at a given size—namely, 0.60 mg. The values are as follows:

TEMPERATURE	TOOTH NUMBER
14°	9.44
19°	9.65
29.5°	10.83

There is clearly a difference in tooth number as the temperature increases. If size were playing any large role in the observable temperature effect on the primary sex-combs, we should expect all four lines in figure 3 to lie as near or nearer together as those for 14° and 19° . Their lying apart from each other indicates that sex-comb differences at various temperatures are largely independent of body size, most strikingly so in the higher part of the temperature range. If size were constant at all temperatures, the apparent effect of temperature would be considerably greater.

NEEL (1940a) has shown in his study of the correlation of body size with the expression of the mutants *Dichaete* and *polychaetoid* that in both cases there is a direct relationship between body weight and the number of dorsocentral bristles per side per fly. In his evaluation of the separate roles of size and temperature in the determination of bristle number he found for *Dichaete* that when size is held constant over a 15° temperature range, there is no consistent change in bristle number. Hence body size is an important factor in the temperature effect on the expression of this mutant. For *polychaetoid*, however, he found that the role of the size factor

is not the same for all temperature intervals but is greatest at high temperatures and least at low. It has now been demonstrated for engrailed that the size factor in the determination of the number of teeth in the primary sex-comb is important only at the lower temperatures.

Secondary sex-combs.—The secondary sex-combs present a strikingly different picture from the primaries. Instead of the uniformly positive relations for weight-tooth number there is a positive slope of the line at one temperature and negative slopes for the others. This is shown in figure 4. Data for 29.5° are not given, since secondary sex-combs are rarely present at this temperature. At 14° the data show as good an approximation to linearity as at any of the temperatures in figure 3. The slope is not so great and the intercept is smaller. At 19° and 25° there is obviously another sort of process involved as judged from the negative slope of the curves. The straight lines are clearly not a correct expression of the relationship of size to secondary sex-combs at these temperatures. There is a decided drop after an initial high tooth number for the smaller size groups, followed by a slight rise—whether significant or not is difficult to say, but the data at 19° strongly suggest that it is.

A comparison between the sizes of the secondary sex-combs in flies of the same body weight at different temperatures may be made as for the primary sex-combs. Flies which weighed 0.77 mg at 25° had an average of 3.45 teeth in their secondary sex-combs; flies weighing 0.78 mg at 19° had a mean of 5.50 teeth ($\epsilon_d = 0.13$). Between some other points in figure 4 there are not such great differences, but in these cases—namely, small flies at 14° and 25° and large individuals at 14° and 19° —the two temperatures involved behave differently in their weight-tooth number relationships. Comparisons have not been made between values calculated from the regression equations for 19° and 25° , since the lines are only very poor approximations to the data.

The correlation of increased size of an organ with decreased size of the whole organism is not a common one. Two other such cases, however, have recently been described for *D. melanogaster*. BODENSTEIN (1939) starved larvae of a stock carrying the mutant Bar in order to ascertain the correlation of increased larval life with the number of facets in the eye. The starved flies had a smaller tibia length and hence a smaller body size than their normal controls but the males had a much higher facet number. Body size is probably not the decisive factor here, since when larvae were completely starved they pupated earlier than the controls and had a lower facet number. CHILD (1939) working with the vestigial mutant also used starvation to increase the time of development at a constant temperature and obtained flies which were smaller but which had larger wings than the unstarved controls. However, it should be pointed out that the

production of vestigial wings is accomplished by a degeneration of the wing tissue rather than by a failure of it to develop. Thus this case is probably not comparable to the former ones.

Effect of intersexuality on the sex-combs

In attacking the problem of the sex-limited expression of engrailed it was sought to bring this gene into a genetic environment which would modify the sex of the individuals concerned. Such an environment is found in intersexes—individuals which have both male and female characteristics. It is known that sex-combs are present in most wild type triploid intersexes ($2X+3A$), but no data were available as to their size in intersexes compared to wild type diploid males. It is of interest to discover whether the secondary sex-comb occurs in intersexes homozygous for engrailed or whether the departure from absolute maleness suppresses or modifies the appearance of this sex-limited structure, and also whether the degree of intersexuality influences the expression of either or both primary and secondary sex-combs.

As a group intersexes are not uniform in appearance, especially in respect to the genitalia. Some show predominantly male structures, others female, and still others have neither typical male nor female parts. DOBZHANSKY (1930) has grouped intersexes into five classes, as follows: class 1, extreme male type; external male genitalia present; penis and genital arch symmetrical; class 2, external male genitalia or rudiments present; penis and genital arch asymmetrical, reduced in size or rudimentary; class 3, neither male nor female external genitalia present; class 4, external female genitalia present at least in rudimentary form; vaginal plates asymmetrical; class 5, extreme female type; external female genitalia present; vaginal plates symmetrical. He has shown that the type of intersex is influenced by genetic modifiers, since by selection for male or female type he could establish lines in which the percentage of either type was high with a corresponding decrease in percentage of individuals of the opposite type.

Intersexes homozygous for purple engrailed were obtained by repeated backcrossing of wild type triploid females to engrailed males. A homozygous triploid engrailed stock was thereby established. Engrailed intersexes are easily recognized. In them the scutellum is split characteristically, and the secondary sex-comb is commonly found. The wings of the majority are considerably shortened and rounded. In any doubtful cases—namely, extreme male types—intersexuality may be checked by an examination of the cells of the wing. In triploid females and intersexes the increased size of these cells is a clear diagnostic character.

The triploid engrailed strain and a wild type triploid stock were bred

on standard culture medium at 25°. All the cultures involved in the synthesis of the homozygous triploid engrailed stock were likewise bred under these conditions. Intersexes were collected from both triploid stocks over a period of two and one-half years, and counts were made of the number of teeth in their sex-combs in the manner previously described. The data for each intersexual class were kept separately. The mean and standard deviation together with their standard errors were calculated for each class as well as for each group as a whole.

The data obtained are recorded in tables 4 and 5. They show several important facts. First, the number of teeth in the sex-combs of intersexes

TABLE 4

Mean number of teeth in the sex-combs of wild type intersexes (N-number of sex-combs).

CLASS	N	MEAN	σ
1	116	7.76 $\pm$ 0.09	0.95 $\pm$ 0.06
2	110	7.93 $\pm$ 0.10	1.08 $\pm$ 0.07
3	40	8.13 $\pm$ 0.10	0.86 $\pm$ 0.10
4	132	7.80 $\pm$ 0.09	1.02 $\pm$ 0.06
5	88	7.66 $\pm$ 0.10	0.96 $\pm$ 0.07
Σ	486	7.82 $\pm$ 0.05	1.01 $\pm$ 0.03

is decidedly lower than in diploid males. COMBS (1937) reports mean numbers of teeth of 9.80 and 10.23 at 17° and 27°, respectively, for wild type sex-combs. The mean value for primary sex-combs of engrailed has been

TABLE 5

Mean number of teeth in the sex-combs of engrailed intersexes.

CLASS	N	PRIMARY		SECONDARY	
		MEAN	σ	MEAN	σ
1	170	8.12 $\pm$ 0.07	0.95 $\pm$ 0.04	2.57 $\pm$ 0.15	2.04 $\pm$ 0.11
2	196	8.33 $\pm$ 0.07	0.91 $\pm$ 0.05	1.76 $\pm$ 0.15	2.03 $\pm$ 0.10
3	28	8.54 $\pm$ 0.15	0.79 $\pm$ 0.11	1.25 $\pm$ 0.25	1.30 $\pm$ 0.17
4	80	8.50 $\pm$ 0.10	0.87 $\pm$ 0.07	1.71 $\pm$ 0.23	2.04 $\pm$ 0.16
5	44	8.27 $\pm$ 0.15	0.97 $\pm$ 0.10	3.02 $\pm$ 0.37	2.47 $\pm$ 0.26
Σ	518	8.29 $\pm$ 0.04	0.96 $\pm$ 0.03	2.10 $\pm$ 0.09	2.10 $\pm$ 0.06

reported above as 11.04 (25°). The means of 7.82 and 8.29 found in tables 4 and 5 obviously represent a real decrease in the size of the sex-comb. Second, in neither the wild type nor the primary engrailed sex-combs is there a significant difference between the mean number of teeth in the sex-combs of any of the five classes of intersexes. The degree of intersexuality, at least that to which the external genitalia are an index, seems to have no effect on the size of the sex-combs, since an intersex of the extreme

female type may have as many teeth in its sex-combs as one of extreme male type. Intersexes are then a uniform group in regard to their primary sex-combs.

When a comparison between the mean number of teeth in the sex-combs of wild type intersexes and the primary sex-combs of engrailed is made, it is seen that the former is significantly smaller than the latter ($d=0.37$, $\epsilon_d=0.06$). This might be accounted for in one of two ways—either the engrailed gene is directly responsible for increasing the number of teeth in the sex-comb or this increase is due to various other genetic modifiers which were brought into the wild triploid stock in the synthesis of the homozygous triploid engrailed strain. In order to test these possibilities the teeth in the sex-combs of intersexes collected from cultures used in the

TABLE 6
Mean number of teeth in the sex-combs of intersexes of intermediate genotypes.

CLASS	N	MEAN	σ
1	134	8.52 ± 0.09	1.03 ± 0.06
2	122	8.24 ± 0.09	1.04 ± 0.07
3	44	8.48 ± 0.14	0.96 ± 0.11
4	74	8.61 ± 0.11	0.89 ± 0.07
5	48	8.33 ± 0.15	1.01 ± 0.10
Σ	422	8.43 ± 0.05	1.02 ± 0.04

synthesis of this strain were counted. Such intersexes would have genetic constitutions of various intermediate conditions between those of the wild type and engrailed intersexes, and could not be distinguished phenotypically from the former. If the mean tooth number for this group should turn out to be the same as that of the wild type intersexes, it would indicate that the larger sex-comb in engrailed intersexes is due to a specific effect of the engrailed gene. If on the other hand the mean tooth number of the intermediate group should be intermediate between the other groups or as high as the engrailed intersexes, then we would conclude that the larger sex-combs in the latter have been produced by genetic modifiers brought into the triploid stock when it was outcrossed. The data in table 6 show that the latter interpretation is the more correct, since the mean number of teeth in the sex-combs of the intermediate group was actually a little higher than the mean of the engrailed intersexes, although not differing significantly from it ($d=0.14$, $\epsilon_d=0.06$). There remains the untested possibility of engrailed acting in heterozygous condition.

Further evidence for the independence of the primary sex-combs and the degree of intersexuality may be found in the data presented. If the percentage of individuals in each of the intersexual classes is computed for the

three genetically different groups of intersexes, we note that in the transition from wild type to engrailed there is an increase in male types and a corresponding decrease in female types (table 7). Class 3, which rises and then falls, is represented in each case by a rather small number of individuals. It is interesting to note that in the intermediate group there are percentages midway between wild type and engrailed, whereas these same intersexual classes were not intermediate but resembled engrailed in the condition of their sex-combs. These shifts cannot be attributed to any specific action of the engrailed gene but must be assigned to the changed genetic constitution as a whole.

The mean size of the secondary sex-combs in all five classes of engrailed intersexes was found to be less than that of diploid males reared at 25°.

TABLE 7
Percentages of individuals in intersexual classes.

CLASS	WILD TYPE	INTERMEDIATE	ENGRAILED
1	23.8	31.8	32.8
2	22.6	28.9	37.8
3	8.2	10.4	5.4
4	27.2	17.5	15.4
5	18.2	11.4	8.5

The mean for the former was 2.10 teeth, for the latter 3.57. There is a significant difference between the members of various classes—class 1 and class 5 differ from classes 2, 3, and 4. Classes 1 and 5 do not differ significantly from each other, nor do classes 2, 3, and 4. It seems strange that, if there are any differences among the classes, the extreme female type intersexes should have sex-combs of a size comparable to the most extreme male type. However, it has just been shown that genetic modifiers were responsible for the increase in number of teeth of the primary sex-combs of engrailed intersexes over that in wild type. On a more labile structure such as the secondary sex-comb it might be expected that more extreme effects of such genetic factors would be found. The combined effects of those modifying the number of teeth in the sex-comb and those which modify the degree of intersexuality may serve to produce the observed phenotype.

In the light of MRS. CURRY'S demonstration of the effect of engrailed on the genitalia, the question arises as to the accuracy of the classification of the intersexes. There is a possibility that, due to rotation of the genitalia, individuals of class 1 (extreme male type) have been erroneously placed in class 2 (abnormal male type). This, however, would not influence the conclusion that the degree of intersexuality has no effect on the size of the

primary sex-comb, nor does it affect the conclusion that there are shifts in the proportion of male and female type intersexes (table 7). Furthermore the findings regarding secondary sex-combs are not appreciably affected even if MRS. CURRY'S impression should turn out to be true that there may be an increase in the size of the secondary sex-comb in males with extremely rotated genitalia. In intersexes with abnormal genitalia this is not the case since the secondary sex-combs of class 1 were significantly larger than those of class 2 (table 5). If there were any errors in classification, they would have tended to lessen this difference.

Correlation coefficients for the number of teeth in the primary and secondary sex-combs of the engrailed intersexes were calculated and are given below. In contrast to the correlation in diploid males (table 3), these

CLASS	r
1	-0.32 ± 0.07
2	-0.46 ± 0.07
3	-0.38 ± 0.16
4	-0.49 ± 0.09
5	-0.60 ± 0.10

are all negative and, except for class 3, are all significant. There is thus a dependence of the two sex-combs on the foreleg of an intersex not found in a diploid male. Discussion of this interesting fact is reserved for a later section.

SCUTELLUM

Effect of temperature on the scutellum

Methods

Scutellar data were obtained from the same flies the study of whose sex-combs has already been discussed. In addition to 14° , 19° , 25° , and 29.5° , five other temperatures were employed— 22° , 26° , 27° , 28° , and 29° . The method of rearing the flies was identical for all and has been described. For examination the adult flies were etherized and, under a binocular dissecting microscope, sorted into classes according to the extent of the cleft in the scutellum. Four classes were arbitrarily designated as follows (illustrated in fig. 5): class I, the scutellum completely divided into two separate halves;

FIGURE 5.—Types of scutellar classes, I-IV. Explanation in text.
Camera lucida drawings, $160\times$.

class II, a cleft extending beyond a line connecting the posterior scutellar bristles, but not entirely separating the two halves of the scutellum; class III, a smaller cleft, extending no farther than a line connecting the posterior scutellar bristles; class IV, no visible cleft in the scutellum but a flattening of its posterior border; completely wild type scutella with pointed posterior borders are not found. The order of these classes presumably represents the degree of fusion attained by the imaginal discs during development. There is some variability within each class, but a sharper dis-

FIGURE 6.—Effect of temperature on percentage of individuals in the four scutellar classes. At each temperature classes I-IV read from left to right except for females at 14° where class I is not represented.

inction is difficult to make, and the chance of error in placement becomes larger with a greater number of classes.

Results

The effect of temperature on the expression of the split condition of the scutellum is graphically represented in figure 6. Males and females were considered separately, since they are affected differently as determined by χ^2 calculations (table 8). In all cases, except at 29.5° and possibly at 14°, the P values are very low and thus indicate that males and females represent different populations with respect to their scutella and hence that temperature affected the cleft differently in the two sexes. In view of the sex-limited expression of the extra sex-comb it is interesting to find a sexual difference in another aspect of the expression of the engrailed gene. GOLD-

SCHMIDT (1938) has pointed out in this connection that the sex-determining mechanism of one X chromosome in the male and two in the female sets up different developmental systems in the two sexes. Therefore, not only sex-linked genes but autosomal genes as well may have a different effect in males and females. Basic developmental rates may differ or the speed of

TABLE 8
Difference of distribution of males and females into scutellar classes. ($n = 3$ degrees of freedom except at 14° where $n = 2$.)

TEMP.	SEX	CLASS I	II	III	IV	Σ	χ^2	P
14°	Male	1	12	108	11	132	12.19	0.01
	Female	0	3	145	25	173		
19°	Male	20	42	544	85	691	27.24	0.00
	Female	22	89	585	34	730		
22°	Male	33	25	289	34	381	69.94	0.00
	Female	68	84	276	7	435		
25°	Male	273	78	378	36	765	58.82	0.00
	Female	278	185	295	17	775		
26°	Male	34	22	133	15	204	22.15	0.00
	Female	51	48	107	2	208		
27°	Male	40	23	140	22	225	20.61	0.00
	Female	51	54	156	6	267		
28°	Male	29	16	333	64	442	34.03	0.00
	Female	40	38	342	20	440		
29°	Male	37	6	114	33	190	16.90	0.00
	Female	30	19	163	17	229		
29.5°	Male	12	7	196	72	287	2.00	0.74
	Female	12	15	233	89	349		

certain auxiliary processes may be modified in one sex more than in the other.

It must be emphasized again that the system of classification of the split scutella is an artificial one in that it leaves out of account the variability within each class. This is particularly true for classes II and III. It also divides up arbitrarily the progressive separation of the two halves without considering that this continuous series may not represent the result of a growth process whose rate is always the same. The divisions between the groups, therefore, may not coincide with the points of acceleration or of other change in the developmental reaction.

Inspection of the data in figure 6 reveals immediately that at all temperatures class III is the largest group. The distributions are different at the various temperatures except those at 26° and 27°, which are similar for both males and females. Classes I and II tend to become larger up to 25–26° and then grow smaller, while the tendency in classes III and IV is to decrease and then increase. In order to obtain a quantitative expression which would represent the average condition of the scutellum at a given temperature, the following arithmetical procedure was carried out. For each temperature the percentage of individuals in class I was multiplied by 1, that in class II by 2, and so on. The sum of these products was divided by 4 and this quotient designated as a "class average" for males and females at each temperature. These are plotted in figure 7. Treatment of the data in this way shows that there is a drop from a high class average at 14°

FIGURE 7.—Effect of temperature on cleft in scutellum as indicated by class averages.

and 19° to a low at 25° and then a gradual rise from 25° to 29.5°. The females give lower values than the males except at the extreme limits of the temperature range. A high class average indicates that the tendency toward an extreme cleft in the scutellum is low in the group as a whole; a low class average shows that the mean amount of splitting is somewhat higher. It should be realized that the comparatively simple picture presented in figure 7 obscures some of the enigmatical but significant fluctuations of the various classes as shown in figure 6.

When these experiments were first performed, the temperature of 22° was not employed. Later it was thought desirable to conduct a series at this temperature to determine the trend in the seemingly important interval between 19° and 25°. This was done, with a control series at 25°. The latter gave results different from those previously obtained ($\chi^2 = 94.22$, $P = 0.00$). There was a smaller percentage of both males and females in class I, and hence the class averages are higher. During the interval genetic modifiers in the purple engrailed stock had arisen by mutation or become recombined in such a way that the temperature response of the scutellum was

changed. For this reason the class averages obtained at 22° and for the controls at 25° are plotted in figure 7 but not connected to the curve drawn. These results, however, do not alter the trend of the distributions, since 22° is intermediate between 19° and 25° , and the low point of the curve still lies around 25° . The interpretation of these data will be discussed later in this paper.

Relation of body weight to scutellum

It was demonstrated earlier in this paper that body size is an important factor in the temperature effect on the sex-combs. A similar examination of the role of size in the expression of the cleft in the scutellum at a constant temperature is accordingly in order.

The flies whose body size-sex-comb relationship has already been discussed were used also for this study. Their scutellar classes were determined

FIGURE 8.—Relation of mean body weight to extent of cleft in scutellum of males at four temperatures, as indicated by class averages for each weight group.

after weighing. Class averages for each weight group are plotted in figure 8. The significance of differences between the points at each temperature was tested by χ^2 determinations.

It was found that the rise in the curves at 14° and 19° is a significant one. Hence the scutellum tends to be less split in the larger flies at these temperatures than in the smaller individuals. At 25° the rise is not so marked, but between the low and high points there is still a significant difference and therefore still a tendency for larger flies to have more "normal" scutella. At 29.5° the nearly straight line shows that there is no effect of size on the expression of the cleft in the scutellum.

Thus the same relationship between body size and temperature effect that was found for the sex-combs holds for the scutellum. For the mutant engrailed at low temperatures there is no appreciable effect of temperature apart from its action through the body size of the fly, but at higher tem-

peratures the role of size becomes of less importance while some other phase of the temperature effect gains greater prominence in the development of the phenotype.

WINGS

Examination was made of the wings of male and female flies reared under conditions previously described at 14°, 19°, 25°, and 29°. To do this the wings were removed from the thorax and mounted directly in Canada balsam. Camera lucida drawings were then made. A study of these drawings revealed that at 14° and 19° the wings are very similar, having either large, irregular gaps in the fourth and fifth longitudinal veins or complete absence of these veins. At 25° the wings resemble those at 14° and 19° except for their smaller size. However, at 29° there is marked similarity to the wild type wing, only small gaps in the veins being occasionally found. There is thus an approach toward wild type expression in the wings with

TABLE 9
Comparison of the variability of the sex-combs and scutellum.

TEMP.	N	C	χ^2	n	P
14°	90	0.39	4.12	3	0.25
19°	324	0.14	1.53	6	0.96
25°	311	0.24	4.86	8	0.77
29.5°	283	0.30	1.39	3	0.71

increase in temperature. No quantitative study of the wing venation was attempted, since the great irregularity in the expression of the abnormalities does not lend itself readily to simple measurement.

RELATIONSHIP BETWEEN THE MULTIPLE EFFECTS OF ENGRAILED

The question now arises as to whether the changes with temperature in sex-combs, scutellum, and wings are correlated with each other. The answer to this question is a prerequisite to possible generalizations concerning the manner of action of engrailed in producing its multiple effects. Only the secondary sex-combs are used in this comparison, since the temperature response of the primaries is very slight.

In order to test for a correlation between a set of quantitative data, such as that for the sex-combs, and a set of qualitative data, as for the scutellum, the coefficient of mean square contingency (C) was calculated. The values obtained are found in table 9. There is some association between the two characters, but the coefficients are not high. It seemed desirable to determine, in this case, not how great the correlation is between the sex-combs and the scutellum, but rather how great is the lack of correlation. The χ^2 test was accordingly applied. The resulting P values are high (table 9) and

thus indicate that the observed distribution is not significantly different from a random distribution—namely, that no great degree of correlation exists between the number of teeth in the secondary sex-comb and the extent of the cleft in the scutellum at each temperature.

At 29.5° engrailed flies are most nearly wild type with respect to all three characters studied. A simple trend from extreme expression of the several effects of the mutant at low temperatures to an approach to the wild type condition at high is not evident, however. The sex-combs are most “abnormal” at 14°, the scutellum at 25°, and the wings are quite similar at 14°, 19°, and 25°.

Two mutants which resemble engrailed in their pleiotropic effects may be mentioned. The first is Polyphaen in *D. funebris*, described by H. A. TIMOFEEF-RESSOVSKY (1931), which also exhibits a lack of correlation between its various effects and a difference in the influence of temperature upon them. The second is short-wing in *D. melanogaster*, another temperature-sensitive mutant (EKER 1939). Comparison of short-wing and engrailed shows that the latter is better expressed at low temperatures, the former at high. Both show sexually dimorphic expression in some phase of the gene's effect. Whereas short-wing overlaps wild type considerably, engrailed always has 100 percent penetrance.

THE MANNER OF ACTION OF ENGRAILED

Sex-combs

The most interesting problem posed by the engrailed gene is the production of a secondary sex-comb in a specific region of the forelimb. A possible mechanism for the formation of an extra sex-comb might involve an excess either of material which can induce the development of sex-combs or of material which actually becomes incorporated in them. The disposition of this excess is such that instead of a single large sex-comb being formed a secondary sex-comb appears on the opposite side of the foreleg. There are thus specific regions in which sex-combs may arise. In this way the concept of an embryonic field may be introduced. Such a field is defined as the totality of those factors which direct development in compliance with a specific pattern.

In order to make the field concept apply to the case at hand, it may be assumed either that the sensitivity of cells to the field stimulus varies in flies of different types (as determined by genetic constitution and external environment) or that the strength of the field itself varies from type to type. In elaborating the first hypothesis it became apparent that a constant sex-comb field demands variable degrees of sensitivity of cells in different regions of the same foreleg or, in other words, two fields, one of sex-comb stimulation and one of sensitivity. The second hypothesis involves

constant sensitivity for cells of all types in all regions of the foreleg but demands various absolute strengths of the sex-comb field and also various relative strengths of its two peaks. While in reality both alternatives may contain elements of truth, only one of them, the second, will be dealt with in some detail.

This field concept is illustrated in figure 9 in which a constant threshold of sensitivity is crossed by various types of sex-comb fields. If the forelimb

FIGURE 9.—Sex-comb fields with constant threshold of sensitivity represented by the heavy horizontal line. The numbers at the left indicate for each field the number of teeth in primary and secondary sex-combs. Further explanation in text.

of an engrailed male were split along its longitudinal axis and laid out flat, the two sex-combs would appear as structures in parallel planes. Similarly a cross section of the distal end of the first tarsal joint could be laid out, and the regions of sex-comb development would lie along two parallel lines perpendicular to the plane of the cross section. For diagrammatic purposes the field may be depicted as a curve with two somewhat conical peaks. The height of the curve at any point determines the strength of the field so that the height above the threshold of sensitivity may be used as an index of the number of teeth in the sex-comb. The area beneath the curves represents the total amount of sex-comb forming or inducing substance available.

By using a diagram such as figure 9 it is possible to give specific interpretations to the whole array of data involving different genetic constitutions—namely, femaleness, maleness, and intersexuality combined with engrailed or wild type and reared under various environmental conditions. It will be sufficient to treat only a few of these types. Wild type males

have less sex-comb forming substance than engrailed males. In the latter the added substance is distributed in such a manner that the secondary rather than the primary field is strengthened. This is also true of wild type compared to engrailed intersexes. Engrailed males differ from engrailed intersexes in that the latter have less sex-comb forming substance. While engrailed intersexes and wild type males have about the same amount of substance, its distribution is different in these two types. Females may or may not possess a sex-comb field, but if they do, as assumed in this scheme, its strength is insufficient for actual sex-comb production.

The sex-comb field for engrailed males reared at 25° varies in configuration depending on body weight. It may similarly be shown that the sex-comb field of engrailed males varies with temperature due to the amount of sex-comb forming material present. This is evident if a comparison is made of the sums of the mean number of teeth in both sex-combs at the four temperatures which are respectively 16.68, 16.22, 14.61, and 11.05 at 14°, 19°, 25°, and 29.5°. Again not only the general field strength but also the relative height of the two peaks would be affected.

It has been previously pointed out that there is no correlation between the sizes of the two sex-combs on the same foreleg in engrailed males (table 3). In intersexes, on the other hand, there is a significant negative correlation between these sex-combs, such that when one sex-comb is large the other is small. Both of these relations may be explained by assuming that the two sex-combs depend on the same material for their formation. For diploid males the total amount is great enough so that the two sex-combs may make use of it independently of each other. In intersexes the amount of material is reduced with the result that there is competition for it in the formation of the sex-combs and thus a negative correlation between their sizes.

In conclusion it may be said that whereas the above suggestions and diagram may be considered as no more than hypotheses, they may help to visualize the place of gene action in developmental processes. Definite information as to the validity of the hypothesis might be gained from observation of gynandromorphs in which a foreleg is a mosaic of male and female tissue. If a sex-comb should appear in a region composed of female tissue but surrounded by male tissue, then it might be said that a sex-comb field was present and sex-comb formation persisted in spite of the female constitution of the responding tissue. A search for such material has thus far revealed no crucial case.

Scutellum

In order to understand the complex variability of the scutellum in engrailed, an accurate knowledge of its development will be necessary. Lack-

ing such a study, only a general discussion will be attempted. We may consider two processes which are possibly concerned in the abnormal development of the scutellum—the growth of the imaginal discs and the formation of the chitinous exoskeleton. If it is assumed that these processes are dependently controlled,—that, for instance, the latter proceeded faster than the former,—the scutellum would become chitinized before its two halves were able to grow together. If growth of the imaginal discs proceeded more rapidly than chitinization, then a more complete fusion of the halves would be possible. Superimposing upon these processes (which may have different temperature coefficients as well as different rates) a variety of incubation temperatures, it is still conceivable that a fairly simple interpretation may be given for the results obtained. For instance, it may be assumed that the rate of growth of the imaginal discs increases with increasing temperature either linearly or logarithmically while the rate of chitinization increases more rapidly and reaches a maximum at 25°. This combination would produce a larger number of individuals with no cleft or only a small one at the extreme temperatures and greater splitting in a majority of those at intermediate temperatures.

The pleiotropism of engrailed

The developmental basis of pleiotropism is still largely unknown. While it may be possible to show sometimes that manifold effects are consequences of one ontogenetic mechanism ("spurious" pleiotropism, GRÜNEBERG 1938), it does not follow that those cases are "genuine" in which the reduction of apparently different effects to a common process has not been accomplished. It may be tempting to regard on the one side the effect of engrailed on both wing and scutellum as correlated ontogenetically in view of their development from the same imaginal disc and on the other side to regard the effect on the sex-comb system as independent in view of its origin from a different disc. However, at present it seems purely speculative to interpret the association data given earlier in terms of primary action of the engrailed gene.

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SUMMARY

The principal effects of the mutant "engrailed" in *Drosophila melanogaster* are described, including a hitherto unreported character produced by this gene—an extra sex-comb on the lateral side of the first tarsal joint

of the foreleg. All of these are variable in their expression. An analysis of this variability shows that:

The mean number of teeth in the primary sex-comb is little influenced by temperature while that of the secondary sex-comb decreases with increasing temperature.

There is no correlation between the primary and secondary sex-combs on the same foreleg and low or insignificant right-left correlation between the number of teeth in the primary and secondary sex-combs.

At a constant temperature the primary sex-combs increase in size with the size of the fly in a linear fashion; the secondary sex-combs do likewise at 14°, but at 19° and 25° smaller flies have a larger mean tooth number, and the decrease in size of the sex-comb with increase in body size is not linear.

The extent of the cleft in the scutellum is influenced by temperature, with the greatest amount of separation of the two halves occurring at 25° and less splitting at the extreme temperatures.

There is less separation of the halves of the scutellum in large flies than in small ones, except at 29.5° where body size has no effect.

There is no appreciable correlation between the trend of the variability of the sex-combs and the scutellum.

Studies of sex-combs in intersexes revealed that:

The number of teeth is lowered in both wild type and engrailed.

The degree of intersexuality, as indicated by the external genitalia, has no influence on the number of teeth in the wild type sex-comb or on the primary of engrailed, but there are significant differences in mean tooth number between some of the intersexual classes in the secondary sex-combs.

There are significant negative correlations between the primary and secondary sex-combs on the same foreleg in four of the five intersexual classes.

Studies of the scutellum show that:

There is sexual dimorphism in the response to the temperature effect on the scutellum.

The influence of a body size factor in the temperature effect is greatest at low temperatures. At higher temperatures its influence is less apparent. This is true likewise for the primary sex-combs.

The concept of a developmental field is introduced as a basis for an interpretation of the effects of engrailed on the sex-combs.

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