THE INTERRELATIONS OF TEMPERATURE, BODY SIZE, AND CHARACTER EXPRESSION IN DROSOPHILA MELANOGASTER[1]

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INTRODUCTION

DURING the past half-century, numerous investigations have shown that the exact expression of many wild-type and mutant characteristics of diverse organisms depends upon the temperature at which development takes place. Concurrently, studies on relative growth have frequently demonstrated a regular relation between the size of the whole individual and the degree of development of certain of its organs. Between these two fields of investigation, which have grown up quite independently, there is a bridge. It is supplied by the fact that in some organisms, such as the insects, adult size is related to the temperature prevailing during development. Consequently, the results of any study of the effects of temperature upon character expression in these forms are possibly complicated by the failure of size to remain constant at all temperatures.

The present paper constitutes a step in an analysis of the interrelations of temperature, body size, and character expression in an insect, *Drosophila melanogaster*. It will be shown that in the cases of polychaetoid and Dichaete, two "temperature-responsive" mutants of this species, a large part of the apparent "temperature effect" is under the conditions of these experiments bound up with changes in body size attendant upon the temperature change. Were the flies of the various temperature series of equal size, then the so-called "temperature effect" would be greatly reduced.

The principal conclusions of this paper were summarily reported at the Seventh International Genetics Congress.

THE POLYCHAETOID MUTANT

Flies homozygous for this mutant (*pyd*, 3–39 ± 2) are characterized by the presence of extra bristles at or near the various normal bristle loci. Supernumerary macrochaetae are particularly likely to occur in the dorsocentral and scutellar regions. A detailed description of the mutant will be given by NEEL (1940). The stock employed in this investigation had been inbred in small mass cultures (three-four pair matings) for two years prior to the beginning of the experiments.

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[1] This and a following paper (Genetics, in press) have been submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Rochester.

The size of the fly and the expression of polychaetoid, at constant temperature

The relation between the size of *pyd* flies and the number of bristles present has been investigated in several different ways. Thus, in a first approach to the problem, groups of *pyd* flies were raised under such unlike nutritional conditions as to result in differences in average fly size from group to group, and the relation between mean size, as expressed by body weight, and the mean number of dorsocentral bristles present per side per fly (M dc) established. As a standard procedure, two or three pairs of flies were introduced into half-pint milk bottles containing approximately 50 cc of corn-meal agar and allowed to remain there seven to eight days. The bottles were kept in an incubator similar to the two-shelf type described by BRIDGES (1932), in which temperature stayed within 0.2° of 24.0°C. When the offspring began to emerge the flies were collected at three day intervals. From one to four collections were made from a set of bottles, and thus groups of flies developing at different nutritional levels of the cultures and hence of different mean sizes were secured. The flies of each collection period were given unlimited access to food during the intervals between collections. Immediately after collection, all the males were etherized and weighed together, and the average body weight established. Only males were used in the weight determinations, since their weight is less subject to fluctuation than that of the females. For all groups the time elapsing between removal from food, etherization, and weighing was made as short as possible (ca 10–15 minutes). This minimized errors due to dehydration of the flies. The M dc was then calculated. A total of ten groups of flies, representing a range in average fly weight of from 0.600 ± 0.011 to 0.953 ± 0.004 mg, and in M dc of from 2.322 ± 0.052 to 3.470 ± 0.034, was thus secured. The mean number of males weighed in a group was 98.7; an average of 139.6 half thoraces from these were used in the M dc determinations. The complete data for this experiment and for the similar experiments to be reported below have been filed with GENETICS.

A simple expression for the relation between average body weight and M dc was desired. It was found that while a plot of M dc against average body weight yielded an approximation to a straight line, a better approach to linearity was secured with a double log plot (figure 1). This indicates that the data can be treated as an expression of the power function

$$y = bx^k$$

recognized by biologists as the relative growth function. In this case, y corresponds to M dc, x to mean body weight, and b and k are constants.

The ranges of the variables x and y are towards the lower limits of valid applications of logs.

The line which has been fitted to the points of figure 1 is the regression line of log M dc on log mean body weight, as determined by the least squares method with unweighted ordinates. The equation of this line is

$$Y = 0.713X + 0.577$$

where Y corresponds to log M dc and X to log mean body weight. The aberrant point seen in the lower left hand corner of the figure has not been included in the fitting of the line, since for reasons unknown it is in such obvious disagreement with the others. The flies from which the extreme right hand point in the figure was derived were raised under somewhat different conditions than the rest, namely, from eggs laid over a two to six hour period and allowed to develop in finger bowls of 10.5 cm diameter containing approximately a one centimeter layer of agar, one hundred to a bowl.

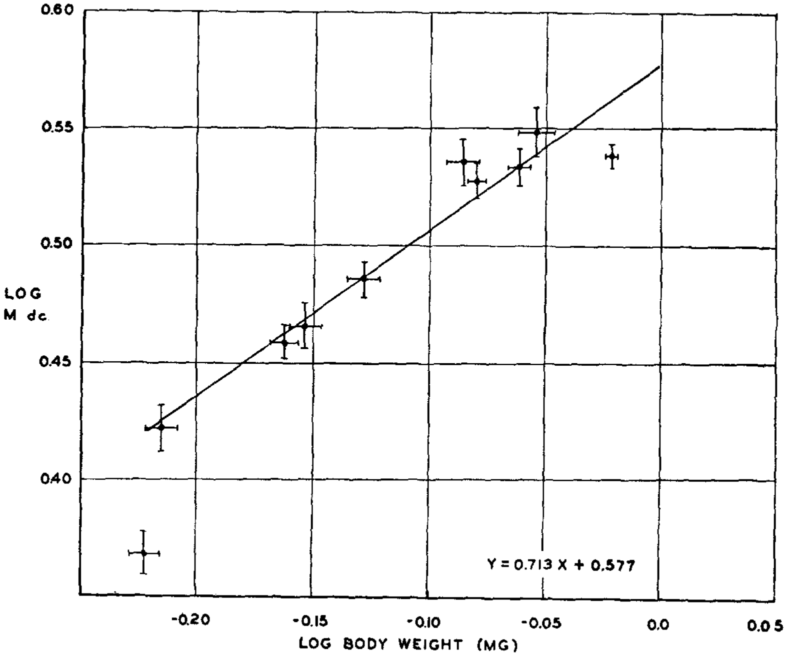

FIGURE 1.—The correlation, in groups of male *pyd* flies raised at 24.0°C at various nutritional levels, between log average body weight and log M dc. The vertical lines attached to each point represent the limits of $\pm\sigma_m$ for the M dc. The horizontal bars indicate the calculated magnitude of $\pm\sigma_m$ for the average weight. All logs are to the base 10. Details in text.

Each of the points shown in figure 1 is based upon two means, M dc and mean body weight. The determination of these means involves both methodological and sampling errors. In consequence of the simplicity of the operations involved (counting bristles and weighing groups of ca 50–150 flies on a Becker Chainomatic Balance with a sensitivity of 0.2 mg), methodological errors are small. Sampling errors are of somewhat greater magnitude. The sampling error for bristle number is given by the standard error of the M dc (σ_m). The upper and lower limits of the vertical bar attached to each point of figure 1 are respectively M dc$+\sigma_m$ and M dc$-\sigma_m$.

The evaluation of the sampling error for weight, which is the standard error of the mean body weight of a group, is somewhat more difficult. In the course of the experiments reported in this paper, two different methods have been used in raising groups of flies of different average sizes. a) The one method, used in securing the flies incorporated into figure 1, involved the withdrawal from culture bottles of flies emerging during successive three day periods, and has already been described in some detail. b) The other method, used in the derivation of the points of figures 3, 4, 5, and 6, was to place varying numbers of eggs from two to eight hour egg-laying periods on a given quantity of food (amount contained in a one centimeter layer of corn-meal agar in a finger bowl of 10.5 cm diameter). It seems that the first method should lead to a somewhat greater variation in body weight within the group than the second. Accordingly, the means of estimating the weight error which follows is based upon flies raised by the first procedure; this should be adequate for flies raised by the second method.

One hundred male flies composing a portion of the first three days emergence from culture bottles subjected to standard conditions were weighed individually on a microbalance with a sensitivity of 0.001 mg. Repeated weighings of the same fly showed that the accuracy of the weight determination was within one percent. For these 100 flies, the mean body weight (M) was 0.881 mg; the standard deviation (σ) was 0.089 mg; the standard error of the mean (σ_m), 0.009 mg; and the coefficient of variability (CV), 10.1. From these determinations, an estimate of the error of the weight determination for each group can be made. It is assumed that from group to group, no matter what the average body weight, CV remains approximately constant and equal to the value found in these 100 flies. Hence in any other group, σ_m may be reasonably estimated as equal to $.01\ M/\sqrt{N}$, since $\sigma_m = \sigma/\sqrt{N} = (CV \times M)/100\sqrt{N}$. The standard errors so estimated are indicated in figure 1 by horizontal bars.

Some of the points in figure 1 deviate significantly from the fitted line, where the criterion of significance is deviation by an amount equal to three times the standard error of body weight or M dc. Similar deviations will be noted in later figures. Possible causes for these deviations are as follows: 1) The relation between the two sets of values studied may not be strictly linear. Thus, HUXLEY (1932) has pointed out that in cases of insect heterogony, at large body sizes points usually fall below the straight line fitted to a double log plot of the data. A deviation which may be of this nature is shown in the figure. 2) Experience has shown that the weight of a given group of flies may from time to time vary considerably, depending on feeding conditions. In this work, an attempt was made to give each group of flies the same free access to food, prior to weighing. It is possible that this attempt was not entirely successful, and that therein lies the cause of at least part of these deviations.

A second determination of the relation between size and dorsocentral number consisted in deriving the correlation in individual flies between femur length and total number of dorsocentrals present. Femur length has been used as an index to body size by a number of investigators (cf BREHME 1939), but the exact relation between these two variables has not been established. Since an investigation of this point seemed desirable, the right fore-femurs of the males composing each of the ten samples incorporated into figure 1 were mounted, and the mean femur length of each sample determined from camera lucida drawings of the mounted femurs. Over a range in mean femur length from 516.0 ± 4.55 to $582.0 \pm 3.16\mu$ and in mean body weight from 0.600 ± 0.011 to 0.953 ± 0.004 mg, these two variables appeared to be linearly related. It seems probable that with uniform age and feeding this group relation would hold good for the individual flies which compose the group.

Male flies were collected at intervals over a period of three weeks from cultures kept at 24.0°C, in order to get a considerable distribution in size. The right fore-femur and the dorsum of the thorax of each fly considered were mounted together on a slide. For this population ($N = 134$), the correlation between femur length and total number of dorsocentral bristles present on both sides of the fly was 0.619 ± 0.053. There was a still higher higher correlation between femur length and the total length of the dorsocentrals present (0.705 ± 0.044). To determine this latter measurement the bristles were drawn by camera lucida (see NEEL 1940). It seems probable that slight errors of measurement tend to obscure an even greater natural correlation between these variables. A similar strong correlation between the size of individual flies (as measured by over-all length) and dorsocentral number, in an extra-bristled stock, has been reported by MACDOWELL (1915).

The third method of evaluating the relation between size and bristle number consists in correlating the weight of the individual fly with the number of dorsocentrals present on both sides of the fly. In the sample of 100 *pyd* males which were weighed individually to determine the standard error of mean body weight (p. 228), the correlation was 0.278 ± 0.092. An insignificantly higher value (0.308 ± 0.090) is obtained when the logs of the two variables are correlated. This correlation between size and bristle number, although significant, is much looser than that indicated by the other two approaches. There are two outstanding reasons for this. 1) The range in body size in these 100 flies was not as great as that utilized in the other two experiments. Since a correlation between two variables becomes more apparent as the range of either is extended, the smaller size distribution probably contributes to the relatively low correlation observed. 2) The flies weighed were those emerging in the first three days from a set of bottles subject to conditions described above. The weighings required two days. Although every effort was made to give the flies unlimited access to food, microscopic investigation at the time of weighing revealed considerable differences from fly to fly in the degree of distension of the abdomen. These are in part due to age differences. Because of this variation in abdominal contents, body weight must be regarded as a less accurate index of innate fly size than femur length.

TABLE 1

The relation between length of egg-larval life and number of dorsocentral bristles present in male pyd flies, when size is held constant.

a. Femur length between 569 and 581μ

Interval after oviposition at which puparium formation occurred (hours at 21°C)	132.0–139.9	140.0–147.9	148.0–155.9	156.0–163.9
Number of flies	28	35	59	19
Mean total number of dorsocentrals $\pm\sigma_m$	6.71 ± 0.21	6.68 ± 0.15	6.68 ± 0.12	6.64*

b. Femur length between 582 and 594μ

Interval after oviposition at which puparium formation occurred (hours at 21°C)	132.0–139.9	140.0–147.9	148.0–155.9	156.0–163.9
Number of flies	26	77	41	8
Mean total number of dorsocentrals $\pm\sigma_m$	7.11 ± 0.21	6.72 ± 0.11	7.00 ± 0.12	6.75*

* No σ_m calculated because of the small number of flies involved.

In a group of flies, random weight fluctuations from fly to fly would be expected to cancel out, so that average body weight for the group, as compared with that for other groups, would probably be an accurate index of mean size. This appears a reasonable explanation for the fact that for different groups of flies the correlation between average body weight and M dc is quite high (figure 1), although for individual flies the correlation between weight and total number of dorsocentrals is much lower.

It is apparent from each of these three procedures that at a constant temperature bristle number is linked with body size; an estimate of the closeness of the association depends upon the particular approach used.

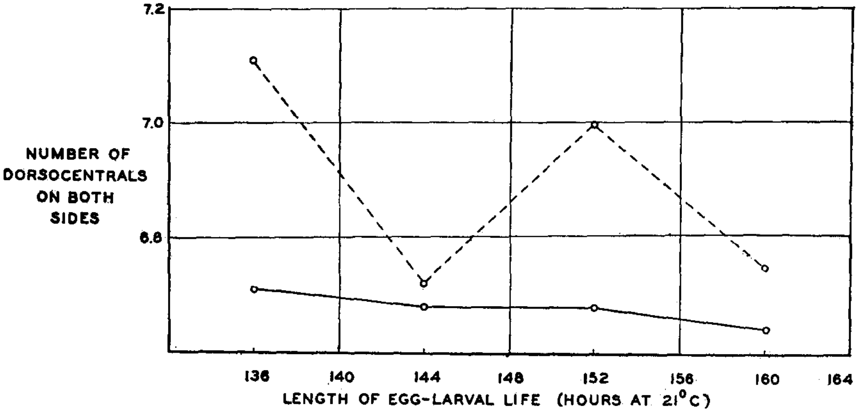

FIGURE 2.—The lack of correlation of length of egg-larval life with the total number of dorsocentral bristles present in *pyd* flies. The circles connected by the broken lines represent groups of flies with femurs 582–594μ long, while those connected by solid lines are based on flies with femurs 569–581μ in length. Details in text.

Independence of M dc and length of larval life, at constant temperature

Groups of flies of different average sizes have been obtained by varying cultural conditions. Thus, some of the groups used in deriving the regression equations developed at quite low nutritional levels. A concomitant of a restriction on food intake such as practiced here is an increase in the average duration of larval life. It seemed necessary to test for a possible correlation of this prolongation of the larval stadium with bristle number, for the existence of such a relation would complicate any interpretation of the size-bristle number relation. Accordingly, an experiment was designed which would permit a comparison of the total number of dorsocentrals in equal-sized flies whose larval lives had been of unequal lengths.

Eggs laid by *pyd* females over a two-hour period were transferred to finger bowls containing yeasted corn-meal agar; each bowl received sufficient eggs that the resulting larvae developed under sub-optimum food conditions. This insured a considerable distribution in the time of puparium formation. Development took place at 21°C. The great majority of puparia were formed from 132 to 164 hours after oviposition. New puparia were isolated every four hours during this period. When the flies emerged, the males of each collection period were classified as to the total number of dorsocentrals present and the right fore-femur length. A total of 527 flies was treated in this manner. Some males with femurs between 569 and 594μ in length were found in each collection. The mean total number of dorsocentrals of flies having femurs 569 to 581μ long was calculated for each four-hour group. A similar calculation was made for flies whose femurs ranged in length between 582 and 594μ. Since this subdivision of the data meant that the number of cases in any four-hour group was small, adjacent groups have been combined, so that there are available four eight-hour periods.

The data are given in table 1. In figure 2 for each of the two femur length ranges (= fly sizes) mean number of dorsocentrals has been plotted against length of larval life. For each eight-hour group, the length of larval life has been treated as the mid-point of that period. The broken line connects circles representing groups of flies with femurs 582–594μ long; the circles connected by the solid lines are based on flies with femurs 569–581μ in length. No significant trend in the data is evident. This suggests that the inequalities in length of larval life observed at the various nutritional levels are of no significance in the determination of bristle number.

The relation between temperature and M dc

To test the effect of temperature upon fly size and M dc, well fed *pyd* females were allowed to oviposit at room temperature on food exposed in small rectangular metal containers. The eggs obtained in a two to eight-hour egg-laying period were divided into lots of 100 and placed on well yeasted corn-meal agar contained in finger bowls of 10.5 cm diameter. The bowls were then put at the various experimental temperatures. Such a procedure should insure each larva a plentiful supply of food. Subsequent to puparium formation, the puparia were placed in vials furnished with enough food that the flies could feed freely after emergence.

TABLE 2

The effect of temperature upon bristle number and body weight in pyd Drosophila melanogaster males. M dc stands for the mean number of dorsocentrals present per side per fly.

TEMPERATURE (°C)	M dc $\pm\sigma_m$	NO. HALF THORACES	AVERAGE BODY WEIGHT (MG $\pm\sigma_m$)	NO. FLIES WEIGHED
14.0°	4.533 ± 0.033	542	1.061 ± 0.006	276
19.0°	4.213 ± 0.023	754	1.062 ± 0.005	441
24.0°	3.470 ± 0.034	536	0.953 ± 0.004	500
29.0°	3.058 ± 0.022	740	0.784 ± 0.003	396

The temperatures at which the flies were raised and the M dc and average fly weight at each temperature are given in table 2. A 15°C increase in temperature is associated with a pronounced decrease in the M dc, from 4.533 ± 0.033 at 14°C to 3.058 ± 0.022 at 29°C. Simultaneously, however, mean weight is decreasing. In view of the above-demonstrated correlation between fly size and M dc, a question immediately arises. To what extent is this effect of temperature upon bristle number concerned with some bristle-forming process within the body not related to fly size, and to what extent is the effect mediated either through size or some processes common to size and M dc?

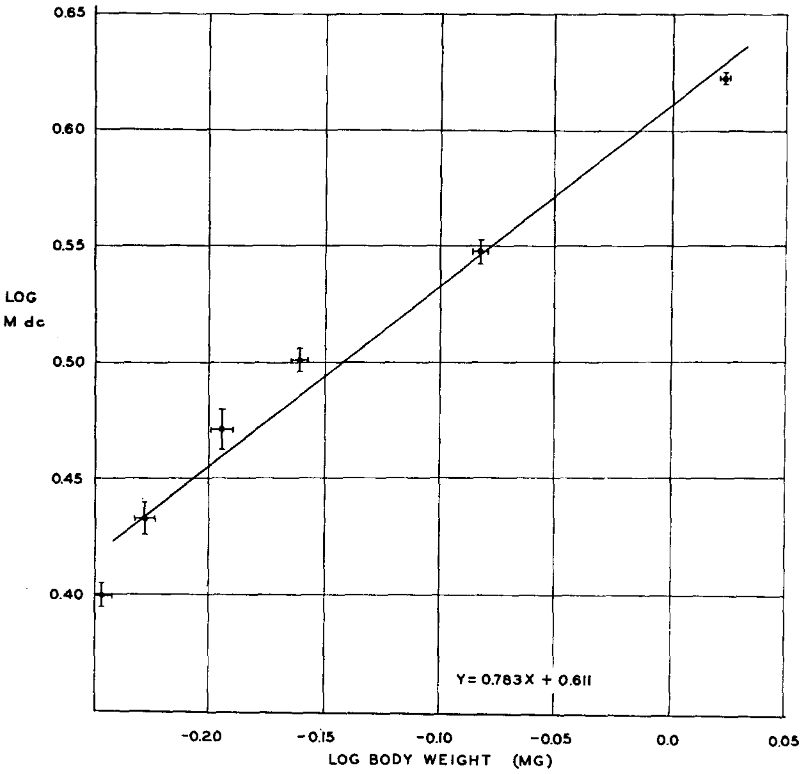

FIGURE 3.—The relation, in groups of *pyd* flies raised at 19.0°C at various nutritional levels, between log average body weight and log M dc.

An evaluation of the manner of action of temperature

Given that there is a change in the M dc from one temperature to another, then let a direct effect of temperature upon bristle number be defined as one which is independent of the size differences at the various temperatures, while an indirect effect is one which under these experimental conditions is bound up with a size change. The question raised in the last paragraph may be restated thus: what are the relative roles of these two effects in the total temperature effect?

To answer this question, equations for the regression of M dc on weight at each of the three lower temperatures employed are necessary. This equation has already been obtained at 24.0°C (p. 227). The relation at the other two temperatures was determined by placing egg samples from two to eight-hour laying periods under various food conditions (different numbers of eggs per finger bowl containing food). Groups of flies showing wide differences in average body weight were secured.

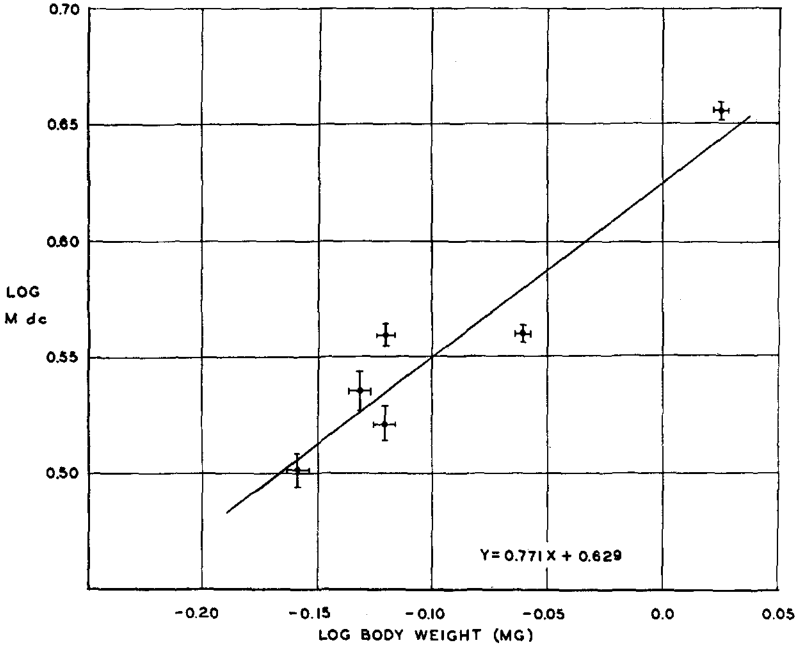

FIGURE 4.—The correlation between log average body weight and log M dc, in groups of *pyd* flies raised at 14.0°C at different nutritional levels.

Six lots of flies were raised at 19.0°C. The highest mean body weight secured for any group was 1.062 ± 0.005 mg; this was accompanied by an M dc of 4.213 ± 0.023. These were the flies which have already been discussed, raised under optimum conditions and listed in table 2. The lowest mean body weight was 0.568 ± 0.005 mg; the corresponding M dc was 2.508 ± 0.035. In agreement with the 24.0°C data, a working approximation to a linear relation is observed when log M dc is plotted against log average body weight (figure 3). The equation for the regression of log M dc on log weight is

$$Y = 0.783X + 0.611.$$

Six groups of flies, varying in weight from 1.061 ± 0.006 to 0.694 ± 0.007 mg and in M dc from 4.533 ± 0.033 to 3.175 ± 0.049, were raised at 14.0°C. The distribution of the data is such that it would be difficult to determine the exact nature of the regression. However, on the assumption that it is not different from that believed to obtain at 24.0° and 19.0°C, a straight line of best fit to a double log plot of the results has been obtained (figure 4.) Its equation is

$$Y = 0.771X + 0.629.$$

Possible causes for the significant departure of some points from the fitted line have already been discussed.

These regression equations make possible a determination of the relative importance of direct and indirect temperature effects. Two methods of evaluation have been employed. The first is summarized in table 3. In column A of the table the six temperature combinations possible from these data have been listed. Column B shows the M dc at the lower of the two temperatures composing a given combination, while column C gives the M dc at the upper of the two. Column D, showing the difference B − C, gives the total temperature effect on the M dc over the given temperature range. From the regression equations which have been developed, it has been calculated what the M dc would be at each of the lower temperatures, if average fly size were equal to that obtaining at the higher temperatures. The results of these calculations are entered in column E. The difference between the entries in columns B and E, given in column F, is the change in M dc correlated under these conditions with the size change

TABLE 3

An evaluation of the relative importance of direct and indirect temperature effects in the expression of the pyd mutant of Drosophila melanogaster.

A	B	C	D	E	F	G	H
TEMPERATURE RANGE	OBSERVED M dc AT LOWER TEMPERATURE	OBSERVED M dc AT HIGHER TEMPERATURE	DIFFERENCE B−C	CALCULATED M dc AT LOWER TEMPERATURE*	DIFFERENCE B−E (=INDIRECT TEMPERATURE EFFECT)	DIFFERENCE D−F (=DIRECT TEMPERATURE EFFECT)	RATIO OF F/G
29°–24°	3.470	3.058	0.412	3.177	0.293	0.119	2.46
29°–19°	4.213	3.058	1.155	3.373	0.840	0.315	2.66
29°–14°	4.533	3.058	1.475	3.532	1.001	0.474	2.11
24°–19°	4.213	3.470	0.743	3.936	0.277	0.466	0.59
24°–14°	4.533	3.470	1.063	4.102	0.431	0.632	0.68
19°–14°	4.533	4.213	0.320	4.457	0.076	0.244	0.31

* If size equals maximum size at higher temperature.

(indirect effect). The difference between the entries in columns D and F (or E − C), given in column G, is the change in M dc independent of a size factor (direct effect). Finally, the ratio of the entry in column F to that in G (column H) is the ratio of indirect to direct temperature effects over the given range. It appears that under these experimental conditions the major portion of the effect of temperature upon the M dc is indirect. The role of the indirect effect is not the same at all temperatures, but is greatest at high temperatures (where the M dc is changing most rapidly) and least at low.

In the above procedure, in columns B and C have been listed the actually observed M dc at the observed maximum size. This M dc does not always correspond to that expected on the basis of the regression equation. This is of course due to the circumstance that not all points fall right on the regression line. In this evaluation it is possible to use, not the actual observed M dc at maximum size, but the M dc which as calculated from the appropriate regression equation should accompany this size. This modification of the procedure results in a slightly higher average value for the ratio of indirect to direct effects. If the deviation below the line of the terminal points in the 24.0° and 19.0°C results is a systematic rather than a random occurrence, then this modification is less valid than the original procedure.

A second approach to this evaluation problem has been developed by Dr. D. R. Charles of this laboratory, who has kindly given me permission to incorporate the method and its results into this paper.

Eigenbrodt (1930) has shown that as temperature decreases, weight increases, down to ca 17°C; "below 17°C there is very little increase in weight" (p. 395). Similarly, Riedel (1934) has found that the tibia length of wild-type flies increases with temperature decrease until the 15–16°C point is reached. With further reduction in temperature, tibia length now decreases. The data contained in the present paper (see also below) tend to confirm these observations, that in the neighborhood of 15°C fly size is at a maximum. On the assumption that weight is an exponential function of temperature deviation from 15°C, the following equation has been found to describe satisfactorily the observed relation of temperature to body weight, under optimum larval feeding conditions:

$$\log W = 0.0373 - 0.0007\,|T - 15|^{2.041}. \qquad (1)$$

In this equation, W corresponds to body weight and T to temperature.

The M dc is a function of both size and temperature. The equation derived from the preceding data which relates these three variables is:

$$\log B = 0.640 + 0.800 \log W - 0.0069[T - 15]^{0.990}. \qquad (2)$$

Here W and T have the same significance as in (1), and B represents M dc.

Equations (1) and (2) may be written as

$$W = 1.0898\ e^{-0.0015|T-15|^{2.041}} \tag{3}$$

and

$$B = 4.365\ W^{0.800}e^{-0.0159[T-15]^{0.990}}. \tag{4}$$

For convenience in the following discussion, these equations will be represented by

$$W = k_1\ e^{k_2|T-15|^{k_3}} \tag{5}$$

and

$$B = k_4\ W^{k_5}e^{k_6[T-15]^{k_7}}. \tag{6}$$

From equation (5) it follows that at any given temperature, the rate of change in body weight is given by

$$\frac{dW}{dT} = k_1k_2k_3|T - 15|^{k_3-1}e^{k_2|T-15|^{k_3}} \tag{7}$$

and the amount of change, ΔW, over a small temperature interval, ΔT, is

$$\Delta W \doteq k_1k_2k_3|T - 15|^{k_3-1}e^{k_2|T-15|^{k_3}}\Delta T \tag{8}$$

which by substitution from (5) becomes

$$\Delta W \doteq k_2k_3|T - 15|^{k_3-1}W\Delta T. \tag{9}$$

Equation (6) is of the form $B = f(W, T)$. As a result of partial differentiation of this equation with respect to W, it is seen that as temperature changes, at any instant the rate of change in the M dc linked with the size change may be expressed as

$$\frac{\partial B}{\partial W} = k_4k_5W^{k_5-1}e^{k_6[T-15]^{k_7}} \tag{10}$$

and the amount of change in the M dc linked with the size change (ΔB_W) as

$$\Delta B_W \doteq k_4k_5W^{k_5-1}e^{k_6[T-15]^{k_7}}\Delta W. \tag{11}$$

This by substitution from equation (9) and then (6) and simplification becomes

$$\Delta B_W \doteq k_2k_3k_5|T - 15|^{k_3-1}B\Delta T. \tag{12}$$

Similarly it is seen that the rate of change in the M dc due directly to the effect of temperature change may be expressed by

$$\frac{\partial B}{\partial T} = k_4k_6k_7W^{k_5}[T - 15]^{k_7-1}e^{k_6[T-15]^{k_7}} \qquad (13)$$

while the amount of change in the M dc due directly to the temperature change (ΔB_T) is

$$\Delta B_T \doteq k_4k_6k_7W^{k_5}[T - 15]^{k_7-1}e^{k_6[T-15]^{k_7}}\Delta T. \qquad (14)$$

This by substitution from equation (6) becomes

$$\Delta B_T \doteq k_6k_7[T - 15]^{k_7-1}B\Delta T. \qquad (15)$$

Over a vanishingly small temperature interval, the ratio of the amount of change due to an indirect effect of temperature to the amount due to a direct effect is

$$\frac{\Delta B_W}{\Delta B_T} = \frac{k_2k_3k_5|T - 15|^{k_3-1}B\Delta T}{k_6k_7[T - 15]^{k_7-1}B\Delta T} = \frac{k_2k_3k_5}{k_6k_7}|T - 15|^{k_3-k_7}. \qquad (16)$$

After substitution of the numerical values of the above constants, which are given in equations (3) and (4), equation (16) reduces to

$$\frac{\Delta B_W}{\Delta B_T} = \frac{|T - 15|^{1.05}}{6.25}. \qquad (17)$$

In order to evaluate the ratio of indirect to direct temperature effects over an infinitesimal temperature range at any given temperature, it is necessary only to substitute that given temperature for T of the equation. This substitution has been carried out at 1°C temperature intervals over the whole range employed (14.0–29.0°C). The results are given below.

T	14°	15°	16°	17°	18°	19°	20°	21°	22°	23°
$\frac{\Delta B_W}{\Delta B_T}$	0.16	0.0	0.16	0.33	0.51	0.69	0.87	1.05	1.23	1.42

24°	25°	26°	27°	28°	29°
1.61	1.80	1.98	2.17	2.36	2.56

Here again, in agreement with the results given in table 3, it is found that under these conditions the role of the indirect effect is greatest at high temperatures. The chief difference between the results of the two evaluations is that the second method consistently assigns to the indirect effect a smaller value than does the first.

THE DICHAETE MUTANT

These findings concerning the role played by a size factor in the *pyd* temperature effect made it desirable to determine whether similar relationships exist for other temperature-responsive mutants. After a preliminary survey of the literature the *Drosophila melanogaster* mutant Dichaete (*D*, 3-40.4-41.0±) was chosen for an analysis of the type conducted on *pyd*. BRIDGES has shown that this mutant is associated with a short inversion of 3L (MORGAN, BRIDGES, and SCHULTZ 1937). The character is dominant, lethal when homozygous, and in addition to other morphological effects is characterized by a reduction in the number of chaetae present. The dorsocentral bristles are particularly likely to be affected. Either one or both of the two normally present on a side may be missing. When only one is absent, it usually corresponds to the anterior dorsocentral. Occasionally, when one bristle is missing, the remaining does not correspond strictly to either the anterior or posterior dorsocentral, but is located at some point between the usual positions of these two. The character was studied in a balanced Lyra/Dichaete line.

PLUNKETT (1926) found that with an increase in temperature there is a marked decrease in the mean number of posterior dorsocentrals present per side per fly (M p dc) in Dichaete flies. At 24.5°C, where the duration of the egg-larval-pupal period is 9.5 days, temperature was reported to have an effect upon bristle number from the second to the eighth days. Puparium formation was found to mark the approximate mid-point of the temperature-effective period.

TABLE 4

The relation of bristle number and body weight to temperature in Dichaete Drosophila melanogaster males. M dc represents the mean number of dorsocentral bristles present per side per fly.

TEMPERATURE (°C)	M dc $\pm\sigma_m$	NO. HALF THORACES	AVERAGE BODY WEIGHT (MG)	NO. FLIES WEIGHED
14.0°	1.530±0.025	268	0.914	137
19.0°	1.557±0.014	788	0.922	417
24.0°	1.356±0.018	480	0.864	243
29.0°	1.213±0.015	632	0.725	325

In the present work the criterion of size and temperature effects has been the mean frequency of occurrence of both the anterior and posterior dorsocentrals (M dc), rather than the mean frequency of occurrence of the posterior dorsocentral alone (M p dc) studied by PLUNKETT. This is due to the occasional difficulty, when only one dorsocentral is present, in definitely identifying it as anterior or posterior.

The relation between temperature and M dc

Lyra/Dichaete flies of a long-inbred strain were raised at four different temperatures. The procedure was as described for the similar *pyd* experiment (p. 232). Table 4 shows that the M dc increased from 1.213±0.015 to

1.557 ± 0.014 when the temperature decreased from 29.0° to 19.0°C. However, an additional 5°C decrease, from 19.0° to 14.0°C, was without significant effect upon bristle number. It is of interest to note that the 19.0°C flies are 27 percent heavier than the 29.0°C group, and also slightly heavier than the 14.0°C group. Already in these data there are indications of a parallelism between size and M dc. PLUNKETT (1926) found that in his stock a temperature decrease over the lower part of the range employed did have an effect upon M dc, since the M p dc at 20.0°C was .439 ± 0.013, at 17.0°C 0.581 ± 0.023, and at 14.0°C 0.690 ± 0.036.

The correlation between fly size and Dichaete expression, at constant temperature

Groups of *D* flies were raised at a constant temperature but at different nutritional levels and the relation between M dc and average weight determined. The *D* relation appears to be different from that observed for *pyd*. Whereas in the latter instance an approximation to a straight line was obtained when log M dc was plotted against log average body weight, in the former a straight line relation was observed with a semi-log plot. This is evident from figure 5, where for eight groups of flies of different average sizes raised at 24.0°C, M dc has been plotted against log mean body weight. The equation of the regression line fitted to these points is

$$Y = 3.606X + 1.637$$

where Y corresponds to M dc and X to log mean body weight. The meaning in this apparent difference in the relation of M dc to weight in *pyd* and *D* is not at present clear.

In figure 6 is shown the 19.0°C regression of M dc on body weight. As at 24.0°C, so here an approximation to a straight line is obtained when log weight is plotted against M dc. The equation of the line of best fit to these data is

$$Y = 3.337X + 1.705.$$

The standard error of mean body weight has not been indicated in these figures. The calculation of this constant by the method given earlier demands data on the CV of individual body weight, and such data were not available for Lyra/Dichaete flies.

The flies employed in this work grew very poorly at 14.0°C. Although a number of different egg samples were started under various cultural conditions, only two yielded adults in sufficient numbers to give a reliable M dc and mean body weight. This circumstance makes impossible the derivation of a regression equation at 14.0°C. One of the two groups which did come through at this temperature developed under very favorable conditions; the M dc and weight for these flies has been given in table 4. By a fortunate coincidence, flies of the other group, grown under less favorable conditions, had an average body weight of 0.726 mg, which is almost identical with the body weight of the 29.0°C flies. The M dc for this group was 1.139 ± 0.021.

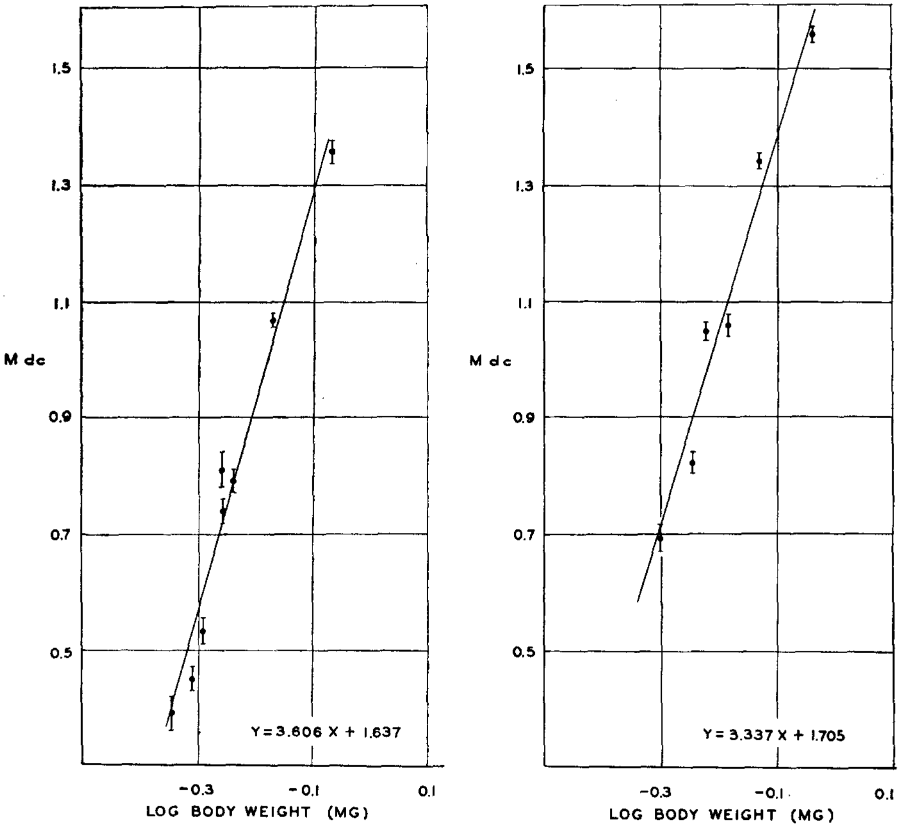

FIGURE 5 (left).—The relation, in *D* fles, between M dc and log average body weight. Each point represents a group of flies raised at a particular nutritional level at 24.0°C. The limits of plus or minus one times the standard error of the M dc are indicated by vertical bars, as before.

FIGURE 6 (right).—As above, but at 19.0°C.

The relation between M dc and length of larval life, at constant temperature

As in the *pyd* case, so for *D* it is necessary to determine whether the increase in length of larval life at lower nutritional levels is correlated with the M dc. PLUNKETT (1926) noted that in *D* flies malnutrition resulted in a decrease in the M p dc, that males have a smaller M p dc than females, and that the introduction of a Minute mutant into a *D* line resulted in a lowered M p dc. Each of these three factors (malnutrition, maleness, Minutes) was observed to "decrease the size of the flies as well as the rate of development and the mean bristle numbers" (p. 229). Although it was recognized that the data then available were inadequate to analyze the interrelations among these three phenomena, it was thought more probable that the observed decrease in the M p dc was correlated with the increase in the length of developmental time than with the size decrease.

Lyra/Dichaete eggs laid over a two-hour period were placed at 19.0°C under somewhat overcrowded conditions. When puparium formation began, new puparia were isolated every four hours. Each fly after emergence was classified as to femur length and total number of dorsocentrals, and the bristle numbers of equal sized flies compared. The data are given in table 5. Within the limits of this experiment there is no evidence that length of larval life is correlated with the number of dorsocentrals present in *D* flies.

TABLE 5

The absence of correlation between length of egg-larval life and total number of dorsocentral bristles in Dichaete flies, when size is held constant.

INTERVAL AFTER OVIPOSITION AT WHICH PUPARIUM FORMATION OCCURRED (HOURS AT 19°C)	SEX	FEMUR LENGTH	NUMBER OF FLIES	TOTAL NUMBER OF DORSOCENTRALS $\pm\sigma_m$
194.0–210.9	♀♀	580–606μ	28	2.964±0.131
211.0–234.9	♀♀	580–606μ	23	3.087±0.168
194.0–210.9	♂♂	561–587μ	37	2.432±0.096
211.0–234.9	♂♂	561–587μ	26	2.269±0.164

An evaluation of the manner of action of temperature

The roles of indirect and direct temperature effects may now be briefly evaluated (figure 7). The upper, solid line of figure 7 connects circles showing the values obtained for the M dc at each of the four experimental temperatures when fly size was maximum at that temperature. Circles connected by the lower, broken line indicate M dc values to be expected at the same four temperatures if flies were the same (29.0°C) size at all temperatures. The four points connected by this lower, broken line are: 1) The M dc obtained for the 29.0°C flies, 2) the M dc which the regression equation indicates would be found at 24.0°C if average weight were equal to that of the 29.0°C flies, 3) the M dc which from the regression equation would be expected at 19.0°C if average weight were the same as observed at 29.0°C, and 4) the M dc actually obtained at 14.0°C when weight equalled that found for the 29.0°C flies. At 24.0° and 14.0°C this M dc is somewhat lower than that observed at 29.0°C; at 19.0°C, somewhat higher. In no case is there a wide departure from the 29.0°C value. It cannot be told at present whether the irregularities in the values are of some significance or whether they represent chance deviations from a straight line whose slope may be small and positive, small and negative, or of zero value. Yet it is quite apparent that in *D*, under these experimental conditions the effect of temperature upon the M dc would either be small or non-existent if the flies raised at the different temperatures all had the same average weight.

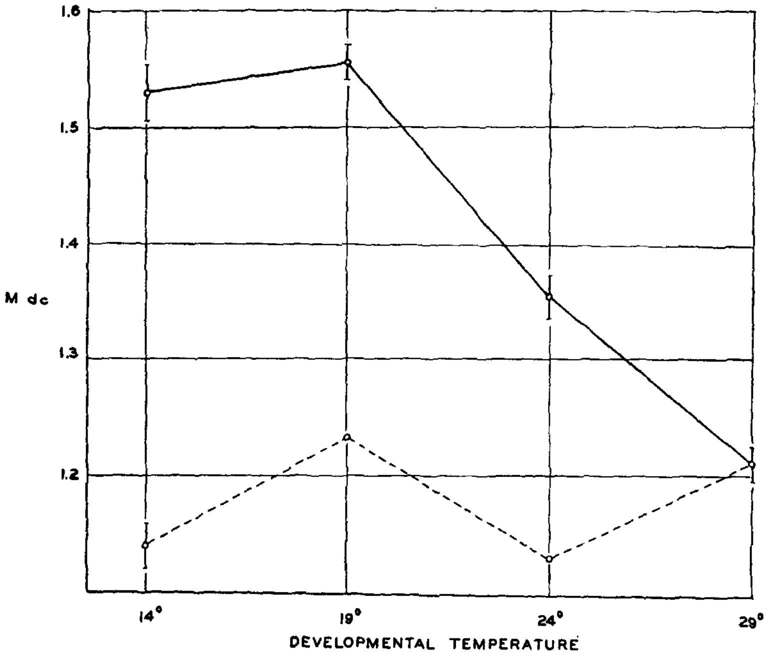

FIGURE 7.—The effect of temperature upon the M dc of *D* flies. The upper, solid-line curve shows the effect of temperature upon the M dc when body size changes at the various temperatures are uncontrolled. The lower, broken line shows the calculated effect of temperature if size were the same at all temperatures. Details in text.

The effect of temperature during the pupal period

PLUNKETT reported that the temperature-effective period in *D* extends over the major portion of larval and pupal life. It was found that when larval life was spent at 24.5°C and pupal at 17.0°C, the M p dc was the same as when larval life passed at 17.0°C and pupal at 24.5°C, that is, "the effect of a higher temperature is the same whether applied to the larval period alone or the pupal period alone" (p. 206). Later work (ROBERTSON 1936, NEEL 1940) has shown that at 24.5°C morphological differentiation of the bristles in wild-type flies begins early in the second day of pupal development. In view of the difficulty of reconciling this finding with that of PLUNKETT concerning the duration of the temperature-effective period, a repetition of the experiment was undertaken.

Larvae were raised at 19.0° and 29.0°C. When puparium formation began, puparia were collected every four hours. Those formed at 19.0°C were divided into two lots, one of which continued to develop at 19.0°C, and another which completed development at 29.0°C. Similarly, one half of the puparia formed at 29.0°C completed development at this temperature, while the others were placed in the 19.0°C incubator. The results of this treatment are shown in table 6. No effect of temperature during the pupal period is evident. This lends further confirmation to the idea of temperature working largely through its effect on size or a factor common to size and M dc. No reason for the discrepancy between these and PLUNKETT'S results can be advanced. (The M dc values given here are considerably lower than those given in table 4 for *D* flies raised under optimum food conditions. This is due to the higher larval densities per unit food in these experiments).

TABLE 6

The effect of temperature during larval and pupal life upon the mean number of dorsocentral bristles per side per fly, in Dichaete males.

LARVAL STAGE AT:	PUPAL STAGE AT:	NUMBER HALF THORACES	M dc$\pm\sigma_m$
19.0°	19.0°	310	1.197±0.019
19.0°	29.0°	206	1.247±0.026
29.0°	19.0°	218	1.000±0.027
29.0°	29.0°	176	0.994±0.030

DISCUSSION

Differences in the expression of some characteristic from one temperature to another are frequently treated as due to a direct effect of temperature upon some processes resulting in this characteristic, as these are contrasted with other processes affecting the rest of the imago. A temperature increase is thought of as differentially accelerating some one or few of the many organismic reactions taking place during ontogeny. But temperature, whatever else its effects may be, is a size-altering agent. This fact has frequently been overlooked or disregarded in studies of temperature effects, in spite of a large literature on heterogonic growth. It would therefore seem to be a matter of primary importance, wherever a "temperature effect" is involved, to evaluate in so far as possible the relation of size changes to this effect. In this paper an attempt has been made to do this for two mutants under certain conditions. The Dichaete data, although presenting certain irregularities commented on above (p. 243), indicate a negligible effect of temperature on mutant expression when there is no size change. In the polychaetoid case, analysis shows that only a minor portion of the temperature effect is due to a direct and differential action of temperature upon some bristle-forming processes, and that the role of this direct effect, relative to the total M dc change, is greatest at the low temperatures.

How general is the situation described for these mutants? In a survey of the Drosophila literature, a number of cases were found where the correlations of both size and temperature with the expression of a given character are known. These cases are summarized in table 7. While the list makes no pretense to completeness, it is probably representative. Some of the characters treated there are wild-type and some mutant. The correlation of decreased size with character expression is not always clearly stated as such in the literature. Thus, it is not uncommon to find references to a "starvation effect" or a "crowding effect" (where excess food is not supplied) in character expression. Obviously in these cases decreased size is involved.

In most of the eleven cases listed in the table we find that the characters studied are oppositely correlated with increased size and with increased temperature (attended as the latter is by a size decrease). The two clear exceptions to the general rule are infra-bar and number of teeth in the wild-type sex-comb.

Is this general agreement in the literature a mere coincidence, or is there a causal relation, namely, is the effect of temperature in part at least through the medium of body size or some factors common to size and the characteristic? Certainly an answer to this question cannot be given at present. For none of the cases listed can conclusions be drawn until it is known what effect temperature has when body size is held constant at the various temperatures, and further, what role increase in length of larval life plays. It is to be expected that the part played in the temperature effect by size factors will be found to vary from mutant to mutant, just as it seems to differ for the *pyd* and *D* cases reported here.

A large amount of work has been done towards determining, for various mutants, the time during development at which temperature exerts its effect upon mutant expression. To cite one example, CHILD (1935b) has reported that in the case of the scute-1 mutant, over a temperature range from 18° to 30° "the temperature-effective period in any one fly lies entirely between the time when 89.3 percent and the time when 96.8 percent

Table 7

A comparison of the correlation between character expression and a) increased temperature and b) increased body size. The symbol (+) in column 2 indicates that the expression of the normal or mutant character is better (more pronounced) at a high temperature, while a (−) indicates a poorer manifestation at the high temperature. The symbol (+) in column 4 indicates that the wild-type or mutant character is better expressed at large sizes, while (−) indicates poorer manifestation with increasing size.

CHARACTER	HOW CORRELATED WITH INCREASED TEMPERATURE	INVESTIGATOR	HOW CORRELATED WITH INCREASED SIZE	INVESTIGATOR
wild-type wing length	−	Alpatov and Pearl (1929), Alpatov (1930), Eigenbrodt (1930), Hersh and Ward (1932), Imai (1933), Stanley (1935)	+	Alpatov (1930), Gause (1931)
fore-femur length	−	Alpatov and Pearl (1929), Imai (1932), Neel (unpub.)	+	Neel (this paper)
number of teeth in wild-type sex-comb	+	Combs (1937)	+	Castle, Carpenter, Clark, Mast, and Barrows (1906)
Bar series of eye shape alleles				
a. Bar and double-bar	+	Seyster (1919), Krafka (1920), Zeleny (1923), A. H. Hersh (1924), E. C. Driver (1926, 1931)	±	Margolis (1935), Bodenstein (1939)
b. infra-bar and double infra-bar	−	Luce (1926), O. W. Driver (1931)	−	Luce (1931)
vestigial wings	−	Roberts (1918), Harnley (1930), Stanley (1931), Li and Tsui (1936), Riedel (1937)	+	Harnley (1930) Child (1939)
scute bristles	some bristles increase in frequency, others decrease	Child (1935a)	some bristles increase in frequency, others decrease	Child (1936)
the *D. funebris* mutants		Neel (1937)		Neel (1937)
ascute	+		−	
evaginated	+		−	
interrupted	+		−	
Missing	+		−	

of the egg-larval development has been completed" (p. 154). Other cases where temperature is reported to have an effect only during a brief portion of the egg-larval-pupal life are numerous.

Available evidence indicates that the effects of temperature on size are distributed over a considerable portion of larval life (IMAI 1937). How is this evidence to be reconciled with the usual relative briefness of the temperature-effective period, if a size factor generally plays a role in temperature effects? As a possible answer to this question, capable of experimental tests, it is suggested that both gross size and the expression of a given character may depend upon some such factor as metabolic level. But whereas size is influenced by the metabolic level of the entire larval period, character expression is primarily dependent upon metabolic level at the time of active differentiation of the character. This time may be only a minor portion of the larval period, and would correspond to the temperature-effective period. Changes in the metabolic level at this time would have a much more profound effect upon character expression than on size.

The present work has shown that under a given set of experimental conditions elimination of size changes is attended by a reduction in temperature effects on bristle numbers. It remains to be determined whether this correlation ever breaks down, and if so, to what extent. From the reasoning of the above paragraph it appears possible that there exist periods in development where a decrease in nutritional level would have an effect upon the expression of a given character out of proportion to the effects on gross size.

At the end of the embryonic determination period of any part, the "size-effective" period for that part must come to an end. Accordingly, the exploration of the role of a general size factor in character expression should involve a measurement of larval or pupal size during and at the end of the embryonic determination of the character. This would not only involve working with larvae, but would necessitate an exact knowledge of the determination period. The assumption is therefore implicit in all this work that adult size is proportional to larval size at the time of determination.

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SUMMARY

1. At a constant temperature the expression of the polychaetoid mutant

of *Drosophila melanogaster* is correlated with fly size. This is shown in three ways:

a. When groups of male flies are raised at varying nutritional levels, a double log plot of the mean number of dorsocentral bristles present per side per fly (M dc) against average body weight yields a straight line.

b. In individual flies, the correlation between femur length and the total number of dorso-centrals present on both sides of the fly or the total length of all dorsocentrals present is high.

c. In individual flies, the correlation between weight and total number of dorsocentrals is significant.

2. Increase in length of larval life is not correlated with the M dc of polychaetoid flies.

3. A decrease from 29.0° to 14.0°C in the temperature at which development proceeds results in an increase in the M dc, from 3.058 ± 0.022 to 4.533 ± 0.033. But at the same time there is an increase in mean weight, from 0.784 ± 0.003 to 1.061 ± 0.006 mg.

4. Equations for the regression of log M dc on log mean body weight at the various experimental temperatures employed are established. From these it is shown that under the present experimental conditions the greatest part of the total M dc change observed over the entire temperature range is correlated with the effect of temperature upon imaginal size.

5. In groups of Dichaete males of different average sizes raised at the same constant temperature, the relation between M dc and log average body weight appears to be linear.

6. No evidence is found for a correlation between length of larval life and the M dc of Dichaete flies.

7. When temperature is decreased from 29.0° to 14.0°C, the M dc increases from 1.213 ± 0.015 to 1.530 ± 0.025. However, over the same temperature range average body weight increases from 0.725 to 0.914 mg.

8. It is shown that if the size of Dichaete flies did not change from one temperature to another, there would be no or only a very slight change in the M dc.

9. The application of these findings in the interpretation of other data concerning the effect of temperature upon character expression is discussed.

LITERATURE CITED

ALPATOV, W. W., 1930 Phenotypical variation in body and cell size of *Drosophila melanogaster*. Biol. Bull. **58**: 85–103.

ALPATOV, W. W., and PEARL, R., 1929 Experimental studies on the duration of life. XII. Influence of temperature during the larval period and adult life on the duration of life of the imago of *Drosophila melanogaster*. Amer. Nat. **63**: 37–67.

BODENSTEIN, D., 1939 Investigations on the problem of metamorphosis. V. Some factors determining the facet number in the Drosophila mutant Bar. Genetics **24:** 494–508.

BREHME, K. S., 1939 A study of the effects on development of "Minute" mutations in *Drosophila melanogaster.* Genetics **24:** 131–161.

BRIDGES, C. B., 1932 Apparatus and methods for Drosophila culture. Amer. Nat. **66:** 250–273.

CASTLE, W. E., CARPENTER, F. W., CLARK, A. H., MAST, S. O., and BARROWS, W. M., 1906 The effects of inbreeding, crossbreeding, and selection upon the fertility and variability of Drosophila. Proc. Amer. Acad. Arts Sci. **41:** 731–786.

CHILD, G. P., 1935a Phenogenetic studies on scute-1 of *Drosophila melanogaster.* I. The associations between the bristles and the effects of genetic modifiers and temperature. Genetics **20:** 109–126.

1935b Phenogenetic studies on scute-1 of *Drosophila melanogaster.* II. The temperature-effective period. Genetics **20:** 127–155.

1936 The effect of starvation on the mean bristle numbers of *sc-1* in *Drosophila melanogaster.* Amer. Nat. **70:** 43–44.

1939 The effect of increasing time of development at constant temperature on the wing size of vestigial of *Drosophila melanogaster.* Biol. Bull. **77:** 432–442.

COMBS, J. D., 1937 Genetical and environmental factors affecting the development of the sex-combs of *Drosophila melanogaster.* Genetics **22:** 427–433.

DRIVER, E. C., 1926 The temperature effective period—the key to eye facet number in Drosophila. J. Exp. Zool. **46:** 317–332.

1931 Temperature and gene expression in Drosophila. J. Exp. Zool. **59:** 1–28.

DRIVER, O. W., 1931 Temperature effective periods in reciprocal crosses in *Drosophila melanogaster.* J. Exp. Zool. **59:** 29–44.

EIGENBRODT, H. J., 1930 The somatic effects of temperature on a homozygous race of Drosophila. Physiol. Zool. **3:** 392–411.

GAUSE, G. F., 1931 Über den Einfluss verkürster larvaler Ernährungszeit auf die Eiergrösse von *Drosophila funebris* und *Drosophila melanogaster.* Biol. Zbl. **51:** 209–218.

HARNLEY, M. H., 1930 A critical temperature for lengthening of the vestigial wings of *Drosophila melanogaster* with sexually dimorphic effects. J. Exp. Zool. **56:** 363–368.

HERSH, A. H., 1924 The effect of temperature upon the heterozygotes in the Bar series of Drosophila. J. Exp. Zool. **39:** 55–71.

HERSH, A. H., and WARD, ESTHER, 1932 The effect of temperature on wing size in reciprocal heterozygotes of vestigial in *Drosophila melanogaster.* J. Exp. Zool. **61:** 223–244.

HERSH, R. K., 1924 The effect of temperature upon the full-eyed race of Drosophila. J. Exp. Zool. **39:** 43–53.

HUXLEY, J. S., 1932 Problems of relative growth. xix+276 pp. New York: The Dial Press.

IMAI, TAKEO, 1933 The influence of temperature on variation and inheritance of bodily dimensions in *Drosophila melanogaster.* Arch. EntwMech. Org. **128:** 634–660.

1937 Influence of temperature on the growth of *Drosophila melanogaster.* Science Reports Tohoku Imp. Univ. **11:** 403–417.

KRAFKA, J. JR., 1920 The effect of temperature upon facet number in the Bar-eyed mutant of Drosophila. J. Gen. Physiol. **2:** 409–464.

LI, J. C., and TSUI, Y. L., 1936 The development of vestigial wings under high temperature in *Drosophila melanogaster.* Genetics **21:** 248–263.

LUCE, W. M., 1926 The effect of temperature upon infra-Bar, an allelomorph of Bar eye in Drosophila. J. Exp. Zool. **46:** 301–316.

MACDOWELL, E. C., 1915 Bristle inheritance in Drosophila. Extra bristles. J. Exp. Zool. **19:** 61–98.

MARGOLIS, O. S., 1935 Studies on the Bar series of Drosophila. I. The effect of the gene vestigial on facet number in Bar. Genetics **20:** 156–171.

MORGAN, T. H., BRIDGES, C. B., and SCHULTZ, JACK, 1937 Constitution of the germinal material in relation to heredity. Carnegie Instn. Wash. Year Book **36:** 298–305.

NEEL, JAMES, 1937 Phenotypic variability in mutant characters of *Drosophila funebris*. J. Exp. Zool. **75**: 131–142.

1940 The pattern of supernumerary macrochaetae in certain Drosophila mutants. (In Press.)

PLUNKETT, C. R., 1926 The interaction of genetic and environmental factors in development. J. Exp. Zool. **46**: 181–244.

RIEDEL, H., 1934 Der Einfluss der Entwicklungstemperatur auf Flügel und Tibialänge von *Drosophila melanogaster* (wild, vestigial und die reziproken Kreuzungen). Arch. EntwMech. Org. **132**: 463–508.

ROBERTS, E., 1918 Fluctuations in a recessive Mendelian character and selection. J. Exp. Zool. **27**: 157–192.

SEYSTER, E. W., 1919 Eye facet number as influenced by temperature in the Bar-eyed mutant of *Drosophila melanogaster* (*ampelophila*). Biol. Bull. **37**: 168–182.

STANLEY, W. F., 1931 The effect of temperature upon vestigial wing in *Drosophila melanogaster*, with temperature-effective periods. Physiol. Zool. **4**: 394–408.

1935 The effect of temperature upon wing size in Drosophila. J. Exp. Zool. **69**: 459–495.

ZELENY, CHARLES, 1923 The temperature coefficient of a heterozygote with an expression for the value of a germinal difference in terms of an environmental one. Biol. Bull. **44**: 105–112.