# STUDIES ON THE BAR SERIES OF DROSOPHILA IV. THE TEMPERATURE-EFFECTIVE PERIOD FOR FACET DETERMINATION IN THE WILD TYPE

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## INTRODUCTION

THE effect of temperature on facet number in wild type Drosophila has been investigated by R. K. HERSH (1924) who showed that the facet-temperature relation is similar to that in Bar, involving a decrease in facet number with increase in temperature. The effect of temperature on the wild type eye, however, is not nearly as marked as in Bar.

The time during which temperature can produce any change in facet number in Bar and its alleles is limited to a definite portion of the larval stage, falling mainly within the third quarter of the egg-larval period (KRAFKA 1920; E. DRIVER 1926, 1931; LUCE 1931; O. W. DRIVER 1931; MARGOLIS 1935b). This period has been designated the temperature-effective period for facet determination (T. E. P.). Knowledge of the T. E. P. for the different Bar alleles has been useful in attempts to explain the facet-temperature relations observed, and in working out the time course of facet determination (HERSH 1934).

It has been suggested by one of us (MARGOLIS 1935a), that facet number in the Bar series is determined by two sets of opposing processes:

1) those processes leading to formation of facet-forming material and found in the wild type as well as in Bar and its alleles, and

2) those processes leading to removal or diminution of facet-forming material and found in members of the Bar series.

A consideration of the temperature characteristics of these processes and of the processes governing the duration of the T. E. P. offers a formally consistent explanation of the facet relations observed.

Since it has not been possible experimentally to dissociate these two sets of processes in Bar, the determination of the T. E. P. in the wild type was undertaken in order to secure an independent body of data on one of the two postulated sets of facet determining processes.

## STOCKS AND CULTURE METHODS

A highly inbred wild type stock was used in the experiments. This stock is one which was derived from the 47th generation of brother-sister matings of a Bar and a vestigial stock. The Bar and vestigial stocks had previously been rendered isogenic with the Oregon wild stock of POWSNER

(1935). The details concerning the breeding methods have been given elsewhere (BARON 1935, MARGOLIS 1936). The procedure followed insures a high degree of genetic homogeneity and at the same time a close relationship to POWSNER'S Oregon stock.

As in previous experiments, a two percent banana-agar preparation was used as the culture medium. The medium was poured into 1×4 inch shell vials which were slanted at approximately 45° thereby exposing a larger surface for egg-laying and yeast growth. Twelve to twenty-four hours before experiments began the medium was seeded with a single drop of a thick yeast suspension (1 cake per 100 cc water).

The flies used for egg-laying were aged from 3 to 5 days before experiments were begun. In all experiments 10 pairs of flies were placed in each experimental vial and egg-laying took place for one hour. The parent flies were then transferred to fresh vials at hourly intervals until a sufficient number of vials for the experiments was obtained. Under these conditions the great majority of the vials contained from 20 to 40 eggs, a number which gives very good culture conditions. If one may draw conclusions from experiments with Bar, this number of flies per vial should not give crowding effects. Vials in which less than 20 or more than 45 flies emerged were not used for facet counts.

The type of thermostat used in temperature work in this laboratory has been described in other publications (BRIDGES 1932, POWSNER 1935, HARNLY 1936).

Throughout the course of the experiments temperature fluctuations did not exceed ±0.1°C. in any one thermostat, with one exception. In the experiment at 16° the temperature rose to 18° for about 12 hours during the larval period due to a brief rise in the temperature of the cold room. It is doubtful whether this relatively short temperature fluctuation markedly affected the facet counts at this temperature since, as will be seen later, the T. E. P. covers approximately the entire egg-larval period.

### *Facet Counts*

The large size of the wild type eye precludes the counting of facets directly as is done with the members of the Bar series. The method we have adopted represents a modification of a method used previously in counting eyes of large size (MARGOLIS 1934).

All flies are preserved in 95 percent alcohol until such time as facets are to be counted. The corneas are then dissected free of the head by means of fine needles on which cutting edges have been ground. This dissection is facilitated by first severing the head and then, beginning on the posterior aspect, carefully running the needle along the margin of the cornea and separating it from the chitin of the head. The corneal margin serves as a

natural line of separation so that clean dissections can be made in a very high percentage of cases. The dissected corneas are then placed for clearing in a depression slide containing a saturated solution of NaOH. In this way clearing is accomplished within less than half an hour. The cleared corneas are then placed in a drop of water on a slide and several small radial incisions made in order to flatten the normally convex surface. Slight pressure on the cover slip is sufficient to insure flattening.

This method of dissection represents a distinct advance over the earlier method in that removal of the cornea is easier in flies which have not previously been cleared. The presence of pigment in the uncleared eye decreases the probability of losing facets during dissection. Moreover, the time required for clearing is greatly reduced.

TABLE 1

*Recounts on individual eyes to determine error in counting.*

| OBSERVER A | OBSERVER B | DIFFERENCE | OBSERVER A | OBSERVER B | DIFFERENCE |
|---|---|---|---|---|---|
| 832 | 832 | 0 | 772 | 776 | 4 |
| 744 | 736 | 8 | 756 | 760 | 4 |
| 739 | 745 | 6 | 761 | 753 | 8 |
| 803 | 815 | 12 | 743 | 744 | 1 |
| 795 | 808 | 13 | 822 | 833 | 11 |
| 728 | 736 | 8 | 838 | 839 | 1 |
| 759 | 761 | 2 | 834 | 840 | 6 |
| 803 | 787 | 16 | 760 | 759 | 1 |
| 823 | 826 | 3 | 783 | 783 | 0 |
| 739 | 728 | 11 | 718 | 737 | 19 |
| 753 | 750 | 3 | 750 | 747 | 3 |
| 767 | 778 | 11 | 742 | 763 | 21 |

Facet counts were made by projecting the dissected corneas on an improvised projection apparatus consisting of a horizontally placed microscope with side arm prism. A carbon arc was used as the source of illumination. A 10× ocular and 16 mm objective gave a convenient magnification of approximately 225 diameters when the ocular was located about 20 inches from the table which served as a counting surface.

The facets in the wild type eye, apart from occasional roughness, are aligned in perfectly straight rows. This fact made facet counting much simpler, since it was possible to draw a number of parallelograms by running parallel lines between rows of facets at five facet intervals along any two dimensions of the eye. The parallelograms formed by the intersecting of the two sets of parallel lines thus include areas, each containing 25 facets. Since the parallelograms at the periphery of the eye are incomplete, there remains a number of marginal facets which must be counted individually.

In most of the eyes counted 500 to 700 facets were included within the parallelograms, and 100 to 200 marginal facets were counted individually.

It was found that this method of counting increased not only the speed but the accuracy as well. Personal error was checked by having one investigator recount eyes counted by the other. This was done in the case of twenty-four eyes selected entirely at random throughout the course of the investigation. The results are given in table 1. The columns headed A

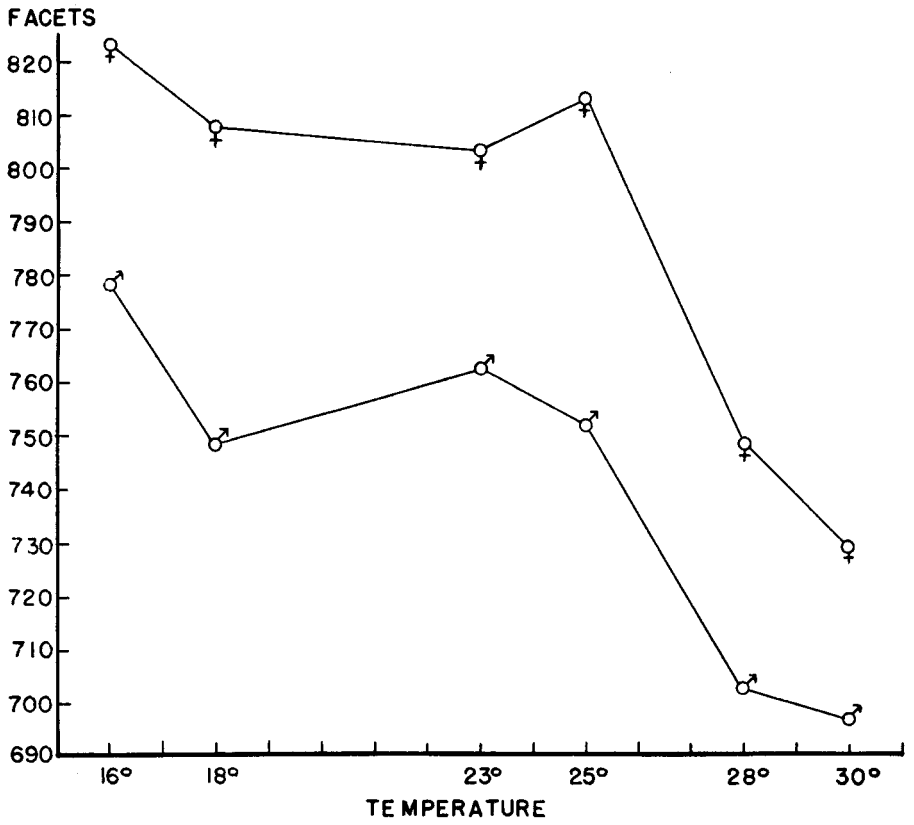


FIGURE 1.—The effect of temperature on facet number.

and B give the facet numbers for the initial counts and the recounts, respectively. The difference in the means for the two series of counts is 3.0 facets, a difference which is slightly less than 0.4 per cent. The results indicate clearly that this method of facet counting introduces a negligible error in the experiments.

## EFFECT OF TEMPERATURE ON FACET NUMBER

The data of R. K. HERSH (1924) on facet number in the wild type demonstrate a systematic decrease of facet number with increase in tempera-

ture over the temperature range 15° to 31°. Our own data, presented in table 2 and plotted in figure 1 also show an inverse relation between facet number and temperature when the temperature range is considered as a whole. There is, however, an interval between 18° and 25° in which temperature has no appreciable effect on facet number. There are, it is true, certain differences in the mean facet values at 18°, 23° and 25° for both sexes, but only in the case of males at 18° and 23° are these differences statistically significant. Since the females at these two temperatures give the same mean facet number within the limits of sampling error, one is led to question the significance of the difference in the males. Data at other temperatures are desirable before concluding definitely that temperature has no effect on facet number in this range.

TABLE 2

*Effect of temperature on facet number.*

| | MALES | | | | FEMALES | | | |
|---|---|---|---|---|---|---|---|---|
| T | M | σ | v | n | M | σ | v | n |
| 16° | 778.6±4.26 | 21.3 | 2.74 | 25 | 823.3±5.02 | 24.1 | 2.93 | 23 |
| 18° | 747.8±3.60 | 18.0 | 2.41 | 25 | 808.2±4.30 | 21.5 | 2.66 | 25 |
| 23° | 762.5±3.04 | 14.6 | 1.91 | 23 | 804.5±9.62 | 43.0 | 5.34 | 20 |
| 25° | 752.4±5.54 | 28.6 | 3.80 | 23 | 811.8±4.39 | 20.6 | 2.54 | 22 |
| 28° | 702.7±6.56 | 32.8 | 4.67 | 25 | 749.0±6.22 | 31.1 | 4.15 | 25 |
| 30° | 696.7±6.51 | 31.9 | 4.58 | 24 | 729.8±5.61 | 25.7 | 3.52 | 21 |

Below 18° there is an increase in facet number and above 25° a decrease. It is, perhaps, significant that in Bar there has been described a critical temperature for facet determination in the vicinity of 27° (KRAFKA 1920). This critical temperature is possibly related to our observation that the most marked change of facet number with temperature in the wild type occurs between 25° and 28°. By parity of reasoning one should expect to find another critical temperature below 18° in a Bar stock isogenic with the wild type used in our experiments, since this stock shows an increase in facet number below this temperature. In other Bar stocks no such critical temperature has been described.

The difference in facet number for the sexes is of some interest since the females consistently show a higher count in our data, differing in this respect from Bar. R. K. HERSH, on the other hand, found a small but significant difference in favor of the males at all temperatures. The data of STURTEVANT (1925) on the wild type at 25° agree with our own in this respect, while those of KRAFKA (1920), although extremely meager, indicate the possibility of a change in sex dimorphism with temperature. Such differences in sex dimorphism are not surprising if facet number in the wild type is the resultant of the effects of many genes distributed throughout both the autosomes and sex chromosomes. These differences do, however,

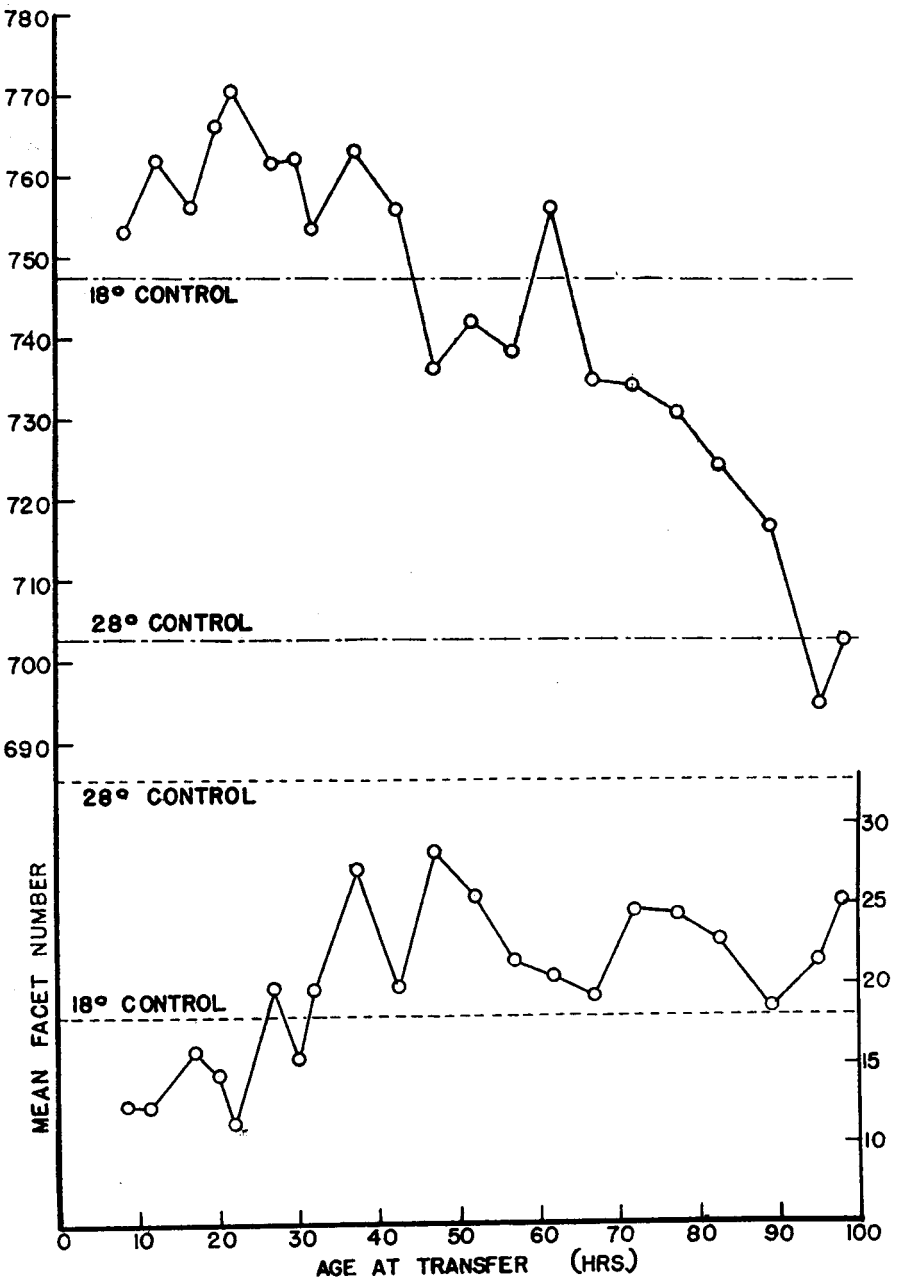


FIGURE 2.—Upper curve: Males transferred at successive intervals from 28° to 18°. Lower curve: Standard deviations of the above groups of data.

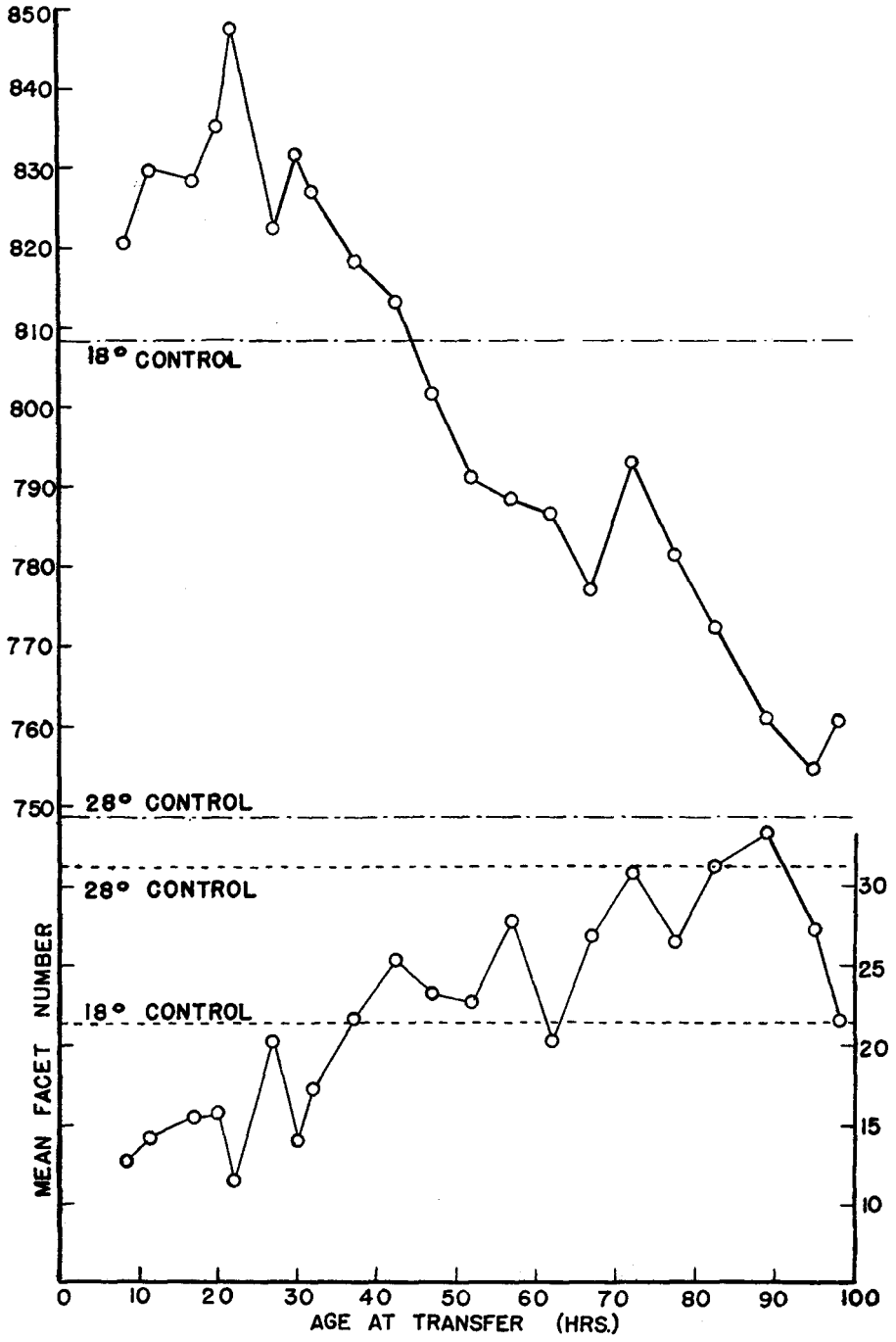


FIGURE 3.—Upper curve: Females transferred at successive intervals from 28° to 18°. Lower curve: Standard deviations of the above groups of data.

raise serious doubts concerning the wild type as a stable biotype which may be used as a reliable standard in comparing the developmental effects of mutant genes. It becomes increasingly clear that phenotypic resemblance is not an adequate measure of genetic similarity, and that a character susceptible to quantitative estimation over the range of some environmental variable, serves as a more rigorous test of relationship. It is for this reason that certain investigators in our laboratory have adopted the Oregon wild type used in Powsner's experiments (1935) as a "standard" wild type. A number of mutant stocks isogenic with this "standard" wild type are now available.

*The temperature-effective period*

The time involved in dissection and counting of the large number of eyes necessary for the reliable determination of the T. E. P. made it necessary to limit our investigation to a single temperature, 28°. Beginning at 8.5 hours after the middle of the egg-laying period and continuing up to 98 hours from the middle of this period, two or three culture vials were transferred from 28° to 18°. The transfers were so arranged that nearly the entire egg-larval period was involved at intervals of from two to six hours. We did not consider it necessary to transfer any cultures after 98 hours of development since at this time approximately half of the larvae had formed puparia. There is some evidence that the ommatidia are already differentiated as visible units at the time of puparium formation (KRAFKA 1924, CHEN 1929) although ROBERTSON (unpublished) does not find the ommatidia differentiated until some time later.

The data on mean facet number in the various transfer groups are presented in table 3, and are plotted in figures 2 and 3 for males and females respectively. The lower curves in each figure show the standard deviations for the different transfer groups. The control values for the mean and $\sigma$ at 28° and 18° respectively are indicated by the horizontal lines. It is evident from the figures that at 8.5 hours, the time at which the first group was transferred, there is already an effect of temperature. This effect of temperature may, as appears probable from the data, extend back to the very beginning of development of the zygote. The effect of transfer from 28° to 18° on the first few groups is to bring about an increase in facet number beyond the 18° control value, although one is led to expect a decrease in view of the facet-temperature relation observed in the stock. CHILD (1935b) in studying the T. E. P. for various bristles affected by the scute gene of Drosophila, observed a similar effect in the case of the anterior notopleural bristles when the flies were transferred from a lower to a higher temperature. In the reciprocal transfers no such stimulating effect was detected.

Following the initial rise in facet number in the early transfer groups, there is a tendency for a progressive decrease in facet number in succeeding transfer groups. There are, however, a number of significant irregularities in the curves for both males and females. The general downward trend is interrupted at several points by significant increases in facet number, notably at 60 hours in the males and at 72 hours in the females. These irregularities are very clearly not sampling errors, nor have we been able to trace them to any possible source of error in the experiments.

TABLE 3

*Facet number in flies transferred from 28° to 18° at successive intervals during the larval period.*

| AGE AT TRANSFER (HOURS) | MALES M | σ | v | n | FEMALES M | σ | v | n |
|---|---|---|---|---|---|---|---|---|
| Total development at 18° | 747.8±3.60 | 18.0 | 2.41 | 25 | 808.2±4.30 | 21.5 | 2.66 | 25 |
| 8.5 | 753.2±2.50 | 12.5 | 1.65 | 25 | 820.8±2.53 | 12.7 | 1.54 | 25 |
| 11.5 | 762.0±2.48 | 12.4 | 1.63 | 25 | 829.6±2.84 | 14.2 | 1.71 | 25 |
| 17.0 | 756.1±3.86 | 15.9 | 2.13 | 17 | 828.2±3.12 | 15.6 | 1.88 | 25 |
| 20.0 | 766.2±2.90 | 14.5 | 1.89 | 25 | 835.2±3.16 | 15.8 | 1.89 | 25 |
| 22.0 | 770.4±2.28 | 11.5 | 1.48 | 25 | 847.6±2.34 | 11.5 | 1.35 | 24 |
| 27.0 | 761.8±3.98 | 19.9 | 2.61 | 25 | 822.2±4.02 | 20.1 | 2.44 | 25 |
| 30.0 | 762.3±3.27 | 15.4 | 2.01 | 22 | 831.8±2.80 | 14.0 | 1.68 | 25 |
| 32.0 | 753.8±3.94 | 19.7 | 2.61 | 25 | 827.0±3.44 | 17.2 | 2.08 | 25 |
| 37.3 | 763.4±5.42 | 27.1 | 3.55 | 25 | 818.2±4.34 | 21.7 | 2.65 | 25 |
| 42.3 | 756.0±4.56 | 19.9 | 2.63 | 19 | 813.3±5.16 | 25.3 | 3.11 | 24 |
| 47.0 | 736.2±5.64 | 28.2 | 3.83 | 25 | 801.8±4.68 | 23.4 | 2.92 | 25 |
| 52.0 | 742.2±5.10 | 25.5 | 3.44 | 25 | 791.4±4.56 | 22.8 | 2.88 | 25 |
| 57.0 | 738.5±4.48 | 21.5 | 2.91 | 23 | 788.6±5.56 | 27.8 | 3.53 | 25 |
| 62.0 | 756.2±4.10 | 20.5 | 2.71 | 25 | 786.8±4.06 | 20.3 | 2.58 | 25 |
| 67.0 | 735.0±3.84 | 19.2 | 2.61 | 25 | 777.4±5.38 | 26.9 | 3.46 | 25 |
| 72.0 | 734.2±4.92 | 24.6 | 3.35 | 25 | 793.4±6.16 | 30.8 | 3.88 | 25 |
| 77.5 | 731.0±4.86 | 24.3 | 3.32 | 25 | 781.8±5.32 | 26.6 | 3.42 | 25 |
| 82.5 | 724.2±4.56 | 22.8 | 3.15 | 25 | 772.2±6.22 | 31.1 | 4.03 | 25 |
| 89.0 | 716.7±3.80 | 18.6 | 2.60 | 24 | 761.0±6.66 | 33.3 | 4.38 | 25 |
| 95.0 | 694.6±4.28 | 21.4 | 3.08 | 25 | 754.6±5.46 | 27.3 | 3.62 | 25 |
| 98.0 | 702.6±5.04 | 25.2 | 3.59 | 25 | 760.6±4.32 | 21.6 | 2.84 | 25 |
| Total development at 28° | 702.7±6.56 | 32.8 | 4.67 | 25 | 749.0±6.22 | 31.1 | 4.15 | 25 |

There is some indication in the case of the females that the T. E. P. is not quite completed at 98 hours. The males, on the other hand, appear to have passed through the T. E. P. at about 95 hours.

The curves showing the relation of $\sigma$ to time of transfer have been presented because of the apparently peculiar behavior of this statistic. There is some indication of periodicity in the graphs for both males and females although the apparent differences for the two sexes make it impossible to draw a definite conclusion. There are, however, certain features in the behavior of $\sigma$ common to the two sexes. The values of $\sigma$ for the early transfer

groups are all smaller than the values for either of the control temperatures. This appears in some way related to the fact that these early transfer groups all show significantly larger means than any of the control means. For example, we find the highest mean and lowest value of $\sigma$ in the 22 hour transfer group for both sexes. This inverse relation between $\sigma$ and the mean in these early transfer groups is very probably significant, although we can offer no explanation for the relation at this time.

Other investigations on the T. E. P. for various mutant characters affecting wing size, bristle number, and facet number, indicate that during the T. E. P. there is a progressive increase in variability and then a decrease. The increase in variability has been shown, in part at least, to be due to individual differences in time of occurrence of the processes characterizing a particular T. E. P. (CHILD 1935b, MARGOLIS 1935b). Individuals of a transfer group will have completed varying portions of the processes affecting the character measured so that increased variability for the population is to be expected. The possible bearing of this fact on the relation of $\sigma$ to time of transfer which we find for the wild type eye will be discussed below

## DISCUSSION

The wild type eye, unlike the members of the Bar series, is affected by temperature throughout the egg-larval period. In the Bar series the T. E. P. falls mainly within the third quarter of the egg-larval period in all stocks so far studied. Within these time limits, differences in relative time of occurrence and duration have been found in different stocks and at different temperatures. Moreover, in the different Bar alleles the T. E. P. curves display a regularity which is to be expected if we are dealing with a single process or a series of processes affecting facet number, all having the same temperature characteristic.

Attention has already been directed to the irregularities in the T. E. P. curves for the wild type in figures 2 and 3. These irregularities do not appear to be due to errors of random sampling and may be construed as representing a series of separate processes, both constructive and destructive, leading to facet determination in the wild type. While the present data do not make possible a conclusive demonstration of this interpretation, a number of processes are clearly indicated. These processes seem to follow the same general course in the two sexes, as evidenced by a comparison of the T. E. P. curves. As a first approximation we may single out four segments in the T. E. P. curves as representing different processes involved in facet formation. The first process is initiated at the time of beginning of development of the zygote and continues to about 22 hours of development in both sexes. The fact that this period is approximately the duration of embryonic development in this stock (POWSNER 1935) may be

significant. A second process then sets in and occupies the period from 22 hours to 57 hours in the male and from 22 hours to 67 hours in the female. The third process occupies the period from 57 to 62 hours in the male, and from 67 to 72 hours in the female. The fourth process extends from 62 hours to 95 hours in the male, and from 72 hours to beyond 98 hours in the female.

The above dissection of the curves is by no means rigorous and is based solely on recording the most conspicuous discontinuities in the curves. While this analysis serves to illustrate the complexity of the processes leading to facet formation, there is clear indication that the situation may be even more complicated. For example, segment 2 in the T. E. P. curves shows irregularities, especially in the males, indicating that this segment may represent a period during which more than a single process affecting facet number is occurring.

Based upon the consideration that progressively longer exposures to the higher temperatures during the embryonic stages leads to an increased facet number, and longer exposures during the larval period to a reduction in facet number, we may provisionally assume that the earlier processes in facet determination are constructive in nature while destructive processes predominate during the later stages. A more thoroughgoing study of the various segments of the T. E. P. curve is desirable in order to single out the various processes involved in facet determination for identification and separate study. An experiment involving the transfer of flies in the same stage of development at one temperature to a series of other temperatures for limited periods of time and then returning these cultures to the original temperature to complete their development, should make it possible to single out the various processes involved in facet determination.

The apparent periodicity in the values of $\sigma$ for successive transfer groups becomes intelligible if the foregoing interpretation of the T. E. P. curves is correct. Since variability in the population increases during the course of any process affecting facet number due to the fact that at the time of transfer, individuals of the population will have completed varying portions of the process; followed by decreasing variability as more and more members of the population complete the process, we may expect to find some periodic fluctuation in $\sigma$ if a number of facet determining processes take place during the T. E. P.

The bearing of our results on interpretations of the kinetics of facet determination in the Bar series deserves brief comment. Our data suggest that facet number in the wild type eye is the resultant of a number of processes, some tending toward facet formation and others toward facet "destruction." The facet number in the adult fly is therefore determined by the relative rates and durations of these different processes. From this

point of view, the facet reduction brought about by Bar and its alleles may be interpreted as due to an acceleration of some facet "destroying" process of the wild type through the catalytic action of the Bar gene or one of its products. This interpretation is simpler than the interpretation offered earlier (MARGOLIS 1935a) in that it does not assume the introduction of a new process by the Bar gene, and yet is equally consistent with experimental data.

## SUMMARY

Data on the effect of temperature on facet number in a highly inbred wild type of *Drosophila melanogaster* are presented. In the temperature range 16° to 30° there is a slight decrease in facet number with increase in temperature, although the temperature effect is not systematic. In the interval 18° to 25° there is no clearly perceptible effect of temperature.

The temperature-effective period for facet determination (T. E. P.) was investigated at 28°. In both sexes the T. E. P. is initiated with the beginning of development of the zygote and terminates at 95 hours of development in the male, and shortly after 98 hours in the female. The termination of the T. E. P. coincides approximately with the mean time of puparium formation in this stock.

A number of significant irregularities appear in the T. E. P. curves. These irregularities are attributed to the occurrence of a number of separate facet-determining processes having different temperature characteristics. Certain ones of these are considered as facet-forming in character and others as "facet-destroying." An experimental method for the investigation of these processes is suggested and the bearing of the experimental observations on facet formation in Bar is discussed.

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