STUDIES ON THE BAR SERIES OF DROSOPHILA MELANO-
GASTER. II. THE TEMPERATURE-EFFECTIVE
PERIOD FOR FACET DETERMINATION
IN BAR AND BAR VESTIGIAL

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In an earlier paper of this series (MARGOLIS 1935), it was shown that the gene *vg* effected a small but significant decrease in facet numbers in Bar-eyed flies of *Drosophila*. The present paper deals with the results of an investigation of the temperature-effective period for facet determination in *B* and *B vg* stocks. The early investigations of SEYSTER (1919) and KRAFKA (1920) served to localize the temperature effect on facet number entirely within the larval period of development. More recently, DRIVER (1926, 1931) and LUCE (1931) succeeded in setting more definite limits for the T.E.P. in *B* and *Bⁱ* flies at different temperatures. DRIVER, especially, has called attention to the necessity of precision in determining temperature-effective periods as a tool in analyzing the kinetics of facet determination in the Bar series. The results of these investigators will be discussed in greater detail in relation to the results obtained by the writer.

For expediency, the temperature-effective period for facet determination will be referred to as the T.E.P.

EXPERIMENTAL

The method employed in T.E.P. studies consists in transferring flies from one temperature to another at successive intervals throughout a portion of larval development. The transferred individuals then complete their development at the second temperature. Facet counts are made on the adult flies in the various transfer groups.

The stocks used and the general methods employed in this investigation have been discussed fully in the paper previously cited. Since a short egg-laying period is desirable in an investigation of this type, 20 to 25 pairs of flies were permitted to lay eggs for a 1-hour period. Five to 7 successive batches of eggs were obtained from each group of parents. In a few cases, where an insufficient number of eggs had been laid, a 1.5 hour egg-laying period was used. Egg-laying took place at room temperature (c. 22°C). The experimental vials were then immediately placed at 28°C to begin their development. Beginning at about the 40th hour from the midpoint of the egg-laying period, a suitable number of cultures were transferred at intervals to 22° to complete their development. The experiment was so arranged that each transfer group covered a range of 5 to 7 hours, at one

hour intervals. The total range of ages of larvae at time of transfer was from 40 to 80 hours. *B* and *B vg* flies were reared at the same time, under identical culture conditions, and in the same thermostats. In order to accelerate the attainment of the new temperature equilibrium at transfer,

FIGURE 1.—Mean facet numbers in *B* flies transferred at successive intervals from 28° to 22°.

a water bath a few tenths of a degree below 22° was used. By this method only 7 to 8 minutes were required for temperature equilibrium to ensue.

Due to low fertility and viability, very few flies were obtained from some of the transfer groups. It was found necessary, in some cases, to combine the data of two or three successive transfer groups in order to reduce sampling errors in the facet counts. When this was done, a mean time of

transfer was calculated. The data on the T.E.P. are presented in tables 1 and 2 and in figures 1 and 2. Males and females have been recorded separately, both in the tables and the figures, because of sexual dimorphism in facet number. Although the points in the figures show a fairly large scatter, it is apparent that the general trend is sigmoid in character. Most investigations on the T.E.P. for facet determination, as

FIGURE 2.—Mean facet numbers in *B. vg* flies transferred at successive intervals from 28° to 22°.

well as those for other characters show this relation. The significance of this point will be discussed later.

In establishing the limits of the T.E.P. a method frequently used is to take the first point at each end of the curve which shows a statistically significant deviation from the control line. For example, in the case of the *B* males, the first significant deviation from the 22° control line is at

48.5 hours. Since the beginning of the T.E.P. must be somewhat earlier, the mean between 48.5 hours and the point preceding it (46.5 hours) is taken as marking the beginning of the T.E.P. Similarly, the end of the T.E.P. may be found. Since there is very little difference between males and females in the duration of the T.E.P., the data for the two sexes are here taken together. There is, however, some slight indication that the females may enter the period a little earlier. Delimiting the T.E.P. as indicated above, we find that in *B* the period begins at 48.5 hours and ends at 74.5 hours; in *B vg* the period begins at 47.6 hours and ends at 76.2 hours. These values, although by no means conclusive, indicate that the T.E.P. may be slightly longer in *B vg* than in *B*. It will be shown later that this method of determining the period involves considerable error.

A somewhat better method for establishing the duration of the T.E.P. is based upon the trend of the points. We may take the first point showing the beginning of the trend away from the 22° control line as a marker. Taking, as before, the mean of this point and the one preceding it, we consider the value so obtained as the beginning of the T.E.P. Similarly, the end of the T.E.P. may be found. By this method, it may be seen from figures 1 and 2 that the period in *B* begins at 46.6 hours and ends at 76.6 hours; in *B vg* the period begins at 45.7 hours and ends some time after 80 hours. It appears that the method of trends gives a more accurate value of the limits of the T.E.P. than does the estimation based upon statistically significant deviations. When sampling errors are relatively large, as is the case in these data, real individual differences may be obscured. The general trend of the points is affected to a lesser extent, and consequently gives more accurate results.

The values for the duration of the T.E.P., as determined by either of the methods considered above, have little meaning, except qualitatively. In establishing the limits of the T.E.P. we have really determined the time at which the first few individuals of the population enter their respective T.E.P.'s, and the time at which the last few individuals emerge. This will represent the mean value of the T.E.P. of all the individuals only in the limiting case when the temporal position of the T.E.P. in relation to the whole period of development is the same in all individuals, that is, when every individual enters the T.E.P. at the same time, and emerges at the same time. We should certainly not expect this to be actually true. From tables 1 and 2 we see that the coefficients of variability for transfer groups which have entered the T.E.P. show a marked increase. As soon as the T.E.P. is completed the value falls to the average level. This is true in *B*, but not in *B vg*, indicating that the latter had not completed the T.E.P. Clearly then, some factor is contributing to this additional variability in

TABLE 1
Temperature-effective period (28° to 22°) Bar.

HRS. AT 28° BEFORE TRANSFER	M	MALES PERCENT V	n	M	FEMALES PERCENT V	n
0.0	112.9±1.6	17.5	155	107.1±1.4	14.5	118
39.5	112.3±3.3	12.7	20	103.8±4.5	16.3	14
41.3	111.2±2.9	16.7	42	100.5±2.4	14.4	37
43.7	108.0±2.3	18.7	79	105.2±2.9	17.6	41
44.8	113.2±1.9	15.5	83	107.4±1.8	13.7	66
46.5	112.7±2.7	18.4	59	103.3±2.7	16.7	42
48.5	106.2±2.7	19.7	57	102.0±3.3	17.6	30
50.8	105.0±2.1	18.6	84	99.4±1.9	16.6	73
53.0	106.5±3.4	17.4	29	105.2±2.8	13.6	27
54.5	100.4±2.2	17.9	69	92.5±1.8	15.5	64
57.7	93.5±2.5	17.6	45	88.7±2.6	18.7	42
60.3	87.1±2.1	21.8	84	88.3±2.6	22.3	57
62.3	77.0±2.1	23.5	75	75.5±2.3	20.7	45
65.1	68.4±2.3	24.0	51	62.7±1.7	16.3	36
67.4	69.1±2.0	24.5	70	64.2±1.4	15.6	54
69.3	56.0±1.2	17.1	65	55.4±1.6	18.8	41
71.8	53.5±0.9	13.1	61	50.0±0.7	10.4	60
73.6	54.1±0.6	9.3	60	53.3±0.7	10.7	65
75.4	54.3±1.2	12.3	29	52.5±0.7	9.3	51
77.8	51.5±0.7	10.9	64	49.5±0.6	8.1	39
Total time	52.1±0.4	10.0	149	51.6±0.4	9.3	122

TABLE 2
Temperature-effective period (28° to 22°) B vg.

HRS. AT 28° BEFORE TRANSFER	M	MALES PERCENT V	n	M	FEMALES PERCENT V	n
0.0	102.1±1.9	15.3	66	93.4±1.9	16.1	64
40.5	103.3±3.7	15.7	19	93.9±2.2	15.4	45
42.6	102.8±3.1	21.8	53	92.2±2.1	13.0	31
44.4	102.0±2.7	16.7	38	93.5±2.9	16.0	27
45.2	105.0±4.5	19.5	20	89.5±2.2	11.3	21
47.7	100.3±2.7	17.4	42	87.0±1.8	13.2	39
49.6	91.5±1.9	14.6	50	87.3±1.8	12.1	33
52.8	92.9±2.5	18.8	51	84.7±2.0	15.5	41
54.7	89.6±2.2	18.6	60	87.5±2.2	14.3	32
56.4	89.6±2.6	20.6	51	85.4±2.2	15.8	39
58.9	80.7±1.7	13.3	38	79.8±2.0	12.9	25
61.4	80.5±3.8	27.3	34	71.5±3.5	19.3	16
64.2	79.7±3.5	27.1	37	65.5±2.9	23.4	28
66.3	75.2±2.2	23.3	61	65.7±1.7	20.5	62
68.0	67.0±3.9	30.3	27	63.0±4.4	31.9	21
70.4	60.6±2.5	24.6	36	57.5±2.4	23.0	30
72.4	55.4±1.5	16.9	37	53.1±2.3	22.6	28
74.2	54.2±2.0	19.2	28	51.5±2.1	22.3	30
78.3	52.0±3.3	30.8	23	51.3±3.6	24.9	13
80.0	51.5±3.3	23.9	14	45.8±3.2	31.2	20
Total time	47.0±1.0	13.6	38	44.5±0.7	8.1	25

populations which have entered the T.E.P. If individuals of any one transfer group enter their respective T.E.P.'s at different times, an increase in the coefficient of variability is to be expected, since the transfer group is then a heterogeneous population in respect to the temperature effect on facet number. It may be demonstrated that this is actually the case.

Since flies show variability in the length of the egg-larval period, we may segregate individuals at the time of puparium formation, and determine the correlation coefficient between facet number and length of egg-larval period. This was done at several temperatures. The data are presented in table 3. With the exception of females at 22.7°, no significant

TABLE 3
Correlation between length of egg-larval period (x) and facet number (y).

T	r	BAR MALES	M _x	M _y
22.7°	-0.049±0.088		135.0±0.48	95.4±1.40
27.0°	-0.193±0.130		97.7±0.50	58.1±0.88
28.0°	-0.142±0.137		96.2±0.54	49.9±0.81
29.0°	0.020±0.143		96.5±0.71	41.8±0.53
		FEMALES		
22.7°	-0.240±0.077		134.3±0.50	93.6±1.02
27.0°	-0.009±0.147		97.2±0.53	55.2±0.94
28.0°	-0.212±0.155		96.3±0.85	48.4±1.00
29.0°	-0.101±0.162		95.8±0.92	40.1±0.59

correlation was found. It should be noted, however, that with a single exception (males at 29.0°) all the values of r are negative, although not significant statistically. The writer has found that under crowded culture conditions there is a significant negative correlation between facet number and length of the egg-larval period. Since crowding tends to increase the time of development, this result indicates that those flies which are delayed the most show the smallest facet counts. The fact that the values of r in table 3 are consistently negative may indicate slight crowding effects in the cultures, which are too small to manifest themselves in the mean facet counts. In relating these facts to the problem of the T.E.P. we have the following considerations. We should in general expect those individuals which complete the egg-larval period first, to have completed other developmental phases relatively earlier than those individuals which develop more slowly. If this is true then those individuals in the transfer groups which pupate first will have passed a longer portion of their T.E.P.'s at 28° than those pupating later, and will consequently have smaller facet numbers. This relation is the converse of that found in flies raised at a single temperature under crowded conditions.

This point was tested by determining the correlation coefficient between

facet number and length of egg-larval period for a group of *B* flies transferred from 28° to 22° at approximately the middle of the T.E.P. The values obtained for the correlation coefficients are:

$$\text{males} = 0.27 \pm 0.071 \text{ (n = 172)}$$

$$\text{females} = 0.28 \pm 0.066 \text{ (n = 197)}$$

These values are positive and statistically significant and show that different individuals of the same age as expressed in hours of development, are in different stages of their individual T.E.P.'s.

The T.E.P. which we actually determine experimentally is then composite of two quantities, (1) the mean length of the T.E.P.'s of the individuals, and (2) the mean range of variation in time at which the T.E.P.'s of the individuals occur. For convenience, in the following we shall adopt the terminology used by CHILD (1935) who studied the T.E.P. for bristle determination in scute.

Let P = the length of the T.E.P. of the whole population as determined experimentally (apparent T.E.P.)

D = the mean length of the individual T.E.P.'s

V = the mean range of variation between the midpoints of the individual T.E.P.'s (D)

From the conditions set down it is apparent that we have the following relation: $P = D + V$. It is apparent that several possible relations may subsist between D and V . The value of V may equal 0, so that $P = D$. We have already seen that this is not the case, since a positive correlation between facet number and length of the egg-larval period is obtained in the transfer groups. The value of D may equal 0, so that $P = V$. If this were true the facet distributions in all the transfer groups would be bimodal, especially since the two distributions do not overlap. Those flies which had not yet been affected by temperature at the time of transfer would show the 22° facet distribution, while those which had been affected would show the 28° distribution. Since none of the transfer groups shows definite bimodality, this possibility may also be dismissed. We are left then with the third possibility that D and V both have finite values.

The problem with which we are concerned is the evaluation of D , in order to ascertain whether *vg* has any effect on the duration of the T.E.P. for facet determination in the individual. This problem will be considered fully in a later section.

Duration of Egg-Larval Period in Transfer Groups

The length of the egg-larval period was determined in a number of the groups transferred from 28° to 22° at intervals during the larval period. LUDWIG (1933) has shown that when *Drosophila* pupae are transferred back and forth between two temperatures, the effects of each temperature

on length of the pupal period are, in general, additive. The data of table 4 give the mean values for length of the egg-larval period for different transfer groups. No attempt was made to keep separate records for males and females since earlier data showed no sex difference. The calculated values are based on the assumption that the effects of the two temperatures are additive. In general there is a fairly good agreement between the observed and calculated values. This agreement is apparently much better for *B vg* than for *B*. The fact that the effects of the two temperatures on length of the egg-larval period are additive, shows that no effect of the first temperature is carried over to development at the second temperature after the flies are transferred.

TABLE 4
Duration of egg-larval period in flies transferred from 28° C to 22° C.

HRS. AT 28° BEFORE TRANSFER	<i>B</i>		<i>B vg</i>	
	M (obs.)	M (calc.)	M (obs.)	M (calc.)
0.0	143.1±0.26	..	150.3±0.98	..
40.9	120.4±0.55	122.8	129.8±1.70	129.0
45.8	117.0±0.56	120.2	..	..
48.0	..	..	125.6±1.08	125.4
59.8	111.0±0.56	113.3	..	..
61.8	109.4±0.64	112.3	..	..
65.0	..	..	115.2±1.05	116.6
66.8	109.4±0.39	109.7	..	..
70.0	..	..	113.0±0.96	114.0
72.9	108.0±0.45	106.7	113.6±1.56	112.5
73.9	104.2±0.52	106.3	112.6±1.23	112.0
75.8	105.0±0.44	105.2	..	..
77.9	100.0±0.66	104.1	109.8±1.30	110.0
79.9	102.2±1.0	103.3	108.8±1.24	109.0
Total time	95.4±0.19	..	98.9±0.51	..

THEORETICAL

Analysis of the temperature-effective period

It has been shown that the T.E.P. for the population as a whole (P) is composite of two factors:

- (1) the mean length of the individual T.E.P.'s (D), and
- (2) the mean range of variation of the midpoints of the D's (V).

Since $P = D + V$,

it is clear that if we can evaluate V, we can readily determine D, assuming that the value of P, as experimentally determined, is correct. It was thought that V could be evaluated from a comparison of the facet distributions in the transfer groups with those of the controls at 22° and 28°. A consideration of the manner in which the facet frequency distributions in the transfer groups are built up will illustrate the nature of the problem.

Let us assume that we know the form of the distribution of times at which various fractions of the population enter the T.E.P., and that a fraction a_1 enters at t_1 , a_2 enters at t_2 , a_n enters at t_n . We shall assume, moreover, that the time-facet function is known for 28° and 22° . By time-facet function, as used here, is meant the relation describing the course of the facet-forming processes in time. If now, we transfer the population from 28° to 22° at time t_x —and for simplicity we shall assume that at time t_x the whole population is actually in the T.E.P.—we observe the following condition of the population in respect to facet formation at the completion of development. A fraction of the population a_1 will have formed x_1 facets at 28° and the remainder at 22° ; a_2 will have formed x_2 facets at 28° and the remainder at 22° ; a_n will have formed x_n facets at 28° and the remainder at 22° . At the completion of development each fraction of the transfer group, $a_1, a_2, \dots a_n$, will show a mean facet number and a particular facet distribution. The mean facet number for any fraction of the population ($a_1, a_2, \dots a_n$) is uniquely defined by the position of that fraction on the facet axis of the time-facet curve at 28° and 22° at the time of transfer. We shall assume that the actual facet distribution for any fraction ($a_1, a_2, \dots a_n$) of the population having a given mean is known. We may then very easily calculate the mean facet number and facet distribution for the whole group transferred at t_x . Similarly we may find the mean facet number and facet distribution for a group transferred at any time t . Plotting the means obtained in this way against the time of transfer we get a curve in which each point is descriptive of the relative numbers of facets formed at 28° and 22° , respectively, for the population as a whole. It is this relation which, in fact, has been obtained experimentally for B and Bvg , respectively (figures 1 and 2).

The problem with which we are concerned at present, however, is not in building up the frequency distributions of the transfer groups and determining their moments, but rather in breaking them down into their components, thereby obtaining values for V and D . Having seen that a knowledge of the time-facet function and of the variation in time of the individual T.E.P.'s is essential to building up these distributions analytically, it is clear that without independent information concerning one or the other of these factors, no rigorous solution can be had. We may, however, through simplifying assumptions make some estimate of the number of individuals entering and emerging from the T.E.P. at different times. Plotting the percentage of the population in a transfer group which has entered the T.E.P. for each transfer group, we may obtain a curve which is really the integral of the frequency distribution of the beginning of the T.E.P. in the population.

Since those groups which have been exposed to 28° for progressively

longer periods of time will tend more toward the 28° distribution, we find in successive transfer groups greater proportions of the population at the low end of the distribution. Taking the 22° control median facet number as point of reference, we find in successive transfer groups that increasing numbers of individuals assume values less than the median at 22° . Let x = the percentage of the population of a transfer group having facet numbers less than the median value of the 22° control. Since in the control 50 percent of the population have values less than the median, $(x \text{ percent} - 50 \text{ percent})$ = excess percentage of individuals in the transfer group having values less than the median. These individuals clearly are ones which have entered the T.E.P. In addition, an equivalent proportion $(x \text{ percent} - 50 \text{ percent})$ of the transfer group which, if unaffected by the 28° temperature, would have developed facet numbers less than the 22° median, will also have entered the T.E.P. Hence $2(x \text{ percent} - 50 \text{ percent})$ of the transfer group have entered the T.E.P. This method involves the assumption that any individual which enters the T.E.P. is immediately shifted a sufficient amount along the facet axis of the time-facet curve to cross the median value of the 22° controls. This assumption is very probably incorrect, and consequently this method gives, in any transfer group, a *minimum* number of individuals which have entered the T.E.P. By a similar method we may estimate the proportion of the population of a transfer group which has emerged from the T.E.P. Taking the median value of the 28° facet distribution as a point of reference, we shall assume that only individuals which have emerged from the T.E.P. will show smaller facet numbers. If these represent x percent of a transfer group, then, since an equal proportion of flies having facet numbers greater than the 28° median will also have completed the T.E.P., the total proportion emerged will equal $2x$ percent. Since, of course, some flies which had not yet completed the T.E.P. at the time of transfer may have facet numbers less than the median of the 28° control, the value of x calculated in this way gives a *maximum*.

The percentage of individuals in different transfer groups which have entered into, and emerged from, the T.E.P. was calculated in the manner described above, and the relation is shown in figure 3 for B and $B\text{ }vg$, respectively.

The 50 percent points on the curves in figure 3 represent the mean time of beginning and end of the T.E.P. in the two stocks under consideration. These mean values were calculated directly from the data before drawing the curves. The curves were then fitted to the points by eye in such a manner as to pass through these means and at the same time give a good fit to the largest number of points in each series. Originally separate

calculations were made for males and females for each point. Since these values showed a random scatter about a single curve, they were combined to give a weighted mean for the two sexes. The vertical lines through the points in the figure represent the magnitude of plus or minus twice the standard errors of the points. These errors were calculated from the expression: $E = \sqrt{\frac{pq}{n}}$, where p represents the chance of an individual entering or emerging from the T.E.P. at a particular time; $q = (1 - p)$;

FIGURE 3.—Probability integrals for individual variability in time of occurrence of beginning and end of T.E.P.
Left-hand curves represent beginning of T.E.P.
Right-hand curves represent end of T.E.P.
Solid line = B ; broken line = $B\text{ }vg.$

and n is the size of the population. The value of p for each point was taken directly from the appropriate curve.

We have seen that the assumptions leading to the calculation of the points in figure 3 give *minima* for the points representing the percentage of flies which have entered the T.E.P. and *maxima* for the percentage of flies emerged. This means that the true integral curves for the distribution of the beginning of the T.E.P. will lie to the left of the calculated curves. Conversely, the true integral curves for the distribution of the end of the T.E.P. will lie to the right of the calculated curves. The difference between the 50 percent points in the two sets of curves therefore gives a *minimum*

value for D , the mean length of the T.E.P. in the individual. This value is 15.0 hours for B and 15.6 hours for $B\ vg$. The difference between the two values, 0.6 hours although quite small is very probably significant, especially since the 50 percent points on the curves represent means calculated from all the points and consequently have very small sampling errors. Moreover, a much greater increase in the duration of the T.E.P. in $B\ vg$ is hardly to be expected since vg increases the total duration of larval life at 28° by only 2.4 hours (MARGOLIS 1935). We have seen that the assumptions leading to the calculation for the points in figure 3 give minima for the values of D for both B and $B\ vg$. It should be pointed out, however, that the difference between the values of D in the two stocks is not a minimum, but represents very probably a correct value within the limits of experimental and sampling error. This follows from the fact that the errors introduced by the assumptions inherent in the method of calculation affect the probability integral curves for the two stocks in the same way, if vg affects facet number in B by prolonging the duration of the T.E.P. and not by altering the time-course of facet determination. Although the data cannot conclusively establish the absence of any effect of vg on the form of the time-facet function, they do demonstrate an effect on the duration of the T.E.P. Inspection of the curves in figure 3 shows that the effect of vg in increasing the length of the T.E.P. occurs primarily in delaying the end of the period. Although it appears that vg does to some extent retard the beginning of the T.E.P., the effect on the end point is greater. This fact is confirmed by the results obtained on the "apparent" T.E.P. (P) estimated by the method of trends, and is consistent with the interpretation of the effect of vg on facet number in B presented in an earlier paper.

An estimate of P , the "apparent" T.E.P., may also be obtained from figure 3. This is done simply by determining the distance from the 0 percent points on the left-hand curves to the 100 percent points on the right-hand curves along the abscissa, for B and $B\ vg$, respectively. These values of P are 36.6 hours for B and 39.0 hours for $B\ vg$. Apart from any consideration of errors of extrapolation the value of P as estimated above is a minimum, theoretically, because of the assumptions inherent in the method of calculating the points for the curves. It can be demonstrated, however, that these assumptions affect the first flies to enter the T.E.P. and the last to emerge to only a very slight extent, and consequently introduce no great error in the above calculation. The fact that P as calculated above, although a theoretical minimum, is much greater than P calculated either by the trends of the points in figures 1 and 2, or by significant deviations from the control means, gives us some idea of the magnitude of the errors incurred by either of these methods.

It may be seen from figure 3 that the curves for the beginning of the

T.E.P. for both stocks show a much larger range of variation than the curves for the termination of the T.E.P. This difference in the two sets of curves seems too large to be fortuitous and must be accounted for by the factors which operate to determine the beginning and end of the T.E.P., respectively. For this purpose it is necessary to review briefly the mechanism postulated by the writer to explain the kinetics of facet determination in the *Bar* series. The following diagram is a schematic representation of the processes concerned.

Reaction 2 represents the facet-forming processes in the wild-type resulting in the production of F, whose quantity at a particular time determines the number of ommatidia. These processes also operate in *B* stocks. Reaction 3 represents the processes initiated by the *Bar* gene. These result in the production of D, a substance which either combines with F to form X, or catalyzes the conversion of F to X. Reaction 1 represents the general processes of development which determine the time at which the region containing F is converted into ommatidia.

The time limits of the T.E.P. have been identified with the beginning and end of the reaction $F + D \rightarrow X$. It was assumed that F is formed at some early period in development so that the reaction $F + D \rightarrow X$ begins as D is being formed. The reaction stops when F is converted into ommatidia. The latter is governed by reaction 1, when the quantity of E attains a certain level. If these relations presented as a formal explanation of the facts known in the *Bar* series are essentially correct, we may readily account for the difference in variability in the beginning and end of the T.E.P. shown in figure 3. The beginning of the T.E.P. is determined by reaction 3 and represents a mutant process. The termination of the T.E.P., on the other hand, is governed by reaction 1, representing a group of wild-type processes. PLUNKETT (1932) and MULLER (1932) have pointed out that quite generally mutants show a much wider range of variability than the wild-type. The results obtained here are entirely consistent with this view.

CHILD (1935) in studying the T.E.P. for bristle determination for various bristles in *scute-1*, has found that the T.E.P. for the individual (D) is much shorter than the period determined experimentally (P). CHILD's data show far more strikingly than those of the writer that individual variability in the temporal position of the T.E.P. is a large

source of error in experimental determinations of the T.E.P.

In earlier investigations on the T.E.P. in the Bar series, variability in the temporal position of the T.E.P. in different individuals was not taken into consideration in the analysis of the data. The T.E.P. as measured by other investigators consequently represents the "apparent" T.E.P. for the population as a whole (P), rather than the mean length of the period per individual (D). The original tables of DRIVER (1931) on B , and of LUCE (1931) on B^i have been obtained through the courtesy of Dr. A. H. HERSH. Inspection of DRIVER's data shows clearly that the factor of individual variability plays an important part in the duration of the T.E.P. as measured experimentally. At all temperatures used by DRIVER (except 22°), the coefficients of variability for facet number show definite, and in some cases, marked increases in those groups which were in the T.E.P. at the time of transfer. This increased variability, as pointed out earlier in this paper, is most easily accounted for by variability in the temporal position of the T.E.P. in individual flies of the population. The exception at 22° is especially interesting since it is consistent with the fact that at 22° the T.E.P., as determined by the writer from DRIVER's data, is relatively shorter than at other temperatures. It should be pointed out that the duration of the T.E.P. as established by the writer from these data is not fully in agreement with the conclusions of DRIVER, based upon the same data. There is, of course, a large subjective element entering into the determination of limits for the T.E.P. In general, the T.E.P.'s calculated by the writer from the data are longer than those presented in DRIVER's table 14. The T.E.P. curves for DRIVER's data show the typical sigmoid relation commonly found in data of this type.

LUCE's (1931) data on the T.E.P. in B^i differ in certain important respects from the data of either DRIVER or the writer, on B . For B^i , the beginning and end points of the T.E.P. can be selected with a high degree of precision. For the most part, LUCE's T.E.P. curves are not sigmoid in character and show quite definite intercepts with the control lines, rather than the asymptotic approaches which characterize the data on Bar. Correspondingly, only a very slight increase—in some cases no increase—in the coefficients of variability for facet counts is found in those groups transferred during the T.E.P. These results indicate that individual variability is not a very large source of error in LUCE's T.E.P. studies on B^i . The difference between B and B^i in individual variability in the T.E.P. is extremely interesting. This difference may be due to an inherent difference in the kinetics of facet determination in the two stocks. On the other hand, it may possibly be accounted for by greater uniformity, both genetic and environmental, in LUCE's experiments. Unfortunately, the data are inadequate for establishing the latter point.

The sigmoid character of the T.E.P. curves found in B has already

been emphasized. The fact that these curves are composite of both the time-facet function and the probability integral for individual variability in the temporal position of the T.E.P., has been demonstrated. We do not, of course, know the form of the time-facet function, but it is apparent that the superposition of the probability integral curves upon the time-facet curves is of itself adequate to account for the observed sigmoid relation. Moreover, if the interpretation of the kinetics of facet determination in *B*, presented earlier and reviewed here is correct, the time-facet curves will themselves be sigmoid.

GAUSE and ALPATOV (1932) have fitted logistic curves to the data of DRIVER (1926) on the T.E.P. in *B*. These writers have interpreted the curves as representing the time-course of facet determination. Although the logistic curve does give a fairly good fit for these data, it is at once apparent from the discussion presented earlier in this paper that the experimental curves obtained in the T.E.P. studies are composite curves and do not in any sense represent the real time-facet curve.

HERSH (1934) has recognized the compound nature of the T.E.P. curves, insofar as each point on the curve is composite of the number of facets determined at the beginning of the T.E.P. at one temperature and of the number of facets determined during the remainder of the T.E.P. at the second temperature. By a painstaking dissection of DRIVER's 1931 data on facet counts in different transfer groups in *BB* (double-bar, or ultra-bar), HERSH has found that a symmetrical sigmoid curve representing the equation for an autocatalytic irreversible unimolecular reaction gives a satisfactory description of the time-course of the facet-determining reaction at each temperature studied. The observed points are in good agreement, within statistical limits, with the points calculated theoretically. HERSH points out that caution must be exercised in concluding from an analysis of this type that the calculated curve gives the true form of the time-facet function. He states, "Since consistent results are obtained a presumption is created that the assumptions made are to be accepted as being in agreement with the facts but one may not conclude that the resulting equations necessarily give the form of the true function. To draw such a conclusion it would need to be shown further that the form of function which gives consistent results is the only one that could possibly apply."

Apart from the exacting mathematical requirements which must be satisfied before any conclusions may be drawn relating to the form of the time-facet function, it is apparent from the considerations developed here that this type of analysis does not suffice to give a complete answer. To this end it is necessary to develop methods, either analytical or experimental, which make possible the resolution of the experimental T.E.P. curves into their component elements.

It was hoped that the general interpretation of the effect of *vg* on facet

number in *B* presented earlier might be tested quantitatively. For this purpose, exact information on the form of the time-facet function and the mean length of the T.E.P. in the individual is necessary. It has been demonstrated that these cannot be obtained from data of the type presented in this paper. Some contribution in this direction will be attempted by the application of new experimental methods, coupled with an extension of the types of experiments described here.

The results of the investigation on the effect of *vg* on facet number in *B* may be summarized as follows:

- (1) The gene *vg* effects a small but significant diminution in facet number in Bar-eyed flies.
- (2) Concurrently, *vg* effects a slight increase in the duration of the temperature-effective period for facet determination.
- (3) The time limits of the temperature-effective period have been identified with the limits of a reaction which results in the decrease of the amount of material available for facet formation.
- (4) The increase in the duration of the T.E.P. in *B vg* flies is adequate, qualitatively, to account for the decreased facet number, as compared with *B*.
- (5) No quantitative test of the above interpretation has been possible due to limitations in the nature of the experimental methods which have been applied. The interpretation is, however, formally consistent with all the data available for the Bar series.

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