STUDIES ON THE BAR SERIES OF DROSOPHILA.

I. THE EFFECT OF THE GENE VESTIGIAL ON FACET NUMBER IN BAR

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A general theory specifying the action of the genes in development was first proposed by GOLDSCHMIDT (1920), as the result of a series of studies on intersexuality in *Lymantria dispar*. The theory, elaborated more fully in 1927, considers the genes as affecting the rates of developmental processes. Since an effect on the rates of some processes limits the durations of others, the varying grades of expression of any character of the organism may be interpreted as the result of some alteration in either the rate or duration of one or more processes in the developmental chain leading from the genes to the character studied. As a formal explanation of the facts, there can be little doubt that the theory is tenable. It lacks, however, any extensive quantitative experimental verification.

Any direct attack upon this problem necessarily involves the possibility of studying directly one or more of the processes in the chain leading from the genes to the adult characters. The difficulties attendant upon such an attempt are apparent. In a form like *Drosophila*, a direct attack appears impracticable at the present time. We may, however, approach the problem from the standpoint of interaction of genetic factors with each other, and with environmental agents known to affect the expression of various characters of the organism. Since the characters of an organism are the resultants of an interplay of its genes with environmental agents acting upon it throughout the course of development, we may, within certain limits, alter the course of the developmental processes by varying these two kinds of factors either independently or jointly. In working with different genotypes, preferably with biotypes differing only in respect to a single gene, quantitative results relating the character studied to the environmental variables may be obtained. Knowing the functional relations between character and environmental agent for a variety of genotypes, we then have data which may be used for the beginning of an analysis of the action of the gene.

Numerous investigations of this type have been carried out on *Drosophila melanogaster*. A variety of characters which lend themselves to quantitative estimation, for example, facet number, wing area, bristle number, and others, have been studied in different mutant stocks. For the most part, temperature was the environmental agent used in the experiments. In an investigation on bristle number in *Dichaete* flies, PLUNK-

ETT (1926) analyzed his data in terms of conventional chemical kinetic concepts. In virtue of its peculiar temperature characteristic PLUNKETT was able to single out a "bristle-destroying" reaction which simulated the kinetics of an irreversible unimolecular reaction. This reaction was identified as the destruction of a thermolabile bristle-forming catalyst. An exhaustive quantitative analysis of the data showed that the relations observed were, within the limits of experimental precision, in excellent agreement with results predictable from theory. PLUNKETT concluded that the difference between *Dichaete* and wild-type, in respect to bristle production, was attributable to an acceleration of the above reaction in *Dichaete* flies. We find in PLUNKETT's work the first attempt at application of a simple kinetic theory to data amenable to quantitative treatment. These results are especially interesting since they led PLUNKETT, quite independently, to the same conclusions as GOLDSCHMIDT in respect to the action of the genes in development.

The Bar series of alleles in *Drosophila*, have been investigated more than any other genes in relation to the effect of temperature on a character susceptible to quantitative estimation, in this case, facet numbers. These studies have afforded investigators a considerable body of data, both on the facet-temperature relation and on the duration of the temperature-effective period, for a whole series of alleles. A number of explanations of the action of the Bar gene in relation to facet production have been proposed. For the most part, these explanations serve adequately as formal explanations of the action of the Bar gene for a limited group of data. None, however, is consistent with the whole body of data on facet number in the Bar series, now available. This question will be considered more fully, later.

In the investigation here presented, the effect of the gene, vestigial (*vg*), on facet number in Bar-eyed (*B*) flies was studied. Apart from its conspicuous effect in reducing the size of the wing, *vg* also effects an increase in the duration of development, especially in the egg-larval period. Since an increase in the total time of development may to some extent alter the relative durations of various developmental processes, *vg* may be expected to affect the somatic expression of a variety of characters in some degree.

In addition to the major problem considered, a preliminary investigation on wing area in *vg* and *Bvg* flies at 27°, showed no significant difference. Although this result does not preclude a possible effect of *B* on wing area in *vg* at other temperatures, this phase of the problem was not continued. The plan of experimental procedure in determining the effect of *vg* on facet number in *B* is the following:

1. A comparison of facet numbers in *B* and *Bvg* flies over a range of temperatures.

2. A determination of the length of the period during which temperature is effective in determining facet number in *B* and *Bvg* flies.

The results of the first phase of the investigation are presented here. The results on the temperature-effective period investigation will be considered separately.

EXPERIMENTAL

Materials and Methods

Experimental Stocks

Three mutant races of flies were studied: (1) *B*, (2) *vg*, and (3) *Bvg*. These three groups were rendered isogenic for possible modifiers through inbreeding by pair brother to sister matings over a period of 14 months before the experiments were begun, and involving some 30 to 35 generations. The system of inbreeding used was the following:

P₁. *B* ♂ × *vg* ♀

P₂. Inbreeding of F₁ sibs

P₃. *B* ♂ × *vg* ♀ (obtained from F₂)

In this way the cycle of inbreeding was repeated every two generations. Throughout the inbreeding, selection for low facet number and general vigor was practiced. Unfortunately, selection for the latter was not successful, as the stocks which were finally obtained showed very low fertility and fecundity, and poor viability as evidenced by a fairly high mortality in pupal stages. This was especially true of the *vg* and *Bvg* stock, and necessitated the use of large numbers of flies in all the experiments where short egg-laying periods were required.

Thirty generations of inbreeding should insure a high degree of genetic uniformity in the stocks. An attempt was made to test this point by use of the parent-offspring correlation, in respect to facet number. This proved unsuccessful at first due to the difficulties attendant upon the use of pair matings in these stocks. A second parent-offspring correlation was tried on the *B* stock, a year after inbreeding had been discontinued. This time a sufficient number of offspring was obtained from 20 pairs of parents to make the test possible. The value of the coefficient for male and female respectively was found to be:

$$r \text{ ♂} = 0.17 \pm 0.067, n = 210$$

$$r \text{ ♀} = 0.25 \pm 0.065, n = 210$$

These values are quite small but statistically significant, although the small number of parents on which the values are based, leaves the question of a real correlation open to doubt. However, certain other data indicate that the correlation may be real. In table 1, are given the values for facet

counts on *B* at 28°, for a series of six experiments. This temperature was used as a control throughout the investigation. The measure of sampling errors used throughout the investigation is the standard error, $\frac{\sigma}{\sqrt{N}}$. The individual means scatter quite at random about the grand mean, and for the most part show no significant deviations. The largest difference occurs in the males of experiment 5. Here the deviation is equal to 4.2 times its standard error. This difference, however, does not represent a single difference between two samples, but rather the largest deviation in a group of 12. Here the simpler tests of significance cannot be used. For a group of ten means, a single deviation as great as 3.2 times its standard error lies on the 5 percent level of significance (TIPPET 1931).

TABLE 1
Facet counts at 28° Bar.

EXPERIMENT	M	MALES			FACET RANGE
		σ	v	n	
1	51.7±0.69	7.14	13.8%	106	39-68
2	49.9±0.80	5.70	11.4%	51	41-67
3	49.2±0.95	5.48	11.2%	33	40-59
4	52.0±0.48	5.80	11.2%	146	39-74
5	54.3±0.78	6.20	11.4%	64	41-70
6	47.7±0.69	7.46	15.9%	119	31-77
Mean	50.8±0.29	6.52	12.8%	519	
FEMALES					
1	46.1±0.88	8.0	17.4%	85	33-68
2	48.6±0.96	5.9	12.2%	38	37-63
3	49.8±1.20	6.7	13.4%	32	37-63
4	51.9±0.50	5.5	10.6%	119	38-68
5	51.9±0.94	6.2	11.9%	44	38-64
6	48.3±0.72	7.2	14.9%	101	35-67
Mean	49.4±0.33	6.69	13.5%	419	

According to this criterion the deviation under consideration still appears significant. However, considering the fact that the mean for the females in experiment 5 shows no significant departure from the grand mean, we must conclude that we are probably dealing solely with sampling errors. A comparison of the range of the facet counts in table 1 shows in general no trend, with the exception of the males in experiment 6. The ranges of the facet counts are quite uniform in the different experiments. This constancy is more strikingly illustrated by the coefficients of variability (*v*). Neglecting the data for experiment 6, since the results of this experiment are not included in this paper, it seems clear that no perceptible amount of mutation affecting facet number had occurred during the course of the experiments. If, then, the parent-offspring correlations found are

real, some slight genetic heterogeneity must have been present in the stocks at the completion of inbreeding.

The question may be considered from another point of view. PLUNKETT (1926) pointed out that the absence of a right-left correlation for any character indicated a complete uniformity of all factors affecting that character in the population studied. In the bristles which he was studying, no right-left correlations appeared, showing that all factors which might affect bristles were acting uniformly on all individuals. In table 2, right-left correlations for facet number at a series of temperatures are tabulated.

TABLE 2
Left-right correlations for facet number.

STOCK	T	MALES		M (left)	M (right)
		r	n		
<i>B</i>	20°	0.776±0.080	49	123.9±2.52	123.9±2.66
<i>B</i>	22°	0.677±0.077	49	105.2±2.34	106.0±2.40
<i>B</i>	25°	0.678±0.073	51	78.8±1.19	77.2±1.19
<i>B</i>	27°	0.583±0.067	98	58.9±0.72	58.5±0.70
<i>B</i>	27.8°	0.570±0.060	146	52.1±0.52	52.0±0.48
<i>Bvg</i>	22°	0.669±0.088	39	102.8±2.19	104.2±2.23
FEMALES					
<i>B</i>	20°	0.721±0.070	47	111.2±2.29	113.0±2.17
<i>B</i>	22°	0.577±0.096	48	95.4±2.00	98.7±2.11
<i>B</i>	25°	0.643±0.088	45	76.3±1.66	75.3±1.48
<i>B</i>	27°	0.437±0.100	65	55.9±0.87	56.0±0.92
<i>B</i>	27.8°	0.596±0.059	119	52.2±0.48	51.9±0.50
<i>Bvg</i>	22°	0.828±0.042	55	91.1±2.22	91.3±2.21

At all temperatures the correlations are high. Assuming that the parent-offspring correlations found are real, we should then expect some right-left eye correlation. Precisely what the right-left correlations should be for varying magnitudes of the parent-offspring correlation, is not apparent. An analytical investigation of this question has not been undertaken. In this connection, however, certain data of PLUNKETT (1926) are interesting. In his "*D6*" stock which showed a parent-offspring correlation of 0.412, PLUNKETT found that the right-left correlations between four different bristle pairs were of approximately the same magnitude as the parent-offspring correlation. The mean of the four values was 0.386. It appears then that the right-left correlations on facet number found here are too high to be accounted for by slight genetic heterogeneity, or by differential action of environmental agents on individual flies. In this event the high right-left correlations found would in some measure be due to another factor or factors.

It has been shown that, in general, various parts in *Drosophila* develop

independently. The evidence for this comes largely from the work of REITH (1925), and from the many observations on localized effects of the genotype in the development of various parts in mosaics. It is, however, entirely possible that some structures may show some degree of interdependence in development. There is evidence for this in the case of vermilion eye color (STURTEVANT 1920 b). Moreover, in *Dichaete*, PLUNKETT (1926) found definite associations between certain bristles on the same side of the fly. These associations clearly indicate some type of unilateral developmental dependence.

A mechanism which would explain the interdependence of the two eyes may be formally presented by the assumption that certain substances entering into the formation of the eye are elaborated medially at some early developmental stage, and then distributed to each side. A condition of this type is entirely possible in *Drosophila*, where the primordium of the optic discs appears at first as a single structure located medially and later gives rise to the two frontal sacs which develop into the optic discs (CHEN 1929). The earliest appearance of the optic primordium in *Drosophila* was not actually observed by CHEN, but was established by analogy with *Rhagoletis* (SNODGRASS 1924). Further speculation on this point would not be profitable since no critical evidence is at present available. It should be pointed out, however, that although the absence of any right-left correlation definitely indicates uniformity of both genetic and environmental factors affecting the character in question, the presence of a correlation does not necessarily imply a lack of uniformity of these same factors.

Methods

The culture medium used in all experiments was the usual banana-agar preparation, consisting of equal parts of water and banana, and 2 percent by weight of the total of agar-agar. An effort was made to secure bananas of the same degree of ripeness for all experiments. Four by one inch shell vials were used in all the experiments. Approximately 15 cc of food was placed in each vial and then inoculated with a drop of a thick yeast suspension (one cake of yeast to 100 cc of water). The food was never used until the day following its preparation, in order to insure an adequate yeast growth. Ten pairs of flies per vial were permitted to lay eggs over a period of two hours. It should be pointed out that the use of a large number of flies for egg-laying tends to minimize any differential effects resulting from genetic differences which may be present in the individual flies. Flies of 4 to 5 days of age were used for egg-laying as this is very near the optimum age for egg-production. To avoid possible crowding effects only vials in which less than 25-30 flies developed were used. LUCE (1931)

reported that crowding causes a marked diminution in facet number in infra-bar, an allele of Bar. In my own experiments, a parallel, though much smaller effect, was found. As a rule, less than 50 flies developing per vial does not produce any decrease in facet numbers in Bar.

All the temperature experiments were carried out in incubators in which temperature variations were limited to a range of $\pm 0.1^\circ$. For most temperatures the variation was much less. In the few cases in which temperature fluctuation was known to be of a magnitude sufficient to account for aberrant results, the experiments were discarded.

FIGURE 1
The facet-temperature relation ($\sigma^7 \sigma^7$)
B—solid line
Bvg—broken line

At most temperatures, facets were counted directly under a Bausch and Lomb dissecting microscope, using a $17\times$ ocular and 25 mm objective. At 13.7° , 15° , and 16° , dissections of the cornea were made and the facets counted on a projecting apparatus, as the eyes were too large to count directly. Error in counting was checked by making recounts of a number of flies at different temperatures. In all cases the personal error was somewhat less than 1 percent.

Temperature Experiments

Effect of Temperature on Facet Number

The effect of temperature on facet number in *B* and *Bvg* was studied at 1° or 2° intervals over the complete range of temperatures in which the flies are able to develop. The essential statistics on facet number at different temperatures are presented in table 3. The data are recorded separately for males and females, since there is definite sexual dimorphism for facet number as, in fact, for most other characters of *Drosophila* which have been studied quantitatively. In figures 1 and 2, the facet-temperature

FIGURE 2
The facet-temperature relation (♀♀)
B—solid line
Bvg—broken line

relation is shown graphically. The effect of *vg* on facet number in *B* is apparent. At all temperatures (with exception of *Bvg* males at 18°) *vg* effects a small but consistent reduction of facet numbers. The effect is somewhat greater in the females than in the males. In the males the facet differences between *B* and *Bvg* are not statistically significant at temperatures below 22° or at 30.4°, but with one exception noted above, the differences are always in the same direction. The facet differences between *B* and *Bvg* in the females are not statistically significant at 13.7° or at 20°, but are always in the same direction. It must be concluded that the effect

of *vg* on facet number in *B*, though small, is real. MAY (1917) in selection experiments on facet number in *B* and *Bvg* flies, observed that, in general, facet number was slightly lower in *Bvg*. Since his stocks were not isogenic with each other, and temperature was inadequately controlled, his observations can hardly be considered as supporting the results presented here, though they are quite in agreement. The reason for the departure of *Bvg* ♂♂ and ♀♀ at 24° from the general trend of the curves is not clear. Some experimental error is probably involved.

The facet-temperature relation in the different members of the Bar series has been extensively investigated. SEYSTER (1919), KRAFKA (1920), and DRIVER (1931) have all observed an inverse relation between temperature and facet number. There are, of course, differences in the form of the curves obtained by these investigators. These, no doubt, are due to genetic differences in the stocks used. The temperature-facet relation presented here for *B*, conforms most nearly to that found by KRAFKA. LUCE (1926) found that infra-bar, an allele of *B*, behaves quite differently in respect to temperature. Facet number increases in infra-bar with increase in temperature, showing a maximum between 27° and 30°, and then decreases. This fact is pointed out here since it is of considerable importance in any interpretation of the action of the *B* gene, as will be shown later.

The statistics other than the mean in table 3 are of some general interest. The coefficient of variability (*v*) which we have taken as σ/M expressed as a percent, is a good measure of the relative variability of a population. The values of *v* (table 3) for male and female show no systematic differences. This is true, also, for *B* as compared with *Bvg*. Moreover, there is no indication of a trend in *v* with temperature. In respect to this last point, these data differ from those of KRAFKA (1920) who finds a systematic increase in *v* with increase in temperature. On the other hand, DRIVER's data (1931) show that *v* is constant at all temperatures except 30°. The relative constancy of *v* for facet number in the data of table 3 is emphasized here, since it bears upon certain considerations in the investigation on the temperature-effective period.

Duration of Egg-Larval Period

The criterion used in determining the end of the larval period was the eversion of the anterior spiracles. Individuals which had completed the egg-larval period were transferred to agar slants at two hour intervals to complete their development. At 16.1°, a four hour interval between observations was used. The sex ratios were determined at the time of emergence from the pupa cases.

The data on duration of the egg-larval period for *B* and *Bvg* are presented in table 4. The general relation between temperature and length

TABLE 3

T	MALES				FEMALES			
	M	σ	v	n	M	σ	v	n
13.7°	260.7±4.4	32.4	12.4%	55	253.2±9.1	43.7	17.3%	23
15°	218.8±5.1	32.3	14.8%	41	215.9±4.1	24.2	11.2%	35
16.1°	180.0±2.7	26.9	14.9%	97	175.5±4.1	22.3	12.7%	30
18°	146.6±3.5	22.7	15.5%	43	150.4±4.6	26.7	17.8%	33
20°	126.9±3.2	19.1	15.1%	35	122.5±2.8	14.3	11.7%	30
22°	107.8±1.4	18.3	17.0%	176	102.4±1.4	14.8	14.5%	106
24°	84.0±1.3	11.4	13.6%	74	68.1±1.5	8.3	12.2%	30
25°	78.2±1.2	10.3	13.2%	75	71.7±1.3	7.5	10.5%	33
25.8°	70.1±1.8	11.4	16.3%	78	60.2±1.1	7.1	11.8%	39
26.8°	58.9±0.6	7.5	12.7%	143	54.4±0.8	5.7	10.5%	55
28°	50.8±0.3	6.5	12.8%	519	47.1±0.6	6.0	12.7%	117
29°	41.8±0.5	3.7	8.9%	48	39.9±0.7	3.8	9.4%	31
30.4°	32.7±0.9	4.4	13.3%	26	32.4±0.8	3.0	9.3%	14
13.7°	205.8±3.9	28.9	14.0%	56	195.7±7.8	39.9	20.4%	26
15°	190.5±5.3	28.3	14.9%	29	165.7±4.5	18.6	11.2%	17
16.1°	154.4±2.3	22.4	14.8%	94	138.4±2.9	15.6	11.3%	28
18°	130.6±3.2	17.0	13.0%	28	123.7±3.3	19.0	15.4%	33
20°	112.8±2.2	16.4	14.5%	55	109.1±2.3	14.0	12.9%	36
22°	102.4±1.2	15.1	14.7%	150	92.4±1.4	15.7	17.0%	119
24°	82.1±1.1	12.3	15.0%	120	63.2±0.7	5.7	9.0%	64
25°	77.2±1.3	10.6	13.7%	72	66.1±1.1	6.8	10.3%	40
25.8°	70.9±1.6	11.2	15.8%	47	56.5±1.1	5.9	10.4%	28
26.8°	56.3±0.7	7.5	13.3%	106	52.0±0.8	6.1	11.7%	55
28°	49.4±0.3	6.7	13.5%	419	45.3±0.6	5.2	11.5%	86
29°	41.2±0.5	3.6	8.8%	61	37.0±0.7	4.2	11.4%	36
30.4°	32.9±0.6	3.5	10.5%	35	29.3±0.5	2.7	9.2%	26

TABLE 4

Duration of egg-larval period.

T	MALES				FEMALES			
	M	σ	v	n	M	σ	v	n
16.1°	297.3±1.40	11.6	3.9%	69	297.1±1.57	13.5	4.5%	74
22.0°	143.6±0.38	6.0	4.2%	247	142.6±0.33	4.9	3.4%	212
27.0°	97.7±0.50	3.6	3.7%	53	97.2±0.53	3.6	3.7%	47
28.0°	95.4±0.44	3.1	3.2%	49	95.6±0.72	4.5	4.7%	39
29.0°	93.9±0.66	3.8	4.0%	35	95.5±0.52	3.2	3.4%	39
*30.2°	98.8±0.21	2.6	2.6%	176				
<i>Beg</i>								
*22°	150.3±0.98	8.1	5.4%	68				
27°	101.9±0.66	4.2	4.1%	41	102.2±0.63	3.9	3.9%	39
28°	97.7±0.57	3.4	3.5%	36	98.0±0.98	4.7	4.8%	23
29°	96.7±0.52	3.0	3.1%	33	96.6±0.68	3.4	3.5%	26

* Males and females were not recorded separately.

of the egg-larval period is the same as that found by other investigators. Time of development decreases with increase in temperature, with a minimum at about 29°. Beyond this point the time increases slightly. It may be seen, further, that *vg* increases the time for egg-larval development. HARNLY (1929) found that *vg* increased the duration of the egg-larval period about 5.5 hours at 25°C. He found, also, that there is little, if any, effect of *vg* on the pupal period. Although no data for 25° are available in table 4, the values at 22° and 27° for *B* and *Bvg* are of a magnitude entirely consistent with HARNLY's results.

No sexual difference in duration of the egg-larval period was found at any temperature in the present experiment. In this respect the data confirm the observations of LUCE (1931) on *infra-bar*. They differ, however, from the observations of other investigators. POWSNER (unpublished), and CHILD (1935) working in this laboratory on *Drosophila* stocks unrelated to each other or to mine, found that at all temperatures the males have a shorter egg-larval period than the females. On the other hand, BONNIER (1926) observed that at 30° the egg-larval period for the females was shorter, but found no sexual difference at 25°. The disparity in the results of different investigations is, no doubt, attributable to genetic differences in the stocks.

Discussion

Any interpretation of the effect of *vg* on facet number in *B*, necessitates first a consideration of the action of the *Bar* gene itself in facet production. From the observed facts on facet number in *B*, at least two simple possibilities are indicated. (1) We may visualize the processes initiated by *B* as forming part of the general facet-producing system, but resulting in a slower production of facets. (2) Alternatively, *B* may initiate processes which act entirely independently of the facet-producing mechanism. These processes may act in a manner which results in the "destruction" of facet-forming material. Either of these systems equally well explains the facet decrease in the presence of *B* or any of its alleles. Moreover, either system can be adjusted to explain the facet temperature relations observed in the *B* series. However, a consideration of the possible effects of factors which may alter the duration of the facet-determining processes, shows a significant difference in the two systems. According to (1) an increase in the duration of the processes would effect an increase in facet number, while in (2) an increase in duration would result in a decrease. As will be shown, (2) is consistent with all the known facts relating to the *Bar* series, and lends itself, moreover, to an interpretation of the results presented here.

KRAFKA (1920) explained the facet-temperature relation in *B* on the basis of two independent sets of processes occurring in facet determination.

One is a facet-producing system, and the other a facet-reducing system. The facet number which develops in *B* is then the resultant of these two sets of processes. In explaining the temperature effect on facet number, it was assumed that the temperature coefficient of the second set of processes was greater than that of the first. This explanation was entirely in accord with the facts then known. It does not, however, take into consideration the question of duration of the processes involved in facet formation. ZELÉNY (1923) in discussing the effect of temperature on facet number, states, "The change in ommatidial number with change in temperature can be explained most satisfactorily by assuming a differential effect of temperature upon the physiological processes involved in ommatidial production as opposed to other physiological processes. In view of the fact that temperature is effective only during a few hours of larval life, it may be considered that the initial steps in the formation of ommatidia are confined to a definite embryological period. The length of this period is determined by the general physiological processes of the larva, while the rate of formation of ommatidia during the period is a function of special processes which have a different coefficient. It is evident that under these circumstances two different temperatures must give two different ommatidial numbers."

The following simple system formally illustrates the relations of the two sets of processes postulated by KRAFKA, and in addition the role of factors which govern the duration of these processes.

In the above diagram we have three independent sets of processes, which we may consider as irreversible reactions. We are not at the moment, concerned with the exact nature of the three sets of reactions illustrated, but rather with their interrelations. The production of substances E, F, and D, from A, C, and B, respectively, is purely schematic. Quite probably each reaction would be more accurately represented by a reaction chain. 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 facets. The assumption that this reaction occurs in the Bar series is not gratuitous. The work of BRIDGES (1917), and of STURTEVANT (1925), shows that the normal allele of *B* plays no part in facet production, and that consequently other genetic factors are concerned. The broken line from F to ommatidia denotes a morphological transforma-

tion of a region containing F into facets. This transformation, based upon the facts of ontogeny, occurs "instantaneously" in the sense that all facets are visibly differentiated at the same time. Reaction (3) is not present in the wild-type, but is found in members of the Bar series. This reaction initiated by B leads to the formation of a substance D which combines with F to form X . It should be pointed out that for our present purpose D may equally well be considered as a catalyst, catalyzing the reaction $F \rightarrow X$. The combination of F and D is the "facet-destroying" process previously mentioned. We are not concerned with the nature of X , except to note that its production effects a diminution in F . In considering the time relations of reactions (2) and (3), we may assume that F is produced at some early stage of development, and in sufficient quantity to give the facet number of the wild-type. Reaction (3), then, occurs somewhat later so that F combines with D as the latter is being formed. It should be pointed out that the time relations for the reactions here presented are not essential to the general theory, and can only be established by other experimental data. If the conditions postulated are correct, the time-facet relation will be sigmoid in character.

Reaction (1), resulting in the formation of a substance E , represents really the aggregate of all developmental processes which determine the time at which the region containing F is converted into facets. These processes occur, of course, in all stocks, but will vary considerably according to the genetic constitution of the stocks.

It is of interest to note what factors limit the duration of the reaction $F + D \rightarrow X$. We have assumed that F is produced and localized at some early developmental period, that is, prior to the beginning of reaction (3). Consequently, the reaction $F + D \rightarrow X$ begins when D is produced and begins to combine with F . The factors determining the end of the reaction are, as we have seen, entirely independent of D and F , but depend rather on a great many other processes of general development which determine when differentiation of the optic disc into ommatidia may take place. This obviously implies that the reaction $F + D \rightarrow X$ need not go to completion. PLUNKETT (1932) has pointed out that very probably a great many developmental reactions controlled by mutant genes do not actually go to completion, but may be terminated by extrinsic factors.

The facet-temperature relations observed in the Bar series may now be considered in relation to the system of reactions postulated. If the temperature coefficient for reaction (1) is less than that for the formation of X , an increase in temperature will produce a decrease in facet number. This relation is observed in B and BB . If, on the other hand, the temperature coefficient for reaction (1) is greater than that for the formation

of X, facet number will increase with increase in temperature. This is the condition found in B^i and B^iB^i over the greater portion of the temperature range in which the flies are viable. LUCE's observation that B^i shows a maximum facet number between 27° and 30° may very well be related to the fact that time of development increases at temperatures above 29°. As a consequence, in B^i the "facet-destroying" reaction ($F+D\rightarrow X$) should take place over a relatively longer period of time at high temperatures, and result in a decrease of facet number with increase in temperature, above 29°.

The effect of vg on facet number in B may now be interpreted. It has been shown that vg increases the length of the egg-larval period. Since a retardation in the rate of general development will to some extent retard the rates of those developmental processes which determine the time at which F is converted to facets, we should expect the reaction $F+D\rightarrow X$ to take place over a somewhat longer period of time in Bvg than in B . This, clearly, will result in a diminution of facet number in Bvg as compared with B , a fact already observed. It seems quite probable that crowding acts in this same way, that is, by retarding development.

It is, of course, desirable to test more directly the effect of vg on the duration of the reaction $F+D\rightarrow X$. The work of KRAFKA (1920), and especially that of DRIVER (1931), and LUCE (1931) has demonstrated that the effect of temperature on facet determination in the Bar series is restricted to a definite portion of larval life. Moreover, KRAFKA (1924) and CHEN (1929) have shown that the end of the temperature-effective period corresponds very closely to the time at which ommatidia are differentiated as discrete units. These results make possible an identification of the reaction $F+D\rightarrow X$ with the temperature-effective period. We cannot, of course, dismiss the possibility that other reactions involved in facet formation are also affected by temperature. The results of R. K. HERSH (1924) on the temperature-facet relation in the wild-type fly show such an effect of temperature, although much smaller than that found in the Bar series. Speculation on this point is hardly profitable since the validity of the above identification rests to some extent on the correctness of the time relations of the different facet-determining processes here postulated. For this purpose a knowledge of the temperature-effective period for facet determination in the wild-type fly is essential.

If the identification of the reaction $F+D\rightarrow X$ with the temperature-effective period is correct, then we may test the interpretation given here of the effect of vg on facet number in Bar, by determining the length of the temperature-effective period in B and Bvg . The results of this phase of the investigation will be submitted in a separate communication.

SUMMARY

1. An investigation of facet numbers in *B* and *Bvg* flies over a range of temperatures, showed that *vg* effected a small but significant decrease in facet number.

2. Three sets of reactions which formally explain the facet-temperature relations observed in the Bar series are postulated. In this system of reactions, the essential difference between wild-type and Bar is the presence in the latter of a "facet-destroying" reaction, $F + D \rightarrow X$, in which F represents facet-forming material, and D a substance whose formation is initiated by the Bar gene.

3. The effect of *vg* on facet number in *B* is interpreted as resulting from an increase in duration of the reaction, $F + D \rightarrow X$, thereby decreasing the quantity of F available for facet formation.

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