THE EFFECT OF TEMPERATURE UPON FACET NUMBER IN THE BAR-EYED MUTANT OF DROSOPHILA.*

PART II.

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Determination of the Period during Which Temperature is Effective in Modifying the Facet Number.

Preliminary Experiments on Unselected Bar Stock at 30 and 15°.

A preliminary experiment was planned to determine whether temperature had an effect throughout the immature stages or whether it was limited to a specific phase of development. In Table XIV[1] are shown the results of subjecting successive cultures for the 1st day, first 2 days, first 3 days, and so on to 30° before subsequent development at 15°. 3, 2, and 1 days at 30° give the same facet number as those raised at 15° throughout. The early days of larval life may be spent at high temperatures without effect on the facet number.

6 and 7 days at 30° followed by transfer to 15° show that the number of facets had been determined prior to the transfer, as indicated by the fact that all the counts come well within the range of the stock counts at 30°.

Next, if we consider only those counts made on the 1st day of hatching, the bottles that were kept at 30° for the first 4 and 5 days show only the facet counts characteristic of the 30° stocks. The counts made on the flies hatching on the 2nd and 3rd days are inter-

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[1] Krafka, J., Jr., *J. Gen. Physiol.*, 1919-20, ii, 409.

mediate between the 30 and 15° stocks. These intermediates were to be expected. The parents were not removed from the bottles until the end of the first 24 hours. Hence some of the larvæ may have been 24 hours older than others. Those 4 days old had already passed the point X in development during which the facet number-determining reaction is going on. They are also the first individuals in the bottle to hatch. Those hatching later were not so far along in development and hence were affected by the transfer to 15°.

Obviously temperature is not capable of modifying the facet number throughout the immature stages, but is limited to a definite stage in development.

Subsequent experiments on Ultra-bar consider these points more in detail.

Effect of Temperature during the Pupal Period.

Experiments of the same type as the preceding were carried out on Ultra-bar at 27 and 15°. We will consider the effects of temperature during the pupal period first. Subjecting the cultures to 27° for 5, 6, 7, 8, and 9 days before subsequent removal to 15° gave counts which are characteristic of the 27° stocks both in mean and in individual variation. The first four cultures had pupated before the transfer to the colder temperature was made. In the last one the imagos had begun to emerge from pupation as the transfer from 27 to 15° was made. Obviously in the last case a change of temperature would have no effect, since there is no further change in the number of facets after the adult is once formed.

From the distribution of the counts in Table XV it is just as clear that subjecting the immature stage to low temperature after pupation has no effect on the facet number. This is a particularly important point, since the facets themselves first become evident only toward the end of the pupal period. The reaction which has determined the number of facets that are to form has greatly preceded the actual formation of these facets (Fig. 11).

TABLE XV.

Experiment 45, Showing the Distribution of Facet Numbers. Larvæ Were Allowed to Complete Part Development at 27° and Were then Transferred to 15° for the Remainder.

No. of days at 27° before transfer to 15°.....	1		2		3		4		5		6		7		8		9	
Facet class.	Distribution of individuals in separate bottles.																	
	Larval period.								Pupal period.									
7–10	*	*					4	1		1								
11–14							4	2	7	2	2							
15–18						1	8	6	15	8	4	1	7		4	1	6	3
19–22							23	11	19	17	9	5	21	9	14	9	14	6
23–26					2		8	12	7	7	9	15	17	18	21	28	16	17
27–30					7	3	2	6		3		2	2	8	5	12	2	7
31–34					3	3	6	7								1		1
									Upper range of 27° stock counts.									
35–38					6	3	2	2										
39–42		1	2		8	4												
									Lower range of 15° stock counts.									
43–46	5	0	16	2	8	7												
47–50	3	1	12	7	3	4												
51–54	1	2	11	10	3	8												
55–58	1	2	8	16	1	7												
59–62	1	4	1	5		2												
63–66	1	0		6		1												
67–70		1		2		1												
71–74				2		1												
Total No. of individuals....	12	11	50	50	41	45	57	47	48	38	24	23	47	35	44	51	38	34

* The first column represents females; the second, males.

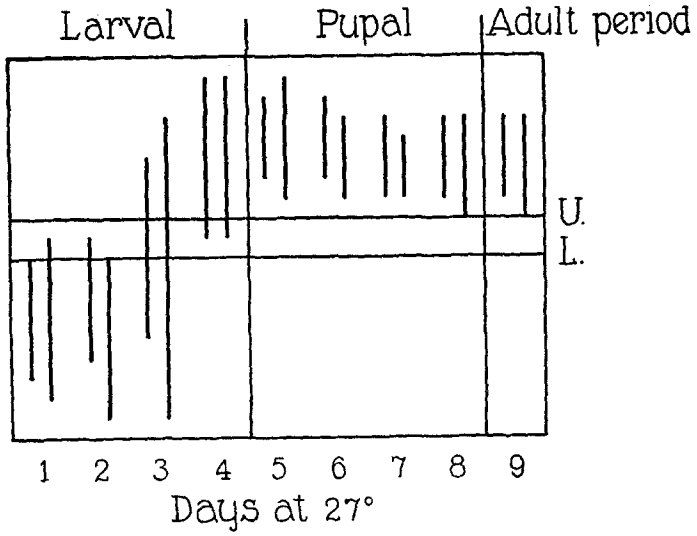

FIG. 11. Effect on the distribution of facet counts as a result of subjecting successive cultures from the same parentage of Ultra-bar to varying periods of time at 27° before subsequent development at 15°. After mating the first bottle was put at 27° for 24 hours. The parents were then removed to the second bottle. The first bottle was allowed to complete development at 15°. At the end of 24 hours, the parents were removed to a third bottle, etc. The second bottle remained at 27° for 48 hours, and completed development at 15°. The third bottle remained at 27° for the first 3 days, etc. The first vertical line of a pair represents the range of the female counts, the second is that of the males. Those cultures to the left of the first full vertical line were in the larval stage when the transfer from 27 to 15° was made. Those between the two full vertical lines were in the pupal stage when the transfer was made (see Table XV). U. is the upper range of 27° stock counts; L., the lower range of 15° stock counts.

Effect of Temperature during the Larval Period. Initiation and Duration of the Effective Period at 27°.

An examination of Table XV shows that a temperature of 27°C. for 1 or 2 days at the beginning of the larval period has no effect on the facet number, as only three individuals out of 123 are slightly outside the range of the stock experiments at 15°.

Of those that passed the first 3 days at 27°, some had developed beyond the point X, at which facet number is determined, since their counts are characteristic of the 27° stock. Some were just in the effective period when the transfer from 27 to 15° was made, as shown by the fact that the counts are intermediate between those characteristic of the 27 and those of the 15° stocks. Some individuals were not quite so far along and hence the whole facet number-determining reaction was carried out at 15°.

4 days at 27° brought nearly all individuals through the effective period. Four individuals out of 104 are slightly above the upper range of the 27° stock counts. We may conclude that the stage in development, at which the facet number is being determined, is passed prior to the end of 4 days at 27°. Likewise this stage is not reached by the end of 2 days at 27°.

To define this period more closely Experiment 59 was planned. After many unsuccessful attempts to get a series in which the eggs were not more than 1 hour apart in age, the present series was carried through. Here the parents were allowed to lay eggs during a period of 12 hours. This series includes the following number of days at 27° with subsequent removal to 15°; 1 day (24 hours), 2, $2\frac{3}{4}$, 3, $3\frac{1}{4}$, $3\frac{1}{2}$, $3\frac{3}{4}$, and 4 days. The results are given in Table XVI and in Fig. 12.

This experiment bears out the previous one in that 1, 2, $2\frac{3}{4}$, and 3 days at 27° did not bring the larvæ up to the point X. This phase of development is initiated between 3 and $3\frac{1}{4}$ days as shown by the intermediate condition of the counts of the latter. These are predominated by the lower temperature. $3\frac{1}{2}$ days at 27° give a preponderance of individuals with the 27° count. $3\frac{3}{4}$ days at 27° have brought all but one individual through the effective period. After 4 days at 27°, all individuals had completed this stage in de-

velopment and further changes in temperature had no effect on the facet number thereafter.

A second culture, allowed to develop 3 days at 27°, demonstrates individual variation in rate among separate bottles. In this culture the effective period was passed by thirteen individuals at 27°,

TABLE XVI.

Experiment 59, Showing Distribution of Facet Numbers. Larvæ Were Allowed to Complete Part Development at 27° and Were then Transferred to 15° for Remainder.

No. of days at 27° before transfer to 15°.	1		2		2½		3 (I)		3 (II)		3½		3½		3¾		4		27° Control.	
Facet class.	Distribution of individuals in separate bottles.																			
15–18													5		2		3		6	3
19–22									2				3	1	8	4	8	5	36	23
23–26									3				7	3	11	11	11	14	37	38
27–30									4		1		4	10	3	7	3	11	19	32
31–34	Upper range of 27° stock.								2	2	4	1	8	5		1			4	3
35–38									4	7	3	1	12	4		1				
39–42					1		1		9	5	3	2	12	9	Lower range of 15° stock.					
43–46	1		1	2	1	1			7	3	7	7	3	5						
47–50	4		6	2	4	1	9		9	4	4	2	2	6						
51–54	17	2	11	3	9	11	9	3	13	7	2	6	2	5						
55–58	12	7	4	4	8	9	5	1	1	3	3	2	2	1						
59–62	6	7	6	6	6	5	2	4	2	4	1	3	0	4						
63–66	4	7	2	3	3	6	2	11	2	3		2	1	2						
67–70	3	10	2	5		2	1	7		3	1									
71–74	3	5		1				4		2		1								
75–78		2																		
79–82								1												

before the transfer was made; and in many others the counts are intermediate showing that the reaction was going on at the time of the transfer from 27 to 15°.

A control mating by the same parentage as the foregoing series was reared at 27°. It shows the normal distribution of the 27° stock.

According to this and the previous experiment, the reaction, which determines the number of facets and whose rate is subject to temperature modification, is initiated at 27° at or near the end of 3 days of development. Furthermore, it is practically over at the end of 3¾ days, making the effective period of less than 18 hours duration.

The actual time during which the reaction which determines the number of facets takes place is doubtless much shorter than this for

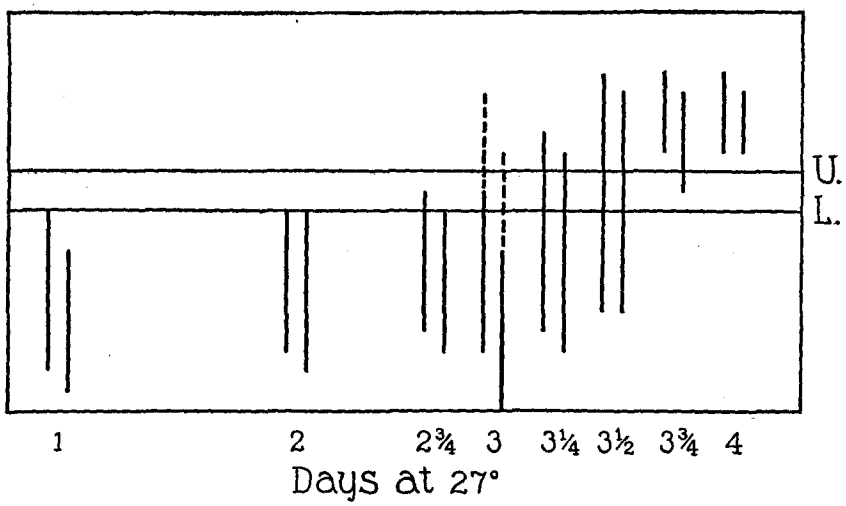

FIG. 12. Effect on the distribution and range in the Ultra-bar as a result of subjecting successive bottles to 27° for 1, 2, 2¾, 3, 3¼, 3½, 3¾, and 4 days, respectively, before removal to 15° (see Table XVI). Broken line represents extension of range in a second culture kept at 27° for 3 days. U. is the upper range of 27° stock counts; L., the lower range of 15° stock counts.

the individual, since under the conditions of the experiment we are dealing with material that may differ 12 hours in age.

Initiation and Duration of the Effective Period at 15°.

Experiment 62, of the same type as the preceding except that the conditions were reversed, was carried out to determine when the effective period was initiated at 15°. The same parents were used as in Experiment 59. The cultures were made up at 27° and left

for 24 hours. The parents were then removed to the next set of bottles, while the bottles containing the eggs and larvæ were transferred to 15°. Here they were left for the number of days indicated in Table XVII and then returned to 27° to complete development.

TABLE XVII.

Experiment 62, Showing Distribution of Facet Numbers. Larvæ Were Allowed to Complete Part of Development at 15° and Were then Transferred to 27° for Remainder.

No. of days at 15° before transfer to 27° *	1		2		3		4		5		6		7		8	
Facet class.	Distribution of individuals in separate bottles.															
11–14			4						2		1		1			
15–18	5	1	8		5	1	7	2	15	5	13	7	3	1		
19–22	15	16	35	16	25	25	23	15	18	21	17	22	4	6		
23–26	23	26	50	63	15	18	26	32	6	18	8	7	3	1		
27–30	2	12	7	21	3	9	4	12		2	1	1		1		
31–34	Upper range of 27° stock counts.				2		1								1	
35–38																
39–42	Lower range of 15° stock counts.														1	
43–46															1	
47–50															1	
51–54															1	
55–58																
59–62																1
63–66																
67–70																1
71–74																
75–78																
79–82																

* The bottles were at 27° the first 24 hrs.; then removed to 15° for the number of days indicated before return to 27°. Parents are the same as in Experiment 59.

It is plainly evident that the effective period is not initiated at the end of 3 days as at 27°. The point X is not reached even at the end of 7 days at 15° plus the 1st day at 27°. In Experiment 62, the change comes on the 8th day at 15° (Fig. 13). Unfortunately this experiment was not planned to cover a longer period; a second experiment was therefore started.

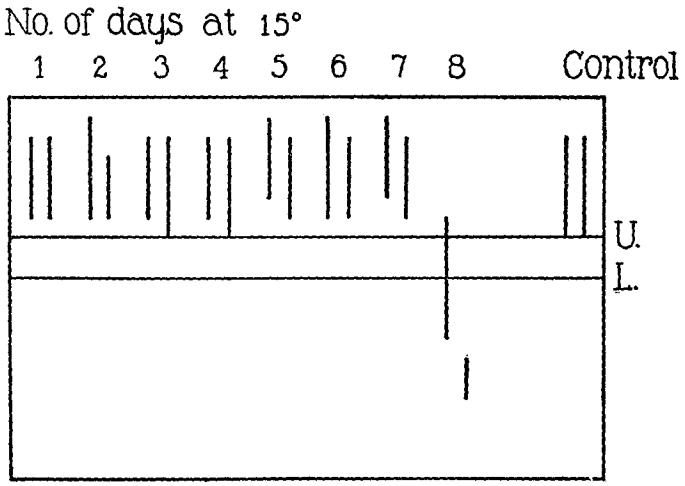

FIG. 13. Effect on the range of facet counts in Ultra-bar as a result of subjecting successive cultures from the same parents for varying periods to 15° before subsequent development at 27° (see Table XVII). U. is the upper range of 27° stock counts; L., the lower range of 15° stock counts.

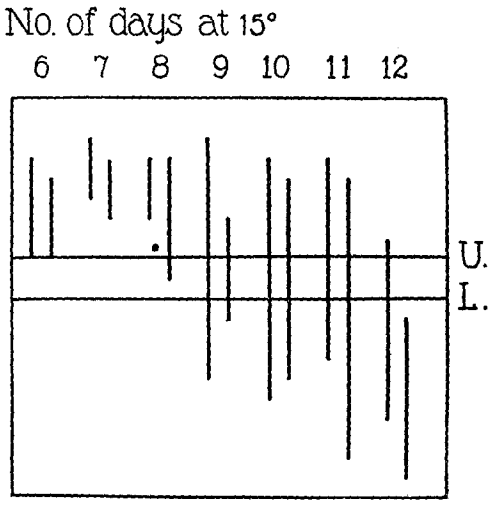

FIG. 14. Duration of the effective period at 15°. Effect on the range of facet counts as a result of subjecting successive cultures from the same parentage for varying periods of time to 15° before subsequent development at 27° (see Table XVIII). U. is the upper range of 27° stock counts; L., the lower range of 15° stock counts.

Experiment 72 supplies the data on the length of the period at 15° (Table XVIII and Fig. 14). As pointed out for Experiment 59 there may be a slight difference in the rates of the individual bottles. Experiments 62 and 72 show such a variation. In Experiment 62, the period was initiated on the 8th day. In Experiment 72, only two

TABLE XVIII.

Experiment 72, Showing the Distribution of Facet Numbers. Larvæ Were Allowed to Complete Part Development at 15° and Were then Transferred to 27° for the Remainder.

No. of days at 15° before return to 27°.*	6	7	8	9	10	11	12	
Facet class.	Distribution of individuals in separate bottles.							
11–14		1		2				
15–18	1	2	3 2 1	1	1	1		
19–22	6 6	4 4 5	3	0	3	1 1	1	
23–26	1 2	2 5	1	2	3	4 3	2	
27–30			2	4 4	2	3 4	3	
31–34	1 1		2	3 2	1	3 2	1	Upper range of 27° stock.
35–38				1 3	1	1 3	1	
39–42			1	2	2	1 2	1 3	Lower range of 15° stock.
43–46				1 1	1	2	3 7	
47–50					2	1	8	3
51–54					2	1 2	2 16	7
55–58				1		2	1 6	8
59–62					1		2 3	14
63–66							•1	6
67–70			1				1	8
71–74							1	1
75–78								1

* All these cultures were at 27° for the first 24 hrs. They were then removed to 15° for the number of days indicated at the top of the table and allowed to complete development at 27°.

individuals had passed the effective period at 15° when the transfer was made from 15 to 27°. After 9, 10, and 11 days an increasing number of individuals show the 15° count, and after 12 days only five of 94 individuals had not completed the facet number-determining reaction when the transfer was made.

As there may be 24 hours difference in age among the individuals in a bottle, the period at 15° is practically limited to the 9th, 10th, and 11th days, or the length of the period at 15° is about 72 hours.

Comparison of the Length of the Period during Which Temperature is Effective on Facet Number with the Total Length of the Immature Stage.

It has been shown that the period during which temperature is effective is initiated at two very remote time intervals when development is carried out at 15 and 27°, respectively. Does this period represent a definite stage in development?

The total number of days required to complete development of the immature stages at 15° is 31.87, while at 27° it is 9.21. This gives a daily rate of 3.13 per cent total development at 15° and 10.86 per cent total development at 27°. With these rates we may calculate the point X.

3 days at 27°	$3 \times 10.86 =$	32.58 per cent
8 " " 15°	$8 \times 3.13 =$	25.04 " "
1 day " 27°	$1 \times 10.86 =$	10.86 " "
		35.90 " "

In other words, the reaction which determines the number of facets that will develop in the adult is initiated at the completion of 32 to 36 per cent of immature development.

Comparing now the length of the periods during which this reaction is going on at 15 and at 27° with the total length of the immature stages at those temperatures, we find 18 hours at 27° and 72 hours at 15° as opposed to 9.21 days at 27° and 31.87 days at 15°. These ratios are a fair approximation considering the experimental conditions.

Expressing the length of the effective period in percentage of the total development and adding the results to those for the initiation of the period, we find that the reaction which determines the number of facets starts at the completion of 32 per cent of development and ends with the completion of 45 per cent.

Thus the length of a particular phase of development is proportional to that of any other phase. Reciprocally, the rate of a given

reaction such as that which determines facet number is proportional to the rate of general metabolism.

In the first part of this study we noted a correlation between facet number and length of the immature stage when the latter was completed at a single temperature. Subsequently it appeared that facet number and the total length of the immature period were independent. Why do we find this apparent correlation?

The facet-determining reaction has been shown to be of relatively short duration. It is obvious that a change in temperature following its completion could affect the total length of the immature period without affecting the facet number.

We may conclude that the number of facets and the length of the immature period are not directly dependent but rather that the former is determined by a specific reaction the rate of which is correlated with that of the general metabolic processes extant while this particular phase of development is going on.