THE EFFECT OF ALTERNATING TEMPERATURES ON THE PUPAL DEVELOPMENT OF DROSOPHILA MELANOGASTER MEIGEN

(One figure)

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THE generalization has frequently been made that varying temperatures have a stimulating effect on development as compared with their mean constant temperature. Shelford (1927) found that variable, as compared with constant temperatures, accelerated all developmental stages of the codling moth. Proportional increases were as noted as follows: eggs, 7 per cent; larvae, 8 per cent; and pupae, 7 per cent. Cook (1927) found that when first instar larvae of the cutworm, *Porosagrotis orthogonia*, were alternated between 8° C. and a high temperature an acceleration of the development resulted. Peairs (1927) observed a definite acceleration in the development of blow fly larvae exposed to temperatures changed at 6–12 hour intervals. Parker (1930) showed that in the nymphs of the grasshopper, *Melanoplus mexicanus*, alternating between 22° C. and a higher temperature such as 27° caused an acceleration of 12.4 per cent. When 12° was used as the low temperature, the acceleration was 45.8 per cent.

A few experiments, however, tend to show that varying temperatures may also produce a retardation of development. Headlee (1914) studied the development of the grain aphis, *Toxoptera graminum*, and found that a constant temperature was more effective in development than a fluctuating temperature with the same mean. In 1929 he obtained similar results with the codling moth, *Carpocapsa pomonella*.

Ludwig (1928) suggested that, in the Japanese beetle, the effect produced by alternating temperatures may depend upon the temperatures involved.

1. If one of the temperatures is above the optimum of development and the other is between the threshold and the optimum, the rate is retarded. He believed that this may be explained by the assumption that, while the organisms are exposed to temperatures above the optimum, their development is retarded to such a degree that the total developmental time is greater than at the corresponding constant temperature.

2. If both of the temperatures used are between the threshold and the optimum of development, neither an acceleration nor a retardation of rate is produced.

3. If one of the temperatures involved is below the threshold of development and the other is between the threshold and the optimum, the rate is accelerated.

The present study is an attempt to test the validity of these postulates by determining the effect of daily alternations between two temperatures on the duration of the pupal stage of *Drosophila melanogaster*.

MATERIAL AND METHODS

The stock of *Drosophila* used in these experiments was secured from Dr. C. R. Plunkett of New York University, and had been inbred for several years. The standard banana-agar culture medium was employed. The cultures were kept in an incubator at approximately 25° C., and development up to the pupal stage was allowed to take place at that temperature. To facilitate the transference of pupae, the flies were cultured in one-half pint mayonnaise jars. Since no filter paper was added, the larvae pupated on the sides of the jar and could be removed very readily. Pupae were collected every hour, so that, when obtained, they were always within the first hour after pupation. When the larvae became quiescent and protruded the anterior horns, they were identified as pupae. Each pupa was placed in a separate test tube, in the bottom of which was a wad of moist cotton. The tubes were then stoppered with cotton, labeled, and placed in a water bath at the proper temperature. Temperatures were controlled to within 0.1 of a degree C. Temperatures below that of the room were secured by running tap water through a coil of copper tubing placed in the water bath. When lower temperatures were desired, the water was cooled by first passing it through a coil placed in an ice bath. When there was any reason to doubt the accuracy of water-bath temperature, the experiment was repeated. Alternations between temperatures were made every twenty-four hours, and a record was kept of the length of time each pupa was exposed to the high and low temperature. Readings on emergence were also made every hour, except at 15° and at alternating temperatures of 10° and 20°, when they were made at eight-hour intervals.

TABLE I

DURATION OF THE PUPAL STAGE OF DROSOPHILA AT CONSTANT TEMPERATURES

Males					Females				
Temperature (Degrees C.)	No. Flies Emerged	Percentage Emerged	Time in Days	Percentage Development per Day	Temperature (Degrees C.)	No. Flies Emerged	Percentage Emerged	Time in Days	Percentage Development per Day
10........	0	0	....	....	10	0	0	....	....
15........	40	96.4	14.15±0.036	7.06	15	42	96.4	13.54±0.022	7.38
20........	92	96.7	6.78±0.008	14.74	20	82	96.7	6.45±0.006	15.29
22.5......	63	98.6	5.27±0.012	18.97	22.5	70	98.6	4.93±0.007	20.28
25........	118	98.2	4.26±0.007	23.47	25	100	98.2	4.02±0.004	24.87
26........	35	100.0	4.00±0.006	25.00	26	42	100.0	3.81±0.004	26.24
27........	108	97.6	3.78±0.003	26.45	27	101	97.6	3.59±0.004	27.85
27.5......	110	99.5	3.65±0.002	27.39	27.5	124	99.5	3.48±0.004	28.73
28........	71	95.9	3.56±0.005	28.09	28	94	95.9	3.37±0.003	29.67
28.5......	47	92.6	3.45±0.004	28.98	28.5	53	92.6	3.27±0.004	30.58
29........	104	99.5	3.40±0.004	29.41	29	102	99.5	3.22±0.003	31.05
29.5......	105	99.9	3.35±0.004	29.85	29.5	110	99.9	3.17±0.003	31.54
30........	66	95.6	3.35±0.003	29.85	30	64	95.6	3.23±0.004	30.96
31........	21	92.7	3.35±0.008	29.85	31	17	92.7	3.21±0.014	31.15
32........	24	78.2	3.50±0.008	28.57	32	24	78.2	3.36±0.010	29.76
33........	17	38.7	3.63±0.016	27.57	33	7	38.7	3.50±0.017	28.57
34........	0	0	....	....	34	0	0	....	....

To determine the rate of development at each temperature, a series of constant temperature experiments was performed. In every case, records were kept of the duration of the pupal stage and the sex of each adult which emerged.

OBSERVATIONS

Effects of constant temperatures.—The results obtained at constant temperatures are shown in Table I and Figure 1. At 10° C., which is below the theoretical threshold for pupal development (the point at which the rate curve crosses the temperature axis), no flies emerged. They appeared to be completely formed, however, within the pupal cases after 40–45 days, showing that development occurred at 10°.

At 34° no flies emerged, although in a few cases they also appeared to be completely formed within the pupal cases. Table I shows that the pupal stage is of longer duration in males than in females. This is in agreement with the work of Bonnier (1926). Between 27° and 30°, readings were made at one-half degree intervals to determine the

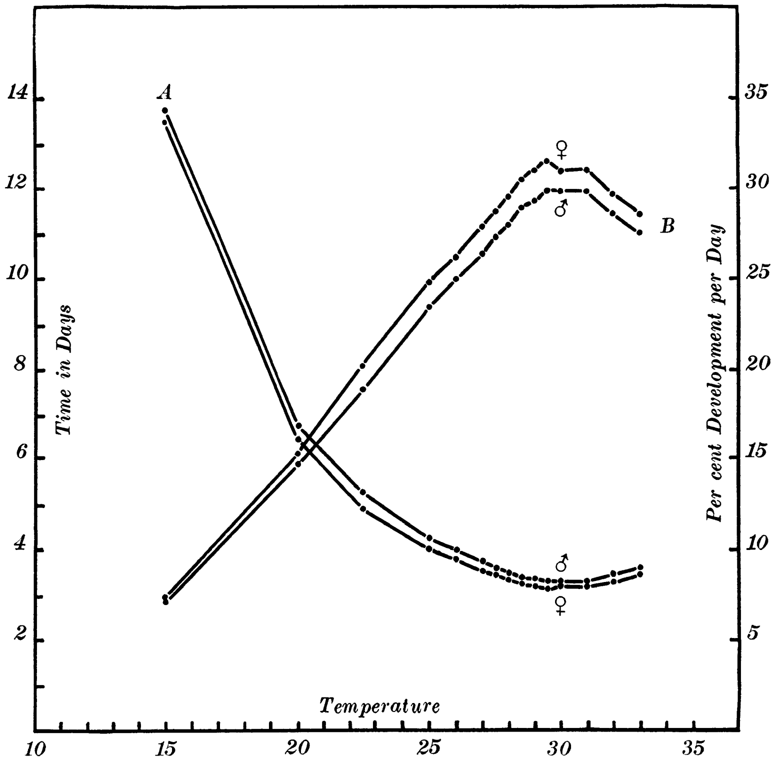

FIG. 1.—Effect of constant temperatures on pupal development. *A*, time curves; *B*, rate curves.

temperature at which development occurs most rapidly. This was found to be 29.5° for both sexes. Table I also contains the percentages of development per day which occur at each constant temperature. These figures are used in evaluating the effects of alternating temperatures.

Effect of alternating temperatures, one of which is above the optimum.—Table II gives the results of daily alternations between two temperatures when one is above the optimum for pupal development. A comparison of the values given in this table with those in Table I (for constant temperatures) will show that in every case there is a retardation of development as compared with the rate at the temperature corresponding to their mean. For example, when placed first at 24° and alternated with 33°, the duration of the pupal stage in the female average 3.88 ± 0.014 days, while at 28.5°, the mean of 33 and 24, it averaged only 3.27 ± 0.004 days. When alternated between 24° and 33° but exposed first to 33°, the time was increased to 4.17 ± 0.019 days. A possible exception to this statement occurred when the pupae were alternated daily between 25° and 30°. When exposed to 25° the first day, it required 3.50 ± 0.004 days for the development of the female pupae as compared with 3.48 ± 0.004 days at 27.5°. However, in this case the pupae were exposed to 30° for 2.04 days and to 25° for only 1.46 days.

The suggestion was made by Ludwig (1928) that this retardation may be due to the slower developmental rate at constant temperatures above the optimum. To test this hypothesis, the percentage of development which occurs at each temperature was calculated by multiplying the time of exposure (in days) by the percentage of development per day at the respective temperatures. By adding the percentages of development at the temperatures used, approximately 100 per cent should be obtained if the hypothesis is a satisfactory explanation. Table II shows that values of approximately 100 are obtained when the pupae are exposed to the low temperature the first day after pupation. When they are exposed to the high temperature first, however, and when a temperature as high as 32° or 33° is used, a definite retardation above that expected on the basis of constant temperature experiments occurs in both sexes. It is interesting to note (Table I) that the percentage of emergence resulting from constant exposure to these temperatures is also lowered. This observation suggested that perhaps the entire pupal stage is not equally susceptible to the retarding influence of exposure to high temperatures.

To determine whether there are differences in susceptibility during the course of pupal development, *Drosophila* pupae were collected and divided into four series. Series I was exposed to 33° during the first 24 hours and then to 25° until the flies emerged. Series II was exposed to 25° for the first 24 hours, to 33° the second 24 hours, and finally to 25° until emergence. Series III was exposed to 25° for the first 48 hours, to 33° for the next 24 hours, and finally to 25° until emergence. Series IV was exposed to 25° for the first 72 hours, then

TABLE II

EFFECT OF ALTERNATING TEMPERATURES ONE OF WHICH IS ABOVE THE OPTIMUM

Temp.* (Degrees C.)	No. Flies Emerged	Time in Days			Percentage of Development				
		Low Temp.	High Temp.	Total	Per Day		Low Temp.	High Temp.	Total
					Low Temp.	High Temp.			
Males									
24 and 33	19	2.10	1.96	4.06±0.016	21.58	27.54	45.31	53.97	99.28
33 and 24	28	1.96	2.36	4.32±0.008	21.58	27.54	42.29	64.99	107.28
24 and 32	40	2.01	1.96	3.97±0.007	21.58	28.54	43.37	55.93	99.30
32 and 24	29	1.96	2.26	4.22±0.007	21.58	28.54	42.29	64.50	106.79
26 and 31	29	2.02	1.62	3.64±0.007	25.00	29.85	50.50	48.35	98.85
31 and 26	29	1.62	2.13	3.75±0.008	25.00	29.85	40.50	63.58	104.08
25 and 30	33	1.46	2.21	3.67±0.006	23.47	29.85	34.26	65.96	100.22
30 and 25	37	2.16	1.79	3.95±0.007	23.47	29.85	50.69	53.43	104.12
20 and 30	36	2.86	2.00	4.86±0.019	14.75	29.85	42.18	59.70	101.88
30 and 20	45	2.00	2.57	4.57±0.011	14.75	29.85	29.50	76.71	106.21
Females									
24 and 33	36	1.92	1.96	3.88±0.014	22.74	28.57	43.66	55.99	99.65
33 and 24	36	1.96	2.21	4.17±0.019	22.74	28.57	44.30	63.13	107.43
24 and 32	34	1.88	1.96	3.84±0.009	22.74	29.76	42.75	58.32	101.07
32 and 24	22	1.96	2.01	3.97±0.029	22.74	29.76	44.30	59.81	104.11
26 and 31	40	1.92	1.62	3.54±0.005	26.24	31.15	50.38	50.46	100.84
31 and 26	32	1.62	1.83	3.45±0.016	26.24	31.15	42.50	57.00	99.50
25 and 30	42	1.46	2.04	3.50±0.004	24.87	30.96	36.31	63.15	99.46
30 and 25	39	2.16	1.54	3.70±0.014	24.87	30.96	53.71	47.67	101.38
20 and 30	25	2.50	2.00	4.50±0.026	15.50	30.96	38.75	61.92	100.67
30 and 20	31	2.00	2.34	4.34±0.009	15.50	30.96	31.00	72.44	103.44

* In the tables of alternating temperature experiments, the manner of exposure is indicated by the order in which the temperatures are given. For example, if the temperatures are stated as 24° and 33°, the pupae were exposed the first 24 hours to the 24° and the second 24 hours to 33°.

to 33° until emergence. The results of this experiment are given in Table III. In both sexes a decided retardation of development is produced when the pupae are exposed to 33° during the first 24 hours of pupal development. When the exposure to 33° occurred later in pupal development, however, the results indicated a sex difference. The males are retarded during the second-day exposure and accelerated during the fourth-day exposure to 33°. This acceleration during the fourth day is sufficient to cancel the retardation resulting from exposure to 33° during the second day. In an alternating temperature experiment between 25° and 33°, when the pupae are placed at 33° the first day and transferred at 24-hour intervals, theoretically there should be a retardation of nearly 7 per cent. Table II shows that a retardation of 7.28 per cent did occur with male pupae in a similar experiment. Exposure of female pupae to 33° on the second,

TABLE III

EFFECT OF A SINGLE 24-HOUR EXPOSURE TO 33 DEGREES C. DURING DIFFERENT PERIODS OF PUPAL DEVELOPMENT, REMAINDER OF TIME AT 25 DEGREES C.

Period at 33 Degrees	No. Flies Emerged	Time in Days			Percentage of Development					
		Low Temp.	High Temp.	Total	Per Day: Low Temp.	Per Day: High Temp.	Low Temp.	High Temp.	Total	Total −100
Males										
First day...	61	3.31	1.00	4.31±0.009	23.47	27.54	77.68	27.54	105.22	+5.22
Second day.	64	3.27	1.00	4.27±0.009	23.47	27.54	76.74	27.54	104.28	+4.28
Third day..	62	3.14	1.00	4.14±0.020	23.47	27.54	73.69	27.54	101.23	+1.23
Fourth day.	66	3.00	0.92	3.92±0.005	23.47	27.54	70.41	25.33	95.74	−4.26
Females										
First day...	39	3.08	1.00	4.08±0.009	24.87	28.57	76.59	28.57	105.16	+5.16
Second day.	53	2.94	1.00	3.94±0.006	24.87	28.57	73.11	28.57	101.68	+1.68
Third day..	58	2.82	1.00	3.82±0.011	24.87	28.57	70.13	28.57	98.70	−1.30
Fourth day.	51	3.00	0.86	3.86±0.007	24.87	28.57	74.64	24.57	99.21	−0.79

third, or fourth days resulted in no appreciable change in the rate of development. In Series IV, this acceleration of male pupae was great enough to eliminate the sex difference in the duration of the pupal stage, male and female flies emerging together.

This experiment was repeated and similar results were obtained a second time.

Effect of alternating temperatures when both are between the theoretical threshold and the optimum.—Table IV gives the results of alternating temperature experiments when each temperature is between the optimum and the theoretical threshold of development. If the total percentage of development is less than 100, an acceleration occurs; if it is greater, there is a retardation of development. With the exception of one experiment, when pupae were alternated between 21° and 29° (exposed to 29° the first day), there was neither an acceleration nor a retardation of development. This exception might possibly be explained by the fact that the values used in calculating the percentage of development per day at 21° were not ob-

TABLE IV

EFFECT OF ALTERNATING TEMPERATURES BOTH OF WHICH ARE BETWEEN THE THEORETICAL THRESHOLD AND THE OPTIMUM

Temp. (Degrees C.)	No. Flies Emerged	Time in Days			Percentage of Development				
		Low Temp.	High Temp.	Total	Per Day: Low Temp.	Per Day: High Temp.	Low Temp.	High Temp.	Total
Males									
21 and 29	35	2.52	2.00	4.52±0.007	16.72	29.41	42.13	58.82	100.95
29 and 21	30	2.00	2.39	4.39±0.004	16.72	29.41	33.44	70.28	103.72
22.5 and 27.5	42	2.31	2.00	4.31±0.010	18.97	27.39	43.82	54.78	98.60
27.5 and 22.5	32	2.00	2.17	4.17±0.012	18.97	27.39	37.94	59.43	97.37
15 and 25	28	3.95	3.00	6.95±0.007	7.06	23.47	27.88	70.41	98.29
Females									
21 and 29	35	2.17	2.00	4.17±0.007	17.61	31.05	38.31	62.10	100.41
29 and 21	27	2.00	2.23	4.23±0.014	17.61	31.05	35.22	69.24	104.46
22.5 and 27.5	29	2.01	2.00	4.01±0.008	20.28	28.73	40.76	57.46	98.22
27.5 and 22.5	26	1.97	2.00	3.97±0.008	20.28	28.73	39.95	57.46	97.41
15 and 25	40	3.38	3.00	6.38±0.016	7.38	24.87	24.94	74.61	99.55

tained by an experiment at 21°, but were calculated from the rate curve based on experiments at other temperatures.

Effect of alternating temperatures, one of which is below the theoretical threshold.—Table V contains the results of alternating temperatures on pupal development when one of the temperatures is below the theoretical threshold. When alternated between 10° and 20° (placed first at 10°) female flies emerged in an average of 11.59±0.030 days, while the average time required at 15° (Table I) was 13.54±0.022 days, and at 20° it was 6.45±0.006 days. If no development occurred at 10° it should have required twice the time necessary at 20°, hence, daily alternations between these temperatures gave an acceleration of from 11 to 14 per cent in female, and 10 to 11 per cent in male, pupae. If it is assumed that development at 10° is completed in 42.5 days, an approximation derived from constant temperature experiments, no acceleration at alternating temperatures of 10° and 20° is obtained.

TABLE V

EFFECT OF ALTERNATING TEMPERATURES ONE OF WHICH IS BELOW THE THEORETICAL THRESHOLD

Temp. (Degrees C.)	No. Flies Emerged	Percentage Emerged	Time in Days		Percentage of Development				
			Low Temp.	High Temp.	Per Day		Low Temp.	High Temp.	Total
					Low Temp.	High Temp.			
Males									
7 and 27......	13	40.6	....	3.75 ± 0.006	....	26.45	....	99.18	....
27 and 7.......	27	96.8	....	3.85 ± 0.004	....	26.45	....	101.83	....
8 and 27......	21	59.2	....	3.71 ± 0.002	....	26.45	....	98.12	....
27 and 8.......	38	100.0	....	3.60 ± 0.004	....	26.45	....	95.22	....
9 and 27......	33	78.2	....	3.67 ± 0.003	....	26.45	....	97.07	....
27 and 9.......	36	97.3	....	3.59 ± 0.007	....	26.45	....	94.95	....
10 and 27......	55	78.3	3.62	3.56 ± 0.011	2.38	26.45	8.61	93.63	102.24
27 and 10......	67	97.3	3.39	3.73 ± 0.017	2.38	26.45	8.06	96.65	104.71
10 and 20......	12	90.2	6.66	6.10 ± 0.040	2.38	14.74	15.85	89.91	105.76
20 and 10......	20		5.77	6.04 ± 0.021	2.38	14.74	13.73	89.02	102.75
20 and 10......	30	76.0	5.22	6.07 ± 0.034	2.38	14.74	12.42	89.47	101.89
Females									
7 and 27......	13	40.6	....	3.64 ± 0.014	....	27.85	....	101.37	....
27 and 7.......	25	96.8	....	3.73 ± 0.004	....	27.85	....	103.88	....
8 and 27......	22	59.2	....	3.68 ± 0.003	....	27.85	....	102.48	....
27 and 8.......	35	100.0	....	3.43 ± 0.007	....	27.85	....	95.50	....
9 and 27......	26	78.2	....	3.43 ± 0.006	....	27.85	....	95.50	....
27 and 9.......	36	97.3	....	3.43 ± 0.007	....	27.85	....	95.50	....
10 and 27......	55	78.3	3.61	3.34 ± 0.013	2.38	27.85	8.59	92.99	101.58
27 and 10......	57	97.3	3.41	3.37 ± 0.007	2.38	27.85	8.11	93.83	101.94
10 and 20......	15	90.2	6.00	5.59 ± 0.030	2.38	15.50	14.28	86.64	100.92
20 and 10......	18		5.03	5.75 ± 0.036	2.38	15.50	11.94	89.12	101.06
20 and 10......	24	76.0	5.00	5.59 ± 0.009	2.38	15.50	11.90	86.64	98.54

Since by a constant temperature experiment at 10°, and by alternating between 10° and 20°, it was found that 10° is above the threshold of development, it was decided to place pupae at different low temperatures alternated with the same high temperature to determine, if possible, the actual threshold of development. In calculating the results of these experiments only the time at high temperatures was used to obtain the total percentage of development. If this value is 100 or above, it is assumed that no development occurs at the low temperature. When pupae were alternated between 7° and 27°, the time spent at the high temperature was the same as the duration of the pupal stage at a constant temperature of 27°. This indicates that all of the development occurred at 27° and that 7° is below the threshold of pupal development. However, when pupae were alternated between 8° and 27° (exposed to 27° first), assuming that no development occurs at 8°, a definite acceleration of nearly 5 per cent occurs in both sexes. This observation indicates that the developmental threshold is between 7° and 8° and that some development does occur at 8°. These experiments further indicate that, in *Drosophila*, the development which occurs between the actual and the theoretical threshold of development is sufficient to account for the apparent acceleration of development when pupae are alternated daily between two temperatures, one of which is below the theoretical threshold of development.

The results in Table V also suggest that in different stages of pupal development there may be a differential susceptibility to low temperatures. When pupae were exposed to 7° the first day, only 40.6 per cent emerged, while when exposed to 27° the first day 96.8 per cent emerged. To determine definitely whether the stage of pupal development determines the ability of pupae to survive after exposure to low temperatures, a number of pupae were collected and divided into three series. Series I, containing 68 pupae, were placed at 10° the first day and then exposed to 27° until the adults emerged. Only 85 per cent emerged. Series II, containing 61 pupae, were placed at 27° the first day, 10° the second day, and then exposed to 27° until emergence. In this series 98.4 per cent emerged. Series III, containing 56 pupae, were exposed to 27° for two days, then to 10° for one day, and then returned to 27° and kept there until emergence. In this series 96.4 per cent emerged. These experiments demonstrate that during the first 24 hours following pupation there is a greater susceptibility to low temperature than during the remainder of the pupal stage.

DISCUSSION[1]

These results, obtained on the pupal stage of *Drosophila melanogaster*, are in agreement with those reported by Ludwig (1928) on

[1] Comprehensive discussions of the effects of constant and varying temperatures on insect development are found in the works of Shelford (1929), Uvarov (1931), and Chapman (1931), to which the reader is referred.

the egg, larval, and pupal stages of the Japanese beetle, *Popillia japonica*. They suggest a possible explanation for some of the apparently contradictory results obtained by many other investigators. It seems quite evident that most of the retarding effects obtained by alternating pupae between two temperatures, when one is above the optimum, are due to the fact that the rate curve at high temperatures no longer follows the straight-line relationship which obtains over the more favorable temperature range. Moreover, at constant temperatures as high as 32° and 33°, the percentage of flies emerging is much reduced, being 78.2 at 32° and only 38.7 at 33°. When alternating temperature experiments were performed involving these temperatures, it was found that the results depended upon the stage of pupal development when exposed to the high temperature. When exposed to 32° or 33° during the first 24 hours of pupal life, the retardation was greater than expected on the basis of constant temperature experiments. This suggests the development in older pupae of a resistance to the retarding effects of exposure to high temperature. Furthermore, a greater mortality was observed when pupae were exposed to a low temperature during the first 24 hours, also suggesting an increase in resistance with age. These results agree with those of Mavor (1927) who described differences in susceptibility of different stages of *Drosophila melanogaster* to X-rays. He found the egg, larva, and early pupa relatively susceptible to X-ray, resistance increasing about 20 hours after pupation. It then increases with great rapidity until the end of the pupal stage. At this time the dose required to produce 50 per cent mortality in 5 days after exposure was 35 times that required at the beginning of the pupal period. Mavor states that this increase in resistance coincides with the later part of metamorphosis after the main organs of the imago had been formed but are still undergoing differentiation.

The retarding effect of a single 24-hour exposure to a high temperature (such as 33°) on the rate of subsequent development at a lower temperature is in agreement with the results of Janisch (1930) on the moth, *Prodenia littoralis*. He found that short exposures to a high temperature retard the subsequent development at the optimum.

Many of the investigations dealing with the effects of varying temperatures on insect development have been made under field conditions where the temperature frequently goes below the threshold of development. This may account for the opinion that varying temperatures accelerate insect development. Cook (1927), in his work on the cutworm, *Porosagrotis orthogonia*, used 8° alternated with different high temperatures. The accelerating effects reported by him may possibly be due to a greater rate of development at 8° than he supposed, since he performed no control experiment at that temperature but calculated the rate of development from his experiments at higher temperatures. The experiments reported in this paper for *Drosophila* pupae suggest that any acceleration produced when pupae are alternated between a temperature below the theoretical threshold and a high temperature is due to a certain amount of development which occurs at the low temperature. No experiments were performed using temperatures between 10° and 15°, but it is possible that accelerations might be produced, especially at the lower temperatures. Krogh (1914) found that in the meal worm, *Tenebrio molitor*, at low temperatures the observed results deviate from the straight line, development occurring more rapidly than is calculated on the basis of experiments conducted at higher temperatures. If one of these is used as the low temperature in an alternating temperature experiment, development would probably be accelerated as compared with the mean constant temperature.

The threshold of development has been defined by Peairs (1927) as the temperature at which, on the descending scale, the development definitely ceases, and at which, on the ascending scale, development is initiated. The results of Krogh (1914), Peairs (1927), and Shelford (1927) indicate that the actual threshold is below the theoretical threshold. Uvarov (1931) noted that "the practical difficulty of determining the exact threshold of development for a given stage of an insect is evidently very great, since it is scarcely possible to register by any method a very small amount of development going on at a very slow rate." Shelford (1929) states that few or no threshold points have been definitely determined but that they have been approximated. He further states that in all cases the use of these approximations have led to erroneous results. One method of calculating the developmental threshold, introduced by Oettingen (1879), is based on the concept of a thermal constant for development. If the time in days required for the completion of a given stage of development is multiplied by the effective temperature, a constant value, the thermal constant, is obtained. This is expressed in day-degrees required for development. The effective temperatures for the stage are obtained by subtracting the threshold temperature from the temperature at which the animals were kept. Oettingen's method consists of making a series of observations on the duration of the developmental period at different constant temperatures. Then several probable values are assumed for the threshold tem-

TABLE VI
DETERMINATION OF THE THRESHOLD TEMPERATURE BY THE METHOD OF OETTINGEN

TEMPERATURE (DEGREES)	TIME IN DAYS	ASSUMED THRESHOLD VALUES			
		8°	9°	10°	11°
		Day Degrees	Day Degrees	Day Degrees	Day Degrees
15	13.79	96.53	82.74	68.95	55.16
20	6.78	81.36	74.58	67.80	61.02
22.5	5.27	76.41	71.14	65.87	60.80
25	4.26	72.42	68.16	63.90	59.61
26	4.00	72.00	68.00	64.00	60.00
27	3.78	71.82	68.04	64.26	60.48
28	3.56	71.20	67.64	64.08	60.52
29	3.40	71.40	68.00	64.60	61.20
29.5	3.35	73.03	68.67	65.32	61.97

perature, and thermal constants are calculated. The value which gives the most constant results over the widest range of temperatures is accepted as the threshold.

This method of obtaining the threshold of development was applied to the results obtained on *Drosophila* pupae at constant temperatures. In Table VI the day-degrees are given for pupal development using temperatures ranging from 8° to 11° as the threshold. This table shows that the most constant values are obtained when 10° is used, although both alternating and constant temperature experiments demonstrated that development does take place at this temperature. Therefore, it must be concluded that the method of Oettingen does not give the correct value for the threshold temperature. The data reported in this paper have indicated that alternating between different low temperatures and a high temperature is a satisfactory experimental method of determining the developmental threshold. In an alternating temperature experiment, if the animals must be exposed to the high temperature the same length of time for the completion of a given stage as is required in a constant exposure to the same temperature, it is obvious that no development occurs at the low temperature. However, if the time at the high temperature is less than is required in a constant exposure to the same temperature, some development occurs at the low temperature which, therefore, is above the developmental threshold.

Peairs (1927) attempted to find the actual threshold of development of blow-fly pupae by placing different groups in low temperature incubators for periods varying from 1 month to 2 years, after which they were kept at higher temperatures until the completion of development. He thought that if any measurable development had occurred while the pupae were exposed to the low temperature, it should be indicated by a corresponding reduction of the time necessary to complete development at the higher temperature. Actually, development was retarded after such prolonged exposure to low temperature, so that any development which may have taken place at the low temperature was not observed. A retardation of developmental rate is not apparent in 24-hour exposures to low temperatures.

These results have no application to experiments on the diapause eggs of grasshoppers. Bodine (1925) on *Melanoplus differentialis* and Parker (1930) on *Melanoplus mexicanus* found that if eggs were allowed to develop for some time at a favorable temperature and then exposed to a low temperature, subsequent development at the high temperature was greatly accelerated. Parker (1930) found that exposure to 0° resulted in a greater acceleration than an exposure to 8°. Bodine (1932) explains the action of cold on the diapause eggs of *Melanoplus differentialis* as follows: "Diapause eggs contain factors (X) which cause diapause in rather definite fashion and at definite stages in the eggs' development. Diapause factors (X) are extremely susceptible to low temperatures (below developmental zero) and can be completely destroyed or inhibited by appropriate exposure to these temperatures."

Hase (1927) with the eggs of the flour moth, *Ephestia kuehniella*, and (1930) with the eggs of the bedbugs, *Cimex lectularius* and *Cimex rotundatus;* Janisch (1930) with the eggs of the moth, *Prodenia littoralis;* and Ludwig (1930) with the eggs of the Japanese beetle, *Popillia japonica*, found that exposure to low temperature delays the time of hatching at the optimum temperature as compared with eggs kept at a constant optimum temperature. In these cases, however, the eggs were not alternated between a low and a high temperature as in the present experiments.

SUMMARY

1. The results of exposing *Drosophila* pupae to daily alternations between two temperatures depend upon the temperatures involved. (*a*) If one of the temperatures is above the optimum of development and the other is between the theretical threshold and the optimum, development appears to be retarded. This retardation may be due to the reduced rate of development at temperatures above the optimum so that the total developmental time is longer than at the mean constant temperature. (*b*) If both temperatures are between the theoretical threshold and the optimum, neither an acceleration nor a retardation is produced. (*c*) If one temperature is between the theoretical threshold and the actual threshold of development, the rate is accelerated. This acceleration is due to development which occurs at temperatures below the theoretical threshold.

2. *Drosophila* is more susceptible to the retarding influence of exposure to high temperatures during the first 24 hours of pupal life than in the remainder of this period. Exposure to low temperatures during the first 24 hours of pupal life also results in a higher mortality than a similar exposure later in pupal development.

3. Alternating *Drosophila* pupae between different low temperatures and a high temperature is a satisfactory method of determining the developmental threshold. In an experiment of this nature, if the pupae must be exposed to the high temperature the same length of time for the completion of a given stage as is required in a constant exposure to the same temperature, no development occurs at the low temperature. However, if the time at the high temperature is less than is required in a constant exposure to the same temperature, some development occurs at the low temperature which, therefore, is above the developmental threshold.

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