THE EFFECTS OF TEMPERATURE ON THE DEVELOPMENT OF AN INSECT (POPILLIA JAPONICA NEWMAN)

(Nine figures)

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INTRODUCTION

MANY investigators of temperature and its relation to growth and development consider growth as a chemical process. Attempts are made to interpret the differences in rates of development associated with different temperatures by applying the rules governing chemical reactions. According to van't Hoff's hypothesis (1884), the rates of chemical reactions are either doubled or tripled for each 10° rise in temperature on the centigrade scale. This is expressed as the constant Q_{10}. Hertwig (1898) was among the first to make a systematic and quantitative study of development at different temperatures and to apply the van't Hoff formula, obtaining a Q_{10} value of more than 2. The value of Q_{10} has been determined for a number of biological processes; and whenever more than 2 is obtained, it is concluded that the process is chemical in nature. Snyder (1911) made a study of Q_{10} values obtained for various biological processes, and showed that they are not constant but decrease as the temperature is increased. Krogh (1914) showed that the Q_{10} values obtained for developing frogs' eggs are not constant but also decrease as the temperature is increased. He showed that the increase in rate of development is in direct proportion to the increase in temperature and can be more accurately expressed by adding a constant, K_{10}, for each 10° C. rise in temperature within certain limits, usually called the limits of normal development. He made no attempt to analyze growth in terms of either chemical or physical processes. Crozier (1924) employed the formula of Arrhenius (1915)

$$\frac{K_2}{K_1} = e^{\frac{\mu}{2}\left(\frac{1}{T_1}-\frac{1}{T_2}\right)},$$

where K_1 is the rate at one temperature, K_2 is the rate at another temperature, e is the base of natural logarithms, μ is a constant, and T_1 and T_2 are the temperatures used, expressed on the absolute temperature scale. According to this method, growth and development are recognized as the result of a complex series of chemical reactions whose rates are governed by the rate of the slowest or "master" reaction. The method is an attempt to analyze the kind of reaction underlying the various complex processes involved, since μ is constant for reactions determined by the same catalyst. Friend (1927) recently applied the Arrhenius formula to the development of the birch-leaf skeletonizer *Bucculatrix canadensisella* and obtained diminishing values of μ as the temperature was increased.

Many of the investigations on the influence of temperature on development deal with only one stage of the life-cycle, such as the egg or the pupa. The question may be asked, "Do all the stages in the life-cycle of an insect react in a similar manner, so that results obtained on one may be used in an interpretation of the reactions of the other stages?" Usually records are made of groups of animals without any consideration of how the individuals composing the groups respond. Frequently, investigators discard from their records those results which are widely different from the average of the group. This is often unjustifiable and might lead to a neglect of facts which may be important in understanding the true relations existing between environmental factors and development.

The discussion which follows deals with the development of the various stages in the life-cycle of the Japanese beetle under controlled temperature conditions. The development of each individual is recorded from the time the egg is laid until the animal emerges as an adult. The results are analyzed according to each of the foregoing methods. Since under field conditions the beetles are subjected to daily varying, rather than to constant, temperatures, experiments were also performed to study the effect of daily alternation of temperatures as compared with the effect of a constant temperature corresponding to their mean value.

ACKNOWLEDGMENTS

The work on this problem was begun while the writer was in the employment of the Japanese Beetle Laboratory, United States

Department of Agriculture, Moorestown, New Jersey. He is indebted to Dr. Henry Fox, of the Japanese Beetle Laboratory, for suggesting the problem, supplying material, and giving helpful suggestions throughout the investigations.

The experiments were performed at the Zoölogical Laboratory, University of Pennsylvania, and the writer wishes to express his appreciation to Dr. C. E. McClung, director of the Laboratory, for placing at his disposal the necessary equipment. The writer is especially grateful to Dr. J. H. Bodine for valuable advice and criticism.

MATERIAL AND METHODS

Throughout the experiments, daily observations and individual records were made. Individuals of each stage, except the egg stage, were placed separately in 1-ounce metal salve boxes. These boxes had been previously sterilized by heating in a hot-air sterilizer for 30 minutes at a temperature of 150°–200° C. Moist plant mold which had been fumigated by treatment with carbon bisulphide was placed in each box. The eggs were also kept in sterile 1-ounce salve boxes but were placed in depressions in moist sterile soil. The humidity was maintained approximately constant (near the point of saturation) by the addition of tap water to the soil or plant mold.

To study the rate of embryonic development at constant and alternating temperatures, a large number of eggs were collected during the summers of 1926 and 1927 from beetles kept in breeding-cages in the laboratory. The soil in the cages was sifted daily so that the eggs, when obtained, were less than 24 hours old. They were divided into series, some of which were used for constant, and others for alternating, temperature conditions. A series was placed at each of the following constant temperatures (expressed in degrees centigrade): $10° \pm 1°$; $13° \pm 0.5°$; $15° \pm 1°$; $17.5° \pm 1°$; $20° \pm 1°$; $22.5° \pm 0.5°$; $25° \pm 0.5°$; $27.5° \pm 1°$; $30° \pm 1°$; $31° \pm 0.5$; $33° \pm 0.5°$; $34° \pm 0.5°$; and $35° \pm 0.5°$. A series was also placed at each of the following alternating temperature conditions: 10 and 20°; 15 and 25°; 20 and 30°; 25 and 35°. For example, by "10 and 20°" is meant that a series was placed one day at 10°, the next day it was removed from 10° and placed at 20°, and the following day returned to 10°, and so on. An effort was made to transfer each series at the same

time each day. No changes were made on Sundays, corrections being made in the calculations of the mean temperature for each series. The number of eggs used at each temperature is given in Table I.

The larvae, as soon as hatched, were transferred from the boxes containing soil to boxes containing moist plant mold and, with the

TABLE I

NUMBER OF INDIVIDUALS USED IN EXPERIMENTS AT EACH TEMPERATURE

TEMPERATURE (C.)	EGGS	LARVAE			PUPAE
		First Instar	Second Instar	Third Instar	
40°	.....	.....	.....	.....	65
37°	.....	.....	.....	.....	55
36°	.....	.....	.....	.....	59
35°	100	45	.....	.....	68
34°	78	.....	.....	.....	.....
33°	93	30	.....	.....	.....
32.5°	.....	.....	.....	.....	70
31°	78	26	24	.....	.....
30°	100	87	54	30	70
27.5°	197	131	111	86	72
25°	359	243	192	160	155
22.5°	100	99	65	61	128
20°	273	210	146	126	167
17.5°	102	116	39	.....	96
15°	50	45	15	.....	64
13°	103	.....	.....	.....	54
10°	50	70	30	.....	39
Alternating temperatures:					
30° and 40°	.....	.....	.....	.....	29
25° and 35°	98	58	.....	.....	37
20° and 30°	100	82	68	61	74
15° and 25°	103	83	67	61	83
10° and 20°	100	73	25	19	41
10° and 15°	.....	.....	.....	.....	57
Total	2,084	1,398	836	604	1,483

few exceptions mentioned below, were continued under the same temperature conditions as the eggs from which they hatched. That is, larvae hatched from eggs which had been kept at 20° C. were also kept at 20° C until the adults emerged. In this way daily records were obtained of the length of the entire developmental cycle at each of the temperatures used at which complete development was possible. At 10° C. no larvae were obtained; at 15° C. the eggs hatched but the larvae did not survive; at 17.5° C. their mortality was very great. To determine if they were able to survive and de-

velop at these relatively low temperatures, eggs were hatched at 25° C., and the larvae hatched from these eggs were transferred to 10° 15°, and 17.5° C. To ascertain if second-instar larvae were also able to survive and develop at 10° and 15° C., larvae which had been allowed to develop to the first molt at room temperature (22°–27° C.) were transferred to these lower temperatures.

The larval period of the Japanese beetle consists of three instars, the last of which includes a prepupal period during which the larva ceases feeding, the food material and fecal matter is discharged from the alimentary canal, the mouth parts and legs become immotile, and the body shrinks within the larval skin. As soon as this skin splits on the dorsal surface, the animal is called a pupa. Records were made of each of the three instars; but since there is no well- defined external change which marks the transformation of the prepupa from the third-instar larva, this larval period is calculated from the second until the third molt. In respect to food conditions, the experiments were divided into (1) larvae which were fed on only plant mold during the first and part of the second instar, when wheat was also included in the diet, and (2) larvae which were fed on plant mold and wheat throughout the entire larval period. Food conditions were controlled by keeping an abundance of food available for the larvae throughout the experiments. A list of the constant and alternating temperatures used in the experiments on the larval period, as well as the number of larvae used at each temperature, is given in Table I.

Pupae were obtained from two different sources: those which had developed from the larvae used in these experiments, and those developed from prepupae collected in the field during the months of June, 1926, and June, 1927. The experiments on the prepupae collected in the field were as follows: The prepupae were divided into series and kept at room temperature (22°–27° C.) until they molted to pupae. The pupae obtained from each series were then placed at one of the following constant temperatures (expressed in degrees centigrade): $10° \pm 1°$; $13° \pm 0.5°$; $15° \pm 1°$; $17.5° \pm 1°$; $20° \pm 1°$; $22.5° \pm 0.5°$; $25° \pm 0.5°$; $27.5° \pm 1°$; $30° \pm 1°$; $32.5° \pm 1°$; $35° \pm 0.5°$; $36° \pm 0.5°$; $37° \pm 0.5°$; and $40° \pm 0.5°$. Other series of pupae thus obtained were placed at one of the following alternating temperature conditions;

10 and 15°; 10 and 20°; 15 and 25°; 20 and 30°; 25 and 35°; 30 and 40°. The number of individuals used at each temperature is given in Table I.

RESULTS

Effects of constant temperatures.—A study of the effects of temperature on the development of different stages in the life-cycle of the Japanese beetle shows that there is a different threshold of development for the egg, larval, and pupal stages. The time required is longer at low temperatures and decreases with higher temperatures until an optimum point is reached. Above this point, it is again increased until the temperature reaches the maximum at which development is possible.

Figure 1 shows that for the egg the time varies from 7.98 ± 0.018 days at 30° C. to 61 ± 0.68 days at 15° C. No eggs hatched at any temperatures used below 15° C.; although at 13° C. some development took place, as shown by the appearance of the mandibles of the larvae through the egg membrane. Above 30° C. the time was again increased to 9 days at 34° C.

The following discussion of the larval period deals only with larvae fed on plant mold during the first instar and part of the second instar, when wheat was also included in the diet. Results were obtained for the total larval period at temperatures between 20° and 27.5° C. Above and below these points the larvae did not transform to pupae. The time required for the total larval period is not influenced greatly by temperature. For example, at 25° C. the average time was 149.2 days, and at 20° C. it was 160.3 days—a difference of only 11 days. However, if the time of development of each of the three instars is considered separately, it is found that the length of each is not only dependent on temperature and food but also on a time factor, which will be discussed later. As shown in Figure 2, the first instar larval period varies from 16.9 ± 0.25 days at 30° C. to 160 days at 15° C. At 17.5° C. it required 101.4 ± 1.6 days; while at 20° C. only 37.6 ± 1.1 days. The time at 17.5° C. is longer than expected on the basis of the time-temperature curve obtained at the higher temperatures. Some factor other than temperature and food must, therefore, influence development at this temperature. This fact becomes more evident when the results obtained

at 20° C. are examined. Here the larvae fall into two groups, one of which passes the first instar in an average time of 30 days and the other in the average time of 80 days. In the first group the time

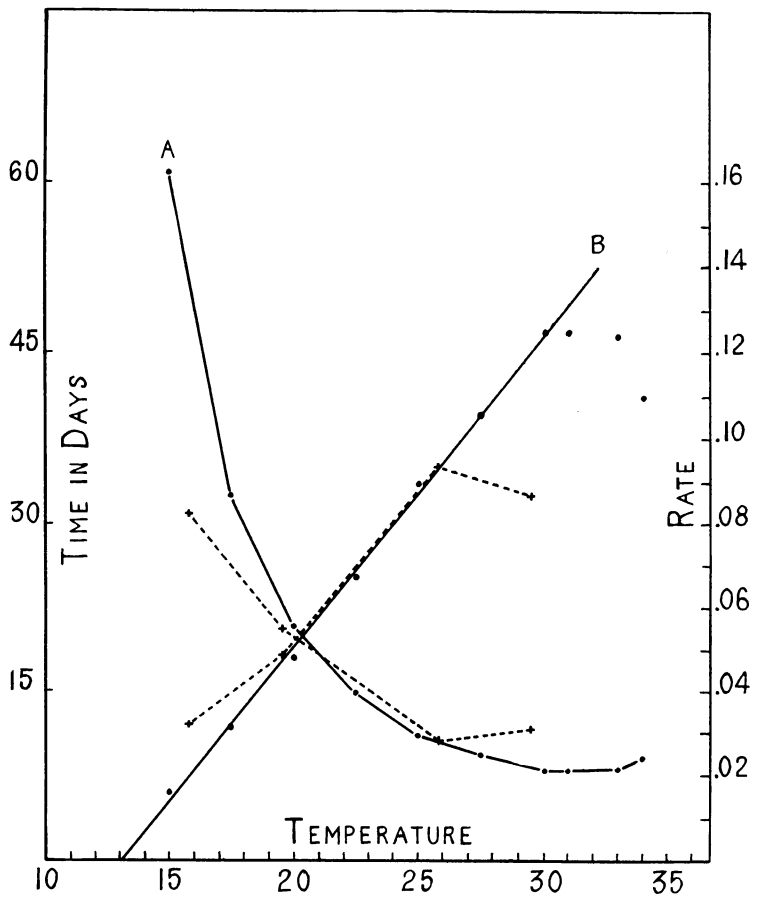

FIG. 1.—Influence of temperature on the development of the egg. *A*, time curve; *B*, rate curve. The solid line represents constant-temperature experiments, while the broken line represents alternating-temperature experiments.

varied from 24 to 48 days, while in the second group it varied from 70 to 101 days.

The length of the second instar larval period was found to depend not only on temperature and food but also on the length of the first instar. Figure 3 shows that it varied from 30.7 ± 0.43 days

at 27.5° C. to 210.5±4.5 days at 15° C. (In this figure the length of the second instar at 15° is not plotted, since it is based on larvae hatched and allowed to develop to the first molt at room temperature,

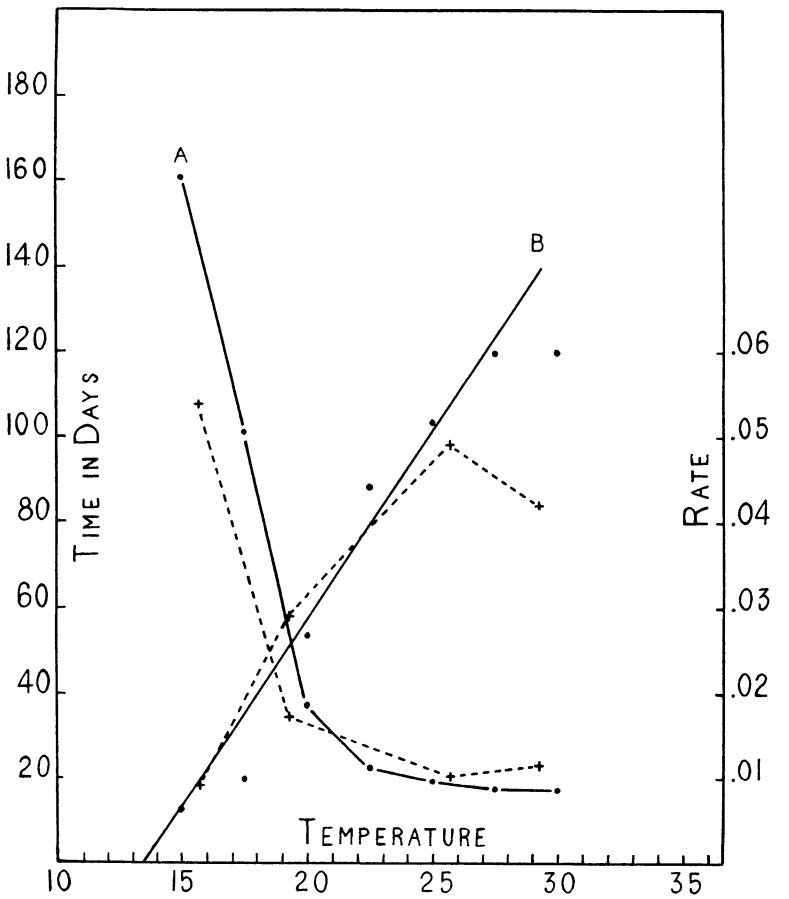

FIG. 2.—Influence of temperature on the development of the first-instar larva. *A*, time curve; *B*, rate curve. The solid line represents constant-temperature experiments, while the broken line represents alternating-temperature experiments.

those obtained at 15° C. having died before completing the second instar.) At 20° C. those individuals which had a short first have a long second instar period, while those which had a long first have a short second instar period. In both groups, the sum of the first and second instars is approximately 108 days. At 22.5° C. the second

instars also fall into two groups, one with a relatively short and the other with a relatively long second instar. In this case there was no sharp demarcation between the individuals of each group.

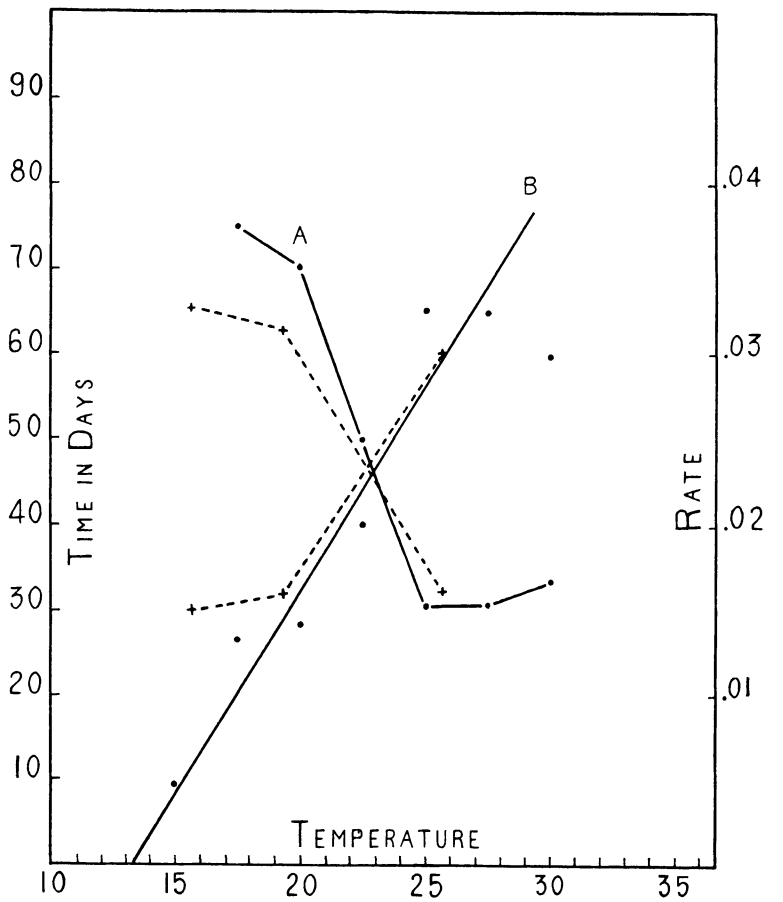

FIG. 3.—Influence of temperature on the development of the second-instar larva. *A*, time curve; *B*, rate curve. The solid line represents constant-temperature experiments, while the broken line represents alternating-temperature experiments. The time at 15° was 210 days. This point is not plotted because the larvae were kept at room temperature (22°–27° C.) until the beginning of the second instar. It is therefore not comparable to the other results plotted.

The time required for the development of the third instar (Fig. 4) was found to be more dependent upon the past history of the larva than any other factor. At 25° C. it was relatively long, being

99.3 ± 1.8 days; at 22.5° C. it was 75.2 ± 2.1 days; at 20° C. it was relatively short, being 52.4 ± 1.6 days. At 22.5° C. those individuals which had a short second have a long third, while those which had

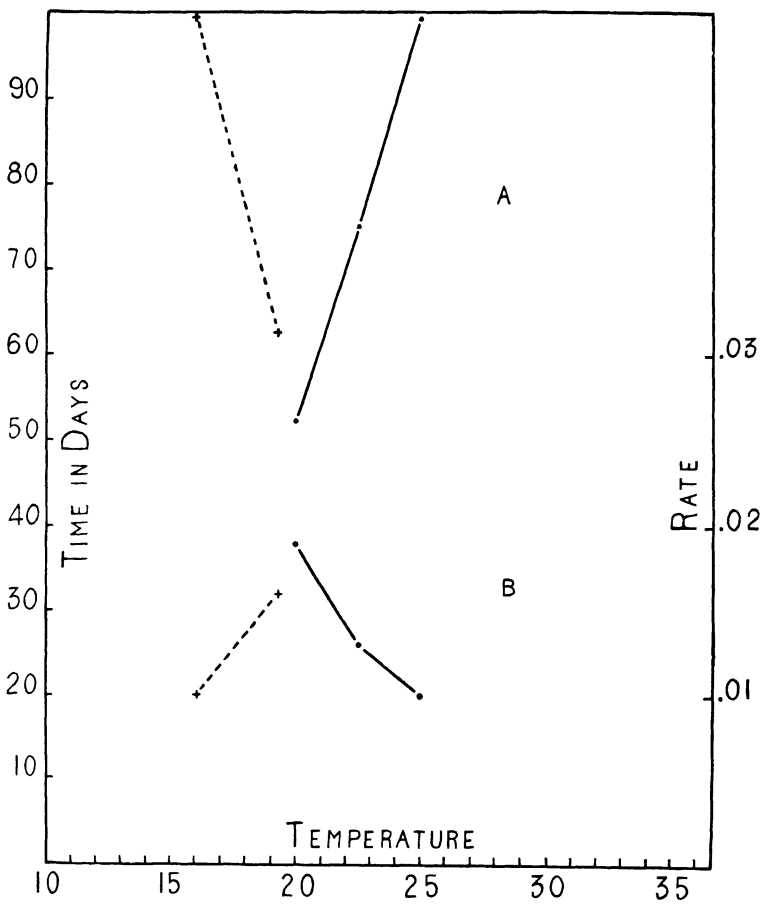

FIG. 4.—Influence of temperature on the development of the third instar larva. *A*, time curve; *B*, rate curve. The solid line represents constant-temperature experiments, while the broken line represents alternating-temperature experiments.

a long second have a short third instar. As a result, the sum of the second and the third instars is also approximately constant at this temperature. These results are shown graphically in Figure 7.

The observation that the third instar period is longer at higher than at lower temperatures led to an experiment to determine if

this stage actually does develop faster at low than at high temperatures or if its length is entirely dependent upon the past history of the individual. Eggs were collected in the laboratory and divided into four series. Series 1 was placed at 25° C. and was kept there until adults emerged. Series 2 was also placed at 25° C. until the larva molted to the third instar, when they were immediately transferred to 20° C. Series 3 was placed at 20° C. and was kept there until the adults emerged. Series 4 was placed at 20° C. until the larva molted to the third instar, when they were transferred to 25° C. In each series the larvae were fed both wheat and plant mold

TABLE II

EFFECT OF TEMPERATURE ON THE THIRD INSTAR WHEN THE PAST HISTORY OF THE LARVA HAS BEEN CONTROLLED

Series	No. of Larvae	First Instar	Second Instar	Third Instar	Total Larval Stage
1.....	50	25° C. 17.4±0.16 days	25° C. 16.9±0.3 days	25° C. 104.8±1.8 days	139.1 days
2.....	45	25° C. 17.9±0.27 days	25° C. 17.2±0.27 days	20° C. 136.6±1.4 days	171.7 days
3.....	45	20° C. 28.1±0.54 days	20° C. 80.5±1.2 days	20° C. 61.9±3.1 days	170.5 days
4.....	50	20° C. 29.9±0.79 days	20° C. 80.8±1.18 days	25° C. 39.4±0.8 days	150.1 days

throughout the entire larval period. The results summarized in Table II show that the length of the third instar is dependent principally upon the lengths of the other larval stages but is also influenced by temperature. In this experiment the past history of the larva as a factor in determining the length of the third instar has been eliminated, leaving temperature as the only factor to be considered. In Series 1 and 2, the first and second instars are both short and the third is long; while in series 3 and 4, the second is long and the third is relatively short. In the former case, the third instar is 31.8 days longer at 20° than at 25° C., while in the latter, the difference is 22.5 days.

The pupal stage is very regular in its response to temperature changes, always developing more slowly at low than at high temperatures (between 15° and 32.5° C.) regardless of the past history

of the individual. Results with pupae which were obtained from prepupae collected in the field were the same as those with pupae which had developed under laboratory conditions, although the for-

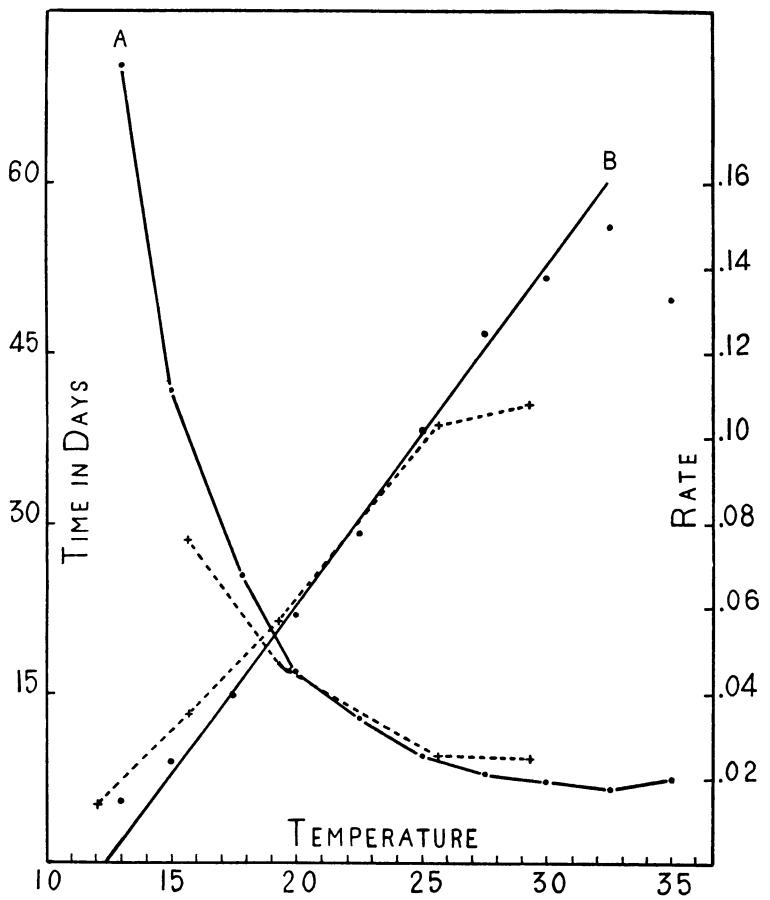

FIG. 5.—Influence of temperature on the development of the pupal stage. *A*, time curve; *B*, rate curve. The solid line represents constant-temperature experiments, while the broken line represents alternating-temperature experiments.

mer had passed the winter as larvae in the field at low temperatures while the latter were kept at controlled high temperatures. The length of the pupal stage (Fig. 5) ranges from 6.6 ± 0.04 days at $32.5°$ C. to 70.4 ± 0.49 days at $13°$ C. Above $32.5°$ C. the time is again increased to 7.5 ± 0.082 at $35°$ C. Figure 6 shows a comparison

of the time required for the development of each stage at the different temperatures used in these experiments.

Effects of food on larval development.—The experiments on the effects of food on the development of the larva are not sufficiently

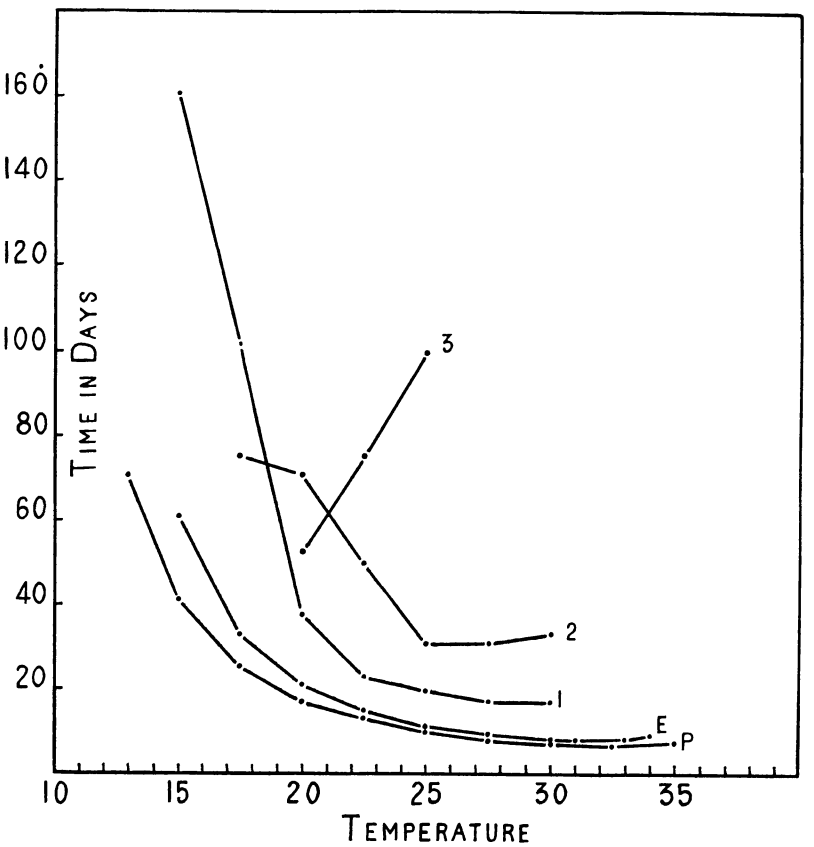

FIG. 6.—Comparison of the time required for the development of each stage. *P*, pupal stage; *E*, egg; (1) first-instar larva; (2) second-instar larva; and (3) third-instar larva.

complete to justify a detailed analysis of results, although a few preliminary statements may be made. The results seem to show that the presence of wheat in the diet hastens the development of those stages not affected by the time factor described above. However, the stage affected by this time factor seems to be correspondingly

longer so that the length of the total larval period is not affected. This can be seen by comparing the results shown in the time-temperature curves for larval development in Figures 2, 3, and 4 with those in Table II, and also by studying the chart of the total life-cycle in Figure 7. The presence of wheat in the diet also seems to reduce the number of individuals at 20° C. which are retarded in the first instar (Fig. 7). At 17.5° C. the first instars of those individuals fed only on plant mold were retarded in each case, while those fed on wheat and plant mold were divided into two groups, some with a long and others with a short first instar. These results may be due to the hastening of development just described. Larvae fed wheat and plant mold seem to be more resistant to extreme temperature conditions than those fed only on plant mold. For example, at 27.5° C. only one pupa was obtained from larvae fed on plant mold although 88 first instar larvae were placed at that temperature. On the other hand, 5 pupae were obtained from 43 larvae fed wheat and plant mold and kept at that temperature.

Toleration of extreme temperatures by various stages.—The experiments at the upper and lower temperature extremes led to a number of interesting observations. Eggs will hatch at temperatures which are too low or too high to permit the larvae, which hatch from them, to survive. Seventy-five per cent of the eggs placed at 33° hatched, but the larvae died within a few days. At 15° C. only 42 per cent of the eggs hatched, the larvae also dying within a few days. However, larvae which hatched at room temperature (22°–25° C.) and were transferred immediately to 15° C. were able to live at that temperature, some developing into second instars, all of which died soon after molting. Second-instar larvae which had hatched and developed to the first molt at room temperature, were able to survive at 15° C. At 17.5° C., 79 per cent of the eggs hatched, but only 21 per cent of the larvae reached the second instar, while 76 per cent of the larvae hatched at 25° C. and, transferred the same day to 17.5° C., were able to develop to the second instar.

The highest temperature at which normal adults were obtained was 35° C. Although pupae placed at 36° C. seemed to develop normally, the adult structures forming within the pupal skin, and the color of the head, thorax, and legs changing to the dark green char-

acteristic of normal pupae just before emergence, no adults were obtained. At 37° C. the pupae lived from 1 to 5 days but never

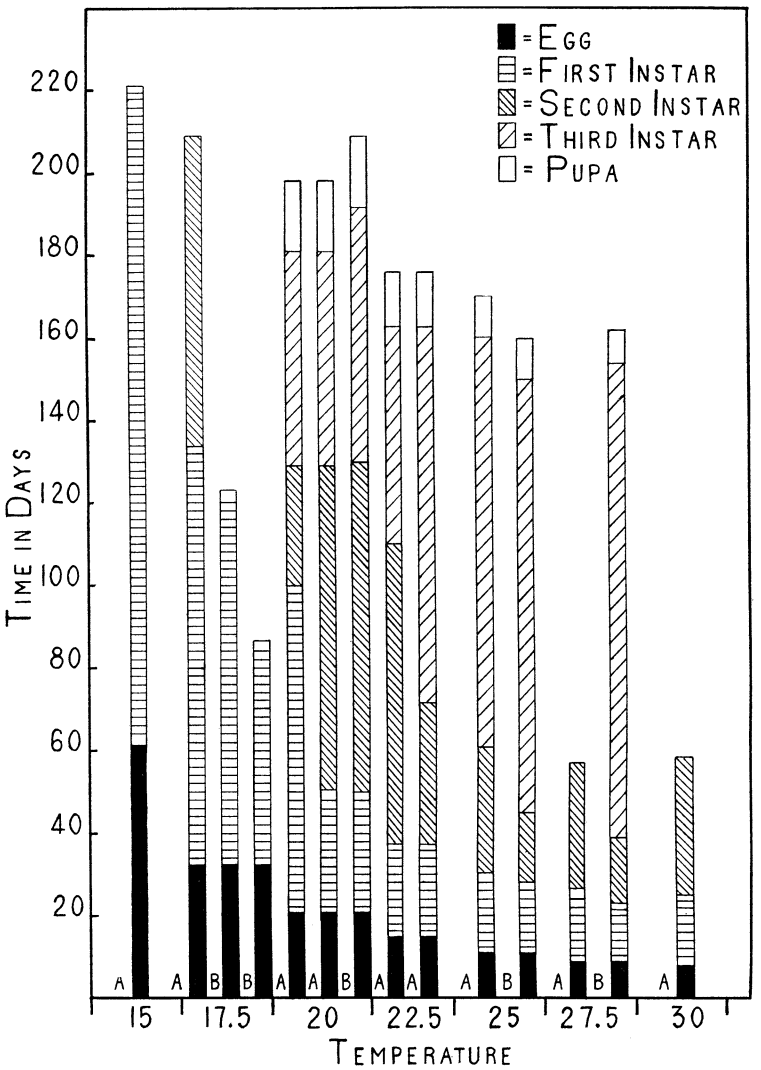

FIG. 7.—Relative lengths of each stage of the life-cycle at different temperatures. *A*, those individuals fed on plant mold; *B*, those fed on plant mold and wheat during the entire larval period. The larvae represented at 15° and at 17.5° (*A*) were not hatched from the eggs kept at those temperatures.

showed any external signs of development. At both extremes of temperature (13° and 35° C.) at which normal adults were obtained, there were also many abnormal individuals which were unable to shed the pupal skin entirely. In these the abdomen failed to diminish in size (as in the normal adult), the wings did not unfold, and the animal resembled a pupa, except that it was able to move its legs and mouth parts.

Effects of alternating temperatures.—The time required for the development of any stage at alternating temperature conditions, compared with the time at a constant temperature which corresponds to the mean of the alternating temperatures, seems to indicate that the effects depend on the temperatures involved. (1) If one of the alternating temperatures is above the optimum of development and the other is between the threshold and the optimum, development is slower than at the mean constant temperature. For example, Figure 1 shows that eggs placed at the alternating temperatures of 25° and 35° C. required 11.5 ± 0.05 days to hatch, while those placed at a constant temperature of 30° C. required only 7.98 ± 0.018 days. Under the same temperature conditions, the first-instar larvae required 23.3 and 16.9 ± 0.25 days (Fig. 2); the pupae required 9.2 ± 0.118 and 7.2 ± 0.054 days (Fig. 5). No readings were made at these alternating temperatures for the second- and third- instar larvae. (2) If both of the alternating temperatures are between the threshold and the optimum, development is neither retarded nor accelerated, as compared with the mean constant temperature. Eggs placed at the alternating temperatures of 15° and 25° C. required 20.4 ± 0.062 days to hatch, while those kept at a constant temperature of 20° C. required 20.9 ± 0.048 days. Under the same temperature conditions, the first-instar larvae required 34.1 ± 0.64 and 37.6 ± 1.1 days; the second-instar larvae required 62.6 ± 1.3 and 70.3 ± 1.56 days; the third-instar larvae required 62.8 ± 1.48 and 52.4 ± 1.6 days; and the pupae required 17.5 ± 0.102 and 17.25 ± 0.071 days. In this case, the first- and second-instar larvae seem to develop faster at the alternating temperatures than at 20° C.; but if the entire larval period be calculated, it is found to be 159.5 and 160.3 days. (3) If one of the alternating temperatures is below the threshold and the other is between the threshold and the optimum,

development seems to be accelerated, as compared with the mean constant temperature. Eggs placed at the alternating temperatures of 10° and 20° C. required 31.15 ± 0.158 days to hatch; while those placed at 15° C. required 61 ± 0.68 days. Under the same temperature conditions, the pupae required 28.6 ± 0.24 and 41.6 ± 0.22 days. Figure 2 shows that the rate of development of the first instar at 10° and 30° C. is not accelerated, as compared with the rate curve obtained for constant temperatures. Undoubtedly, the reason is that under these conditions, development is retarded in the first instar by the time factor, the second and third being correspondingly shortened, as shown in Figures 3 and 4. Comparable readings were not obtained for the second- and third-instar larvae at 15°.

DISCUSSION

Effects of temperature on different stages of the life-cycle.—The results just described show that the different stages of the life-cycle of the Japanese beetle are not affected to the same degree by temperature. The threshold of development differs for the egg, larva, and pupa. To illustrate, pupae transformed to normal adults at 13°, but no eggs hatched at that temperature. Figure 8 shows that the rates of development $\left(\frac{1}{\text{time}}\right)$ of each stage are not accelerated to the same extent by an increase in temperature. The rate of development of the egg and of the pupa is accelerated to a greater extent than that of the larva. The optimum temperature for development also differs with each stage. The optimum for the pupa is between 30° and 32° C.; that for the egg is approximately 30° C., while that for the larva is approximately 27.5° C. Each stage has a different maximum temperature at which development is possible. Pupae transformed to normal adults at 35° C., but no eggs hatched above 34° C., and no larvae transformed to pupae above 27.5° C. Sanderson (1910) discussed the relation of temperature to the growth of insects particularly in respect to threshold temperatures and thermal constants of development in different species and in different stages of the life-history of the same species. He showed that each species of insect has its own threshold temperature and that each stage in the life-cycle may react differently from the other stages, the effect

on the life-cycle being the sum total of the effects on each stage. Bonnier (1926) showed that in *Drosophila melanogaster* an increase of temperature has a different effect on different stages and that it shortens the time during pupation relatively more than the

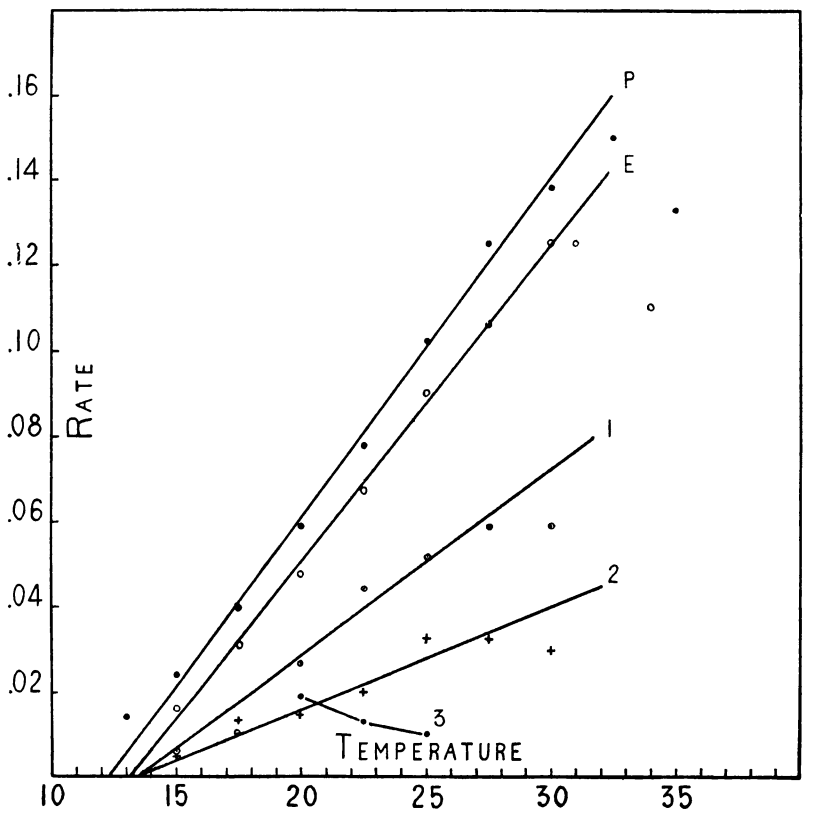

FIG. 8.—Comparison of the rates of development of each stage. *P*, pupal stage; *E*, egg; (1) first-instar larva; (2) second-instar larva; and (3) third-instar larva.

time up to pupation. Shelford (1927) showed that the threshold of development in the codling moth varies with the individual, and also that the thermal constants of development are not the same for different stages.

In the Japanese beetle, the egg and pupal stages are very regular in their responses to temperature; but the larval stages are affected by other factors, such as the past history of the individual. This is

probably associated with the fact that the larval stages, principally the second and third instars, are the over-wintering stages, and under field conditions development is inhibited completely during the winter. Under laboratory conditions with a constant temperature favorable for development, the larva apparently is forced to go through a resting condition, similar to the over-wintering condition in the field, before it can complete development. An examination of Figure 7 shows that at 25° C. this resting stage always occurs in the third instar; at 22.5° C. it may occur either in the second or third; at 20° C. it occurs either in the first or second, but usually in the second; at 17.5° C. it almost always occurs in the first but may occur in the second. This suggests that there is a time cycle or rhythm in development and that the rate of development, which is dependent on the temperature, determines in which stage the resting period will occur. Bonnier (1926) showed that in *Dorsophila melanogaster* the rate of development of the pupa seems to depend not only on the temperature but also on the length of the prepupal stages. He found that in those individuals which required a relatively long time for development up to pupation, this time was compensated by a shorter time during the pupal stage; also, that if the time for development up to the pupal stage was short, the pupal stage was correspondingly longer.

Frequently investigators have studied temperature effects on development, using only one stage, such as the egg or pupa; and from these results they have made generalizations regarding the effects of temperature on the entire development of the organisms studied. The results obtained by Bonnier on *Drosophila* and by the writer on the Japanese beetle indicate a possible interrelationship between development in different stages. One stage cannot be considered separately unless something is known about the other stages.

Analysis of results obtained at constant temperatures.—(1) Van't Hoff's temperature coefficient as applied to growth and development: Van't Hoff's hypothesis that the rates of chemical reactions are either doubled or tripled for each 10° C. rise in temperature may be calculated from the formula

$$Q_{10} = \left(\frac{K_2}{K_1}\right)^{\frac{10}{t_2 - t_1}},$$

where Q_{10} is the temperature coefficient, K is the rate of the reaction at the temperature t_1, and K_2 is the rate of the reaction at the temperature t_2. For many chemical reactions the temperature coefficient is constant for a great range of temperatures, but in some it decreases gradually as the temperature is increased. Van't Hoff suggested that this variation may be due to a change in the viscosity of the solvent associated with the increase in temperature. A number of investigators, Hertwig (1898), Loeb (1908), Loeb and Wasteneys (1911), Krogh (1914), Loeb and Northrop (1917), Krafka (1920) and others, studied the processes of growth and development at various temperatures and calculated the temperature coefficients, obtaining values of more than 2. On this basis,

TABLE III

TABLE OF Q_{10} VALUES

Temperature Range (C.)	Egg	First Instar	Second Instar	Third Instar	Pupa
15°–20°	8.8	19.6	9.0	.....	5.8
17.5°–22.5°	4.7	20.1	2.5	.....	3.8
20°–25°	3.5	3.6	5.1	0.27	3.2
22.5°–27.5°	2.5	1.7	2.5	1.21	2.6
25°–30°	1.9	1.2	0.81	.....	1.8
27.5°–32.5°	1.3	.....	.....	.....	1.4
30°–35°	.....	.....	.....	.....	0.9

Hertwig (1898), Loeb (1908), Loeb and Wasteneys (1911), and Loeb and Northrop (1917) conclude that the processes involved are chemical in nature. On the other hand, Krogh (1914), Krafka (1920), and Chapman (1925) point out that for growth-processes the values of Q_{10} diminish as the temperature is increased and that the change in value is out of proportion for the requirements of van't Hoff's law. Krogh (1914) states, "If the constant is found to vary regularly from the lowest temperatures to the highest it is misleading to speak of it as a constant or to publish the average of a number of determinations over a certain range of temperature as the Q_{10} for the process in question. Such a process does not follow van't Hoff's formula" (p. 165).

The temperature coefficients have been calculated for the development of each stage of the Japanese beetle. The values obtained are given in Table III.

It is very evident that the temperature coefficients diminish quite regularly as the temperature is increased, except in the third instar and in the first and second instars at 17.5° C. The range of variation, for the temperatures at which growth is possible, is so great that one is hardly justified in using an average value as the Q_{10} of the process. Consequently, here also the van't Hoff hypothesis does not apply, and, on this basis, it cannot be assumed that the processes involved are necessarily chemical in nature.

(2) The Arrhenius formula as applied to growth and development: The Arrhenius formula,

$$\frac{K_2}{K_1} = e^{\mu\left(\frac{1}{T_1}-\frac{1}{T_2}\right)},$$

is used to measure the rate of chemical reactions which show a slightly decreasing Q_{10} value as the temperature is increased. In this formula, μ is known as the temperature characteristic, and its values are constant for the same chemical reaction. When the logarithmic value of the rate is plotted against the reciprocal of the absolute temperature, a straight line results. If complex biological processes are governed by a simple chemical reaction (the master reaction), μ may be used as a key to the type of underlying reaction. Rogers (1911) calculated μ for the velocity of the heart beat, obtaining values which were not constant but which decreased as the temperature was increased. Krogh (1914) studied the rate of cleavage of the frog's egg and concluded that the Arrhenius formula does not express the relation any better than van't Hoff's formula. Arrhenius (1915) applied the formula to a few biological processes obtaining fairly constant values of μ. Crozier (1924) analyzed a large number of biological processes by this method and was able to divide them into groups according to their temperature characteristics. Frequently he obtained a break in the curve at certain temperatures. These breaks are explained by assuming that different reactions control the process above than below those temperatures. For many processes he obtained values of 11,500 and 16,700, in which the basic reaction is considered to be probably catalyzed respiratory oxidation. He analyzed the results of Krogh on the development of the frog's egg and obtained values of 10,200 and 22,600. This dif-

ference was explained by assuming that oxidation is not the controlling reaction in growth. Bliss (1926) calculated the temperature characteristics for the prepupal development of *Drosophila melanogaster* and obtained curves which show regularly diminishing values of μ with higher temperatures but which he divided into three straight lines with μ values of 33,000, 16,000, and 7,500. Brown (1926) studied the development of three species of *Cladocera* and obtained values of μ which are of the same magnitude as those obtained by Bliss on *Drosophila* and as those obtained for oxygen consumption in arthropods. Some of his curves also show regularly di-

TABLE IV

VALUES OF μ OBTAINED FOR THE JAPANESE BEETLE

Temperature (C.)	Egg	First Instar	Second Instar	Third Instar	Pupa
13°–15°	.....	.....	.....	.....	44,914
15°–17.5°	43,225	32,707	.....	.....	33,215
17.5°–20°	29,734	66,565	.....	.....	26,065
15°–20°	.....	.....	65,298	.....	.....
20°–22.5°	24,117	35,946	24,462	−27,105	19,934
22.5°–25°	20,352	11,255	33,912	−18,092	19,207
25°–27.5°	11,688	9,175	− 439	.....	14,523
27.5°–30°	12,211	124	−6,175	.....	7,321
27.5°–31°	8,676	.....	.....	.....	.....
30°–32.5°	.....	.....	.....	.....	6,175
30°–33°	− 259	.....	.....	.....	.....
32.5°–35°	.....	.....	.....	.....	−8,909

minishing values of μ with higher temperatures. Friend (1927) studied the development of the birch-leaf skeletonizer *Bucculatrix canadensisella* at different temperatures and obtained values of μ which also decrease as the temperature is increased.

The temperature characteristics have been calculated and the values plotted for the development of each stage of the Japanese beetle. The results are given in Table IV and in Figure 9. A study of the table shows that μ is not constant but is different for nearly every temperature studied. In almost every case the values are higher for lower temperatures, except for the third-instar larva. Correspondingly, Figure 9 shows that there is a break in each curve for nearly every point plotted. It is very evident that if the temperature characteristics can be used as an index to the type of reaction

underlying growth, the reaction is different for each stage of the life-cycle, and for the same stage it is different for nearly every temperature interval studied. Since Bliss (1926) used only the prepupal stage of *Drosophila*, his results cannot be used as an index to the

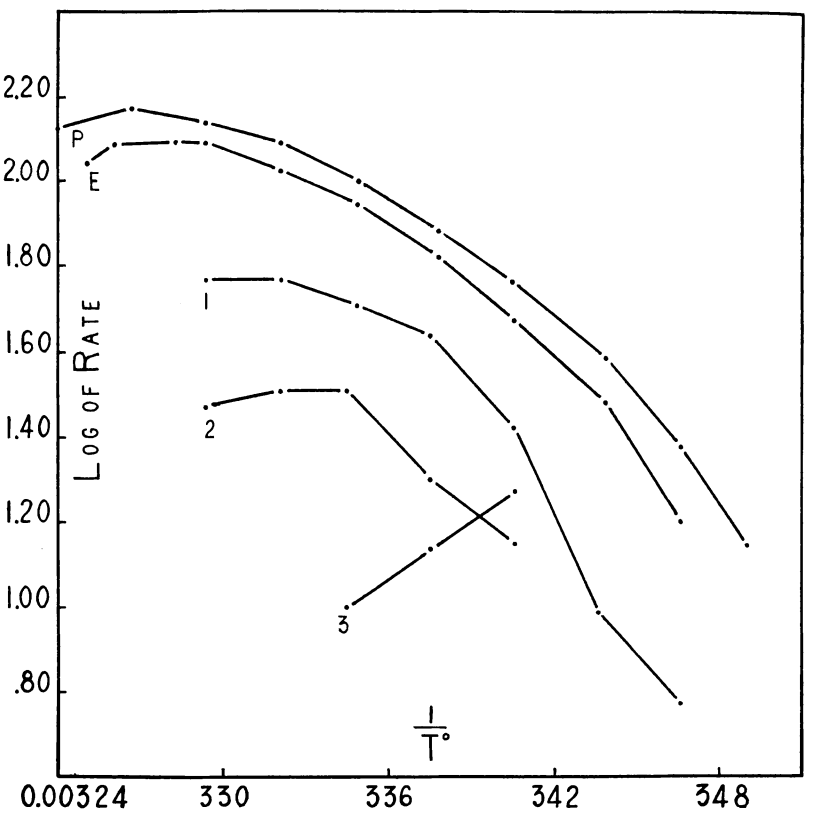

FIG. 9.—Abscissae, reciprocals of the absolute temperatures; ordinates, logarithms 1,000×the rates. *P*, pupal stage; *E*, egg; (1) first-instar larva; (2) second-instar larva; and (3) third-instar larva.

reactions of the other stages. Bonnier (1926) showed that in *Drosophila* there is an interrelationship between the stages of the life-cycle, so that conclusions cannot be reached on the basis of the reactions of only one stage. From these studies it appears that the temperature characteristics of growth cannot be used in an analysis of its nature.

[PHYSIOLOGICAL ZOÖLOGY

(3) Krogh's formula as applied to growth and development: To express the relation between temperature and the rate of development, Krogh (1914) advanced the formula $V_{t+10^\circ} = V_t + K_{10}$, where V_t is the velocity at one temperature and K_{10} is the increase in velocity for each 10° C. rise in temperature. It differs from the van't Hoff formula in that the constant is added, instead of being multiplied, to the rate at one temperature in order to obtain the rate at a higher temperature. This means that within certain limits usually described as the limits of normal development, an increase of 1° always produces the same acceleration in the rate of development. The time-temperature curve is, within these limits, a hyperbola, and the rate-temperature curve is a straight line which crosses the temperature axis near the threshold or zero temperature of development. This is demonstrated by the works of Sanderson and Peairs (1913), Krogh (1914), Peairs (1914), Glenn (1922), Lathrop (1923), and Shelford (1927). Krogh found that at the lower temperatures the observed results deviate from the straight line, producing an actual zero below that calculated from the curve. Chapman (1925) stated that this observed zero cannot be used in calculating the effective temperatures, since it lies off the hyperbola curve. Shelford (1927) concluded that, since the calculated is not the actual zero, it cannot be used in calculating effective temperatures. Krogh (1914) also demonstrated that the straight-line relation holds only for the temperatures below the optimum of development. Above that temperature the rate is retarded. Glenn (1922) assumed that, for the codling moth, the rate above the optimum decreases to the same extent that it increases as the temperature is increased up to the optimum. After making corrections based on this assumption, he obtained results which followed the straight-line rate curve. However, Glenn used data obtained under field conditions where the temperature is variable, and the method may not apply to results obtained under controlled constant temperature conditions. Blackman (1905) and Kanitz (1915) correlated the decrease in rate at higher temperatures with the reduction in the amount of active enzymes present in the tissues; while Gray (1923) associated it with a lack of oxygen in the tissues. Because the observed results deviate from the straight line at the upper and lower extremities, Krafka (1921) assumed that the

relation is not in reality a linear curve but a modified exponential curve. Friend (1927), working on the birch-leaf skeletonizer *Bucculatrix canadensisella*, and Cook (1927), working on the cutworm *Porosagrotis orthogonia*, obtained sigmoid curves when the rates were plotted against the temperature. These investigators do not present sufficient data to determine whether the curves obtained are straight or sigmoid between the upper and lower extremities where the results usually deviate from the straight line.

Peairs (1914), Glenn (1922), Shelford (1927), Korschelt (1924), and others have calculated thermal constants of development from the time-temperature curves by multiplying the number of effective degrees by the number of days required for the development of any stage or stages in the life-cycle at a given temperature, the effective temperatures being calculated by subtracting the threshold temperature for the stage from the temperature at which the animals were kept. The result is the number of day-degrees required for the development of the stage in question. Shelford (1927) made the analysis more accurate by calculating "developmental units." A developmental unit he defined as "the difference in amount of development produced in one hour by a difference of one degree of mean medial variable temperature (other conditions being average), as shown by the difference in time required to complete the stage." These investigators obtained thermal constants of development within the range of the straight line. Friend (1927) stated that, in his experiments, at no two consecutive temperatures were the number of degree-hours equal, or approximately equal, and that the curves show that no thermal constants exist in the development of the larva of the birch-leaf skeletonizer under experimental conditions.

The results obtained for the development of the Japanese beetle were analyzed according to the method of Krogh. The rate curves in Figures 1–5 and 8 show that for the egg and pupa the increase in rate is proportional to the increase in temperature. The rates of the larval stages, being modified by the inception of the resting period and by the food of the larva, do not follow the straight line as well as do the other stages. The first instar has a rate curve which is decidedly sigmoid (Fig. 2), but an examination of the results show that at 17.5° C. the larvae remained in this stage longer than had

been expected from the rates at other temperatures, on account of the inception of the resting period. This is also proven by the fact that the second instar at this temperature was correspondingly shorter, as shown in Figures 3 and 6. The rate for the first instar at 22.5° C. was accelerated more than was expected on the basis of the rates at other temperatures. More recent experiments seem to indicate that the rate calculated from the curve is more accurate than the observed rate at this temperature. The third instar, being

TABLE V

DAY-DEGREES REQUIRED FOR DEVELOPMENT OF EACH STAGE

Temperature (C.)	Egg	First Instar	Second Instar	Third Instar	Pupa
13°	.....	.....	.....	.....	35.24
15°	123.0	240.0	315.7	.....	104.0
17.5°	146.7	454.4	320.0	.....	126.5
20°	146.3	245.1	456.9	340.6	128.2
22.5°	141.5	201.0	448.2	676.8	129.0
25°	133.2	221.9	351.9	1141.9	122.0
27.5°	136.3	238.0	430.9	.....	120.0
30°	135.0	278.8	551.1	.....	126.8
31°	144.0	.....	.....	.....	.....
32.5°	.....	.....	.....	.....	133.0
35°	.....	.....	.....	.....	168.7

dependent on the lengths of the other larval stages, shows an inverse relation (Figs. 4 and 8). Food is a factor in the development of the larval stages which cannot be very accurately controlled because of differences which probably exist in the composition of the plant mold and in the quantity of food eaten by the larvae. All the curves show a decrease in rate above a certain (the optimum) temperature. For the pupa the rate is higher at 13° C. than that calculated from the straight line. The number of day-degrees shown in Table V is approximately constant between 15° and 30° C. for the egg, first instar (except at 17.5° C.), and pupal stages. In making the calculations, the temperature at which the rate curve crosses the temperature axis was used as the threshold temperature. The complete life-cycle, as shown in Table VI, does not have a constant number of day-degrees at the different temperatures, but the number increases with the temperature.

It may, therefore, be said that the formula of Krogh applies to the development of the egg and pupal stages but not to all the larval stages of the Japanese beetle. The method is of practical value, where it applies, because if the rates of development are known at several temperatures, it is possible to predict with accuracy the rates of those stages at other temperatures, within the limits of the straight line. The method explains the actual relations existing between temperature and the rate of development, without attempting to analyze the processes involved. These investigations seem to

TABLE VI

DAY-DEGREES REQUIRED FOR THE COMPLETE LIFE-CYCLE

Stage	20° C.	22.5° C.	25° C.
Egg	146.3	141.5	133.2
First instar	245.1	201.0	221.9
Second instar	456.9	448.2	351.9
Third instar	340.6	676.8	1141.9
Pupa	128.2	129.0	122.0
Total	1,317.1	1,596.5	1,970.9

indicate that, at the present time, growth and development cannot be analyzed in terms of the laws of chemical reactions. Bayliss (1924) and Heilbrunn (1925) point out the dissimilarity between the reactions taking place in a homogeneous solution and a heterogeneous system (such as protoplasm). Bayliss mentions several physical processes, such as diffusion and adsorption, which take place simultaneously with the chemical processes, and states that the velocity of the process as a whole is conditioned by the factor which takes place at the slowest rate, concluding that in many cases it is diffusion.

The effects of alternating as compared with constant temperatures.—The generalization has been frequently made that alternating or varying temperatures have a stimulating effect on development, as compared with their mean constant temperatures. Peairs (1914) advanced the tentative law that the effective temperatures calculated for variable are higher than for constant temperatures. If this is true, the rate of development would be accelerated. Peairs stated that he had no evidence to support the view. Headlee (1914), work-

ing with the insect *Toxoptera graminum*, found that a constant temperature was more effective in development and considerably shortened the growth stages. Chapman (1925) included some of Parker's unpublished data on *Melanoplus atlanis* which seem to support the idea that varying temperatures have an accelerating effect. He stated, "It is evident that the constant calculated from the time and effective temperature has a lower value in the case of varying temperatures than in the case of constant temperatures and that this value is lowest when a temperature below the minimum effective temperature is involved." His constants are only slightly less for varying than for constant temperature, except when a temperature below the minimum of development is involved. Shelford (1927) found in his codling-moth investigations that variable temperatures accelerate the development of the pupa 7 per cent, of the egg 7 per cent, and of the larva 8 per cent, as compared with constant temperatures. However, he also found that "in some exceptional experiments with small numbers of individuals, when the temperature rose suddenly and dropped again within a few hours, the velocity seemed to be decreased as compared with that for the corresponding constant temperatures." His experiments were conducted under the varying conditions found in the field and were not verified by carefully controlled experiments. To compare the effects of alternating with constant temperatures, Cook (1927) conducted a series of experiments on the first-instar larva of the cutworm *Porosagrotis orthogonia* and obtained accelerating effects. He conducted all his experiments using 8° C. as the minimum, alternated for different daily periods with various high temperatures. He did not perform a control experiment at 8° C. but calculated the rate of development from his rate curve at the higher temperatures. This curve, which he assumed to be sigmoid, shows that 8° C. is near the threshold temperature. Consequently, his results cannot be used to generalize in respect to the effects of alternating temperatures; and they are not very conclusive, since he used only eleven individuals for each experiment.

The experiments performed on the Japanese beetle show that the effects produced by alternating temperatures depend on 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. This may be explained by assuming that, while the organisms are exposed to temperatures above the optimum, the development is retarded to such a degree that the total developmental rate is lower than at the corresponding constant temperature. Glenn (1922) assumed that the rate is increased regularly up to an optimum temperature and then is decreased at the same rate. He, therefore, deducted twice the number of day-degrees accumulated above the optimum to correct for their retardation. It is interesting to note that if this correction is applied to these results obtained on the Japanese beetle, the corrected value is equal to that obtained at the mean constant temperature. For example, the number of day-degrees required for the development of the pupa at the alternating temperatures of 25° and 35° C. was 154.5. This number is changed to 122.3 by applying Glenn's correction. Table V shows that this value is the same as those obtained for the constant temperatures between 15° and 30° C. (2) If both the temperatures alternated are between the threshold and the optimum of development, neither an acceleration nor a retardation of the 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. However, if the threshold temperature is used as the minimum in the calculation of the mean constant temperature, the rate falls on the straight line. For example, for the egg the rate $\left(\frac{1}{\text{time}}\right)$ at the alternating temperature of 10° and 20° C. was 0.032 (Fig. 1). The mean constant temperature, calculated between 13.2° (the threshold) and 20° C., is 17.1° C.; and the rate at that temperature, calculated from the rate curve, is 0.030. The rate for the pupa at 10° and 20° C. was 0.035 (Fig. 5), and the rate at 16.8° C. (the mean between the threshold and 20° C.) is 0.035. Just why this condition exists is not evident, since it is assumed that, in this case, development can take place only while the organisms are at 20° C.

These results may help in explaining the apparently contradictory results obtained by various investigators. Most investigations are made under field conditions where the temperature is vari-

able and where it frequently goes below the thresholds of insect development. This may account for the opinion that alternating or varying temperatures accelerate insect development. On the other hand, results obtained on one species may not apply to another, or those obtained on one stage of the life-cycle may not apply to the other stages of the same species. Harrington (1923), working on the use of alternating temperatures on the germination of seeds, found that the seeds of some plants germinate as rapidly at favorable constant temperatures as with any alternation of temperatures, while other seeds require an alternation of temperatures for best germination. A similar condition may be found to exist in the responses of various animals to varying, as compared with constant, factors of the environment.

SUMMARY

1. Different stages of the life-cycle of the Japanese beetle are not affected to the same degree by temperature. The threshold, optimum, and maximum temperatures are different for each stage. The egg and pupal stages are very regular in their temperature responses; but the larval stages are modified by other factors, such as food and the inception of a resting stage. The presence of wheat in the food seems to hasten the development of those stages not affected by the resting stage; but the total larval period is not affected, since the resting stage is correspondingly longer.

2. The temperature coefficients (Q_{10} values) are not constant for any stage. They diminish regularly as the temperature is increased for each stage, except the third-instar larva, which is modified by the behavior of the other larval stages.

3. The temperature characteristics (μ values) as derived from the Arrhenius formula are not constant for any stage. They diminish regularly as the temperature is increased, except for the third-instar larval stage.

4. Krogh's formula, that the rate of development is increased in direct proportion to the increase in temperature (within certain limits), has been found to apply to the egg and pupal stages. The number of day-degrees required for the complete life-cycle is not constant for different temperatures but increases with the temperature.

5. The effects of alternating temperatures, as compared with constant temperatures representing their mean values, depend upon the temperatures involved. (*a*) If one of the temperatures is above the optimum of development and the other is between the threshold and the optimum, development seems to be retarded. (*b*)If both of the temperatures are between the threshold and the optimum, neither an acceleration nor a retardation of rate is produced. (*c*) If one of the temperatures is below the threshold and the other is between the threshold and the optimum, the development seems to be accelerated.

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