An Analysis of Threshold Temperatures for the Development of Oncopeltus and Tribolium Eggs

Author(s): Sping Lin, Alexander C. Hodson and A. Glenn Richards

Source: *Physiological Zoology*, Vol. 27, No. 4 (Oct., 1954), pp. 287-311
Published by: The University of Chicago Press. Sponsored by the Division of Comparative Physiology and Biochemistry, Society for Integrative and Comparative Biology
Stable URL: https://www.jstor.org/stable/30152364
Accessed: 19-06-2019 08:34 UTC

PHYSIOLOGICAL ZOÖLOGY

Volume XXVII OCTOBER 1954 Number 4

AN ANALYSIS OF THRESHOLD TEMPERATURES FOR THE DEVELOPMENT OF ONCOPELTUS AND TRIBOLIUM EGGS[1]

SPING LIN, ALEXANDER C. HODSON, AND A. GLENN RICHARDS
Department of Entomology and Economic Zoölogy, University of Minnesota

THE interest of biologists in temperature thresholds of biological processes dates back to the time when Réaumur (1735; see Bělehrádek, 1935) recognized that there was a quantitative relationship between the activities of organisms and the temperature. This may be expressed in various ways. As deduced by later authors, if y is the time required for complete development at temperature t, the relationship may be expressed as follows:

$$y\,(t - a) = K\,, \qquad (1)$$

where K is called the "thermal constant," and a is another constant representing the minimum temperature for development of the species under consideration. The relationship has also been expressed in another form:

$$v = k\,(t - a)\,, \qquad (2)$$

where v equals the rate of any process at temperature t, while k and a are constants. Since v of equation (2) is represented by the reciprocal of y of equation (1), the two equations will be the same when $k = 1/K$.

When v is plotted against t in equation (2) and a straight line is drawn and prolonged, the intercept on the temperature axis is a, i.e., the theoretical minimum temperature for the process or the so-called "developmental zero." It is the temperature at which development is supposed to cease if the relationship holds precisely. The fulfilment of the foregoing equations requires certain assumptions.

1. The time-temperature curve is a true hyperbola, and hence the rate-temperature curve is a straight line through the whole range at which the process can proceed (since rate is represented by reciprocal of time).

2. The accelerating effect of temperature is the same at all stages in the developmental process.

From these, one can derive the following assumptions:

3. The effect of a constant temperature and its equivalent alternating temperatures should be equal.

[1] Paper No. 3089, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 1, Minnesota. A portion of the work described in the present paper was submitted by S. L. as a thesis in partial fulfilment of the requirements for a Ph.D. degree. Subsequent elaboration of the study was performed under terms of a contract between the Office of the Surgeon General, U.S. Army, and the University of Minnesota. The authors acknowledge with thanks the advice and criticism received from Dr. Sheldon C. Reed, Dr. Eleanor H. Slifer, Dr. H. Burr Steinbach, and Dr. Carroll M. Williams.

4. The constant a is the true value for the threshold of development and hence should be the same under both constant- and alternating-temperature conditions.

Any evaluation of these equations requires determination or estimation of the threshold represented by the constant a. In the past, various attempts have been made to determine, or to justify determining, this threshold by indirect means. One of the earliest, introduced by von Oettingen (1879; see Shelford, 1927), was based on the postulate of a thermal constant for development. In this method, a is taken to be the numerical value which gives most constancy for K when substituting determined values in equation (1). Based on the same concept, but approached from a different point of view, is the graphical method introduced by Sanderson and Peairs (1913). These authors concluded that the time-temperature curve is a true hyperbola and hence that the rate-temperature curve is a straight line; subsequently, Peairs (1927) did acknowledge that there were deviations at both the upper and the lower end of the curve.

In later investigations where more careful techniques were employed, a nonlinear function of temperature to rate of development was obtained, and the calculated a values were invariably found to be higher than the actually determined thresholds (e.g., Shelford, 1927; Ludwig and Cable, 1933; Johnson, 1940; Davidson, 1944). This agrees with Krogh's early report (1914) that the zero of the equilateral hyperbola, to which the time-temperature curve partly conforms, is not the actual threshold of development.

While a large literature deals with the effects of variable temperatures on insect development in general, there are almost no published data on the effect of variable temperatures and humidities on threshold temperatures per se (see Shelford, 1927). In other words, the constant a of equations (1) and (2) has never been evaluated under variable conditions.

The present study was designed to reexamine and extend some aspects of this subject, namely, after determining the threshold temperatures of development of certain insect eggs, to examine the effect of various temperatures and humidities on these thresholds; to estimate development at subthreshold temperatures, with particular reference to temperature summation under alternating temperatures one of which is subthreshold; and to analyze the nature of the threshold-temperature effect.

MATERIAL AND METHODS

Two species of insects were used, the large milkweed bug, *Oncopeltus fasciatus* (Dallas), and the confused flour beetle, *Tribolium confusum* Duval. Only the egg stage was used, and the experiments were usually terminated at hatching. This was partly for convenience and partly to avoid complications arising from the effects of temperature on feeding.

Stocks were maintained in a culture-room at 28° ± 1° C. and about 65 per cent R.H. *Oncopeltus* eggs were obtained by putting cotton or cheesecloth wads into the culture jars and allowing the insects to oviposit in them. *Tribolium* eggs were obtained by introducing adults into whole-wheat flour previously passed through a precision grade No. 40 sieve, which does not permit the passage of eggs; after a chosen time, the flour was passed through a No. 20 sieve to remove the adults and then through a No. 40 sieve to collect the eggs. In the earlier experiments the age of the eggs was known to within ±4 hours, in later experiments it was known to within ±2 or in some cases ±1 hour.

The eggs obtained were examined under a dissecting microscope, and obvious-

ly defective or damaged ones were removed and discarded. After this elimination, lots of 25 eggs were introduced into 18×20-mm. vials. The time lapse from isolation of eggs until placing them in temperature cabinets was about 1.5 hours (time required to sort 1,000 eggs). Since thousands of eggs were required for each series of experiments, there was difficulty in obtaining enough eggs in one collection and in handling them with sufficient rapidity. Accordingly, eggs for the eight replicates usually used for each test were not all obtained at the same time. However, the experiments were so arranged that eggs from each single collection were divided among the replicates of different treatments.

The eggs, 25 per vial, were then subjected to different temperatures, which were controlled to within $\pm 0.1°$ or $0.2°$ C. by means of mercury-toluene thermostats and electronic relays. In the alternating temperature experiments, the change was abrupt. Thermocouple readings under conditions of the experiments indicated that the microclimate surrounding the eggs attained equilibrium within 2–30 minutes after transfer, depending on the direction of transfer and the amount of the temperature gradient (Fig. 1). For the tests, humidity was maintained at about 75 per cent by means of saturated sodium chloride solution in the presence of the solid phase (Solomon, 1951), except when humidity itself was being tested. Lower humidities were obtained with sulphuric acid solutions (Solomon, 1951), and saturated humidity was approximated with distilled water.

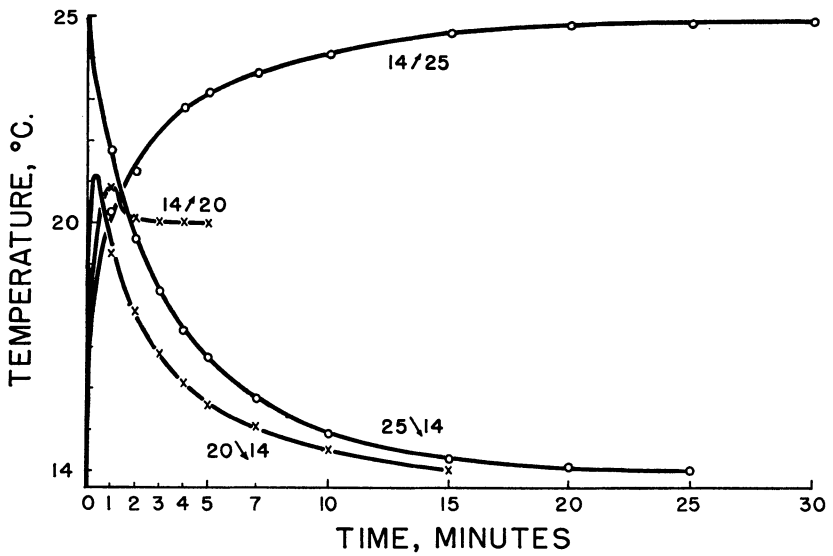


FIG. 1.—Curve showing equilibration times for a small thermocouple in the vessels used for these studies. Arrows between numerals indicate direction of transfer. The small humps on the 14°–20° curves were due to the necessary momentary exposure to the higher room temperature at the time of transfer.

In earlier experiments hatching was checked twice daily. In later experiments where greater precision was desired, hatching was checked every 3 or 6 hours. No observations were made at night. Data from early experiments permitted planning later experiments so that most of the hatching would occur during the day.

The weighted mean time for all the eggs in a lot which hatched at a given temperature was taken as the time required for the eggs to complete development at that temperature. Usually eight replicates (25 eggs each) were run for each treatment; the weighted means from these were averaged to give the mean time for the treatment. The ± values given in the tables and in Figure 10 are the standard errors shown by the replicated lots of 25. In condensing tables for publication, means of repeat tests were again averaged to give the values recorded in Tables 1 and 2. Since the time required for hatching was taken at the end of each interval of observation, it is obvious that the recorded time tends to be somewhat longer than the actual time. It is also obvious that this systematic error, when stated in percentages, would be greater, the shorter the duration of development. The magnitude of this error would be about 1–2 per cent in later experiments in which hatching was checked at 3- or 6-hour intervals.

As a check on the developmental status of the embryos, approximately 20 eggs from each of six to ten stages from selected experimental lots were fixed in hot, freshly prepared acetic acid–absolute ethanol (1:3). The fixed eggs were dechorionated, stained in bulk with borax carmine, dehydrated, and infiltrated with clarite as whole mounts for gross microscopic examination. For sections, paraffin-imbedded eggs were cut at 3 and 5 μ and were stained with either Delafield's hematoxylin and eosin or with Heidenhain's iron-alum hematoxylin.

DEFINITION OF TERMS

Average temperature.—This is the arithmetic average of temperatures over a 24-hour period. Thus (Table 5) a treatment involving 22 hours at 13° and 2 hours at 20° daily gives $(22 \times 13) + (2 \times 20) \div 24 = 13.6°$ C.

Hatched eggs.—These are eggs from which young actually did emerge and expand their appendages. Often, especially at the lower temperatures, the egg shells were broken, but the young could not free themselves therefrom; these eggs were *not* considered as having hatched (see later discussion of this).

Rate of development.—This is taken as the reciprocal of the time T required for development through hatching of the eggs which actually hatched. In the text and figures this rate is expressed as $100/T$ and signifies the average percentage of the total developmental time completed per unit time at any given temperature. It must be emphasized that the rate so calculated is only the *average rate* and is strictly a *time expression.*

Threshold of development.—This is the minimum temperature at which some embryos were able to complete their development through hatching. It is the same as the "developmental hatching threshold" of Johnson (1940). As shown in Tables 1 and 2, the percentage hatching decreases as one approaches the threshold temperature. If one interprets the hatching curves (Figs. 4–9) as indicating a distribution of cold tolerance within the population, then the threshold value would represent one end of a distribution curve.

Total per cent of development.—This is the accumulated product of the average rate $(100/T)$ and the time spent (T) at the corresponding temperatures when the eggs were subjected to more than one temperature. In other words, it is the average per cent of total development (i.e., in terms of developmental time) that should take place during a given time interval at a given temperature or set of temperatures. The resulting figure is nondimensional, and 100 per cent is

taken as standard. A smaller or larger value signifies that more or less development has been accomplished than would have been expected at the corresponding constant temperature, hence an acceleration or a retardation, respectively, in development.

EXPERIMENTAL RESULTS

1. ESTIMATION OF TEMPERATURE THRESHOLD OF DEVELOPMENT

Oncopeltus fasciatus.—Results of the development at various constant temperatures are summarized in Table 1 and presented graphically in Figure 2. The time-temperature curve (Fig. 2, *A*) resembles a hyperbola, in that the two ends of the curve approach the two axes. However, the rate-temperature curve (Fig. 2, *B*), which is the reciprocal of the time-temperature curve, is sigmoid in form instead of linear, as is required for the reciprocal of a true hyperbola. Whether the form of the rate-temperature curve can be expressed in equations of a logistic curve (Davidson, 1944; Browning, 1952) or a catenary curve (Janisch, 1932; Huffaker, 1944) is immaterial to the fact that the curve is definitely not rectilinear.

The survival of eggs (= per cent hatched) decreased at both ends of the effective temperature range (Table 1). Furthermore, abnormal hatching behavior occurred at the extreme temperatures. For example, at 15° C. many of the mature embryos could force open the egg shell but not free themselves from it. Or they could "emerge," but with some appendages stuck to the embryonic cuticle; when only the hind legs were stuck, the nymph could walk with difficulty, dragging the egg shell behind it. If rupture of the egg shell (chorion) were used as the criterion for hatching, successful completion at 15° C. would be about 55 per cent instead of about 24 per cent (i.e., still significantly lower than in the 17°–30° C. range). On the other hand, nymphs hatched at 35° C. often could free themselves from the embryonic membranes but failed to get on their legs and walk;

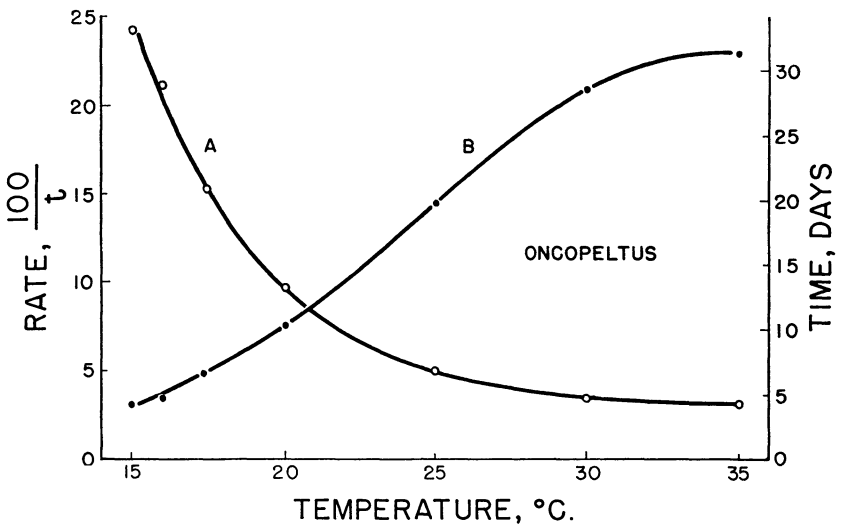


FIG. 2.—Development of *Oncopeltus fasciatus* eggs through hatching at different temperatures. *A*, time-temperature curve; *B*, rate-temperature curve.

they just lay on their backs, moving their legs vigorously in the air. Such nymphs could not walk normally when placed on their legs. Time did not permit following these individuals to maturity; accordingly, we have no data on the relationship between per cent hatching and per cent capable of growing to maturity.

No tests were made in the range between 14° and 15° C., but at 14° no hatching ever occurred (several thousand eggs tested in various experiments). This was true both for the 75 per cent R.H. routinely used and for humidities near saturation. No attempt was made to hatch the embryos artificially, e.g., by breaking the egg shell, but low survival of nymphs reared from eggs incubated at minimal conditions suggests that this would have been unsuccessful (see sec. 6). At 38°–40° C. no sign of development was evident in the eggs.

TABLE 1*

DEVELOPMENT OF *Oncopeltus fasciatus* EGGS AT VARIOUS CONSTANT TEMPERATURES

Temp. (° C.)	No. of Eggs	Per Cent Hatched	Duration in Days (T)	Rate ($100/T$)
40....	200	0	.....	.....
38....	200	0	.....	.....
35....	400	13.2±3.2	4.4±0.03	22.7
30....	1,000	92.3±2.0	4.8±0.01	21.0
25....	800	92.1±2.3	6.9±0.03	14.5
20....	1,200	89.8±2.3	13.2±0.03	7.6
17....	600	81.8±3.8	20.8±0.05	4.8
16....	400	64.8±4.8	28.9±0.06	3.5
15....	740	24.4±3.7	33.1±0.19	3.0
14....	400	0	.....	.....
13....	400	0	.....	.....
10....	200	0	.....	.....
5....	200	0	.....	.....

* Additional values (= increased number tested) are given by the controls for Tables 5–7, as well as by data used for Figs. 4–6.

Tribolium confusum.—Results of the development at various constant temperatures are summarized in Table 2 and presented graphically in Figure 3. Chapman and Baird (1934) recorded 27.5 per cent hatching in 38.8 days at 17°.

At low temperatures, abnormal hatching behavior also occurred with *Tribolium*. The external manifestation of abnormality, however, differed from that of the preceding species. *Tribolium* larvae assumed a head-stand on the floor of the vial, their heads seemingly being glued to the glass by some liquid secreted or ejected during the hatching process. Sometimes the larvae even carried their egg shells on the tips of their abdomens; they then had the appearance of a ball balanced on top of an erect post!

With both these species and all the replicates, hatching was fairly uniform in time, as indicated by the standard errors listed in Tables 1 and 2. Except at the threshold temperature itself in the case of *Tribolium*, the spread in hatching was usually much less than 10 per cent of the developmental period.

TABLE 2*

DEVELOPMENT OF *Tribolium confusum* EGGS AT VARIOUS CONSTANT TEMPERATURES

Temp. (° C.)	No. of Egg	Per Cent Hatched	Duration in Days (T)	Rate ($100/T$)
40.....	200	27.0±7.2	4.9±0.1	20.5
35.....	200	88.5±2.2	4.0±0.01	25.3
30.....	600	80.0±3.1	5.2±0.02	19.4
25.....	400	78.5±3.2	8.1±0.8	12.4
20.....	400	77.5±2.3	18.1±0.1	5.5
17.....	600	21.0±2.9	34.8±0.6	2.9
16.....	200	0	.....	.....
15.....	400	0	.....	.....
10.....	200	0	.....	.....

*Additional data (= increased number tested) are given by the controls for Table 8, as well as by data used for Figs. 7–9.

2. EFFECT OF HUMIDITY ON THRESHOLD OF DEVELOPMENT

Results summarized in Table 3 indicate that the humidities used had only a small effect in altering the threshold temperature or the rate of development. However, low humidities appear to be unfavorable to the eggs, since low or zero per cent hatches were obtained. At 25 and 50 per cent R.H., the threshold tem-

perature for *Oncopeltus* would therefore appear to rise on the temperature scale. The *Tribolium* eggs did not hatch well at saturated humidity, but it is not certain that this is a direct effect of humidity, since these eggs became thickly covered with fungus growth (only a few *Oncopeltus* eggs became moldy).

3. DEVELOPMENT OF EGGS AT AND BELOW THRESHOLD TEMPERATURES

A direct demonstration that some development takes place at subthreshold 14° development appeared to be nearly normal, although more abnormal early embryos were found than at higher temperatures. After 24–27 days at 14°, most of the embryos had fairly well-segmented appendages (= Fig. 29 in Butt, 1949); longer exposures to 14° resulted in some of the embryos' developing "normally" almost to maturity (fully formed and almost fully colored). Observations at room temperature of embryos that had been held at 14° showed that at least some exhibited body movements within

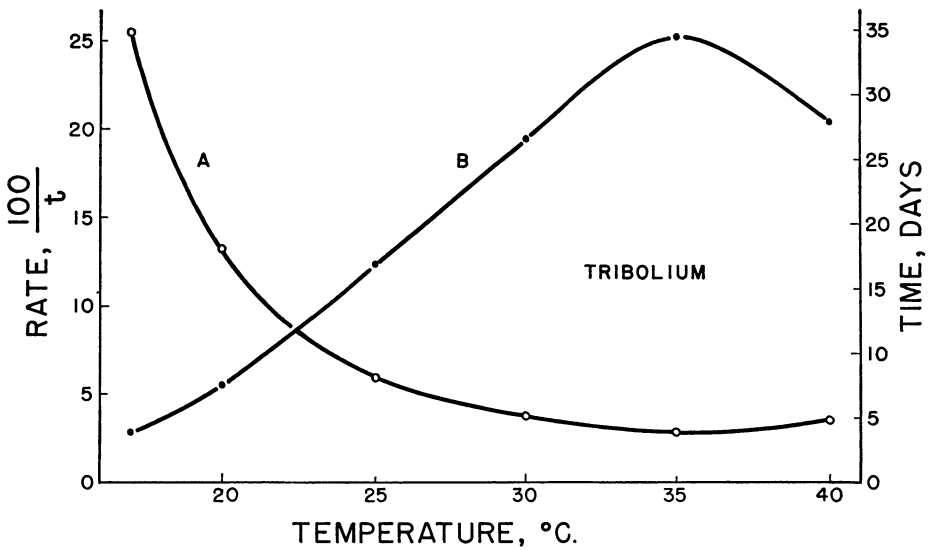


FIG. 3.—Development of *Tribolium confusum* eggs through hatching at different temperatures. *A*, time-temperature curve; *B*, rate-temperature curve.

temperatures can readily be made by gross microscopic examination of stained embryos. *Oncopeltus* eggs held at 5° and 10° C. developed only to the blastoderm stage. Eggs held at 13° for 15–18 days had developed germ bands and the beginning of segmentation (= Fig. 6 in Butt, 1949); longer exposures to 13° usually resulted in the embryos' becoming amorphous clumps of cells, although a few developed into deformed and more or less incomplete advanced embryos. At the egg shell. Cursory examination of stained serial sections from eggs held at 14° for 63 days showed fully formed embryos with various degrees of internal development. The most advanced ones had all appendages and organs fully developed and the various tissues differentiated. Sections of ones segregated as showing movement at the time of fixation were particularly well differentiated, with integument, gut, Malpighian tubules, striated muscle, central nervous

system, and adipose tissue appearing comparable to sections of 20° controls.

In general, a similar picture is shown by *Tribolium* eggs. At 15° C. they develop to segmentation of the germ band in 33 days and continue developing to equal an approximately 70 per cent developed embryo in 60 days (last examination). At 16°, inclosure of the yolk had begun at 28 days, and further development took place if the temperature was maintained.

TABLE 3

EFFECT OF DIFFERENT HUMIDITIES ON THRESHOLD OF DEVELOPMENT ON *Oncopeltus* EGGS
(200 Eggs Used for Each Treatment)

Temperature (° C.)	Relative Humidity (Per Cent)	Per Cent Hatched	Duration (Days)
15	100	39.5±4.8*	33.9±0.2
	75	19.5±2.2†	33.8±0.2
	50	0‡	.....
	25	0	.....
14	100	0	.....
	75	0	.....
	50	0	.....
	25	0	.....
25	100	85.5±4.0	7.0
	75	88.0±3.4	7.0
	50	88.5±2.1	7.0
	25	60.5±5.1	7.0
15	75	22.0±2.4§	32.9
	25	0	.....
15	100	1.5‖	.....
	75	13±3#	.....
	50	0**	.....

* Additional 25.5 per cent burst egg shell.
† Additional 25.5 per cent burst egg shell.
‡ One and a half per cent burst egg shell.
§ Additional 33 per cent burst egg shell.
‖ Additional 12 per cent burst egg shell; 18.5 per cent undeveloped.
Only 100 eggs used. Additional 35 per cent burst egg shell; 17 per cent undeveloped.
** Thirty-nine per cent of eggs undeveloped (usually, only 5–10 per cent showed no development).

Quantitative evaluation was made by experiments presented in Figures 4 and 5 for *Oncopeltus* and in Figures 7 and 8 for *Tribolium*. Figures 6 and 9 serve as controls. The curves labeled *B* in these figures indicate clearly that the eggs require less time to complete development at 30° after initial exposures to the subthreshold temperatures. There is also a reasonably straight-line slope to these *B* curves both when the low temperature is just above the threshold (Figs. 6 and 9) and when it is below threshold (Figs. 4–5 and 7–8). These *B* curves show that *Oncopeltus* eggs tolerated having only the first 27 per cent of development at 13°, or the first 45–50 per cent at 14°. Similarly, *Tribolium* eggs, although more variable, tolerated having only the first 40 per cent at 15°, or the first 45 per cent at 16°. This is obviously not in agreement with the data on embryonic development presented in the preceding paragraph. Hatching or survival curves (curves *A* of Figs. 4–9) were of various shapes, and, as shown in Figure 5, not necessarily repeatable except for end-points. It does not seem possible to analyze these survival curves; all one can say is that, in general, survival decreased as exposure time to a subthreshold temperature was increased (see sec. 6).

We have, then, the seeming discrepancy that neither of these species will tolerate more than approximately the first 45–50 per cent of development at subthreshold temperature, yet embryonic development continues. In *Oncopeltus*, many of the embryos form nymphs which differ from hatched ones only in having slightly paler coloration; yet there was not a single hatched embryo out of a total of 800 eggs held at 14° for more than 50 per cent of the calculated developmental period (this is additional to the several thousand eggs held continuously at 14° in various experiments). At first glance these data look as though we are dealing with complications in the hatching process which are superimposed on true embryonic development, but experiments recorded in section 4 show that

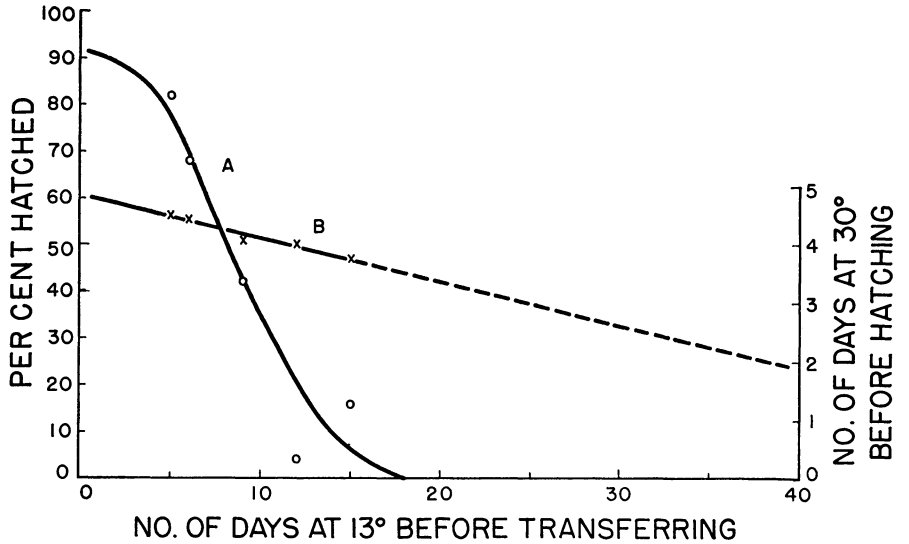


FIG. 4.—Development of *Oncopeltus* eggs at 30° after previous exposures to 13° C. *A*, per cent hatching of the eggs at 30° after various exposures to 13° C.; *B*, time required to complete development after transfer to 30° C. Hypothetical duration of the egg stage at 13° C. estimated from the prolonged straight line as 65.5 days, giving a rate of 1.5 per cent per day.

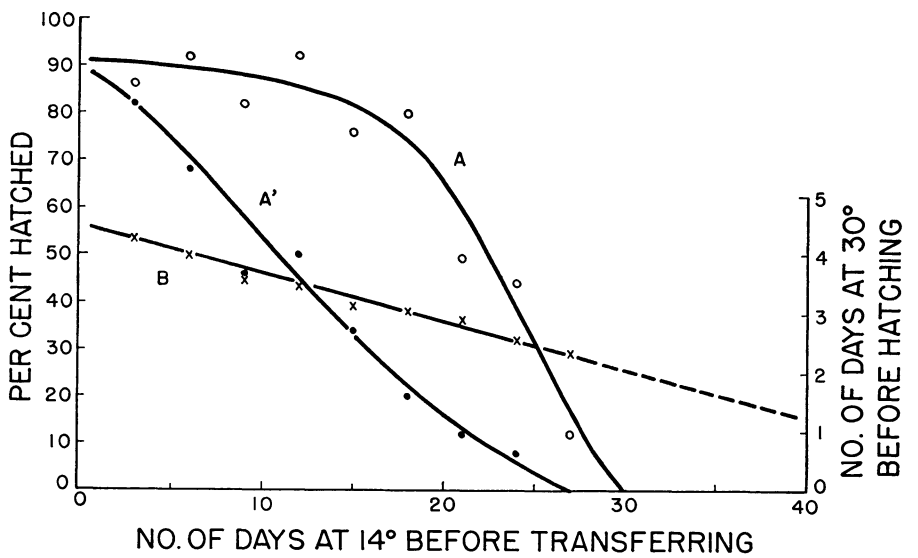


FIG. 5.—Development of *Oncopeltus* eggs at 30° after previous exposures to 14° C. *A* and *A'*, per cent hatching of the eggs at 30° after various exposures to 14° C.; *B*, time required to complete development after transfer to 30°. Hypothetical duration of the egg stage at 14° C. estimated from the prolonged straight line as 55.0 days, giving a rate of 1.8 per cent per day.

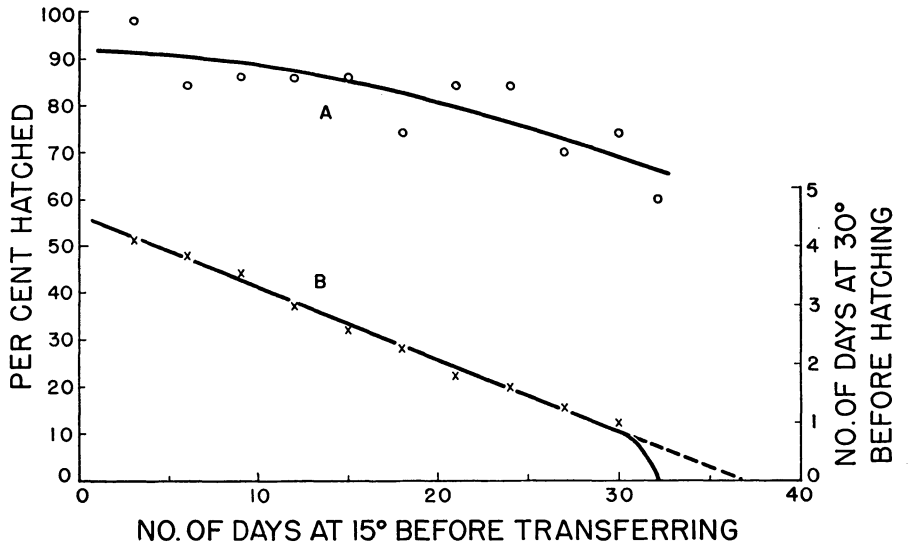


FIG. 6.—Development of *Oncopeltus* eggs at 30° after previous exposures to 15° C. *A*, per cent hatching of the eggs at 30° after various exposures to 15° C.; *B*, time required to complete development after transfer to 30° C. Hypothetical duration of the egg stage at 15° C. estimated from the prolonged straight line as 36.8 days, giving a rate of 2.7 per cent per day. Actual duration of eggs in control was 33.8 days.

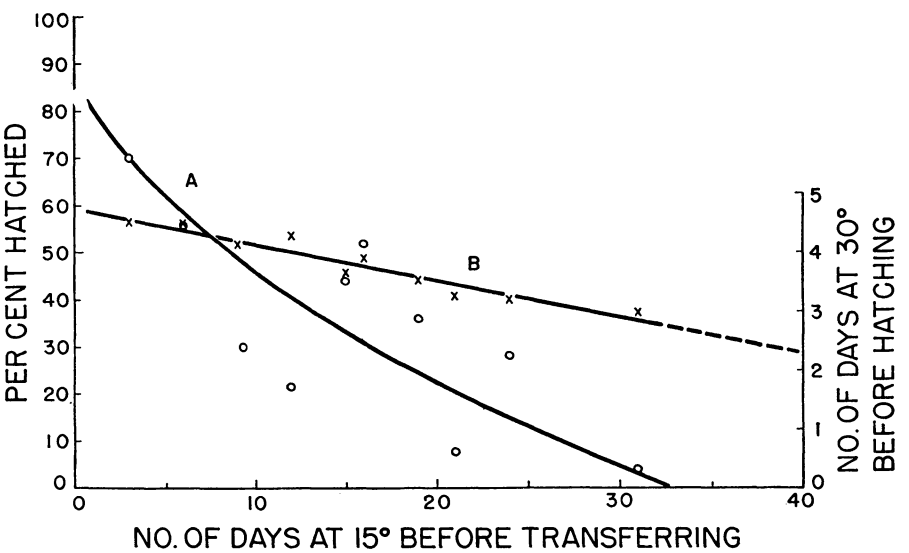


FIG. 7.—Development of *Tribolium* eggs at 30° after previous exposures to 15° C. *A*, per cent hatching of the eggs at 30° after various exposures to 15° C.; *B*, time required to complete development after transfer to 30° C. Hypothetical duration of the egg stage at 15° C. estimated from the prolonged straight line as 77.1 days, giving a rate of 1.3 per cent per day.

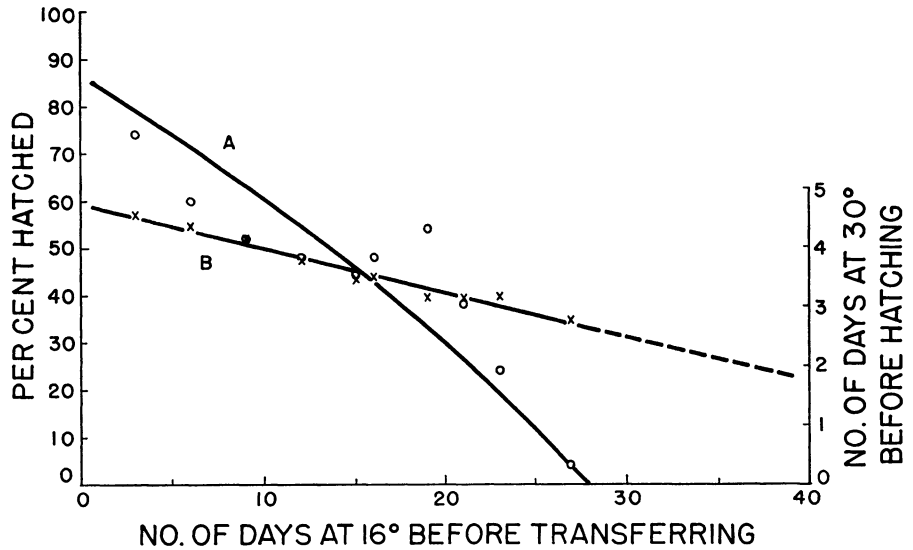


FIG. 8.—Development of *Tribolium* eggs at 30° after previous exposures to 16° C. *A*, per cent hatching of the eggs at 30° after various exposures to 16° C.; *B*, time required to complete development after transfer to 30° C. Hypothetical duration of the egg stage at 16° C. estimated from the prolonged straight line as 62.4 days, giving a rate of 1.6 per cent per day.

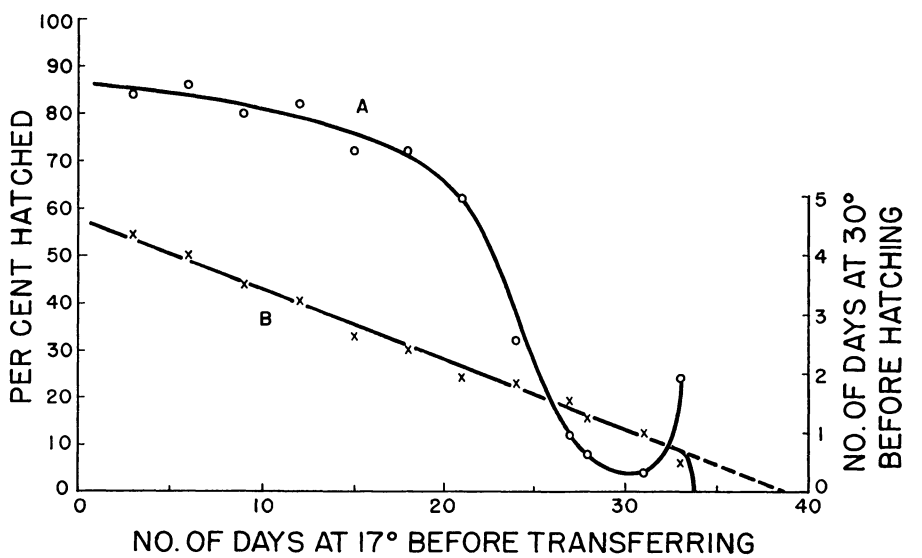


FIG. 9.—Development of *Tribolium* eggs at 30° after previous exposures to 17° C. *A*, per cent hatching of the eggs at 30° after various exposures to 17° C.; *B*, time required to complete development after transfer to 30° C. Hypothetical duration of the egg stage at 17° C. estimated from the prolonged straight line as 38.8 days, giving a rate of 2.6 per cent per day. Actual duration of eggs in control was 33.6 days.

hatching can occur at subthreshold temperatures, and experiments recorded in section 6 make it seem improbable that hatching phenomena are critical.

Since the appearance and slope of the development curves at subthreshold temperatures (curves *B* of Figs. 4–5 and 7–8) are quite similar to those at threshold temperatures (curves *B* of Figs. 6 and 9), it seems justifiable to extend these curves to estimate hypothetical durations of the egg stage at each of the sub-

TABLE 4

SURVIVAL OF *Oncopeltus* EGGS AT 5° AND 10° C., IN PER CENT HATCHED AT 25° C. AFTER PREVIOUS EXPOSURES TO LOW TEMPERATURES IMMEDIATELY AFTER LAYING

(20 Eggs Used in Each Exposure)

TEMPERATURE (° C.)	DAYS EXPOSURE TO LOW TEMPERATURES				
	1	2	3	4	5
5.....	50	35	5	0	0
10.....	80	65	55	15	0

threshold temperatures. A straight line was fitted by the method of least squares to the *B* curves of Figures 4, 5, 7, and 8 and was prolonged to intercept the time axis. By this method, hypothetical durations for *Oncopeltus* eggs were estimated to be 65.5 days at 13° C., and 55.0 days at 14° C.; for *Tribolium* 77.1 days at 15° C., and 62.4 days at 16° C. Corresponding hypothetical rates would be 1.5, 1.8, 1.3, and 1.6 per cent per day, respectively. These calculated hypothetical values are used in analyzing subsequent tests with alternating temperatures (Tables 5–8) but should not be considered as more than approximately correct (see below).

Considerably lower subthreshold temperatures permitted little development (to blastoderm stage) and were tolerated for only a few days (Table 4).

As controls on the tests at subthreshold temperatures, similar tests were made at threshold temperatures (Figs. 6 and 9). Four points seem noteworthy: (1) survival of the embryos decreased later or less steeply throughout the course of development; (2) the numbers employed did not prevent the appearance of anomalies such as the upcurve at the end of curve *A* in Figure 9; (3) survival of *Oncopeltus* eggs was much higher (70 per cent) than for eggs held at the threshold temperature through hatching; and (4) the fitted straight line (*B*) intercepts the time axis at a point beyond the observed hatching of controls. The first two points require no further comment.

Concerning the third point, it was recorded in Table 1 that, at 15° C., hatching of *Oncopeltus* eggs averaged 24.4 per cent, the highest individual value for one test being 33.5 per cent. However, this average value is approximately doubled if, instead of hatching, as that term is used here, one uses rupture of the egg shell. In alternating-temperature experiments to be reported below, hatching usually occurred when the eggs were at the higher temperature; therefore, it seems reasonable to suggest that the higher survival percentage may be due to more ready hatching at 30°.

Concerning the fourth point, explanation of the discrepancy between the observed time of hatching of controls and the time expected on the basis of extending the line *B* in Figures 6 and 9 is more obscure. Table 1 gives a mean duration of 33.1 days for *Oncopeltus* eggs at 15° C., and the control accompanying the data presented in Figure 6 gave a mean duration of 33.8 days; yet prolongation of curve *B* gives 36.8 days. Similarly, for *Tribolium* the estimated value of 38.8 days was 4.0 days longer than the mean in Table 2 and 5.2 days longer than the accompanying control. Another way of

saying this is that it consistently took a somewhat longer time than expected at 30° to complete development. Descriptively speaking, then, the fitted straight line is pulled upward and caused to intercept the time axis beyond the observed point. In effect, there appears to be a slight retardation of development subsequent to the transfer; this is the reverse of what one would expect on the basis of more ready hatchability at higher temperatures. The possible error resulting from the fact that hatching was recorded periodically rather than continuously cannot account for more than about two-thirds of this discrepancy, even if we assume maximum error. It is quite possible that the same sort of discrepancy might be found for the calculation of the hypothetical duration of development at subthreshold temperatures, given in a preceding paragraph, if such were experimentally verifiable.

4. EFFECTS OF ALTERNATING TEMPERATURES ON TEMPERATURE THRESHOLD OF DEVELOPMENT

It would be prohibitively laborious to study all possible combinations of two or more temperatures exploring the full variability in time of exposure. What we desired was to obtain data of use in field ecology. For this purpose two separable situations are of interest: (1) the summation of days when the average temperature is above threshold and (2) the summation of hours above threshold on days when the average is at or slightly below threshold. To obtain valid data for such a comparison, an analysis was made of hourly temperature records of systematically selected days for Minneapolis, Minnesota, in 1949. It was found that in no case did the maximum temperature of the day exceed the mean temperature for that day by more than 11° C. We plotted the temperature-time values (1°–5° C. and 6°–10° C. above the mean) as rectangular geometrical areas with constant values of 5° and 10° above the mean. It was found that, on the average, there were 6 hours during the day having temperatures 5° above the mean, and there were about 2 hours having temperatures 10° above the mean. Accordingly, to approximate the duplication of the natural situation in which the mean temperature is at the threshold value, we used 2-, 4-, and 6-hour exposures at 20° and 25°, the balance of the 24-hour period being at the stated lower temperature (13°–16°).

Results from alternating temperatures on *Oncopeltus* eggs where one of the temperatures was subthreshold are presented in Tables 5 and 6. It can be seen from these data that hatching percentages approximately equal to those obtained at the threshold temperature were obtained with average temperatures as low as 13.6° C. Further, summating the estimated percentages of development expected to occur in the stated times at each temperature gives "total per cent of development" values usually less than 100 per cent, in some cases much less. This would seem to imply a considerable acceleration in development under the stimulus of alternating temperatures, but two reservations should be made in connection with such an interpretation: (*a*) while statistical analysis shows the figures to be highly significant, this is made questionable by the fact that a repeat on the experiment failed to give similar calculated values (Table 5 versus Table 6); and (*b*) there is some indication from calculated versus observed embryonic durations recorded in section 3 that calculated durations are somewhat too long and hence calculated rates at subthreshold temperatures may well be too low. It does not seem possible to account for objection *a* or to correct for objection *b*. Perhaps there really is some acceleration,

TABLE 5*

EFFECT OF ALTERNATING TEMPERATURES ON DEVELOPMENT OF *Oncopeltus* EGGS

Temp. (° C.) and Time at Higher Temp. Daily (Hr.)	Av. Temp. (° C.)	Per Cent Hatched	Duration (Days) (T)	Rate ($100/T$)	Total Per Cent Development
13° and 20°:					
2	13.6	26.5±3.5	43.2±0.1	2.3	88.8±0.2
4	14.2	78.0±3.5	31.2±0.2	3.2	80.5±0.5
6	14.8	88.0±2.3	24.0±0.2	4.2	74.8±0.6
13° and 25°:					
2	14.0	62.0±5.5	37.5±0.1	2.7	100.0±0.4
4	15.0	85.5±3.7	26.5±0.2	3.8	100.7±0.6
6	16.0	89.0±3.4	20.2±0.1	5.0	99.9±0.3
14° and 20°:					
2	14.5	64.0±2.0	34.1±0.1	2.9	79.2±0.3
4	15.0	83.0±1.5	26.8±0.2	3.7	75.6±0.6
6	15.5	90.0±3.0	22.1±0.1	4.5	73.4±0.3
14° and 25°:					
2	14.9	76.5±3.6	31.6±0.2	3.2	92.5±0.5
4	15.8	89.5±1.7	24.3±0.04	4.1	98.3±0.1
6	16.7	85.5±2.6	19.7±0.2	5.1	101.6±0.9
Controls:					
13°, 24		0	(*65.5*)	(*1.5*)	(*100.0*)
14°, 24		0	(*55.0*)	(*1.8*)	(*100.0*)
20°, 24		86.0±2.5	12.7±0.1	7.9	100.0±0.5
25°, 24		91.5±2.3	6.6±0.04	15.2	100.0±0.6

* Age of eggs was known to within 4 hours. Inspections of hatching made twice daily. 200 eggs in 8 replicates were used for each treatment.

TABLE 6*

EFFECT OF ALTERNATING TEMPERATURES ON DEVELOPMENT OF *Oncopeltus* EGGS

Temp. (° C.) and Time at Higher Temp. Daily (Hr.)	Av. Temp. (° C.)	Per Cent Hatched	Duration (Days) (T)	Rate ($100/T$)	Total Per Cent Development
14° and 20°:					
2	14.5	22.0±4.0	40.8±0.1	2.4	94.3±0.2
4	15.0	85.5±2.0	33.6±0.04	3.0	94.2±0.1
6	15.5	90.5±1.1	28.8±0.1	3.5	94.7±0.2
14° and 25°:					
2	14.9	89.0±2.2	32.8±0.1	3.0	93.8±0.2
4	15.8	94.0±1.5	24.1±0.1	4.2	93.8±0.2
6	16.8	95.5±1.6	19.1±0.1	5.2	94.4±0.3
Controls:					
14°, 24		0	(*55.0*)	(*1.8*)	(*100.0*)
20°, 24		92.5±1.8	13.0±0.01	7.7	100.0±0.1
25°, 24		93.5±1.8	6.9±0.01	14.4	100.0±0.2
25°, 24		89.0±3.6	7.0±0.05	14.2	100.0±0.1

* Age of eggs was known to within 4 hours. Inspections of hatching made at 3-hour intervals except during the night. 200 eggs in 8 replicates were used for each treatment.

but we will not claim to have proved it with these data. However, it is clear that development must be accumulated *at both* the above-threshold and the below-threshold temperatures, because, if one accumulates percentages only for the periods above threshold, one finds, for instance, that development which at a constant temperature of 20° requires about 13 days requires only approximately a calculated 3 days in these tests.

The foregoing experiments were all performed with regular alternation, e.g., a certain set of eggs being exposed to a daily 4-hour period above threshold would be at the higher temperature from 10:00 A.M. to 2:00 P.M. every day. To eliminate the possibility that the systematic alternation was affecting the results, a randomized treatment experiment was performed. For this purpose a standard above-threshold treatment of 4 hours at 25° C. was given at intervals of 6–35 hours (average 20 hours), the sequence of below-threshold treatments at 14° C. being the sequence of numbers from 6 to 35, inclusive, taken from a table of random numbers. Five such randomized sets, each on a different set of randomized numbers, plus controls (regular alternation), were set up. With 250 eggs for each set, no signiffcant differences were found between the five randomized sets or between them and the control or previous experiments with regular alternation. Clearly, the treatment does not have to follow a regular daily cycle.

The alternating-temperature experiment with *Oncopeltus* eggs was repeated with temperatures both of which were above the threshold. As shown by the data in Table 7, hatching percentages were consistently high, and the calculated "total per cent of development" indicated little, if any, acceleration.

Similar experiments were performed with *Tribolium* eggs, employing both the threshold temperature (17° C.) and a subthreshold temperature (15° C.). The results, presented in Table 8, show similar "total per cent of development" values for the 17° and 15° C. combinations. In all cases a considerable acceleration is implied by the calculated values, but the remarks made earlier concerning the data on *Oncopeltus* eggs are relevant here too.

With both these species, hatching took place under alternating temperatures where the average temperature was below the minimum for eggs reared under constant temperature. Also the per cent hatching was always higher than for eggs held constantly at the average of the alternating temperatures. Perhaps this may be related to the fact that hatching in the alternating-temperature experiments almost always took place when the eggs were at the higher temperature, despite the fact that for most of the actual time they were at the lower temperature.

Data presented in Tables 5–8 show that hatching could occur when as little as 8 per cent of the total time was above the threshold temperature. Tests were set up to determine what was the minimum time of exposure to an above-threshold temperature. For this purpose *Oncopeltus* eggs were alternated between 14° and 25°, with the exposure to 25° being 1 hour per day (one 1-hour exposure daily or two 3.5-hour exposures weekly), one $\frac{1}{2}$ hour per day (one 3.5-hour exposure weekly or two $1\frac{3}{4}$-hour exposures weekly), and one $\frac{1}{4}$ hour per day (one $1\frac{3}{4}$-hour exposure weekly). Hatching occurred only when exposure to 25° was equivalent to 1 hour per day (38 ± 3.2 per cent in 39.2 days with one 1-hour exposure daily, and 17 ± 3.3 per cent in 41.5 days with two 3.5-hour exposures weekly). When the exposure to 25° was equivalent to $\frac{1}{2}$ hour per day or less, no hatching occurred, although a few per cent burst the

TABLE 7*

EFFECT OF ALTERNATING TEMPERATURES ON DEVELOPMENT OF *Oncopeltus* EGGS

Temp. (° C.) and Time at Higher Temp. Daily (Hr.)	Av. Temp. (° C.)	Per Cent Hatched	Duration (Days) (T)	Rate ($100/T$)	Total Per Cent Development
16° and 20°:					
2	16.3	79.0±2.8	25.8±0.1	3.9	98.3±0.1
2×2	16.7	82.5±3.0	23.5±0.1	4.3	97.4±0.3
4	16.7	83.5±2.4	24.0±0.03	4.2	99.5±0.2
6	17.0	82.0±2.3	22.0±0.1	4.5	98.8±0.2
16° and 25°:					
2	16.8	90.0±2.7	22.9±0.04	4.4	100.0±0.2
2×2	17.5	91.0±1.2	18.9±0.04	5.3	99.4±0.3
4	17.5	86.0±2.4	19.2±0.05	5.2	101.3±0.4
6	18.2	84.0±2.3	16.4±0.1	6.1	100.9±0.4
Controls:					
16°, 24		60.0±4.5	28.9±0.1	3.5	100.0±0.2
20°, 24		89.0±2.8	13.4±0.02	7.6	100.0±0.1
25°, 24		94.5±1.5	7.0±0.03	14.4	100.0±0.4

* Age of eggs was known to within 2 hours. Inspections of hatching made four times daily. 200 eggs in 8 replicates were used for each treatment.

TABLE 8*

EFFECT OF ALTERNATING TEMPERATURES ON DEVELOPMENT OF *Tribolium* EGGS

Temp. (° C.) and Time at Higher Temp. Daily (Hr.)	Av. Temp. (° C.)	Per Cent Hatched	Duration (Days) (T)	Rate ($100/T$)	Total Per Cent Development
15° and 20°:					
2	15.4	0			
4	15.8	2.0±0.8	45.8±1.3	2.2	92.9
6	16.2	33.0±3.9	39.0±0.4	2.6	93.5
15° and 25°:					
2	15.8	26.5±2.9	41.4±0.4	2.4	94.1
4	16.7	76.5±1.9	28.2±0.1	3.5	91.9
6	17.5	79.0±4.6	22.2±0.1	4.5	94.0
17° and 20°:					
2	17.2	65.5±1.8	28.7±0.2	3.5	92.1
4	17.5	72.0±2.8	27.0±0.1	3.7	92.7
6	17.8	70.5±2.9	25.0±0.1	4.0	91.5
17° and 25°:					
2	17.7	80.5±0.9	23.9±0.1	4.2	91.2
4	18.3	76.0±1.7	20.0±0.1	5.0	93.3
6	19.0	79.5±3.3	17.2±0.1	5.8	94.6
Controls:					
15°, 24		0	(*77.1*)	(*1.3*)	(*100.0*)
17°, 24		31.0±3.1	33.6±0.4	3.0	100.0±1.1
20°, 24		87.0±2.5	17.6±0.04	5.7	100.0±0.2
25°, 24		85.0±2.6	7.7±1.7	13.0	100.0±0.2

* Age of eggs was known to within 4 hours. Inspections of hatching made twice daily. 200 eggs in 8 replicates were used for each treatment.

egg shell. Of the unhatched embryos, a large number were deformed. The minimum for total time at 25° when the remainder of development is at 1° below the constant-temperature threshold is, then, about 4 per cent. Actually, these tests do not show whether we have really reached the minimum exposure to 25° or exceeded the maximum tolerable interval between 25° treatments, but, whichever is correct, frequently repeated short exposures to 25° do not have to total more than 4 per cent of the total time.

Since these experiments all employed subthreshold temperatures near the constant-temperature threshold, an additional experiment was performed. At 10° C. *Oncopeltus* eggs showed development only to the blastoderm stage (sec. 3). Accordingly, *Oncopeltus* eggs were alternated between 10° and 25°, with the exposure to 25° being equivalent to 8 hours daily (administered as two $1\frac{1}{6}$-day exposures weekly). In this experiment, 66.5 ± 3.7 per cent hatched in 20.3 days, but an analysis of the time required is consistent with the possibility that no significant amount of development occurred in the long period at 10°; it could have all occurred within the time when the eggs were at 25°. It seems, then, that there are temperatures above the cold death point at which development in all stages is insignificant and may even be zero.

5. EFFECTS OF THRESHOLD AND SUBTHRESHOLD TEMPERATURES ON HATCHING OF *Oncopeltus* EGGS

A series of tests was made to see if hatching per se was the limiting factor in the development-hatching process. For this purpose, eggs of *Oncopeltus* were incubated at 20° C. and then transferred to 13°, 14°, and 15° after the embryos had completed about 70, 75, 80, and 90 per cent of the average duration at 20°. The age of the eggs was known to within 2 hours, and 100 eggs in four replicates of 25 were used in each combination.

At 15°, which is the threshold temperature, one would expect considerable hatching, even without the pretreatment at 20°. It is interesting to note, however, that when the last 10 per cent of development was at 15°, the hatching per cent equaled that of the controls at 20° (93 per cent); but that when the last 30 per cent of development was at 15°, the hatching per cent dropped only to about 60 per cent (i.e., double that of eggs reared continuously at 15° C.).

At 14°, which is just below threshold, good hatching (80 per cent) is obtained if only the last 10 per cent of development is at this temperature; fair hatching (31 per cent) if the last 17 per cent is at 14°; and no hatching if the last 25 or 30 per cent is at this temperature.

At 13° fair hatching (50 per cent) is obtained if only the last 10 per cent of development is at this temperature; a little hatching (6 per cent) if the last 17 per cent is at 13°; and no hatching when the last 25 or 30 per cent of development is at 13°.

Actually, the coefficient of variability between replicates tended to be high, in some cases very high, in these hatching tests at low temperatures. But whatever the explanation of this variability may be, the fact remains that embryos definitely can finish their development and hatch at the subthreshold temperatures of 13° and 14°.

6. ATTEMPT TO LOCATE "SENSITIVE PERIOD" FOR SUBTHRESHOLD-TEMPERATURE EFFECT IN *Oncopeltus*

In general, deleterious temperatures have been found to exert their effects at a particular stage during development, and much literature deals with such "temperature-sensitive periods" for various

systems of insects and other animals. Such sensitive periods are generally considered as marking the "time of determination" of the system affected. Assuming that it would occur in this case, experiments were set up to locate this effective period.

In preceding sections it has been recorded that *Oncopeltus* eggs can hatch at constant temperatures of 15° C. and higher but that they fail to hatch at a constant temperature of 14° C. or lower. However, they can hatch when: (1) the first 45–50 per cent of development is at 14° and the remainder at 30°; (2) the first 75–80 per cent of development is at either 20° or 30° and the remainder at 14°; or (3) the eggs are subjected to regularly alternating temperatures of from above to below threshold, with a mean temperature at or below the threshold temperature and not necessarily more than 4.2 per cent of developmental time being at the above-threshold temperature.

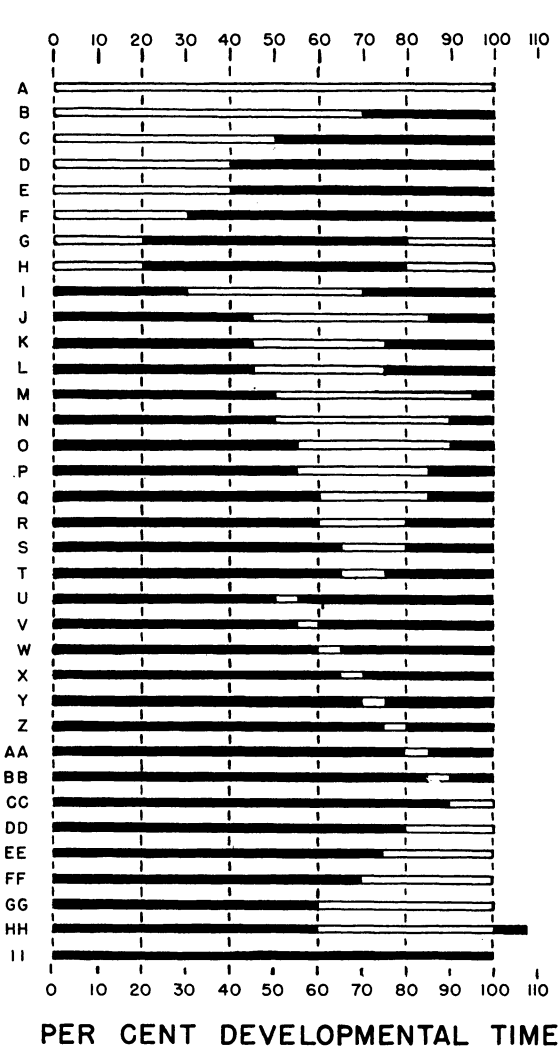
This leaves untested the period between 45 and 50 per cent and 75 and 80 per cent of the developmental time. Accordingly, additional experiments were performed in which the period from 45 to 75 per cent of developmental time was kept at 14°, the preceding and succeeding periods being at 20°. Hatching was 40.0 per cent, i.e., about the same as in eggs held continuously at the threshold temperature of 15°.

Various further experiments on localization of a temperature-sensitive period are summarized diagrammatically in Figure 10. It seems from these data that we are faced with the unexpected situation that the threshold of development temperature is independent of embryonic stage. Not only is there no particular stage especially sensitive to the low-temperature effect, but hatching always occurred at the time predicted by calculations (described in sec. 4), i.e., there is no systematic shift in developmental rate depending on time of exposure to 14°. We know of no similar report in existing literature. The closest thing we have found to an analogous situation is the report that there is no sensitive period for affecting the percentage of expression of the homoeotic mutant "tetraltera" in *Drosophila melanogaster*, although there is a large increase in the percentage of individuals showing this character when rearing is at lower temperatures (Villee, 1942) (see next section).

Incidentally, if rupture of the egg shell be taken as the criterion of successful development, then low percentages of eggs can accomplish this whether the first 70 per cent or the last 30 per cent of developmental time is at 14°, the remainder of development being at 20° or 30°.

7. TEST OF THE POSSIBILITY OF GENETIC DIVERSITY IN *Oncopeltus*

If one ignores the developmental period at which eggs are exposed to the subthreshold temperature of 14° C., and plots per cent hatching against per cent developmental time at the low temperature (Fig. 10), one obtains a curve with a rather sharp break. If the plotting is made on log-log graph paper, the break appears sharpest and looks almost like a cut-off point (Fig. 11). If all the data of Figure 10 were plotted in Figure 11, more scatter would be seen, but the existence of a sloping line in the range of 25–40 per cent time at 14° and of a vertical line below 25 per cent would still be evident. Yet time per se does not seem to be important because experiments which involve shifting to three or four different temperatures (not reported in detail) can be arranged to give identical or different total number of hours; these data showed that, while the per cent of developmental time at the several tem-



	% HATCH	% DIED HATCHING	% DIED PRIOR TO HATCHING
A	0	0	100
B	0	FEW	MOST
C	0	FEW	MOST
D	40 ± 4	13.5	46.5
E	15 ± 3	15	70
F	65	—	—
G	9 ± 2.6	6.5	85
H	2.5 ± 1.3	6	91
I	43 ± 5	40	17
J	7.5 ± 2	38	55
K	44 ± 7	28	28
L	40 ± 6	—	—
M	0	5	95
N	5 ± 2	5	90
O	18 ± 8	18	64
P	75 ± 4	11	14
Q	60 ± 6	17	23
R	79 ± 4	6	15
S	89 ± 3	3	8
T	89 ± 3	5	6
U	94 ± 1	0	6
V	90 ± 3	0	10
W	88 ± 3	0	12
X	96 ± 2	0	4
Y	92 ± 4	1	7
Z	95 ± 3	1	4
AA	94 ± 3	0	6
BB	96 ± 2	0	4
CC	80	—	—
DD	31	—	—
EE	0	FEW	MOST
FF	0	FEW	MOST
GG	0	0	100
HH	2 ± 1.3	7	91
II	90–96	0	4–10

FIG. 10.—Diagrammatic presentation of data on tests attempting to see whether the deleterious effect of subthreshold temperatures is produced at some particular stage during development of *Oncopeltus* eggs. *Open bars*, developmental time at a subthreshold temperature (14° C.); *solid bars*, developmental time at a favorable temperature (20° C.).

peratures was important, the total time elapsing before hatching is not important. No analysis can be made of the curve in Figure 11, but it did suggest the desirability of checking whether genetic diversity within our culture population might result in our obtaining selection of more resistant individuals in experiments with a low percentage of hatching. Accordingly, 27 pairs from our inbred cultures were segregated when they emerged as adults, and their progeny were raised separately under minimal conditions (middle 35 per cent of developmental time at 14° C., remainder at 25° C., intermediate between lines *i* and *o* of Fig. 10).

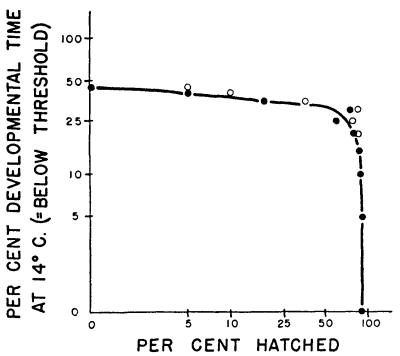


FIG. 11.—Plot of data from one experiment with *Oncopeltus* eggs showing relation between hatching and time at a subthreshold temperature. *Solid circles*, per cent that actually hatched; *open circles*, per cent hatched plus per cent rupturing egg shell.

Individual pairs laid from 48 to 540 eggs over periods of 1–4 days. A total of 9,395 eggs was obtained. Of these, 2,184 (37 per cent) burst the egg shell, but only 1,291 (13.7 per cent) hatched completely. Treating the data by individual pairs, the hatching percentage was distributed throughout the range of 0–24 per cent, with 10 pairs below 10 per cent, 11 pairs in the range of 10–20 per cent, and 6 pairs in the range of 20–24 per cent. There was no correlation between the number of eggs laid and the per cent hatching. Also there was no correlation between early and late eggs and hatching.

Most significant, however, is the subsequent history of individuals that hatched. If they had survived because of being more hardy, they should have grown readily and given one or more stocks of higher resistance. After hatching, the young were placed under standard culture conditions. Of the 1,291 which hatched, only 6 lived past the first instar! The 99 + per cent which died did so throughout the instar without any particular critical period (including the molt itself) being evident. And the 6 which lived were lethargic and less active relative to controls reared continuously at 25° C. Only 3 (all males) reached the adult stage. They were not followed further.

We conclude that the data from experiments giving partial hatching are not due to the eggs from some individuals being "resistant" and those from other individuals being "susceptible" to the treatment. Within the limits of deduction possible from this one test, there is nothing to suggest that genetic diversity within our culture population complicates the data. We are forced to conclude that minimal conditions of rearing give young of such weakened condition that death ensues and that the question of whether death occurs prior to, during, or subsequent to hatching is of little significance.

DISCUSSION

Inspection of the voluminous literature on the subject shows that there is a lack of definiteness to the term "threshold of development." A common point of view in the older literature is that the term "indicates the temperature at which, on the descending scale, de-

velopment definitely ceases and at which, on the ascending scale, development is again initiated" (Peairs, 1927); but "it is scarcely possible to register by any method a very small amount of development going on at a very slow rate" (Uvarov, 1931), and definitions or terms that call for doing this cannot be applied in practice. Some authors have attempted to clarify the situation by substituting a more definite term, e.g., "developmental zero," "alpha value," "temperature minimum," "critical point," etc., but the difficulty is due primarily to the complexity of phenomena involved rather than to the inappropriateness of any particular term.

More recent refinements involve recognizing various finite thresholds. Thus Johnson (1940), working with the bedbug, *Cimex*, recognizes a "developmental threshold" at about 4° C., a "hatching threshold" at 8° C., and a "developmental-hatching threshold" at 13° C. One of the more *useful* definitions, then, is "the lowest temperature at which a given physiological process, or development through a given stage in the life history can be carried through to completion" (Allee *et al.*, 1949). This is usable because it calls for the determination of something tangible. Results are recorded in terms of threshold for the particular process being determined.

Applying this to our data from *Oncopeltus* eggs, some development took place at the lowest temperature tested (5° C.), but development of essentially complete embryos occurred only within a few degrees of the temperature at which some hatching took place. No hatching occurred at a constant temperature of 14° C., but in both external and internal appearance some of the embryos appeared normally and nearly fully developed. Direct experimentation compared with various forms of calculations showed that the threshold for this species could be determined only by experimentation. It seems to us that a still more refined method of stating the results is to incorporate percentage of hatching. Thus, at 75 per cent R.H. and constant temperatures, the percentage of *Oncopeltus* eggs which hatched was about 24 per cent at 15°, about 65 per cent at 16°, about 82 per cent at 17°, and about 90 per cent at 20°–30° C. Unfavorable conditions, such as very low humidity, have the effect of lowering the per cent hatching or of raising the threshold temperature of hatching. On the other hand, alternating temperatures from above to below the threshold value, with the average temperature below the threshold for constant-temperature experiments, has the effect of lowering the threshold—if by "threshold" we mean the average temperature in these tests. Clearly, full specifications of testing conditions are necessary for evaluation of any stated threshold. Also, clearly, the effects of alternating temperatures with a given mean temperature are not the same as the effects of the corresponding constant temperature. With these points in mind, one wonders what degree of accuracy may be obtainable in attempting temperature-summation analyses to predict emergence dates under any except rigorously controlled environmental conditions which are obtainable only in the laboratory. We do not mean to imply that temperature summation under field conditions cannot be used as an *adequate* index to predict hatching, emergence, etc. The widespread use of the method attests its adequacy. Several factors enter into this. Field practices require only approximate, not precise, accuracy. Also seasonal weather is, on the average, sufficiently constant so that development occurring at subthreshold temperatures may be adequately con-

stant and unwittingly included in the initial standardization. Under exceptional season conditions, wide deviations could be encountered. Precise predictions require allowing for development that occurs at all temperatures, both above and below the so-called "threshold" value.

The discovery that development will continue to completion at a lower *average* temperature under alternating-temperature conditions, even though the exposure to the temperature above the threshold is of short duration, has considerable ecological significance. It suggests that a substantial amount of development will be excluded from consideration in temperature-summation analyses if at any time the daily average temperature falls to or a little below the threshold temperature. Such conditions are common during the spring and fall seasons.

The fact that development cannot be completed at a given constant temperature but can be under alternating temperatures having a corresponding mean temperature may be viewed as indicating that component processes respond differently to different temperatures. Thus a component process which cannot be completed at a lower temperature will become limiting under constant temperature, and the whole process will come to a standstill. Under alternating temperatures, however, this component process can be viewed as proceeding at the higher temperature, while other component processes proceed at both lower and higher temperatures. The net result is that development continues to completion at a lower average temperature, although individual processes may necessitate higher temperatures. Tests on the minimum time necessary at the threshold temperature gave the very low value of 4 per cent. However, the absence of any demonstrable "sensitive period" for the low- or high-temperature effect is unexplained and is unexpected, in view of what is known about embryonic development in general.

In retrospect it seems unfortunate that survival and subsequent development of the hatched individuals were not followed routinely. This was not done because time considerations precluded such observation. Some indication of what may be expected can be obtained from the set of tests checking the possibility that our culture of *Oncopeltus* was a genetically diverse population. In these tests at near minimal hatching conditions, a total of 9,395 eggs was obtained; of these, 2,184 burst the egg shell, 1,291 freed themselves completely (= "hatched"), but only 6 survived past the first instar, and only 3 (males) reached maturity. In percentage terms, 37 per cent burst the egg shell, 13.7 per cent hatched, but only 0.03 per cent reached maturity. Otherwise stated, over 99 per cent of the individuals of this species hatching under minimal conditions failed to survive and grow.

When the experimentation reached the stage of showing the absence of any "temperature-sensitive period," we thought that possibly development was independent of the hatching process and that perhaps we were measuring some unknown peculiarity of hatching. This now appears to us unlikely, since eggs from different pairs in the foregoing test gave hatching percentages varying from 0 to 24 per cent (average 13.7 per cent) and only a small fraction of 1 per cent of those hatching survived. It seems to us that we are more likely dealing with some intangible debility, where almost all individuals die under minimal conditions, and that the question of whether death occurs before, during, or after hatching is only a minor detail. In this

connection, Dr. C. M. Williams tells us that if pupae of saturniid moths are kept in the coldroom for many months, they develop a "winter sickness," from which they do not recover on being transferred to a temperature favorable for development. We have had similar experiences with eggs of the grasshopper *Melanoplus differentialis* held in the refrigerator for long periods. Dr. E. H. Slifer tells us that such eggs show considerable reduction in quantity of yolk, and hence possibly are having to expend too much energy for maintenance under conditions which do not permit morphogenesis to proceed. Whether or not the debility is similar in diapausing moth pupae, diapausing grasshopper eggs, and nondiapausing *Oncopeltus* eggs, the exposure of these insects to excessively prolonged subthreshold temperatures leads to a general debility and *subsequent* death, the cause of which is not understood.

Andrewartha (1952), in his review of diapause, distinguishes between physiogenesis and morphogenesis. Physiogenesis underlies morphogenesis and is to some extent dissociable from morphogenesis in diapausing insects. However, our data suggest that growth and viability are inseparable in the nondiapausing eggs of *Oncopeltus*. In other words, maintenance must be accompanied by morphogenesis. On the basis of this interpretation, the repeatability of hatching percentages under a stated set of controlled conditions would have to be interpreted as the repeatable production of a particular degree of debility in the population, which, on the average, allows the stated percentage of the population to survive past the time of hatching. Dr. Williams suggests that the hatching curves (Figs. 4–9) could appropriately be termed "loss of viability curves."

SUMMARY

1. Experiments were performed on the necessary temperature conditions for development and hatching of eggs of *O. fasciatus* and *T. confusum*.

2. The time-temperature curves are not true hyperbolas, and rate-temperature curves are not rectilinear. Hence thresholds need to be experimentally determined. Hatching per cent curves are diverse and variable in course, though fairly constant for end-points; they do not appear amenable to mathematical treatment.

3. Results are comparable only when obtained under rigorously controlled conditions, and values should include a complete statement of these conditions. Thus, under constant-temperature conditions and at 75 per cent R.H., the hatching of *O. fasciatus* eggs was found to average 24 per cent at 15°, 65 per cent at 16°, 82 per cent at 17°, about 90 per cent at 20°–30°, 13 per cent at 35° C., but zero at 14° or lower and at 38° or higher. Under similar constant-temperature and humidity conditions, the hatching of *T. confusum* eggs was found to be 0 at 16°, 21 per cent at 17°, about 78 per cent at 20°–25°, 80 per cent at 30°, 88 per cent at 35°, and 27 per cent at 40° C.

4. When eggs are placed at a temperature below threshold for a part of their development and then shifted to complete their development at a temperature above threshold, or vice versa, a consideration of the time required for hatching shows that development occurs both above and below the constant-temperature threshold. These data permit estimating developmental rates at subthreshold temperatures. The time curves are supported by microscopic observation of embryonic development within the eggs.

5. Summation of temperatures above

a constant-temperature threshold cannot be used to predict precisely the hatching time for these species unless account is taken of fractional percentages of development at the below-threshold temperatures. If the low temperature is near the constant-temperature threshold, a large percentage of development occurs at the low temperature. If the low temperature is considerably below the constant-temperature threshold, the correction becomes small or even negligible.

Low or very high humidities appear to be unfavorable at the threshold temperature. They have the effect of raising the threshold slightly (1°–2° C.).

6. Attempts to determine whether regular alternation or a single shift between two temperatures would accelerate or retard developmental rates gave inconsistent results. When both temperatures were above threshold, no acceleration was observed. When one of the temperatures was below threshold, a calculated acceleration was obtained in some experiments but not in others.

7. Under daily alternations from above to slightly below the constant-temperature threshold, development proceeds to hatching, even when the *average* temperature is more than 1° C. below the constant-temperature threshold. When the *average* temperature equals the constant-temperature threshold, a much higher hatching percentage is obtained.

When the subthreshold temperature is considerably below threshold (e.g., 10° C. for *Oncopeltus*), hatching time is consistent with the possibility that development occurs only when at the above-threshold temperature.

8. Attempts to analyze the nature of the threshold-temperature effect in *Oncopeltus* gave negative results but eliminated certain possibilities. The subthreshold temperature does not block embryonic development or tissue differentiation; neither does it block the hatching process per se. Maintenance is accompanied by morphogenesis. There is no "sensitive period" anywhere throughout development for the subthreshold-temperature effect. Low hatching percentages at and near threshold conditions seem not to be due to selection within a genetically diverse population. If eggs are shifted not more than two or three times between 14° and 20° or 25° C., then somewhat more than 50 per cent of developmental time is required at the higher temperature to complete development. If eggs are alternated daily between the two temperatures, then not more than 4 per cent of the total time is required at 25°. Of the small percentage of eggs which hatch under minimal temperature conditions, less than 1 per cent survive. It seems that minimal conditions give debilitated individuals destined to die before, during, or after hatching.

LITERATURE CITED

ALLEE, W. C.; EMERSON, A. E.; PARK, O.; PARK, T.; and SCHMID, K. P. 1949. Principles of animal ecology. Philadelphia and London: W. B. Saunders Co.

ANDREWARTHA, H. G. 1952. Diapause in relation to the ecology of insects. Biol. Rev., **27**:50–107.

BĚLEHRÁDEK, J. 1935. Temperature and living matter. ("Protoplasma-Monographien," Vol. **8**.) Berlin: Bormtraeger.

BROWNING, T. O. 1952. The influence of temperature on the rate of development of insects, with special reference to the eggs of *Gryllulus commodus* Walker. Australian J. Sci. Res., ser. B, **5**:96–111.

BUTT, F. H. 1949. Embryology of the milkweed bug, *Oncopeltus fasciatus* (Hemiptera). Cornell Univ. Agr. Exper. Sta., Memoir 283.

CHAPMAN, R. N., and BAIRD, LILLIAN. 1934. The biotic constants of *Tribolium confusum* Duval. Jour. Exper. Zoöl., **68**:293–304.

DAVIDSON, J. 1944. On the relationship between

temperature and rate of development of insects at constant temperatures. Jour. Anim. Ecol., **13**:26–38.

HUFFAKER, C. B. 1944. The temperature relations of the immature stages of the malarial mosquito, *Anopheles quadrimaculatus* Say, with a comparison of the developmental power of constant and variable temperatures in insect metabolism. Ann. Ant. Soc. Amer., **37**:1–27.

JANISCH, E. 1932. The influence of temperature on the life-history of insects. Trans. Ent. Soc. London, **80**:137–68.

JOHNSON, C. G. 1940. Development, hatching and mortality of the eggs of *Cimex lectularius* L. (Hemiptera) in relation to climate, with observations on the effects of preconditioning to temperature. Parasitology, **32**:127–73.

KROGH, A. 1914. On the influence of the temperature on the rate of embryonic development. Zeitschr. f. allg. Physiol., **16**:163–77.

LUDWIG, D., and CABLE, R. M. 1933. The effect of alternating temperature on the pupal development of *Drosophila melanogaster* Meigen. Physiol. Zoöl., **6**:493–508.

PEAIRS, L. M. 1927. Some phases of the relation of temperature to the development of insects. West Virginia Univ. Agr. Exper. Sta., Bull. 208.

SANDERSON, E. D., and PEAIRS, L. M. 1913. The relation of temperature to insect life. New Hampshire Coll. Agr. Exper. Sta., Tech. Bull. 7.

SHELFORD, V. E. 1927. An experimental investigation of the relations of the codling moth to weather and climate. Illinois Nat. Hist. Survey, Bull., **16**:315–440.

SOLOMON, M. E. 1951. Control of humidity with potassium hydroxide, sulphuric acid, or other solutions. Bull. Ent. Res., **42**:543–54.

UVAROV, B. P. 1931. Insects and climate. Trans. Ent. Soc. London, **79**:1–247.

VILLEE, C. A. 1942. A study of hereditary homoeosis: the mutant tetraltera in *Drosophila melanogaster*. Univ. Calif. Pub. Zoöl., **49**:125–83.

THE EFFECTS OF X-RAYS ON THE RESPIRATORY METABOLISM OF EGGS AND EMBRYOS OF THE GRASSHOPPER CHORTOPHAGA VIRIDIFASCIATA[1]

S. R. TIPTON AND G. S. ST. AMAND[2]

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, and Department of Zoölogy and Entomology, University of Tennessee, Knoxville, Tennessee

CONSIDERABLE information is available on the respiratory physiology of eggs and embryos of *Melanoplus differentialis* in extensive publications by Bodine and his colleagues. The effect of X-irradiation on the respiration and development of *Melanoplus* eggs and embryos has been investigated by several workers. Although Evans (1934, 1935) showed development to be completely halted by 6,000 r of X-rays, he did not conclusively show immediate respiratory depression of pre- and postdiapause stages. Boell, Ray, and Bodine (1937) studied immediate and delayed effects of X-rays on respiration of both developing and diapause embryos and eggs. A dose of 2,040 r had no effect on respiration of diapause embryos or eggs and no immediate effect on the respiration of postdiapause embryos or eggs. Tahmisian (1949) could demonstrate respiratory inhibition of diapause eggs only with doses of 100,000 r and above. In 1947 Tahmisian and Barron reported an immediate depression of respiration of pre- and postdiapause embryos of *Melanoplus* by as little as 10 r of X-rays. Five hours later, in most cases, respiration had returned to normal.

The nondiapausing grasshopper *Chortophaga viridifasciata* has been used principally for cytological investigations, and little physiological information has been reported. In cytological studies the neuro-

[1] Work performed under Contract No. W-7405-Eng-26 for the Atomic Energy Commission. Contribution No. 100 of the Department of Zoölogy and Entomology.

[2] The authors gratefully acknowledge the valuable advice and criticisms of Dr. Mary Esther Gaulden throughout the course of this investigation.