TEMPERATURE CHARACTERISTICS FOR DURATION OF AN INSTAR IN CLADOCERANS.

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I.

The analysis of growth phenomena through determinations of their relations to temperature requires measurements of the rates of development within stages morphologically well defined and exhibited by individuals genetically uniform and comparable. The duration of an adult instar in Cladocera parthenogenetically produced within a single clone is in several respects a very suitable object for such measurements. It is not altogether clear, however, whether the incidence of ecdysis is directly determined by the growth of the female, or by the full development of her young which ecdysis liberates. It is proposed to investigate this point more fully in subsequent experiments, but the latter interpretation is probably the correct one, for mothers about to release young, if transferred to a considerably higher temperature, will release the young properly but they themselves become caught in the moult which is being cast. In other words the young, at the higher temperature, have reached full development and have apparently caused the mother to moult before the carapace was in proper condition to be cast. For the present it is sufficient to note that the period of growth or development utilized for measurement bears relations to temperature of a kind very closely comparable with those shown by relatively simple vital processes (Crozier, 1924–25, *a, b*), and obtained likewise for the velocity of development within a clearly delimited stage in *Drosophila* (Bliss, 1925–26).

Comparison of several species of cladocerans reared in the laboratory for many generations under similar conditions should reveal spe-

111

cific differences, should such be present, and might be taken to suggest a means for the physiological comparison of related forms.

Three species of Cladocera were employed. Two of these, *Moina macrocopa* and *Simocephalus serrulatus*, belong to the family Daphnidæ; the third species, *Pseudosida bidentata*, belongs to the family Sididæ. A few individuals of *M. macrocopa* and of *P. bidentata* were sent me by Dr. A. M. Banta, both forms having been reared parthenogenetically for many generations in his laboratory. *M. macrocopa* was originally collected near Cold Spring Harbor, Long Island, and it occurs there in small exposed ponds from April through October. The population in the ponds increases rapidly to a maximum in June and the form is found in slightly diminished numbers until the middle of October. The clone of *P. bidentata* that was used was started from individuals collected in Florida in February; I have not taken this form in the north. *S. serrulatus* was collected near Cambridge, Massachusetts, early in October. In general it is a species that is quite wide spread and it may also be taken during any month of the year, usually reaching large numbers towards the last half of April and the first of May, continuing with few individuals during July and August, and reaching a maximum in October.

II.

In the laboratory, reproduction in all species was exclusively parthenogenetic, starting from one female and thus insuring material genotypically identical throughout. The stock cultures were reared at room temperature in an especially devised culture medium (Banta, 1921). The animals used in any one experiment were usually the first or second generation descendants of one individual. Such animals were reared with ample food, so that they would produce average sized to large broods, brood size being a good indication of the vigor of the stock. For precise work it is necessary to study a stage of development having sharply marked beginning and end. In this case one adult instar, usually the second, was chosen as the period to be measured. The successive instars of a given animal, when reared at the same temperature, do not show any appreciable differences in length. The beginning of an adult instar is marked by the release of active young daphnids from the brood chamber of the mother, and the

end of the instar is marked by the release of the succeeding brood of young. In *M. macrocopa* the release of the young, the moulting of the mother, and the passing of a new clutch of eggs to the brood chamber occupies from 2 to 6 minutes at room temperature. The time for this series of events is slightly more variable in *P. bidentata*, but not so variable as in *S. serrulatus* in which the time from release of young to egg laying varies from a few minutes to half an hour in extreme cases.

As the adult females to be tested were nearing the end of an instar they were transferred to individual bottles and placed in constant temperature cabinets. A bottle of food was placed beside a bottle containing a female. The termination of an adult instar, that is, the appearance of young in the bottle, is foreshadowed by the darkening and coalescing of the eye rudiments of the embryos. Observation of the mothers was made at frequent intervals (15 minutes to an hour, depending on the temperature), and the time of the release of the young noted. The mother was transferred to the adjacent bottle and allowed to remain there until the next brood of young was released. When it was impossible to watch for the termination of the instar in question at intervals of an hour or less, those mothers that probably would soon liberate young were examined microscopically and an estimation made of the probable time of release of her young (based upon the condition of the eye pigment of the embryos). This estimated time was further checked by observing the developing eggs of the next brood and noting the stage of segmentation of the egg. If the elapsed time was too long the animal was of course discarded.

Four temperature cabinets were used, in each of which the temperature to be maintained could be raised or lowered at will. The cabinets were heated by carbon filament bulbs controlled by a mercury thermoregulator. The different regions (shelves) of the cabinet varied in temperature depending on the distance from the heating unit, but the temperature of each bottle was taken directly. The extreme variation within a given bottle during an experiment was ± 0.5°C. Slightly different rates of general metabolism are found for the females producing male broods and those producing female broods (Banta and Brown, 1924–25). This has been determined for *M. macrocopa;* the male broods are released on the average later (half an hour, more or
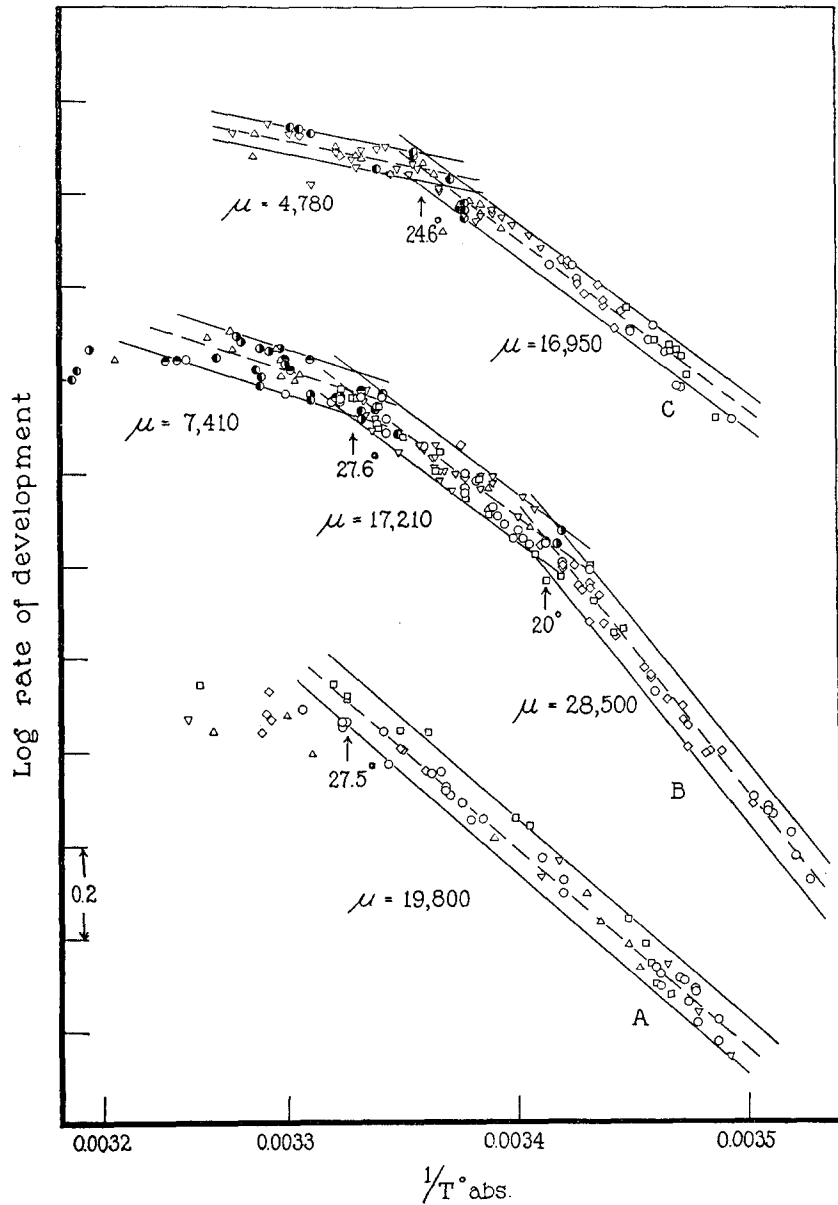
FIG. 1. Graphs for rates of development plotted against reciprocals of the absolute temperatures. *A, Pseudosida bidentata. B, Moina macrocopa. C, Simocephalus serrulatus.* Each point represents an individual animal and the different symbols in each graph denote a single experiment. The values of μ are given opposite the segments of the graphs, and the critical points are indicated by arrows, with the centigrade temperatures given for these points. (Rate of development = 10,000 ÷ time in minutes; one unit on log scale = 0.2.)

114

less) than the female broods.   It has not been possible to employ num-
bers of animals sufficient to remove this source of variation, which
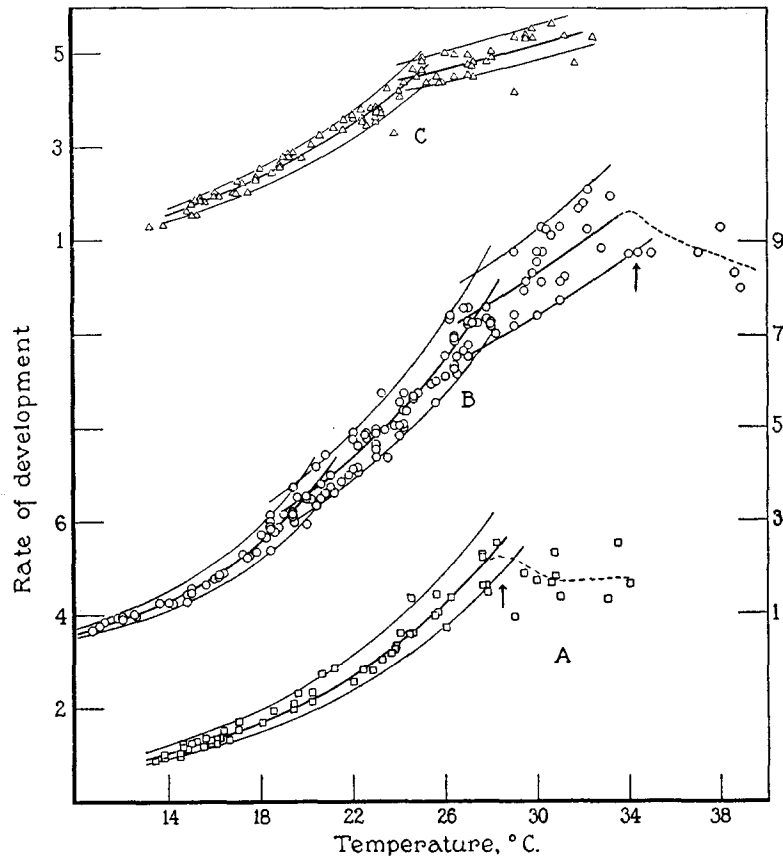would at most amount to between 1 and 2 per cent of the total observed
time.

FIG. 2. *A*, *P. bidentata*.   *B*, *M. macrocopa*.   *C*, *S. serrulatus*.   The rates of
development are plotted against centrigrade temperatures, and the points are
entered without distinguishing individual experiments.   The lighter lines and the
heavier lines are transposed from those in Fig. 1.

III.

Fig. 1 gives the graphs for the rates of development in the three
species.   The logarithm of the rate is plotted against the reciprocal

of the absolute temperature. Each point on the graph represents an individual animal and the different symbols in each graph denote a single experiment. The experiments were conducted at different times between January and May of the same year to examine the possibility of seasonal rhythm. It is clear that there is no secular drift due to such a cause.

The graph for *S. serrulatus* (Fig. 1, *C*) shows a break at 24.6°C. The slope of the line below this temperature gives a temperature characteristic μ = 16,950. In the neighborhood of 15° and below there are insufficient data to determine the exact slope and the presence or absence of a break at this point. At 24°C. there is a point (upright triangle) which has a lower rate. And again at 29°C. there is a point (inverted triangle) which has a lower rate. In neither case was there sufficient latitude in the observations to bring these points within the range of variation of the other points on the graph. These may be individuals abnormal either through starvation or age. The character of the line above 32° has not yet been determined. The slope between 24.6° and 32° yields μ = 4,780.

The graph for *M. macrocopa* (Fig. 1, *B*) is based on more animals than are the other two graphs. There are three evident breaks or critical temperatures. The first break is at 20°, the second at 27.6° and the third somewhere near 33°. The steepest slope, with μ = 28,500, is found between 11° and 20°. The slope of the line between 20° and 27.6° gives μ = 17,210, which agrees fairly well with that for *S. serrulatus* below 24.6°. Above 27.6° there is a distinct lessening of the slope, the value for μ dropping to 7,410. Above 33° the points become irregular and the curve flattens out into an almost horizontal position. It is interesting to note that even at 38° + the females of this species produce apparently normal young. The range of variation, as indicated by the limiting lines, is quite uniform for the different parts of the graph, being slightly greater above 27°; this may be due to the faster rate of development and the correspondingly decreased accuracy of the observations.

The graph for *P. bidentata* (Fig. 1, *A*) is strikingly different from the other two. This graph consists of one straight band, having the value of μ = 19,800, extending from 14° to 27.5°. Beyond 28° the points scatter and the curve assumes a position approximately parallel

to the temperature axis. There is thus but one break, at 27.5°, in the graph for *P. bidentata*.

Inspection of graphs such as that for *M. macrocopa* in Fig. 1 may lead to the superficial suggestion that it is preferable or possible to draw through the plotted points a curve, rather than several straight lines. The fact that it is impossible to fit a single simple curve to the graphs for *S. serrulatus* and *M. macrocopa* is perhaps shown more clearly in Fig. 2. In this figure the rate of development, that is the reciprocal of the time taken to complete an instar, is plotted against centigrade temperature. The lighter limiting lines and the heavier lines in this figure are transposed from those in Fig. 1. It is impossible to fit a single smooth curve through the points for either of these species. The points fall respectively into two and three cusps (when the irregular individuals above 33° for *M. macrocopa* are excluded). The points for *P. bidentata* form a smooth curve from 14° to 27.5°, this whole range yielding a constant value of μ.

The range of variation of course increases with an increase in temperature. It will also be noticed that the latitude of variation for *S. serrulatus* is much smaller than for *M. macrocopa* and for *P. bidentata*, in spite of the fact that the end-points for an instar in *S. serrulatus* are more variable, intrinsically, than in the other two species. This would seem to indicate that the latitude of variation is specifically determined, but is not a property of the system controlled by the nature of the process which determines the temperature characteristic.

IV.

A comparison of the values for μ with similar values obtained for rates of development in other arthropods shows some remarkable similarities, and may be taken to indicate a similar chemical control in the several instances. Bliss (1925–26) obtained a value for μ of 16,850 for the prepupal development of *Drosophila melanogaster*, from 16° to 25°. Crozier (1924–25, *b*), using data from Krogh, obtained a value of 16,850, 16° to 32°, for μ in O_2 utilization of *Tenebrio* pupæ, and Orr (1924–25) obtained a value for μ of 16,800, 1° to 15°, for the O_2 consumption in the prepupa and pupa of *Drosophila*. These values are of the same order as the 16,950 obtained for *S. serrulatus* between 15° and 25°, and the value 17,210 obtained for *M. macrocopa*

between 20° and 28°. The value of μ for *M. macrocopa* below 20° does not correspond to the value obtained by Bliss with *Drosophila* below 16°, but it may be of the same nature (28,500) as the value 27,000 obtained from Krogh's data by Crozier (1924–25, *b*), below 22° in *Tenebrio* pupæ, that for *Drosophila* egg and larva between 10° and 20° and for *Drosophila* pupa at 15° to 20°, both of which gave μ = 27,000 (Crozier, 1924–25, *b;* data from Loeb and Northrop). For *M. macro-copa* the temperature range 28° to 33° yields μ = 7,410, which compares favorably with the value obtained by Bliss for prepupal development in *Drosophila* between 25° and 30° (*i.e.*, 7,100). Also it might be noted that the distribution of the temperature characteristics, with the exception of 4,780 for the upper portion of the graph for *S. serrulatus*, corresponds to peaks in the frequency graph for temperature characteristics given by Crozier (1925–26, *d*).

The breaks in Fig. 1, when rounded off to the nearest whole degree, are located at 15° (?), 20°, 25°, 28°, 30°, and 33°. Crozier has pointed out (Crozier, 1925–26, *c*) that such critical points are usually found in the neighborhood of 4.5°, 9°, 15°, 20°, 25°, 27°, and 30°. Setchell (1925) has pointed out that aquatic and land plants give definite points of critical temperature for anthesis, these points being at approximately regular intervals of 5 degrees from 5° to 30°. The critical points obtaining for the three species of Cladocera thus agree in a rather remarkable way with those found for vital phenomena in general.

A comparison of the three species of Cladocera used in these experiments shows some points of possible significance. The temperatures for *S. serrulatus* above which rate of development is relatively slower, *i.e.* lower value for μ, are 15° (?), 25°, and 30°, while the corresponding temperatures for *M. macrocopa* are 20°, 28°, and 33°. Thus *M. macrocopa* is, upon the basis of rate of development at least, enabled to take advantage of increasing temperature by producing broods in quicker succession than *S. serrulatus*. And conversely, on a lowering temperature, *M. macrocopa* is relatively more slowed up. As previously stated, *M. macrocopa* is typically a summer form while *S. serrulatus* occurs throughout the year but in greater abundance in the spring and autumn. *P. bidentata* cannot be compared so directly. This species maintains a constant μ = 19,800 from 13° to 28°. This

rate of increase with temperature is decidedly greater than that of the other two species above 20°. The distribution of *P. bidentata* is known to be southern and was studied during the winter, but its annual rhythm of abundance is not known at present.

SUMMARY.

1. The temperature characteristics for the rate of development during a well defined instar were determined for three species of Cladocera, and found to be of the same general magnitudes as those obtained for rates of development and of O_2 consumption in other arthropods.

2. Critical temperatures were found to occur at points most frequently critical in quite diverse vital phenomena as determined by abrupt changes in the relationship between rate and temperature.

3. A suggestion is made that, since the values of μ and the positions of critical temperatures obtained for the different species are not the same, some relation may exist between the occurrence of these forms in nature and their relative rates of development as controlled by temperatures.

CITATIONS.

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