

TEMPERATURE CHARACTERISTICS FOR PREPUPAL
DEVELOPMENT IN *DROSOPHILA*
MELANOGASTER.*

By CHESTER I. BLISS.

(From the Zoological Laboratory, Columbia University, New York.)

(Accepted for publication, January 18, 1926.)

Recently attempts have been made to interpret the effect of temperature upon the rate of life phenomena in terms of chemical processes. There are peculiar difficulties, however, in applying such interpretations to development, principally the errors introduced by using an average rate as if it were an instantaneous velocity. This assumes that a single rate-controlling chemical process extends throughout the stage delimited by the markers which time its beginning and end. In one case in which this has been analyzed, the embryonic development of the grape leaf-hopper, a series of successive processes, differing markedly in temperature coefficient, limits the developmental rate (Bliss, 1926). This analysis seemed to warrant further trial under more favorable experimental conditions.

The *Drosophila* pupa which had already been studied by Loeb and Northrop (1917) was chosen as suitable material for testing variations in the temperature characteristic through development and the conclusions therefrom upon the controlling chemical reactions, if chemical. There proved to be several difficulties, however, in using the entire puparial period for the investigation.

Although equal numbers of larvæ formed puparia every hour throughout the day, the adults from these puparia did not similarly emerge in equal numbers.

* The experiments reported here were begun in the Zoological Laboratory at Columbia University and completed at the Marine Biological Laboratory at Woods Hole, Massachusetts, during the summer of 1925. I am especially indebted to Professor T. H. Morgan for suggestions during the course of these studies and to my assistant at Woods Hole, Miss Mary Dunlap.

From about 5 or 6 p.m. until 4 o'clock the next morning in one experiment, for example, the number of adults emerging each hour was less than one-fourth the number of larvæ forming puparia. Beginning at 4 a.m., however, adult flies began to appear in large numbers, and for several hours two to three times as many adults emerged as puparia were formed, this increased emergence continuing until nearly noon. The diurnal fluctuation has appeared regularly, even under constant temperature, light, and humidity conditions. If the minimum time for the stage is used as standard, here 84 to 85 hours at 28.5°C. (female), nearly 75 per cent of the flies did not emerge until from 1 to 9 hours after they had presumably completed development. Accordingly it proved impracticable to use emergence of the adult as an indicator of development.

The puparia, which are more or less transparent, were watched for morphological changes in order to secure markers free from rhythmic variations. The following stages were selected: puparium formation, pupation, first appearance of eye color, change in eye color from orange to red (or first appearance of head bristles which was nearly synchronous at the temperatures used), and emergence of the adult. These stages divided the puparial span into four parts, the first comprising the prepupal period, the last three the pupal stage. The length of each of these stages was then correlated with that of every other one, and the partial correlation coefficients determined. Using the conventional terminology (Yule, 1922), at 30°C.

$$\begin{array}{ll}
 r_{23-14} = -.41 \pm .05 & r_{14-23} = .19 \pm .06 \\
 r_{24-12} = -.35 \pm .05 & r_{13-24} = -.13 \pm .06 \\
 r_{12-24} = -.31 \pm .06 & r_{24-13} = .02 \pm .06
 \end{array}$$

Variations in the length of the successive stages should not influence each other if the markers separated them adequately, but the above results show a marked mutual influence between successive stages. The first appearance of eye color seems the most unreliable marker, followed by change in eye color and pupation. This particular experiment was free from the systematic error in time of emergence. On the basis of these data we cannot judge to what extent the variations in the markers were due to relative independence of the visible change from the rate-controlling developmental systems, and to what extent to experimental errors in recognizing the markers—the order of unreliability parallels that of experimental difficulty. Because of the theoretical and experimental advantages of treating each stage independently, the prepupal period alone will be considered here.

Material and Methods.

A mutant race (sooty) of *Drosophila melanogaster*, inbred by brother-sister matings for over twenty generations, insured relative genetic uniformity. The flies were raised on the usual banana agar in $\frac{1}{2}$ pint milk bottles but without paper. When the larvæ reached maturity, they crawled up the sides of the

bottles and formed puparia on the glass from which they were removed at $\frac{1}{2}$ hour intervals with fine, flexible eye-knives. Under such conditions, two workers could examine 30 to 40 bottles and remove the puparia, usually 25 to 30 of them, in less than 10 minutes time. Small black paper blocks filled with 2 per cent agar-agar, provided a moist dark substratum for the prepupæ and could be easily handled in Petri dishes in the incubators. After an appropriate interval these were examined every $\frac{1}{2}$ hour to determine time of pupation. Following pupation, they were raised at a temperature of 25–28° until the sex combs appeared and the sex of the pupæ could be determined.

The temperature was controlled by means of toluol-mercury regulators. Although the thermoregulation was not as reliable as could be desired, in the experiments included here it probably varied within a range of less than 0.3°C. Thermograph records gave practically straight lines. Each temperature was read several times on a mercury thermometer. Except one graduated to $\frac{1}{4}$ °, the thermometers were graduated to single degrees centigrade, and all were calibrated at the beginning of the experiment with respect to an instrument that had been checked at the Bureau of Standards.

Larvæ which had everted the anterior spiracles and did not crawl upon handling were deemed puparia. Individuals in which the conspicuous, longitudinal, dorsal tracheæ did not extend unbroken from the anterior to the posterior spiracles, or in which a space had appeared between the posterior dorsal surface of the body and the puparium were judged to have completed the prepupal stage. Both of these markers were sharp and unmistakable.

Morphological Changes in the Prepupal Stage.

When the larva has ceased feeding, it crawls out of the food up the sides of the bottle, and, after approximately 2 hours time, it becomes quiescent, unless disturbed, and everts the anterior horns. The larval skin shortens, loses all signs of segmentation, and becomes the puparium from which the adult eventually emerges. This entire period may be called the puparial stage, and is equivalent to the pupal period of *Drosophila* literature. As first formed the puparium certainly does not contain the pupa as the term is generally understood among entomologists, a fact recently emphasized by Snodgrass (1924). The body completely fills the puparium, the larval longitudinal dorsal tracheæ, heart, and fat bodies are very apparent, and there is no external trace of adult structures. It is not until over 11 hours later (at 25°C.) that pupation occurs within the puparium.

Pupation is begun by a contraction of the body away from the posterior-dorsal region of the puparium, so that it occupies only four-fifths of the space. The longitudinal tracheæ break their connections with the posterior spiracles, sink into the body, and disappear. Presently the anterior part of the body pulls away from the puparium and free of the larval mouth armature which is forced flat against the ventral wall of the puparium by the evagination of the head. Synchronously with this comparatively rapid process, the abdomen contracts ven-

trally so that it presses tightly against the dorsal wall of the puparium, leaving the ventral thoracic complex of wing pads and legs and the mouth parts touching the puparium, and, as a whole, clearly distinguishable from the abdomen. At this point in pupation—less than 13 minutes from its start (26°)—the head, thorax, and abdomen are all visible to dorsal view, the abdomen comparatively large. The imaginal disks of the thorax and head have been everted. Presently the legs elongate and become distinct from the wing pads and mouth parts and from one another—about 12 minutes later at 26°. The abdomen shrinks, and the pupa is fully formed, not nearly filling the puparium.

While the internal metamorphosis of *Drosophila* has never been described, it may be inferred from descriptions of the blow-fly, *Calliphora* (Kowalevsky, van Rees, Lowne, Perez,), and of the apple maggot, *Rhagoletis* (Snodgrass). The adult structures are formed from imaginal disks, distinguishable in the embryo, which develop throughout the larval stage as conspicuous internal buds. In the prepupal period of *Drosophila* this development is completed to the point of establishing the adult form.

The prepupal stage is characterized by an intense histolysis of most larval structures, such that the internal contents of the pupa just after pupation are largely fluid. This histolysis is probably nearly completed in the prepupal period. This is indicated by analogy with *Calliphora* (Perez) and by the considerably larger rate of oxygen consumption found by Bodine and Orr (1925) in first day pupæ (puparia) of *Drosophila*. Lack of agreement in temperature coefficient between rate of prepupal development and rate of oxygen consumption in *Drosophila* prepupæ (Orr, 1924–25), however, suggests that histolysis does not control developmental rate. In fact histolysis could exert such an effect only if it were to proceed at so slow a rate that the body fluids failed to supply the imaginal disks with sufficient nutrient, an improbable situation. From this evidence, therefore, and the absence of tissue differentiation upon completion of pupation, we may associate the limiting processes in prepupal development with the rate of growth and cell division in the imaginal disks, including those forming the head. In this latter characteristic, *Drosophila* differs from *Calliphora* and *Rhagoletis*, in which the head is not everted until some time after the thorax and appendages are visible. Evidence will be presented later to show that the imaginal disks probably do not control puparium formation or the rate of larval development.

The Temperature Characteristic for the Prepupal Period.

Two forms of temperature coefficient are in common use, Q_{10} and μ . The former is the ratio between the velocity of a process at temperature t° and its velocity at temperature $t^\circ - 10^\circ$. It is an empirical term without theoretical implications and has been found for many biological processes to decrease as the temperature increases. Although Q_{10} for smaller temperature intervals than 10° can be

TABLE I.
Length of Prepupal Period.

Temperature.	Experiment No.	No. of hrs. isolated.	Mean period.		Curve values.	
			Male.	Female.	Male.	Female.
°C.			<i>hrs.</i>	<i>hrs.</i>		
12.0	107c	2.0	63.00±.29	60.40±.25	63.25	60.94
12.8	106c	4.5	52.50±.09	51.31±.11	53.45	51.87
14.0	103d	2.0	43.45±.08	42.04±.12	41.68	40.37
14.9	105c	2.0	34.44±.07	33.45±.06	34.67	33.65
16.0	104a	1.0	27.22±.11	26.36±.12	27.73	26.92
16.8	107i	1.5	26.56±.07	25.83±.08	25.29	24.66
17.4	102d	1.5	23.89±.07	23.28±.07	23.88	23.23
18.3	106b	4.5	22.13±.04	21.57±.04	21.88	21.23
18.4	102a	2.0	20.81±.06	20.20±.06	21.68	21.04
19.3	103b	3.0	19.29±.05	18.83±.04	19.86	19.23
20.25	102c	3.5	18.55±.04	17.71±.04	18.11	17.54
	103a	3.0	17.63±.06	17.01±.05		
	103g	2.5	18.44±.06	18.01±.05		
	105a	1.5	18.08±.05	17.42±.06		
	105a	5.5	18.57±.03	18.09±.02		
	105b	7.5	18.26±.03	17.60±.02		
	106a	3.0	18.05±.05	17.48±.06		
	106d	1.5	17.90±.08	17.66±.07		
	107a	2.0	17.40±.04	16.96±.05		
21.24	103f	2.0	16.31±.04	15.65±.04	16.48	15.96
22.3	107b	1.5	14.58±.03	14.17±.04	14.93	14.42
23.0	103c	2.5	13.65±.03	13.16±.03	13.96	13.49
23.6	107e	1.5	13.34±.04	12.89±.03	13.21	12.76
	107e	1.0	13.28±.05	13.00±.04		
23.9	106e	4.5	12.98±.03	12.50±.05	12.82	12.39
24.1	102e	2.5	12.95±.04	12.56±.05	12.62	12.19
24.9	102b	1.5	11.79±.03	11.28±.03	11.80	11.25
26.1	103e	2.0	11.46±.03	10.83±.04	11.22	10.71
27.0	104e	2.5	10.55±.03	10.15±.04	10.76	10.30
28.2	104c	1.5	10.19±.04	9.89±.03	10.23	9.79
29.0	106f	4.5	9.78±.03	9.39±.03	9.88	9.49
30.0	104d	2.0	9.61±.05	9.08±.06	9.46	9.10
31.0	105d	3.0	9.40±.04	9.25±.03	9.07	8.73
32.0	107d	2.0	9.77±.03	9.42±.03	8.69	8.38
33.1	108a	3.5	10.20±.04	9.80±.03	8.30	8.02
20.25*	108b	7.0	19.12±.04	18.66±.05	18.11	17.54
24.8*	108c	4.5	13.11±.04	12.67±.02	11.80	11.40

* Temperature prior to puparium formation 16.7°C.

readily calculated, in practice it is seldom used for closer analysis. Recently the constant μ of Arrhenius' equation has come into greater favor both among chemists and biologists. Originally an empirical relation, it has acquired several theoretical explanations which are under active investigation by physical chemists. Aside from its probably possessing a theoretical significance, μ implies no particular

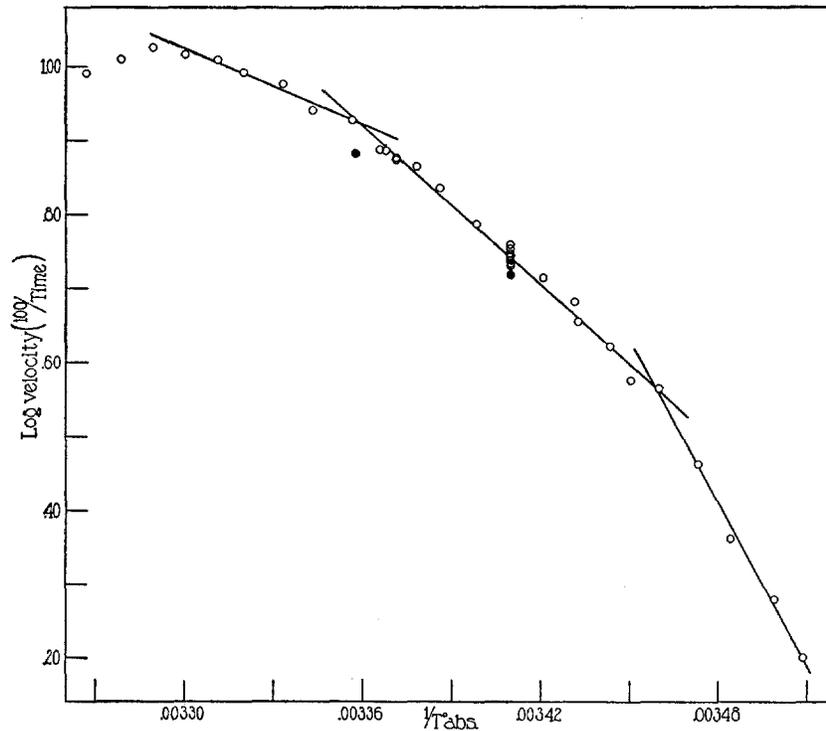


FIG. 1. The rate of prepupal development of the male, data from Table I.

temperature interval, is a much more sensitive measure, and is relatively constant over the temperature range encountered by biological processes. It is the measure here adopted.

The temperature characteristic μ is defined by the equation:

$$\log k_{T_2} - \log k_{T_1} = \frac{\mu}{4.605} \left(\frac{1}{T_1} - \frac{1}{T_2} \right)$$

in which $\log k_{T_2}$ and $\log k_{T_1}$ are the common logarithms of the velocity constants at absolute temperatures T_2 and T_1 respectively, and 4.605 the gas constant 2 multiplied by the modulus. The relation between $\log k$ and $1/T$ is rectilinear, μ giving the slope of the line, and in this form the data of Table I have been plotted, each sex separately, in Figs. 1 and 2. The experimental points very ob-

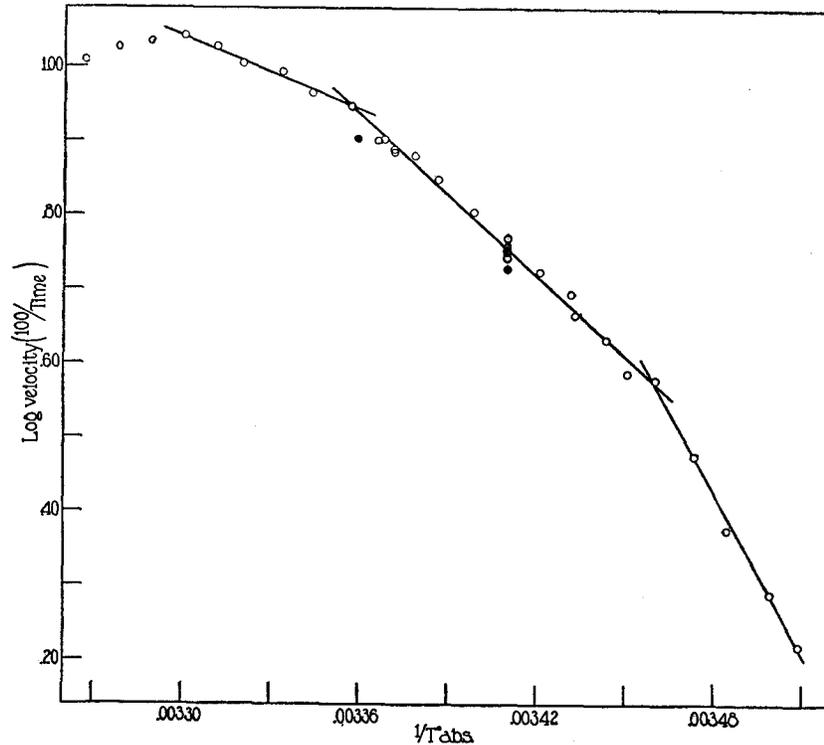


FIG. 2. The rate of prepupal development of the female, data from Table I.

viously do not fall on a single straight line, but instead seem to describe a curve. Were the relation really curvilinear, the temperature coefficient as defined by the Arrhenius equation could not be a constant, but would decrease as the temperature increased. An alternative treatment is to fit several straight lines to the points, representing different values of μ over different temperature intervals. This procedure has been used very effectively by Crozier

(1924-25), and will be followed here, with points of inflection at 16° and 25°C. (Figs. 1 and 2). For each sex separately three straight

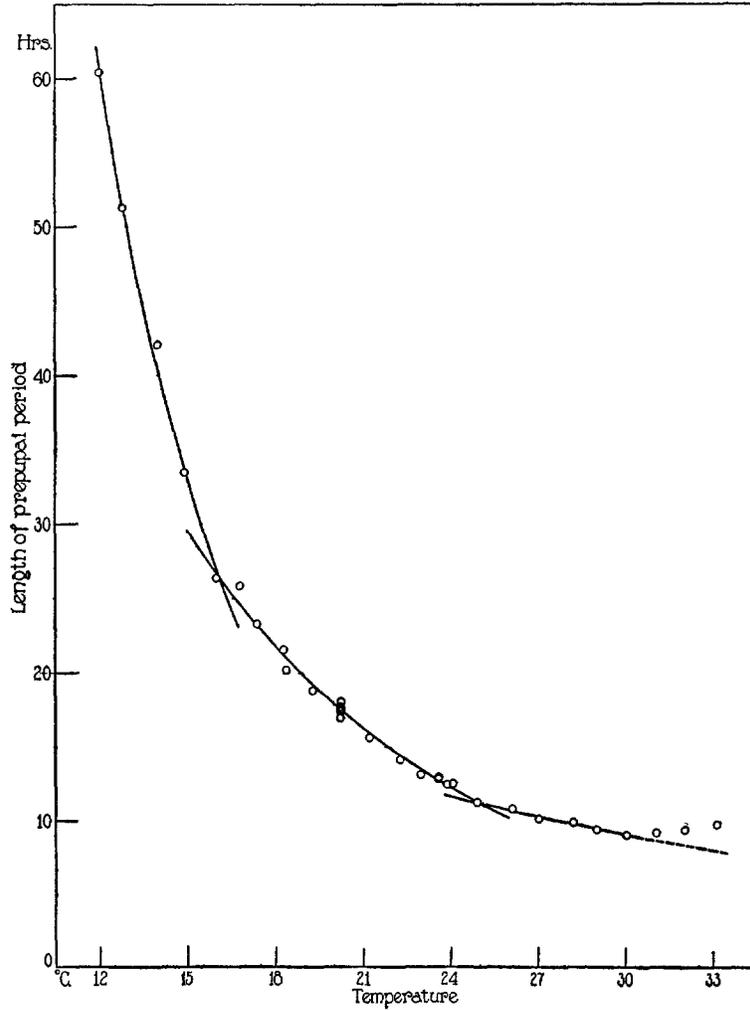


FIG. 3. Length of prepupal period in the female, curves transposed from Fig. 2.

lines have been fitted, weighting each temperature equally and using the principle that the line connecting the partial means passes through the mean of the whole distribution. The experimental values ap-

proximate the lines closely enough to justify this treatment, and compare favorably with most of the cases reported by Crozier.

Each value, however, is a mean of from 18 to 230 individuals (average 60) with its own probable error (Table I). Whether the probable error indicates normal variability may be tested by the coefficient of variation, $v = 100 \sigma/\text{mean}$. If the standard deviation (σ) satisfactorily measures the inherent chance fluctuation, it should maintain a constant ratio to the mean at different temperatures. This it does, the mean coefficient of variation for the 70 cases in Table I being $3.14 \pm .06$ per cent, the two sexes the same within the limits of the probable error ($v \sigma^{\text{♂}} = 3.07$ per cent, $v \sigma^{\text{♀}} = 3.20$ per cent). When the difference between a given mean and the corresponding value on the fitted curve, transposed as in Fig. 3, equals or exceeds three times the probable error of the mean, it may not be attributed to variability of the material. By this test, nearly three-fourths of the means differed from the corresponding curve values by a significant amount, not including temperatures above 30° . Unless these deviations can be further analyzed, the reliability of the values for μ is considerably diminished.

Analysis of Deviations from Expected Curve.

From inspection of Table I, two variables are apparent, (1) the number of hours over which the individuals entering a given mean were isolated, and (2) the order of experimentation in relation to temperature—the successive experiments being numbered serially. If the records from only two or three successive $\frac{1}{2}$ hour isolations are added to give a basic record, the first variable is eliminated and the second variable may be tested. This has been done and the results are given in Table II.

The ratio of the experimental mean period to the curve value for each sex (Table I) gives the proportionate deviation independently of temperature. Since the two sexes show very comparable temperature relations, it seems probable that the two sexes would be affected similarly in any given experiment if the deviations can be analyzed. Correlating the proportionate deviation from male expectation or curve value with the proportionate deviation from female expectation, the correlation coefficient is $.88 \pm .02$, verifying

TABLE II.
Age of Culture and Length of Prepupal Stage.

Experiment No.	Age of culture.	Male.		Female.		Experiment No.	Age of culture.	Male.		Female.	
		Mean period.	No.	Mean period.	No.			Mean period.	No.	Mean period.	No.
	<i>hrs.</i>						<i>hrs.</i>				
102a	3.0	20.68	28	20.08	23	105b	33.0	18.38	25	17.63	31
	4.0	20.88	24	20.27	32		34.2	18.45	41	17.83	36
b	24.8	11.79	62	11.28	47		35.5	18.32	14	17.55	31
c	27.0	18.67	26	18.02	21		36.5	18.02	27	17.41	33
	28.0	17.92	18	17.36	26		37.5	18.30	22	17.61	27
	31.2	18.52	52	17.77	45		38.5	18.18	31	17.78	30
d	39.8	23.89	49	23.28	37		39.5	18.06	17	17.44	41
e	51.5	12.85	20	12.52	20	c	40.5	34.42	31	33.65	24
	52.8	13.02	27	12.60	20		41.5	34.46	34	33.34	44
						d	56.8	9.25	16	9.20	27
103a	1.2	17.52	20	16.98	21		58.2	9.50	26	9.28	37
	3.5	17.74	21	17.06	16						
b	4.5	19.47	15	18.93	15	106a	7.2	18.06	16	17.57	15
	5.5	19.38	12	18.98	21		8.5	18.05	21	17.38	13
	6.5	19.11	22	18.58	19	b	10.2	21.96	13	21.60	24
c	7.8	13.60	39	13.06	35		11.5	22.25	28	21.43	22
	9.0	13.71	28	13.30	23		13.2	22.05	22	21.64	28
d	10.0	43.34	38	41.92	24	c	14.8	52.84	28	51.02	22
	11.0	43.48	27	42.19	18		16.2	52.31	18	51.61	18
e	28.0	11.45	31	10.80	28		17.8	52.20	20	51.37	15
	29.0	11.48	26	10.86	31	d	31.2	17.90	19	17.66	19
f	30.0	16.25	34	15.65	23	e	32.8	13.13	19	12.83	9
	31.0	16.39	28	15.66	29		34.2	12.92	18	12.50	16
g	47.5	18.17	24	17.76	42		35.5	12.92	24	12.36	22
	48.8	18.64	31	18.21	29	f	37.2	9.62	20	9.20	15
							38.8	9.76	33	9.39	44
104a	8.0	27.22	18	26.36	29		40.2	9.96	22	9.50	28
c	32.8	10.19	61	9.89	52						
d	43.5	9.42	20	9.00	14	107a	3.0	17.42	31	16.98	32
	44.5	9.84	16	9.12	24		4.0	17.37	26	16.92	25
e	50.5	10.51	38	10.07	28	b	5.2	14.58	54	14.17	55
	51.8	10.58	38	10.23	31	c	6.5	62.31	29	59.40	20
							7.5	63.95	21	61.20	25
105a	7.2	18.08	36	17.42	38	d	8.5	9.78	27	9.34	28
	13.0	18.88	26	18.14	22		9.5	9.76	29	9.52	24
	14.5	18.42	38	17.89	41	e	22.2	13.34	46	12.89	37
	15.8	18.35	47	17.71	41		29.0	13.28	18	13.00	30
	17.0	18.56	25	18.14	39	i	33.2	26.56	25	25.83	36
	18.0	18.88	26	18.45	30						
						108a	1.5	10.18	22	9.86	38
							3.2	10.21	26	9.74	43

the view that the deviations exceed those expected by random sampling and indicating that their causes affect both sexes equally.

The critical increments are based upon puparia isolated over a period of 2 or 3 days each from six sets of bottles, Experiments 102 to 107 inclusive. Two variables are present: (1) differences in food conditions between the different sets of bottles, none of them being aseptic, and (2) differences correlated with age of the cultures. In no one experiment were the results strikingly different from the others, so that although the first factor possibly contributed to the irregularity in the second, it could not be measured. When the proportional deviations were plotted as a function of the time from the first isolation, the earlier lots of prepupæ were found to have developed faster than those forming puparia somewhat later, although on the second day the trend seemed in the opposite direction. Unfortunately the relation of the first isolation to the beginning of puparium formation was not recorded, but is known to have varied as much as 8 to 10 hours. Accordingly a correction factor of 6 hours for Experiments 105 and 106, and of 2 hours for 102 has been added in each instance on the assumption that the different experiments are really comparable. The results (Fig. 4) are admittedly not very satisfying, although the correlation ratio, η , corrected for too fine a grouping, is $.69 \pm .03$. The curve of the figure is so drawn as to approximate the means calculated in determining η .

There is other evidence, however, that the successively isolated individuals from the same parents show an increasing prepupal period, primarily that of experiments on length of successive puparial stages on which the partial correlations referred to earlier are based. During 14 successive hours (at 30°) the prepupal stage lengthened consistently. In the experiments on diurnal fluctuations in emergence where puparia were isolated and timed for 50 consecutive hours, the mean length of the puparial period, plotted as a function of hour of day of isolation, was consistently longer for those isolated on the 2nd day than for those on the 1st. This result indicates that the delay in the prepupal period is not compensated for in the pupal stage. The possible causes of this effect will be considered in another connection.

Another factor causing the deviation to exceed chance fluctuation

may be inaccurate temperature control both in incubator regulation and during manipulation. While the temperature during the prepupal stage held fairly constant, that during the larval period was not of comparable accuracy, so that all individuals compared had not necessarily reached the same stage in development at the time of puparium formation. Room temperature during isolation averaged 21–22°, while that of the bottles was presumably 27°. The frequent removal of the bottles from the incubator for an appreciable time during manipulation probably caused some variation.

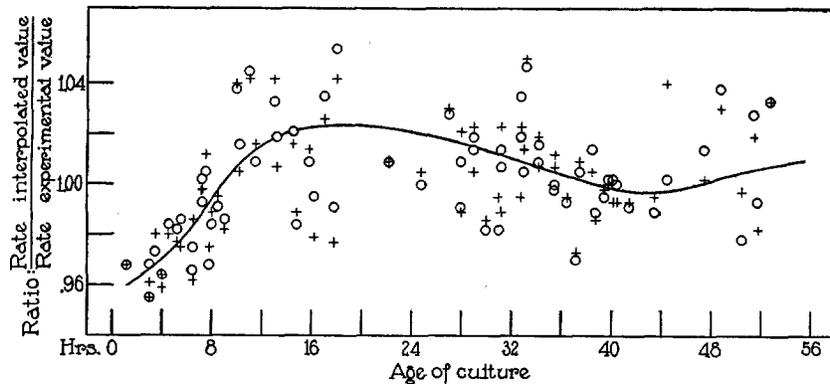


FIG. 4. Relation of proportionate deviations to age of culture, males plotted as crosses, females as circles.

Sex Differences.

As originally determined, the temperature characteristics differ slightly between the two sexes, -0.5 per cent (12–16°), 1.4 per cent (16–25°), and -4.6 per cent (25–30°) on a male basis. If this can be attributed to experimental error, the relative rates of development of the sexes should be constant within the temperature range fitted by a single straight line. If they show a shift with temperature which exceeds the normal variability, the difference in the critical increments is probably significant.

The ratio between the rate of female development and rate of male development, calculated from Table II, has been plotted in Fig. 5 as a function of temperature. Although the diagram shows great variability at all temperatures, a slight increase is apparent in

the developmental rate of the female relative to the male as the temperature rises. Whether this is significant within a temperature range fitted by a single value of μ or only between such ranges, if even then, is the determining factor. The weighted mean values of the ratio for the lower and upper parts of each temperature interval are given in Table III. The differences within the lower (12–16°) and upper (25–30°) ranges are as expected from the determinations of μ , but they fall within the limits of the probable error of the ratio for each total range (p.e. = .002+). The corresponding values of μ ,

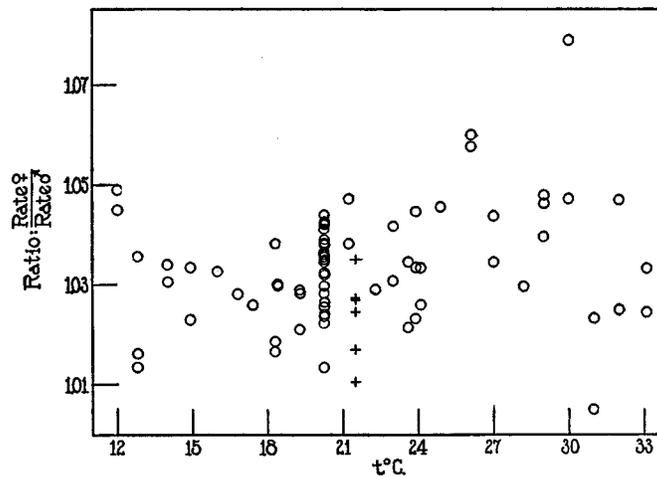


FIG. 5. Developmental ratio of the sexes.

therefore, are not significantly different. Within the longer middle range the result is less certain: the differences exceed their probable errors but not significantly. The larger value of the ratio in the upper as compared with the lower temperature zone (difference = $.015 \pm .004$), however, may be connected by a continuously increasing velocity of female development in the intermediate interval. The data are inconclusive, but for conversion to a male basis in the next section, the shift will be assumed.

The difference between males and females in developmental rate, long familiar to *Drosophila* geneticists, is here shown to appear as early as the prepupal stage. The evidence is clear-cut, all but 4 out

of 80 cases falling within a range in developmental ratio of 1.01 to 1.05, a variation of but 4 per cent.

Corrected Temperature-Development Curve.

The deviations of the experimental points from the curves of Figs. 1 and 2 have been correlated with age of the cultures at the time of puparium formation. If this factor were eliminated, would the points approximate more nearly to three straight lines when plotted as in Figs. 1 and 2? It is not unreasonable to apply such a correction to the present data for two reasons. (1) As analyzed in Table II, 37 per cent were means of prepupæ raised at 20°. This proportion

TABLE III.
Mean Developmental Ratio of the Sexes for Different Temperature Intervals.

Temperature range.	Mean ratio.	Temperature range.	Mean ratio.	Temperature range.	Mean ratio.
°C.		°C.		°C.	
12-13	1.033	16-19	1.027	25-27	1.048
14-16	1.031	20	1.033	28-30	1.046
12-16	1.032	21-25	1.035	25-30	1.047
		16-25	1.032	30-33	1.036

would probably suffice to scatter the points even more than they are if the true correction were very different from one based on Fig. 4. Means deviating widely from the curve of Fig. 4, when corrected from the curve, might very possibly give a poorer fit to the Arrhenius equation at critical points than do the crude data. (2) Granting that the correction is rough and uncertain, it is much superior to no correction. As pointed out by statisticians, crude data which include a known error are not unweighted, but are weighted inaccurately, more inaccurately than those treated with the roughest sort of correction.

Before applying the correction for age of culture, however, all the female records of Table II have been converted to a male basis by means of the mean developmental ratio of the sexes, thus doubling the number of cases. Following this the partial means of Table II have been corrected for the age of culture error from Fig. 4, and the

results plotted in Fig. 6, males as crosses, females as circles. In order to calculate the temperature characteristics, a weighted final mean for each temperature was determined (plotted in Fig. 7). These means should give the most reliable values of μ which can be secured from the data. In lieu of the reciprocal of the probable error, each value was weighted by the square root of the number of cases on

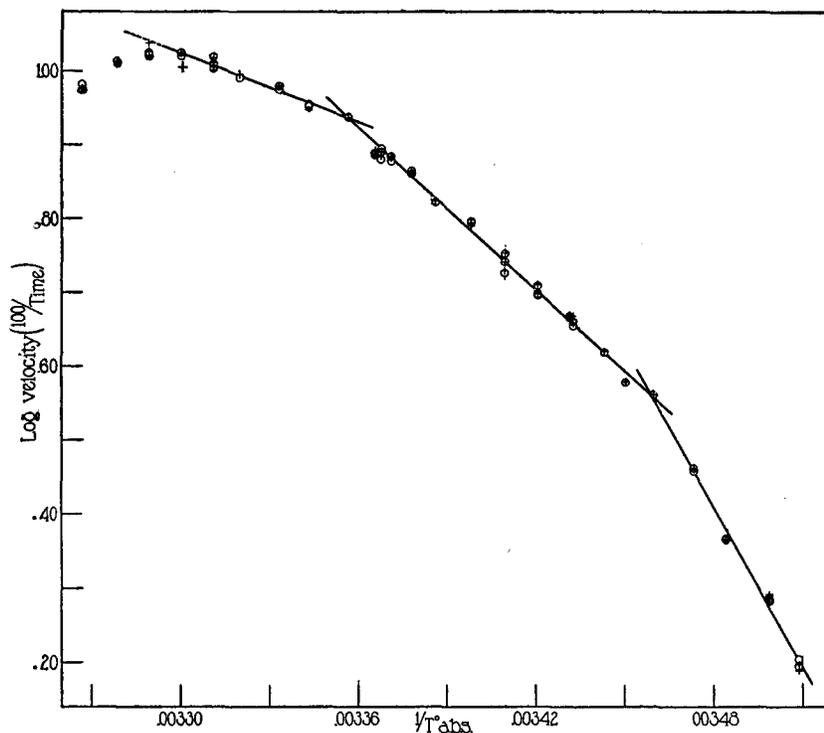


FIG. 6. Final curve for rate of prepupal development, showing partial means.

which it is based, and the μ calculated. The method, the same as that used before, may be described in more detail. Each temperature range is divided into an upper and lower part and the weighted mean values for logarithm of the velocity and reciprocal of the absolute temperature determined for each part. From these two values, μ can be calculated arithmetically and the line placed on the diagram. Since all the points did not fall on the line, the value for

μ was determined twice, in one case with the mid-value of a given interval in the upper part mean, in the other case with it in the lower part mean, and the two averaged. The values for 16° and 25°, the critical points, were included in calculating the μ for the temperature ranges both above and below them.

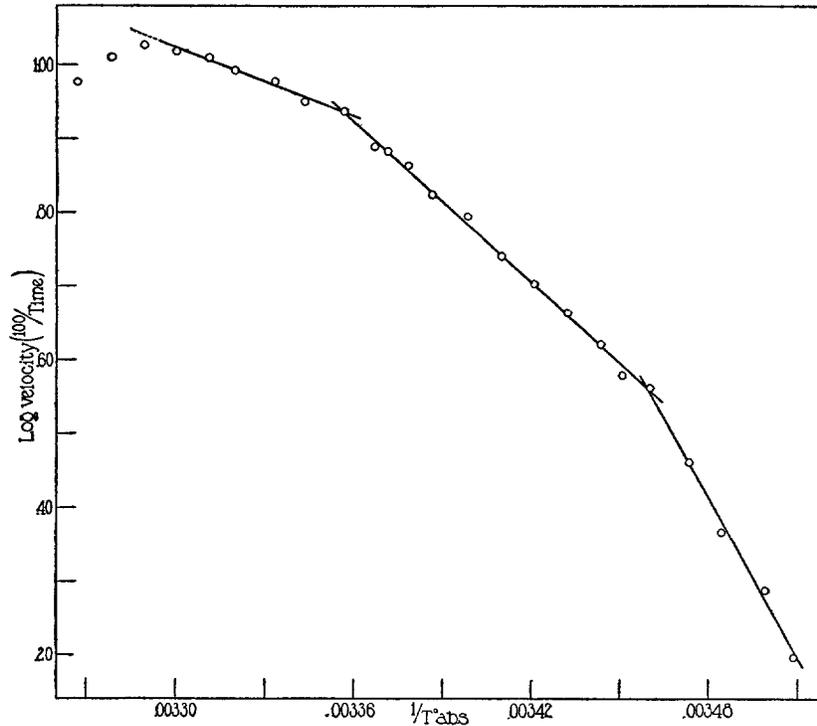


FIG. 7. As Fig. 6, but with final weighted means from which the curve was calculated.

The final temperature characteristics are given in Table IV, and apply to either sex, except in the middle temperature region where the value of μ for the female is 0.7 per cent greater than for the male (the one given). Probable errors would be of doubtful value since the error in the age of culture correction is unknown. Accordingly Table IV does not tell us how significant are the differences between the critical increments. The only index to the excellence of fit is by

examination of the figures. The experimental values up to 30° seem to me sufficiently well described by the Arrhenius equation to warrant its use. Any interpretation of the processes controlling the developmental rate of the *Drosophila* prepupa must be consistent with this relation.

Does a Given Critical Increment Apply throughout the Prepupal Period?

The Arrhenius equation has been applied to the development of the prepupa under constant temperature conditions, and suggests that a single rate-limiting master process, characterized by its critical thermal increment, may extend throughout the period at any given temperature. If this is true, the μ for a part of the period should

TABLE IV.
Temperature Characteristics for the Prepupal Period.

Temperature range. °C.	Original data.		Corrected data.
	Male.	Female.	
12-16	33,850	33,680	33,210
16-25	16,440	16,670	16,850
25-30	7,880	7,520	7,100

agree quantitatively with that for the whole. This was tested in two preliminary experiments similar to those described in an earlier paper.

In the first experiment with 20° as the standard temperature, the prepupæ consisted of two groups. Those of one group were exposed for 10 hours at 14° in three sets at the (1) beginning, (2) middle, and (3) end of the prepupal stage. For the rest of the period they were kept at the standard temperature and the time of pupation noted in each case. The other group was similarly treated to 3 hours exposure at 26°. In each case the temperature difference included a break in the curve, a fact not appreciated at the time. The controls developed at constant temperatures of 14°, 20°, and 26°.

In computing the μ for the different parts of the period, the length of exposure to 14° or 26° was compared with the length of the equiva-

lent stage in those kept constantly at 20°. This assumes that the stages passed at 20° in the experimental animals lasted as long as equivalent stages in the controls. Then

$$M_c - (M_x - D_x) = D_c$$

when M_c = mean prepupal period of control (at 20°), M_x = mean period of experimental lot (at 14° or 26°), D_x = duration of exposure to changed temperature, and D_c = duration of equivalent stages at control temperature (20°). Substituting the reciprocals of D_x and

TABLE V.

First Experiment on Temperature Characteristics for Parts of Prepupal Stage, Standard Temperature 20°.

Treatment.		Mean period.		Thermal increment.	
		Male.	Female.	Male.	Female.
<i>hrs.</i>	<i>°C.</i>				
1-10	14	24.347 ± .052	24.056 ± .051	26,800 ± 520	29,000 ± 560
6-15	14	24.227 ± .068	23.815 ± .053	25,940 ± 590	27,160 ± 550
11-21.8	14	25.666 ± .091	24.766 ± .118	28,340 ± 680	26,330 ± 780
11-20.2	14	24.921 ± .086	24.159 ± .088	29,960 ± 790	28,560 ± 800
Entire period at	14	45.156 ± .150	44.000 ± .108	25,690 ± 120	25,650 ± 100
	20	18.190 ± .049	17.609 ± .050		
	26	12.214 ± .068	11.849 ± .034	11,650 ± 190	11,590 ± 120
1-3	26	16.394 ± .066	15.672 ± .049	13,700 ± 500	14,550 ± 410
6-8	26	16.100 ± .051	15.794 ± .042	15,440 ± 410	13,820 ± 390
6-8.5	26	15.929 ± .086	15.393 ± .080	14,560 ± 510	14,320 ± 480
11-13	26	16.603 ± .059	16.122 ± .058	12,400 ± 490	11,750 ± 500

D_c in the Arrhenius equation, the temperature characteristic can be readily determined. Since both M_x and M_c are averages with probable errors, the probable error of D_c is readily secured. Two values of μ were determined in each case, one from $D_c + p.e.$, the other from $D_c - p.e.$, and the mean of these taken as the true value.

The results of the experiment (Table V) are quite erratic, and most of the differences of questionable significance. Much of this can be attributed to experimental error, the numbers being small and the marked variation correlated with age of the culture uncorrected. The

sex differences fluctuate so irregularly as to be meaningless. When compared with the standard curve of Fig. 7, the developmental rates of the controls were all markedly low, and unequally so, giving smaller values of μ for the upper temperature range and larger values for the lower range than standard. Yet for both groups, the temperature characteristic for the whole as determined from the controls was less than that for any of the parts.

TABLE VI.

Second Experiment on Temperature Characteristics for Parts of Prepupal Stage, (No. 107e to i), Standard Temperature 23.6°.

At 16.8°	Age of culture.	Mean period.		Average period. Male basis.	No.	Age correction.	Final mean, M_x .	D_c for D_x = 6 hrs.	μ
		Male.	Female.						
<i>hrs.</i>	<i>hrs.</i>								
0-6	25.5	16.61	16.21	16.615 ± .010	88	1.012	16.429 ± .031	2.694 ± .042	20,220 ± 390
	32.0	16.95	16.50	16.932 ± .019	41	1.028			
5.5-11.5	27.5	16.63	16.06	16.542 ± .029	101	1.014	16.270 ± .058	2.853 ± .064	18,770 ± 570
	30.0	16.37	16.09	16.428 ± .025	35	1.019			
9-15	23.8	16.10	15.93	16.211 ± .095	71	1.012	16.009 ± .080	3.114 ± .085	16,570 ± 690
	31.0	16.37	15.94	16.349 ± .018	39	1.023			
23.6°*	22.2	13.34	12.89	13.283 ± .023	83	1.011	13.123 ± .028		16,870 ± 60
	29.0	13.28	13.00	13.308 ± .015	48	1.016			
16.8°*	33.2	26.56	25.83	26.514 ± .027	61	1.036	25.593 ± .027		

* Controls, temperature constant throughout.

The second experiment proved much more satisfactory. The standard temperature was 23.6° and treatment lasted 6 hours at 16.8°. Thus the experiment fell within the range characterized by $\mu = 16,850$. The pronounced age of culture error could be corrected because of the continuity of the data: the developmental ratio of the sexes was sufficiently uniform that a weighted mean ratio (1.025) permitted conversion of all records to a male basis: and fair sized numbers reduced the probable error to within bounds. The calculations followed the same principles as in the preceding case; the results are given in Table VI.

The differences in μ are probably significant. If verified, they would indicate that several successive reactions limit the rate of development, and that μ for the whole averages a time series. The thermal increment is greatest, however, during the first part of development and least at the end. Further, μ for the control is not intermediate in value between the lowest and the highest, but within the limits of the probable error is identical with that for the end of prepupal development. This does not agree with the view that the critical increment for the whole averages a time series. Nevertheless, the differences in duration of the prepupal stage due to time of incidence of the lower temperature are probably real.

The discrepancy may arise from the basic assumption upon which the μ are calculated; *i.e.*, that the effect of the lower temperature is confined to the stages exposed to it. Instead the effect of the lower temperature may persist after the prepupæ are returned to 23.6°. The stages actually exposed to the low temperature would then be delayed only as much as the value of μ determined for the whole period would demand. The mechanism for producing such an effect is not evident from the data.

We may conclude from these experiments that while fluctuations in end-point as functions of time of exposure to a changed temperature are real, they indicate only that the temperature characteristic for the whole period cannot be the simple average of the μ for a series of successive reactions differing in their critical increments. This type of experiment, however, seems more likely to provide an analysis of dynamic interrelations in development than constant temperature studies.¹

¹ In conversation, Dr. Crozier has suggested that if the velocity constant of the autokinetic growth reaction were the sum of two factors, one the constant characteristic of the main reaction, the other that expressing the catalytic effect of a resultant, the velocity curve for the entire process would have different forms at different temperatures (unless the temperature coefficients of the two components were identical). This would explain the shift in end-point described here when the relative period of incidence of a lowered temperature is changed. Although the interpretation is complicated, data secured under constant temperature conditions might still apparently agree with the Arrhenius equation within the limits of the experimental error.

Effect of Temperature Prior to Puparium Formation.

Inaccurate temperature control prior to puparium formation has been named as one source of experimental error. How important is this factor? A set of culture bottles, raised at the standard temperature of approximately 27°, were put at 16.7° when they started to produce puparia. After 22 hours at the lower temperature prepupæ were isolated at $\frac{1}{2}$ hour intervals for 11 hours. The first of these passed the prepupal stage at 20.25°, the last at 24.8°: the results are given at the end of Table I and plotted in Figs. 1 and 2 as solid black circles. While the length of the prepupal period is shown to be conditioned by the temperature prior to its onset, the results are not sufficiently accurate to determine the temperature characteristic of this delay. The greater difference from standard of the 24.8° group is probably caused by their longer exposure to the low temperature before puparium formation.

This experiment puts one on guard in dealing with markers of development. Whether one would find the same increments and critical temperatures for the prepupal period following some temperature other than 27° cannot be predicted. Nor are such data comparable with those in which the larval period is passed at the same temperature as the pupal period, as in the studies by Loeb and Northrop.

The delay in the prepupa following the lower temperature at the end of the larval stage is not unexpected. If puparium formation is conditioned by larval growth independently of imaginal disk development, a smaller temperature coefficient for larval growth than for imaginal disk development would give the observed results. The puparium would form before the disks had reached as advanced a stage as under a larval temperature 10° higher. Published data on *Drosophila* larvæ are not detailed enough to decide the point.

This supposed independence of larval and imaginal processes is a very suggestive viewpoint. It is consistent with Baumberger's results (1919) on the effect of concentration of yeast in larval food upon length of the larval and pupal periods. Although he concludes that the two stages are independent, his Fig. 8 seems to show a negative correlation between them. This would be expected if the de-

ficient food supply at low yeast concentrations were not strictly apportioned between larval growth and the much smaller requirements for imaginal disk development.

The lengthening of the prepupal period as the culture from which the puparia are isolated grows older seems inconsistent. Baumberger found that larger pupæ were formed from better fed larvæ, which in turn developed faster. The first puparia to form in a culture are largest, and on this ground should be the best nourished, have the shortest larval period, and consequently the longest prepupal period if the age change can be attributed to nutrition. The food conditions in the cultures change from day to day and would seem to furnish the necessary mechanism. J. C. Li² has shown, however, that larvæ from the first eggs to be laid had the longest larval period, while those from later eggs required a constantly decreasing time to complete their growth over a 6 day oviposition period. The length of the puparial stage of the same individuals, however, increased to a corresponding degree, agreeing with the condition in the prepupæ. Li has further demonstrated that this change was not correlated with the condition of the culture medium of the larva, which was constant for all individuals, but must be traced back to conditions under which the successive eggs are developed in the mother. The age of culture change is consistent, therefore, with the explanation adopted here. If it is sufficient alone, the age change in the larva must be completely accounted for in the prepupal stage, a demonstration that has yet to be made.

If the effect of a lowered temperature at the start of the prepupal period holds over after return to a higher temperature, there is no reason why such a change just before the prepupal period starts should not have the same effect. The effect of this hold-over is quantitatively not sufficient, however, to account completely for the results obtained.

DISCUSSION.

The interpretation of temperature characteristics similar to those presented here is not self-evident. Crozier has frequently used the catenary system of limiting reactions $A \xrightarrow{k_1} B \xrightarrow{k_2} C$ to explain his results.

² Unpublished data.

Each reaction in such a series is characterized by a value of μ determined experimentally. At the critical temperature, where a break occurs in the plotted data, the velocity coefficients k_1 and k_2 are equal. At higher temperatures the reaction with the smaller thermal increment is the slower, and as the limiting process its temperature characteristic is that of the process as a whole. As the temperature falls below the critical point, however, its velocity does not diminish as rapidly as that of the reaction characterized by a larger value of μ . This latter is then the limiting reaction, and its characteristic represents the process as a whole. He further assumes "that the critical increment refers to . . . the formation of active molecules or ions of a catalyst." The velocity of the biological process on this basis is that of the limiting reaction, which proceeds in the direction of the process.

A recent paper by Janisch (1925), however, interprets the decreasing acceleration at higher temperatures on quite a different basis. He postulates a reverse reaction, opposite to the anabolic process that dominates insect development at lower temperatures. His experimental data are inadequate for a critical test, however, and his analysis is not based on physicochemical principles. In 1914, Pütter proposed the same idea that the velocity of a biological process at the higher temperatures is a resultant of two opposed reactions, one promoting the function, the other retarding it. Pütter's analysis has been corrected and extended by Hecht (1918-19), who used it very successfully in explaining the effect of temperature upon the photosensory latent period of *Mya*. However, the hypothesis as corrected by Hecht has not been applied to insect development which both Pütter and Janisch explain on this basis.

For temperatures from 25-30°, I have assumed that the rate of prepupal development is adequately described by the fitted line, and, using Hecht's analysis, have determined the corresponding velocity constants for the reverse process (k_2). Let us assume in the reactions $A \xrightarrow{k_1} B, B \xrightarrow{k_2} C$ that a certain intracellular concentration of B must be reached before any given cell in the imaginal disks will divide, and that its formation is characterized by $\mu = 16,850$. Above 25° we will postulate a destructive process which breaks B down into an

innocuous substance C. The formation of B in a concentration necessary for initiating cell division is thus delayed. The resultant of the two might possibly give an apparent increment of 7,100, which has so far been treated as typifying an irreversible anabolic process rather than an equilibrium. If the thermolabile component, $B \rightarrow C$, were a valid postulate, however, its temperature characteristic would be a constant. As shown in Table VII, this is not the case. If the experimental points give a rectilinear relation on the ordinates used here, the rate of the process cannot be controlled by an equilibrium between such opposing reactions. This fact has already been pointed out by Crozier, but does not seem to be appreciated sufficiently. Above 30° , however, where the relation is not linear with these coordinates, this explanation may very well apply.

TABLE VII.

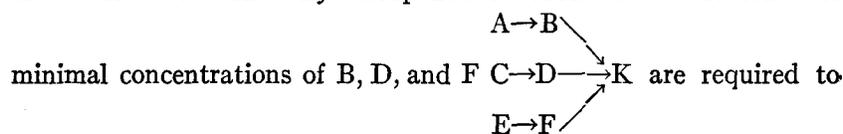
Velocity Constants, k_2 , and Thermal Increments, μ , for Hypothetical Thermolabile Reaction.

Temperature.	k_2	μ
27	.00130	
28	.00197	76,300
29	.00271	57,900
30	.00348	45,400

The thermal increments characterize three distinct anabolic processes which probably limit the rate of cell growth and, possibly, cell division in the imaginal disks of the head and thorax. Certainly one cannot invoke a mechanism, such as Robertson's autocatalytic theory of growth, which postulates a reversible limiting reaction that progresses to an equilibrium. The limiting reactions need not be specific determiners of pupation which terminates the prepupal period. More probably pupation is initiated by pressure or other mechanical forces due to crowding of the cells in the invaginated imaginal disks. Since these are already well developed by the end of the larval period, the prepupa represents a relatively uniform stage of development. Even so, the short exposures to a lowered temperature already described showed that the successive parts were not entirely equivalent in their temperature relations.

A catenary chain of consecutive simultaneous reactions has been used to explain the three values of μ : $A \rightarrow B \rightarrow C \rightarrow D$. Whether such a system could give the observed results is questionable. As suggested by C. R. Plunkett,³ at temperatures such that $B \rightarrow C$ (or $C \rightarrow D$) is the slowest reaction of the series, B would tend to accumulate faster than it would transform to C, and thereby accelerate $B \rightarrow C$ by the law of mass action. The temperature characteristic of the entire process in this case could not be that of the component $B \rightarrow C$, but would be governed primarily by $A \rightarrow B$. By postulating that each reaction to the left of the slowest member is reversible, this difficulty may be partly obviated. At temperatures where $A \rightarrow B$ is the slowest component, B would be removed as rapidly as formed, so that $B \rightarrow A$ would be negligible. At other temperatures where $B \rightarrow C$ is the slowest component, $A \rightleftharpoons B$ would maintain B in constant concentration dependent upon the temperature, so that $B \rightarrow C$ could not be continuously accelerated by mass action. The temperature coefficient, however, would average the μ for $B \rightarrow C$ and the effect of temperature upon the equilibrium $A \rightleftharpoons B$. It could not have a relatively simple meaning, and it is doubtful if the Arrhenius equation would fit it.

As an alternative, the temperature characteristic may be attributed to a series of relatively independent reactions.⁴ Here constant



complete the cycle between equivalent stages in successive cell divisions. Then the three reactions might exhibit so loose an interdependence that the rate of the whole would be that of the slowest component and would be characterized by its value of μ . This proposal seems to avoid the dynamic difficulties inherent in the others and suggests a morphological basis. During interkinesis, to which the limiting processes have been assigned, a large number of more or less independent processes must reach a given stage before the mitotic

³ Personal communication.

⁴ This possibility, I find, has already been suggested by Crozier (1924-25, p. 213) in somewhat different form.

mechanism is released. Thus the formed bodies of the cytologist, particularly the chromosome elements, are double in the early pro-phases. Accordingly the limiting reactions may be attributed to those three (in this case) which require the most time to grow duplicates of themselves.

The values of μ obtained here may be compared with those of other processes (Table VIII). The prepupal stage does not agree either in values of μ or in critical temperatures with determinations for the

TABLE VIII.
Temperature Characteristics of Some Related Phenomena.

Object.	Observer.	Temperature range.	μ
		°C.	
<i>Drosophila</i> prepupa (rate of development).	Bliss.	12-16	33,210
		16-25	16,850
		25-30	7,100
<i>Drosophila</i> pupa* (rate of development).	Loeb and Northrop.	15-20	27,000
		20-30	10,000
<i>Drosophila</i> egg* + larva (development).	Loeb and Northrop.	10-20	27,000
		20-30	10,000
<i>Drosophila</i> prepupa and pupa (O ₂ consumption).	Orr.	1-15	16,800
		15-30	11,500
<i>Tenebrio</i> pupa* (rate of development).	Krogh.	14-22	27,000
		22-30	10,000
<i>Arhacia</i> egg* (rate first cleavage).	Loeb and Wasteneys.	7-11	41,000
	Loeb and Chamberlain.	11-19	21,000
		20-27	12,400

* Determinations of μ quoted from Crozier.

entire puparial period of which it is a part. The increment for the puparial period therefore averages a time series, but the number of determinations is so small that the temperature characteristics based upon them are of little significance. The same is true for the egg + larval stage. When a process is known to consist of several, quite different, successive phases, apparent agreement with the Arrhenius formula is no assurance that the μ derived applies to a single extended limiting reaction. The pupal period of *Tenebrio* (Krogh, 1914), which gives a beautiful fit below 22° to the Arrhenius equation, is probably equally heterogeneous. At least it includes the same high

initial CO₂ production (Krogh's figures) demonstrated by Bodine and Orr in *Drosophila* and here identified with the prepupal period.

A more curious circumstance is the quantitative agreement between the critical increments for O₂ consumption of the *Drosophila* pupa (Orr, 1924-25) and rate of prepupal development. However, the former holds *below* 15°, and the latter *above* 16°; at equivalent temperatures the two are distinctly different. That the same reaction limits the one process of respiration below 15° and the other of speed of development above 15° is questionable.

Roughly, the increments for the prepupa form a geometrical series, a puzzling relationship that is not uncommon; for example, the values for the first cleavage of *Arbacia* (Loeb and Wasteneys, 1911; Loeb and Chamberlain, 1915).

SUMMARY.

1. Diurnal fluctuations in emergence of the adults and negative correlation between the length of successive stages in the puparium made it desirable to restrict study of relation of temperature to development to the prepupal stage.

2. On morphological grounds, the formation of the puparium, which starts the prepupal period, seemed to be determined by the stage of larval development; pupation, which terminates the prepupal stage, by imaginal disk development.

3. The rate of prepupal development may be represented by the Arrhenius equation relating velocity of an irreversible chemical reaction with temperature. The data gave three values for the critical increment over different temperature intervals, corresponding to three straight lines of different slope. When deviations of the points from these lines were compared with their probable errors, however, in nearly three-fourths of the cases the difference was significant.

4. Analysis of these deviations showed them to be due primarily to changes in the extent of imaginal disk development at the time of puparium formation. These, in turn, were correlated with age of the culture.

5. The two sexes differed in developmental velocity, such that the rate of female development was about 1.03 times as great as rate of male development. For the upper temperatures this ratio was

greater than for the lowest of the three temperature ranges, the intermediate zone possibly varying between the two.

6. A final curve relating prepupal development to temperature has been calculated after (1) converting all female records to a male basis, (2) applying a correction for age of culture error, and (3) weighting each point by the square root of the number of cases upon which it was based. This yielded the following values for the temperature characteristic μ ; namely, 33,210 from 12–16°, 16,850 from 16–25°, and 7,100 from 25–30°. Above 30° the data could not be fitted by the Arrhenius equation.

7. By treating prepupæ in different developmental stages to brief exposures at a lower temperature, pupation was more delayed by treatment at the beginning than at the end of the prepupal stage. From these data, μ for parts of the stage were calculated on the assumption that the effect of temperature did not persist after return to the standard temperature. Since the μ thus secured were greatest for the beginning and least for the end, and none were less than that for the whole, the interrelations of the successive stages are probably more complex than they were assumed to be in making the calculations.

8. Lowering the temperature prior to puparium formation lengthened the prepupal stage. Puparium formation, therefore, was not conditioned by imaginal disk development, but by larval processes possessing a lower temperature coefficient than did the imaginal disks.

9. Possible physicochemical mechanisms for producing these results are discussed, and the observed temperature characteristics were finally attributed to three relatively independent anabolic processes which limit the rate of cell growth in the imaginal disks.

10. Both the thermal increments and the critical temperatures for the prepupal stage differed markedly from those reported by Loeb and Northrop for the entire development within the puparium. Since the prepupa forms part of the latter period, temperature characteristics for extended developmental phases known to be heterogeneous are of doubtful significance.

CITATIONS.

- Baumberger, J. P., 1919, A nutritional study of insects, with special reference to microorganisms and their substrata, *J. Exp. Zool.*, xxviii, 1.
- Bliss, C. I., 1926, Variation in the temperature characteristic of embryonic development of the grape leafhoppers, *J. Agric. Research*, xxxii (in press).
- Bodine, J. H., and Orr, P. R., 1925, Respiratory metabolism, *Biol. Bull.*, xlvi, 1.
- Crozier, W. J., 1924-25, On biological oxidations as function of temperature, *J. Gen. Physiol.*, vii, 189.
- Hecht, S., 1918-19, The effect of temperature on the latent period in the photic response of *Mya arenaria*, *J. Gen. Physiol.*, i, 667.
- Janisch, E., 1925, Über die Temperaturabhängigkeit biologischer Vorgänge und ihre kurvenmässige Analyse, *Arch. ges. Physiol.*, ccix, 414.
- Krogh, A., 1914, On the rate of development and CO₂ production of chrysalides of *Tenebrio molitor* at different temperatures, *Z. allg. Physiol.*, xvi, 178.
- Loeb, J., and Chamberlain, M. M., 1915, An attempt at a physicochemical explanation of certain groups of fluctuating variation, *J. Exp. Zool.*, xix, 559.
- Loeb, J., and Northrop, J. H., 1917, On the influence of food and temperature upon the duration of life, *J. Biol. Chem.*, xxxii, 103.
- Loeb, J., and Wasteneys, H., 1911, Sind die Oxydationsvorgänge die unabhängige Variable in den Lebenserscheinungen? *Biochem. Z.*, xxxvi, 345.
- Lowne, B. T., 1890-92, Anatomy, physiology, morphology, and development of the blow fly, London, i.
- Orr, P. R., 1924-25, Critical thermal increments for oxygen consumption of an insect, *Drosophila melanogaster*, *J. Gen. Physiol.*, vii, 731.
- Perez, C., 1910, Metamorphose des Muscides, *Arch. zool. exp. et gén., series 5*, iv, 1.
- Pütter, A., 1914, Temperaturkoeffizienten, *Z. allg. Physiol.*, xvi, 574.
- Snodgrass, R. E., 1924, Anatomy and metamorphosis of the apple maggot, *Rhagoletis pomonella* Walsh, *J. Agric. Research*, xxviii, 1.
- van Rees, J., 1889, Beiträge zur Kenntniss der inneren Metamorphose von *Musca vomitoria*, *Zool. Jahrb., Abt. Anat. u. Ontog.*, iii, 1.
- Yule, G. U., 1922, An introduction to the theory of statistics, London, 6th edition.