TEMPERATURE AND TIME OF DEVELOPMENT OF THE TWO SEXES IN *DROSOPHILA*

By GERT BONNIER.
(From the Zootomical Institution, University of Stockholm.)

(*Received 28th May* 1926.)

(With Four Text-figures.)

1. INTRODUCTION. METHODS.

ALL who have worked with *Drosophila* know that the females are the first to hatch in the culture-bottles. This fact makes it very probable that the females develop in a shorter time than do the males. However, it is not *a priori* necessary that it must be so. It could also possibly depend upon a highly selective mortality among the first eggs from each female. In order to study this question in more detail the following investigation was undertaken.

The flies used belong to the yellow stock. The sex-linked mutant yellow has a viability almost as great as that of the wild type flies. The cause why these latter flies were not used, was that I possessed a culture of triploids carrying yellow in their X-chromosomes, and that it was my intention to compare the time of development not only of the two normal sexes but also of the intersexes. Unfortunately, I have until now not succeeded with the intersexes. Before the experiments were begun a rather great number of ordinary culture-bottles (about 50) were made up and five to eight pairs of flies were put in each of them. There they were left for about three days in order to make it probable that all the females had been fertilised. Next, the same number of culture-bottles was made up, but contrary to the ordinary method, to these bottles no paper was added. The flies from the old bottles were then transferred to the new ones where they were left for two hours only. When the two hours had elapsed, the bottles were emptied and left in the incubator until pupation began. In bottles without paper pupation takes place either on the surface of the food or on the walls of the bottle, and since my bottles are cylindrical in shape and only contain 100 c.c. each, it is quite impossible for the pupae to escape observation. During the whole period of pupation the bottles were controlled every second hour, and the pupae, which had pupated during the last 2-hour period were transferred to vials containing moistened filter-paper. These vials were then labelled with the pupation-period and left in the incubator until the beginning of hatching. As in the case of pupation, the vials were controlled every second hour during the whole time of hatching.

By this method it is possible to be informed—for each individual female and each individual male—upon how many periods of 2 hours it takes, (1) from the laying of the egg up to the moment of pupation, and (2) from this moment up to the moment of hatching. There is one possible source of error due to the fact that the eggs sometimes develop to some extent before laying. I think, however, that the error does not influence the averages very much, and since the aim of this investigation is to compare the time of development of different groups of animals, the error may be left out of consideration by this comparison.

As stated above, the eggs for which the time of development have been calculated, have all been laid within a period of only 2 hours. This means that it is very much a matter of chance if we get many or few individuals upon which to control the time. The experiments, which have been carried out in an incubator, have been made at two different temperatures, viz. 25° C. and 30° C. In the first case I got a quite sufficient number of individuals, viz. 1083. In the second, however, I got only 77 individuals. This low figure is almost certainly due to the heavy mortality at the high temperature of 30° C. Since this last experiment nevertheless gave some decisive results and since the experiments are rather laborious (it is necessary to control the cultures for 36 hours or more) they were not repeated.

2. THE EXPERIMENTS.

In the calculation of the time of development up to the moment of pupation, we may imagine that all the eggs have been laid just at the middle of the 2-hour period. And likewise, in the calculation of the pupal time it is imagined that all the pupae have pupated just at the middle of the different 2-hour periods. This approximation is necessary for the statistical treatment of the problem and does not involve any error since, on an average, the same number of eggs have been laid before as after the mid-point of the period and the same number of pupae have pupated before as after the mid-point.

Experiment at 25° C.

(*a*) *Time from egg-laying up to pupation.* The distribution of this time for the different individuals is shown in Tables I and II and in Fig. 1. From the tables we find the mean and its mean error to be

For the females ... ... 116·62 ± 0·19 hours,
For the males ... ... 116·78 ± 0·20 hours.

The difference between these two times is 0·16 hours and its mean error is 0·28 hours. And as 0·16 : 0·28 = 0·57 it is obvious that the difference between the time of development of the females and of the males is without any statistical significance.

(*b*) *Time during the pupal stage.* This time is to be found in Tables I and II and in Fig. 2. Here we have the mean and its mean error:

For the females ... ... 111·36 ± 0·15 hours,
For the males ... ... 115·46 ± 0·13 hours.

The difference of these times and its mean error are respectively 4·10 hours and 0·20 hours; and as 4·10 : 0·20 = 20·5 it is correct to conclude that the males are significantly slower in their development during the pupal stage than are the females.

Table I.

Showing the number of Females *distributed according to their time of development at* 25° C.; (1) *up to the moment of pupation, and* (2) *during the pupal stage.*

Time in hours during the pupal stage	Time in hours up to the moment of pupation																
Hours	99–101	101–103	103–105	105–107	107–109	109–111	111–113	113–115	115–117	117–119	119–121	121–123	123–125	125–127	127–129	129–131	Totals
101–103	.	.	.	.	.	.	.	2	7	.	.	.	.	.	.	1	10
103–105	.	.	.	.	6	3	6	8	4	.	.	.	1	1	1	1	31
105–107	.	.	.	.	1	4	5	4	7	.	.	.	2	7	4	.	34
107–109	.	1	.	.	1	2	3	1	2	1	3	13	9	10	1	.	47
109–111	1	.	.	2	1	3	4	1	1	9	32	25	6	1	.	.	86
111–113	.	.	.	1	.	5	9	25	28	26	38	4	.	.	.	.	136
113–115	.	.	.	1	1	8	20	44	31	28	7	.	1	.	.	.	141
115–117	.	.	.	.	.	4	23	8	11	5	1	.	.	.	.	.	52
117–119	.	.	.	.	.	1	4	3	3	1	.	.	.	.	.	.	12
Totals	1	1	0	4	10	30	74	96	94	70	81	42	19	19	6	2	549

Table II.

Showing the number of Males *distributed according to their time of development at* 25° C.; (1) *up to the moment of pupation, and* (2) *during the pupal stage.*

Time in hours during the pupal stage	Time in hours up to the moment of pupation													
Hours	105–107	107–109	109–111	111–113	113–115	115–117	117–119	119–121	121–123	123–125	125–127	127–129	129–131	Totals
105–107	.	.	.	.	.	.	.	.	.	.	.	1	2	3
107–109	.	.	.	.	.	.	.	.	.	1	7	4	2	14
109–111	.	.	.	.	.	.	.	.	.	6	11	6	.	23
111–113	1	.	.	.	.	1	3	20	11	7	2	2	1	48
113–115	1	8	7	4	21	18	19	20	16	2	.	.	1	117
115–117	1	4	9	28	37	43	26	20	7	.	.	.	.	175
117–119	.	1	7	28	22	25	15	9	2	.	.	.	.	109
119–121	.	.	4	8	6	8	10	2	.	.	.	.	.	38
121–123	.	.	.	2	1	1	1	.	.	.	.	.	.	5
123–125	.	.	.	.	.	.	.	.	.	.	.	.	.	0
125–127	.	.	.	1	.	.	.	.	.	.	.	.	.	1
127–129	.	.	.	1	.	.	.	.	.	.	.	.	.	1
Totals	3	13	27	72	87	96	74	71	36	16	20	13	6	534

Table III.

Showing the number of Females *distributed according to their time of development at* 30° C.; (1) *up to the moment of pupation, and* (2) *during the pupal stage.*

Time in hours during the pupal stage	Time in hours up to the moment of pupation								
Hours	91–93	93–95	95–97	97–99	99–101	101–103	103–105	105–107	Totals
73–75	2	.	1	5	.	.	1	.	9
75–77	.	.	.	5	.	.	1	.	6
77–79	.	.	.	1	5	1	.	.	7
79–81	.	.	.	1	5	3	.	1	10
81–83	.	.	.	.	1	2	1	1	5
83–85	.	.	.	.	1	.	.	.	1
85–87	.	.	.	.	.	.	.	1	1
Totals	2	0	1	12	12	6	3	3	39

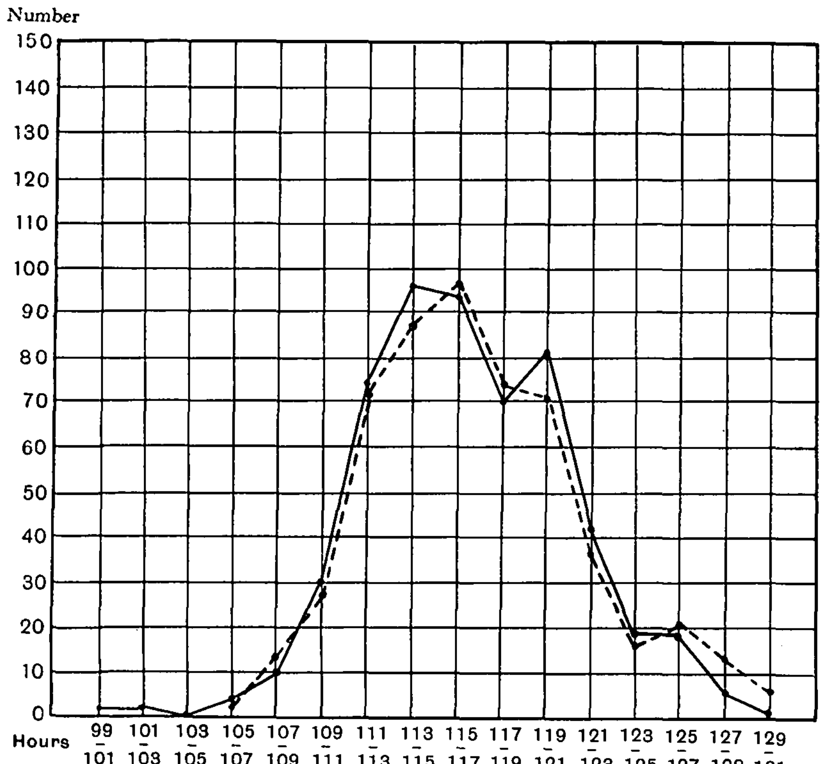

Fig. 1. Time of development up to the moment of pupation at 25° C. of *females* ——— *and males* — — —.

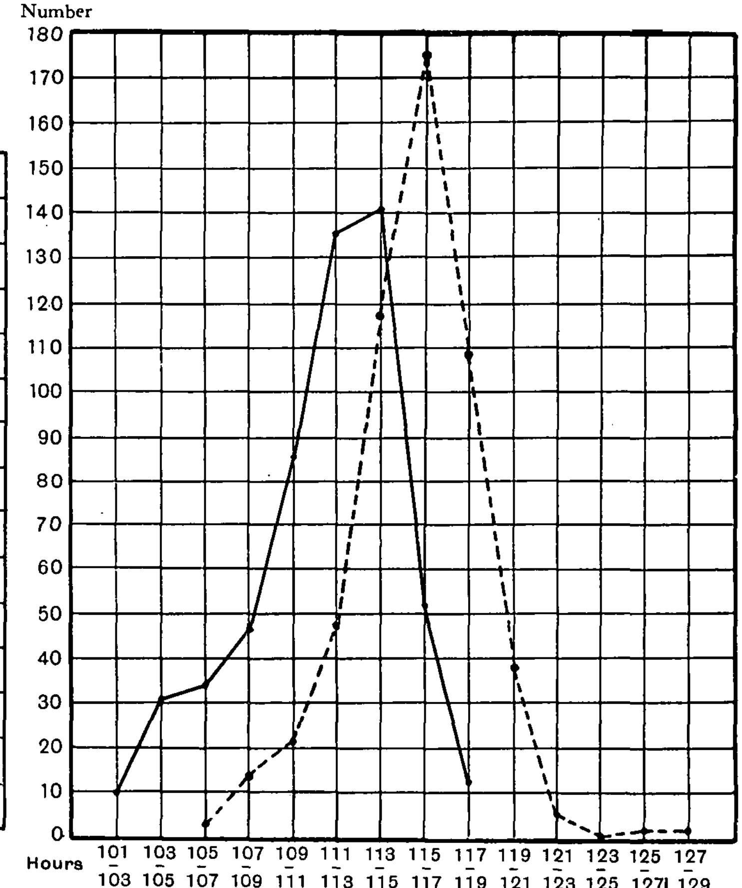

Fig. 2. Time of development during the pupal stage at 25° C. of females ——— and males — — —.

Experiment at 30° C.

(*a*) *Time from egg-laying up to pupation.* The means are (Tables III and IV and Fig. 3):

For the females ... ... 99·95 ± 0·49 hours,
For the males ... ... 103·37 ± 0·43 hours.

The mean error of the difference is 0·65 hours, and since (103·37 − 99·95) : 0·65 = 5·26 it is very probable that the difference between the time of development of the two sexes is of a real significance.

Table IV.

Showing the number of Males *distributed according to their time of development at* 30° C.; (1) *up to the moment of pupation, and* (2) *during the pupal stage.*

	Time in hours up to the moment of pupation							
Hours	95–97	97–99	99–101	101–103	103–105	105–107	107–109	Totals
Time in hours during the pupal stage: 79–81	1	.	.	.	.	.	.	1
81–83	.	.	.	.	3	4	.	7
83–85	.	.	.	9	7	2	2	20
85–87	.	.	5	2	.	.	1	8
87–89	.	.	.	.	.	.	1	1
89–91	.	.	.	.	.	.	.	0
91–93	.	.	.	1	.	.	.	1
Totals	1	0	5	12	10	6	4	38

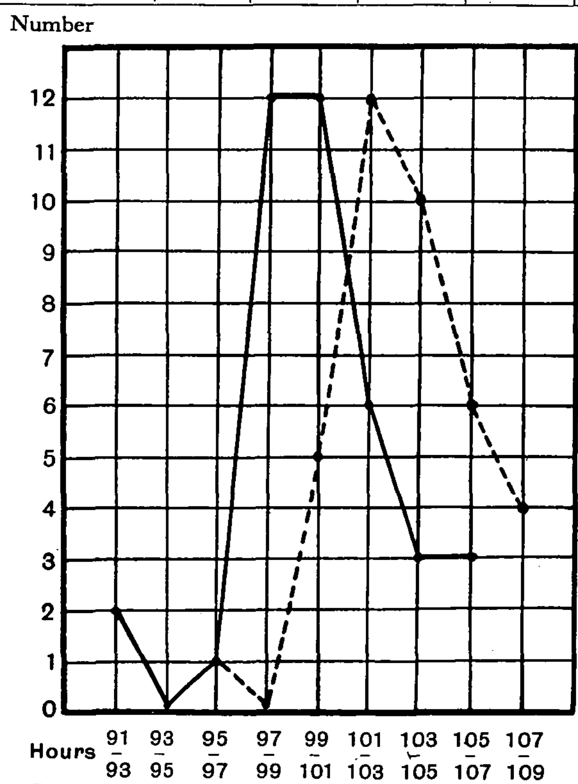

Fig. 3. Time of development up to the moment of pupation at 30° C. of females ——— and males — — —.

(*b*) *Time during the pupal stage.* The means for this period are (Tables III and and Fig. 4):

For the females ... ... 78·15 ± 0·50 hours,
For the males ... ... 84·26 ± 0·34 hours.

The difference and its mean error being respectively 6·11 and 0·60, we have 6·11 : 0·60 = 10·18, and the difference must thus be of a real significance.

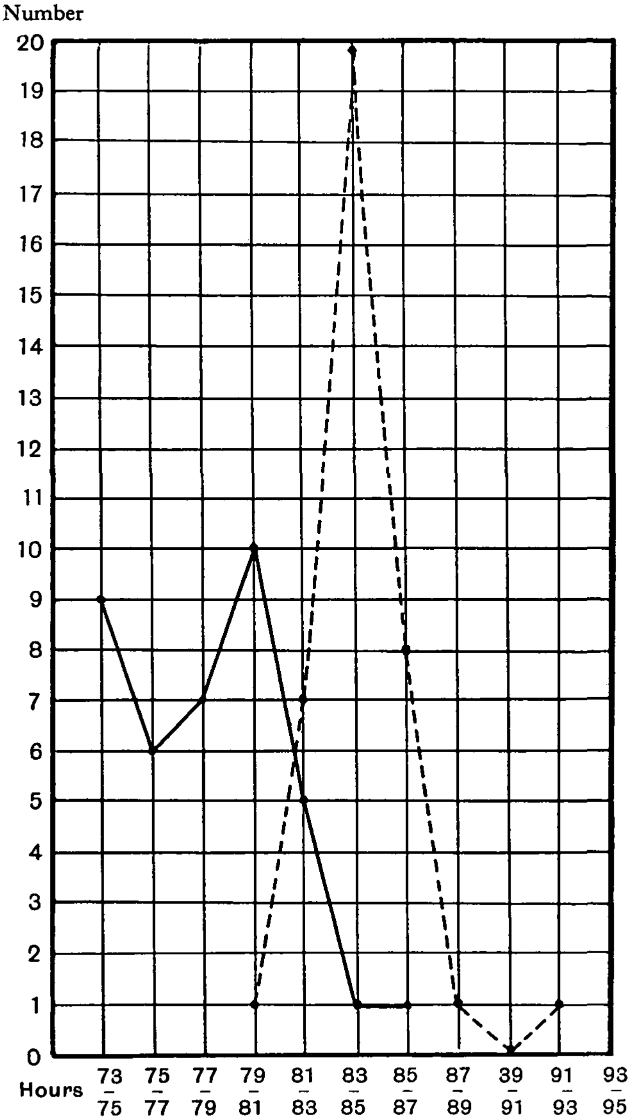

Fig. 4. Time of development during the pupal stage at 30° C. of females ——— and males — — —.

3. DISCUSSION.

As may be seen from the above statements, there is—at 25° as well as at 30° C.—a markedly longer time of development for the males than for the females. At 25° C., however, this lengthening is confined to the pupal stage, the time up to pupation being the same for the two sexes. It seems not unlikely that this may be correlated with the fact that the essential parts of the secondary sexual characters are developed during the pupal stage. But if this correlation is a true one, then probably we also may suppose that at 30° C. these characters develop earlier, not only in an absolute sense, but also in a relative one, since namely the figures show that here the time up to pupation as well as that during the pupal stage is longer for the males than for the females. This supposition must, however, be confirmed by an embryologic investigation.

If we look at the tables, especially Tables II and IV, it is seen that the figures to a great extent are distributed along the diagonal ending in the upper right corner. This fact indicates that there is some kind of correlation between the time up to pupation and the time during the pupal stage. From Tables I and II (25° C.) we also find the coefficient of correlation, r, to be (according to Bravais' formula):

For the females $r = -0{\cdot}256 \pm 0{\cdot}040$,

For the males $r = -0{\cdot}607 \pm 0{\cdot}027$.

It is therefore probably correct to suppose that—at least for the males—for those individuals which have had a considerably long time of development up to the moment of pupation, this long time *as a rule* is compensated by a shorter time during the pupal stage and *vice versa*.

That the time of development is shorter at the higher than at the lower temperature is quite conforming with what we know about the influence of temperature on development. But there is a very interesting fact to note, namely, that the shortening of the time at 30° C. as compared with that at 25° C. is much more pronounced for the time during the pupal stage than during the time up to pupation. As a coefficient of the shortening we may introduce the quotient between the time at 30° C. and that at 25° C. It is obvious, then, that the smaller this coefficient is, the greater is the shortening. In order to make the comparisons more clear, let us introduce a notation. Let the means of the times up to pupation be M_{25} and M_{30} and their mean errors m_{25} and m_{30}. Let, likewise, the means of the times during the pupal stage be P_{25} and P_{30} and their mean errors p_{25} and p_{30}. Let, finally,

$$\alpha = \frac{M_{30}}{M_{25}}$$

and

$$\beta = \frac{P_{30}}{P_{25}}.$$

We have then to compare α and β and to make this comparison for the two sexes separately. From the figures of the different tables we find that

for the females $\alpha = 0{\cdot}8571$ and $\beta = 0{\cdot}7018$,

for the males $\alpha = 0{\cdot}8852$ and $\beta = 0{\cdot}7298$.

Let now the mean error of α be μ and the mean error of β be ν. Since M_{30} is independent of M_{25} and P_{30} is independent of P_{25}, μ and ν must satisfy to the close-approximation formula:

$$\mu^2 = \left(\frac{\partial \alpha}{\partial M_{30}}\right)^2 m_{30}^2 + \left(\frac{\partial \alpha}{\partial M_{25}}\right)^2 m_{25}^2$$

and

$$\nu^2 = \left(\frac{\partial \beta}{\partial P_{30}}\right)^2 p_{30}^2 + \left(\frac{\partial \beta}{\partial P_{25}}\right)^2 p_{25}^2$$

and thus

$$\mu = \frac{\sqrt{m_{30}^2 M_{25}^2 + m_{25}^2 M_{30}^2}}{M_{25}^2}$$

and

$$\nu = \frac{\sqrt{p_{30}^2 P_{25}^2 + p_{25}^2 P_{30}^2}}{P_{25}^2}.$$

With the aid of the foregoing figures we therefore find that

for the females $\mu = 0{\cdot}0045$ and $\nu = 0{\cdot}0046$,

for the males $\mu = 0{\cdot}0039$ and $\nu = 0{\cdot}0031$.

The mean error of the difference between α and β is, as usual, $\rho = \sqrt{\mu^2 + \nu^2}$. Hence, this difference and its mean error have the values:

For the females $0{\cdot}1553 \pm 0{\cdot}0064$,

For the males $0{\cdot}1554 \pm 0{\cdot}0050$.

And, finally, we find the values of the quotient $\frac{\alpha - \beta}{\rho}$ to be

For the females 24·27,

For the males 31·08.

We may therefore without hesitation conclude that the more pronounced influence of the raising of the temperature upon the pupal stage as compared with the influence upon the pre-pupal stages is of a real and absolute significance.

The method commonly used when studying the influence of temperature on development is to observe the time from the beginning of the development up to a certain stage and to compare for a number of different temperatures. The results are often plotted on a scale and it is usually tried to find a mathematical expression which fits the curve thus obtained. But it may be asked if these studies are of more than of a purely descriptive value, or if they really inform us on the processes of development. As we have seen, an increase in the temperature has a different influence on different stages of the development. In fact, it shortens in *Drosophila* the time during the pupal stage relatively more than it shortens the time up to pupation. But if we not only consider *Drosophila* but the organisms in general, there may exist some maximum temperature above which the development is not accelerated but retarded. Since, however, different stages are differently susceptible to the influence of temperature, this maximum temperature may very well be a different one for different stages. Imagine, then, that a raising of the temperature accelerates one part of the development and retards one other part, but in such a way that the total time of development is unaltered. From a graphical or mathematical treatment we would then have concluded that temperature is without influence on the time of development but in reality it had been an *obscured* but nevertheless real influence of temperature. As the above data show, the time up to pupation in *Drosophila* is the same for the two sexes at 25° C. but probably not the same at 30° C. As, however, the time up to pupation involves such different phases of the development as the embryonic phases—phases of differentiation—and the larval stages—phases of growth—it may possibly only be the *total* time up to pupation in 25° C. which coincide for the two sexes, whereas for instance one part may be slower for the males and one other part slower for the females.

It seems, therefore, to me that when studying temperature-coefficients and other figures of that kind, the methods ofted used are too rough to be of a greater value. If we wish to be able to conclude anything about the physical and physico-chemical nature of the processes of development from the variation of the time of this development in different temperatures, it is necessary to study short intervals of the development separately, and to choose the intervals in such a way that we may infer that there is about the same kind of processes going on during the whole of each separate interval. The difficulties here are, however, of a practical kind: it is not easy to find suitable objects for such a study. The best objects are probably those with transparent eggs where it is possible to follow each morphological stage. The methods then to be followed are the ordinary ones: for each separate interval the time of development is noted for a number of different temperatures and the corresponding curve is traced. Conforming then with the facts, shown above, to hold good in *Drosophila*, it is probable that the *rule* should be that curves, corresponding to different intervals, would be of a quite different kind, indicating different natures of the processes during the various intervals.

4. SUMMARY.

1. The time of development at 25° C. up to the moment of pupation is found to be for females and males respectively 116·62 ± 0·19 and 116·78 ± 0·20 hours. During the pupal stage the two times are 111·36 ± 0·15 and 115·46 ± 0·13 hours.

2. At 30° C. the corresponding figures are (in the same order): 99·95 ± 0·49, 103·37 ± 0·43, 78·15 ± 0·50 and 84·26 ± 0·34 hours.

3. These figures show that there is a statistical significance in the differences of the times of development of the two sexes for both the periods at 30° C. but only for the pupal stage at 25° C. It is pointed out that the fact that the longer time of male development as compared with female development at 25° C. is confined to the pupal stage, may be correlated with the other fact that the essential parts of the secondary sexual characters are developed during this stage.

4. It is shown that there is a negative correlation between the pre-pupal and pupal times of development, indicating that the longer the first time is, the shorter is, as a rule, the other time and *vice versa*.

5. With the aid of statistical methods it is shown that the shortening of the time of development at 30° C. as compared with the time at 25° C. is much more pronounced for the pupal than for the pre-pupal stage.

6. This last fact is discussed and it is emphasised that the ordinary methods of studying the influence of temperature on development are too rough to be of more than of a descriptive value, the only way of getting a deeper insight into the processes of development by temperature studies being the separate studies of a number of short intervals.