

GROWTH AND VARIATION OF THE LARVAE OF
DROSOPHILA MELANOGASTER

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ELEVEN TEXT FIGURES AND TWO PLATES (SEVENTEEN FIGURES)

I

It is well known that *Drosophila*, the favorite object of modern genetics, has been extremely little investigated from the point of view of its natural history. Many other insects have been carefully described in special monographs with reference to their anatomy, physiology, and biology. Practically all the known data on the general biology of the fruit fly have been collected twice: first, by A. H. Sturtevant(23) in a chapter devoted to behavior, physiological studies, parasites and enemies of *Drosophila*, and anatomy; secondly, by Morgan, Bridges, and Sturtevant(11) on the biology of *Drosophila* in the book, "The Genetics of *Drosophila*." The disproportion between these small chapters and the tremendous material which has accumulated on the genetics of this form furnishes an entertaining commentary on the present trend of biological fashions.

A comparatively restricted number of contemporary papers deal with the problem of growth in insects. Wolfgang Ostwald(13) simultaneously with T. B. Robertson attempted to consider the growth of living organisms as an autocatalytic process. In his paper he discusses the comparatively old data of Luciani and Lo Monaco(10) on the growth of the caterpillar of the silkworm. The curve of the growth in weight shows the existence of five cycles alternating with

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four molts. After each molt there may be noticed a period of slow growth and even a decrease in weight, which Ostwald interprets as the result of suspended feeding during molting. Although the Italian authors did not weigh the molting stages of caterpillars separately, they indicate approximately the times of molting. That suggested to Przibram the possibility of calculating the probable number of cell divisions connected with each of the moltings. In his paper written in collaboration with Megušar(17) he states (p. 709): "Im ganzen repräsentieren die beobachteten Häutungsintervalle zusammen $4 + 3 + 2 + 2 + 1$ Teilungsschritte." His original material on *Sphodromantis* shows, with perfect evidence, that: "Auch die Gewichte eben gehäuteten Gottesanbeterinnen weisen also von Häutung zu Häutung eine Verdoppelung auf" (p. 689), and farther: "in manchen Fällen tritt an Stelle einer Verdoppelung des Gewichtes von einer Häutung zur anderen eine Vervierfachung (Quotient 4) welche dann von einem Stillstand (Quotient 1) während einer nächsten Häutungsperiode gefolgt sein kann." Concerning the increase in size, which has for us a special interest, Przibram writes: "Demnach können wir sagen, dass die Längenzunahme des Halsschildes von einer Häutung zur anderen durchschnittlich in Kubikwurzel von 2 erfolgt" (p. 684).

In general, the growth curve for *Sphodromantis* according to Przibram's estimation is an S-type which can easily be compared with the autocatalytic curve. Stjern's(24) and Titschack's(27) data confirm Przibram's findings. G. Teissier's(25, 26) preliminary publications dealing with *Dixippus morosus*, *Notonecta glauca*, *Galleria*, and *Calliphora* are in agreement with the conclusions of previous authors. Teissier states that *N. glauca* doubles its weight with each molting stage. This author thinks that the cycles in mammal growth curves may be compared with cycles between molting stages in insects.

Turning our attention to the growth of dipteran larvae, we find that this question has been touched only occasionally in papers devoted to different problems relating to the nutrition

of such larvae. Kunkel(8) gave some very scanty data on the variation of flesh-fly larvae at different periods of their life, keeping them on different kinds of food (meat and thyroid glands). It is not perfectly clear what method of fixation was used in collecting the material, taking into account the author's statement on page 257: "In order to measure the larvae, they were killed by immersion in boiling water or 95 percent alcohol. Both these agents seemed to cause very little contraction or distortion." We cannot agree with this opinion. While the boiling water kills the larvae instantly and seemingly without any contraction, the action of alcohol is extremely slow, the animals not dying sometimes for fifteen to twenty minutes, and giving as a result badly distorted corpses. Baumberger's(1) extensive paper on nutrition in insects deals in part with *Drosophila*. Unfortunately, the technique of age determination and of measurement is far from what must be regarded as elementary requirements of exactness. There are no data as to the determination of the moment of eclosion of larvae from the egg shell—moment of birth. The purpose of this author was to obtain sterile larvae and therefore he was obliged to put in vials sterilized pupae which gave origin to his parental flies. These parents did not lay eggs only at one particular moment. The larvae therefore hatched at different times, and gave naturally a very heterogeneous group in respect of the age distribution of the population. The measuring was done, according to the author's statement, in the following manner:

The size of the larvae on different media was determined by placing the tubes and a millimeter scale on the stage of a binocular microscope and measuring the length of five to ten of the larger specimens while 'crawling' at full length. The larger specimens were selected for measurement because although female adults were allowed to oviposit for only one day, the eggs showed considerable variability from one to three days in their date of hatching, depending on the readiness with which the female oviposited on the medium.

There is a certain discrepancy between the statement that "about five or ten specimens" were measured and a later

statement (p. 12, footnote) "that each point on a curve of growth is an average of the whole culture of larvae, i.e., usually twenty or more individuals. Thus a single curve has considerable weight."

Three papers of Romeis and Dobkiewicz(20), Reznitschenko(18), and Dobkiewicz(3) have a certain interest from the point of view of the technique of dipteran culture. The first of them deals with the size of the larvae of *Calliphora* fed on meat and on thyroid gland. The authors reproduce pictures of very well-preserved larvae, but do not give any information concerning either the method of fixation or the number of specimens on which the averages are based. This makes the findings very doubtful.

The paper of Reznitschenko(18) has also other important defects. As can be understood from the Russian text, the females were allowed to oviposit twenty-four hours. The English summary contains very probably a mistake, indicating that "the experiment has been performed on a group of larvae, hatched from a lot of eggs, laid by a female *Drosophila* which was fed with thyroidine during 24 hours." Records were taken at three periods in the life-cycle: "The laying of eggs, the pupation of the larvae, and the emergence of the flies out of the pupae." According to the Russian text, these records were taken three times in twenty-four hours at definite hours.

Dobkiewicz's last paper is chiefly a repetition of Reznitschenko's work on *Drosophila* fed with thyroid glands. The methods of marking the moment of the beginning of the development and its different steps are even more inaccurate than in the work done by Reznitschenko. The parents were allowed to oviposit thirty-six hours and the emergent flies were counted once a day. We are convinced that the scale of time measurement adopted by all these authors is too rough, and therefore the conclusions must be considered as very doubtful.

II

In working out a method of obtaining larvae of the same age, the following remark of Huettner(6) was kept in mind. This author writes concerning the time of development of eggs of *Drosophila* as follows: "When deposited the eggs may be in any stage of development. I have obtained newly laid eggs in which the sperm is still visible, i.e., before conjugation of the two pronuclei has taken place, and at the other extreme have obtained eggs from which a larva emerged within five minutes after deposition." According to our own observations, the number of eggs so far-advanced in their development is usually very limited, and the whole mass of eggs ecloses, on the average, about eighteen to twenty-four hours after being oviposited. This is true, of course, for a temperature of 28°C., at which the eggs were kept, and for the wild strain (line 107) of *Drosophila melanogaster* cultivated in the fly laboratory of the Institute for Biological Research. Slightly different material was used in the first and second of our experiments. The first preliminary experiment started on the 17th of April, 1928. At 2.30 on that day, females belonging to a culture of wild-type (line 107) *Drosophila melanogaster*, kept during fourteen generations at the temperature of 28°C., were put in eight jars containing a thin layer of synthetic *Drosophila* food without yeast. The culture was started with two generations of brother-sister matings, was run at 28°C., and at a density of 5♀ × 5♂ parental flies per half-pint bottle. The flies in six jars were kept one and one-half hours. It was found by trial that a young female (three to four to five days after emergence), being kept in a crowded condition and then transferred to fresh food, is able to produce 2 to 2.5 eggs an hour. The next morning at 8.30, a few larvae hatched during the night were removed and at noon the collecting of larvae for the experiment began. This operation took three hours, twenty one-half-pint bottles containing 100 cc. synthetic food, with yeast put on the medium the day before, being populated with twenty-five larvae per bottle. The exact time the larvae were

put on the food was marked on each bottle. In that way we had larvae one to two hours old and records of when they began to get food. The killing of the larvae took place at the desired number of hours from the moment of populating the bottle.

The parental flies for the second series of experiments (started on April 24th) were taken from the mass culture of wild *Drosophila melanogaster* (line 107) developed at 25°C. The parental females were allowed to oviposit about fifteen hours on synthetic medium poured on Petri dishes. In order to avoid the killing of larvae at nighttime as we were obliged to do, one-half (eleven) of the bottles of the second experiment was populated twelve hours after the first half. In this way we had in hand two groups of larvae differing in age—which gave us the possibility of obtaining larvae of desired ages at the most convenient times. The collecting of larvae for the second experiment was done a little differently from the first one. A small amount of water was poured on the surface of the medium. The larvae were in this way washed off and could be collected with exceptional ease by means of a glass pipette. This accelerated the whole operation of populating the bottles, and in the second experiment the average age of larvae put on the food was not more than one-half to three-quarters of an hour. The growth of the yeast was much slower in the second experiment than in the first, which can be partially explained by the fact that in the second experiment the yeast was put on the medium only a few (five) hours before the larvae. As will be shown later, the fully grown larvae of the second experiment did not attain the size of the larvae of the first one.² In order to have a larger material for statistical study in the last experiment, each bottle

² It is very likely that the density of larval population plays an important rôle in the process of growth. In one of our bottles eighty larvae, instead of forty, were placed by mistake. At the age of sixty-three hours they were 15 per cent smaller than the corresponding larvae of the bottles with twice as low a density.

was populated with forty larvae.³ In all experiments the growing larvae were kept in the incubator running at 28°C. It was found that the only method of obtaining perfectly straight larvae is the application of boiling water as a method of killing and fixation (figs. 1, 2, and 3). The following procedure proved to be extremely satisfactory. The pieces of synthetic medium were taken from the bottle and placed in a dish with water. The larvae were collected in a watch-glass and the latter dropped into a porcelain cup containing boiling water. The immediately killed larvae were collected and preserved in 70 per cent alcohol. The larvae were measured on hollow slides in alcohol. The zero- to twenty-hour larvae were measured under a Spencer microscope with a no. 3 Leitz objective, twenty- to twenty-four-hour-old ones with a Reichert 1 objective, and the older ones with a Spencer binocular objective of 48 mm. In all of these three cases an ocular micrometer with a one-hundred-division scale was placed in a no. 2 ocular. In measuring the mouth parts we used a 4-mm. Spencer objective. All the measurements in this article are in millimeters.

III

The first difficulty which had to be overcome was the separation of our material into stages. According to Keilin (7), Leuckart already knew in 1861 that the larvae of certain *Diptera cyclorrhapha*, for instance of *Calliphora vomitoria* and *Lucilia caesar*, have three larval stages separated by two molts. But, strange to say, *Drosophila* larvae have never been described in detail. Keilin gives a picture of the first stage of *D. ampelophila*, and writes that: "La larve de *Drosophile* (saprophages) a pendant trois stades, une même structure de l'appareil bucco-pharyngien. Seulement au stade I on voit bien le crochet median dorsal." Sturtevant,

³ The special purpose of the third experiment started on the 19th of June was the obtaining of more detailed data on growth of the third larval stage and particularly of its beginning. The parents of the larvae belonged to line 107 kept at room temperature. During the period which interested us particularly, material was collected at two-hour intervals.

giving a picture of the third stage, confesses not having examined the two younger stages and the transitions between them. De Meijere gives only one picture of the mouth armature of *Drosophila obscura* Fall.

Concerning the other important changes which characterize different larval stages, the presence of prothoracal spiracles must be mentioned. Data concerning this organ are also not complete enough. The authors do not distinguish the appearance of definite spiracles and their rudiments.

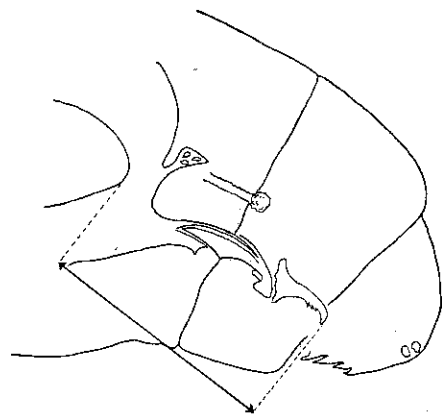


Fig. 4 Schematic representation of the head of the larvae of the second stage. Above the mouth parts is situated the rudiment of the anterior spiracle. The arrows indicate the points of measurement of the 'length' of the mouth parts.

According to our observations, the rudiments of anterior spiracles (fig. 4) appear after the first molt and hence characterize the second larval stage. The fully developed anterior spiracle can be observed only on the larvae of the third stage. They consist usually of eight tracheal ramifications. Figure 5 represents a case in which the number of ramifications is limited to seven.

B. Thompson Lowne(9) expresses the opinion that the anterior spiracles are functionally inactive in the blowfly. We have observed that larvae of the third stage placed in boiling water discharge small bubbles of air also through the anterior spiracles. This phenomenon could not be observed

in larvae of the second stage, in which the openings are still obliterated.

The second important character which permits the separation of stages is the structure of the mouth parts. It is not our purpose to go into a discussion of morphological details of the mouth parts. Our microphotographs (figs. 6, 7, and 8) show quite clearly the successive changes of different parts of the mouth armature.

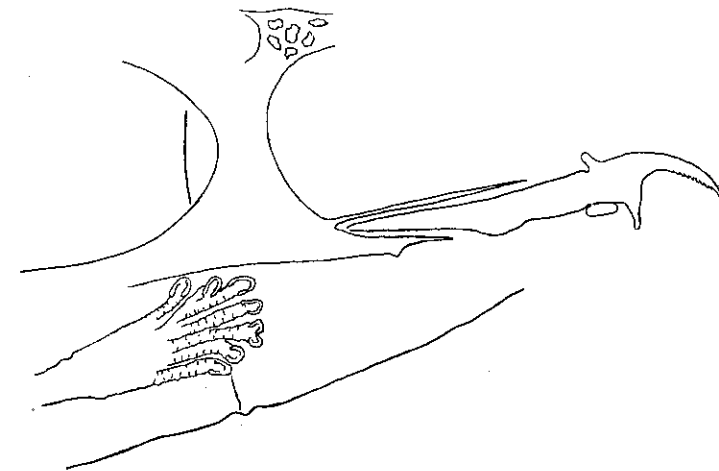


Fig. 5 Definitively developed spiracles of the larvae of the third stage.

We succeeded in finding quantitative differences between larvae belonging to different stages—differences which allow us to separate the stages without any hesitation. The first characteristic is the number of small teeth on the front part of the armature called by Lowne 'great hooks' and 'Aussenslade der Maxille' by Meijere.⁴ Tables 1 and 2 show the average number of teeth of larvae killed at different stages during the second experiment. The averages and the curves of figure 9 show that the stages are perfectly characterized by the number of teeth and that there is even no overlapping in the variation, the curves being not transgressive. Besides

⁴The teeth were counted, as a rule, on one of the hooks.

counts of the number of teeth, there was measured the distance between the top of the hook and the incision in the hypostomial sclerite on the same larvae, boiled for that purpose in a 5 per cent solution of KOH. (The points of measure-

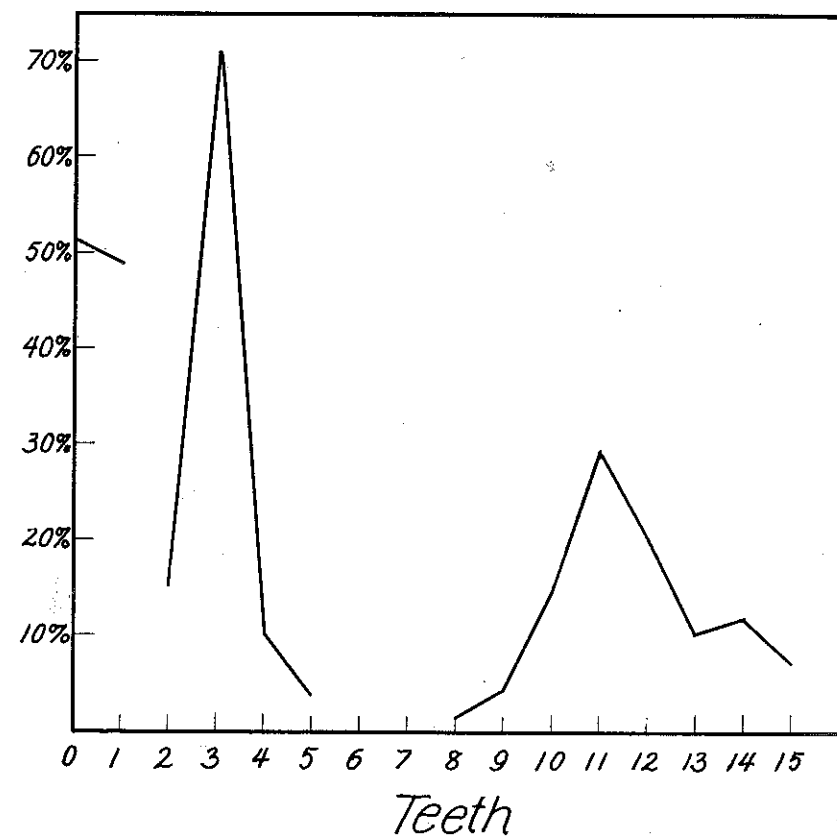


Fig. 9 Variation curve of the frequency of occurrence (expressed in per cent) of the number of teeth on the 'great hooks' in the mouth parts of the first, second, and third stages (from left to right).

ments are indicated in fig. 4 by arrows.) In table 1 averages of the number of teeth and 'length' of armature of larvae of different ages are given. We can see that there is no important change in the averages between larvae of different ages but belonging to the same stage. We have summed up all our

measurements and calculated biometrical constants for each of the three stages. The summary is given in tables 2 and 3.

TABLE 1

Number of teeth and length of the mouth armature in millimeters. Beneath each average is indicated the number of cases

HOURS	STAGE I		STAGE II		STAGE III	
	Length of the armature	Number of teeth	Length of the armature	Number of teeth	Length of the armature	Number of teeth
9	0.1185	0.4				
	11	20				
15	0.1221	0.68	0.1812	3		
	19	19	1	1		
21	0.1132	0.5	0.1998	2.83		
	2	2	16	17		
27			0.2006	2.95		
			17	20		
33			0.1864	3.26		
			20	19		
39			0.2014	3.12	0.3093	13.3
			16	17	3	3
45			0.2034	2.67	0.3131	11.5
			2	2	12	4
51			0.2014	3	0.3150	11.53
			7	2	17	17
57					0.3110	12.17
					18	18
72					0.3037	11.56
					18	16

TABLE 2

Average length of mouth armature in millimeters

	STAGE I	STAGE II	STAGE III
Mean	0.1200±.0007	0.1962±.0008	0.3095±.0013
Standard deviation	0.00611	0.00965	0.01549
Coefficient of variation	5.09±.43	4.92±.27	5.01±.29
Number of cases	32	73	68
Calculated mean	0.1236		0.3115
Difference calculated, absolute	0.0036		0.0020
Difference in per cent of calculated	2.91		0.64

TABLE 3
Average number of teeth on mouth hooks

	STAGE I	STAGE II	STAGE III
Mean	0.537 ± .052	3.025 ± .048	11.78 ± .13
Standard deviation	0.497	0.637	1.642
Coefficient of variation	92.6 ± 11.2	21.1 ± 1.1	13.9 ± .82
Number of cases	41	79	68

Figure 10 shows that the variation curves are not transgressive, i.e., that by measuring the length of the mouth parts we can determine to which stage the given specimen belongs.

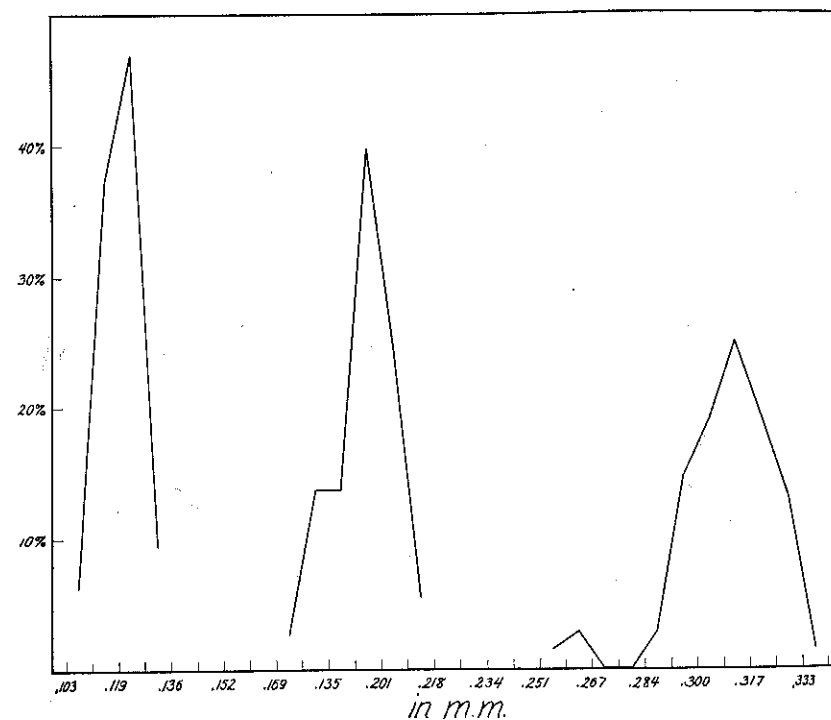


Fig. 10 Frequency distribution (expressed in per cent) of the 'length' of mouth parts of the three stages of larvae.

If we remember the rule established by Przibram, that in the case of *Sphodromantis* the size of the prothorax increases

from one molt to another in proportion to the cube root of 2, i.e., $\sqrt[3]{2} = 1.26$, and apply this rule to our material, we can see that multiplying the length of stage II two times by 1.26 and dividing it twice by 1.26 we get the number 0.1236 mm. and 0.3115, which are extremely close to the actual averages obtained from observations. The need for a double division and multiplication by 1.26 indicates that each of the moltings in *Drosophila* larvae is connected with two cell divisions. It is very likely that the limited number (two) of moltings during the larval life of *Diptera cyclorrhapha* is a process of secondary reduction, and that in an earlier phylogenetic stage the larvae were characterized by four moltings, each of them linked with one cell division.

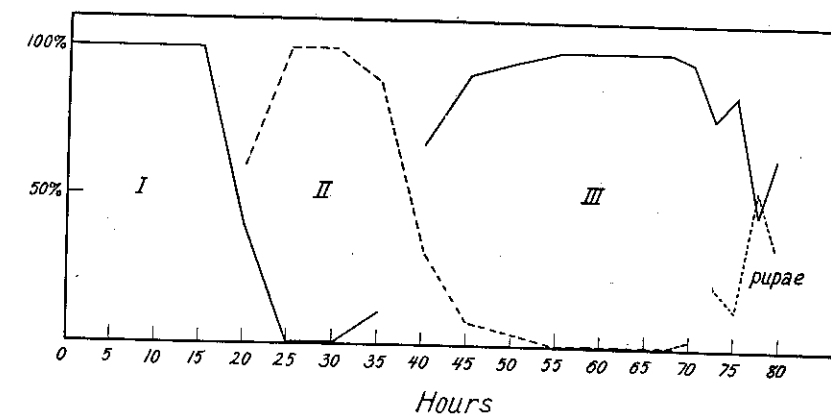


Fig. 11 Graphs of the passage of one larva from one stage to another. First experiment. Based on data of table 4.

Figures 11 and 12 show graphically the distribution of stages along the time scale. The number of larvae of a given age is expressed in per cent of the whole number of larvae collected in the bottle at a certain hour. We can see that the development was a little faster in the first experiment than in the second one. It is difficult to determine the reason for such a difference. The most probable explanation is the slower growth of yeast in the second experiment, especially at the moment when the newly born larvae were put in bottles.

Table 4 and figures 13 and 14 give averages of the length of larvae at different moments of their growth. Even the distribution on figure 14 smoothed by free hand gives an indication of the composite character of the growth curve. From figure 14, in which the data for each larval stage are plotted separately, we see very definitely three growth-cycles corresponding to three larval stages. It must be mentioned that there are cases when single individuals remain far behind their sibs in their development. So, for instance, one individual 0.711 mm. long remained a larva of the second stage

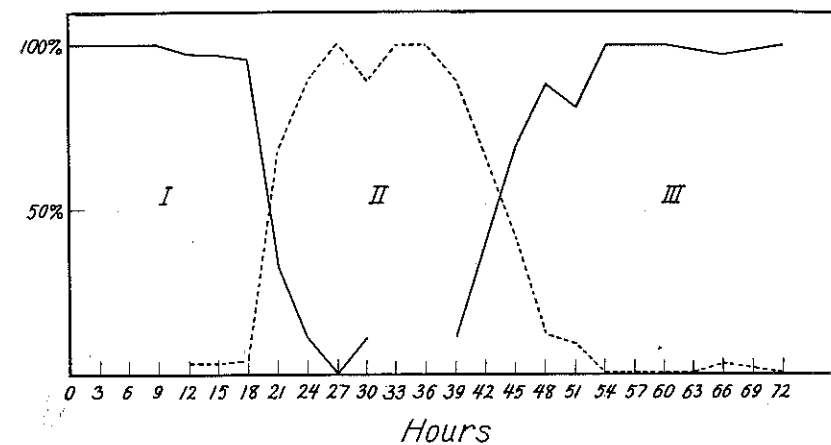


Fig. 12 The same as figure 11, but based on data of the second experiment.

until 72.5 hours of age. But, in general, the change of the stage occurs at nearly the same time in all the larvae. The biometric constants characterizing the size of larvae of different ages collected during the second experiment are presented in tables 5, 6, 7, and 8. The material was classified in frequency rows, and usual formulae were applied. In calculating the probable errors of the mean when the number of observations was less than fifteen, we used the following formula:

$$P.E._{mean} = 0.67449 \frac{\sigma}{\sqrt{n-3}}$$

The coefficients of variation are low at the beginning of the growth. From the age of fifteen hours they begin to move

up and down without any regularity. The existence of three periods of growth is perfectly clear on the curves of figure 15, due to the short intervals used in the collection of the material.

The observational data on the growth during the three larval stages were graduated with logistic curves. There is no

TABLE 4
Averages of the length of the body of *Drosophila* larvae. First experiment

STAGES	I		II		III		I + II + III		III		NUMBER OF PUPAE
	hours	N	hours	N	hours	N	hours	N	On the surface of the food and walls	In the food	
Newborn	0.831	40					0.831				
5	1.026	25					1.026				
10	1.185	25					1.185				
15	1.435	20					1.435				
20	1.465	10	1.854	15			1.698				
25			2.077	19			2.077				
30			2.562	20			2.562				
35	1.466	2	2.801	16			2.653				
40			2.587	8	3.196	17	3.001				
45			2.499	2	3.884	24	3.777				
50			2.916	1	4.185	23	4.132				
55					4.979	25	4.979				
60					4.855	25	4.855				
65					5.114	28	5.114				
67.7					4.925	22	4.925	5.066	8	4.845	14
70		1.700	1	4.938	30	4.830	4.995	17	4.856	12	
72.5				4.679	14	4.679	4.679	14			
75				4.858	18	4.858	4.846	16	4.964	2	4
77.5				4.758	12	4.758	4.803	12	4.269	1	3
79.5				4.790	15	4.790	4.903	14	3.218	1	14
											8

need to go into the details of these fitting operations because of the broad development of logistic curves for fitting different growth phenomena made by R. Pearl, chiefly in his work on population growth. The growth curves are evidently symmetrical ones. The $\frac{k-y}{y}$ data were plotted on arithlog paper, and by using the method of a graphical fitting there have been found the straight-line equations placed as exponents of e.

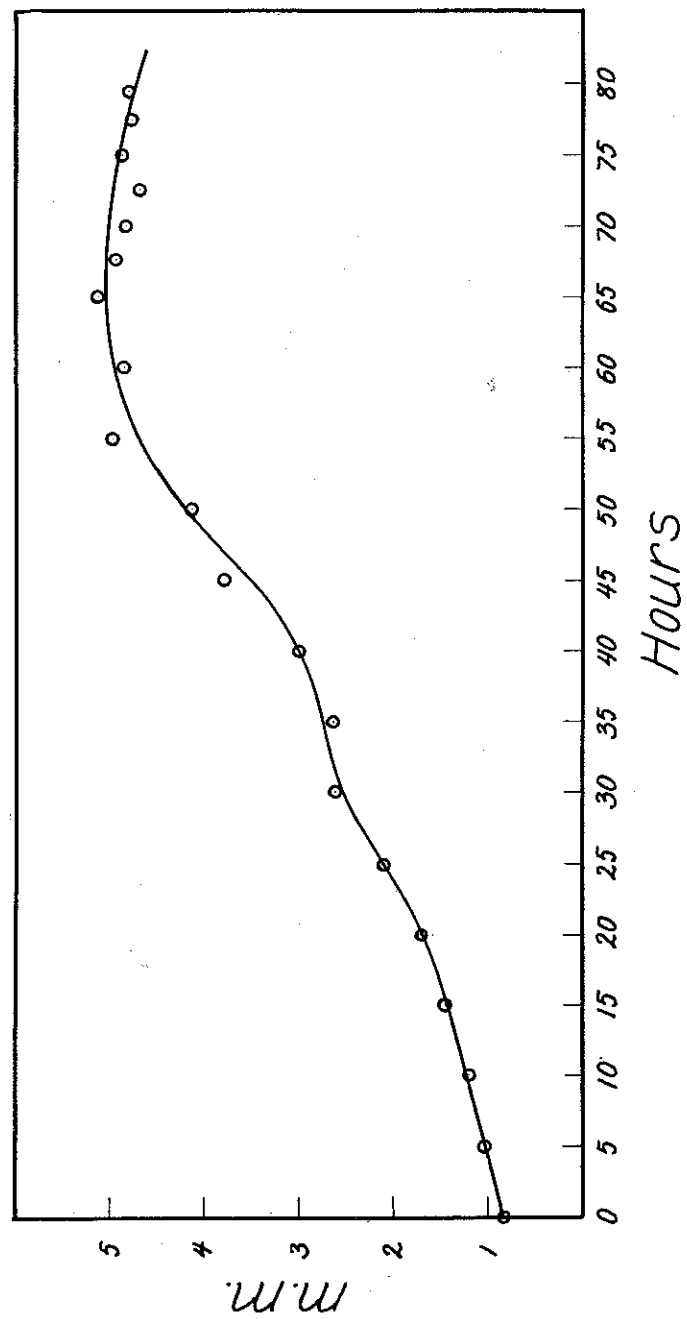


Fig. 13 Growth curve of the larvae without separating them in stages. First experiment.

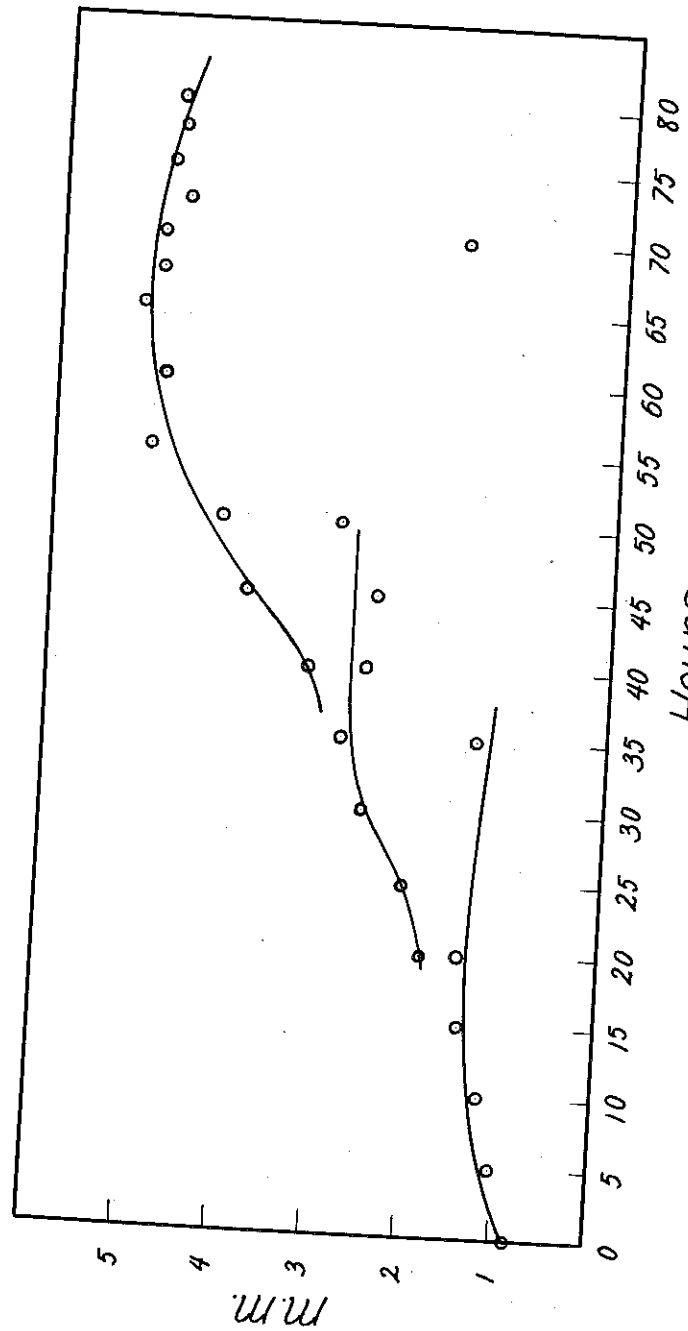


Fig. 14 Growth curves of the larvae of the first, second, and third stages. Curves fitted by free hand. First experiment.

The curves of figures 16 and 17 show that the growth of larvae of different stages advanced with different rates of speed. The smallest slope is in the curve of the first stage, and the most rapid that of the third. It must be pointed out that the logistic expression of the growth of the first stage can only be attained with the assumption that the period begins before

TABLE 5

Biometrical constants for the length of the body of *Drosophila* larvae. Second experiment. All stages

HOURS	MEAN OBSERVED	STANDARD DEVIATION	COEFFICIENT OF VARIATION	N
0	0.861 ± .008	0.0668	7.76 ± .68	30
3	1.041 ± .009	0.0716	7.06 ± .65	27
6	1.165 ± .010	0.0860	7.38 ± .59	36
9	1.179 ± .013	0.1052	8.92 ± .79	29
12	1.314 ± .012	0.1067	8.12 ± .66	34
15	1.406 ± .020	0.1737	12.35 ± 1.01	35
18	1.425 ± .027	0.2137	15.00 ± 1.38	28
21	1.813 ± .026	0.2309	12.73 ± 1.03	36
24	1.935 ± .035	0.3129	16.17 ± 1.32	36
27	2.391 ± .023	0.2001	8.37 ± .66	37
30	2.488 ± .044	0.3881	15.60 ± 1.27	36
33	2.701 ± .026	0.2085	7.64 ± .68	29
36	2.851 ± .021	0.1682	5.90 ± .52	29
39	3.009 ± .037	0.3268	10.86 ± .87	37
42	3.083 ± .056	0.4343	15.13 ± 1.30	32
45	3.372 ± .024	0.2313	6.86 ± .50	42
48	3.725 ± .048	0.4713	12.65 ± .93	43
51	4.205 ± .062	0.5481	13.03 ± 1.07	35
54	4.601 ± .024	0.2043	4.44 ± .32	34
57	4.520 ± .038	0.3309	7.32 ± .60	34
60	4.548 ± .033	0.2815	6.19 ± .51	34
66	4.595 ± .079	0.6725	14.64 ± 1.24	33
69	4.607 ± .054	0.4830	10.70 ± .84	38

the eclosion. It is very likely that the trend of increase of living matter during the egg development is only stopped for the moment when the egg is covered with the shell. Immediately after eclosion, the growing larva continues the increase of its body with the pre-eclosion speed. The fact just discussed can be observed even in the curve based on the data of the first experiment.

TABLE 6

Biometrical constants for the length of the body of *Drosophila* larvae. Second experiment. Stage I

HOURS	MEAN OBSERVED	MEAN CALCULATED	STANDARD DEVIATION	COEFFICIENT OF VARIATION	N
-24		0.056			
-21		0.092			
-18		0.138			
-15		0.202			
-12		0.290			
-9		0.405			
-6		0.545			
-3		0.702			
0	0.861 ± .008	0.863	0.0668	7.76 ± .68	30
3	1.014 ± .009	1.014	0.0716	7.06 ± .65	27
6	1.165 ± .010	1.144	0.0860	7.38 ± .59	36
9	1.179 ± .013	1.248	0.1052	8.92 ± .79	29
12	1.304 ± .011	1.326	0.0911	6.99 ± .58	33
15	1.390 ± .019	1.383	0.1681	12.09 ± 1.00	34
18	1.402 ± .023	1.422	0.1793	12.79 ± 1.19	27
21	1.554 ± .032	1.448	0.1427	9.18 ± 1.26	12
24	1.466 ± .035	1.469	0.0532	3.63 ± .87	4
27		1.478			
30	1.504 ± .104	1.485	0.1549	10.30 ± 2.48	4

TABLE 7

Biometrical constants for the length of the body of *Drosophila* larvae. Second experiment. Stage II

HOURS	MEAN OBSERVED	MEAN CALCULATED	STANDARD DEVIATION	COEFFICIENT OF VARIATION	N
12	1.636	1.902			1
15	1.914	1.909			1
18	2.069	1.924			1
21	1.942 ± .019	1.964	0.1389	7.15 ± .70	12
24	2.030 ± .033	2.056	0.2807	13.83 ± 1.19	32
27	2.391 ± .023	2.233	0.2001	8.37 ± .66	37
30	2.612 ± .020	2.476	0.1718	6.58 ± .55	32
33	2.701 ± .026	2.687	0.2085	7.64 ± .68	29
36	2.851 ± .021	2.809	0.1682	5.90 ± .52	29
39	2.905 ± .013	2.864	0.1095	3.77 ± .32	33
42	2.857 ± .047	2.887	0.3187	11.16 ± 1.18	21
45	2.773 ± .063	2.895	0.2955	10.66 ± 1.43	13
48	2.670 ± .145		0.3041	11.39 ± 2.45	5
51	2.648		0.2716	10.26 ± 2.84	3

TABLE 8
Biometrical constants for the length of the body of *Drosophila* larvae. Second experiment. Stage III

HOURS	MEAN OBSERVED	MEAN CALCULATED	STANDARD DEVIATION	COEFFICIENT OF VARIATION	N
21		3.102			
24		3.105			
27		3.111			
30		3.123			
33		3.148			
36		3.201			
39	3.873 ± .153	3.301	0.2256	5.83 ± 1.39	4
42	3.525 ± .052	3.478	0.2167	6.15 ± .88	11
45	3.614 ± .025	3.740	0.2010	5.56 ± .49	29
48	3.871 ± .026	4.047	0.2361	6.10 ± .47	38
51	4.352 ± .032	4.308	0.2661	6.11 ± .52	32
54	4.601 ± .024	4.490	0.2043	4.44 ± .32	34
57	4.520 ± .038	4.595	0.3309	7.32 ± .60	34
60	4.548 ± .033	4.650	0.2815	6.19 ± .51	34
63		4.676			
66	4.663 ± .065	4.689	0.5445	11.68 ± 1.00	32
69		4.695			
72	4.607 ± .054	4.698	0.4930	10.70 ± .84	38

TABLE 9
Equations for larval growth of different stages of the second experiment

STAGES	LOWER ASYMPTOTE	UPPER ASYMPTOTE	K	EQUATION OF THE LOGISTIC CURVE
I	0	1.5	1.5	$y = \frac{1.5}{1 + e^{-0.202547 - 0.144102x}}$
II	1.9	2.9	1	$y = 1.9 + \frac{1}{1 + e^{9.680024 - 0.242501x}}$
III	3.1	4.7	1.6	$y = 3.1 + \frac{1.6}{1 + e^{11.898182 - 0.255294x}}$

¹This equation was calculated assuming that the 39 hours' moment is equal to one, 42 to two, etc.

IV

During the last few years a pronounced tendency is observable to connect data accumulated in two fields of biological research, namely, in genetics and in physiology of development. As an illustration we may refer to the opinion expressed by Morgan(11). He writes: "One of the central

TABLE 10
Averages of length of the body of *Drosophila* larvae. Third experiment

STAGES	I		II		III		CALCULATED FOR STAGE III, $y = 3.1 + \frac{2.1}{1 + e^{8.408565 - 0.181892x}}$
	hours	N	hours	N	hours	N	
10	1.293	17					
20	1.652	8	1.985	14			
30	1.324	1	2.728	20	4.144	1	3.236
32			2.794	50			3.294
34	1.128	1	2.968	23	3.467	1	3.373
36			3.063	51	3.272	17	3.477
38			3.104	29	3.518	29	3.610
40	1.395	1	2.963	8	3.508	43	3.772
42			2.968	3	4.058	54	3.957
44					4.225	51	4.145
46					4.449	65	4.355
48			2.282	2	4.443	23	4.538
50					4.752	27	4.698
52					4.839	28	4.830
54					4.933	30	4.930
56					5.226	33	5.010
58					5.296	33	5.066
60					4.988	28	5.107

problems of embryology will be the discovery of methods by which the genes affect the development of the characters of the individual" (p. 83), and in another place(12):

The study of the fundamental problems of embryology by experimental methods had almost come to a standstill until two new methods of procedure appeared above the horizon—one the direct application of physico-chemical methods to the developing organism; the other, the application of genetics to problems of development. The combination of these two methods holds for us, at present, I believe, the most promising mode of attack on the problems of developmental physiology.

There have been already several attempts to interpret in terms of developmental physiology the problem of the manifestations of genes. We have in mind investigations such as those of Plunkett(16), Driver(4), and others. Goldschmidt's quantitative theory of the origin of intersexes is based on the assumption of interaction of 'Geschlechtsdifferentiatoren' which are equivalent to sex genes. The growth of sex-deter-

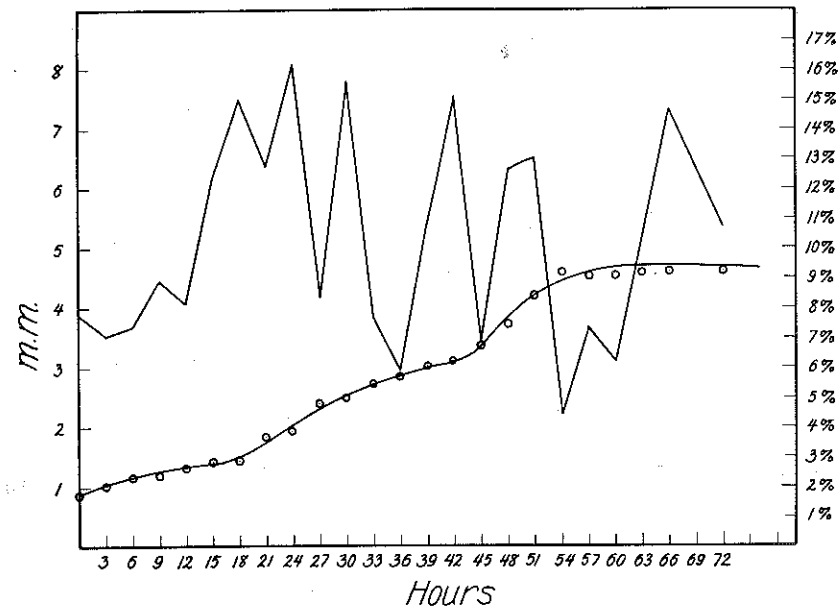


Fig. 15 Growth curves of larvae of the second experiment. The scale on the right side of the diagram relates to the curve of the coefficient of variation of the larvae of different ages.

mining products is expressed in forms of curves which remind one of exponential curves. After reaching a maximum point, the curves very often drop. In other cases the growth of this sex-determining product is expressed as straight lines. Already, Schmalhausen(21) has attempted to compare the exponential growth curve with Goldschmidt's hypotheses. But we cannot agree with Schmalhausen that the exponential ($v = mt^k$) formula is a good one to express the growth of a

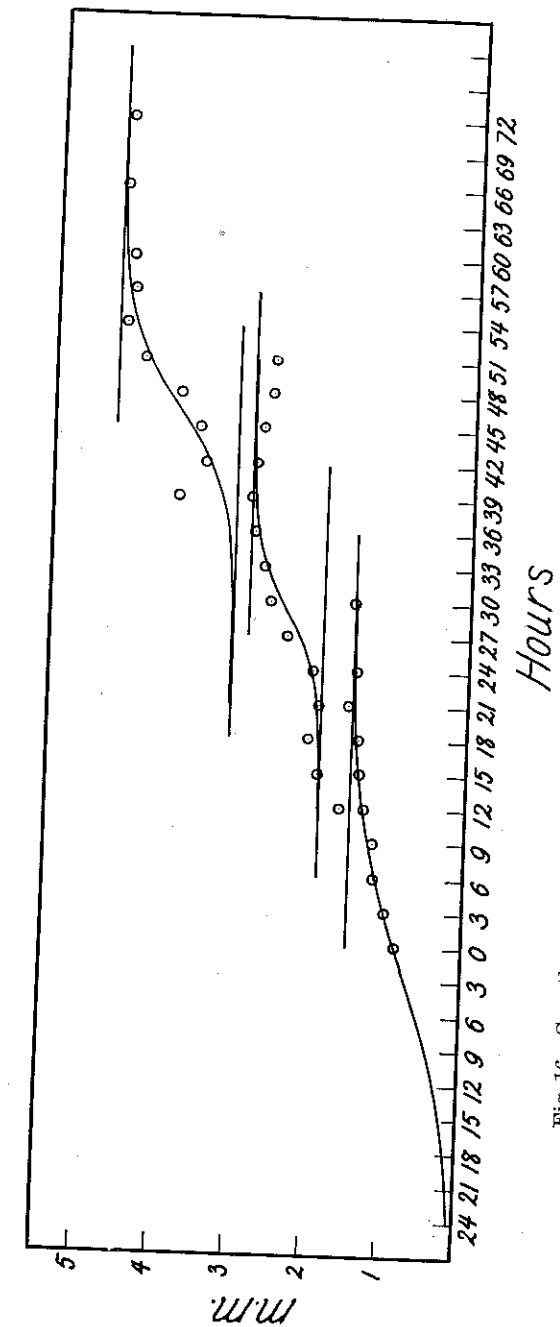


Fig. 16 Growth curves of larvae of the second experiment fitted with logistic curves.

living organism. The logistic law of growth is without any doubt a better description of growth phenomena. It is very probable that the accumulation of sex-determining substances follows the trend of a logistic curve.

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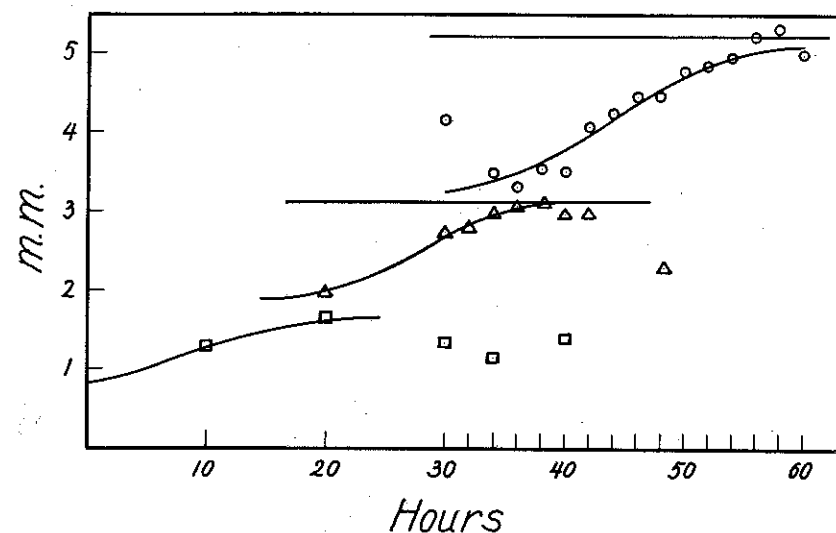


Fig. 17 Growth curves of larvae of the third experiment. The curve for the third stage is fitted with a logistic curve; those for the first and second are fitted by curves drawn by free hand.

SUMMARY

Larvae of *Drosophila melanogaster* have been studied from the point of view of their morphological changes as well as of the growth of the body length. The method of fixing the animals by boiling water has been found very satisfactory. Three sharply defined instars characterized by the structure of the mouth parts and respiratory organs (anterior spiracles) have been observed. The relations between the linear

dimensions of the mouth parts of two successive stages can be expressed accurately enough by the coefficient $(\sqrt{2})^2$. It is very likely that each of the two larval moltings is accompanied by two divisions of the body cells. Each stage has its own cycle of growth, expressed well by a curve of the logistic type. The first cycle of growth begins apparently as the growth of the egg cell in the ovary of the female.

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PLATES