FACTORS AFFECTING OVA DEVELOPMENT IN ANOPHELES ATROPARVUS

by

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ABSTRACT

FACTORS AFFECTING OVA DEVELOPMENT
IN ANOPHELES ATROPARVUS

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There are certain environmental factors which have been reported to affect ova development in anophelines. Data are needed to quantify these observations. The purpose of this research was to obtain data to confirm the reported effect of temperature, light, blood source, and feeding time on ova development in Anopheles atroparvus.

The pattern of ova development in all the experiments conducted was as follows: first there was a slow elongation up to the first peak, then there was a rapid growth period during which the ova reached their maximum lengths, second peak. Shortly after the maximum length was reached a decrease in length due to bending of the ova followed. Critical length in this research was determined to be the maximum length, and it was used as a means of comparison among the experiments.

The results in this research indicate that at a higher temperature ova reach the maximum length faster than at a lower temperature. Optimal lighting condition for the ova development is twelve hours of light and twelve hours of dark. There is a change in the rate of ova development when a different host is offered. Ova in mosquitos offered a guinea pig host reach the maximum length faster than when

human host is offered. Feeding time may have an effect on ova development. A.M. feeding may be preferable to P.M. feeding. A very high temperature causes some reduction in egg viability. This probably is not advantageous to the organism.

ACKNOWLEDGEMENT

I wish to express my grateful appreciation to Dr. Richard D. Kreutzer, who advised and directed my research and gave editorial criticism of the thesis.

To my family for their constant encouragement, I am most grateful.

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LIST OF SYMBOLS

SYMBOL	DEFINITION
C	Control
$\bar{x}$	Mean
$\bar{\bar{x}}$	Mean of means
1A-5C	Experiments explained in Chapter 1

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CHAPTER I

INTRODUCTION

Certain environmental factors have been reported to affect ova development in anophelines. These reports have been qualitative, and data are needed to quantify these observations.

The purpose of this research was to obtain data to confirm the reported affect of light, temperature, host type, and feeding time on ova development in Anopheles atroparvus.

Female Reproductive System

In anophelines the two ovaries lie dorso-laterally in the posterior portion of the abdomen. They are connected by lateral oviducts to a common oviduct which opens into the genital chamber or vagina; Figure 1.

In the ovaries the ovarioles radiate from an extension of each lateral oviduct called the calyx; Figure 2.

Each ovariole consists of an anterior germarium, a posterior vitellarium, and two sheaths, the tunica propria and the ovariole sheath. The germarium contains a central mass of eight nuclei, seven of which are nurse cell nuclei and one the oocyte nucleus, and a peripheral layer containing nuclei which will give rise to follicular epithelium.

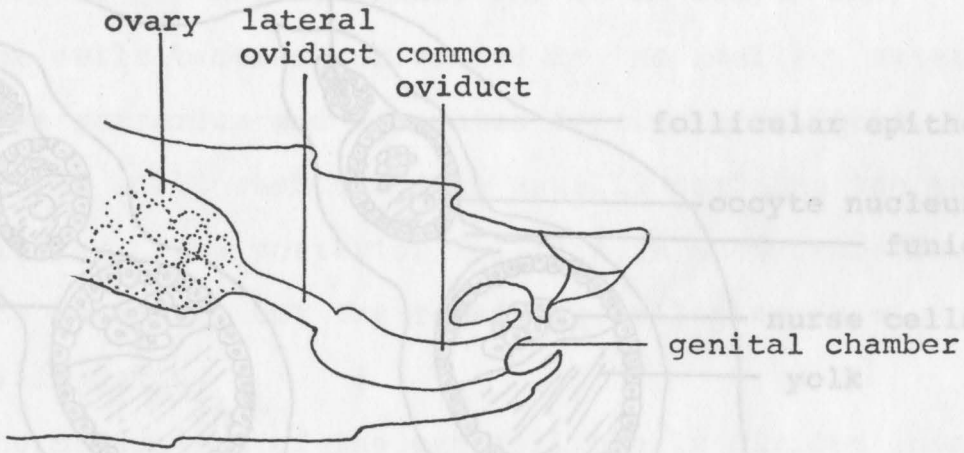

Fig. 1. Lateral view of the internal reproductive organs of a female anopheline.

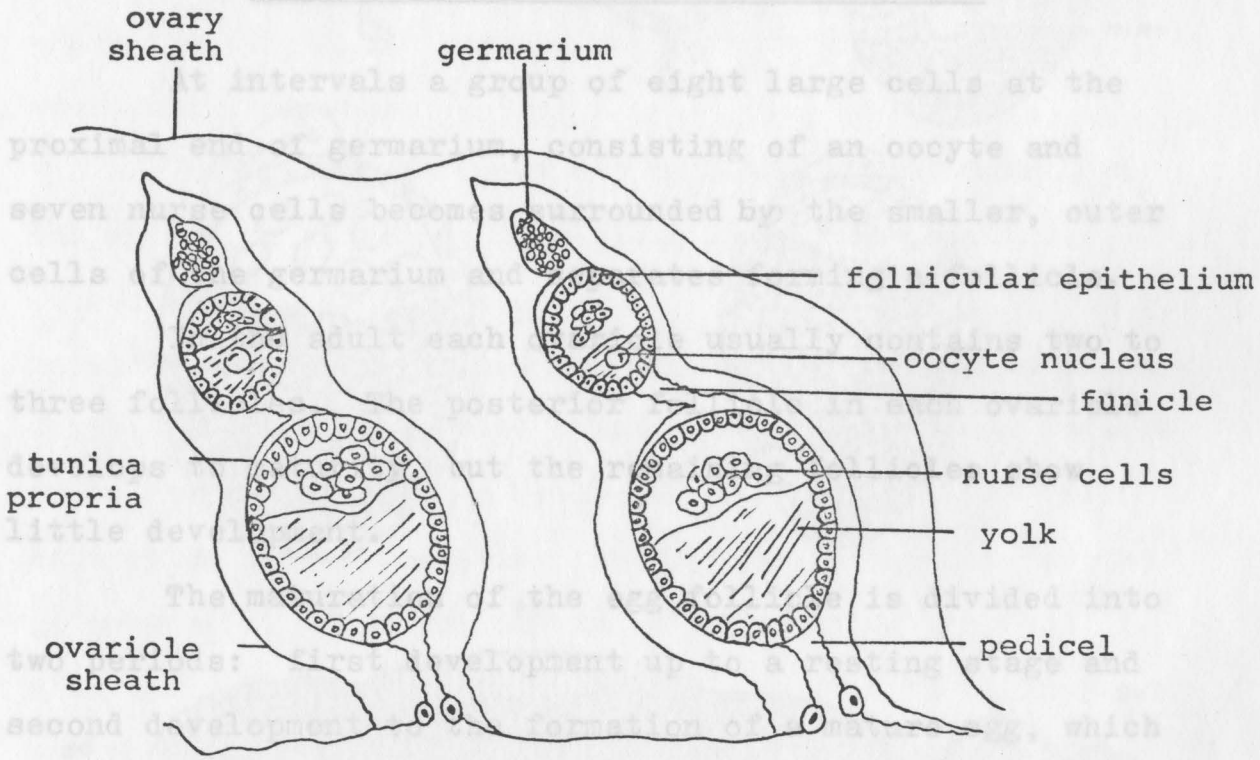

Fig. 2. Two ovarioles from a nulliparous female in longitudinal section.

The follicles are connected to the calyx by a narrow tubular region of the tunica propria called the funicle or pedicel.

Normal Egg Development in Anophelines

At intervals a group of eight large cells at the proximal end of germarium, consisting of an oocyte and seven nurse cells becomes surrounded by the smaller, outer cells of the germarium and separates forming a follicle.

In the adult each ovariole usually contains two to three follicles. The posterior follicle in each ovariole develops to maturity, but the remaining follicles show little development.

The maturation of the egg follicle is divided into two periods: first development up to a resting stage and second development to the formation of a mature egg, which does not normally start until the mosquito has taken a blood meal, figure 3.

The cytoplasm of the oocyte and nurse cells increase as the follicle starts to grow, and a small amount of yolk is deposited around the oocyte nucleus before it enters the resting stage. During the first gonotrophic cycle deposition of yolk can be achieved either at the expense of reserves accumulated during larval life or from sugar or blood ingested by the adult.

In each subsequent gonotrophic cycle, as one oocyte attains full development, the oocyte lying distal

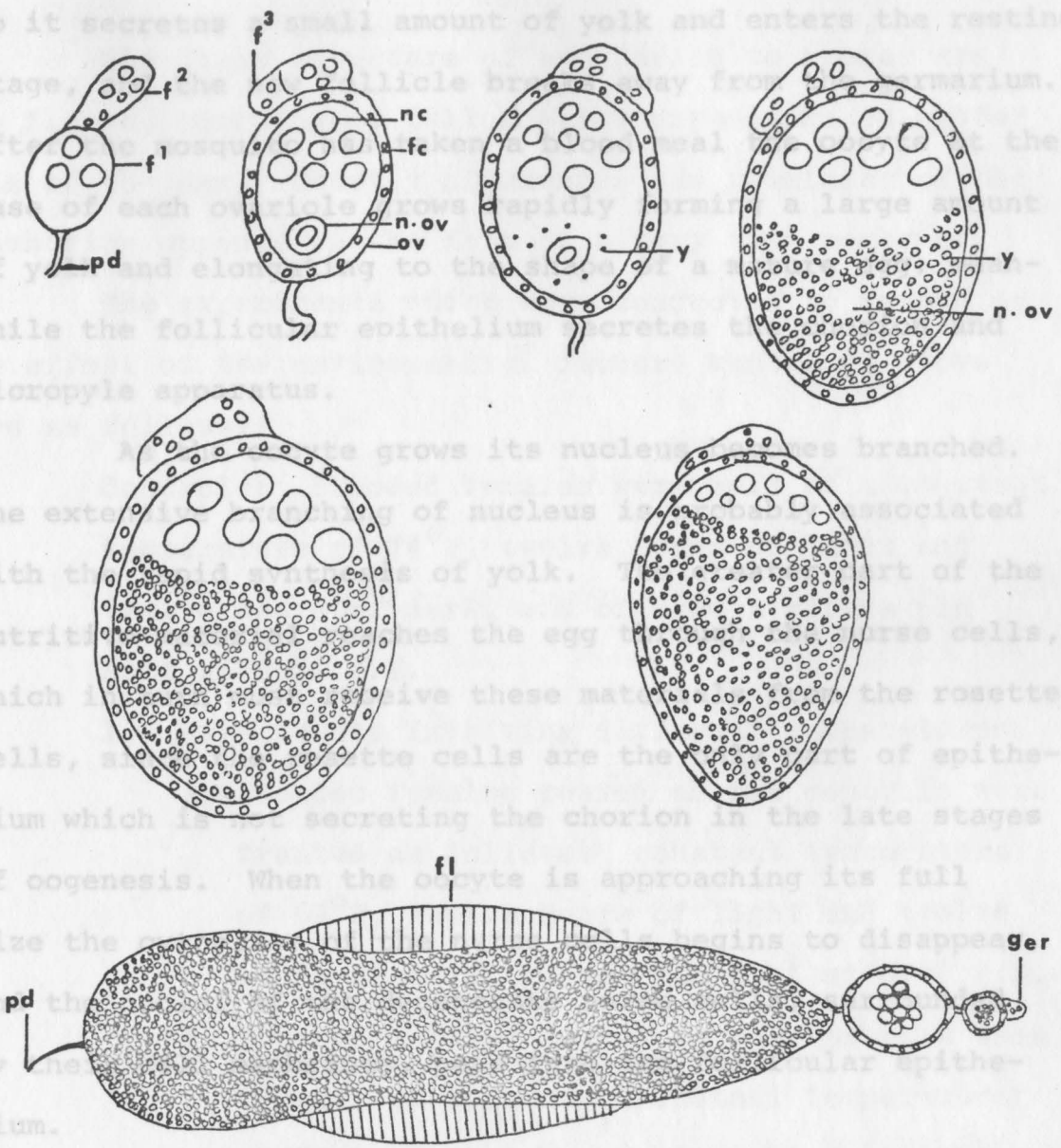

Fig. 3. Diagrammatical representation of ova development.

f^1, f^2, f^3.	Follicles of different ages.
fc.	Layer of follicular cells.
ov.	Ovum.
nc.	Nurse cells.
y.	Yolk granules.
n. ov.	Nucleus of ovum.
pd.	Pedicel.
fl.	Float.
ger.	Germarium.

to it secretes a small amount of yolk and enters the resting stage, and the new follicle breaks away from the germarium. After the mosquito has taken a blood meal the oocyte at the base of each ovariole grows rapidly forming a large amount of yolk and elongating to the shape of a mature egg; meanwhile the follicular epithelium secretes the chorion and micropyle apparatus.

As the oocyte grows its nucleus becomes branched. The extensive branching of nucleus is probably associated with the rapid synthesis of yolk. The greater part of the nutritive material reaches the egg through the nurse cells, which in turn must receive these materials from the rosette cells, since the rosette cells are the only part of epithelium which is not secreting the chorion in the late stages of oogenesis. When the oocyte is approaching its full size the cytoplasm of the nurse cells begins to disappear, and the nuclei of the degenerate nurse cells, surrounded by their cell membranes, pass into the follicular epithelium.

The egg shell or chorion consists of two layers, the endochorion and exochorion. When the oocyte has reached about one third of its final size the follicular epithelium secrets globules which fuse forming a coat around the entire egg except for a circular area below the future mycropyle. This layer is endochorion and remains soft until after the egg has been deposited, when it hardens to form a thin dark membrane.

The first structure of exochorion to appear are the floats, corrugated, balloon-like expansions on either side of the egg. Shortly afterwards the remainder of the exochorion appears in the form of a very thin membrane.

The experiments which were conducted to determine the effect of the environmental factors mentioned above were as follows:

Controls: Blooded females were held at a constant temperature of 74°F, twelve hours of light and twelve hours of dark, and offered a guinea pig host at 8:00 A.M.

1. Experiments involving different temperatures:
   A. Blooded females reared as the controls were treated as follows: constant temperature of 69°F, twelve hours of light and twelve hours of dark, guinea pig host at 8:00 A.M.
   B. Blooded females reared as the controls were treated as follows: constant temperature of 83°F, twelve hours of light and twelve hours of dark, guinea pig host at 8:00 A.M.
   C. Blooded females reared as the controls were treated as follows: constant temperature of 94°F, twelve hours of light and twelve hours of dark, guinea pig host at 8:00 A.M.
   D. Blooded females reared as the controls were treated as follows: 74°F day temperature and 69°F night temperature, twelve hours

of light and twelve hours of dark, guinea pig host at 8:00 A.M.

E. Blooded females reared as the controls were treated as follows: 83°F day temperature and 69°F night temperature, twelve hours of light and twelve hours of dark, guinea pig host at 8:00 A.M.

F. Blooded females reared as the controls were treated as follows: 83°F day temperature and 74°F night temperature, twelve hours of light and twelve hours of dark, guinea pig host at 8:00 A.M.

G. Blooded females reared as the controls were treated as follows: 94°F day temperature and 69°F night temperature, twelve hours of light and twelve hours of dark, guinea pig host at 8:00 A.M.

H. Blooded females reared as the controls were treated as follows: 94°F day temperature and 74°F night temperature, twelve hours of light and twelve hours of dark, guinea pig host at 8:00 A.M.

I. Blooded females reared as the control were treated as follows: 94°F day temperature and 83°F night temperature, twelve hours of light and twelve hours of dark, guinea pig host at 8:00 A.M.

2. Experiments involving light:
   A. Blooded females reared as the controls were treated as follows: sixteen hours of light and eight hours of dark, constant temperature of 74°F, guinea pig host at 8:00 A.M.
   B. Blooded females reared as the controls were treated as follows: eight hours of light and sixteen hours of dark, constant temperature of 74°F, guinea pig host at 8:00 A.M.
   C. First instar larvae from eggs that were reared as controls were treated as follows: sixteen hours of light and eight hours of dark, constant temperature of 74°F, guinea pig host at 8:00 A.M.
   D. First instar larvae from eggs that were reared as controls were treated as follows: eight hours of light and sixteen hours of dark, constant temperature of 74°F, guinea pig host at 8:00 A.M.
3. Experiment involving blood type:
   A. Blooded females reared as the controls were treated as follows: human host at 8:00 A.M., constant temperature of 74°F, twelve hours of light and twelve hours of dark.

4. Experiment involving feeding time:

   A. Blooded females reared as the controls were treated as follows: 8:00 P.M. feeding on guinea pig host, constant temperature of 74°F, twelve hours of light and twelve hours of dark.

5. Egg viability experiments:

   A. Blooded females reared as the controls were treated as follows: constant temperature of 94°F, twelve hours of light and twelve hours of dark, guinea pig host at 8:00 A.M.

   B. Blooded females reared as the controls were treated as follows: 94°F day temperature and 83°F night temperature, twelve hours of light and twelve hours of dark, guinea pig host at 8:00 A.M.

   C. Blooded females reared as the controls were treated as follows: constant temperature of 83°F, twelve hours of light and twelve hours of dark, guinea pig host at 8:00 A.M.

CHAPTER II

MATERIALS AND METHODS

A. Laboratory rearing technique

The mosquitos used in this study have been maintained in the laboratory for over twenty years. Strickberger estimates that a sample population of fifty Drosophila reaches 100% homozygosity after inbreeding for five hundred generations. As this strain probably originated from fewer than fifty mosquitos and has been inbred for over four thousand generations, it can be assumed homozygous at nearly all loci; therefore all mosquitos, control and experimental, taken from this stock are assumed to be genetically identical.

Eggs were collected in a petri dish rimmed with filter paper. After hatching first instar larvae were placed in an 8" x 10" pan containing water one half inch deep. Larvae were fed once a day on a mixture by weight of one third Kellogg's Concentrate, one third dry yeast, and one third wheat germ. Pupae were collected in a glass jar containing some water, and placed in a 12" x 12" x 12" stock cage. Humidity was maintained by placing a wet sponge at the top of the cage. Adult's diet consisted of honey. As an energy source for egg development females were offered a guinea pig host.

Controls and all experimentals except those in experimental group 2C and 2D were reared as the stock with the

exception that blood females aged seven to ten days were transferred to a half gallon paper container and were placed in a Sherrer environmental chamber.

B. Special rearing techniques of some experimental females:

Certain experiments required females reared under other than control conditions. In experiments 2C and 2D first instar larvae were collected from stock reared ova, put in a pan and transferred to the environmental chamber set at sixteen hours of light and eight hours of dark, or eight hours of light and sixteen hours of dark respectively. With these exceptions the rearing and handling procedure was the same.

C. Preparation of ova for measurement:

In all the experiments except the ones that involved egg viability, starting at four hours after the blood meal and continuing at four hour intervals until they deposited eggs females were disected and their ova measured.

Females were etherized, their ovaries were removed and placed on a siliconized glass slide in a dilute solution (approximately 0.15N) of saline, and the individual ova were seperated. Before placing a siliconized cover slip over the specimen two pieces of broken glass were placed to the sides of saline drop. An ocular micrometer was used to measure the ova. Eleven point six units was equal to 0.1 m.m.

D. Computational procedure:

At each reading the lengths of ten ova chosen at random were measured. The mean of ten readings for each hour was computed. Each experiment was repeated at least five times. Then the mean of means for each hour was computed and used for graphing.

E. Additional equipment:

In this experiment a Sherrer environmental chamber was used. Temperature and light can be adjusted. As a second check for temperature a thermometer was placed in the chamber.

CHAPTER III

RESULTS

The pattern of ova development in the control and experimental groups 1A through 4A was as follows: first there was a steady, slow elongation up to a certain point, first peak, then there was a rapid growth during which the egg almost doubled in length and reached the maximum length, the second peak. Shortly after the maximum length was reached a decrease in length due to bending of the ova followed. It was usually during the short decrease period that float formation was completed and the eggs were ready to be deposited.

In this project the maximum length was considered to be the critical length. The hour at which the maximum length was reached was used as a means of comparison among the experiments.

Table 1 shows the results of ova development of the control group. According to these data the egg reached the maximum length at sixty hours after the female had taken a blood meal. There is a graphical representation of these data in Figure 4.

In experimental group 1, involving ova development under different temperatures, the results indicate that as temperature (constant or alternating) increases the amount of time needed for ova to reach maximum length decreases, Table 2.

The results for experiments 1A, 1B, and 1C are graphically shown in Figure 5. According to these results, ova of 1A experimental females reached maximum length at a hundred hours after feeding, 1B at fifty two hours, and 1C at forty hours. Figure 6 graphically shows the results of experiments 1D through 1I (alternating temperatures). According to these results, ova of 1D experimental females reached maximum length at seventy six hours after feeding, 1E at fifty six hours, and 1F at fifty six hours, 1G at fifty two hours, 1H at fifty two hours, and 1I at forty eight hours.

Experimental group 2 involved the effect of light on ova development. Table 3 and Figure 7 show that ova in 2A experimental females reached maximum length at seventy two hours after feeding. Table 3 and Figure 7 show that the maximum length in 2B experiment was reached at seventy six hours. In Table 3 and graphically in Figure 8 ova in experimental females 2C and 2D both reached the maximum length at seventy two hours.

Experimental group 3 involved the effect of blood type on ova development. The results for this experiment are listed in Table 4 and graphically shown in Figure 9. Results indicate that ova reached the maximum length at seventy six hours after feeding.

Experiment 4 was conducted to see whether or not feeding time had any effect on ova development. The results for this experiment are listed in Table 5 and graphically shown in Figure 10. They indicate that maximum length of ova

was reached at sixty four hours after feeding.

The results of experiment 5 on egg viability are listed in Table 6. According to these data the control group had 94.8% hatch, 5A experimental females had 66.4% hatch, 5B experimental females had 70.3% hatch, and 5C experimental females had 92.3% hatch.

TABLE 1

CONTROL

Hour	Replicate $\bar{x}$*					$\bar{\bar{x}}$
	1	2	3	4	5	
4	12	9	12	12	10	11
8	13	12	12	12	12	12
12	14	14	14	13	16	14
24	21	17	19	21	18	19
28	22	21	26	22	21	23
32	23	24	25	23	24	24
36	26	27	25	26	26	26
48	38	30	34	36	34	35
52	57	58	57	53	63	58
56	61	59	61	63	60	61
60	60	63	64	61	62	62
64	58	62	59	61	60	60
68	59	59	60	58	59	59
76	59	59	59	58	59	59

* Each replicate consists of ten individual observations.

TABLE 2

EXPERIMENTS INVOLVING TEMPERATURE

Hour	Control and Experimental $\bar{\bar{x}}$*									
	C	1A	1B	1C	1D	1E	1F	1G	1H	1I
4	11	10	12	12	11	10	10	12	12	12
8	12	11	14	14	12	12	12	14	14	14
12	14	--	15	17	14	15	15	17	18	19
24	19	16	23	28	16	20	22	19	24	24
28	23	17	25	32	18	24	25	23	27	31
32	24	18	30	34	20	26	30	28	31	42
36	26	18	32	52	23	30	29	35	36	48
40	--	--	--	61	--	--	--	--	--	--
48	35	25	60	58	29	42	41	55	55	62
52	58	27	62	--	31	55	58	62	63	58
56	61	31	60	--	36	62	63	59	63	--
60	62	32	58	--	36	60	60	--	59	--
64	60	--	--	--	--	--	--	--	--	--
68	59	--	--	--	--	--	--	--	--	--
72	--	35	--	--	54	--	--	--	--	--
76	59	--	--	--	65	--	--	--	--	--
80	--	55	--	--	63	--	--	--	--	--
84	--	57	--	--	60	--	--	--	--	--
96	--	63	--	--	--	--	--	--	--	--
100	--	71	--	--	--	--	--	--	--	--
104	--	66	--	--	--	--	--	--	--	--
108	--	58	--	--	--	--	--	--	--	--

* Each $\bar{\bar{x}}$ represents the $\bar{x}$ of five replicates.

TABLE 3

EXPERIMENTS INVOLVING LIGHT

Hour	Control and Experimental $\bar{\bar{x}}$*				
	C	2A	2B	2C	2D
4	11	11	10	--	--
8	12	12	12	--	--
12	14	14	14	10	13
24	19	18	18	13	18
28	23	20	19	16	20
32	24	20	23	19	20
36	26	--	--	--	--
48	35	33	29	28	29
52	58	35	36	32	30
56	61	51	36	37	32
60	62	57	48	52	54
64	60	--	--	--	--
68	59	--	--	--	--
72	--	62	59	62	62
76	59	59	63	61	58
80	--	--	58	59	--

* Each $\bar{\bar{x}}$ represents the $\bar{x}$ of five replicates.

TABLE 4

EXPERIMENTS INVOLVING BLOOD TYPE

Hour	Control and Experimental $\bar{\bar{x}}$*	
	C	3A
4	11	11
8	12	12
12	14	12
24	19	17
28	23	18
32	24	20
36	26	21
48	35	27
52	58	31
56	61	32
60	62	39
64	60	--
68	59	--
72	--	52
76	59	61
80	--	59

* Each $\bar{\bar{x}}$ represents the $\bar{x}$ of five replicates.

TABLE 5

EXPERIMENTS INVOLVING FEEDING TIME

Hour	Control and Experimental $\bar{\bar{x}}$*	
	C	4A
4	11	--
8	12	--
12	14	13
16	--	16
20	--	19
24	19	20
28	23	--
32	24	--
36	26	28
40	--	31
44	--	32
48	35	34
52	58	--
56	61	--
60	62	59
64	60	66
68	59	61
76	59	--

* Each $\bar{\bar{x}}$ represents the $\bar{x}$ of five replicates.

TABLE 6

EGG VIABILITY EXPERIMENTS

	C	5A	5B	5C
Total	2154	2194	2019	2204
Hatch	2041	1457	1420	2035
Embryonated	4	57	38	9
Unembryonated	101	680	561	160
% Hatch	94.8	66.4	70.3	92.3

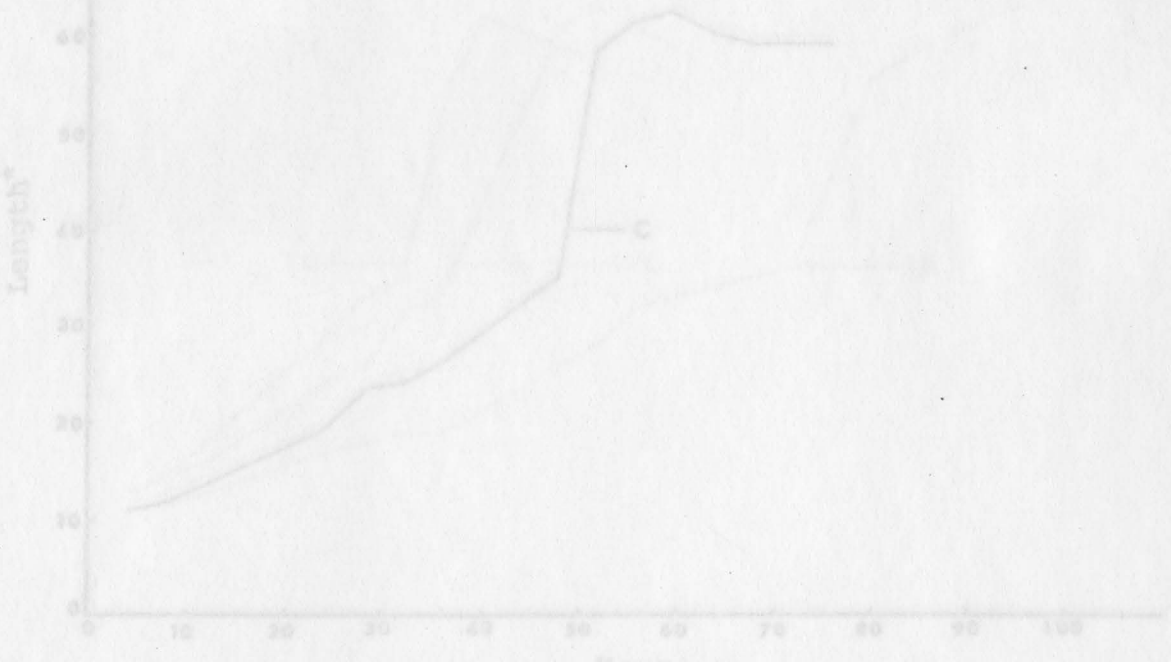

Fig. 4. Graphical representation of ova development in Control Group.

*Eleven point six unit length is equal to 0.1 m.m.

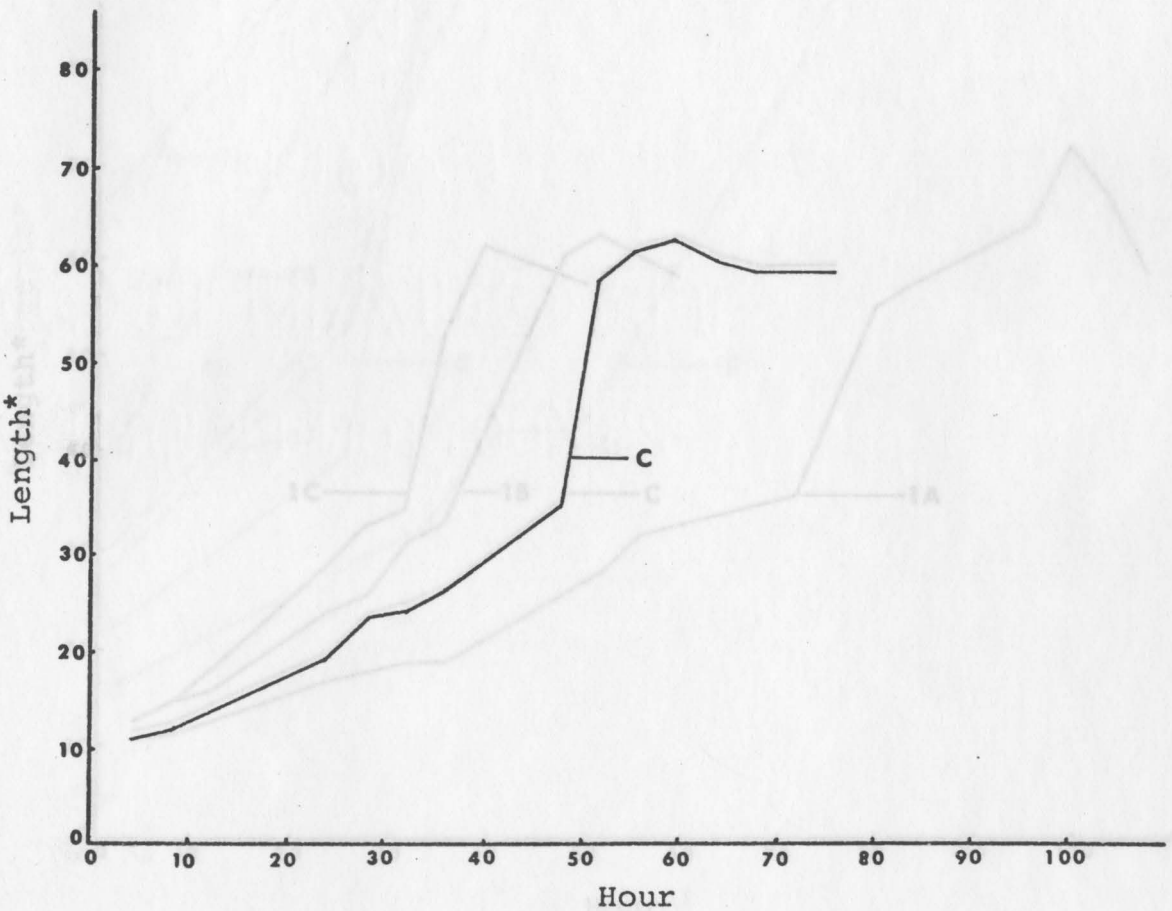

Fig. 4. Graphical representation of ova development in Control Group.

*Eleven point six unit length is equal to 0.1 m.m.

Fig. 5. Graphical representation of ova development in Control and Experimental Groups 1A, 1B, and 1C.

*Eleven point six unit length is equal to 0.1 m.m.

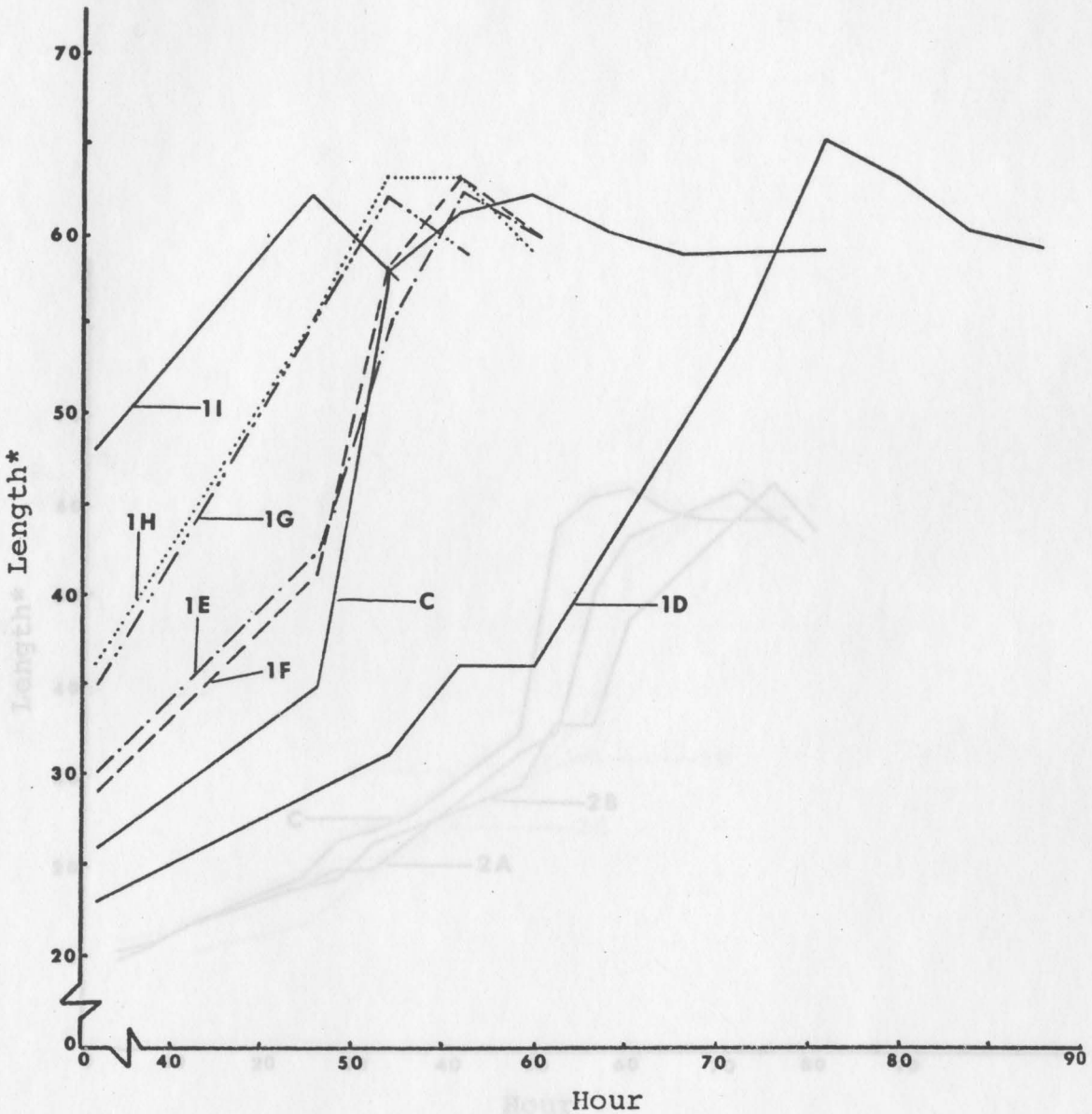

Fig. 6. Graphical representation of ova development in Control and Experimental Groups 1D, 1E, 1F, 1G, 1H, and 1I.

*Eleven point six unit length is equal to 0.1 m.m.

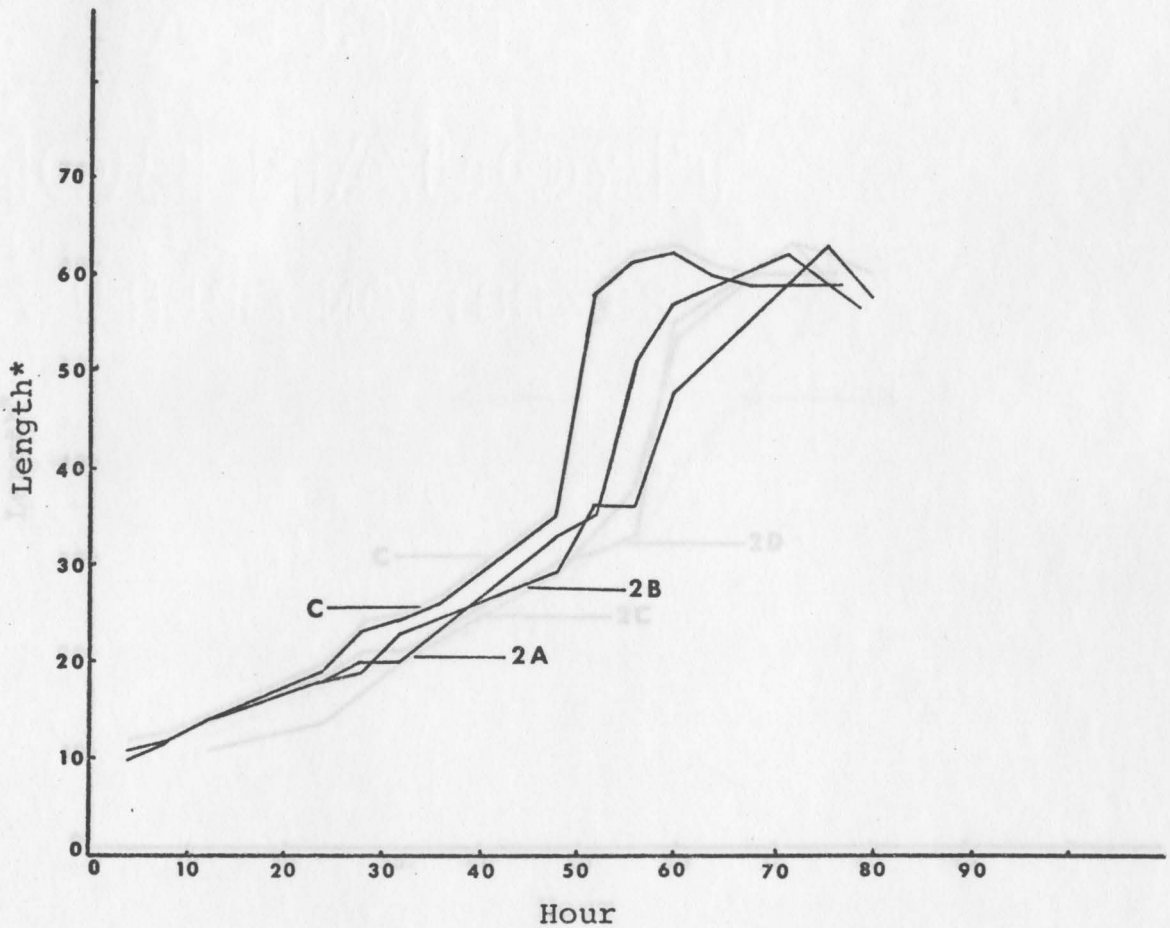

Fig. 7. Graphical representation of ova development in Control and Experimental Groups 2A and 2B.

*Eleven point six unit length is equal to 0.1 m.m.

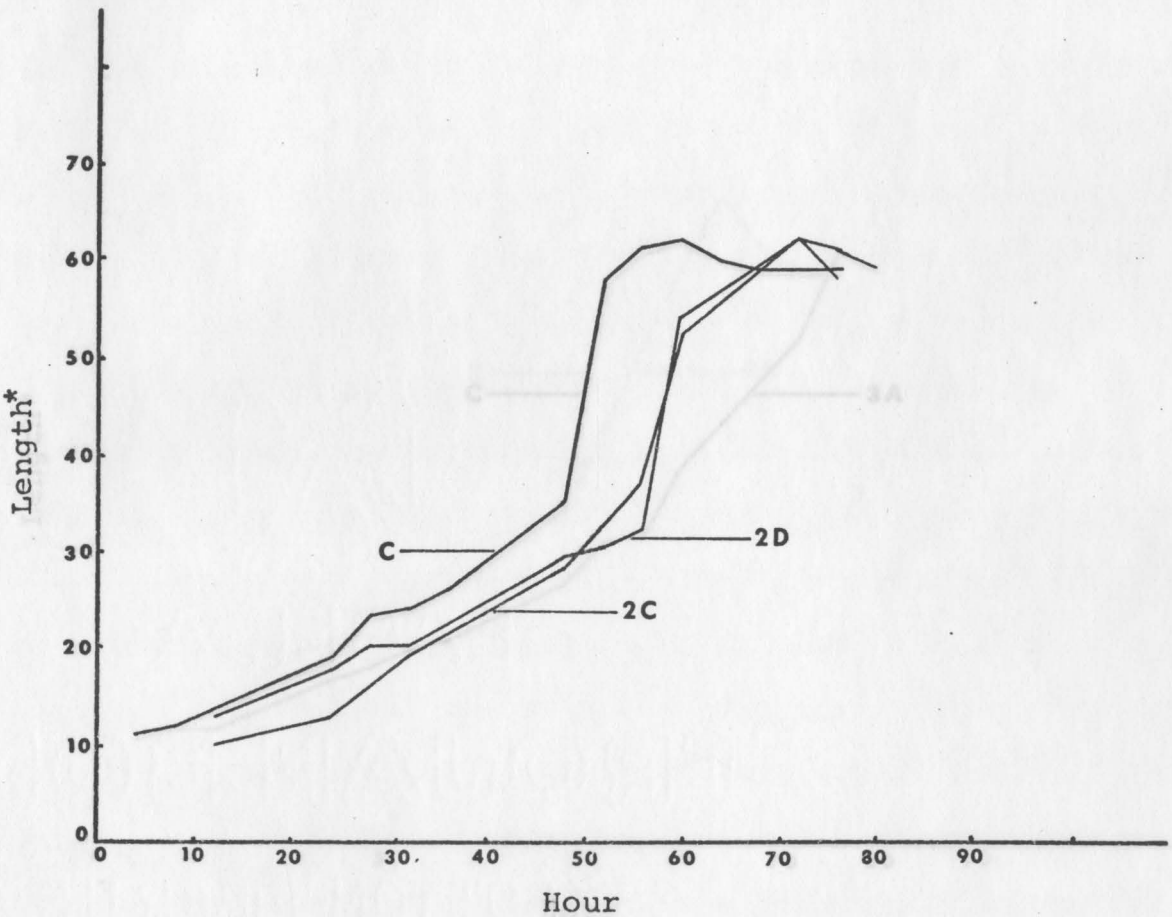

Fig. 8. Graphical representation of ova development in Control and Experimental Groups 2C and 2D.

*Eleven point six unit length is equal to 0.1 m.m.

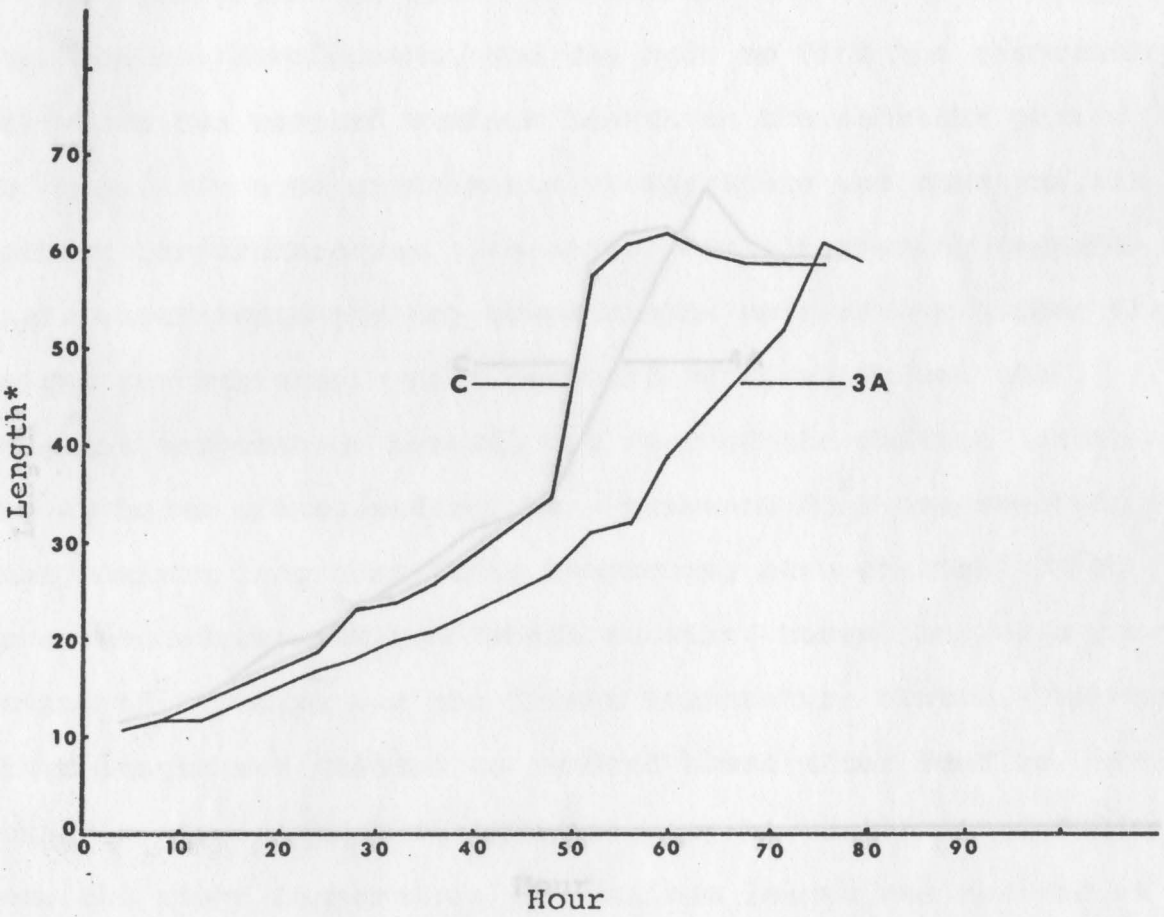

Fig. 9. Graphical representation of ova development in Control and Experimental Group 3A.

*Eleven point six unit length is equal to 0.1 m.m.

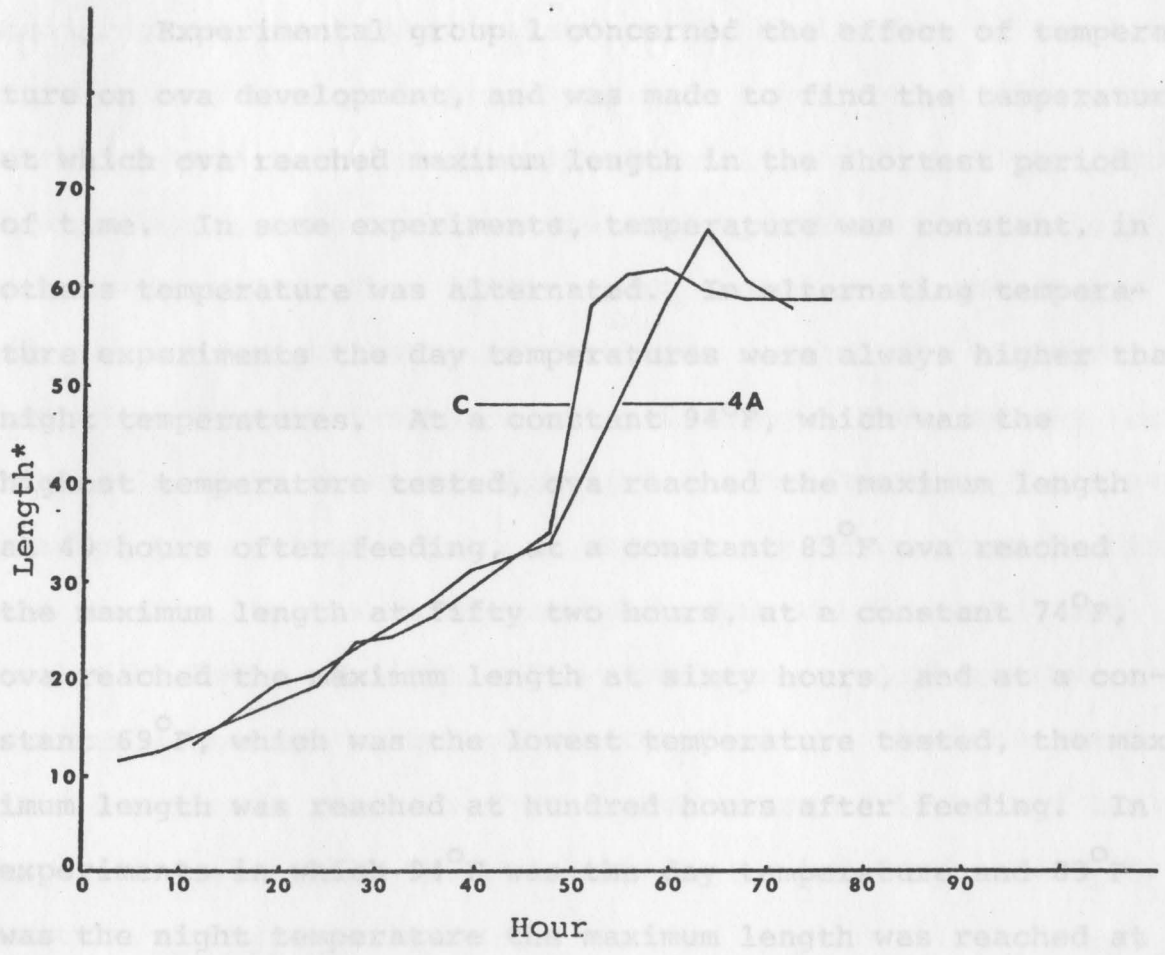

Fig. 10. Graphical representation of ova development in Control and Experimental Group 4A.

*Eleven point six unit length is equal to 0.1 m.m.

CHAPTER IV

DISCUSSION

The purpose of this research was to test the effects of certain environmental factors on ova development in Anopheles atroparvus.

Experimental group 1 concerned the effect of temperature on ova development, and was made to find the temperature at which ova reached maximum length in the shortest period of time. In some experiments, temperature was constant, in others temperature was alternated. In alternating temperature experiments the day temperatures were always higher than night temperatures. At a constant 94°F, which was the highest temperature tested, ova reached the maximum length at 40 hours ofter feeding, at a constant 83°F ova reached the maximum length at fifty two hours, at a constant 74°F, ova reached the maximum length at sixty hours, and at a constant 69°F, which was the lowest temperature tested, the maximum length was reached at hundred hours after feeding. In experiments in which 94°F was the day temperature and 83°F was the night temperature the maximum length was reached at forty eight hours after feeding. In other experiments involving temperature variability ova reached the maximum length after a longer period of time. These results indicate that as temperature increases, the amount of time needed for ova development decreases. Among all the temperatures tested ova under a constant 94° F reached the maximum length in

the shortest period of time.

Experimental group 2 was conducted to show the effect of light on ova development in Anopheles atroparvus. The control group was raised under equal periods of light and dark (12 and 12), whereas the experimental groups were exposed to different periods of light and dark. Whether the experimental groups were raised as the control up to the time of the experiment and then exposed to experimental light and dark, or were raised under experimental conditions from first instar larvae, the results were a slow down in the rate of ova development. When females were reared in sixteen hours of light and eight hours of dark, ova reached the maximum length at seventy two hours after feeding. When females were reared in eight hours of light and sixteen hours of dark, ova reached the maximum length at seventy six hours after feeding; and finally the ova in the control group which were raised in twelve hours of light and twelve hours of dark reached the maximum length at sixty hours after feeding. The results in this experiment indicate that twelve hours of light and twelve hours of dark is the optimal lighting period for ova development in atroparvus.

The results from experiment 3 involving the effect of blood source on ova development indicate that there is a change in the rate of ova development when a different host is offered to the female mosquito. When the human host was used, ova reached their maximum length sixteen hours later than when a guinea pig host was offered.

Experiment 4 was conducted to determine the effect of the time of feeding on ova development. Ova of the females in the experimental group which were fed at 8:00 P.M. reached the maximum length four hours later than the control group. These data show that the time of feeding may have some effect on ova development

The data thus far presented show that the optimal conditions for the growth of Anopheles atroparvus are a high constant temperature of 94°F, twelve hours of light and twelve hours of dark, guinea pig host, and 8:00 A.M. feeding. Although rapid ova development must be important to the organism, more important would be the viability of the ova produced. Because temperature has been reported to affect egg viability in other dipterans, the viability of ova deposited at the experimental and control temperatures was determined. The temperature at which ova development was most rapid was 94°F; however, the viability at this temperature was 66.4%. Egg development at 83°F was more rapid than at 74°F, and there was no significant reduction in viability at these temperatures. These data indicate that although the ova development is more rapid at 94°F, the slower development at 83°F is more advantageous to the organism.

The data presented in this thesis indicate that the optimal conditions for ova development in Anopheles atroparvus are a constant 83°F temperature, twelve hours of light and twelve hours of dark, guinea pig host, and 8:00 A.M. feeding.

These are the conditions of experiment 1B in which ova did reach their maximum length with greatest viability in the shortest period of time.

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