A MORPHOLOGICAL STUDY OF THE RESPIRATORY HORNS ASSOCIATED
WITH THE PUPARIA OF SOME DIPTERA, ESPECIALLY
OPHYRA ANESCENS (WIED.)

Dissertation

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LEON RAMON RODDY, B.S., M.S.
The Ohio State University

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Approved by: Wah. Johnson
Advisor AK

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	11
I- INTRODUCTION.....	1
II- REARING AND HANDLING FLIES.....	3
<u>Ophyra anescens</u> (Wied.).....	3
<u>Lucilia sericata</u> (Meig.).....	5
<u>Musca domestica</u> (Linn.).....	6
Procurement of Puparia.....	7
III- STRUCTURE AND DEVELOPMENT OF THE RESPIRATORY HORNS...	9
Orthorrhaphous Pupae.....	9
Cyclorrhaphous Pupae.....	11
Time of Emergence.....	13
Location.....	14
Size.....	15
Effects of Temperature and Humidity.....	15
IV- FUNCTION OF THE RESPIRATORY HORNS.....	17
Spiracular Plugging.....	18
Table I.....	19
Dissection.....	19
Dye Penetration.....	20
V- RELATIVE RATE OF RESPIRATION IN PUPARIA.....	21
The Microrespirometer.....	22
Procedure.....	23
Photographs.....	24
Table II.....	26
VI- DISCUSSION.....	27

VII-	SUMMARY.....	29
VIII-	LITERATURE CITED.....	33
IX-	ABBREVIATIONS USED IN FIGURES.....	35
X-	EXPLANATION OF PLATES.....	36
XI-	PLATES 1, 2, 3.....	37

INTRODUCTION

Respiratory horns is the common name given to the pair of prong-like structures which occur on the dorso-lateral aspect of the cephalic half of a number of pupae and puparia of Diptera especially among Cyclorrhapha. This study attempts to explain their structure, development, time of appearance, function and certain other factors associated with them. De Meijere (1902) made the first systematic attempt to describe the structure of the respiratory horns in various families of Diptera, drawing information both from his own observations and from the scattered descriptions in the literature. Many interesting examples of respiratory horns in pupae have been described and figured in several papers dealing with the morphology of larvae and pupae of Diptera, especially in papers by De Meijere (1902), Alexander (1920), and Johannsen (1934, 1935, 1937 and 1937a). The figures in these papers give a clear idea of the great variations in size, shape and structure of the respiratory horns often described as prothoracic horns, trumpets, spiracles, siphons or gills. Their size varies from short, sessile and hardly perceptible to long and distinctly prominent projections in some pupae.

This study was restricted to the puparia of Ophyra anescens (Wied.), Lucilia sericata (Meig.) and Musca domestica (Linn.), however, much of the information was

ascertained from Ophyra anescens (Wied.). The species named are closely related in that they belong to the same large group (Calyptrate Muscoidea), yet the larvae and puparia are distinctly different. The same is true for the respiratory horns that appear on the puparia of these species.

The purpose of this study was to ascertain the following facts regarding the respiratory horns: structure and development, time of emergence, location, size, the effects of temperature and humidity on their development, their function in respiration, and the relation between the size of the horns and the respiration rate.

The results obtained from this study are derived primarily from the puparia of three species of Diptera, however, the information presented may be helpful to other workers in the study of respiratory horns of dipterous pupae among other genera, species or families.

REARING AND HANDLING FLIES

Ophyra anescens (Wied.)

The culture of Ophyra anescens was obtained from Dr. F. M. Snyder of the Chemical Corps Laboratory at Army Chemical Center, Maryland. The rearing methods developed by F. M. Snyder were followed with only minor variations.

Equipment: The rearing equipment consisted of battery jars (9" high and 6" in diameter), stender dishes, plastic refrigerator dishes (2.5" high and 3" in diameter), cellucotton, Petri dishes (1.5 cm. high and 9.5 cm. in diameter), adult screen cages (10" x 12" x 13") with a wood bottom and back.

Larval Medium: This medium consisted of the following ingredients:

200 gm. whole milk (Klim)

200 gm. brewer's yeast (Fleishmanns)

15 gm. bacto-agar

2 liters of boiling tap water

The dry ingredients are mixed and poured into the boiling tap water and stirred until the medium begins to stiffen. It is then transferred to battery jars, covered, and allowed to cool for several hours. The medium should be prepared in the late afternoon of the day previous to egg collection so that eggs may be transferred immediately

to the medium.

Oviposition Medium: This medium consisted of moist ground dog biscuits placed in plastic refrigerator dishes. The cracks and crevices, which appear on the surface of the medium when dry, is a preferred egg laying situation. The medium should be watched carefully to see that it does not become too dry, otherwise the eggs will dessicate.

Eggs: Eggs are collected for a period not exceeding 24 hours from the oviposition medium in cages containing flies not more than 6 days old. Three to four hundred eggs are collected from the medium and buried just beneath the surface of the larval medium. The jar is then covered, dated and placed in an incubator with a temperature of 82°F. and relative humidity approximately 50%.

Puparia: When the larvae mature (about 4 days), small strips of paper towel are placed on the surface of the medium for the larvae to crawl onto where they transform into puparia.

All of the puparia collected on a given day are combined into one lot. Each lot is placed in a shallow dish which is, in turn, placed in an adult cage.

Adult Fly Food: Food for the adults consists of granulated sugar sprinkled over wet cellucotton. The cellucotton is removed every three days which keep mold growth to a minimum. It is essential that satisfactory food be available to the flies at all times.

Lucilia sericata (Meig.)

Equipment: The same as for Ophyra anescens (Wied.) with the exception of a No. 2 tin can.

Larval Medium: This medium consisted of lean ground beef (approximately $\frac{1}{2}$ pound) placed in a No. 2 tin can which is placed in a battery jar and covered with crumpled paper towels.

Oviposition Medium: This medium consisted of two or three slices of lean beef placed in a Petri dish and inserted in the cage with flies.

Eggs: The fresh medium is placed in the adult cage in the late afternoon and the eggs are collected the following morning. After the daily collection of eggs they are placed in the larval medium and incubated at a temperature of 76°F. and an air relative humidity of approximately 40%. The larvae hatch in 36 hours.

Puparia: Larvae are mature 4-5 days after the eggs hatch. The mature larvae migrate to the paper towels located in the bottom of the jar and transform to puparia. The puparia are collected daily from the jar and placed in Petri dishes. If flies are not needed at once the puparia may be stored in a refrigerator at a temperature of 20°F. for two weeks or more.

Adult Fly Food: The food for the adults consist of cubes of granulated sugar placed in Petri dishes inside the cage. Water is provided by filling a beaker and

inverting it on filter paper in one-half of a Petri dish within the adult cage.

Musca domestica (Linn.)

Equipment: The same as used for Ophyra anescens (Wied.).

Larval Medium: The medium used to rear this fly consisted of the following ingredients:

340 gm. C.S.M.A. (N.A.I.D.M.) media

15 gm. moist yeast cake

10 cc. Non-diastatic Dimalt

740 cc. of tap water

The above ingredients are mixed thoroughly until a loose, fluffy mixture is obtained. It is then transferred to a battery jar without packing, and covered with cloth. The amounts of ingredients listed above are enough for one battery jar.

Oviposition Medium: This medium consist of 1 part skim milk and 1 part water. A plastic refrigerator dish is filled with cellucotton and the diluted milk is poured over the cellucotton until it becomes saturated. The adult flies deposit their eggs on the cellucotton adjacent to the side of the dish. The medium should be changed daily to prevent souring and hatching of eggs.

Eggs: Eggs are collected for a period not longer than 16 hours from the oviposition medium in cages containing mature flies not more than 8 days old. After eggs are collected they are washed in tap water at room temperature.

This is done by placing the eggs in a dark porcelain pan containing a small amount of water. They are then collected with the aid of a small pipette and transferred to the larval medium and buried just beneath the surface of the medium. The cover is then placed on the jar and the jar stored in the rearing room. The eggs hatch within 36 hours. The temperature inside the jar (3 days later) must not exceed 130°F., otherwise the larvae will die.

Puparia: Mature larvae migrate to the top portion of the medium and transform to puparia within 5-7 days after the eggs hatch. After the puparia are formed, the top portion of the medium containing puparia may be loosened, poured into a shallow tray, and the puparia collected.

Adult Fly Food: This is the same as the oviposition medium.

Procurement of Puparia

In order to obtain puparia of a known age, a method similar to the one used by Robertson (1936) was employed.

When a larva is full grown and ceases to feed, it leaves the food and crawls up the side of the culture jar where it may move about for several hours, with the oral hooks continually in motion. After locating a place for pupariation, it gradually becomes less and less active until finally the anterior spiracles project cephalad, not laterad as in the maggot. Within a very short time the maggot begins to shorten in length, principally by

retraction of the larval head and most of the prothoracic segment. This marks the beginning of the puparial stage, and the process is termed either pre-pupation or pupariation. In this report the latter term is being adopted.

The term "pupariation" should not be confused with the term "pupation." The actual period of pupation does not occur until 10-12 hours after pupariation. This can be determined by noting the time of the appearance of the respiratory horns within the puparium. In Ophyra anescens (Wied.) the respiratory horns begin to appear inside the puparium within 10 hours after pupariation.

When the puparia are formed they are transferred individually to Petri dishes. The time is checked and written on the top of the Petri dish.

Thus the age of the puparium is calculated in hours from the beginning of pupariation until the adults emerge.

STRUCTURE AND DEVELOPMENT OF THE RESPIRATORY HORNS

There are many variations in the size, shape and detailed structure of the respiratory horns of dipterous pupae. They may be very short, sessile and hardly perceptible as in Musca domestica (Plate 1, Fig. 10), or they may be distinctly prominent and in some pupae (Psychodidae) reach a very great length (Plate 2, Fig. 16). In the pupae of some Ptychoptera only one spiracle develops into a horn while the second spiracle is more or less atrophied.

Orthorrhaphous Pupae

Among the Orthorrhaphous pupae, the respiratory horns of Psychoda alternata (Say) will serve as a type.

The horn (Plate 2, Fig. 17) has the form of a curved tapering cylinder narrowed at the base, where an annulated region consisting of four or five thickened rings occurs. Within the horn is a tube lined with chitinous felt, which De Meijere (1902) terms the "felt chamber" (F.C.). The felt chamber meets the wall of the horn at a number of thin circular regions. These regions De Meijere terms the pits (P), owing to their likeness to similar structures in plant cells. The pits are irregularly arranged and communicate directly with the felt chamber.

The felt chamber communicates, through the annulated base of the horn, with a small tube shaped chamber which

has a characteristic spiral thickening. This is termed the tracheal extension (T.E.).

The side of the tracheal extension is fused with the body wall, the line of fusion being marked by an elongate scar. At the lower end, the tracheal extension passes into the spiracular chamber (Sp. C.). The elongate scar of the tracheal extension terminates in a dark sclerotized plug, the closed pupal spiracle, which De Meijere terms the outer stigmatic scar (E.S.). It is connected to the spiracular chamber by a short chitinous thread, the scar strand (S. St.), which is the shrunken remnant of the tube through which the larval trachea were withdrawn. At the juncture of the scar strand with the spiracular chamber is a smaller inner stigmatic scar which connects the scar strand to the internal surface of the spiracular chamber.

From the spiracular chamber a trachea is given off that enters the prothoracic spiracle of the imago. This spiracle is the type found in most insects and consists of two semi-circular sclerotized lips which can be closed by the contraction of a band of muscle running between them. This constitutes the closing mechanism of the imago trachea. At the upper end of the tracheal extension is a heavily chitinized lever (L.) which constitutes the closing mechanism of the pupal trachea. This closing mechanism is narrowed basally, but broadens apically and bifurcates into two short arms which partially encircle the tracheal extension.

At the tip of the lever is a closing muscle which by contraction and expansion opens and closes the trachea.

Cyclorrhaphous Pupae

The pupal stage of Cyclorrhaphous Diptera is always enclosed within the cuticle of the last larval instar. After it shortens, it becomes a distinctly brown case which is called a puparium. Often the space between the pupae and the puparium, especially in the anterior region, is filled with air. In the majority of the acalyptrates the pupae have a pair of prothoracic spiracles which are enclosed within the puparium and are known as internal spiracles. Each spiracle is composed of a more or less protruding felt chamber covered with numerous spiracular papillae. In the great majority of the calyptrates the felt chambers of the prothoracic spiracles of the pupa, in addition to the internal spiracle (Plate 1, Figs. 4, 8, 12. I.S.), develops into a more or less sclerotized horn (R.H.) covered with spiracular pupillae. These protrude through small circular apertures in the wall of the puparium (C.S.).

The detailed development of the respiratory horns was studied only in the pupae of *Ophyra anescens* (Wied.). One of the most fascinating phenomena observed in this study was the protrusion of the horns through the hardened cuticle of the puparium. Protrusion is possible because

of the presence in the wall of the puparium of two small circular clear spots (Plate 1, Fig. 1. C.S.) where the cuticle is thin and fragile. These spots are light brown in color and surrounded by a brown ring. They first appear in 3-4 hour puparia and persist throughout the pre-pupal or puparial stage.

Dissection of a 10 hour puparium shows that the respiratory horns are developed and lie parallel to the length of the body (Plate 3, Fig. 24) whereas, after protrusion, they are more nearly perpendicular to the body.

About 18 hours after pupariation the anterior end of the pupa contracts leaving a free space. On each side of the pupa the partially evaginated respiratory horns (R.H.), which, according to Keilin (1944), emerge from apertures of their imaginal disks. These horns are light brown and show distinctly small spiracular papillae. Approximately 25 hours after pupariation the horns assume their final shape and structure. Three to four hours later the pupa contracts drawing the respiratory horns posteriorly, away from the anterior end, until their tips appear just beneath the two circular spots. During this period the pupa breaks away from the anterior tracheal tubes of the larva (Plate 3, Fig. 26.). The pupa then makes a few forward and backward movements, pushing the tips of the horns against the thin chitinous membranes of the circular spots until the membranes are broken.

Further movements of the pupa push the respiratory horns through the broken membranes. Soon they are completely outside of the puparium where they are readily visible to the naked eye. The whole process is completed within 1 or 2 minutes.

According to Keilin (1944) the contraction of the pupa is due mainly to the evagination of the imaginal disks of the head. It is remarkable to note that the length of the respiratory horns and the predetermined clear spots on the 1st abdominal segment of the puparium are so adjusted that the tips of the horns having moved backward about 2mm. are brought directly against the two fragile spots, through which they will emerge. In spite of this delicate adjustment, the extrusion of the respiratory horns is almost always successful. However, there are times when one or both horns fail to perforate the fragile spots and slip forward remaining within the puparium. If one horn is extruded the pupa may develop normally, but if both fail to emerge, the pupa does not develop into an adult fly. This may be due to the lack of oxygen which results in the death of the pupa.

Time of Emergence

The time of emergence of the respiratory horns on the exterior of the puparium is variable in the three species studied. In Musca domestica (Linn.), reared at

a temperature of 80°F. and a relative humidity around 50%, they appear externally 18-20 hours after pupariation and are visible in profile as tiny nodules. In Lucilia sericata (Meig.), the appearance of the horns is quite variable even under optimum rearing conditions (temperature 78°F. humidity 40%). In some of the puparia they began to emerge 20-25 hours, and in others from 30-50 hours after pupariation. In unusual cases the horns emerged just a few hours before emergence of the adults.

The emergence of the respiratory horns is not so variable in Ophyra anescens (Wied.). On the average, they began to emerge between 28-30 hours after pupariation. No further emergence of these horns were observed beyond 30 hours after pupariation. Isolated 30-50 hour-old puparia devoid of horns do not develop further. Dissections revealed that the pupae within were dead.

It is evident that in the three species studied, the horns do not emerge for sometime after pupariation, but their site can always be determined by a pair of pale circular spots on the latero-dorsal surface of the puparia. These are visible immediately after the puparia are formed.

Location

The location of the respiratory horns on the puparium is approximately the same for each species studied. They appear in the conjunctiva between the 5th and 6th visible

segments halfway between the meson and the lateral margin on the dorsal aspect. Several investigators have reported that these structures are located between the 4th and 5th segments; however, this may be due to the fact that only 11 segments are recognizable on the puparium because the pseudocephalon of the larva is completely withdrawn. Actually there are 12 segments in each puparium.

Size

The size of the horns afford a good basis of comparison among these species. They are largest in Ophyra anescens (Linn.) and can easily be seen with the naked eye. They are from 0.5-1.0 mm. in length and approximately 0.25 mm. in width, with blunt ends and directed anteriorly (Plate 1, Fig. 2). They are much smaller in Lucilia sericata (Meig.) and cannot be seen readily without the aid of a microscope. They range in size from 0.188-0.25 mm. in length and approximately 0.141 mm. in width. They are widest at the base and taper anteriorly (Plate 1, Fig. 6). In Musca domestica (Linn.) they resemble those found in Lucilia sericata, but are decidedly smaller and can be seen only with the aid of a microscope. They are approximately 0.188 mm. in length and 0.105 mm. in width.

Effects of Temperature and Humidity

During this investigation it was noted in several instances that the appearance of the respiratory horns on

the puparia was often delayed. Occasionally they did not emerge during the course of pupal development. The reasons for this situation were investigated. After checking the conditions under which the flies were being reared, it was thought that the failure of emergence of these horns might be due to a decrease in relative humidity within the culture jars at the time of pupation, for it was noted that the relative humidity inside the jars during this period was less than 45%. Measures were taken to increase the humidity in the jars, especially among the puparia of *Ophyra anescens* (Wied.).

To increase the humidity in the culture jars, a super saturated solution of calcium hydroxide was poured into a small beaker and placed on the top of the paper towels inside the culture jars. The jars were then covered with a piece of muslin cloth and placed in a constant temperature cabinet with a temperature of 82°F. Later examinations showed that the relative humidity had increased to 58% and the horns had developed on most of the puparia. At this point it was assumed that the delay or failure of the horns to appear was due to a decrease of relative humidity in the culture jars at the time of pupation. To test the validity of this assumption, other cultures of high relative humidity were set up under the same conditions except that they were placed in the temperature cabinets where the failure of the respiratory

horns to develop was first noticed. In these cabinets it was noted that the temperature fluctuated between 75° and 89°F. The results from these cultures were the same as in previous cultures, namely, no horns emerged on the puparia.

At this point it was decided to test the effect of both temperature and humidity on the development of the respiratory horns. This was done by placing a culture jar of first instar larvae containing the super saturated salt solution and one without a salt solution in constant temperature cabinets (82°F.). the results from this test revealed that the puparia in both culture jars developed respiratory horns at approximately the same time.

Thus from this experiment the results indicate that a fluctuating temperature rather than a decrease in relative humidity influenced the development of the respiratory horns on the puparia.

FUNCTION OF THE RESPIRATORY HORNS

It has long been suspected that the respiratory horns are the sole means of pupal respiration in those forms that possess them. To determine the function of these horns in pupal respiration the following tests were made: (1) spiracular plugging, (2) dissection, and (3) dye penetration of the tracheal system.

Spiracular Plugging: In this test three methods of plugging were used: (1) plugging the anterior and posterior spiracles before the respiratory horns had emerged on the puparia, (2) plugging the anterior and posterior spiracles after the respiratory horns had emerged, and (3) plugging the respiratory horns after emergence.

The material used for plugging was Duco household cement. Its toxicity to the developing pupa was tested previous to the experiment by placing a small drop of the cement on parts of the body other than the respiratory parts to be tested. It proved to be non-toxic for the adults emerged and without any noticeable effect.

The results from these tests are shown in the following table:

Table 1. Effects of Spiracular Plugging on Emergence.

Spiracular Horns Undeveloped

No. of puparia	Spiracles plugged	Stage	No. emerging	No. not emerging
16	Ant. and post.	3 hr.	1	15
16 (check)	Cement applied elsewhere	3 hr.	15	1

Spiracular Horns Developed

No. of puparia	Spiracles plugged	Stage	No. emerging	No. not emerging
16	Ant. and post.	44 hr.	15	1
16	Cement applied elsewhere	44 hr.	16	0

Spiracular Horns Developed

No. of puparia	Spiracles plugged	Stage	No. emerging	No. not emerging
16	Respiratory horns	30 hr.	0	16
16	Cement applied elsewhere	30 hr.	15	1

Dissection: Two stages of the puparia were used primarily for dissection: those that had not developed the respiratory horns, and those that had well developed horns. Before dissections were made the puparia were placed on a small drop of glue on a microscope slide and the glue allowed to dry. The slide was then placed in a Petri dish containing 50% alcohol. Then with the aid of a microscope the dissections were made.

In young puparia, that is, those devoid of horns, the

tracheal system of the last instar larvae still functions. The tracheal tubes can be seen through the cuticle of the puparia and are connected to the developing imago. In later puparia, those with well developed horns, the larval tracheal system no longer functions and can be seen pressed against the inner surface of the puparium (Plate 3, Fig. 26). Thus it appears that the respiratory horns are the single means of pupal respiration. From each of the horns a large tracheal trunk leads downward to the prothorax of the developing imago. If the pupa is removed from the puparium after the horns develop, the entire respiratory apparatus can be seen, for the respiratory horns remain attached to the prothorax of the pupa (Plate 1, Figs. 3, 7, and 11).

Dye Penetration: In this method of determining the function of the respiratory horns in respiration, the puparium with well developed respiratory horns is placed in a small Petri dish (1.2 x 2 cm.) and just enough water soluble dye (methylene blue) is poured over it to cover the tips of the respiratory horns. At the end of 2 minutes the puparium is removed from the dye and washed in tap water to remove any dye that remains on the external surface. Next, the puparium is placed in a small drop of glue on a microscope slide and the glue allowed to dry. After drying it is then placed under the microscope and dissected to show the tracheal system.

The results of this method show whether the dye is in the tracheal trunks leading from the respiratory horns or from those connected to the anterior and posterior spiracles. In the earlier puparia, the dye penetrates the entire length of the developing imago, but in later puparia (those with horns), the dye is found only in those tracheal tubes leading from the respiratory horns.

RELATIVE RATE OF RESPIRATION IN PUPAE

After the various experiments had been performed to determine the function of the respiratory horns, it was decided to test the effect of the size and structure of the horns on the respiration rate in the various stages of pupal development. It was suspected that the difference in the size and structure of the horns in the three species studied, Ophyra anescens (Wied.), Lucilia sericata (Meig.), and Musca domestica (Linn.), was due to the difference in the rate of respiration. As a means of testing this hypothesis, a Misco type microrespirometer was used as a device for measuring the rate of respiration. This is a highly sensitive apparatus and since the size of the insects under consideration is small one of this nature is required.

The Microrespirometer

The Misco microrespirometer, manufactured by the Microchemical Specialities Company, Berkeley, California, is a differential respirometer. The essential feature of the respirometer is the presence of two similar chambers, one of which contains the active biological system in its medium, the other an equal volume of the medium alone or medium plus inactivated biological system. The two chambers are connected by a length of capillary tubing carrying a colored index drop of some liquid of relatively low viscosity and vapor pressure. During the period of measurement the chambers and the connecting tubing constitute a closed system. The consumption of gas in one chamber creates a difference in pressure in the chambers. Equilibrium is restored by the movement of the index drop, which can be measured quantitatively in millimeters. For measurement of respiration this arrangement is advantageous, for it permits a cancellation of errors arising from the medium alone, and is independent of external temperature fluctuations, provided that such fluctuations induce the same temperature changes in both chambers.

Careful temperature control is not necessary since changes in external temperature will effect both chambers equally since the chambers are bored into a single aluminum

block. Errors arising from the presence of the medium are unlikely, since liquid-gas volume ratios are the same in the two chambers.

Procedure

Prior to a trial, the assembled respirometer is equilibrated with the environment for a period of 30 minutes. At the end of this time, the capillary portion of the apparatus was removed and the insect to be tested is installed in the chamber on the right. (See photographs). The left chamber is left as a blank. The principle underlying these two chambers is that as oxygen in the right chamber is consumed, the reduced pressure causes the index droplet in the capillary tube to move toward the right thereby giving a reading in millimeters for a given amount of oxygen consumed in a given time. Any carbon dioxide produced during respiration was absorbed in the excess (5 drops) of saturated potassium hydroxide present in the chamber below the specimen. By using capillaries of different bores, different degrees of sensitivity are achieved.

After the chambers are assembled, the index droplet is installed with a finely drawn pipette. Deobase mixed with Sudan black to give color was used for the index droplet. The drop can be placed directly in the calibrated portion of the capillary, or it can be drawn in from the end after lightly touching the opposite end with a hot object.

THE MICRORESPIROMETER

Photographs

A- Top view of the assembled apparatus

B- Top view of the disassembled apparatus

The capillary tube is then put in place and allowed to stand 15 minutes before the first reading. At the end of this time, readings were recorded at 2 fifteen minute intervals.

Six stages of development in the three species of flies were tested with this apparatus. They were the last instar larvae, pre-puparia (when the larvae begin to shorten in length), 3-hour puparia, 28-hour puparia, 46-hour puparia, and 75-hour puparia.

The results of this test are shown in table II.

Table II. Oxygen consumption of several stages of development for three species of Diptera.

Lucilia sericata (Meig.)

<u>Stage of Development</u>	<u>Number of Replicates</u>	<u>Oxygen used, scale readings in mm.</u>		<u>Average amount of oxygen used in mm. 15 minutes.</u>
		<u>Scale differences, 2 successive readings 15 minutes apart</u>		
		<u>1st Period</u>	<u>2nd Period</u>	
A	Four	13.4	13.6	13.5
B	"	4.8	4.4	4.6
C	"	2.6	2.4	2.5
D	"	2.2	1.8	2.0
E	"	2.0	1.6	1.8
F	"	1.8	1.8	1.8

Musca domestica (Linn.)

A	"	4.2	3.8	4.0
B	"	2.8	3.2	3.0
C	"	0.9	0.7	0.8
D	"	0.6	0.8	0.7
E	"	0.7	0.7	0.7
F	"	0.8	1.2	1.0

Ophyra anescens (Wied.)

A	"	2.6	2.4	2.5
B	"	2.1	1.9	2.0
C	"	1.6	1.4	1.5
D	"	1.2	0.8	1.0
E	"	0.6	0.4	0.5
F	"	0.5	0.5	0.5

EXPLANATION OF STAGES

A- Last instar larvae	D- 28-hour puparia
B- Pre-puparia	E- 46-hour puparia
C- 3-hour puparia	F- 75-hour puparia

From Table II it is evident that the difference in the size and structure of the respiratory horns in the three species do not influence the respiration rate. If the hypothesis was true, the rate of respiration in Ophyra anescens (Wied.) would be much greater than the respiration rate in the other two species since the respiratory horns are much larger and all three species are about the same weight. The marked decrease in respiratory rate in passing from the larval to pupal stage reflects the decrease in metabolic rate which is the general rule for all holometabolic insects during this period.

DISCUSSION

It is of interest to compare the structure of the respiratory horns of Orthorrhaphous pupae with those of Cyclorrhaphous pupae. In dealing with such a problem, it is well to remember the following points concerning Orthorrhaphous pupae: (1) the thoracic portion of the pupae is marked by its bulk and its structural differentiation, (2) the orientation of the pupae within its environmental medium must be such as to favor the emergence of the adult fly which is purely a terrestrial organism, and (3) this orientation is facilitated by the fact that the pupae takes no food so that its respiratory requirements are completely independent of a nutrient medium. It is therefore not surprising that the orientation of the respiratory system of

the pupa, especially in a fluid medium, is usually the reverse of the larva, namely, the predominant spiracles in the pupa are the prothoracic pair and not the posterior abdominal pair, as is usual in the case of the larva.

In the pupa of Psychoda alternata (Say), the prothoracic spiracle is fused to the long felt chamber by means of a tracheal extension, enlarged in its lower part to form a spiracular chamber. A long chitimized horn covers the felt chamber and communicates with it by means of a series of irregularly arranged pits. There are two closing mechanisms associated with the respiratory horn. One located in the prothoracic spiracle, consisting of a band of muscle and the other located at the upper end of the tracheal extension in the form of a heavily chitimized lever. The respiratory horn is distinctly prominent.

The pupae of the Cyclorrhaphous Diptera are always enclosed within the last larval skin, sclerotized into a hard and dark case known as a puparium. In the majority of the acalyptrates the pupa has a pair of prothoracic spiracles which are enclosed within the puparium and are known as internal spiracles. Each internal spiracle is composed of a felt chamber covered with numerous papillae. In the majority of the calyptrates the felt chamber of each prothoracic spiracle of the pupae, is drawn out into a sclerotized horn which projects through a small circular aperture in the wall of the puparium on the 1st abdominal segment.

The differences between the respiratory horns of Cyclorrhaphous pupae and Urghorrhaphous pupae may be summed up as follows: (1) the felt chamber of Cyclorrhaphous pupae is an extension of the prothoracic spiracle and is connected directly to the spiracle, (2) there is no tracheal extension leading to a spiracular chamber, (3) there is no closing mechanism in the trachea, and (4) the respiratory horns in general are much smaller and located on the first abdominal segment of the puparium.

De Meijere (1902) proposes the term pitted stigma (Tupfelstigma) for the respiratory horns of dipterous pupae. This does not appear to be a legitimate use of the term stigma. In Psychoda alternata (Say), the respiratory horn is not the site of an ectodermal invagination giving rise to a trachea, nor does it have an open communication with the atmosphere. The term therefore seems unsuitable. In this investigation the term "respiratory horns" is used to describe this organ because it has no precise meaning and does not define a particular type of structural entity.

SUMMARY

1. The pupae of Cyclorrhaphous Diptera are always enclosed within the last larval skin, the puparium. The pre-pupal (pre-puparial) stage is defined as that stage when the larvae ceases to feed, becomes quiescent, and

the prothoracic spiracles of the larva project cephaled. The term pupariation is used to describe this process.

2. As a representative of Orthorrhaphous pupae, the respiratory horns of the pupa of *Psychoda alternata* (Say) are described. Within each tubular horn lies the felt chamber, which meets the horn wall at a number of circular regions, the pits, and communicates, through the annulated base of the horn, with the tracheal extension. This is continuous, at the lower end, with the spiracular chamber, from which a trachea leads to the prothoracic spiracle of the developing imago. At the upper end of the tracheal extension lies the closing mechanism consisting of a highly chitinized lever attached to a band of muscle. Another closing mechanism is found in the prothoracic spiracle of the developing imago.

3. In the pupae of Cyclorrhaphous Diptera there are a pair of prothoracic spiracles enclosed within the puparium and are known as internal spiracles. Each prothoracic spiracle is composed of a more or less protruding felt chamber covered with spiracular papillae. In the majority of the calyptrates the felt chamber of the prothoracic spiracle is drawn out into a well developed sclerotized horn covered with spiracular papillae and protrude through a small circular aperture in the wall of the puparium.

4. An account is given of the development of the respiratory horns of *Ophyra anescens* (Wied.). The horn

apparently arises as an outgrowth from the prothoracic imaginal disk of the developing imago. Fully developed horns at first lie parallel to the length of the body, whereas, after protrusion they are perpendicular to the body. About 18 hours after pupariation the pupa contracts, which is apparently due to evagination of the imaginal disks in the head. This contraction of the pupae brings the tips of the horns backward so that they lie directly beneath the two fragile spots in the wall of the puparium. Backward and forward movements of the pupa forces the horns through the two fragile spots in the wall of the puparium. The horns are completely developed and extruding from the puparium 26-30 hours after pupariation.

5. The results from the study of the respiratory horns found in the pupae of Ophyra anescens (Wied.), Musca domestica (Linn.) and Lucilia sericata (Meig.), may be summarized as follows: (1) the time of emergence of the respiratory horns is somewhat constant in the puparia of O. anescens and M. domestica, but quite variable in L. sericata, (2) the location of the horns is the same for each species, namely, the conjunctiva between the fifth and sixth visible segments, on the dorsal side, (3) there is considerable difference in the size of the horns, being largest in O. anescens, intermediate in L. sericata and smallest in M. domestica, (4) the results obtained from spiracular plugging of the tracheal system, dissections, and dye penetration of

the tracheal system indicate that the respiratory horns are the sole means of pupal respiration, (5) the size and structure of the respiratory horns has no influence on the respiration rate in the developing imago, for results show that although the respiratory horns found in the puparia of O. anescens are much larger than those found in the other two species, the respiration rate is less, and (6) it appears that fluctuating temperature rather than humidity effects the development of the respiratory horns, especially in O. anescens.

6. The structure of the horns found in Cyclorrhaphous pupae differ from those found in Orghorrhaphous pupae in that, (1) the felt chamber of Cyclorrhaphous pupa is an extension of the prothoracic spiracle and is connected directly to the spiracle, (2) there is no tracheal extension leading to a spiracular chamber, (3) there is no closing mechanism in the trachea, and (4) the respiratory horns in general are much smaller and located on the 1st abdominal segment of the puparium.

7. The use of the term "respiratory horns" for these organs is discussed.

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ABBREVIATIONS USED IN THE FIGURES

A.C.	-----Anterior Chamber
A.R.	-----Annulated Ring
A.T.	-----Anterior Tracheal Tube
B.PH.	-----Buccopharyngeal Armature
C.S.	-----Circular Spots
E.S.	-----External Stigmatic Scar
F.C.	-----Felt Chamber
I.S.	-----Internal Spiracle
L.	-----Closing Lever
MEM.	-----Membrane
P.	-----Pits
R.H.	-----Respiratory Horns
SP.C.	-----Spiracular Chamber
S.ST.	-----Scar Strand
T.	-----Trachea
T.E.	-----Tracheal Extension

EXPLANATION OF PLATES

Plate I

- Fig. 1. Dorsal View of a 4-hour puparium showing the circular apertures (C.S.). Ophyra anescens (Wied.) X 20.
- Fig. 2. Dorsal view of a 30-hour puparium showing respiratory horn. O. anescens. X 20.
- Fig. 3. Lateral view of a 30-hour pupa dissected from puparium. O. anescens. X 27.
- Fig. 4. Lateral view of the respiratory horn. O. anescens. X 120.
- Fig. 5. Dorsal view of a 4-hour puparium of Lucilia sericata (Meig.) X 18.
- Fig. 6. Dorsal view of a 30-hour puparium with respiratory horns. L. sericata. X 18.
- Fig. 7. Ventral view of a 30-hour pupa dissected from puparium. L. sericata. X 20.
- Fig. 8. Lateral view of respiratory horn. L. sericata. X 140.
- Fig. 9. Dorsal view of a 4-hour puparium of Musca domestica. X 20.
- Fig. 10. Dorsal view of a 30-hour puparium of M. domestica. X 20.
- Fig. 11. Lateral view of a 30-hour pupa dissected from puparium. M. domestica. X 22.
- Fig. 12. Lateral view of respiratory horn of M. domestica. X 160.

PLATE 1

Plate II

- Fig. 13. Culicidae, Aedes aegypti (L.). A lateral view of a yellow fever mosquito pupa showing the respiratory horn. X 30.
- Fig. 14. Enlarged view of the respiratory horn of A. aegypti pupa. X 82.
- Fig. 15. Ventral view of a crane fly pupa showing respiratory horns. Tipulidae, Tipula sp. X 8.
- Fig. 16. Enlarged view of the respiratory horn of Tipula sp. X 20.
- Fig. 17. Ventral view of Psychoda pupa. Psychoda alternata Say (Psychodidae) X 8.
- Fig. 18. Enlarged view of respiratory horn of P. alternata. X 29.
- Fig. 19. Dorsal view of a salt marsh fly pupa Ephydriidae, Brachydentera argenata Loew. X 15.
- Fig. 20. Enlarged view of the respiratory horn of B. argenata. X 60.
- Fig. 21. Dorsal view of the puparium of Criocera claripennis (Tachinidae). X 12.
- Fig. 22. Enlarged view of the respiratory horn of C. claripennis. X 175.
- Fig. 23. Lateral view of the puparium of Meredon equestris F. (Syrphidae). X 9.
- Fig. 24. Enlarged view of the respiratory horn of M. equestris. X 55.

PLATE 2

Plate III
Ophyra anescens (Wied.) Anthomyidae

- Fig. 25. Dorsal view of a 10-hour pupa within the puparium. Note position of the respiratory horns (*Ophyra anescens* Wied.) X 20.
- Fig. 26. Enlarged view of the anterior portion of a 10-hour pupa. X 45.
- Fig. 27. Dorsal view of a 28-hour pupa immediately before emergence of the respiratory horns. Note position of the horns. X 20.
- Fig. 28. Dorsal view of a 30-hour pupa showing the respiratory horns immediately after emergence from puparium. X 20.
- Fig. 29. Enlarged view of Fig. 27. X 45.
- Fig. 30. Enlarged view of Fig. 28. X 45.
- Fig. 31. Enlarged ventral view of Fig. 28. X 45.
- Fig. 32. Enlarged view of the anterior portion of a 90-hour pupa. X 45.
- Fig. 33. Dorsal view of a puparium immediately after emergence of adult. Note horns attached to wall of puparium. X 20.
- Fig. 34. Enlarged ventral view of a puparium after emergence of adult. X 45.

PLATE 3

AUTOBIOGRAPHY

I, Leon Ramon Roddy, was born in Whitewright, Texas, June 16, 1921. I attended the public schools of that state, graduating from Fred Douglas High School in May 1939. My undergraduate training was obtained from Texas College, Tyler, Texas, from which I received the Bachelor of Arts degree in Biology in May 1943. Upon graduation, I entered the United States Army, serving with this service until December 1945. In the fall of 1947, I entered The University of Michigan and received the degree of Master of Science in Zoology in 1949. In the summer of 1949, I entered The Ohio State University. In the fall of the same year, I accepted a teaching position at Tillotson College, Austin, Texas. In December 1951, I resigned from my teaching position to return to The Ohio State University and complete requirements for the degree Doctor of Philosophy in entomology. In the summer of 1952, I was appointed a Research Fellow in The Ohio State University Research Foundation on a project sponsored by the United States National Institute of Health, which position I held while completing the requirements for the degree Doctor of Philosophy.