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The Posterior Spiracles of *Hypoderma Lineatum* de Villers (Diptera: Hypodermatidae) Larvae : Their Morphology and Development

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THE POSTERIOR SPIRACLES OF HYPODERMA LINEATUM DE VIL-
LERS (DIPTERA: HYPODERMATIDAE) LARVAE
THEIR MORPHOLOGY AND DEVELOPMENT

By
Neal O. Morgan

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science at South Dakota
State College of Agriculture
and Mechanic Arts

December 1957

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THE POSTERIOR SPIRACLES OF HYPODERMA LINEATUM LARVAE
THEIR MORPHOLOGY AND DEVELOPMENT

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser //

Head of the Major Department

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NOM

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INTRODUCTION

During the late nineteenth and early twentieth centuries morphologists assembled insect groups according to structural characters with the morphology of the spiracle being one of the primary criteria. Krancher (1881) listed five spiracular types based upon variations of the stigmal apertures, whereas De Meijere (1895) used only two categories - opened and closed stigmas.

There is little published material available concerning cattle grub spiracles other than descriptions of the external surface of the stigmal plate. The representative species of Oestroid larvae studied by the early European morphologists was the northern cattle grub, Hypoderma bovis (L.), and their published conclusions of its spiracular exploration were meager. Major components of the spiracle such as common atrium, stigmal plate and the hollow plates connecting these two structures were described to the extent necessary to establish differences between larval groups. Contemporary morphologists have concerned themselves with the common cattle grub, Hypoderma lineatum (De Vill.), as well as with H. bovis, but have added very little to the previous information.

Two questions of interest are: how do the posterior spiracles of H. lineatum larvae differ from those of other parasitic and non-parasitic dipterous larvae; and what char-

acteristics relate the common cattle grub spiracles to those of other dipterous larvae. This thesis is an attempt to provide some information that may help to answer these questions through observations of prepared microscope slides and whole mounts of H. lineatum. Comparisons with published descriptions of other dipterous larval spiracles are by reference in the Review of Literature.

REVIEW OF LITERATURE

Historical

The greatest advances in the morphological studies of dipterous larvae were made during the late nineteenth and early twentieth centuries. Although the contributions of contemporary morphologists have been expanding and improving the concepts of morphology as described by their predecessors, the number of contributors and the volume of their published material have decreased noticeably during the past forty years. Among the few who have recent publications concerning the respiratory systems of dipterous larvae are Snodgrass (1924), Townsend (1935) and Peterson (1951).

Soon after the turn of the nineteenth century, many insect morphologists concentrated their studies on the structures of the respiratory systems, especially those structures which permit passage of gasses. Dufour, in 1825, discussed the role of spiracles of Hippoboscidae, and in 1849, published on the methods of respiration of aquatic insects.

Several other morphologists published accounts of their studies of specific stigmata, and Krancher (1881) summarized much of this information and classified the stigmal forms. His classification was based upon three stigmal variables: the presence of lips, the number of stigmata,

and the shape of the lips. According to his analysis, Diptera lacked lips and the plates consisted of series of minute platelets, the internal openings of which unite to form common atria.

De Meijere (1895) proposed a classification for spiracles of dipterous larvae. His classification was much more specific than that of Krancher (1881) for he used only two groupings - opened and closed stigma, and listed variations for many genera within each major group. Hypoderma was placed with those having a closed stigma, many small "buds" and a branched "felt-chamber", and the stigmal scar lying outside the stigma.

The number of larval instars occurring in H. bovis and H. lineatum was considered by many morphologists to be four or five for several years following the turn of the century. Riley (1892) described a newly hatched H. lineatum and later claimed that there was sufficient difference between the newly hatched and the esophageal stages to classify them as two distinct instars. With the common acceptance of two instars occurring in the backs of animals he decided that there must be a total of four larval instars. Laake (1921) described H. lineatum as having another instar, making the total five. The theories of the authors were tested and supported by numerous morphologists. In 1935, Knippling presented his conclusions concerning the

number of larval instars. He described three distinct stages - the newly hatched and esophageal, the post-esophageal or penultimate and the ultimate. His writings have been accepted by the author for this thesis.

A general nomenclature of spiracular structures was suggested for H. bovis by De Meijere (1895) and was generally accepted thereafter. Townsend (1935) and a few other authors have added technical descriptions; but the original terms have been altered very little.

Since the common cattle grub became a major pest in North America, entomologists have published accounts of its life cycle and general descriptions of its spiracular plates. Among them are Hadwen (1919), Bishopp et al. (1926) and Scharff (1950).

Description of Dipterous Larval Spiracles

Many cyclorrhaphous larvae have posterior spiracles bearing a pair of sclerotized plates, the distinct structures of which are commonly used as distinguishing characteristics. A general rule applicable to Cyclorrhapha is that the stigmal plates bear two or three apertures per plate, dependent upon the larval stage of development. The apertures are either pyriform, straight slits, or sinuous slits with each mounting inwardly projecting sclerotized rods over an internal system of branched trabeculae, the

combination of which forms an effective filter (Imms, 1948). A non-parasitic representative of the group supporting the rule is Rhagoletis pomonella which has three straight slits lined with the rods in the second and third larval instars, and only two of such apertures in the first (Snodgrass, 1924). Parasitic representatives include the ectoparasite Stomoxys calcitrans which has three semi-sinuous slits formed around a central scar, and the endoparasite Guterbra uniculti which has three sinuous-cribriform platelets forming each plate (Peterson, 1951).

Hypodermatidae (Entomological Society of America, 1955) is an exception to the rule, and H. lineatum is a representative species. The pattern of its apertures is not as simple as is that of the earlier mentioned representatives. The pattern shows loosely scattered buds contained within a thin peritreme in the second instar larva of H. lineatum. The third instar is irregular with the stigmal plate giving the appearance of being sectored by radiating grooves which originate around the stigmal scar (Pl. I, Fig. 1). Each sector may contain eight to fifty buds. Other larvae having these characteristics are H. bovis, Oedomagena tarandi, and Oestrus ovis, and all of these are endoparasites. There is a minor difference which separates even members of the same genus, for the stigmal surface of H. lineatum is flat whereby that of H.

bovis is concave (Peterson, 1951). Other marked differences between the stigmal plates of the two species would include coloration, size and number of buds, and the number and shape of the surface grooves.

Townsend (1935) referred to the form of spiracular openings of Hypoderma as "reticulate", for the mass of apertures tended to form a network, whereas the larvae with stigmal plates bearing three "opened" apertures were referred to as "blocklike". De Meijere (1895) referred to buds as having semipermeable membranes forming the external limit of the bud and to the ramifications of the felt-chamber (atrium) as "hollow plates" (hohlen Platten). Keilen (1944) referred to the ocdysial tube, after the scar tissues formed at the terminals, as the "scar filament", and usually it was represented by a withered filament.

A comparison of spiracular profiles tends to group the ectoparasitic with the non-parasitic dipterous larvae, for usually they have at most three tubules connecting the apertures with the atrium and the tracheal trunk. The endoparasitic Oestroids have eight to twelve hollow plates connecting the buds to the atrium (De Meijere, 1895).

MATERIALS AND METHODS

All specimens were obtained within the state of South Dakota. First instar larvae were obtained from beef esophagi which were collected at the John Morrell Company, Sioux Falls. These animals were presumed to have been raised in the upper midwest. An attempt was made to obtain newly hatched larvae by a method described by Weintraub (1956); however due to uncontrollable microclimatic factors, the attempt failed. The first instar larval specimens used were esophageal.

The second and third instar larvae were extracted from hypodermal cysts in the backs of beef animals.

All specimens were identified as H. lineatum and age was determined by examination of stigmatal arrangements and/or mouth-hooks, as described by Bishopp, et al. (1926) and Knipling (1935). All abnormal specimens (deformed) and those injured during extraction were discarded. The remaining specimens were killed and fixed in Petrunkevitch's cupric-paranitrophenol and 20 percent formalin.¹ The specimens were bisected transversely, and the posterior

¹Petrunkevitch's cupric-paranitrophenol and 20 percent formalin formula:
 60 percent alcohol 100 cc Cupric nitrate cp 2 gm
 Nitric acid cp 3 cc Paranitrophenol
 Ether 5 cc crystals cp 5 gm
 The 20 percent formalin was added just before the fixative was used.

portions bearing the spiracles were retained for further sectioning. Serial sections, cross and longitudinal, were made by a rotary microtome. The thickness of sections varied from seven to thirty-five microns, depending upon the hardness of the spiracular plate. Soaking the specimens in five percent formalin for six to twenty-four hours prior to final dehydration softened the sclerotization considerably, permitting thinner sections to be made. The sectioned material was stained with Harris' hemotoxylin and counterstained with eosin.

Whole mounts were prepared, showing entire spiracles and portions of the connecting tracheal trunks. The mounts were carved from specimens embedded in paraffin, and were cleared in xylol prior to mounting on slides. Only the first instar larval whole mounts were stained and counterstained.

The prepared specimens were examined under magnifications varying from 30x to 970x (with immersion oil). The drawings, unless otherwise stated, were produced with the aid of camera lucida. The measurements of sectioned material were taken from a micrometer, and those of whole mounts were taken with a transfer caliper and read from a metric scale.

Technical Problems

When making micro-sections of first and second instar larvae, shattering of the internal structures was frequent with the atria disintegrating, but the stigmal plates remained intact. The use of cedarwood oil, shortening of the xylol series, soaking the embedded specimens in water, and soaking in 4 percent formalin were methods used to improve the quality of material for sectioning. Specimens soaked in 4 percent formalin for 24 hours or more shattered least upon sectioning of all three larval stages. Excessive soaking in formalin caused the internal structures to become very soft and were crumpled rather than sectioned by the microtome blade. The specimens fixed with paranitrophenol and stored in 70 percent alcohol were transferred from the storing alcohol to 4 percent formalin and remained there for 6 to 24 hours. The specimens thus treated prior to final dehydration sectioned without shattering.

The use of the posterior one-half of the larva for sectioning evolved from tests conducted with whole and portioned larvae. The two categories were given the same treatment prior to sectioning with one exception; the whole larvae were either pierced or incised to enhance infiltration. The whole larvae continually failed to become completely infiltrated, whereas those that were only posterior portions were completely infiltrated and sectioned easily.

The timing requirements for the stains used depended upon the thickness of the sectioned material. The 12 micron sections indicated the best definition and differentiation when stained for 45 seconds and counterstained for 8 seconds. As a comparison, the 25 micron sections required staining for two minutes and counterstaining for 8 seconds.

The felt lining and the membranous bud-discs did not take either the hematoxylin or the eosin stains, rather they remained pale yellow and orange, respectively.

DATA

One hundred and fifty-one specimens were prepared and examined for this thesis. Of these, fifty-two were first, sixty-one were second and thirty-eight were third instar larvae. All measurements, unless otherwise indicated, are in microns.

The Exterior of the Spiracle

Of the material examined the stigmal plate of the first instar larva is composed of a single bud. All that is externally visible is the membranous covering of the bud and a dark, sclerotized peritreme. The average diameter of the buds is 14.6 microns. The peritreme extends from the external surface of the bud, inward, to midway along the atrium, and expands laterally to three or four times its thickness at the external surface.

The stigmal plate of the second instar larva is composed of several buds which are loosely grouped and are surrounded by a thin, brown sclerotized peritreme (Pl. II, Fig. 1). The peritreme extends from the surface, inward to beyond the base of the buds to a constricted locus of the atrium, an area to be discussed later. The measurements of the stigmal plates are variable with the average length and width being 238.8 and 152.9 microns respectively, and

PLATE I

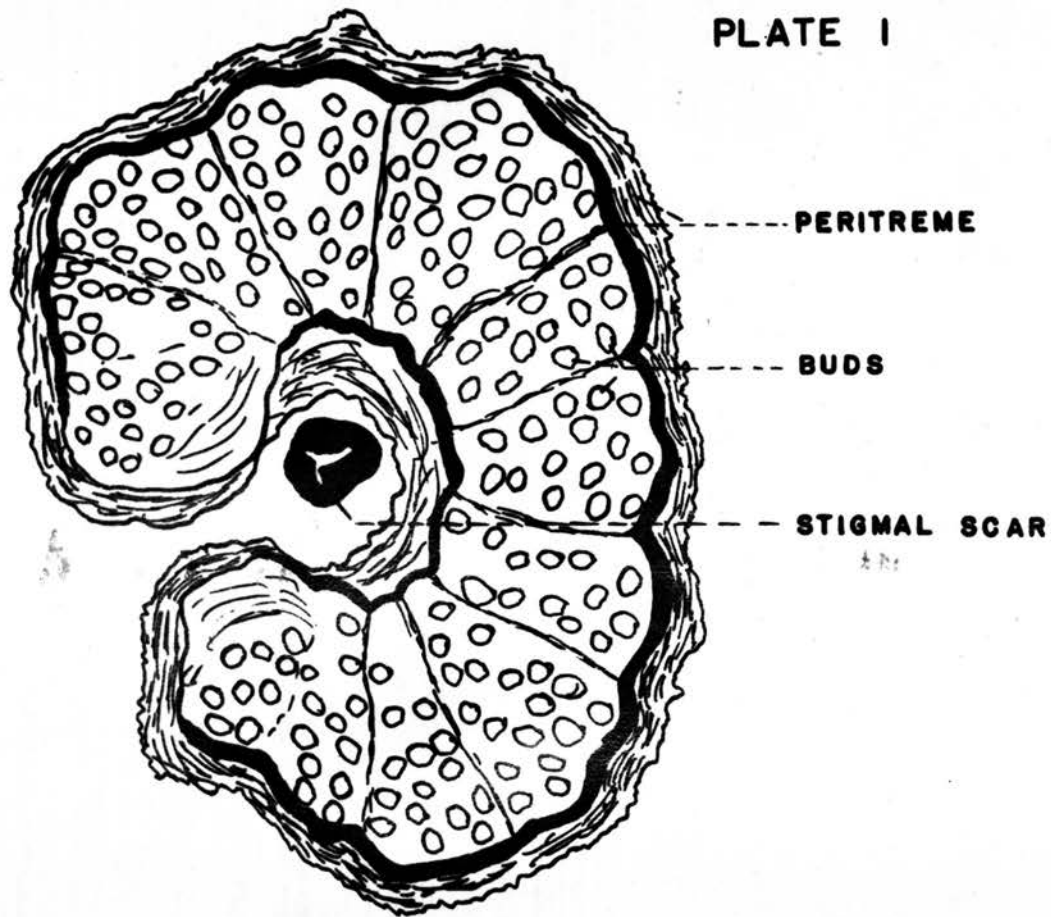


Fig. 1. External view of a stigmal plate and scar of third instar larva. X30.

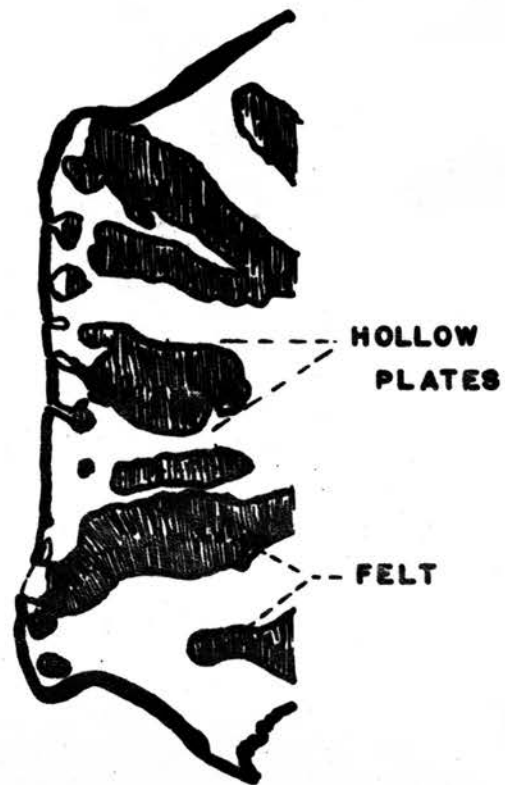


Fig. 2. Section through a stigmal plate and hollow plates. X30.

PLATE II

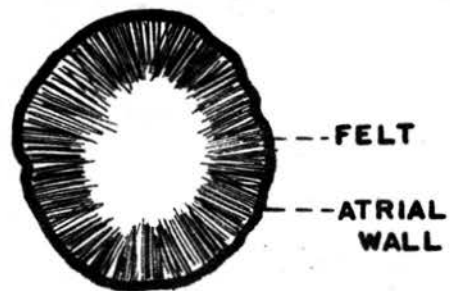
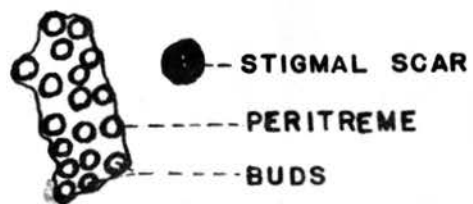


Fig. 1. Second stage stigmal plate and scar. X430.

Fig. 2. Section through hollow plates and scar-plug. X430.

Fig. 3. Section through atrium. X430.

the diameters of the buds average 27.9 microns. Located median to the stigmal plate is a circular, sclerotized stigmal scar, the average diameter of which is 34.5 microns.

The stigmal plate of the third instar larva is surrounded by a heavy, dark brown, sclerotized peritreme which extends, in depth, from the surface of the stigmal plate through the posterior one-third of the hollow plate area. The measurements of the stigmal plates are variable with the average length and width being 960.9 and 830.5 microns respectively, and the diameters of the buds average 37.6 microns. The surface of the plate is generally level. The sclerotization which separates the buds appears as various hues of yellow and brown. Only a slight groove separates the peritreme from the stigmal scar, the focal point for radiating lines that transect the sclerotized plate. These lines separate groups of 8 to 50 buds and indicate possible internal spiracular divisions. The scar, all but surrounded by the stigmal plate, is singly indented, and the diameter measurements average 253.3 microns. (Pl. I, Fig. 1)

The atrium of the first instar larva is a continuation of the main tracheal trunk with no obvious difference in diameter except for that portion which joins the peritreme. The average length and diameter is 91.0 and 28.4 microns respectively. (For more inclusive measurements, see Table 1.)

The shape of the second instar larval atrium is similar to that of the first, for there is no lateral expansion beyond the tracheal diameter; however, a constricted area encompasses the atrium in the region of the peritremal base. The constricted area is reduced to approximately one-half the remaining atrial diameter, the average measurement of which is 141.0 microns. The average length of the atria is 399.0 microns.

The ultimate larval spiracle is, again, similar to that of the first instar larva, but the diameter is noticeably larger than that of the trachea. The constricted area is present and extends from the base-line of the buds to one-third the length of the atrium, and reduces the diameter of the atrium by one-third of the remainder. The diameter measurements average 431.0 microns and the length 644.0 microns.

The Interior of the Spiracle

Longitudinal sections of a first instar larval stigma reveal an extremely heavy, sclerotized peritreme which appears to form the wall of the posterior half of the atrium as well as the bud wall (Pl. III, Fig. 1). The interior of the atrium, hereafter referred to as the felt-chamber, is slightly constricted at the junction with the peritreme. The dendricular processes, or felt, which line the felt-chamber are sparse in the posterior half, form a thick

PLATE III

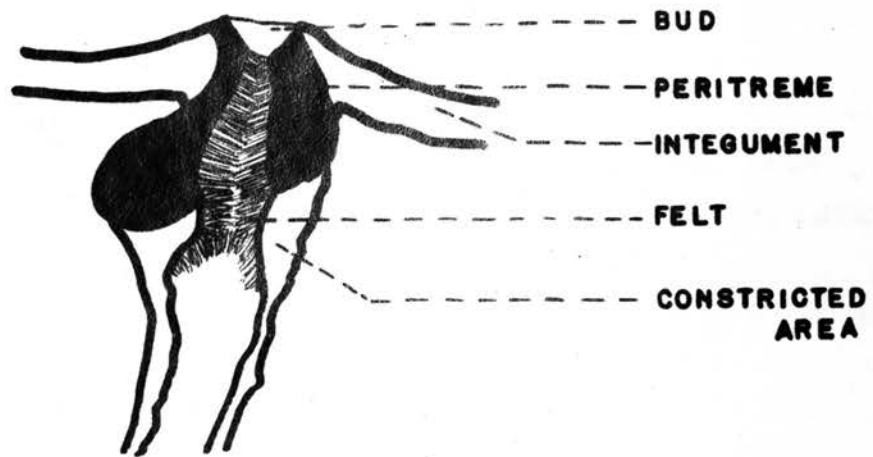


Fig. 1. Section of a first instar larval spiracle. X430.

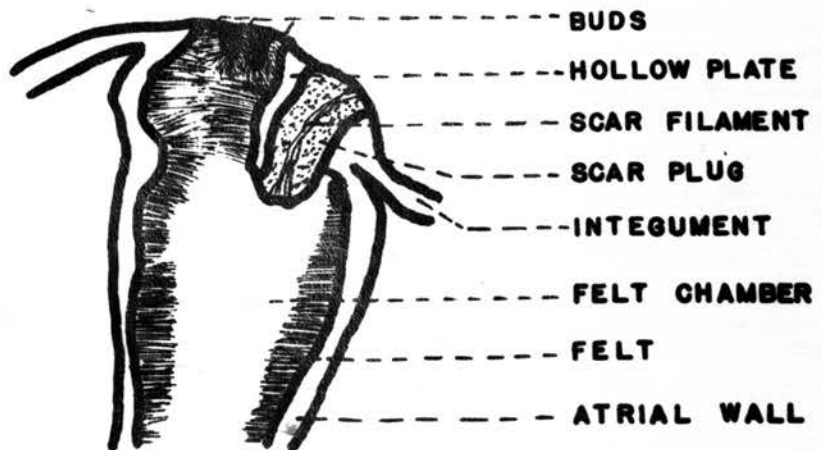


Fig. 2. Section of a second instar larval spiracle with hollow plates and scar-plug. X30.

PLATE IV

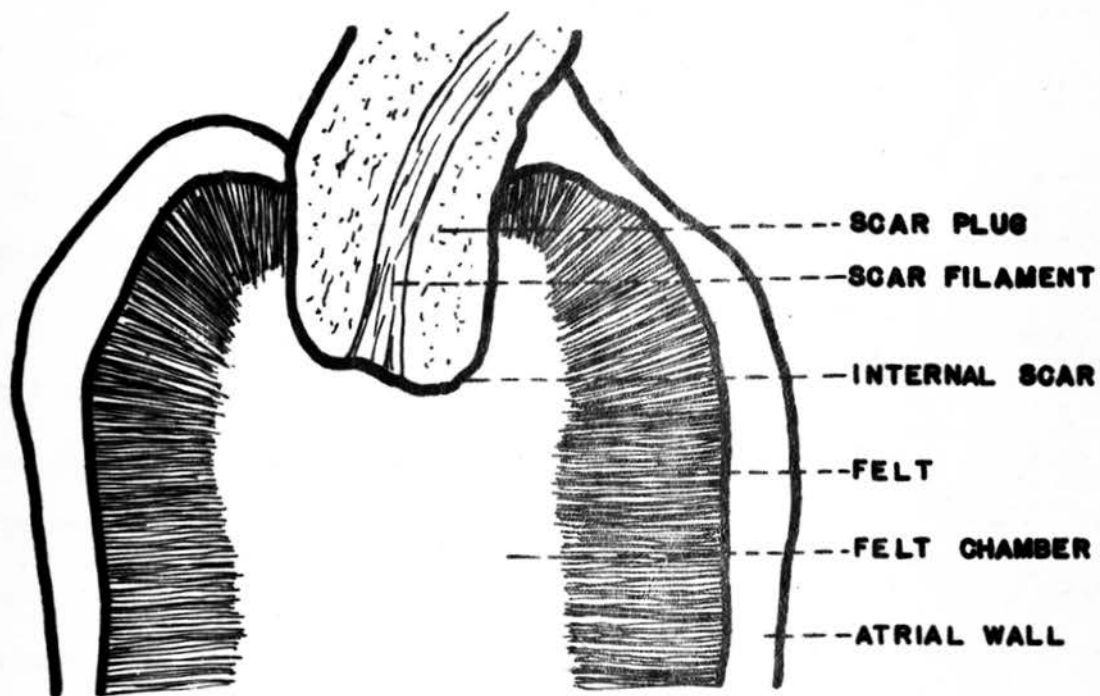


Fig. 1. Section through posterior one-half of felt-chamber of a second instar larva. X970.

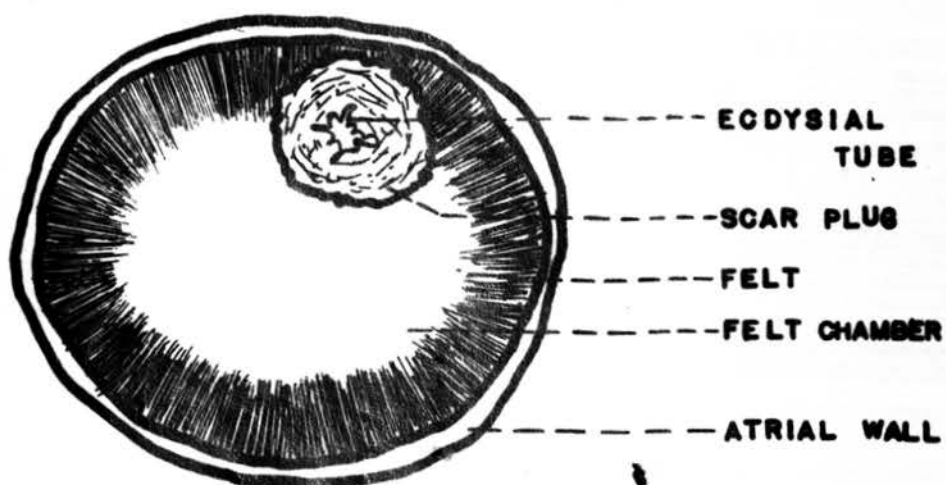


Fig. 2. Cross section through posterior one-half of felt-chamber of second instar larva. X970.

ring at the constriction, remain thick along the constricted area and diminish towards the anterior end of the felt-chamber. The average length or thickness of felt is 11.2 microns.

Sections of a second instar larval stigma reveal four major ramifications of the posterior one-third of the felt-chamber (Pl. II, Fig. 2). Each ramification is further ramified for each bud; hence, a tube is formed from the buds, through the various ramifications to the non-ramified felt-chamber and then to the trachea. The felt within the ramifications all but fills the buds, leaving an open space anterior to the cover, equivalent to three-fourths the diameter of the bud. The average length or thickness of felt within the felt-chamber is 53.8 microns. In the ramified areas the felt meets in the center of the chamber, whereas the non-ramified chamber is open in the center, a space equal to two and one-half times the thickness of the felt. The internal stigmal scar is recessed in a pouch on the posteromedial wall of the non-ramified felt-chamber (Pl. III, Fig. 2 and Pl. IV, Fig. 1 and 2). The pouch appears to be a plug which extends into the felt-chamber a distance equal to one and one-half times the length of the felt. From the internal scar, extending posteriorly through the center of the pouch, passes a thin, bent tubule which terminates at the external

stigmatal scar.

Sections of a third instar larval stigma reveal ten major ramifications of the posterior one-third of the felt-chamber, each of which is further ramified two or three times with each minor ramification connecting several buds with the organized felt-chamber. Cross sections through the ramified felt-chamber reveal a thin, central tube within a thick sclerotized mass. The atrial ramifications appear to radiate from the perimeter of the mass. The average length or thickness of felt is 58.9 microns. As observed in the second instar larva, felt fills the atrial ramifications and one-fourth of each bud, with the open spaces appearing anterior to the bud-discs and in the center of the non-ramified felt-chamber.

A minute, irregular aperture is observed in the center of each bud-disc of the second and third instar larvae. The apertures are best observed in longitudinal sections of buds which, also, reveal the extent of the discs as forming the lining of the posterior three-fourths of each bud (Pl. V, Fig. 1 and 2). The composition of the discs appears similar to that of the dorsal spines.

PLATE V

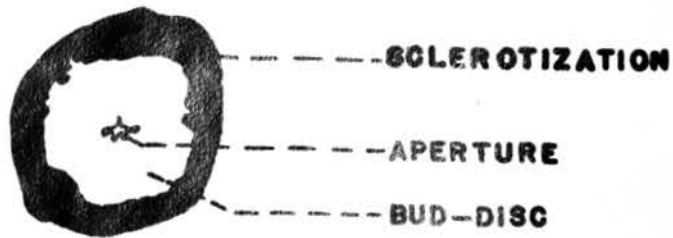


Fig. 1. External view of a stigmal bud of a third instar larval spiracle. X970.

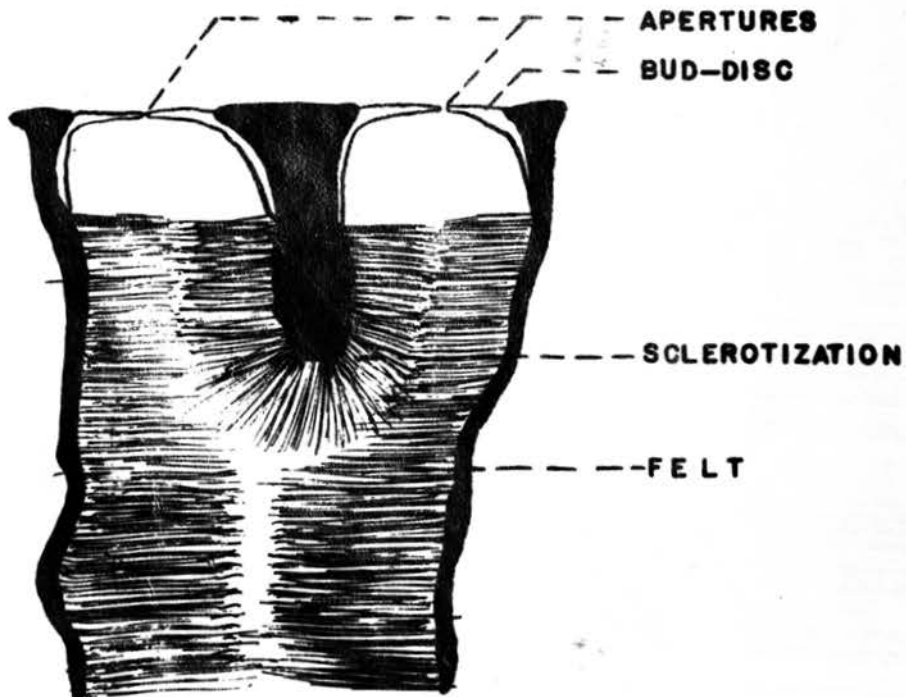


Fig. 2. Longitudinal section of hollow plate and buds of a third instar larval spiracle. X970.

Table 1. Comparative sizes of spiracular structures as measured in microns. Twenty to thirty specimens were measured for each subject.

Subject	First instar			Second instar			Third instar		
	Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.
Buds (diameter)	11.4	18.0	14.6	22.8	32.3	27.9	28.5	45.6	37.6
Hollow plates									
Length	26.6	41.8	35.9	114.0	323.0	215.3	285.0	351.5	306.0
Width	22.8	36.1	30.4	47.5	66.5	60.2	57.0	66.5	63.6
Atrium									
Length	55.1	154.9	91.0	256.5	475.0	399.0	532.0	712.0	644.0
Diameter	20.9	35.2	28.4	114.0	237.5	141.0	408.5	446.5	431.0
Peritreme									
Length	34.2	38.0	36.1	228.0	257.5	238.8	849.3	1059.3	960.9
Width	32.3	33.3	32.8	136.8	178.6	152.9	702.1	940.5	830.5
Felt (length)	9.5	12.4	11.2	38.0	57.0	53.8	47.5	66.5	58.9

DISCUSSION

The findings of this study reveal several previously unmentioned or little known loci of the spiracle, as well as confirm many previously described structures.

The Exterior of the Spiracle

Bishopp et al. (1926) described the second instar larva as having 12 to 30 buds per stigmal plate. This study confirms, in part, that description with the finding of 12 to 35 buds being more inclusive, and adds that over 200 buds have been observed of third instar larval stigmal plates. Each bud is comprised of a sclerotized disc with a centrally located aperture, a space beneath the disc, and a felt-lined tubule. The apertures are irregular openings in the center of the discs, and do not appear as slits as was suggested by morphologists of the early twentieth century.

The external stigmal scar, centrally located in the stigmal plate of the third instar larva of H. lineatum, is located medial to the group of buds of the second instar larva, a position similar to the location of the scar of the tri-apertural stigmal plates as described by Keilin (1944).

This study indicates that the third instar larva of H. lineatum usually has ten hollow plates or major ramifi-

cations of the felt-chamber, each of which may be further ramified 2 to 25 times. Each minor ramification terminates with the connection to one to four buds.

As is indicated in Tables 1 and 2, the spiracular measurements increase more rapidly, proportionate to body growth, during the first ecdysis than during the second.

Keilin (1944) described three spiracular types according to the location of the external ecdysial opening or scar relative to the stigmal plate. In type I the ecdysial opening becomes the spiracular aperture in successive stages. In type II the ecdysial opening is obliterated and a stigmal plate with radial clefts, or spiracular papillae with clefts, is formed around the external stigmal scar. Type III is common among the majority of *Cyclorrhapha* in which the external stigmal scar is present but not surrounded by the stigmal plate. Hypodermatid larvae are included in type III, although the stigmal plates appear to indicate otherwise. In these studies it is found that hypodermatid stigmal plates of ultimate larvae are "C" shaped with the two points of the letter often being separated by merely a double thickness of compressed peritreme. In *H. lineatum* the separation is more pronounced in the external view, but the difference is negligible. The line of separation is, also, noticeable below the stigmal plate in the constricted portion of the

Table 2. Comparative measurements of larval bodies (in millimeters). Twenty to thirty specimens were measured for each subject.

Subject	First instar			Second instar			Third instar		
	Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.
Body									
Length	4.5	14.7	10.7	11.0	14.9	13.1	15.0	22.2	19.4
Width	0.7	2.6	1.8	4.5	6.2	5.3	5.1	9.7	9.7

atrium, and ceases at the edge of the non-constricted atrium.

The Interior of the Spiracle

As previously mentioned, several buds may be connected to one minor ramification, or branch of a hollow plate. A partition, between two adjacent buds of one branch, may be a sclerotized cone projecting from the stigmal surface for twice the width of a bud, or a wall of the hollow plate with the outer edge of the anterior base becoming a part of the non-ramified felt-chamber. The remaining portion of the base is similar to the cone and extends towards the center of the felt-chamber. From the space (lacuna) beneath a bud-disc through the hollow plate the felt is so thick that only a trace of a clear channel is detected in even the wider plates.

The area of the hollow plates corresponds to the external area of the spiracle that is constricted. The non-constricted area is the non-ramified felt-chamber. The constricted condition appears to result from the infolding of the felt-chamber in forming the hollow plates. The infoldings are radial and utilize the entire posterior portion of the felt-chamber except the location of the internal ecdysial scar. The hollow plates appear to enfold the ecdysial tube; however, as mentioned previously, a

distinct space from the stigmal surface to the anterior wall of the non-ramified felt-chamber separates the hollow plates and the two points of the "C".

Reference to the ecdysial tube, by Keilin (1944), as being a "scar filament" connecting the internal and external stigmal scars is confirmed by this study as being a valid description. In this study it is found that in H. lineatum, the scar filament appears to be closely surrounded by hollow plates, and only the medial separation of the spiracle interrupts the enclosure. The internal scars, of both the second and third instar larvae, extend into the non-ramified felt-chamber a short distance, appearing as plugs. The scar filaments extend anteriorly through the center of the plug to its rounded surface.

The felt lining of the anterior portion of the atrium is as thick as is that of the hollow plates; however, the union of the atrium with the tracheal trunk is felt-free. Immediately posterior to the union the thickness of the felt is minute and increases in thickness towards the posterior of the spiracle. The maximum thickness of felt, usually, is lining the posterior half of the non-ramified felt-chamber.

Spiracular Development Through Larval Growth

It appears that no ecdysis occurs until after H. lineatum reaches the back of the host (Knipling, 1935), after

which two moults occur. During the period, from the entrance of the larva into the host until the larva reaches the back of the host, the larva grows, the flexible integument expands and the posterior spiracles remain approximately the same. The slight increase in the diameter of the stigmal plate is due to sclerotization of brownish material around the yellowish bud-disc.

Keilin (1944) declared that the spiracles of the second and third instar larvae never appear independent of the corresponding spiracle of the previous stage. The succeeding trachea and atrium are formed about the functional members. Here it is noted that the new stigmal plate may be observed, just prior to ecdysis, below and lateral to the functional plate with the median margin of the new plate being beneath the lateral margin of the other. During ecdysis, the atrium and hollow plates pass through the external ecdysial opening of the succeeding stigmal plate. The remainder of the tracheo-spiracular system passes through the internal ecdysial opening in the posterior wall of the new non-ramified felt-chamber, through the ecdysial tube, and follows the old spiracle outward. Once the old tracheo-spiracular system has been shed, the ecdysial tube withers to form the scar filament, the internal opening is closed by the formation of a scar-plug, and the external opening closes to leave a sclero-

tized "button" or scar. According to Keilin (1944), it should be noted that the ecdysial tube separates the ecdysial fluid from the perivisceral fluid.

The ecdysic procedure previously outlined occurs during both moults in precisely the same manner. The stigmal plates of both the second and third stages are easily observed through the clear integument adjacent to the previous stage stigmal plate just prior to ecdysis.

After the second ecdysis, the sclerotized portion of the stigmal plate, excluding the peritreme, is a yellowish-brown and darkens as the larva matures. This was described by Bishopp et al. (1926) and is confirmed by this study. Here it is noted that during the third larval stage radiating furrows appear, transecting the stigmal plate at various intervals. The number of furrows corresponds to the number of intervals between the hollow plates beneath.

SUMMARY

The peritreme and one bud of the first instar larva constitute the stigmal plate. The peritreme encloses one-half of the atrium and is very thick at the anterior limit, causing a constriction of the felt-chamber. The felt varies in thickness throughout the felt-chamber.

The peritreme of the second instar larva is very thin and pale brown. The peritreme and an average of 19 buds, constitute a stigmal plate. The peritreme extends from the external surface to the atrial constriction. The atrium is ramified posteriorly with the ramifications arched lateral to the internal ecdysial scar-plug. The scar filament extends from the inner surface of the scar-plug to the inner surface of the external ecdysial (stigmal) scar. The thickness of felt is uniform throughout the felt-chamber.

The peritreme, over 200 buds, and the sclerotizations between the buds of the third instar larva constitute a stigmal plate. The peritreme extends from the external surface to the base-line of the buds. The posterior one-third of the atrium is constricted and includes the hollow plates which appear to radiate from the scar filament. The thickness of felt is uniform throughout the felt-chamber.

LITERATURE CITED

- Bishopp, F. C., E. W. Laake, H. M. Brundrett and R. W. Wells. 1926. The cattle grubs or ox-warbles, their biologies and suggestions for their control. U.S.D.A. Dept. Agr. Bull. 1369. 1-119 p.
- De Meijere, J. C. H. 1895. Ueber zusammengesetzte Stigmen bei Dipterenlarven, nebst einem Beitrag zur Metamorphose von Hydronyza livens. Tijdschrift voor Entomologie 38:65-100.
- Dufour, L. 1825. Recherches anatomiques sur l'Hippobosque chevaux. Ann. Sc. Nat. 6:299-322.
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 _____ De diverses modes de respiration aquatique chez les insectes. Compt. rend. Acad. de Sc. Paris. 29:763-770.
- Entomological Society of America. 1955. Common names of insects approved by the Entomological Society of America. Ent. Soc. Amer. Bull. 1(4):33.
- * Hadwen, S. 1919. Warble flies. Dept. Agr. Canada Sci. Series No. 27.
- Imms, A. D. 1948. Textbook of entomology. 7th ed. New York, E. P. Dutton Co., Inc. 727 p. 1948.
- Keilin, D. 1944. Respiratory systems and adaptations. Parasit. 36(1):1-66.
- Knipling, E. F. 1935. The larval stages of Hypoderma lineatum DeVillers and Hypoderma bovis DeGeer. Jour. Parasit. 21(2):70-82.
- Krancher, O. 1881. Der Bau der Stigmen bei den Insekten. Zeitschr. wissensch. Zool. 35:505-574.
- Laake, E. W. 1921. Distinguishing characteristics of the larval stages of the ox warbles Hypoderma bovis and Hypoderma lineatum, with description of a new larval stage. U.S.D.A. Jour. Agr. Research 21: 439-457.
- Peterson, A. 1951. Larvae of insects. Ann Arbor, Edward Brothers, Inc. 2:219-349.

- Riley, C. V. 1892. The ox bot in the United States, habits and natural history of Hypoderma lineata. U.S.D.A., Div. Ent., Insect Life 4:302-317.
- X X Scharff, D. K. 1950. Cattle grubs. Montana Agr. Exp. Sta. Bull. 471.
- Snodgrass, R. E. 1924. Anatomy and metamorphosis of the apple maggot, Rhagoletis pomonella Walsh. Jour. Agr. Research 28(1):1-32.
- Townsend, C. H. T. 1935. Manual of myiology. Sao Paulo, Brasil, Charles Townsend and Filhos.
- Weintraub, J. 1956. The mating, oviposition and other activities of warble-fly adults (Hypoderma spp.) under laboratory conditions. Abs. 10th Inter. Congr. of Ent., Sec. 12 Med. and Vet. Ent. Montreal, Canada.