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THE STRUCTURE AND FUNCTION OF THE RESPIRATORY HORNS OF THE EGGS OF SOME FLIES

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[Plate 3]

CONTENTS

Eggs of flies of the families Sepsidae, Drosophilidae, Sphaeroceridae, Muscidae, and Cordiluridae may have one or more tubular or flattened projections from the anterior part of the shell. These projections are called respiratory horns. The eggs are laid in decomposing vegetable or animal matter. The respiratory horns project above the surface of the material in which the egg is embedded. The surface of most of each horn consists of an open hydrophobe meshwork of chorionin that provides a large water/air interface when the egg is submerged. The layer of air held in the meshwork is retained even when subjected to a considerable pressure. This layer of air is thus a plastron. The plastron found most resistant to wetting by excess pressures was that of *Drosophila funebris*, which resists 1·3 atm. The plastron air is continuous with the layer of air held in the shell. When it rains, a layer of well-aerated water flows over the eggs, and the plastron of the respiratory horn serves to extract oxygen from that dissolved in the ambient water.

The plastron of all species resists wetting by excess pressures considerably greater than those to which it is likely to be exposed in nature. To be an efficient respiratory structure the plastron must also resist wetting by surface-active substances, the concentrations of which are likely to be high in the environments in which the eggs are normally laid. Any change in the geometry or the nature of the surface of the plastron meshwork that increases its resistance to wetting by surface-active substances also increases its resistance to wetting by excess pressures, since wetting by excess pressures always occurs before there is a mechanical breakdown of the meshwork. Thus selection for greater resistance to wetting by surface-active substances results in an increase in resistance to wetting by excess pressures. These facts provide an explanation of the paradox that the plastron of some terrestrial eggs is more resistant to wetting by excess pressures than is the plastron of many wholly aquatic insects.

INTRODUCTION

The possible function of the conspicuous projections, sometimes called wings and sometimes respiratory horns, near the anterior end of the eggs of some flies has for long attracted attention. Perhaps the first to speculate upon their function was Réaumur (1738), who said of *Scopeuma stercorarium*, 'Why does the egg have these wings? It cannot be without necessity since they must make the laying of the egg more difficult. We shall see the true reason for the wings being attached to the egg, and we shall admire at the same time the precautions which the Author of nature has taken to preserve species of animals which to the ordinary man are beneath notice.' Réaumur came to the conclusion that the wings prevent the fly from burying the egg too far into the dung, '...just as a clove is stopped by the head when one sticks it into a lemon'. Amongst later writers it was generally recognized that the projections were concerned in respiration, and they were therefore often referred to as respiratory horns: it was implied, where not explicitly stated, that oxygen entered through holes in the distal end of the horn. Some writers have suggested that the respiratory horns of *Drosophila* and other flies become attached to the surface when the egg is flooded; in this way they function as floats. These structures are said, in addition, to convey oxygen from the ambient air to the air spaces of the shell when the egg is submerged. But, apart from the fact that the eggs of *Drosophila* and those of other flies with respiratory horns are normally stuck to the substrate, they are heavier than water even when the shell and horns are air-filled: they do not float if they are flooded, when under natural conditions they might be washed away from the larval food supply.

As I have already shown in a preliminary note (Hinton 1959), fly eggs with respiratory horns, such as those of *Drosophila* and *Scopeuma*, breathe by means of a plastron when they are immersed in water. The term plastron has been restricted to describe a thin layer of air of constant volume and an extensive water/air interface that is held by hydrofuge structures against an appreciable pressure difference. In all species of eggs examined, the surface of most of the respiratory horn consists of an open hydrofuge meshwork of chorionin that provides a large water/air interface. This meshwork resists the penetration of water under considerable pressure. At the base of the respiratory horn, the air in the plastron meshwork is always continuous with the layer of air contained in the shell.

In well-aerated water a plastron enables an insect to remain immersed indefinitely, when it obtains the oxygen it requires from the ambient water. But a plastron is also an efficient means of extracting oxygen from the tissues if the oxygen pressure of the environment falls below that of the tissues. The plastron method of respiration therefore seems at first sight unlikely to be utilized by insects living in decomposing vegetable and animal matter where reducing conditions often occur, and, indeed, all previously known examples of insects with plastrons are restricted to well-aerated water such as rapidly flowing streams. However, the horns bearing the plastron meshwork project above the substrate and provide a direct route for the entry of oxygen into the layer of air held in the relatively impermeable shell.

The significance of the plastron immediately becomes apparent when the eggs are observed in their natural environment when it is raining. At such times a layer of well-aerated water flows over the surface of the dung or piece of carrion in which the eggs are embedded and over the projecting respiratory horns. The plastron of insect eggs thus appears to be primarily an adaptation to rainy periods. The incubation period of the eggs with respiratory horns is not more than 2 or 3 days in warm weather, and in rainy periods much of it may be passed beneath a layer of water.

In this paper the structure of the plastron meshwork and that of the respiratory system of the shell of species in five families of Cyclorrhapha is described. Only species with respiratory horns have been considered, and these have been chosen to illustrate the widest range of structure. The oviposition habits of the species are briefly summarized.

Materials and methods

Most of the eggs used were collected in the field or bred in the laboratory. The identity of the eggs collected in the field was established by comparison with eggs laid by isolated females in the laboratory. The eggs of *Drosophila gibberosa*, *D. virilis*, *D. subobscura*, *D. pseudoobscura*, and *D. andalusica* were obtained from cultures kept in the Institute of Animal Genetics, Edinburgh.

The structures of the plastron and the respiratory system of the shell were examined by means of whole mounts and serial sections. Electron micrographs of the plastron of the horn and the respiratory system of the shell of one of the species revealed the fact that all essential features of these systems can be resolved with the light microscope.

A column of water was used to study the resistance of the plastron to a hydrostatic pressure of 7 cm Hg. Its resistance to higher pressures was tested in a rectangular chamber made of sheets of polished Perspex. The chamber was connected to a mercury reservoir by a long piece of rubber tubing. The pressure required was obtained by raising the mercury reservoir the appropriate distance above the chamber. The specimens in the chamber were examined with a dissecting microscope, and visibility through the walls of the chamber was improved by mounting a cover-slip on a film of water. Owing to the small size of the plastron area and to the fact that there is often little difference between the colour of the air-filled and the water-logged plastron, the eggs of most species were transferred for examination to water in a watch-glass without at any time being exposed to the air. In the recorded results no allowance is made for changes in atmospheric pressure during the course of each experiment, but these changes never exceeded about 1 cm.

In nearly all experiments live eggs were used. The figures for the resistance of the plastron to excess pressures are therefore minimum figures. Any oxygen uptake by the egg produces a pressure gradient in the plastron and a fall in the back pressure of the system. The maximum possible fall in the back pressure of the system due to respiration of the egg would be equivalent to a rise in the hydrostatic pressure of about 16 cm Hg. However, as in all experiments the total volume of the eggs used was small in comparison to that of the well-aerated water in the chamber, and most experiments lasted only ½ h, it is probable that the actual hydrostatic pressures were at most only a few centimetres greater than those recorded.

Sepsidae

The egg of *Sepsis violacea* Meig. (figure 1*A*) is 0·70 to 0·75 mm long, and the single anterior respiratory horn is five to six times as long as the egg. The horn is about 25 μ at its base, and it is gradually narrowed towards its apex, which is about 5 μ wide. It is everywhere circular, or nearly circular, in section. A central tube (figure 1*B*), circular in section, extends from the base nearly to the apex of the horn. The tube is always full of air. The central tube is gradually narrowed towards the apex so that its external diameter is everywhere about one-third that of the horn. Radiating from the surface of the central tube, in a plane normal to it, are numerous struts that gradually become more slender and shorter from the base to the apex of the horn. About 0·4 mm from the base of the horn (figure 1*B*) the struts are 1 to nearly 2 μ thick, about 5 μ long, and their bases are usually separated by 2 to 3 μ. Branches from the apices of the struts form a close network on the surface of the horn (figure 1 *C*). The plastron is held between this network and the central tube, the water/air interface of the plastron being limited to the interstices between the distal branches of the struts. At the base of the respiratory horn the plastron is continuous with the air spaces in the chorion and with the air spaces contained in the slightly raised ridge that extends on each side from the base of the horn to the posterior two-thirds of the egg (figure 1 *A*). The hatching line is along the side of each ridge. The micropyle is at the dorsal base of the horn, as it is in *S. punctum* F. (Leuckart 1855). The respiratory system of the chorion is similar to that of other Diptera. It consists of an air film between the outer and inner laminae of the chorion, except for a small area at the posterior pole which is without air. The laminae are separated by vertical columns arranged in somewhat irregular hexagons, the boundaries of which probably correspond to the boundaries of the follicular cells. At the boundaries of the hexagons the vertical columns are absent so that there is a continuous hexagonal network of air canals (see figure 8 *A*). Nearly all of the hexagons have a central area of varying extent in which there appear to be no air spaces. An air film does not appear in these areas even when the chorion is thoroughly dried. It is the absence of air from the central areas of the hexagons that makes the hexagonal network of air canals so conspicuous in this species.

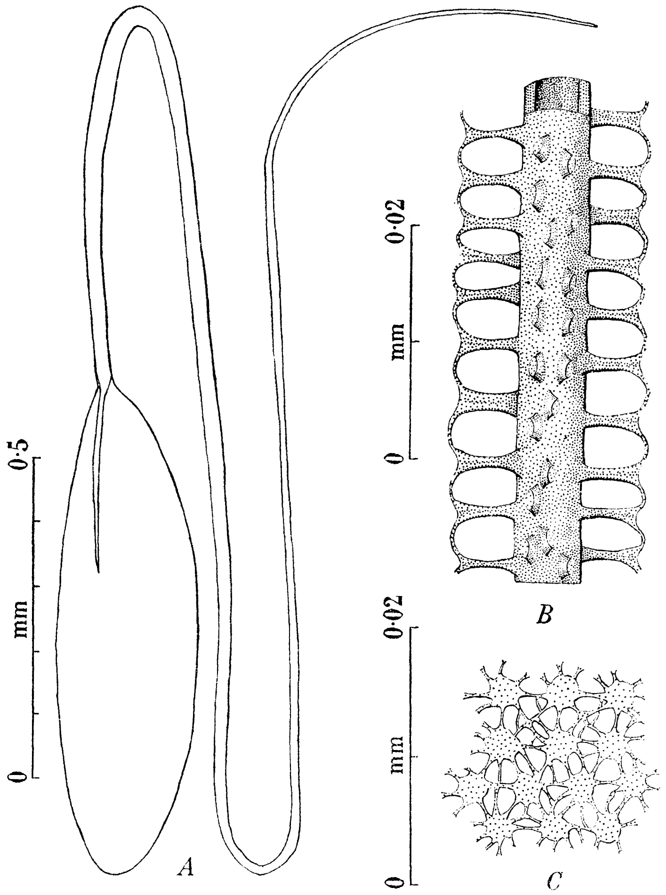

FIGURE 1. *Sepsis violacea.* (*A*) Right side of egg. (*B*) Optical section of respiratory horn 0·4 mm from its base. (*C*) Surface of respiratory horn at about the middle of its length.

The respiratory horn of *Sepsis* is most unusual in that it has two independent respiratory systems that communicate only at the base of the horn and perhaps also at the extreme apex. At first sight it appeared that the struts were hollow and conveyed air to the central tube and were thus analogous to the aeropyles of the respiratory horns of some tipulid pupae, for example, *Lipsothrix* and *Taphrophila* (Hinton 1957), in which fine aeropyles convey air from the surface to the spiracular atrium contained within the horn. However, the air in one system can be dissolved by means which do not affect, at any rate for a short time, the air in the other system, and an examination of the way in which air disappears in the two systems separately showed that the struts contained no air.

When specimens in water are subjected to an excess pressure of 60 cm Hg for 30 min, the plastron of the basal third or so of the horn is lost in many specimens, but in some of these the column of air in the central tube remains. When such specimens are examined with an oil-immersion lens, the sharply defined sides of the column of air in the central tube are straight and there are no outward projections of the column opposite the points of origin of the struts, such as might be expected if some or all of the struts contained air. If specimens treated as described above are mounted in Euparal, the column of air shrinks only from one or both ends; it is not interrupted from the sides as might be expected if the struts served to convey the fluid into the central tube. By the same token it would appear that there are no holes in the walls of the central tube, and no holes could be observed with the light microscope. When specimens with an intact plastron are mounted in Euparal, the plastron disappears sufficiently slowly to observe the manner of its disappearance; it disappears locally along a broad and generally even front momentarily leaving behind no air pockets that correspond with the struts and that might be expected to be left occasionally if the struts were hollow and contained air.

Sepsis violacea lays in cow pats within a few minutes of the time they are deposited. It also frequently lays in cow pats a few hours old. Laying usually occurs while the female is mounted by the male, but many unmounted females have been seen to lay. The egg is inserted just below the surface of the dung so that all of the respiratory horn but its extreme base lies on the surface of the dung. According to Hammer (1941), *S. cynipsea* L. buries its egg so that the basal fourth or even third of the respiratory horn is concealed. The number of eggs in 27 batches collected in the field varied from 4 to 19 with a mean of 10·4. The respiratory horn is pulled out during oviposition so that it extends away from the site of insertion of the egg. The respiratory horns sometimes radiate along the surface in all directions from the site of insertion of the batch, but are more usually confined to a small arc so that, especially near the point of insertion, they are often clumped together.

The selective value of the enormously long respiratory horn is not clear to me. In eggs of other families the required surface area of the plastron is achieved by structures that do not so materially increase the length of the diffusion path. When the egg is placed in a dish of water, part of the very flexible horn, usually the apical part, often becomes attached to the surface film so that, as in eggs that are not covered by water, oxygen can enter the horn directly from the ambient air. To test the possibility of this occurring in the field, the surfaces of cow pats containing numerous batches of eggs were flooded with water. In no instance did a horn become attached to the surface film. The reason for this is that the drying surface of the dung acts like a cement and firmly binds the horn to the dung. Nevertheless, after a prolonged rain that dissolved part of the surface of the dung the horns might become unstuck and sometimes attached to the surface film of the small pools of water that remain for a time on the cow pat. Some other species of *Sepsis*, as well as other genera of Sepsidae with long respiratory horns, deposit their eggs in beetle tunnels and in the deepest natural recesses in the dung (Hammer 1941). In species with such habits the advantage of a long respiratory horn is more evident, and it may be that *S. violacea* occasionally oviposits in a similar manner or did so normally in the recent past.

When the eggs are immersed in 9% *iso*butyl alcohol, the plastron of only about the apical fifth of the horn and an occasional patch elsewhere is wetted. Most of the plastron thus resists wetting even when the surface tension of water is reduced to about 25 dyn/cm. The plastron extends to, or very nearly to, the base of the horn as nearly as can be judged by the light microscope. No difference that appeared relevant was detected between the plastron of the apical fifth of the horn and the more proximal plastron that is not wetted by 9% *iso*butyl.

TABLE 1. EFFECTS OF EXCESS PRESSURES UPON THE RETENTION OF THE PLASTRON OF *SEPSIS VIOLACEA* (18 TO 20 °C)

number	pressure (cm)	time (h)	% retaining over 90% of plastron
10	7	24	100
10	20	½	100
32	20	26	100
10	30	½	100
10	40	½	100
27	40	6	41
14	60	½	44
14	76	½	0

In all other eggs examined, paraffin oil readily penetrated the plastron. In *S. violacea*, however, there were great individual differences in this respect. When paraffin was applied to the apical fourth of the horn of twenty-six eggs it did not penetrate in five; when applied to the basal half of the horn of twenty-six eggs it did not penetrate in sixteen. In order to reduce the chances of mechanical damage to any part of the plastron, the eggs were not touched, and only eggs laid in cow dung in the laboratory were used. The irregular results obtained with paraffin oil may perhaps be due to contamination of the surface of the horns with lipophobe particles from the dung. About twenty eggs were carefully washed in water, then in 90% ethanol, and then dried. The plastron of the basal half of the respiratory horn of only three of these eggs resisted the entry of paraffin. When a further eighteen eggs were similarly treated, paraffin immediately penetrated all parts of the plastron of every egg. These results appear to confirm the suggestion that it is contamination by the dung that is responsible for the irregularity of penetration of unwashed eggs, although there is no evidence that the plastron escaped all mechanical damage from the washing.

In aerated tap water the plastron of *S. violacea* resists higher excess pressures than the plastron of most of the eggs laid in cow dung (table 1). In this connexion it is interesting to note that the plastron is in more intimate contact with the dung and therefore more exposed to the action of surface-active substances than is the plastron of most other species.

The egg of *Saltella scutellaris* Fall. is similar in structure to that of *Sepsis*. It is 1·4 to 1·5 mm long and 0·60 to 0·65 mm wide. The single anterior respiratory horn is 2·0 to 2·3 mm long. At its extreme base the respiratory horn is broadly elliptical in section and 15 to 20 μ wide. Elsewhere it is much flattened, wider, and more or less parallel-sided to about 0·20 mm from its apex. About 0·20 mm from its apex it is about 30 μ wide, and from here it is gradually narrowed to the apex which is about 10 μ wide. A parallel-sided central tube (figure 2*B*), circular in section, extends from the base to nearly the apex of the horn. The tube is always full of air. The central tube is one-sixth as wide as the horn instead of one-third as in *Sepsis*. Radiating in all directions from the surface of the central tube, in a plane approximately normal to it, are numerous struts that are joined to form an irregular meshwork from which groups of more slender struts extend to the surface of the horn in a plane normal to the long axis of the central tube (figure 2*A*). Each of these groups of slender struts is joined to a thin diamond-shaped or irregularly hexagonal sheet of chorion (figure 2*B*), and it is these sheets of chorion that constitute the outer surface of the horn. There are no holes in the individual sheets of chorion, and the water/air interface of the plastron is confined to the spaces between the sheets. From five to eighteen, usually ten to twelve, slender struts support each sheet of chorion. An occasional strut bridges the space between two sheets at the surface, but there does not appear to be a regular system of bridges between the individual sheets, as was expected by analogy with similar structures. The spaces between the sheets are usually about 0·7 μ wide but are occasionally more than 1 μ wide.

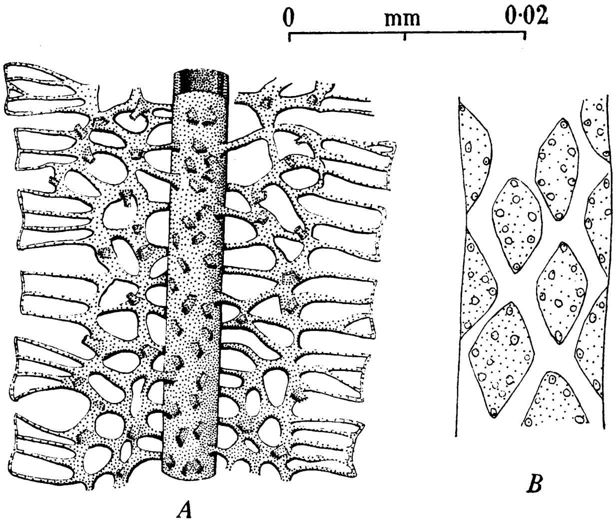

FIGURE 2. *Saltella scutellaris*. (*A*) Optical section of respiratory horn 0·4 mm from its base. (*B*) Surface of respiratory horn about 100 μ from its apex.

In both *Sepsis* and *Saltella* the apex of the central tube may have openings into the plastron, an impression reinforced by the speed of penetration into the tube of various substances. However, there is no communication between the air in the central tube and the plastron in the remainder of the horn. The probable significance of the central tube is to provide a support of great mechanical strength and flexibility for the plastron.

The structure of the shell is very similar to that of *Sepsis*. The micropyle is on the dorsal base of the respiratory horn. On each side on the anterior third is a distinct ridge with a hatching suture along its side. The hexagonal network of air canals extends nearer to the posterior pole than it does in *Sepsis*, and most of the central area of nearly all hexagons is without air spaces.

All of the eggs of *Saltella* that have been available were found in a single cow pat, or were laid in the laboratory by individuals collected on the same cow pat. In the field the eggs were laid in a deep but open crevice in the dung. The respiratory horns were arranged much as in *Sepsis*, but those in each batch were more clumped together at their bases than is usual in *Sepsis*. When the batches of eggs were flooded with water *in situ*, it was found that in every instance a large bubble of air was trapped between the bases of the horns. Part of the surface of the air bubble was in contact with the water and thus provided an important but temporary addition to the water/air interface available to each egg. The batches were flooded with water shortly after they were laid and before the surface of the dung was viscous enough to prevent the surface tension of the water from moving the bases of the horns closer together. Nevertheless, even when the surface was allowed to dry, a smaller bubble was in every instance trapped between the bases of the horns. When *Sepsis* eggs are flooded after the surface of the dung is dry, a bubble is much more rarely trapped and in all instances was a smaller bubble.

TABLE 2. EFFECTS OF EXCESS PRESSURES UPON THE RETENTION OF THE PLASTRON OF *SALTELLA SCUTELLARIS* (18 TO 20 °C)

number	pressure (cm)	time (h)	% retaining over 90 % of plastron
51 unhatched	7	24	0
60 unhatched	14	½	83
42 unhatched	20	½	0
62 hatched	7	21	100
50 hatched	20	½	100
50 hatched	60	½	72

The capacity to trap a bubble of air can hardly be other than a selective advantage, an advantage that accrues to eggs of the sepsid type of structure only when they are laid in close batches. The bubble functions as a temporary physical gill. Oxygen withdrawn from the bubble lowers the partial pressure of oxygen and raises the partial pressure of nitrogen in the bubble. Equilibrium tends to be restored by oxygen diffusing into the bubble from the surrounding water rather than by nitrogen diffusing out because of the much greater solubility of oxygen. In time, however, enough nitrogen will diffuse out to render the surface area of the bubble negligible for respiration.

The plastron of *Saltella* is not wetted by 9 % *iso*butyl alcohol. Results of experiments on the resistance of the plastron to excess pressures are recorded in table 2. The results of the first experiments, in which unhatched eggs were used, were most unexpected. After these results were obtained a further three experiments were made with the remaining eggs, which in the meantime had all hatched. The resistance of the plastron in the hatched eggs is similar to that of *Sepsis* (table 1), and I have little doubt that the results of the first three experiments are artifacts; they are not, however, due to contamination of the pressure chamber by surface-active substances, against the possibility of which great care was taken. The differences between the results obtained with hatched and unhatched eggs cannot possibly be accounted for even by making the impermissible assumption that the unhatched eggs had consumed all of the oxygen in the plastron and surrounding layer of water, since this could not have had an effect greater than raising the hydrostatic pressure by 16 cm Hg. Repeated attempts to obtain a further supply of eggs have been unsuccessful.

Drosophilidae

The general appearance of the eggs of many species of *Drosophila* has been described by other writers, but details of the structure of the respiratory system have been given only for *D. melanogaster* by Wigglesworth & Beament (1950) and for the same species and *D. gibberosa* by Hinton (1959). In the latter paper the plastron of *Drosophila* is described for the first time, and reasons are given for not accepting the account given by Wigglesworth & Beament of the respiratory structures of *D. melanogaster*.

All known species of *Drosophila* have at least one pair of respiratory horns, many have two pairs, and a few have more than two pairs, for example, *D. coracina* Kikkawa and Peng with four pairs (Kikkawa & Peng 1938). *D. lebanonensis* Wheeler is exceptional in having three to five pairs and sometimes seven or nine horns (Wheeler 1949). The respiratory systems of three species with two pairs of horns (*D. gibberosa* Patterson and Mainland, *D. virilis* Sturt., *D. funebris* F.) and four species with one pair of horns (*D. melanogaster* Meig., *D. subobscura* Collin, *D. pseudoobscura* Frolowa, *D. andalusica* Strobl) have all been examined.

In all seven species the respiratory horns are alike in essential particulars. A few specimens of *D. virilis* have either the right or the left large horn branched near the apex. Similar branching of normally simple horns has been noted also in *D. funebris* and in other species by other writers. Most of the surface of each horn consists of an open hydrofuge meshwork resembling that shown in figure 3*B*. Near the base of the horn the meshwork is replaced by a continuous wall (figure 3*C*). The extent of the basal area of the horn without an open surface meshwork varies not only with the species but also with the pair of horns. For instance, in *D. gibberosa* a continuous wall is present on about the basal two-fifths of the anterior and about the basal third of the posterior horns. Such differences are possibly related to differences in the angle and depth to which the different species bury their eggs: it would be disadvantageous if the plastron were in direct contact with a reducing medium. The air held in each horn is continuous at the base of the horn with the layer of air held between the vertical columns in the shell (figure 4*B*). As in most dipterous eggs, the vertical columns in the shell are arranged in somewhat irregular hexagons bounded by lines from which the vertical columns are absent, so that there is a continuous and conspicuous hexagonal network of air canals. The chorion over these air canals is slightly raised above the general surface of the shell (figure 4*B*), so that the outer surface of the shell has a hexagonal network of distinct ridges, the crests of which may be evenly convex or, as in *D. gibberosa*, asymmetrically enlarged.

Apart from the differences already noted in the number of respiratory horns and the extent of the open network on the surface, conspicuous specific differences exist in the relative lengths of the horns. In the species with one pair of horns they are approximately 0·13 mm in *D. andalusica*, 0·25 mm in *D. pseudoobscura*, 0·26 mm in *D. melanogaster*, and 0·28 mm in *D. subobscura*. In the species with two pairs they are approximately 0·40 and 0·30 mm, respectively, in *D. virilis*; 0·46 and 0·32 mm, respectively, in *D. funebris*; and 0·50

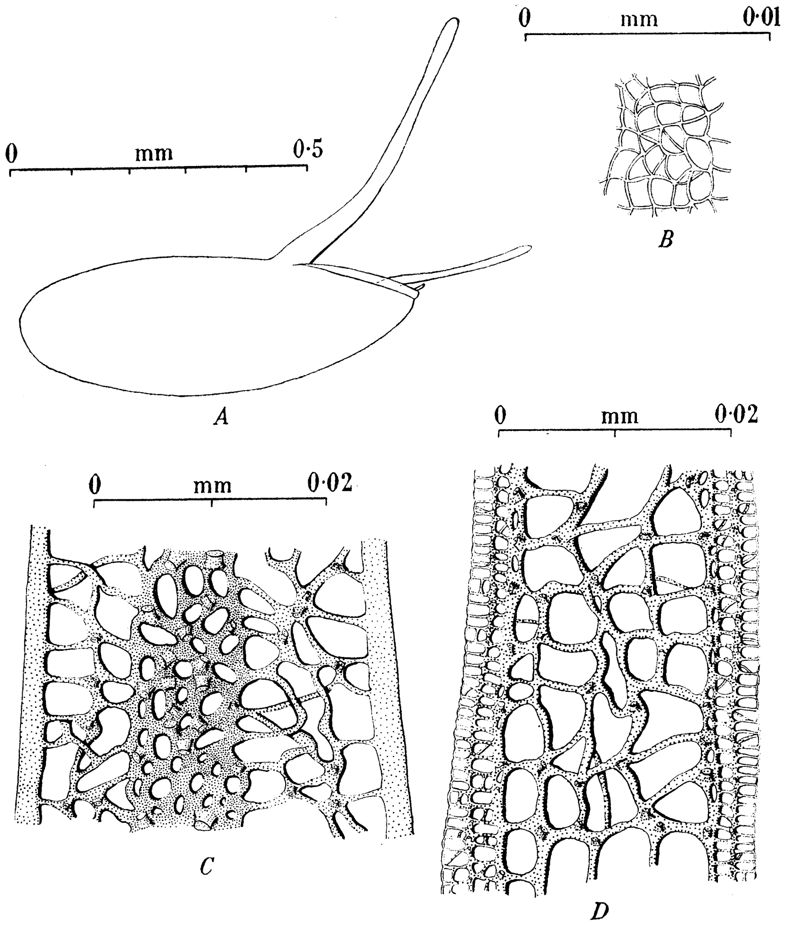

FIGURE 3. *Drosophila gibberosa*. (*A*) Right side of egg. (*B*) Surface network of posterior respiratory horn at apical two-fifths. (*C*) Optical section of posterior respiratory horn about 100 μ from its base. (*D*) Optical section of posterior respiratory horn at apical two-fifths.

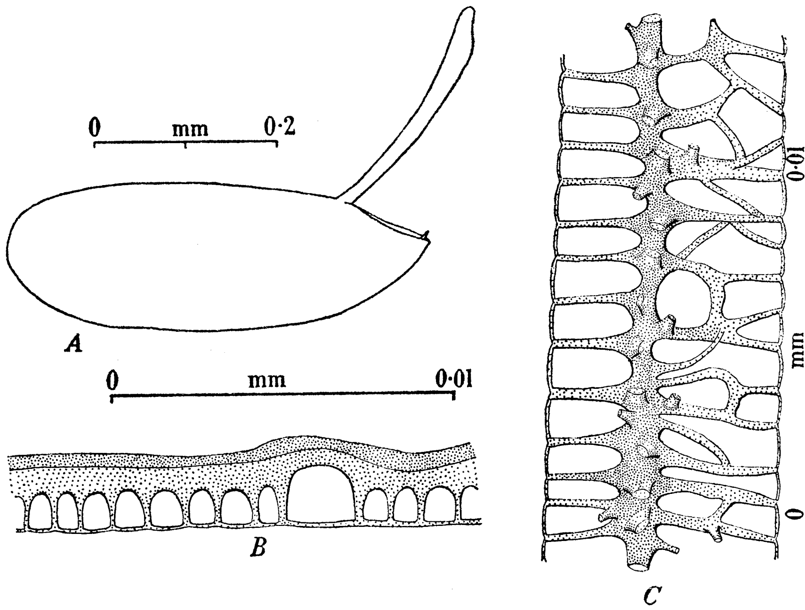

FIGURE 4. *Drosophila melanogaster*. (*A*) Right side of egg. (*B*) Optical section of chorion. (*C*) Optical section of edge of dilated part of respiratory horn about 60 μ from its apex.

and 0·26 mm, respectively, in *D. gibberosa*. The eggs with two respiratory horns are 0·46 to 0·50 mm long and those of the two species with four horns are 0·53 to 0·65 mm long. There seems to be no close relation between the size of the eggs and the area of the water/air interface of the plastron, as might have been expected. *D. melanogaster* and *D. andalusica* have embryos of approximately equal size, but the area of the water/air interface of the plastron of *D. melanogaster* is about four times greater than that of *D. andalusica* even where no allowance is made for the apical dilation of the horn of the former species. If, as appears likely, the rate of oxygen uptake is approximately the same per unit weight in the different species of *Drosophila*, the area of the plastron must be determined by factors other than the oxygen uptake of the insect.

TABLE 3. EFFECTS OF EXCESS PRESSURES UPON THE RETENTION OF THE PLASTRON OF *DROSOPHILA* (18 TO 22 °C)

species	number	pressure (cm)	time (h)	% retaining over 90 % of plastron
gibberosa	10	7	48	100
	10	30	12	100
	10	48	½	100
	10	60	½	100*
	10	60	¾	100*
	10	76	½	100*
	10	76	½	100*
	17	76	½	94*
	16	76	½	75*
melanogaster	8	76	½	75
	16	76	½	66
funebris	29	48	17	100
	10	76	½	100
	10	76	8	100
	10	100	½	100
	14	100	11	100
	12	152	½	33
	10	152	2	20

*See text.

The plastron of both *D. melanogaster* and *D. gibberosa* resists wetting by 9 % *iso*butyl alcohol; after prolonged immersion only a few small patches are wetted in both species. The resistance of the plastron of these two species and *D. funebris* to excess pressures is shown in table 3. The experiments with *D. gibberosa* were made with eggs that had been preserved in Pampel's fluid for some days but still retained the plastron. The eggs were washed, dried, and then immersed in water for 1 h before being placed in the pressure chamber. The experiments with *D. melanogaster* and *D. funebris* were done on fresh eggs.

In both large and small horns of *D. gibberosa* there is a basal longitudinal strip, as shown in figure 3*C*, of much finer openings and much coarser struts. This strip contributes to the rigidity of the horns, which are much more rigid than those of *D. melanogaster*. Towards the apex, the meshwork of the strip becomes more open and more or less indistinguishable from the general meshwork of the interior of the horn. At excess pressures of 60 to 76 cm Hg for 30 min, water displaces the air on either side of the median longitudinal strip but not the air contained in the strip nor, in most specimens, that contained in the apical surface meshwork of large and small horns. The water enters the horn through minute areas in the plastron, as is readily seen by examination with an oil-immersion lens after pressure treatment. Even when water has penetrated into the basal part of the horn, in a high proportion of specimens the air in the median strip appears to be continuous apically with the plastron air and so the diffusion path is not completely interrupted by a layer of water. The plastron thus often remains functional despite the entry of some water into the horn, since proximally the air in the median strip is continuous with the air in the chorion.

The fact that the plastron of the species of *Drosophila* resists excess pressures appreciably greater than those tolerated by the plastron of the cow-dung species is hardly likely to reflect differences in the depth of water to which the eggs of the two groups may be occasionally subjected: it is probably related to differences in the extent to which the eggs of the two groups are likely to be exposed to surface-active substances (see p. 72). For instance, in aerated solutions of 2% *iso*butyl alcohol there is a steep fall in the resistance of the plastron to wetting by hydrostatic pressures: at an excess pressure of 76 cm Hg for 1 h only two of ten eggs of *D. funebris* retained over 90% of the plastron; at 100 cm for 45 min only two of fourteen eggs of the same species retained over 90% of the plastron.

DRYOMYZIDAE

The egg of *Dryomyza flaveola* F. (figure 5) is 1·4 mm long and 0·5 mm wide. There are two anterior respiratory horns about 0·8 mm long and a median projection about 0·26 mm long. The horn is cylindrical with the apex dilated. On the ventral side on about the apical third there is a groove which becomes deeper distally and ends in a deep pocket in the apical dilation. At the base of the horn the walls are thick and have numerous air spaces that do not, however, open to the exterior (figure 6). The middle part of the horn consists of a coarse and irregular meshwork (figure 6). Distally the dorsal and lateral walls of the horn become a fine meshwork that supports an open hydrofuge network at the surface (figure 7*B*). The structure of the ventral wall, however, remains the same so that neither the surface of the groove nor that of the apical pocket has openings to the exterior, as is evident from microscopical observation (figure 7*A*) and from electron micrographs (figure 15*C*, plate 3).

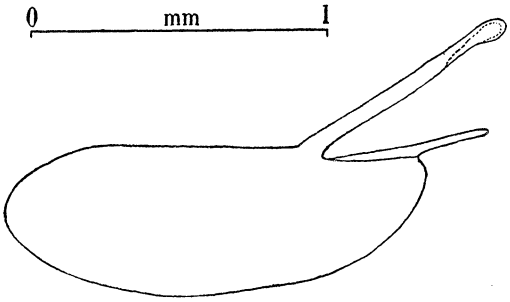

FIGURE 5. *Dryomyza flaveola*, right side of egg.

The median area in front of the horns and the median projection consists of a very fine meshwork that rests upon a continuous sheet of chorion. Numerous pockets 10 to 25 μ long extend more or less vertically into the meshwork. The diameter of the pockets

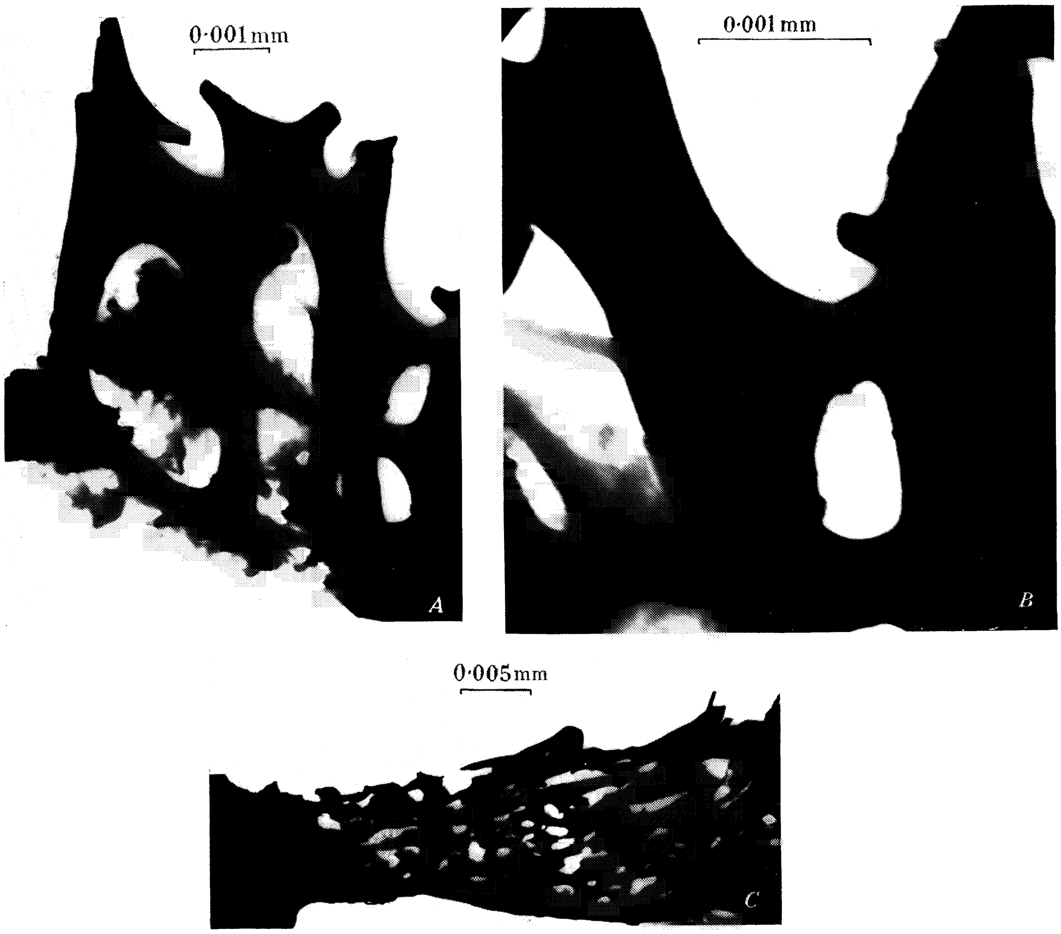

FIGURE 15. Electron micrographs of the respiratory horn of *Dryomyza flaveola*. (*A*) Plastron meshwork of middle layer of apex of horn, see figure 7*A*. (*B*) The same at a higher magnification. (*C*) A part of the inner wall of the apical pocket, see figure 7*A*.

is about 2 to 3 μ at the surface but nearly twice as great near their ends. The micropyle is just behind the ventral base of the median projection.

The air in the shell is contained in the spaces between vertical columns that in most parts of the shell are grouped in hexagons bounded by lines from which the vertical

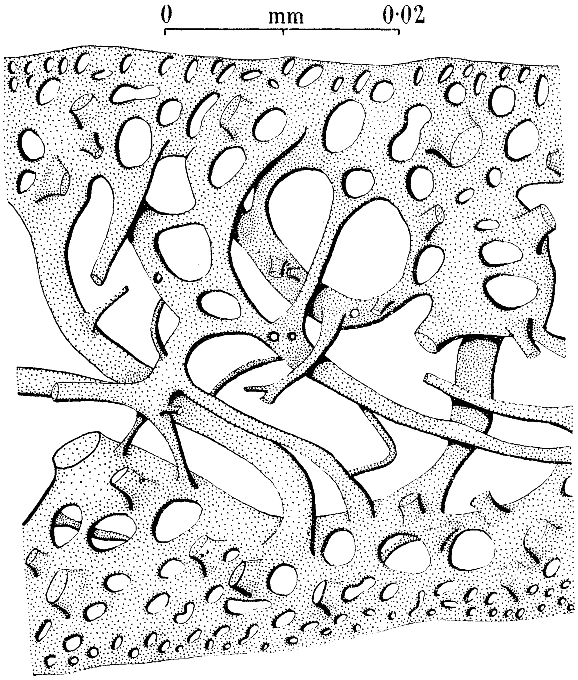

Figure 6. *Dryomyza flaveola*, longitudinal section of respiratory horn 30 μ from its base.

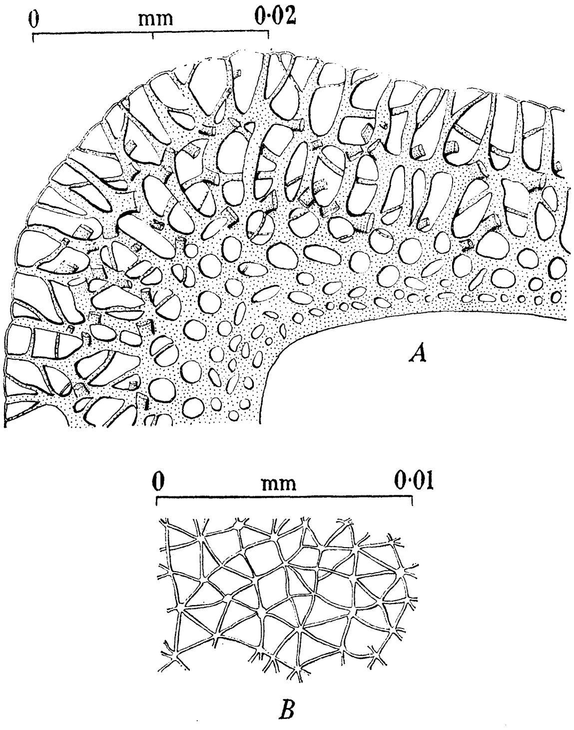

Figure 7. *Dryomyza flaveola*. (*A*) Longitudinal section of apex of respiratory horn. (*B*) Surface network near apex of respiratory horn.

columns are absent (figure 8*A*). The lines from which the vertical columns are absent form a continuous hexagonal network of air canals over most of the shell. The chorion over the air canals is slightly convex (figure 8*B*). The inner apices of the vertical columns are joined to a very thin sheet of chorion that appears to be continuous in all parts of the shell including the posterior pole. At the posterior pole the shell is much thicker, and the vertical columns are replaced by a coarse meshwork, as shown in figure 8*C*. Over an extensive area at the posterior pole this meshwork is open to the exterior (figure 8*D* and *E*).

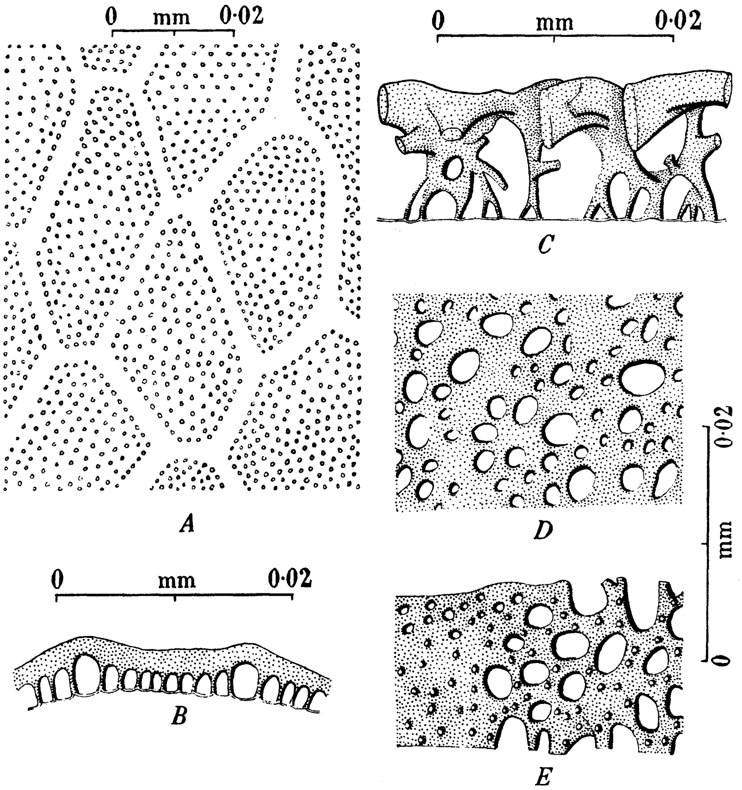

Figure 8. *Dryomyza flaveola*. (*A*) Surface view of shell showing the vertical columns grouped in irregular hexagons. (*B*) Transverse section through wall of shell near the point of convergence of two of the air canals that form the hexagonal network. (*C*) Transverse section through wall of shell at the posterior pole. (*D*) Surface view of shell at the posterior pole showing the holes through the outer wall. (*E*) Inner view of outer wall of shell at the zone of transition between the polar area with holes in the outer wall and the adjacent area without such holes.

The egg is unusual in that it has three distinct routes for the entry of oxygen into the layer of air in the shell: (1) through the respiratory horns; (2) through the aeropyles in the fine meshwork in front of the horn and in the median anterior projection and (3) possibly through the surface openings at the posterior pole. It is the only egg known to me with respiratory horns in which there is a specialized area at the posterior pole that provides a direct route for the entry of oxygen into the layer of air held in the shell. However, amongst eggs without respiratory horns there are many in which the layer of air in the chorion communicates directly with the ambient air, for example, *Trichiaspis equina* Fall. (Sphaeroceridae), *Orthellia* (Muscidae), and all of the syrphid eggs that I have seen (*Helophilus*, *Eristalis*, *Chrysogaster*, *Rhingia*, *Syrphus*). In the drawing of the chorion of *Syrphus luniger* Meig. by Wigglesworth & Beament (1950, figure 7*A*) the outer part of the chorion is incorrectly drawn as a continuous and uninterrupted sheet.

Eggs of *Dryomyza* were found in the field from November to the middle of March. They were usually laid on the vertical sides of cow pats that were in the shade at the edges of fields or in woods. The egg is pushed into the dung so that all of the respiratory horns and the median anterior projection are above the surface. The egg is cemented to the dung with a mucous-like substance that covers all of the shell in contact with the dung. Hatching was not observed, but from the position of the egg it would seem that the larva must hatch on the surface of the dung. The experiments on the resistance of the plastron to wetting by hydrostatic pressures were too few to make profitable a comparison with the other species described in this paper.

Muscidae

The egg of *Musca autumnalis* Deg. (figure 9) is 3·0 to 3·2 mm long, including the respiratory horn, and 0·50 to 0·57 mm wide. There is a single anterior respiratory horn that projects beyond the anterior pole of the egg for about 0·8 mm. The ventral side of the respiratory horn is convex and the dorsal side concave with the upper edges slightly to strongly turned inwards (figure 9). The horn may be quite straight or somewhat twisted either to the right or to the left. All surfaces of the horn have an open hydrofuge network. The chief supporting structure of the horn is a very close layer of meshwork which is much nearer to the dorsal than to the ventral surface (figure 10*B*), and the struts supporting the network on the ventral surface are longer than those supporting the network on the dorsal surface and edges.

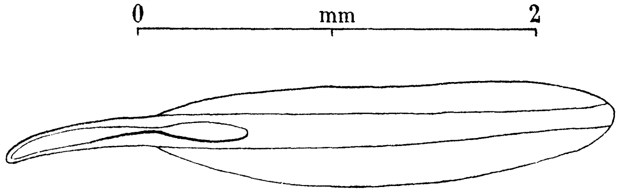

Figure 9. *Musca autumnalis*, dorsal side of egg.

The hatching lines are on each side of the dorsal surface and run more or less parallel to each other from the anterior to the posterior poles, the plane of the hatching line (figure 10*A*) being normal to the long axis of the egg. When the larva hatches, the chorion usually splits along only one of the hatching lines, and in the eggs examined the split was as often along the right as along the left line. The structure of the median area between the hatching lines is similar to that of the horn. The layer of coarse and close meshwork that gives rigidity to the median area is near the ventral surface posteriorly but near the dorsal surface anteriorly. The median area is concave near the anterior pole (figure 9), and the edges of the concavity are turned inwards and are continuous with the inwardly turned edges of the horn, which would appear to be evolved as an anterior production of the median area. The surface of both the outer and inner sides of the median area consists of an open network.

The micropyle is behind the ventral base of the horn. The structure of the shell (figure 10*C* and left side of *A*) is unusual amongst Diptera in that it consists of a middle layer of very thick and short struts in a plane parallel to the surface. Vertical columns from this layer project upwards and join the outermost layer of the chorion, which consists of a continuous sheet without air spaces. Vertical columns from the same layer project inward. The latter are similar to the vertical columns in most dipterous egg shells except that they do not join a thin but continuous inner sheet of chorion. Instead, they are branched at their apices in a plane normal to their long axes. The branches of adjacent columns anastomose to form an open inner network. That the inner surface of the shell has an open network instead of a continuous sheet as usual in the Diptera seems fairly clear from microscopical examination. Also, small drops of paraffin oil placed on the inner surface immediately displace the air between the vertical columns, whereas oil does not do so in those Diptera, for example *Scopeuma*, in which the innermost sheet or lamina of the chorion is continuous. The vertical columns are, as usual, grouped in hexagons bounded by lines from which they are absent. These lines from which the vertical columns are absent form a continuous hexagonal network of air canals in most of the shell. These hexagonal air canals are also evident on the lower side of the median area.

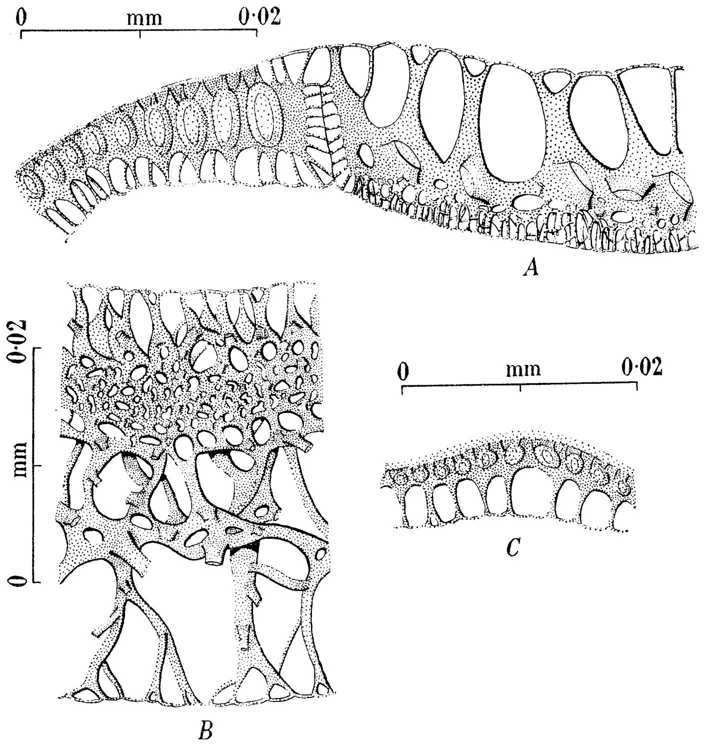

FIGURE 10. *Musca autumnalis*. (*A*) Transverse section through the dorsal wall of the shell showing the structure of the chorion and that of the median area between the hatching lines. (*B*) Transverse section through the respiratory horn at the middle of its width and the middle of its length. (*C*) Transverse section through shell.

The chorion of *M. autumnalis* contains two distinct air layers. The interstices between the stout struts of the middle layer serve as air canals between the thin outer and the thick inner layer. This type of chorion structure has been found in the few other Muscinae examined, for example *Orthellia*, where the outer layer is in direct communication with the ambient air. It is quite unlike the chorion structure of any of the Phaoniinae examined.

Collin (1948) appears to have been the first to draw attention to the fact that in the subfamily Phaoniinae the chorion on either side of the median area is always elevated to form flanges or 'wings', whereas it is never so elevated in the Muscinae. This distinction between the two subfamilies obtains in all the eggs examined by me.

The females begin to arrive on the cow pat a few minutes after it has been deposited. According to Hammer (1941), the greatest number of females occur on pats 10 to 20 min old or slightly older. Females are occasionally attracted to pats 24 h or more old. The female is not accompanied by the male during oviposition. The eggs are laid singly but usually several eggs to as many as twenty or more will be found close together and, when the fly is abundant, over two hundred eggs have been seen on a single cow pat. The eggs are pushed more or less vertically into the semi-liquid dung or through the recently formed thin and soft crust. The surface of the shell is coated with a mucous-like substance, presumably from the accessory glands, which serves to cement it to the dung. The respiratory horn is always free of this material. The tip of the horn usually projects slightly above the surface, but the proximal part is, nevertheless, normally not in contact with the dung because the female usually leaves a narrow crater in the dung above the egg through which the horn projects to the surface. Unlike many of the other species with respiratory horns, the larva always hatches beneath the crust of the dung, and it does not have to crawl over the surface looking for a place soft enough to penetrate.

TABLE 4. EFFECT OF EXCESS PRESSURES UPON THE RETENTION OF THE PLASTRON OF *MUSCA AUTUMNALIS* (18 TO 22 °C)

number	pressure (cm)	time (h)	% retaining over 90 % of plastron
10	7	24	100
10	14	12	70
10	20	½	100
10	30	½	90
10	30	½	80
10	40	½	0
3	76	¼	0

When the egg is flooded with water, a bubble of air is often trapped and held in the hollow of the median area and the basal part of the horn. The air in the bubble is in direct communication with the layer of air held by the plastron meshwork. Much of the surface of the bubble is in contact with the water and so provides the egg with a large water/air interface. The bubble functions as a temporary physical gill: it does not persist against a pressure difference, and it becomes smaller and eventually disappears as nitrogen diffuses out of it into the ambient water. The capacity to trap a bubble of air that acts as a temporary physical gill would seem to be the only functional significance of the hollow in the median area and the respiratory horn.

The resistance of the plastron of the respiratory horn to excess pressures in aerated tap water is shown in table 4. When the eggs are subjected to an excess pressure of 30 cm Hg for 30 min, the meshwork of the upper layer of the median area becomes water-logged, but the layer of air in the lower or inner meshwork remains more or less intact.

The egg of *Hebecnema umbratica* Meig. is 1·1 to 1·2 mm long and about 0·3 mm wide. The flanges are strongly produced. They are normal to the surface posteriorly but are usually inclined outwards anteriorly. They are nearly parallel, converging only slightly towards the posterior pole. Each flange is produced anteriorly to form a long lateral respiratory horn (figure 11*A*). Short respiratory papillae arise from both the flanges and the lateral respiratory horns, as shown in figure 11*A* and *B*. The structure of the lateral respiratory horns is shown in figure 11*B*. Each consists of a wide strip of chorion with relatively few air spaces. Radiating from the surface of this strip in a plane normal to it are numerous struts which support an open network at the surface. The denser supporting strip becomes gradually narrowed towards the apex where it is often branched, and one or more of these branches may be continued as the median strip of one of the respiratory papillae (figure 11*D*).

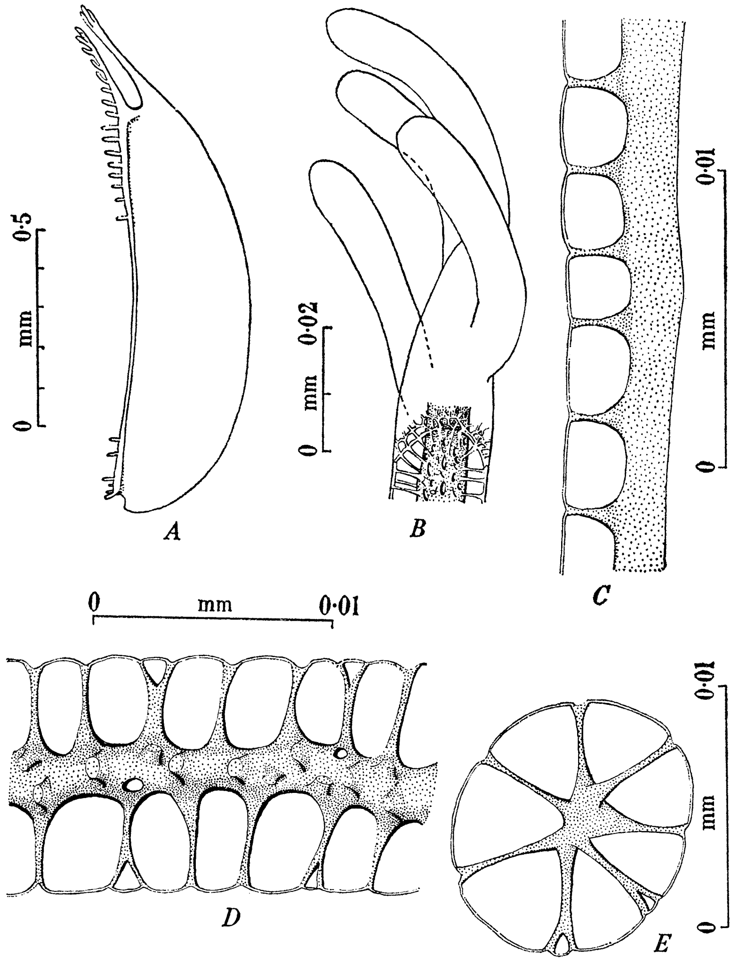

FIGURE 11. *Hebecnema umbratica*. (*A*) Right side of egg. (*B*) Lateral view of apex of right respiratory horn. (*C*) Transverse optical section through shell. (*D*) Longitudinal optical section of a respiratory papilla from the apex of a respiratory horn. (*E*) Transverse optical section of same.

Closely applied to the outer ventral surface of each lateral horn is a slender, flattened projection from the anterior lower apex of the shell. This projection is narrowed anteriorly and only extends to about the apical two-fifths of the horn. It consists of a hydrofuge meshwork that holds a film of air that is continuous with the air held in the remainder of the horn. Near the base of this projection from the lower part of the shell there is a median strip similar in structure to the supporting strip of the horn.

The area between the flanges is produced anteriorly to form a median respiratory horn at the apex of which there are usually seven respiratory papillae. No papillae are present on the proximal part of the median horn. This structure consists of a hydrofuge meshwork without a supporting denser strip of chorion. Closely applied to its ventral surface is an anterior projection from the middle lower apex of the shell. This ventral part of the median horn consists of a hydrofuge meshwork supported by a broad strip of chorion with relatively few air spaces. The supporting strip is narrowed towards its apex, which may be simply pointed or forked. The micropyle is behind the base of the median projection from the lower part of the shell.

Both lateral and median respiratory horns of *H. umbratica* are thus double structures. The rigidity of the lateral horns is chiefly due to the denser strip of chorion contained in that part that is a continuation of the flange, whereas the rigidity of the median horn is chiefly due to that part that is produced from the lower apex of the shell. When the larva hatches, the two components of both lateral and median horns are partly or entirely separated. The hatching lines are continued dorsally on the outer side of the crest of each flange. Normally, the shell is split on hatching for a short distance anteriorly along both hatching lines. Posteriorly, however, the shell usually splits along only the right or only the left dorsal hatching line.

The structure of the respiratory papillae of both lateral and median horns and those of the flanges is identical. Each is a short cylinder with a central longitudinal strip of chorion that extends nearly to its apex. This strip has relatively few air spaces (figure 11*D*). Numerous struts radiate from the central strip in a plane normal to its long axis, as shown in figure 11*D* and *E*. Each strut branches at the surface of the papilla, and its branches anastomose with those of adjacent struts so that an open network is formed similar to that of the surface of the respiratory horns and the inner and dorsal sides of the flanges. The layer of air contained in a respiratory papilla is of course continuous with the layer of air contained in the respiratory horns and the flanges, which in turn is continuous with that contained in the shell.

Each flange proximal to the lateral respiratory horn consists of a flattened sheet of chorion with relatively few air spaces. From this sheet arise numerous struts. On the inner and dorsal sides each strut branches, and its branches anastomose with those of adjacent struts to form an open network at the surface. The effective plastron area of the egg thus includes not only the surface of the respiratory horns and the papillae but also the inner and dorsal surfaces of the flanges themselves. The surface between the flanges consists of a thick sheet of chorion with sharply defined round or broadly oval holes which are usually 1·2 to 1·4 μ in diameter and are separated from one another by slightly less than their diameters. This area is broken up into hexagons by narrow ridges. Beneath the surface sheet is a fine meshwork into which the holes open. The area between the flanges is hydrophilic, and the air it contains when dry is immediately displaced if a drop of water is placed on its surface. Water contained in the hydrophilic area is quickly lost, when the ambient air is not saturated with water. The structure of the remainder of the shell is as usual in the Diptera: air is contained in the spaces between the vertical columns which are arranged in hexagons bounded by lines from which the vertical columns are absent, so that there is a continuous and conspicuous hexagonal network of air canals.

The females of *H. umbratica* often arrive on the cow pat very shortly after it is deposited, and they may continue to arrive for some hours afterwards. According to Hammer (1941), the maximum number of females, five to ten, are found on a cow pat about 10 min after it is deposited. The females walk about alone on the pat. They lay their eggs chiefly on the vertical or overhanging sides but sometimes also on the surface, as has been noted by Hammer. The eggs are laid singly, as is general for species with predaceous larvae, and usually about two to six eggs but sometimes more are found fairly close together. The egg is pushed into the dung usually at an angle of 15 to 30° but sometimes as much as 45°. The respiratory horns and the anterior part of the flanges are above the surface. The posterior part of the flanges and the posterior respiratory papillae are normally in contact with the dung. Because of the position of the egg, the larva emerges on the surface of the dung. It crawls about on the surface until it finds a crevice or some other area where the dung is still soft enough for it to penetrate.

The resistance of the plastron to excess pressures is somewhat less than that of the plastron of any of the other cow-dung species tested (table 5). The figures for the survival of the plastron recorded in table 5 refer to the whole plastron and not only to that of the respiratory horns.

TABLE 5. EFFECTS OF EXCESS PRESSURES UPON THE RETENTION OF THE PLASTRON OF *HEBECNEMA UMBRATICA* (18 TO 22 °C)

number	pressure (cm)	time (h)	% retaining over 90 % of plastron
10	7	24	100
10	20	$\frac{1}{2}$	100
10	30	$\frac{1}{2}$	30
10	40	$\frac{1}{2}$	20
10	50	$\frac{1}{2}$	0

A single egg of *H. affinis* Malloch has been available. It was laid by a captive female, and I do not know where the egg is normally laid. It is very similar to that of *H. umbratica*. Both the posterior and anterior ends of the flanges are slightly produced, the anterior ends a little more than the posterior but not enough to be called horns. The median area between the flanges is not produced anteriorly, as it is in *H. umbratica*. On the crest of each of the flanges there are about forty-five respiratory papillae which are very similar in structure to those of *H. umbratica*. These papillae are long at both the anterior and posterior ends of the flanges but are elsewhere shorter than those of *H. umbratica*. As *H. affinis* has nearly twice as many respiratory papillae as *H. umbratica* the difference in the surface area of the plastron of the two is not as great as appears at first sight because of the absence of respiratory horns in the former species. On the anterior middle margin of the median area there is a single respiratory papilla. The structure of the median area between the flanges is as in *H. umbratica*, but the ridges that delimit each hexagon have at each corner a short vertical projection no thicker than the ridge itself. The structure of the shell is like that of *H. umbratica*. The position of the micropyle and hatching lines are as in the latter species.

The egg of *Mydaea urbana* Meig. is 3·1 mm long and about 0·8 mm broad. It is very similar in shape and structure to that of *Hebecnema umbratica*: the lateral and median respiratory horns are double structures and both have numerous respiratory papillae which are also present on the flanges, and the micropyle and hatching lines are similar as is the structure of the shell of the two species. The respiratory papillae are considerably shorter than those of *Hebecnema*; they are longest on the apices of the horns and on the posterior ends of the flanges, but even here the longest are scarcely 25 μ and are about as broad as long. On most of the crest of each flange they are distinctly shorter but nearly as broad. The papillae of the two species are similar in structure, but those of *Mydaea* lack the well-defined central supporting strip although the meshwork in the centre of each papilla is closer. The median respiratory horn differs from that of *H. umbratica* in having usually six or seven respiratory papillae along each lateral margin and three or four apical papillae. Most of the median area between the flanges lacks the hexagonal network of ridges present in *H. umbratica*; these are present but indistinct at the extreme posterior end. The holes through the thick sheet of chorion to the fine inner meshwork are very much larger, frequently 20 to 30 μ, and are round to narrowly oval. A few holes are as little as 5 to 7 μ in diameter except on the posterior middle third of this area, where most holes are 3 to 5 μ and many are smaller.

According to Hammer (1941), the female of *Mydaea urbana* usually arrives on the cow pat within 15 min of its deposition, and it is not attracted to the pat when it is more than about 30 min old. Thomson (1937), however, says that the females show no marked preference for freshly dropped dung and may be found on pats 24 h old. The egg is pushed much more vertically into the dung than is that of *Hebecnema*, and its respiratory horns project up into the air. The larva emerges on the surface of the dung and must then penetrate into it. Only eleven eggs of this species were found. These were subjected to an excess pressure of 7 cm Hg for 28 h, and no part of the plastron of any of the eggs was wetted.

The egg of *Mydaea pagana* F. is known to me only by the outline drawing given by Hammer (1941). It differs from *M. urbana* in almost precisely the same way as the egg of *Hebecnema affinis* differs from that of *H. umbratica*. In *Mydaea pagana* the anterior end of neither the flange nor the median area is sufficiently produced to be called a horn. The short respiratory papillae appear to be confined to the anterior ends of the flanges. This species lays in cow pats.

CORDILURIDAE

The egg of *Scopeuma stercorarium* L. is 1·3 to 1·6 mm long and 0·4 to 0·5 mm wide. The respiratory horns are the modified flanges. They are confined to the anterior third of the egg. They diverge towards the apex and are produced beyond the apex (figure 12*A* and *B*). Each horn is more or less normal to the surface posteriorly but towards the apex it gradually becomes larger and is curved outwards (figure 12*A*). The inner and dorsal surfaces of the respiratory horn are convex and the outer surface is concave. Each horn consists of two quite different components, the hatching line running between them.

The inner component of the respiratory horn consists of a hydrofuge meshwork which in section appears as a fine inner layer and a distinctly coarser outer layer. These two layers are separated and supported by a much denser, more or less median layer (figures 12*C*, 13*A*). The surface of the inner layer consists of an open hydrofuge network (figure 13*E*). The surface of the outer layer of meshwork consists of a similar but coarser hydrofuge network.

The outer component of the respiratory horn is a projection from the more ventral part of the shell. It is very closely applied to the outer surface of the inner component and it breaks away from the latter when the larva hatches. The outer component of the horn has the same basic structure as the shell, as may be clearly seen in figure 13*A*. The struts or vertical columns support an open meshwork that constitutes the inner wall of the outer component.

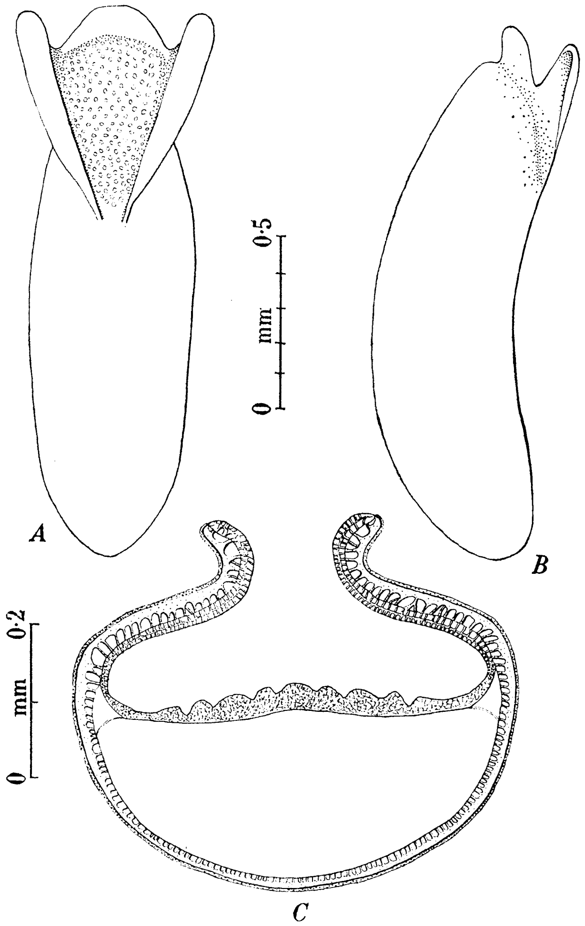

FIGURE 12. *Scopeuma stercorarium*. (*A*) Dorsal side of egg. (*B*) Left side of egg. (*C*) Transverse section through egg behind the base of the median anterior projection.

The layer of air held in the inner and outer components of the respiratory horn is continuous. This layer of air is also continuous with that held in the hydrophilic median area (figure 12*C*) and with that held between the vertical columns of the shell (figure 13*A*). The water/air interface of the plastron is provided only by the inner and dorsal surfaces of the inner component of the horn.

The apex of the egg is broadly but not sufficiently produced to be called a respiratory horn (figure 12*A*). This produced portion consists of two parts: (1) an upper lip formed by an extension of the median area between the horns, and (2) a lower lip formed by an extension of the more ventral part of the shell. The lower lip is closely applied to the ventral surface of the upper lip. At the apex of the median structure the lower lip is usually flexed upwards to form a slight ledge upon which the anterior margin of the upper lip rests. When the larva hatches, the upper and lower lips come apart, the hatching line on this median structure being nearly in the same plane as that of the hatching line on the apex of the horns. The structure of the upper lip (figure 13*C*) is very similar to that of the inner component of one of the respiratory horns, whereas the structure of the lower lip resembles that of the outer component of a horn. The layers of air in the upper and lower lips are continuous, and this air is also continuous with the air contained in the respiratory horns and with that normally contained in the finer meshwork of the hydrophilic area between the horns. The water/air interface of the plastron of the median structure is the open network of the dorsal surface of the upper lip.

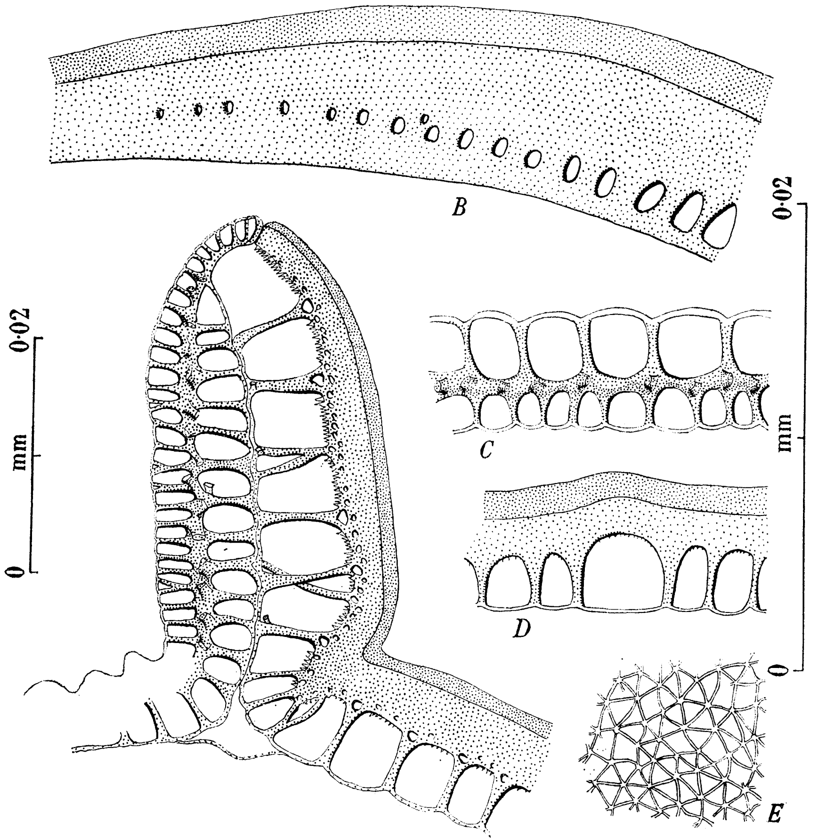

FIGURE 13. *Scopeuma stercorarium.* (*A*) Transverse section through the right respiratory horn near its posterior end. (*B*) Transverse section through shell near the micropyle. (*C*) Longitudinal section through upper lip of the median anterior projection about 70 μ from the fine hydrophilic meshwork. (*D*) Transverse section through chorion showing one of the air canals. (*E*) Surface network of inner side of the inner component of the respiratory horn.

The median area between the respiratory horns consists of a thick layer of very fine meshwork into which open shallow to deep pits usually about 6 to 10 μ wide and usually separated by about their own diameters (figure 12*C*). This median area is hydrophilic. A drop of water placed on its surface will displace the air it normally contains, and it is always completely wetted when the egg is immersed in water. The water contained in the hydrophilic meshwork is fairly quickly lost when the ambient air is dry.

The structure of the shell is as usual in the Diptera: air is contained in the spaces between vertical columns that are grouped in hexagons bounded by lines from which vertical columns are absent. These lines from which the columns are absent form a continuous and conspicuous hexagonal network of air canals over most of the shell. The chorion over the air canals is slightly raised above the general surface of the shell (figure 13*D*), so that the shell has a hexagonal network of low ridges. The micropyle is behind the base of the ventral lip of the median anterior projection. Behind the ventral lip near the micropyle the vertical columns are replaced by almost solid chorion in which the air spaces are small and widely separated so that there is here no continuous layer of air (figure 13*B*), but at the sides the layer of air held in the lower lip is continuous with the layer in the shell.

The water/air interface of the plastron of *Scopeuma* is provided by the inner and dorsal sides of the inner component of the horns and by the dorsal surface of the upper lip. When the egg is out of water, these structures provide a direct route for the entry of oxygen into the shell as does also the hydrophilic area between the horns. Experiments with paraffin oil support the observations made with the microscope. Small drops of oil rapidly penetrate when placed on all those structures seen to have an open network at the surface: all surfaces of the inner component of the respiratory horns but only the inner surface of the outer component; the dorsal and ventral surface of the upper lip but only the dorsal surface of the lower lip; and the hydrophilic area between the horns. The surface of the extreme anterior margin of the median structure is the ventral surface of the upwardly flexed lower lip. Oil does not penetrate here, nor is the surface an open network. However, for a distance of about 50 μ or so behind the anterior margin, the dorsal surface of the upper lip behaved irregularly towards oil; oil rapidly displaced the air in the lip in seventeen of forty-six eggs tested but not in twenty-nine. All specimens examined appeared to have an open network on the whole of the dorsal surface of the upper lip. It was therefore thought that in some specimens the upwardly flexed part of the lower lip might extend posteriorly over the upper lip, but no specimens have been found in which the lower lip covers the upper sufficiently to account for the differences noted. Drops of oil do not penetrate through the outer surface of the unbroken shell, and, when the posterior part of the egg is immersed in oil almost up to the level of the respiratory horns, the air in the shell is not displaced. Drops of oil placed on the inner walls of the shell after the removal of the embryonic cuticle do not displace the air in the shell, nor do they displace the air in the meshwork of the hydrophilic area when placed on the inner wall of this area.

Scopeuma begins to arrive on cow pats almost as soon as they are deposited, and, unlike many of the other cow-dung species, the female will ovipost on pats that are many hours and sometimes even more than a day old. According to Cotterell (1920), they sometimes lay on pats several weeks old. The females usually, but not always, lay while mounted by the males. The eggs are inserted separately into the dung, but large numbers may be laid very close together. During seasons when *Scopeuma* is abundant, single cow pats commonly have several hundreds to over 1500 eggs. These are laid in all parts of the upper surface and sides of the dung. According to Hammer (1941), oviposition occurs, '...all over the smooth areas in not too warm and not too windy weather, whereas on warm and windy days flies deposit their eggs very densely in crevices and rough places or on the vertical sides in the shade sheltered from the wind'. The eggs are pushed into the dung so that the bases of the flanges are level with the crust or even slightly covered by the crust. The angle at which the eggs are pushed into the dung varies considerably, usually 10 to 25° and rarely more than 45°. The resistance of the eggs to desiccation at various relative humidities is described by Larsen (1943). The eggs appear to be an important scource of food for various Staphylinidae especially species of *Philonthus*, *Quedius*, and *Staphylinus*, which may be seen eating them on the surface of the pat. The larvae hatch on the surface of the dung and then crawl about until they find some crevice or other place where the dung is still soft enough for them to penetrate.

The plastron of some eggs resisted prolonged immersion in 9 % *iso*butyl alcohol, whereas that of other eggs treated in the same way and with the same previous history was partly or entirely wetted by this strength of *iso*butyl. The reason for this variation was not found.

TABLE 6. EFFECT OF EXCESS PRESSURES UPON THE RETENTION OF THE PLASTRON OF *SCOPEUMA STERCORARIUM* (18 TO 22 °C)

number	pressure (cm)	time (h)	% retaining over 90 % of plastron
143	7	26	88
25	14	2	80
59	20	2	54
51	30	2	23
67	40	2	6
76	20	½	100
58	30	½	93
84	40	½	37
62	48	½	3
54	48	¾	0
18	76	½	0
22 (5 % *iso*butyl)	30	¼	0
67 (2 % *iso*butyl)	14	½	91
69	20	½	19
66	20	½	11
79	25	½	24
71	25	½	17
100	30	½	0

It was not due to differences in the condition of the hatching line. As already noted, both the inner surface of the outer component of the horn and the dorsal surface of the lower lip have an open meshwork with larger interstices than those of the plastron surface. Any opening along the hatching line therefore results in the plastron being wetted by surface-active substances of lower strength than normally required. Similarly, any opening along the hatching line causes the plastron to be wetted by hydrostatic pressures lower than those ordinarily required.

The resistance of the plastron to excess pressures in aerated tap water is shown in table 6 and figure 14. At 7 cm for 26 h 88 % retained 90 to 100 % of the plastron. In some of those that lost more than 10 % of the plastron, the hatching line was slightly open. As shown in table 6 and figure 14, surface-active substances reduce the hydrostatic pressure necessary to wet the plastron. Since the plastron resists hydrostatic pressures in clean water considerably greater than any it is likely to be subjected to under natural conditions, it seems possible that this resistance is in part the result of selection for resistance to wetting by surface-active substances, such as organic acids, that may be dissolved out of the dung in which the egg is embedded: any change in the geometry or nature of the surface that increases resistance to wetting by surface-active substances also increases resistance to wetting by hydrostatic pressures and vice versa (see discussion pp. 70–73).

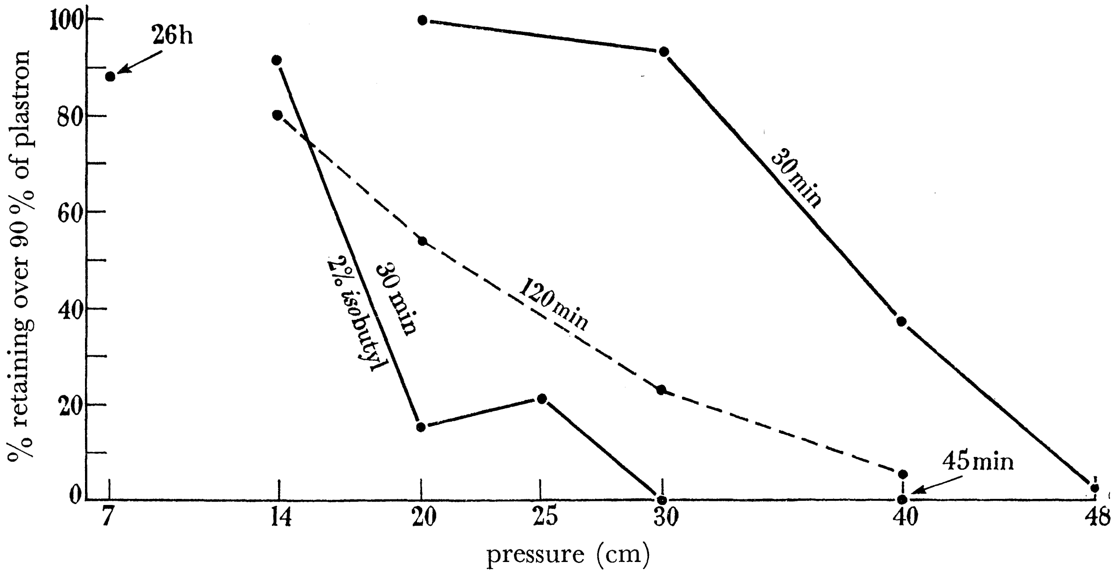

Figure 14. *Scopeuma stercorarium.* Effect of excess pressures (cm Hg) upon the retention of the plastron in aerated tap water and in an aerated 2 % solution of *iso*butyl alcohol (*ca.* 46 dyn/cm).

Discussion

In *Sepsis*, *Saltella*, *Drosophila*, *Musca*, *Hebecnema*, *Mydaea*, *Scopeuma*, and in many other genera of flies not dealt with in this paper, for example, *Trichiaspis*, *Orthellia*, *Dasyphora*, *Myospila*, *Morellia*, *Phaonia*, *Azelia*, it was found that the respiratory system of the egg becomes filled with air while the egg is still bathed in fluid in the common oviduct, as has been noted by Leydig (1867) in *Tachina* and by Wigglesworth & Beament (1950) in *Drosophila*. The latter writers point out that under such circumstances air filling of the respiratory system involves the active absorption of the contained fluid, which would reduce the pressure in the lumen and cause bubbles of gas to be formed. The formation of bubbles would be assisted by any increase in the hydrophobe properties of the chorionin such as accompanies tanning.

The plastron method of respiration has been evolved independently in a few groups of adult insects, expecially beetles (Thorpe 1950) and no less than seven times in the pupae of the Diptera (Hinton 1958). The air-filled ribbon of silk spun by the larva of the parasitic wasp, *Agriotypus armatus* Curtis, functions as plastron (Thorpe 1950). It is probable that the layer of air contained in the cocoons of many beetles, Lepidoptera, and other insects also functions in a similar way. Amongst insect eggs the existence of a plastron has been definitely established only in the Diptera–Cyclorrhapha.

There would appear to be little doubt that a plastron has been evolved independently in the families Sepsidae, Drosophilidae, Dryomyzidae, Sphaeroceridae, Muscidae, and Cordiluridae and that it has been evolved more than once within the Muscidae and Sphaeroceridae. Most of the examples of plastron respiration in the Muscidae are found in the subfamily Phaoniinae (*sensu* Collin 1948). In this subfamily the flanges are always well developed and frequently if not always bear a plastron on their inner sides. Thus within the Phaoniinae a plastron may have been evolved but once. Nevertheless, respiratory horns bearing a plastron have been evolved from the flanges on numerous occasions as is evident from such genera as *Hebecnema* and *Mydaea*, each of which contains both species without respiratory horns and species in which the flanges are produced anteriorly to form long respiratory horns. In the subfamily Muscinae, the plastron-bearing horn of *Musca autumnalis* is so different from any of the structures to be found in the Phaoniinae that it is difficult to suppose that it was not evolved independently, a supposition supported by the absence of respiratory horns in some species of *Musca* and in other genera of Muscinae. The plastron-bearing structures of such Sphaeroceridae as *Coprophilla*, *Chaetopodella*, and *Limosina* are sufficiently different strongly to suggest more than one origin within the family.

If the plastron of an egg is to be an efficient respiratory structure it must resist wetting by the pressures exerted by raindrops falling on it. That it does so has been determined by direct observations on *Hebecnema umbratica* and *Scopeuma stercorarium*. These two species have plastrons that are amongst the least resistant to wetting by excess pressures. Assuming a rain drop to be spherical, the pressure it exerts on striking the plastron is equivalent to a head of water about 1000 times its diameter. A large rain drop of, say, 4 mm diameter will thus, discounting the effect of the air cushion, be equivalent to an excess pressure of about 31 cm Hg. But this pressure is only exerted for a minute fraction of a second, and it therefore appears that the species with the least resistant plastrons are more than adequately protected against being struck by rain drops.

According to the resistance of the plastron to excess pressures, the species described in this paper belong to two very distinct groups: (*a*) Those that normally lay in cow dung (*Musca*, *Hebecnema*, *Scopeuma*, *Sepsis*, *Saltella*) and (*b*) those that normally lay in decomposing vegetable and animal substances (*Drosophila*). In the first group the resistance of the plastron to wetting falls off rapidly on exposure to excess pressures of over 30 to 40 cm Hg for 30 min, whereas the second group resists excess pressures of 60 to 100 cm for 30 min or more.

The incubation period of the eggs of the species of both groups is 1 to 2 days in warm weather. The plastron of the cow-dung species resists 3 ft. of water for the whole of the incubation period, and that of *Sepsis* and *Saltella* resists 8 ft. of water for a similar period. Under natural conditions it must be very exceptional indeed for the eggs to be covered for long periods by as much as 3 ft. of water, and the capacity to resist 8 ft. of water would seem to have little or no selective value but be, perhaps, related to some other attribute of selective value. The impression gained from considering the resistance to pressure of the plastron of the cow-dung species is confirmed when the enormously greater resistance to pressure of the plastron of the species of *Drosophila* is examined. The resistance of the plastron of *Drosophila* to wetting by excess pressures (table 3) so much exceeds anything it is ever likely to be called upon to resist under natural conditions that some other explanation for this extraordinary capacity seems likely.

As has been previously noted, if a plastron is to be an efficient respiratory structure, it must, apart from having a suitable surface area, resist wetting by the hydrostatic pressures and the surface-active substances to which it is likely to be subjected. Contamination of the water by any surface-active substance lowers its angle of contact and therefore reduces the hydrostatic pressure required to wet the plastron. This is clearly shown in figure 14 and table 6. When it rains, the plastron of both groups of eggs will be immersed in a layer of water contaminated by organic acids and other surface-active substances formed during the decomposition of the substrates in which the eggs are embedded. The danger of wetting by surface-active substances may be expected to be particularly acute for a short period after it has stopped raining. For instance, after it has stopped raining small pools of water are present on the surface of many cow pats. These soon evaporate, but while they are evaporating the concentration of surface-active substances dissolved out of the dung will reach higher levels than any reached when the layer of water over the eggs is continuously renewed.

Any change in the geometry or the nature of the surface of the plastron meshwork that increases its resistance to wetting by surface-active substances also increases its resistance to wetting in clean water by excess pressures, since wetting at excess pressures always occurs before there is a mechanical breakdown of the plastron meshwork. It thus follows that selection for greater resistance to wetting by surface-active substances inevitably results in an increase in the resistance to wetting by excess pressures. These facts seem to provide a satisfactory explanation of the paradox that the plastron of the egg of *Drosophila* is more resistant to wetting by excess pressures in clean water than is the plastron of many wholly aquatic insects, for example, the adults of some beetles (Thorpe & Crisp 1949) and the pupae of the Simuliidae (Hinton 1960) and some Tipulidae (Hinton 1957): under natural conditions the plastron of *Drosophila* is often exposed to concentrations of surface-active substances that rarely if ever occur in the streams in which the beetles and pupae live.

As has been shown, the dipterous eggs that have been examined can be placed in two groups according to the resistance of their plastrons to excess pressures, the cow-dung species and the species of *Drosophila*. Since any change in the resistance to wetting by excess pressures necessarily involves a change in the same direction in the resistance to wetting by surface-active substances, the one is a measure of the other. Under natural conditions there would appear to be no great difference in the maximum depths of water to which the two groups of eggs may be subjected, and, indeed, the eggs least resistant to pressure appear more than adequately protected. It thus appears that in so far as differences in the resistance of the plastron to wetting are related to outward circumstance, they are related to environmental differences in surface-active substances rather than hydrostatic pressures.

Most of the material in cow dung consists of lignin and undigested cellulose, and these decompose very slowly. It seems likely that the concentrations of organic acids and other surface-active substances in cow dung do not reach the levels to be found in liquifying flesh or liquifying vegetable materials from which most of the fats and proteins have not been removed. The surface tension of a temporary pool of rain water on the surface of a cow pat was found to be 50 dyn/cm. Under comparable conditions water standing on decomposing flesh has its surface tension reduced to 39 or 40 dyn/cm. Dilution has a relatively slight effect on surface tension. For instance, when the liquid collected from the surface of the cow pat was diluted with tap water to half its strength, the surface tension was increased to 53·5 dyn/cm and when diluted to a quarter of its strength the surface tension increased to 55·5 dyn/cm. In this connexion it is of interest to note that of all the cow-dung species examined it is only the Sepsidae that sometimes lay in decomposing animal matter, and it is the Sepsidae that have the most resistant plastrons.

It seems very probable that of the many species of flies that lay in or on decomposing vegetable and animal matter likely to be rich in surface-active substances, some will be found to have plastrons that are much less resistant to excess pressure than are those of the Sepsidae and Drosophilidae and thus be less well adapted to one of the contingencies of their environment. For instance, the eggs of all species of *Phaonia* that I have seen have a plastron on the inner surface of the flanges. Many of the species lay near or on decomposing animal matter, but the plastron of the few species tested does not resist excess pressures of as much as 30 cm Hg for 30 min. However, Thomson (1937) has found that *P. variegata* Meig. sometimes lays not on the larval food material but on moss near to it. Many of the eggs of *Phaonia* found by me in the field have not been in or on the larval food material but on stones and dry sticks near to it, that is, on materials that are not or only very slowly decomposing and likely to be very poor in surface-active substances. Thus more has to be known of the oviposition habits of *Phaonia* before it can be said that their eggs are not so well adapted to their environment as are those of *Drosophila*.

I am indebted to Mr E. A. Fonseca and Dr J. C. Hartley for the hours they spent kneeling over cow pats searching for eggs for me, to Mr E. B. Basden for the eggs of several species of *Drosophila*, to Dr L. Frost for cultures of *D. melanogaster*, to Mrs Joyce Ablett for technical assistance, and to Miss Elaine Harris for taking the electron micrographs.

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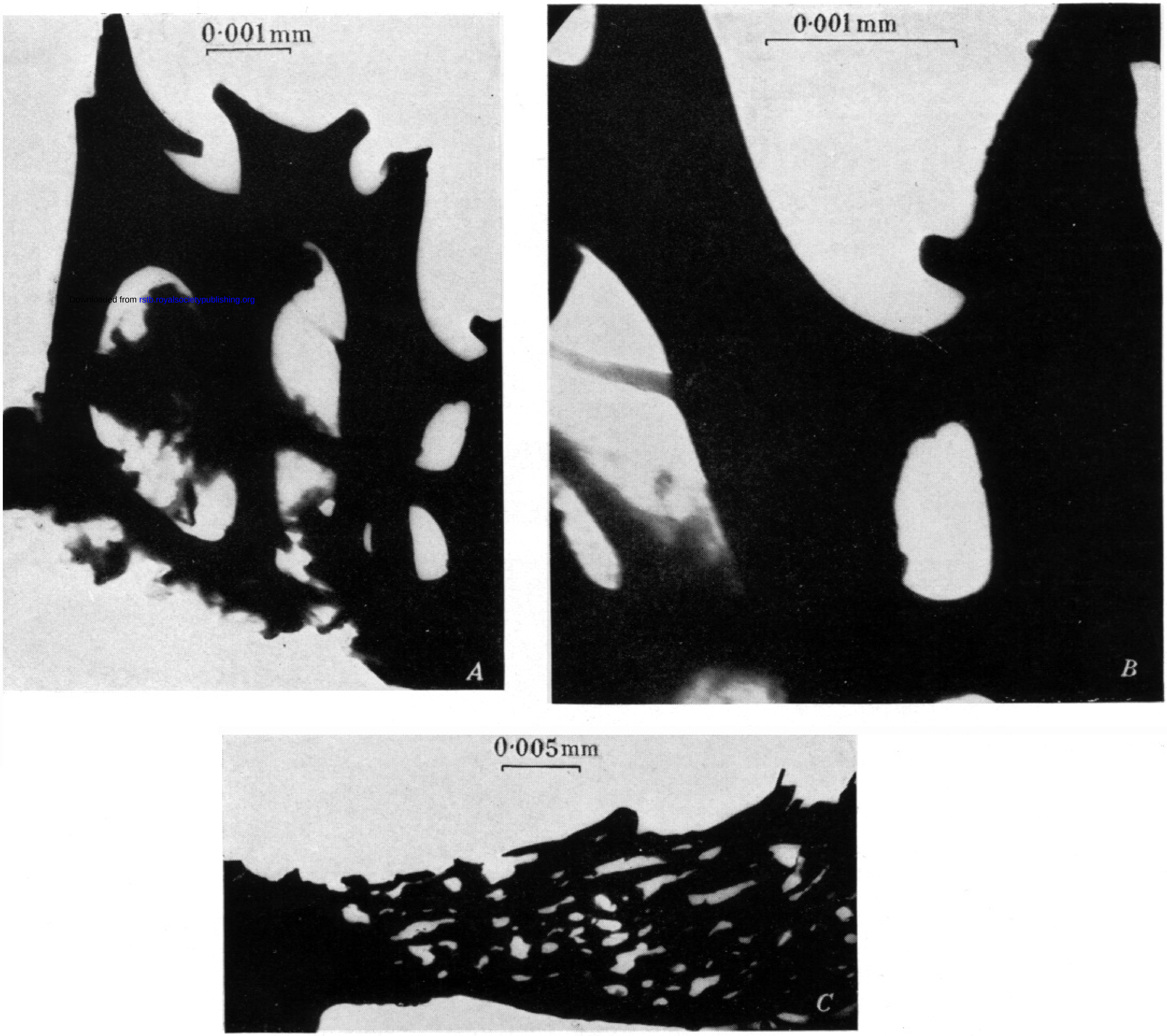

FIGURE 15. Electron micrographs of the respiratory horn of *Dryomyza flaveola*. (*A*) Plastron meshwork of middle layer of apex of horn, see figure 7*A*. (*B*) The same at a higher magnification. (*C*) A part of the inner wall of the apical pocket, see figure 7*A*.