The fine structure and biology of the egg-shell of the wheat bulb fly, *Leptohylemyia coarctata*

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With 3 plates (figs. 3 to 5)

Summary

The egg-shell of *Leptohylemyia coarctata* consists of 3 meshwork layers. The outermost wall is a sheet with perforations that are usually 5 to 10 μ wide. Across each of these perforations is a very fine network, the interstices of which are 0.1 to 0.2 μ wide. The middle meshwork layer is 2 to 7 μ thick. It is chiefly responsible for the mechanical strength of the shell. The inner meshwork layer is usually 2 to 4 μ thick. It consists of vertical columns extending inwards from the middle layer. The apices of the columns are branched in a plane normal to their long axes. These horizontal branches anastomose to form an open network, which is the innermost wall of the shell. The struts of all meshworks consist of fibrils 1 to 1.5 μ in diameter. The fibrils form a meshwork, the interstices of which are usually 2 to 5 μ wide.

When the egg is submerged in water, a film of air is held by the hydrophuge inner meshwork. This film of air functions as a plastron, but as a relatively inefficient one because above it there is a stationary layer of water 8 to 15 μ thick held by the 2 upper meshwork layers, which are hydrophil. Between the shell and the subchoral cuticle there is a waxy membrane. This membrane is closely attached to the inner face of the shell. It plays an important part in the resistance of the egg to desiccation.

Introduction

*Leptohylemyia coarctata* Fallén (Muscidae, Anthomyiinae) is an important pest of wheat and rye. The egg is laid in late July or early August on or just below the surface of the soil. Embryonic development is rapid, but the first instar larva diapauses within the shell. It does not hatch until the end of January or early March of the following year. During this long time it is exposed to a succession of dry and wet periods, and it may be immersed beneath a layer of water for long or short intervals. The complex structure and attributes of the shell are best understood in relation to the contradictory demands imposed upon the egg by an environment that is alternately dry and flooded. When the shell is seen from this point of view many of its features take on a new interest in that their functional significance becomes comprehensible.

Many previous descriptions have appeared of the egg-shell of the wheat bulb fly, but these are chiefly concerned with its general appearance and contain little or no structural detail. Until now, the most complex type of shell known in the higher flies (Cyclorrhapha) was that of the Muscinae (Hinton, 1960a). The shell of *Leptohylemyia* is as complex as that of the Muscinae, and many of its structures are similar.

Structure of egg-shell

The egg is 1·3 mm long and 0·37 mm broad. The anterior pole is slightly dilated (fig. 1, B), and the single micropyle is at the centre. Distinct hatching lines, such as are present in other Muscidae (Hinton, 1960a, fig. 2; 1960b, fig. 10), are absent: the larva uses its mandibles to cut its way out of the shell, as noted by Way (1959). The chorion consists of 3 distinct meshwork layers (fig. 2),
the outer one of which forms the longitudinal ridges that are so conspicuous in surface view. These ridges are usually more or less parallel, but they frequently anastomose, and in some restricted areas, especially near the anterior pole, they often form an hexagonal pattern.

The outer wall is a sheet of chorionin with more or less rectangular perforations about 5 to 10 μ wide (fig. 1, a). Around the posterior pole (fig. 1, c), and occasionally elsewhere, the perforations are more oval than rectangular. In surface view, mounts of the shell appear to have numerous holes between the large perforations, but this appearance is brought about by light internally reflected in the vertical columns or struts that support the outer wall. A very fine network extends across each perforation. This fine network was not distinctly resolved with the light microscope: electron micrographs show that the holes in the fine network are usually 0.1 to 0.2 μ wide. Drops of water applied to the outer surface of this network immediately displace the air below. The behaviour of drops of paraffin oil was much less regular. When they displaced the air below, they did so much more slowly than water, but they often rested for a considerable time on the surface without penetrating. In some eggs, the air below could not be displaced by drops of paraffin oil applied to the surface. In a few preparations, especially those of areas near or at the posterior pole, elliptical holes 2 to 4 μ long are present in the fine network extending across the coarse perforations (fig. 1, c). These holes do not appear to be artifacts. The general structure of the outer meshwork layer is shown in figs. 2 and 3, a, b. This layer is supported by more or less vertical struts of different sizes that arise from the middle layer.

The middle meshwork layer varies in thickness from about 2 to 7 μ. It consists of a dense meshwork, the interstices of which vary considerably in width: many, perhaps the majority, are between 0.15 and 0.3 μ (figs. 2; 4, a), but many are as wide as 0.5 μ and others are only 0.05 μ (fig. 4, b). This layer is chiefly responsible for the mechanical strength of the shell. It is strongly hydrophil.

The inner meshwork layer consists of vertical columns or struts that extend inwards from the lower part of the middle layer (figs. 2; 3, a, b). The apices of the struts are branched in a plane normal to their axes (fig. 5, b, c). These horizontal branches connect the apices of adjacent columns and also anastomose to form an open network. In transverse sections it is not possible to resolve the branches of this network with the light microscope, and it therefore appears as a continuous edge, as shown in fig. 2. However, the network can be resolved with the light microscope in a view normal to the plane of the inner surface. The vertical columns are usually 3 to 4 μ long, but in some parts are only about 1.5 μ long (fig. 5, b). Around the anterior pole a short distance from the micropyle the vertical columns are as much as 10 μ long. The density of the vertical columns is shown in fig. 3, c. The inner meshwork layer of most but not all eggs (see discussion below) is hydrofuge in contrast to the outer and middle layers of the shell, which are apparently always hydrophil.

The struts of all meshworks are composed of fibrils. These are about
FIG. 2. Transverse section through middle region of shell of L. coarctata.
FIG. 3

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1 to 1.5 μ wide, and they may thus consist of one or only a few polypeptide chains. The arrangement of the fibrils is not clear. They appear to form a meshwork (fig. 5, A), the interstices of which are usually about 2 to 5 μ wide. Since these spaces are an order of magnitude below the mean free path of the oxygen molecule, oxygen passing through these 'solid' struts of insect egg-shells can play no significant part in respiration, as previously noted by Hinton (1960c).

Water relations of egg-shell

When an egg is in contact with water or immersed in it, the 3 layers of the shell are affected in different ways. Water enters the outer layer but does not immediately displace all of the air in it unless it is subjected to a considerable excess pressure. A variable amount of air is trapped in the longitudinal ridges. Under natural conditions this air probably has little if any significance in respiration: it is separated from the air film of the inner meshwork by the layer of water contained in the middle meshwork layer. When much air is trapped in the outer layer, the egg floats; and if the air so trapped has a selective value it is probably to do with floating rather than respiration. Of the 124 eggs subjected to excess pressures of 7 to 40 cm Hg (table 1), 15 had the inner layer intact. In these eggs the percentage of air trapped in the longitudinal ridges varied from 10 to 80 (mean, 49%).

The middle meshwork layer of the shell is hydrophil. Its interstices were completely filled with water in all eggs subjected to excess pressures (table 1). But there need be no pressure difference for the middle layer to become water-logged: it will fill with water if the egg is immersed in a drop of tap-water or even if part of the surface of the shell is in contact with a thin film of water. When the film of air in the innermost layer of the chorion remains intact, the disappearance of air from the middle layer is very difficult to observe except either by splitting the chorion to obtain an edge-on view, or by dissecting off the chorion and examining a water mount under high power, preferably oil immersion.

Under natural conditions, the middle layer of the shell will be full of water whenever the chorion is in contact with free water. This water is at no point in contact with the embryo or subchoral membranes, but it is separated from them by a continuous air film 2 to 4 μ thick. Any transfer of water from the middle layer to the embryo or larva must therefore in the first instance occur in the gas phase. This is in striking contrast to the eggs of some other insects, e.g. the Nepidae (Hemiptera). In the Nepidae there is a specialized area of the chorion, called the chorionic hydropyle, that is concerned with the transfer of liquid water through the shell, and early in embryonic development a

Fig. 3 (plate), A and B, photographs of transverse sections through middle region of shell of Leptohylemyia coarctata. C, photograph of inner wall of shell from a view normal to the plane of the surface. Light internally reflected in the vertical columns of the inner meshwork layer makes the tips of the columns appear as bright spots.
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serosal hydropyle is formed against the inner face of the chorionic hydropyle (Hinton, 1961).

**Resistance of inner meshwork layer to flooding**

When an air-filled shell is immersed in water, the film of air held in the interstices of the inner meshwork usually remains intact despite the displacement of much or all of the air in the outer layer and all of the air in the middle layer. That this is not an effect of merely trapping the air in the inner layer is evident from the fact that the air layer here remains intact even when the outer layers are gradually filled by adding small drops of water to one end of the shell.

The inner gas-containing layer is limited above by the meshwork of the middle layer. At this level the interstices of the middle layer are not usually less than about 0.15 to 0.2 µ broad. The failure of water to penetrate into the inner layer and displace the air in it must be due to the hydrofuge nature of either the lowermost struts of the middle layer, or the vertical struts of the inner layer, or both. When fragments of shell are immersed in water, the air film of the inner layer usually remains intact or nearly so, which suggests that the failure of water to enter this layer from the middle layer is at least in part due to the hydrofuge surface of the vertical struts.

**Table 1**

Effects of excess pressure upon the retention of the air contained in the innermost meshwork layer (20° to 21° C).

<table>
<thead>
<tr>
<th>No. of eggs</th>
<th>Pressure (cm Hg)</th>
<th>Time (h)</th>
<th>% retaining over 90% of air in innermost layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>7</td>
<td>24</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>384</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>10</td>
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<td>50</td>
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<tr>
<td>10</td>
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<td>10</td>
<td>30</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>40</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
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<td>40</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

The eggs are frequently subjected to flooding in nature. The resistance of the innermost layer of the shell to wetting by excess pressures is shown in table 1, which is based upon eggs exposed to tap-water of a surface tension of 72.8 dyn/cm. It seems probable that under natural conditions the eggs

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**Fig. 4 (plate).** A and B, electron micrographs of different parts of the middle meshwork layer of the shell of *L. coarctata.*
FIG. 5
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will rarely be subjected to pressures of much more than about 7 cm Hg and then only for relatively short periods. The variation shown in the resistance to wetting is somewhat surprising, especially in view of the fact that all eggs were 47 to 50 days old and had the same previous history. The air film held in the inner layer of the shell functions as a plastron: it is constant in volume, resists a pressure difference, and has an extensive water-air interface. It is, however, a very inefficient type of plastron in that a stationary layer of water that forms a diffusion boundary about 8 to 15 μ thick is held above it by the meshworks of the middle and outer layers of the shell.

The eggs are laid from late July to August and hatch from the end of January to early March. During most of this time they are in diapause, when it may be safely assumed, by analogy with other eggs in diapause, that their oxygen uptake is very low. Flooding of the innermost layer, if ever critical, is probably so only before diapause is initiated and after diapause is broken, when the oxygen uptake will be very much higher.

The shell and the resistance of the larva to desiccation

In considering the resistance of the egg to drying a distinction must be made between the tolerance of the tissues to dehydration and the capacity of the chorion and subchoral and larval cuticles to prevent dehydration. Bardner and Kenten (1957) have shown that eggs flatten and collapse within 6 days when kept at a relative humidity of 34%, but that nearly 20% hatch in due course if transferred to a water-saturated environment after an exposure to 34% r.h. for 13 days. These and other figures given by Bardner and Kenten suggest that the eggs can, as they point out, recover from considerable temporary dehydration.

At first sight it would appear that the shell is of little importance in preventing dehydration, since its 3 layers are open meshworks. The water contained in the interstices of the outer and middle meshworks is lost very readily: a water-filled chorion loses all of its water in a few minutes when exposed to about 60% r.h. The meshworks of the shell hold a relatively stationary layer of air which will delay the time taken for equilibrium to be reached between the moisture content of the tissues and the drier ambient air. The chorion is itself hygroscopic, and so may to a slight extent serve to damp down short-term humidity fluctuations.

In preliminary experiments intact eggs were exposed to 0% r.h. at 25° C for periods up to 12 h. At the end of 12 h there was no, or no obvious, shrinkage of the larvae. Because of the open nature of the shell wall, it would seem that this high resistance to water-loss was due chiefly to the impermeability of the subchoral and larval cuticles and possibly not to any attribute of the shell. To test this, the amount of shrinkage of 3 groups of 10 larvae, each exposed

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*Fig. 5 (plate).* Electron micrographs of the shell of *L. coarctata.* A, a single strut from the middle layer. B, middle and inner layers of the shell. C, middle and inner layers of the shell.
to 0% r.h. at 25°C for 12 h, was compared. In the first group the shell was intact, in the second group the anterior end of the shell immediately around the micropyle was removed, and in the third group larvae enclosed in their subchoral cuticles were taken out of the shell without damaging the subchoral cuticle. As in the preliminary experiments, the larvae of the first group showed little or no shrinkage. Those of the second and third groups were distinctly though variably shrunken, those of the third group being on the average slightly more shrunken than those of the second group.

The results of the experiments could be accounted for if there were a very thin but relatively impermeable membrane adhering to the inner face of the shell wall. Attempts to detect such a membrane microscopically were not successful, and the electron micrographs (fig. 5, B, C) show no trace of such a structure. However, the material prepared for the electron microscope was at one stage washed in wax solvents that might well have removed a delicate waxy membrane. Drops of water applied to the inner face of the shell do not spread and do not displace the air in the inner meshwork. Drops of an aqueous solution of malachite green were added to the inner face until there was a heavy deposit of pigment in the form of a ring. The shells were then washed in ethanol, and the rings of pigment could be lifted off the surface with a pin. None of the pigment had penetrated into the inner meshwork layer. The same results were obtained when the experiment was repeated with an aqueous solution of eosin. Drops of butyl alcohol and paraffin oil spread but do not displace the air. However, when the shell was treated with a variety of wax solvents, including boiling for 10 min in chloroform, the butyl alcohol and paraffin oil immediately displaced the air in the layer, but the drops of water behaved as before. These results, together with those of the desiccation experiments, find a ready explanation if there is indeed a thin waxy membrane attached to the network that forms the inner wall of the shell. The behaviour of water on the surface after the shells were treated with wax solvents is consistent with the view that the chorionin of the inner meshwork, unlike that of the middle and upper meshworks, is itself hydrofuge. The existence of waterproofing waxy layers or membranes attached to the inner face of the chorion was first convincingly demonstrated in the egg of *Rhodnius prolixus* by Beament (1946, 1949).

A few irregularities in behaviour have to be noted. In nearly 10% of the shells that had not been treated with wax solvents, drops of water, butyl alcohol, and paraffin oil readily penetrated the apparently undamaged inner face and displaced the air in the inner meshwork. The cause of this variation is not known. The larvae appeared to be healthy and were in the expected stage of morphological development. In the first experiment listed in table 1, one of the 10 eggs had no air left in the inner layer and another had only about 5%. Thus here again some shells appeared to have the inner meshwork layer hydrophil instead of hydrofuge. In a few of the shells treated with boiling chloroform, butyl alcohol and paraffin oil failed to penetrate rapidly through restricted areas of the inner face. This could be due to a failure of the solvent
to remove all of the waxy membrane, or, more likely, to the inner face having a continuous sheet of chorionin occasionally replacing areas of network.

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References

— 1961. Ibid., 7, 224.