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EGGS OF BRITISH MENISCUS MIDGES (DIPTERA: DIXIDAE) OBSERVED BY SCANNING ELECTRON MICROSCOPY

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Introduction

In their early stages, meniscus midges are truly inhabitants of the water-film, in a variety of habitats from still to flowing water. Larvae dislodged into the water from their usual inverted-U-shaped position in the film drawn up around emergent leaves and stems, carry a bubble of air between their infolded bristle-fringed posterior paddles and soon surface again; they may make S-shaped curves across the surface of the water before taking up position in the meniscus again. The pupa also lies in the water-film drawn up by some emergent substratum, receiving air through two characteristic respiratory trumpets, and remaining motionless unless disturbed. Stillness is in fact a feature of this Family of non-biting midges, and the adults, especially the females, stand for hours in a vertical position, head up, motionless or slowly waving the third pair of long legs. A unique type of semaphore, however, is demonstrated by both males and females of one species of *Dixella,* by rapid, or sometimes more prolonged, wing flexes in which two, three or more individuals may take part in turn. Occasionally this is an obvious preliminary to mating. In all the species of *Dixella* studied so far, mating can readily be observed in laboratory cultures, and it is usually followed by oviposition within the next two days. The gelatinous egg clutches are laid in the water-film around emergent plants or pebbles, or on the side of the container, where they are more easily seen; the clutches of *Dixella filicornis* and *D. amphibia* DeGeer are less compact than those of the other species of *Dixella,* and may be loosely entwined among the stems and leaves of a marginal plant. For study purposes, cultures were contained in cylinders of clear plastic six inches in height and in diameter, as previously described (Goldie-Smith 1989a). The cylinder was inserted into a plastic base and a half petri dish containing appropriate water, sediment and vegetation was placed inside for the growing larvae. Small drops of honey, jam and/or marmalade were later placed on strips of paper hung over the side of the container; adults were often seen to tilt over at an angle of 45 degrees to suck the juice; the strips could easily be removed if they became mouldy. The needle-perforated cover of cling-film had to be ample enough to enclose the wrist while manipulations were being carried out within the cylinder.

Previous work (Goldie-Smith 1989a, b, c) utilizing light microscopical techniques, revealed the eggs of Dixidae to be unexpectedly complex structures, comparable with those of the closely related Family, Culicidae. In the present study, eggs were cultured (by E. K. G-S.) from ten species identified with Disney's (1975) key, and prepared for scanning electron microscopy (SEM; by J. R. T.). Among the ten species, of the fourteen* known from Britain, three types of eggs can be distinguished. Two of these are characteristic of the genus *Dixella,* and the third type is characteristic of *Dixa* (Table 1). The following account of the previously-undescribed egg of *Dixella martinii* Peus, illustrates Type I in particular. It also serves as the basic pattern of the dixid egg in general.

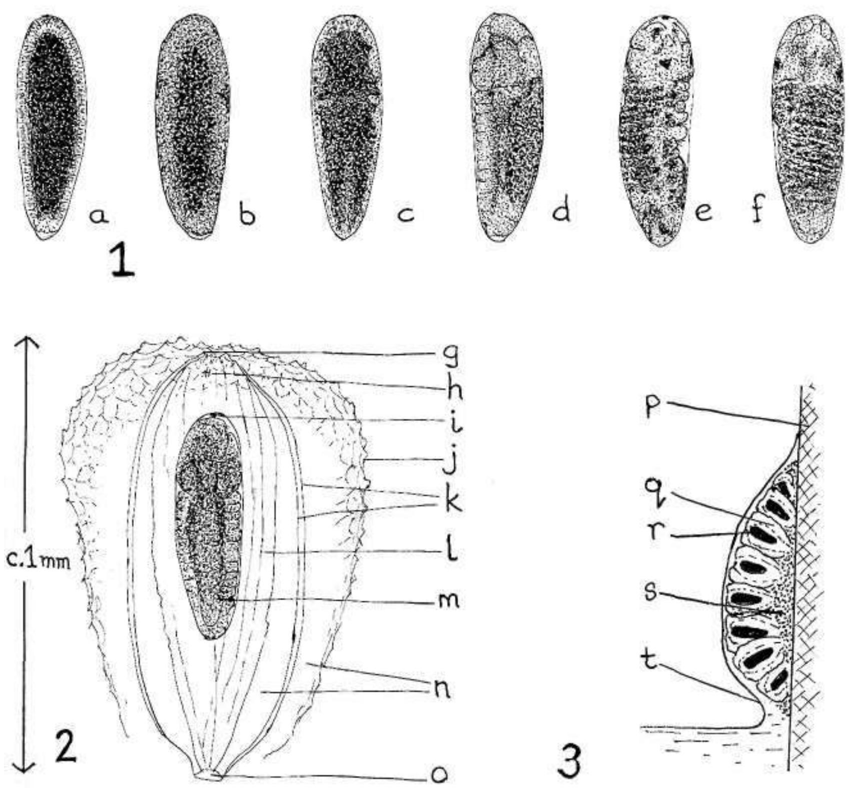
Table 1. Three types of eggs in Dixidae.

Type	*Description*	*Species*
I	Bulbous, meshed	*Dixella aestivalis* Meigen *Dixella attica* Pandazis *Dixella autumnalis* Meigen *Dixella martinii* Peus *Dixella obscura* Loew
II	Streamlined, smooth	*Dixella amphibia* DeGeer *Dixella filicornis* Edwards
III	Streamlined, minutely spiculated	*Dixa nebulosa* Meigen *Dixa nubilipennis* Curtis *Dixa submaculata* Edwards

*(**Note added in proof.** Dr R. H. L. Disney has identified *Dixella graeca* Pandazis among the specimens examined by E. K. G-S from the NCC East Anglia Survey (A. P. Foster), bringing the British list to fifteen species.)

The egg of Dixella martinii (Figs 1-3, 6)

The embryo, usually just under 0.5mm long, with a broad, blunt apex and a more pointed posterior end, is enclosed in a bulbous envelope of transparent jelly within a thin skin. The latter is conspicuously meshed over the upper third or half, decreasing towards the base. These meshes are characteristically hexagonal, but may be five- or seven-sided (Fig. 6). The apex opens into a comparatively shallow micropylar funnel and there is less of a tendency for the meshes to be drawn out into a more elongated shape around the opening than in *D. autumnalis* (Fig. 4).



FIGS 1-3. *Dixella martinii* Peus. **1a-f**, successive stages in embryo development: a, "hyaline rim" stage; b, early differentiation; c, cephalic groove appearing; d-f, advanced differentiation, d, dorso-lateral view; e, ventro-lateral view; f, dorsal view, eye rudiments apparent. **2**, egg: g, shallow micropylar cup; h, seminiferous residue; i, micropylar spot; j, meshed outer skin; k, outer and inner layers of "capsule"; l, refractive "strings"; m, differentiating embryo; n, transparent jelly; o, "hyaline plug." **3**, diagrammatic representation of typical position of egg clutch of this or any species of *Dixella* Type I: p, emergent leaf or stem; q, gelatinous envelope of egg; r, embryo; s, gelatinous matrix; t, water film.

Within the meshes the surface is smoothly rippled, and thus comparable with that of *D. attica.* This outer skin terminates by gradually merging into the common gelatinous matrix which holds all the eggs together, at least at the base, and attaches the clutch to the substratum. An inner "capsule", however, bounded by a double membrane in this species, is closed at the base by a refractive thickening termed the "hyaline plug". At its upper end the capsule unites with the outer skin to form the micropylar opening. Thin refractive "strings" traverse the capsule from this basal plug to the apex. A tiny mass of seminiferous residue lies between the micropylar opening and the embryo, and a dark micropylar spot at the top of the embryo indicates where the sperm entered the egg. The whole egg is about 1 mm in length.

The development of the embryo takes about five days under normal laboratory conditions. Under the light microscope, it can be observed through the transparent gelatinous envelope. The pattern in *D. martinii* (Fig. 1 a-f) is very similar in all the *Dixella* species studied. The hatching larva leaves yet another delicate membrane behind, torn and crumpled to different degrees in different species. Normally the larvae readily develop in the laboratory, but in this study they were frequently infected with a very persistent virus (Goldie-Smith 1987).

A typical position of a clutch of *Dixella* eggs, Type I, is as shown in Fig. 3. The whole cluster, of up to a hundred or more eggs but usually fewer in our cultures, lies in the water film drawn up by an emergent leaf or stem or other substratum (such as a pebble). This protects the eggs from drying out during development. A vertical join in the side of a plastic container provides sufficient capillarity to attract a female seeking a site to deposit her eggs.

Preparation of eggs for SEM

Eggs were cultured as described by Goldie-Smith (1989a). Each *Dixella* clutch was carefully transferred to a petri dish and allowed to hatch. Still containing jelly, the eggs were washed in distilled water and then transferred to a 5% glutaraldehyde fixative solution for three hours, before thorough rinsing in distilled water and storage at 4°C until being further processed for SEM. As the *Dixa* eggs were not viable (see below), the whole egg, including the embryo, received the same treatment as the *Dixella* egg from which the larva had emerged.

Subsequently in the laboratory, the eggs were post-fixed in osmium tetroxide, dehydrated in an ethanol series and critical-point dried (via liquid $C0_2$) before sputter-coating with gold and examination with a Jeol 100C electron microscope fitted with an ASID-4D ultra-high resolution scanning attachment.

Egg Type I: bulbous, meshed (Figs 2, 4-7, 11)

In *Dixella autumnalis* (Figs 4, 5) the minute dotting of the entire surface over the meshes is due to the presence of closely-packed tiny refractive rods. This effect is much more pronounced in the somewhat more elongated eggs of *D. aestivalis,* resulting in a scintillating surface "bloom" when viewed under the light microscope. The knobs or pegs causing this are seen from the underside of the outer skin, in Fig. 11. No such dotting has been observed in *D. martinii* (Fig. 6) or *D. attica* (Fig. 7); in both these species, but especially in *D. attica,* the ridges of the mesh are so strongly developed that they cause a holly-leaf effect in a side view of the whole egg (Fig. 2). Within the meshes the surface is rippled (Fig. 6) or slightly ridged (Fig. 7).

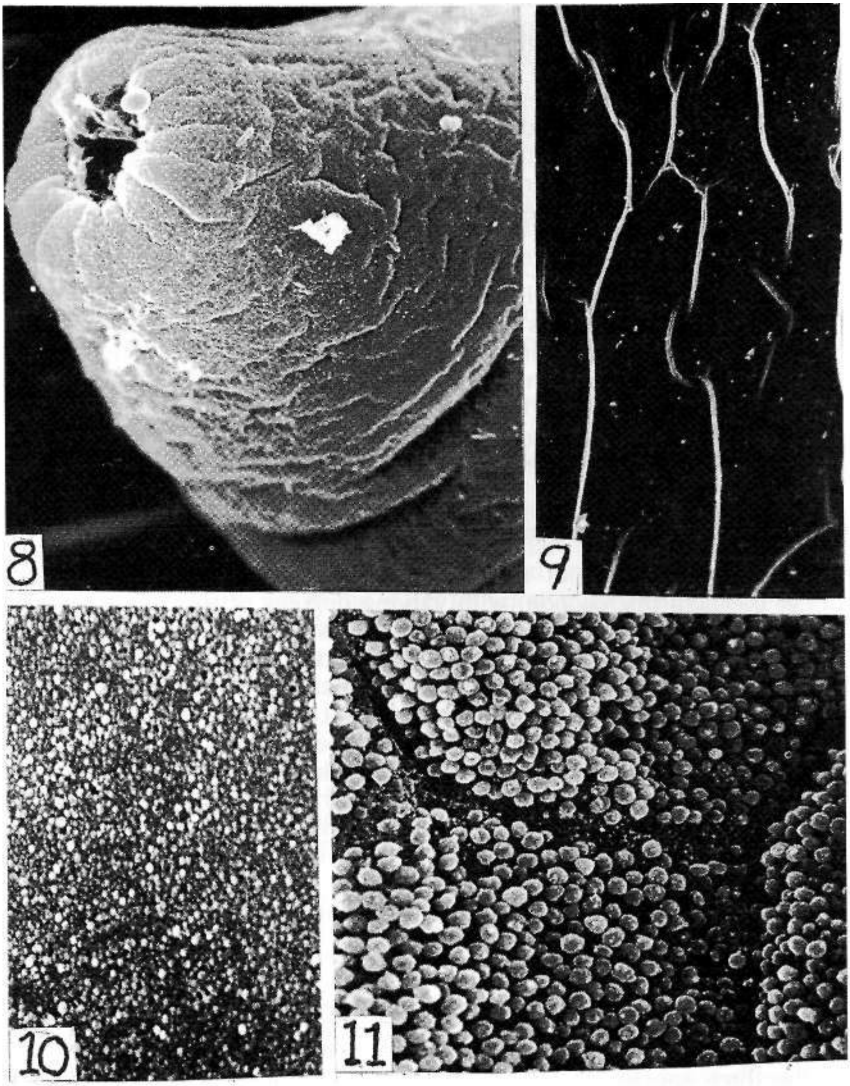
The egg of *D. obscura* is the most bulbous, almost spherical (Goldie-Smith 1989c) and scanning electron micrographs (not shown here) corroborate the observation that it is also meshed, though apparently this is less pronounced than in the other Type I species. More detailed study of this uncommon species is desirable, especially as it differs in additional structural features from the other Type I species.

Egg Type II: streamlined, smooth (Figs 8, 9)

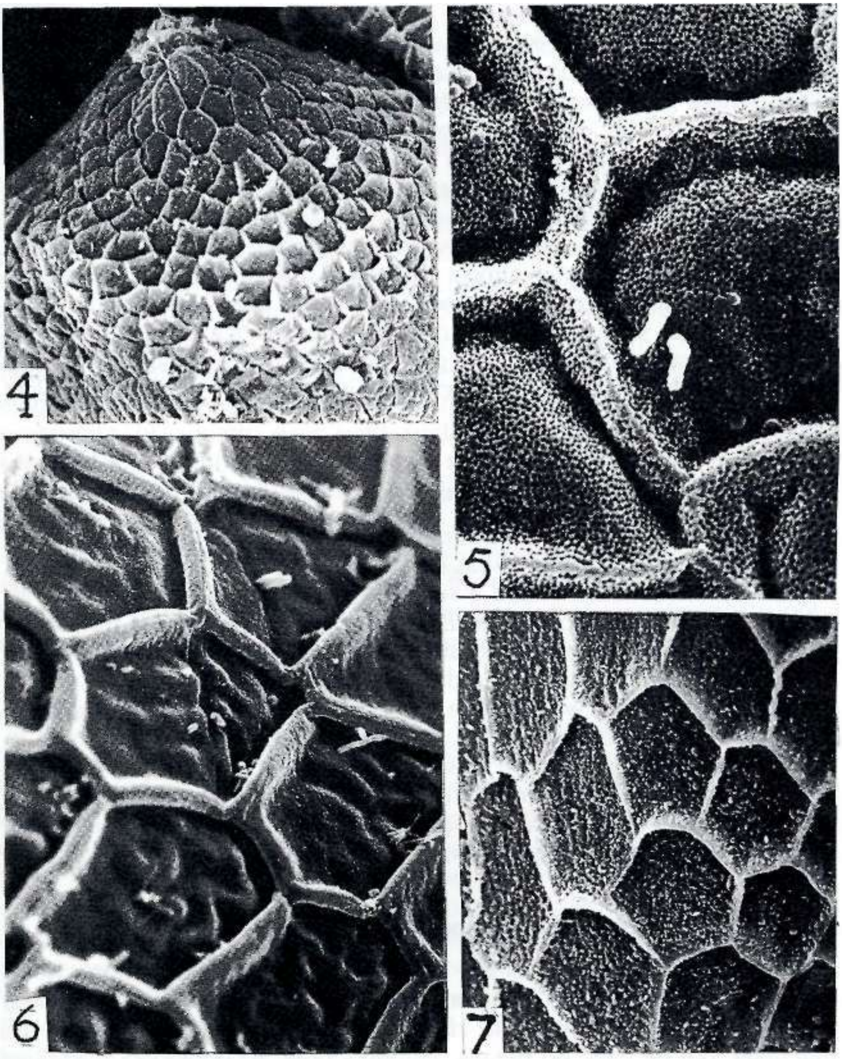
The streamlined eggs of *Dixella amphibia* appear generally smooth under the light microscope, with varying degrees of short transverse or oblique folds. However, Fig. 8 clearly shows a pattern of irregular folds or wrinkles, which become more regular around the micropylar opening. An appearance of extremely faint meshing has sometimes been observed in the lowermost third. A similar effect over most of the surface of the *Dixella filicornis* egg has sometimes been seen with the light microscope. No meshing has been revealed by the SEM in either species (Figs 8, 9) and subsequent studies suggest that the effect is due, not to meshing in the outer coat, but to a chunkiness of the jelly within, enhanced by the narrow lines of wall thickenings which occur in certain areas of the eggs of both species. In general structure and development, however, these eggs closely resemble those *oi* the other species of *Dixella* which have been studied.

Egg Type III: streamlined, minutely spiculated (Fig. 10)

Although following the same basic pattern as in *Dixella* species, the eggs of the three species of *Dixa* that have been studied show outstanding differences from those of *Dixella.* Under the light microscope they have quite a strong brown colour *(Dixella aestivalis* eggs may be brownish, but are never more than slightly tinged), frequently with irregular



FIGS 4-7. Scanning electron micrographs of *Dixella* eggs. **4, 5,** *D. autumnalis*: 4, upper third (x 300); 5, detail of mesh (x 2,400); **6,** *D. martinii* mesh (x 2,400); **7,** *D. attica* mesh (x 1,200).



FIGS 8-11. Scanning electron micrographs of eggs of Dixidae. **8,** *Dixella amphibia,* apex of egg, with micropylar funnel (x 960); **9,** *Dixella filicornis,* delicate grooving of surface (x 4,800); **10,** *Dixa nebulosa,* spicules of outer skin (x 2,400); **11,** *Dixella aestivalis,* "knobs" seen from under the outer skin (x 6,000).

markings of a very characteristic, almost magenta, red, which has never been seen in *Dixella. Dixa* clutches are found more often at the surface of the water or only just above it, rather than higher up the substratum, and the jelly matrix is dense and tough. These differences are probably related to the more common occurrence of *Dixa* species in running water, where they may be subjected to currents, eddies and spray. The difference in habitat, in general, may also account for the more robust texture of *Dixa* eggs, in which the surface "skin" is packed with extremely minute refractive rods or spicules (Fig. 10) which contrast with the "knobs" of *Dixella aestivalis* (Fig. 11). The length and very close packing of the *Dixa* spicules give the covering of the egg a tough consistency and render it almost opaque. Furthermore, the otherwise streamlined *Dixa* egg is flattened over the apex and drawn out posteriorly to a sharper point than in the *Dixella* egg, and the embryo itself is characteristically more pointed posteriorly in *Dixa* than in *Dixella.* Under the conditions of culture in this study, none of the *Dixa* eggs developed further.

Egg production and viability in culture

The production of eggs of Dixidae in culture was first reported for *Dixella autumnalis* (Peach 1983; Peach & Fowler 1986). Subsequently, Goldie-Smith (1989a-c) made a series of studies of this and nine other species. In the seven species of *Dixella* studied, mating was frequently seen in the cultures, and the resultant eggs developed and hatched. No mating of the adults was seen in the cultures of the three species of *Dixa* studied, and the eggs which were found did not develop. The tiny mass of seminiferous residue visible in each *Dixella* egg was not observed in *Dixa* (although *Dixa* eggs are less transparent, such masses would have been discernible). It is assumed that the *Dixa* eggs were unfertilized and that the adults require more specialized conditions for mating than those of *Dixella;* perhaps aerial swarming is essential for *Dixa.*

Eggs of Dixidae are similar to those of Culicidae

Among the scanty records of the eggs of Dixidae in the literature, Rolle (1928) noted a marked difference in the eggs of various species, mentioning *Dixella amphibia, D. aestivalis, Dixa nebulosa* and D. *maculata,* but he gave no details. Hubert's brief account (1953) implies that he considered the egg to include the gelatinous covering, but most older records treat the embryo simply as the egg. Recent studies reveal the dixid egg to consist of the embryo, surrounded by a complex gelatinous envelope, and contained within an outer "skin" which is more or less sculptured. Thus defined, although the gelatinous envelope is unique, the egg of Dixidae appears similar to that of Culicidae, especially in the types of sculpturing and variations in different regions of the egg (Goldie-Smith 1989b).

Application of the plastron theory to dixid eggs

Hinton (1968) warned of the pitfalls in trying to trace homologies between the various layers of the outer coats of different insect eggs. It seems possible, however, that his "plastron" theory may be applicable to Dixidae. "A plastron is a gas film of constant volume and an extensive water-air interface. Such films are held in position by hydrofuge hairs or hydrofuge meshworks of various kinds . . ." (Hinton 1981, p. 108). Hinton (p. 96) also states: "It now seems that the majority of terrestrial eggs have meshworks in the chorion that hold a layer of gas. The proportion of aquatic eggs with such meshworks is evidently much smaller." A number of structural types are described, including meshworks and one in which "a continuous film of air is held between the vertical columns in the inner part of the chorion. The columns are arranged in irregular hexagons". It seems reasonable to suggest that the fine sculpturing and meshwork seen in the eggs of Dixidae may serve the function of a plastron or physical gill, ensuring that a continuous supply of oxygen is available to the developing embryo.

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