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[SHORT COMMUNICATION]

A New Technique for Elucidating the Fine Morphological Structures of Animals, Applied to the Analysis of the Micropylar Canal Passage in a Snakefly, *Inocellia japonica* Okamoto (Insecta: Neuroptera, Raphidiodea)

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The eggs of the snakefly *Inocellia japonica* Okamoto, the embryogenesis of which we have been studying, are cylindrical in shape (Fig. 1). At the anterior pole of the egg is a knob-like projection (Fig. 2), around the base of which micropyles open (Woglum and McGregor, 1959; Aspöck and Aspöck, 1991). However, nothing definite is known about the internal structure of the projection, such as the passage of the micropylar canal, because it is too complex to comprehend using ordinary methods. Thus, we developed a new method, to grasp the complex passage of the micropylar canal of the egg. This method may be helpful for understanding the other fine internal structures of animal tissues or organs. In this report, we give details of the procedure.

1. Eggs of *I. japonica* were punctured with a fine needle and fixed with alcoholic Bouin's fluid for 3 h, rinsed and stored in 70% ethyl alcohol.
2. Fixed eggs were orientated by embedding then into agar, dehydrated through a graded ethyl alcohol series, and replaced in acetone. Then, eggs were embedded in epoxy resin, Quetol 651. We also used another epoxy resin, Spurr, as a substitute.
3. Specimens were processed into 1–2 μm serial sections with a semi-thin microtome equipped with a diamond knife.
4. The sections were serially stuck to a cover glass coated in advance with albumin-glycerin. Then, the resin was removed with a Maxwell solution for Epon [Saturated potassium hydroxide absolute methyl alcohol + propylene oxide (2:1) solution] (Maxwell, 1977) for 2 min, and residual resin was rinsed with absolute methyl alcohol. For the eggs embedded in Spurr, another Maxwell solution for Spurr [Saturated sodium hydroxide absolute ethyl alcohol + propylene oxide (1:1) solution] (Maxwell, 1977) was used for the removal of resin (5 min), and residual resin was rinsed with absolute ethyl alcohol.
5. Then, sections were dried using a *t*-butyl alcohol freeze dryer, coated with gold and observed under a TOPCON SM-300 scanning electron microscope.

The sections thus prepared are shown in Figure 3A, B. The direction of the micropylar canal is clearly demonstrated, and its passage can be grasped by carefully comparing the serial sections.

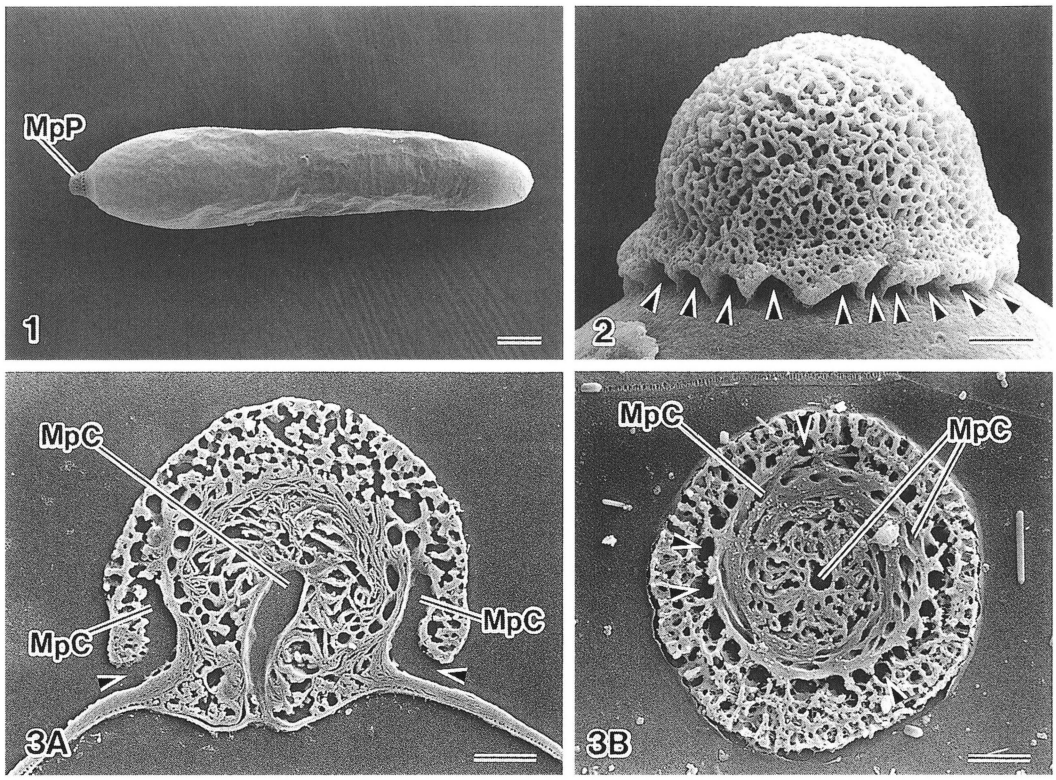

Fig. 1 An egg of the snakefly *Inocellia japonica* Okamoto. The anterior pole is to the left, where there is a micropylar projection (MpP). Scale = 100 μm.

Fig. 2 Enlargement of the micropylar projection. Micropyles (arrowheads) open around the base of the projection. Scale = 10 μm.

Fig. 3 SEMs of longitudinal (A) and cross (B) sections of the micropylar projection, from which epoxy resin was removed. The passage of the micropylar canal (MpC) is clearly demonstrated. Arrowheads show micropyles. Scales = 10 μm.

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