Scanning Electron Microscopy of the Egg Attachment Structures of *Electrogena zebrata* (Ephemeroptera: Heptageniidae)[1]

ELDA GAINO AND MASSIMO MAZZINI

Istituto di Zoologia, Università di Genova, Via Balbi 5, Genova 16126 and
Dipartimento di Biologia Evolutiva, Università di Siena,
Via Mattioli 4, Siena 53100, Italy

Abstract. Scanning electron microscopy of the chorionic egg attachment structures of *Electrogena zebrata* permitted study of the organization of knob-terminated coiled threads (KCT's). With the exception of the micropyles, chorionic sculptures cover the entire cell surface. Each KCT is composed of many fine fibers tightly spiraled together to form a single thread terminating in a distinct knob. The KCT's are isolated by a ring of protuberances interconnected by thin strands. Available evidence suggests that upon attachment, KCT's extend individual threads and fix their rounded terminal knob to the substrate. A high concentration of KCT's attachment structures may represent a more specialized adhesion mechanism among the Ephemeroptera.

The egg chorion of mayflies frequently has attachment structures, the anatomy and distribution of which may be useful in taxonomy (Kopelke, 1980; Koss, 1968; Koss & Edmunds, 1974; Malzacher, 1982). According to the classification proposed by Koss & Edmunds (1974), the chorionic attachment structures of *Electrogena zebrata* (Hagen, 1864) are considered to be of the type having individually knob-terminated coiled threads (KCT's).

The present paper reports a scanning electron microscopic study of the structural organization of KCT's as anchorage devices on the egg chorion of *E. zebrata*.

MATERIALS AND METHODS

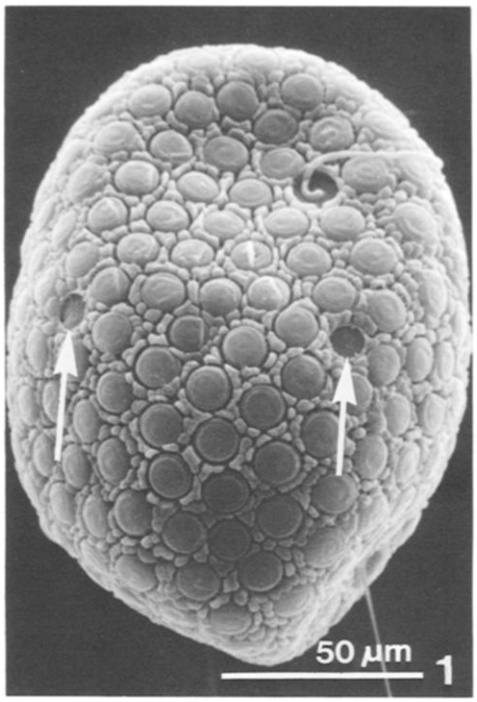
Eggs were removed from nymphs collected from the Catena Stream by S. Gaiter, 15 August 1981, and preserved in 95% ethanol. Eggs were critical-point dried using liquid CO_2 in a Bomar apparatus. Dried specimens were placed in

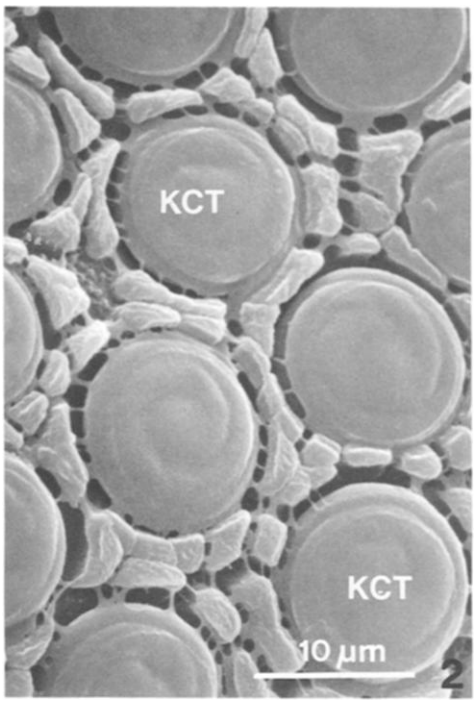
[1] Research supported in part by the Italian National Research Council (CNR) under "Gruppo Nazionale di Biologia Naturalistica," Contrib. No. 3350340, and in part by the Ministry for Public Education (MPI) under "Biogeografia delle terre del Mediterraneo Occidentale."

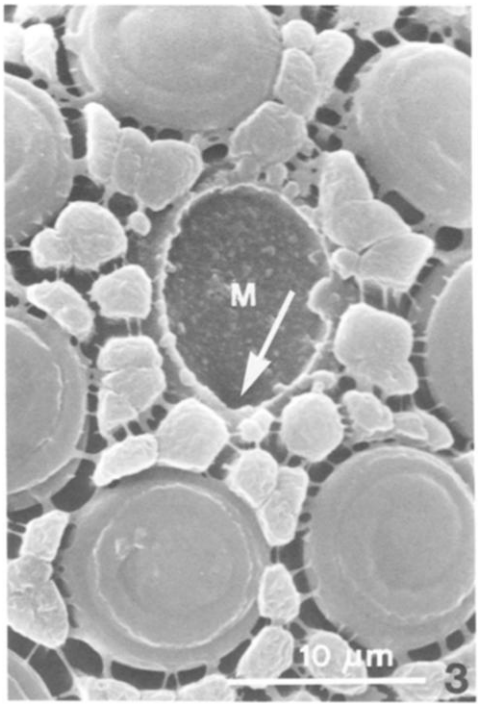
→

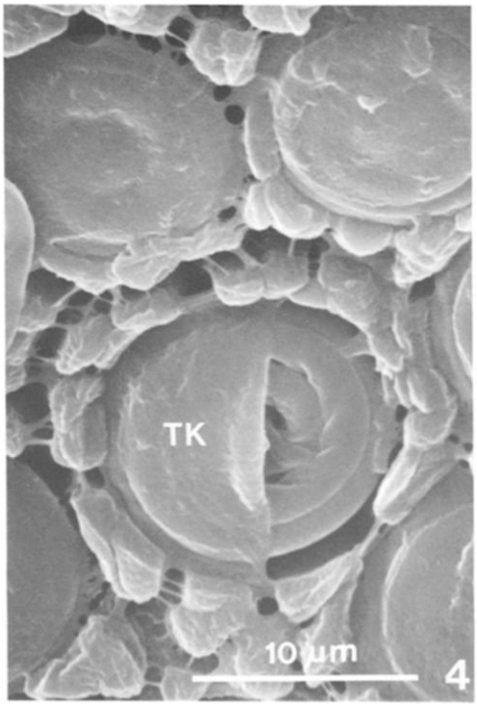
FIG. 1. Overview of the egg of *Electrogena zebrata*. Two micropyles (arrows) are visible. FIG. 2. Chorionic pattern showing the arrangement of knob-terminated coiled threads (KCT's) separated from one another by a ring of protuberances. FIG. 3. Micropyle (M) with ovoid sperm guide and micropylar opening (arrow) among KCT's. Each knob has a transparent covering that reveals its underlying coiled pattern. FIG. 4. A KCT at the beginning of its extension. The terminal knob (TK) is ruptured on one side.

TRANS. AM. MICROSC. SOC., 106(2): 114–119. 1987.


50 µm
1


KCT
KCT
10 µm
2


M
10 µm
3


TK
10 µm
4

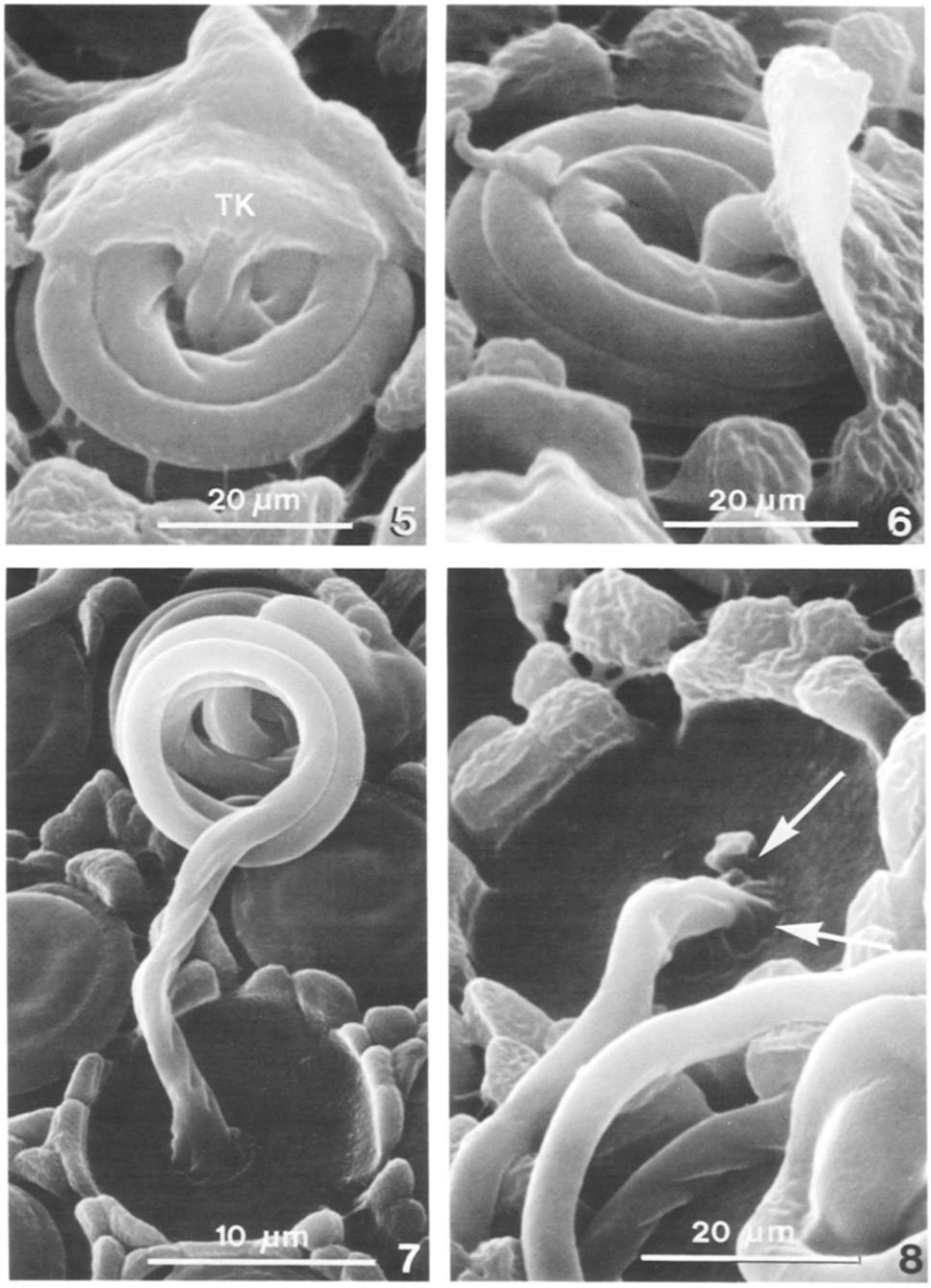


FIG. 5. A KCT of *Electrogena zebrata* with a partially elevated terminal knob (TK) revealing the coiled thread beneath. FIG. 6. Elevated terminal knob partially attached to the chorionic surface. FIG. 7. Coiled thread showing its extended configuration. FIG. 8. An extended thread emerging from the central part of its chorionic recess. The thread is made up of a bundle of fibers at its base (arrows).

a vacuum evaporator and spattered with gold-palladium in a Balzer Union evaporator. Some eggs were studied without critical-point treatment. Observations were made with a Philips 505 scanning electron microscope.

The terminology of Koss & Edmunds (1974) is employed in the present paper.

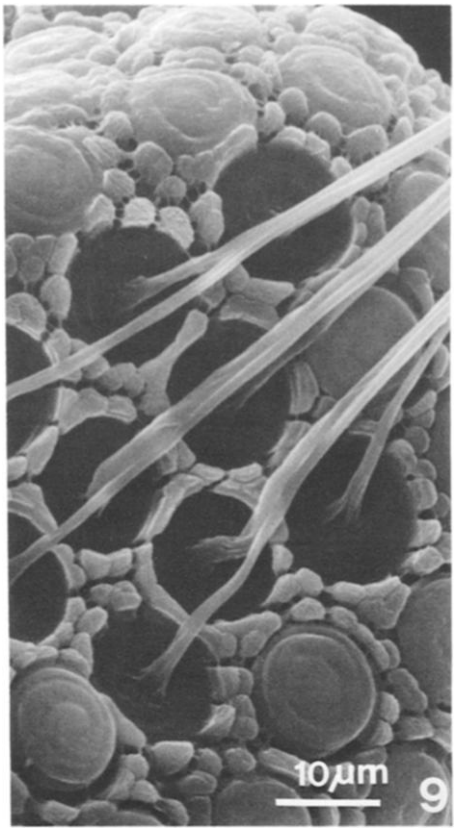
RESULTS

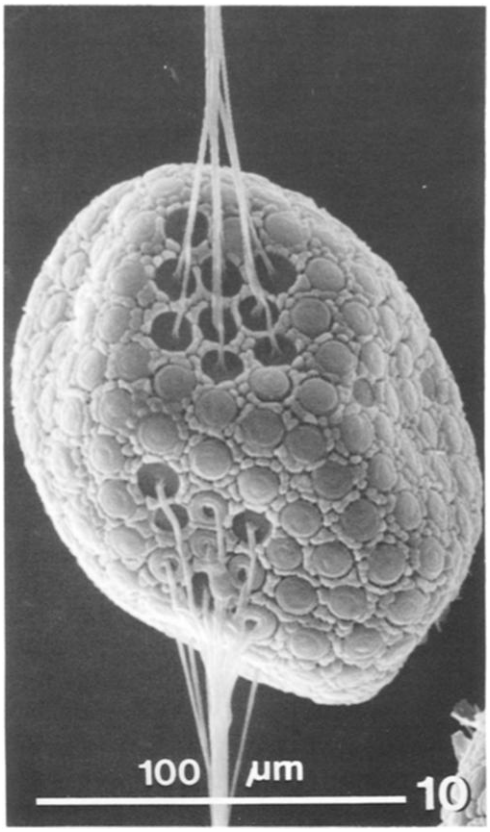
The eggs of *Electrogena zebrata* measure about 150 × 120 μm in diameter and are characterized by a chorionic pattern of geometrically arranged KCT's covering the entire egg surface (Fig. 1). Each of these attachment structures, measuring 12 μm in diameter, is isolated by a ring of protuberances (Fig. 2), 1.6 μm high. The arrangement of these protuberances reveals numerous interconnecting thin strands connecting the KCT's (Fig. 2). The chorionic pattern of KCT's does not persist in the area of the micropyles (Fig. 1). The micropylar sperm guide is ovoid and the micropylar opening is located to one side (Fig. 3), at the junction with the micropylar canal situated more deeply in the egg. Each thread is covered by a transparent terminal knob that reveals its coiled pattern beneath (Fig. 3). These coiled attachment structures extend collectively anchoring the egg to the substratum. The transparent knobs lose their uniform covering, which ruptures toward one side (Fig. 4), to result in gradual uncovering of the coiled threads beneath (Fig. 5). At greater magnification, the knob surface appears rugose, just as do the loosely-arranged surrounding protuberances (Fig. 6). Each knob connects with its own thread that is coiled about its base (Figs. 5, 6). The extended configuration of the threads (Fig. 7) exposes their respective chorionic recesses. Each thread emerges from the central zone of its recess where it originates from many tightly coiled fine fibers (Fig. 8). In their extended configuration, the KCT's appear as elongated threads (Fig. 9), which also may anastomose (Fig. 10); thus, the KCT's project from the chorion and attach to the substratum with their round terminal knobs (Fig. 11). Many extended KCT's adhered to the bottom surface of our specimen holders, causing the eggs to form a clustered pattern.

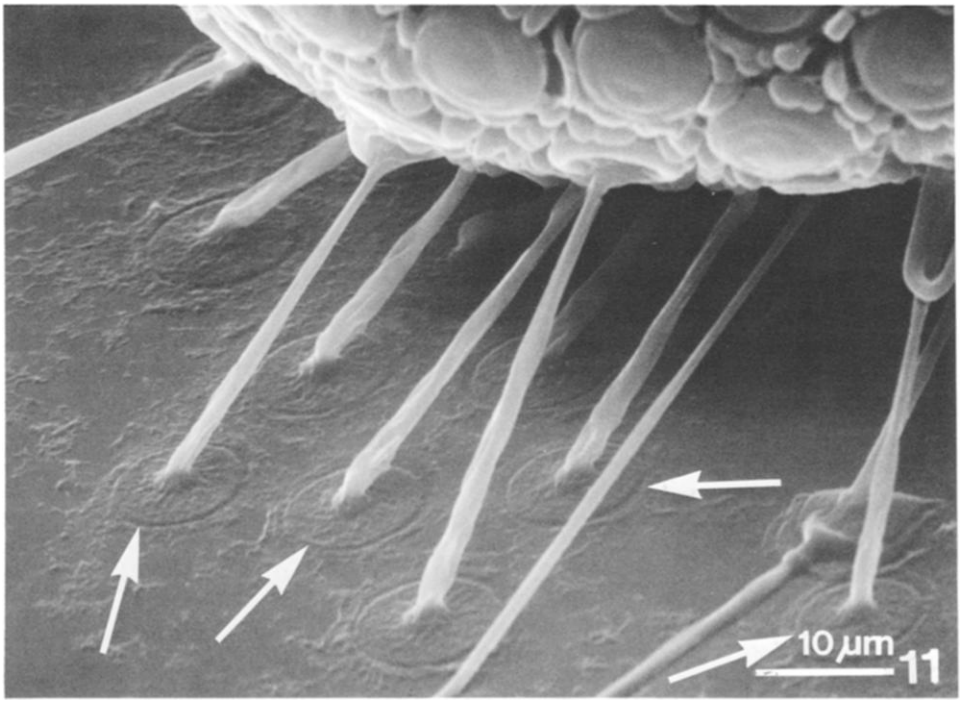
DISCUSSION

The most common type of egg among the Heptageniidae displays a chorionic pattern in which the KCT's are either randomly distributed or concentrated at one or both poles (Flowers, 1980; Koss & Edmunds, 1974). According to Koss & Edmunds (1974), the KCT's are to be considered apomorphic characters, being derived from fibrous attachment devices. Moreover, the KCT's of *Electrogena zebrata* are composed of many fine fibers arising from the point where the threads emerge from the chorion. The occurrence of a well-developed knob is considered to be an advantage because it permits more surface for anchorage. Such structural organization appears to be apomorphic. By contrast, homogeneously distributed KCT's represent a plesiomorphic state, more primitive than the random pattern or the polar concentration on the egg surface (Koss & Edmunds, 1974).

The ability to fix their eggs to a substrate is crucial in the survival of many ephemeropteran species. Most Ephemeroptera oviposit in running water; the


10μm
9


100 μm
10


10 μm
11

possession of effective anchoring structures would seem to augment, in a significant way, reproductive success for many species. Apparently, some species have solved this problem simply by producing an adhesive layer on the egg surface (Degrange, 1960; Kopelke & Müller-Liebenau, 1981 a,b, 1982; Koss, 1968, 1970; Koss & Edmunds, 1974; Mazzini & Gaino, 1985; Soldàn, 1979). Other species have highly specialized structures, such as those described here, suggesting that environmental pressures may be especially influential at this stage of the ephemeropteran life cycle.

As with other types of chorionic sculpturing, the KCT's of *Electrogena zebrata* are formed by follicle cells enveloping the egg during its growth phase. The spring-like uncoiling of threads may be triggered by contact with water after breakdown of the terminal cap, permitting adhesion to the substrate.

LITERATURE CITED

DEGRANGE, C. 1960. Recherches sur la reproduction des Ephéméroptères. *Trav. Lab. Hydrobiol. Piscic. Univ. Grenoble,* 51: 7–193.

FLOWERS, R. W. 1980. A review of the nearctic *Heptagenia* (Heptageniidae, Ephemeroptera). *In* Flannagan, J. F. & Marshall, E. E., eds., *Advances in Ephemeroptera Biology,* Plenum Press, New York and London, pp. 93–102.

KOPELKE, J. P. 1980. Morphologische Studien an den Eiern der Eintagsfliegen (Ephemeroptera) aus der Emergenz des zentralafrikanischen Bergbaches Kalengo. *Mitt. Schweiz. Entomol. Ges.,* 53: 297–311.

KOPELKE, J. P. & MÜLLER-LIEBENAU, I. 1981a. Eistrukturen bei Ephemeroptera und deren Bedeutung für die Aufstellung von Artengruppen am Beispiel der europäischen Arten der Gattung *Baetis* Leach, 1815 (Insecta: Baetidae). Teil II: *rhodani- vernus-* und *fuscatus*-Gruppe. *Spixiana,* 4: 39–54.

1981b. Eistrukturen bei Ephemeroptera und deren Bedeutung für die Aufstellung von Artengruppen am Beispiel der europäischen Arten der Gattung *Baetis* Leach, 1815 (Insecta: Baetidae). Teil III: *buceratus-, atrebatinus-, niger-, gracilis-* und *muticus*-Gruppe. *Dtsch. Entomol. Z. N. F.,* 28: 1–6.

1982. Eistrukturen bei Ephemeroptera und deren Bedeutung für die Aufstellung von Artengruppen am Beispiel der europäischen Arten der Gattung *Baetis* Leach, 1815 (Insecta: Baetidae). Teil I: *alpinus-, lutheri-, pavidus-* und *lapponicus*-Gruppe. *Gew. Abw.,* 68/69: 7–25.

KOSS, R. W. 1968. Morphology and taxonomic use of Ephemeroptera eggs. *Ann. Entomol. Soc. Am.,* 61: 696–721.

1970. Ephemeroptera eggs: sperm guide morphology and adhesive layer formation. *Trans. Am. Microsc. Soc.,* 89: 62–69.

KOSS, R. W. & EDMUNDS, G. F. 1974. Ephemeroptera eggs and their contribution to phylogenetic studies of the order. *Zool. J. Linn. Soc.,* 55: 267–349.

MALZACHER, P. 1982. Eistrukturen europäischer Caenidae (Insecta, Ephemeroptera). *Stuttg. Beitr. Naturk., Ser. A,* 356: 1–15.

MAZZINI, M. & GAINO, E. 1985. Fine structure of the egg shells of *Habrophlebia fusca* (Curtis) and *H. consiglioi* Biancheri (Ephemeroptera: Leptophlebiidae). *Int. J. Insect Morphol. Embryol.,* 14: 327–334.

SOLDÀN, T. 1979. The structure and development of the female internal reproductive system in six European species of Ephemeroptera. *Acta Entomol. Bohemoslov.,* 76: 353–365.

←

FIG. 9. Extended KCT's of *Electrogena zebrata* emerging from the chorionic surface. FIG. 10. Two polar groups of extended KCT's projecting from the chorionic surface. FIG. 11. Terminal knobs (arrows) of extended KCT's adhering to the substratum.