

Legs. Chaetotaxy of the last three segments of Leg IV varies greatly from the other legs (Fig. 9). A double row of simple spinous setae arises along the lateral margins of the ventral surface of the genu and tibia. The setae get longer toward the distal end on each segment, terminating in a clump of six to eight setae. On the dorsal surface of the genu and tibia are four simple setae. The tarsus contains four ventral spinous setae and one dorsal. The number of hair-like setae present per segment of Leg IV is 0-0-0-2-1-(34-40). From eleven to fifteen minute setae are present in a row along each lateral side of the tarsus. Size of the segments of leg IV varies among individuals of the same and of the opposite sex (Table IV).

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EPHEMEROPTERA EGGS: SPERM GUIDE MORPHOLOGY AND ADHESIVE LAYER FORMATION¹

RICHARD W. KOSS²

Department of Biology, University of Utah, Salt Lake City, Utah 84112

KOSS, RICHARD W. 1970. Ephemeroptera eggs: sperm guide morphology and adhesive layer formation. *Trans. Amer. Microsc. Soc.* 89: 295-299. The regular occurrence of a sperm guide in the adhesive layer, the constant alignment of the sperm guide with the micropylar canal, and the presence of sperm guides and adhesive layers on eggs dissected from nymphal stages indicate that in the Ephemeroptera the adhesive layer must be deposited by the follicle cells after these same cells have laid down the chorion. This adhesive layer deposition is thus completed prior to fertilization.

INDEX DESCRIPTORS: Ephemeroptera; Eggs; Morphology; Accessory glands; Sperm guide; Adhesive layer; Micropyles; Insecta.

It is generally believed that the chorion of insect eggs is the last layer deposited by follicle cells, and that accessory (or colleterial) glands are responsible for the deposition of any suprachorionic adhesive material.

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² Present address: Dept. of Geography and Environmental Engineering, 513 Ames Hall, The Johns Hopkins University, Baltimore, Maryland 21218.

There are, however, some references to the contrary. Johannsen & Butt (1935, fig. 26) illustrated a "mucous layer" between the follicle cells and the chorion, but said nothing about it in the text. Hinton (1961) found "viscous material" on eggs in the ovarioles of *Nepa cinerea* L. (Nepidae), and this material reacted differently to identifying reagents than did the accessory gland contents. Discussing Hemiptera, Cobben (1968, p. 259) stated that "evidence strongly suggests that they [suprachorionic layers] are also generally secreted in the ovariole." Sweeney et al. (1968) indicated that in *Lytta nuttalli* Say (Meloidae) "mucilage" is secreted by the lateral oviducts instead of the accessory glands. Brinck (1956), in a detailed study of the reproductive system of Plecoptera, stated that accessory glands were found in one genus only (*Perlodes*). In a similar study of Ephemeroptera, Brinck (1957) stated that accessory glands are absent, although he did not state the source of the adhesive material coating the eggs.

To my knowledge the origin and deposition of the adhesive material found on Ephemeroptera eggs has never been described. The understanding of these phenomena can be of great value to studies of external egg morphology in that such an understanding would aid the interpretation of how other egg structures are formed and how they have evolved.

The purpose of this paper is to report my data and theory relevant to adhesive layer formation in the Ephemeroptera. These data became obvious during a morphological study of eggs of representative genera of all families of Ephemeroptera except the Palingeniidae. The goal of that research was to collect data for a phylogenetic study of the order, and those results will be published at a later date.

MATERIALS AND METHODS

The techniques used were essentially those described by Koss (1968). The eggs of approximately 100 genera and 225 species of exotic and native Ephemeroptera were slide mounted and examined. The eggs were dissected from adult and mature nymphal female specimens, most of which were available at the Entomology Museum of the University of Utah, Salt Lake City. These specimens, together with one set of slides are presently housed in that museum. I have retained the only other set of slides.

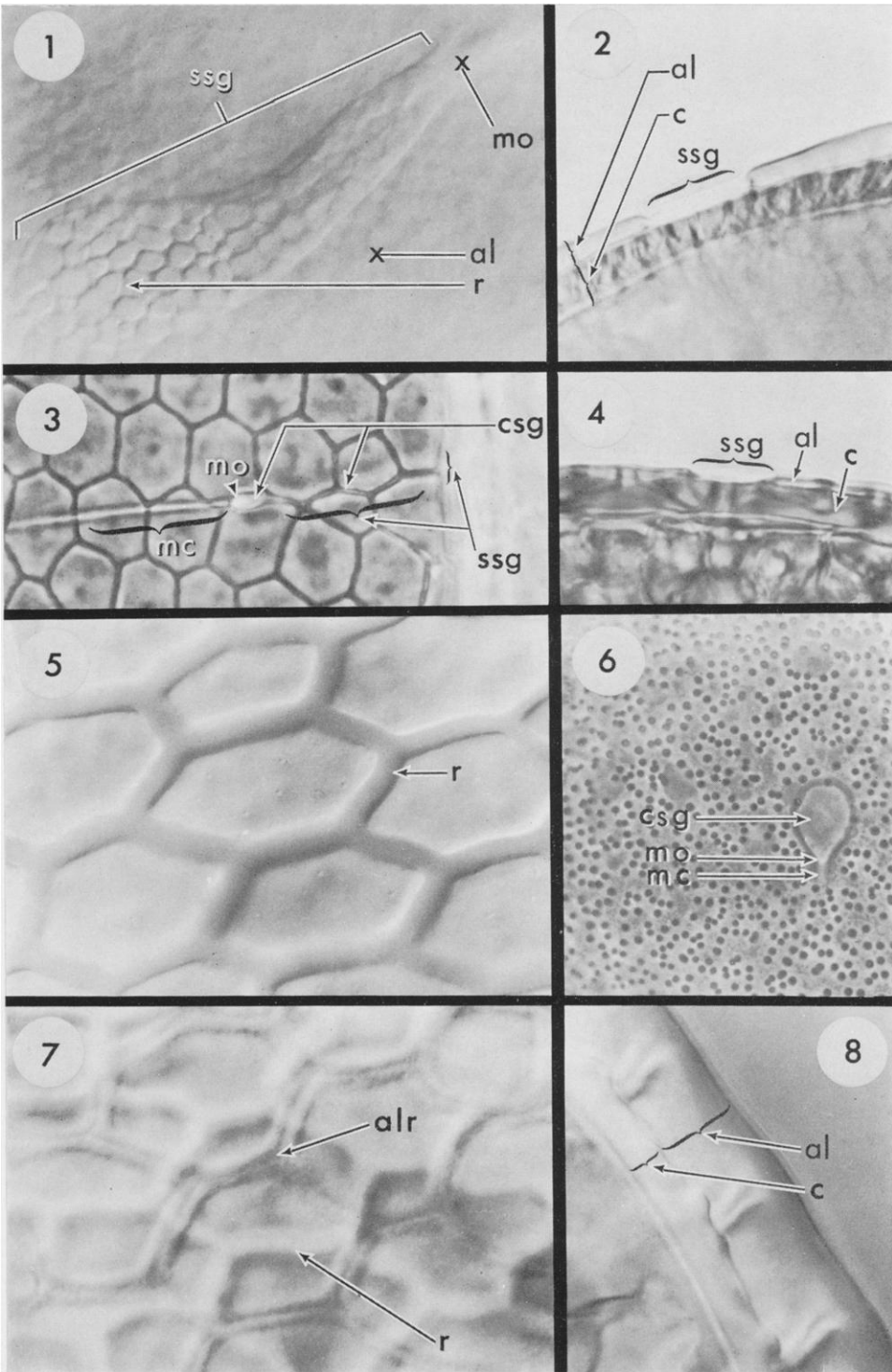
CMC-S was used as a mounting medium, and generally the eggs were mounted whole. CMC-S is a product available from the General Biological Supply House (Turtox), Chicago, Illinois. Some studies were carried out on fresh eggs or on eggs layed in water and kept there for 12 hours before preservation and/or mounting. Most eggs, however, were dissected from specimens that

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Figs. 1-8. Figs. 1,2. *Ephemerella supposita* Eaton, sperm guide. $\times 1120$. 1. Surface view. 2. Tangential view. 3. *Hexagenia bilineata* (Say), micropyle and chorion. $\times 560$. 4. *Hexagenia munda munda* Eaton, tangential view of proximal portion of sperm guide. $\times 700$. 5. *Eatonica schoutedeni* (Navas), surface view of chorion. $\times 1120$. 6. *Heptagenia diabasia* Burks, micropyle and chorion. $\times 800$. Figs. 7,8. *E. schoutedeni*. $\times 1120$. 7. Surface view, focused midway in depth in adhesive layer. Chorionic ridges in background, out of focus; adhesive layer reticulation in foreground, in focus. 8. Tangential view of chorion and adhesive layer.

Abbreviations: al = adhesive layer; alr = adhesive layer reticulation, c = chorion, csg = chorionic sperm guide, mc = micropylar canal, mo = micropylar opening, r = ridge (strand of chorionic reticulation), ssg = suprachorionic sperm guide.

Microscopy: Figs. 3 and 6, phase contrast; other figs., interference contrast. Figs. 1,5, and 7 show relief, and the lines marked "r" (ridge) should appear as raised ridges; the sperm guide in Fig. 1 should appear as a void in the adhesive layer, not as a raised surface. Light projects from top left in the interference contrast pictures.



were preserved in alcohol at the time of collection. Eggs from dried specimens did not respond satisfactorily to mounting in CMC-S, and therefore were not utilized in this study.

A Carl Zeiss Photomicroscope equipped with phase contrast and Nomarski interference contrast attachments was used for observation and photography; the magnification range was from $\times 160$ to $\times 1600$. All photographs were taken with Kodak Panatomic-X film.

OBSERVATIONS

The micropyle (Figs. 3, 6) is the structure which allows sperm to enter the egg, and on Ephemeroptera eggs it normally consists of two parts: the sperm guide and the micropylar canal. The sperm guide is a void or depression in the adhesive material and/or an external chorionic depression usually lacking sculpturing and attachment structures; it leads to and presumably aids in directing sperm to the micropylar canal. It is almost always of a nearly constant shape for any one species. The micropylar canal is a tunnel-like passage leading from the sperm guide, through the chorion, and into the egg; it may terminate at the inner surface of the chorion or continue into the egg for a short distance (see the broken piece of chorion illustrated in Fig. 49 of Koss 1968). Although this micropylar terminology differs slightly from that used by Koss (1968), the other terminology used herein is adequately explained in that publication.

In Ameletopsinae (Siphonuridae), *Stenonema* (except the *interpunctatum* species-group) (Heptageniidae), Behningiidae, and most Ephemeridae (among others), in which polar caps and accessory attachment structures are usually lacking, there is a well-developed adhesive layer external to the chorion. In such groups the sperm guides are developed in the adhesive layer and thus the guides are suprachorionic (Figs. 1, 2). However, in *Ephemerella* (Ephemerellidae) Tricorythidae, Potamanthidae and most Heptageniidae, in which polar caps and/or accessory attachment structures are present, the adhesive layer is absent. In such instances, the sperm guides are developed in the chorion and thus they are chorionic (Fig. 6). Lastly, there are some groups (e.g., *Hexagenia* s.l., Ephemeridae) in which the sperm guide is chorionic as well as suprachorionic (Figs. 3, 4). In all three conditions, the sperm guide has always been observed to be in direct alignment with the micropylar canal.

A very noteworthy observation was that eggs dissected out of the lateral oviducts of mature nymphs as well as adults, are already supplied with the adhesive layer and/or other attachment structures plus sperm guides of both chorionic and suprachorionic types.

The eggs of some Ephemeridae show patterns in the adhesive layer which resemble the sculpturing and thus the deposition of the chorion (Figs. 5, 7, 8). The adhesive layer is copious on eggs of most ephemerid genera, and in *Eatonigenia*, *Eatonica* and *Hexagenia* s.l. it frequently contains a large-mesh reticulation formed by splits (Fig. 7). In some cases the splits are bordered by adhesive layer thickenings (Fig. 8), and in all cases except *Eatonigenia* the adhesive layer reticulation corresponds to a chorionic one (Figs. 5, 7); in *Eatonigenia* the chorion lacks a reticulation.

DISCUSSION

The regular occurrence of a sperm guide in the adhesive layer, and the constant alignment of the sperm guide with the micropylar canal, indicate that in the Ephemeroptera the adhesive layer must be deposited prior to fertilization, and it must be deposited by the follicle cells after these same cells have laid down the chorion. The chorion is secreted around protoplasmic projections of

the follicle cells in order to form the micropylar canals (Korschelt 1884; Johannsen and Butt 1935). I believe that the adhesive layer must subsequently be secreted around the same protoplasmic projections for it to form the adhesive layer sperm guides which are always in alignment with these micropylar canals. There is no apparent reason why suprachorionic sperm guides should be present so persistently or at all if the adhesive layer were secreted by some non-follicular means after fertilization. Furthermore, the presence of adhesive material and sperm guides on eggs dissected from nymphal specimens is conclusive evidence of a pre-fertilization deposition of the adhesive layer.

Additional evidence of the follicle cell role in adhesive layer secretion may possibly be seen on those Ephemeroidea eggs which possess an adhesive layer reticulation. This adhesive layer reticulation could be an indication of secretive activity of the follicle cells, or it may only reflect compaction of the eggs during storage in the lateral oviducts. The lack of a corresponding chorionic reticulation on *Eatonigenia* eggs opposes the latter view, but the incompleteness of the adhesive layer reticulation on many *Hexagenia* s.l. eggs confirms it.

Nevertheless, the evidence gleaned from sperm guide morphology and adhesive layer occurrence on eggs dissected from nymphal stages clearly shows that follicle cells, instead of accessory glands, are responsible for deposition of the adhesive layer on Ephemeroidea eggs. Because of this place and time of deposition, the sperm guide is always in alignment with the micropylar canal whether the guide is in the adhesive layer only or in the adhesive layer and the chorion.

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