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EVOLUTION OF MICROPYLES IN DRAGONFLY EGGS (ANISOPTERA)*

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In a series of Anisoptera eggs examined, the number of micropyles per egg generally decreased from 14 in Petaluridae to 2 in Libellulidae, and a tendency to concentrate micropyles at the tip of a micropylar stalk was noted. Both of these features may allow more efficient and rapid fertilization of eggs as they are laid.

INTRODUCTION

The term micropyle is properly restricted to one of the outermost holes in the chorion of an egg by which sperm gain access to an ovum. The sperm then travel down a micropylar canal which traverses the layers of the chorion, the exochorion and endochorion, until they penetrate the thin vitelline membrane covering the ovum. In some Anisoptera, the micropyles are located at or near the tip of a micropylar stalk. This nipple-like, cone-shaped projection from the anterior pole of the egg has been called a pedicel or peduncle by some authors (e.g. KORMONDY, 1959). The combined micropyles, micropylar canals, and micropylar stalk is termed the micropylar apparatus (DEGRANGE, 1971). In Odonata, this apparatus is formed in the chorion by the follicle cells surrounding the ovum, and the egg is fertilized just prior to oviposition as it travels down the common oviduct.

We became interested in the number and arrangement of micropyles in Anisoptera eggs, and used a scanning electron microscope (SEM) to examine available eggs of selected species in most families of Anisoptera (eggs of Neopeta-

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liidae were not available). The species we examined, with the names of their authors, are listed in Table I. Also listed in Table I are the few previous studies which have given micropyle number in one or more Odonata, including, for comparison, Zygoptera and Anisozygoptera.

The eggs of most exophytically ovipositing Anisoptera possess a gelatinous coat, called spumaline, which is apparently derived from the exochorion on contact with water (MILLER, 1987b). The chemical composition of odonate spumaline is unknown, but it generally must be removed to obtain a good view of the micropyles.

MATERIAL AND METHODS

Eggs from Petaluridae and Aeshnidae were obtained by placing females in a smooth-sided, covered container with a layer of wet paper toweling on the bottom. Cooperative females oviposited in the toweling. Eggs of Cordulegastridae and Macromiidae were obtained by dissection, those of Gomphidae, Corduliidae, and Libellulidae, by holding a female by her wings and dipping her abdomen to the water surface in a small jar. Reluctant females could sometimes be induced to lay eggs by gently squeezing their abdomens. Many of the eggs we examined had been stored in alcohol for up to 10 years. Voucher samples of the eggs used and the females from which they came are in the S.W. Dunkle Collection.

While many features of odonate eggs can be seen with a light microscope, particularly a phase-contrast microscope, SEM gives greater detail. However, preparation of eggs for SEM was difficult, especially the removal of spumaline, and prevention of collapse during dehydration. Eggs without spumaline could be removed from alcohol, air-dried, and mounted on grids. Eggs with spumaline could be teased from the spumaline using fine needles, but the micropylar stalk often broke off. Sonication also breaks off the micropylar stalk, and fixing with glutaraldehyde makes the spumaline more difficult to remove. Commercial proteinase (Protenase K, Sigma Chemical Co.) and 0.2 M HCL had no effect on the spumaline, but it was dissolved by 0.5 M NaOH in 5 min to an hour. Eggs were dehydrated in an ascending ethanol series of 10% increments for 30 min at each change. Best results were obtained when fresh eggs containing full-grown embryos were teased from spumaline, then fixed with both glutaraldehyde (2.5% vol/vol) and osmium tetroxide (1.0% wt/vol, 1 hr in each) before dehydration. To prepare dehydrated eggs for mounting on SEM grids, we tried both critical-point drying and infiltrating the eggs with dilute resins, but the eggs generally collapsed. It was best to place the eggs in hexamethyldisilazane for 5 min (NATION, 1983), air dry them, then mount them on grids with silver paint before sputter-coating them with gold-palladium.

RESULTS

Data on micropyle number of Anisoptera from previous studies and from ours are summarized in Table I. The number of micropyles observed ranged from 8-14 in Petaluridae, 5-10 in Aeshnidae, 5-9 in Gomphidae, 7 in Cordulegastridae, 2 in Macromiidae, 2 in Corduliidae, and 2 in Libellulidae. Thus, the number of micropyles per egg generally decreases from families we consider evolutionarily primitive in adult morphology to those considered more evolutionarily changed or advanced, as in the sequence of families just given. Along with the reduction in micropyle number is a tendency for clustering micropyles near the center of the

Table I

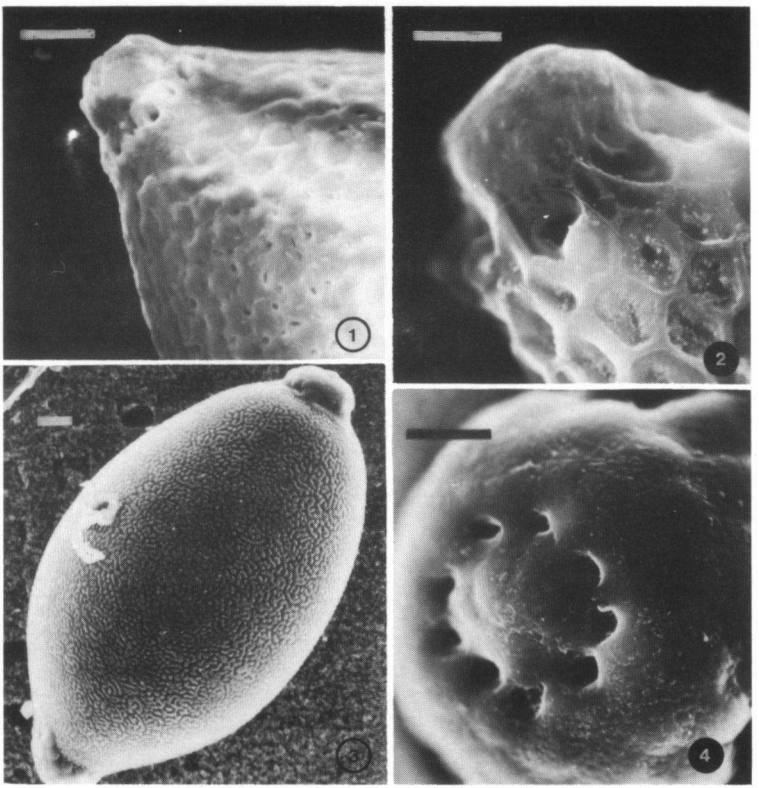
Number of micropyles per egg in Odonata. — References: (A) present study, — (B) ANDO, 1962, — (C) DEGRANGE, 1971, — (D) DEGRANGE, 1974, — (E) GROSS, 1903, — (F) IVEY et al., 1988

Species	No. micropyles	Reference	Species	No. micropyles	Reference
PSEUDOLESTIDAE			*Gomphus exilis*	7-9	F
Rhipidolestes aculeatus	ca 10	B	*Ictinogomphus clavatus*	7-8	B
CALOPTERYGIDAE			*Aphylla williamsoni*	5-6	F
Calopteryx atrata	4-5	B	CORDULEGASTRIDAE		
C. virgo	4	D	*Cordulegaster maculata*	7	A
EPIOPHLEBIIDAE			MACROMIIDAE		
Epiophlebia superstes	12-14	B	*Macromia margarita*	2	A
PETALURIDAE			CORDULIIDAE		
Tachopteryx thoreyi	14	A	*Somatochlora elongata*	2	A
Tanypteryx pryeri	8	B	LIBELLULIDAE		
AESHNIDAE			*Crocothemis servilia*	2	A
Polycanthagyna melanictera	ca 8	B	*Leucorrhinia dubia*	2	D
Aeshna nigroflava	ca 10	B	*Libellula (Ladona) deplanata*	2	A
A. cyanea	7	C	*L. (Belonia) croceipennis*	2	A
A. juncea	6-8	C	*L. saturata*	2	A
A. interrupta	7	A	*L. (Plathemis) lydia*	2	A
Hemianax ephippiger	6-9	C	*L. (L.) auripennis*	2	A
Anax imperator	5-8	C	*L. axilena*	2	A
A. junius	6	F	*L. cyanea*	2	A
A. parthenope	5-6	B, C	*L. flavida*	2	A
GOMPHIDAE			*L. forensis*	2	A
Hagenius albardae	8-9	B	*L. jesseana*	2	A
H. brevistylus	9	A	*L. luctuosa*	2	A
Erpetogomphus designatus	7	A	*L. needhami*	2	A
Onychogomphus forcipatus	7-9	E	*L. pulchella*	2	A
Asiagomphus pryeri	7	B	*L. vibrans*	2	A
			Tramea carolina	2	A

anterior pole of the egg (Figs 1, 2, 5). This trend culminates in 2 micropyles at the tip of a micropylar stalk in the Macromiidae, Corduliidae, and Libellulidae (Figs 7, 8, 9). Note from Table I that some authors found intraspecific variation in micropyle number; this usually refers to variation within a clutch of eggs from a single female. Also note in Table I that within Gomphidae and within Aeshnidae, at least, are separate trends toward reduction of micropyle number, suggesting that numbers of micropyles have become reduced in more than one lineage of the Odonata.

Our results suggest that ANDO (1962) may have been in error on two points. He listed 5-7 micropyles for the libellulid *Crocothemis servilia,* and 9 micropyles at both anterior and posterior poles in the gomphid *Hagenius (Sieboldius) albardae.* We found that 2 micropyles, typical of Libellulidae, were present in *C. servilia. Hagenius (Hagenius) brevistylus* eggs have no micropyles at the posterior end; probably the similarly shaped anterior and posterior poles of *Hagenius* eggs (Fig. 3) led ANDO (1962) to believe that micropyles were present at both ends of *H. albardae* eggs.

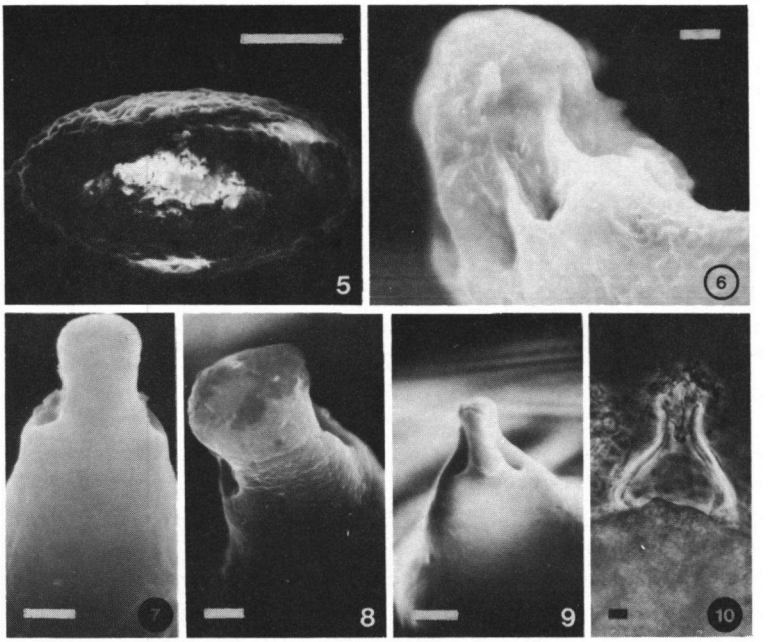
Observations with the phase-contrast microscope indicate that the 2 micropylar canals of libellulids open into a common chamber in the base of the micropylar stalk (Fig. 10), but this should be verified by serial sections.



Figs 1-4. Eggs of (1) *Tachopteryx thoreyi,* anterior pole: ring of 14 micropyles, scattered aeropyles, and scale-like chorionic sculpturing (bar = 50μm); — (2) *Aeshna interrupta,* anterior pole: ring of 7 micropyles and polygonal chorionic ridges (bar = 25μm); — (3) *Hagenius brevistylus,* showing similarly shaped poles and vermiform sculpturing (bar = 50μm); — (4) *H. brevistylus,* anterior pole with ring of 9 micropyles (bar = 25μm).

DISCUSSION AND SYNOPSIS OF MICROPYLE EVOLUTION

During their early evolution, Odonata may have had micropyles scattered over the surface of the egg. Fertilization would probably have occurred by bathing the egg in stored semen as it passed down the common oviduct during oviposition. Such lavish use of semen would probably mean that a female would have had to spend much time looking for mates and in copulation to replenish sperm stores between oviposition bouts. We speculate that egg handling within the female reproductive tract became more efficient so that only a small droplet of stored semen was applied to each egg as it was held in a standardized position. Concomitant with this, the micropyles became restricted to those parts of the egg



Figs 5-10. Eggs of (5) *Cordulegaster maculata,* showing wrinkle-like chorionic sculpturing and micropylar stalk at anterior pole (bar = 250μm); — (6) *C. maculata:* ring of 7 micropyles on a short micropylar stalk (bar = 5μm); — (7) *Macromia margarita,* anterior pole: 2 micropyles near the tip of a micropylar stalk (bar = 5μm); — (8) *Somatochlora elongata,* same as in Fig. 7 (bar = 5μm); — (9) *Libellula deplanata,* anterior pole: micropylar stalk with 2 laterotermínal micropyles (bar = 5μm); — (10) *Libellula needhami,* phase-contrast micrograph of micropylar apparatus, showing 2 terminal micropyles, 2 short micropylar canals separated by a septum, and a possible semen storage chamber in the base of the micropylar stalk. Debris on egg surface are remains of spumaline (bar = 5μm).

contacted by the semen. In all Odonata that have been examined, micropyles are restricted to the anterior pole of the egg. In *Epiophlebia superstes* the anterior pole is blunt (ANDO, 1962), whereas it is more or less pointed in all other Odonata examined. A pointed and tapered anterior pole allows the egg to be accurately positioned at the opening of the bursa. MILLER (1987a) illustrated the postulated position of the pointed anterior pole in the bursal opening at the moment of fertilization in the zygopteran *Ischnura elegans.* Odonata which oviposit endophytically, namely the Zygoptera, Anisozygoptera, and some Anisoptera (Petaluridae, Neopetaliidae, Aeshnidae), have elongate eggs which are relatively easy for the female reproductive tract to keep oriented in a standard way (anterior pole facing anteriorly) as they move along the oviduct. Endophytic oviposition is thought to be the more primitive type in Odonata, in which

egg-laying rates are low (e.g. about 2 eggs/min in *Lestes unguiculatus;* see references in McVEY, 1984) and the ovipositing female is vulnerable to predators for hours. Most other female Anisoptera lay large numbers of ovoid eggs exophytically at a rapid rate, then leave the oviposition site in a few minutes. The fastest oviposition rate, presumably equal to the fertilization rate, so far discovered in Odonata is an incredible 28 eggs/sec at 32°C in *Libellula lydia* (McVEY, 1984). Orientation of the egg for fertilization in such species has been accompanied by clustering micropyles at the extreme anterior end of the egg at the tip of a micropylar stalk, as in some Gomphidae (e.g. *Asiagomphus pryeri,* ANDO, 1962), then reducing the micropyles to one pair as in Macromiidae. The nipple-like micropylar stalk in fast-ovipositing libellulids probably is inserted into the fertilization pore of the bursal opening (see SIVA-JOTHY, 1987) and receives a tiny injection of sperm in an efficient assembly-line process under well-coordinated muscular control. The chamber in the base of the micropylar stalk possibly protects a droplet of semen until a sperm has time to penetrate the ovum, even after the egg has already been deposited in water. The micropylar stalk of libellulids is delicate and easily broken off; probably it is a "throw- away" structure, functioning only at the moment of fertilization and whose fate afterwards does not affect survival. Libellulids generally appear to have thin-shelled eggs, probably so that eggs can be formed as rapidly as possible from limited raw materials, but more research is necessary on this point. Further research may reveal libellulids with the evolution of the micropyles carried to its logical conclusion, one micropyle at the tip of a micropylar stalk.

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