Odonatologica 19(4): 395-401 *December 1, 1990*

EGGSHELLS OF AUSTRALIAN GOMPHIDAE: PLASTRON RESPIRATION IN EGGS OF STREAM-DWELLING ODONATA (ANISOPTERA)

J.W.H. TRUEMAN
Department of Zoology, Australian National University,
P.O. Box 4, Canberra, A.C.T. 2601, Australia

Received June 19, 1990 / Accepted August 2, 1990

Eggshells of 10 *Austrogomphus*, 3 *Antipodogomphus* and 1 *Hemigomphus* species (Gomphinae) were examined using scanning electron microscopy. In 9 *Austrogomphus* spp. the outermost eggshell layers were found to carry a field of prominent pillars supporting a gelatinous outer chorion above the general egg surface. Eggs of *Antipodogomphus* were similar but with pustulate projections in place of pillars. Eggs of 1 *Austrogomphus* and 1 *Hemigomphus* sp. did not carry such structures. The structures are interpreted as respiratory plastrons.

INTRODUCTION

The majority of aquatic insect eggs lack discrete respiratory structures, gas exchange occurring by diffusion across the general egg surface (HINTON, 1969). Many terrestrial insect eggs, in contrast, possess enclosed chorionic meshworks which facilitate gas exchange whilst restricting water loss. Insofar as the chorionic meshwork can act as a plastron, i.e. as a physicall gill of fixed dimensions, eggs of terrestrial type are capable of respiration in well oxygenated water, but at low oxygen pressure reverse diffusion across a plastron decreases oxygen availability and can lead to death of the embryo. Plastron respiration is therefore normally restricted to eggs which have direct access to air or to permanently well oxygenated water (HINTON, 1969; NATION, 1985). Though eggs of the aquatic type respire readily in air, inability to control water loss usually prevents development.

In Odonata, eggs of two libellulids, *Brachythemis lacustris* (Kirby) and *Tholymis tillarga* (Fabricius), have probable plastron capability. Both species oviposit onto leaves on the surface of or overhanging still or slowly flowing waters, and the putative plastrons comprise short chorionic pillars covering the general egg surface and supporting a gelatinous layer conjoined across adjacent eggs (MILLER & MILLER, 1985; MILLER, 1987). The gelatinous layer forms a membranous protection for the eggs and encloses a layer of air. Holes through which some pillars protrude apparently function as aeropyles. Egg plastrons have not been recorded from other odonates, but HINTON (1981, p. 49) suggested they might occur in dragonflies ovipositing exophytically into small, swiftly flowing streams. In such an environment reduction in the partial pressure of oxygen to the point at which reverse diffusion would begin is unlikely, but changing water levels may cause eggs to become subaerial, and an ability to survive both in air and in water would be advantageous.

This paper reports the presence of plastron respiratory structures in eggs of nine species of *Austrogomphus* Selys and three species of *Antipodogomphus* Fraser, Australian endemic gomphine genera. These species inhabit small upland streams and fast flowing rivers and are a group in which egg plastrons would not be unexpected. Eggs from one species of *Austrogomphus* and one species of *Hemigomphus* Selys are found not to contain plastron structures.

SPECIES EXAMINED

Antipodogomphus hodgkini Watson, *A. neophytus* Fraser, *A. acolythus* (Martin); *Austrogomphus amphiclitus* (Selys), *A. australis* Dale in Selys, *A. bifurcatus* Tillyard, *A.* "c" of WATSON (1974), *A. collaris* Hagen in Selys, *A.* "d" of WATSON (1974), *A. guerini* (Rambur), *A. lateralis* (Selys), *A. ochraceus* (Selys), *A. prasinus* Tillyard; *Hemigomphus heteroclytus* Selys. The sample comprises 10 of 18 known *Austrogomphus*, 3 of 6 *Antipodogomphus*, and 1 of 3 *Hemigomphus* species and can be considered representative of these genera.

METHODS

Live eggs of *A. amphiclitus, A. guerini* and *A. ochraceus* were obtained from captured females by dipping the tip of the abdomen into water. Eggs for scanning electron microscopy were preserved in 70% ethanol, dehydrated through graded ethanols, air dried and gold coated for observation in a Cambridge 360 scanning electron microscope. Dried eggs of all species other than *A. guerini* were located attached to terminal segments of pinned adult female specimens in the Australian National Insect Collection (ANIC). Dried eggs were first removed by soaking in water, then dehydrated, mounted and examined as for live eggs.

Available live eggs from *A. guerini* numbered approximately 6000, from 10 females, and about 60 eggs were prepared for scanning electron microscopy. Approximately 100 live eggs were obtained for each of *A. amphiclitus* and *A. ochraceus*, from 1 female of each species, and about 50 of each were prepared for SEM. One egg only was available for each of *A. lateralis* and *A. acolythus*, but between 10 and 50 dried eggs, from single clutches, were available for each of the other species. Two clutches from widely separate locations were obtained for *A. australis*.

Several hundred eggs from each of *A. amphiclitus, A. guerini* and *A. ochraceus* were incubated, some in tapwater and others in a humid atmosphere, and samples of these were examined using light microscopy.

EGG DESCRIPTIONS

Eggs of *Austrogomphus* are characterised by a more or less dense reticulation of chorionic pillars supporting a diffuse, sticky, gelatinous outermost layer above the general egg surface. The overall appearance of the egg and positioning of pillars are shown in Figures 1-3. The precursor of the diffuse jelly layer, in eggs not subjected to wetting, appears as a mucilagous mass concentrated on pillar tops and forming inter-pillar strands (Fig. 4). This gelatinous layer collapses on drying to become a thin and often intermittent covering retaining some strand-like structure (Fig. 5). All *Austrogomphus* eggs examined show either the precursor or the collapsed gelatinous layer, and it can be inferred that the diffuse gelatinous stage occurs under conditions of normal oviposition in those *Austrogomphus* species for which live eggs were unobtainable.

Eggs of *Antipodogomphus* species have a similar structure but with the pillars replaced by a pustulate layer (Fig. 6). Eggs of *Austrogomphus lateralis* and *Hemigomphus heteroclytus* (Figs 7-8) have no pillars or pustules, that of *H. heteroclytus* being entirely without surface markings and without a gelatinous layer.

Eggs are not always identifiable to species level, but are assignable to the following groups.

GROUP 1: EGGS WITH MAXIMAL PILLAR DEVELOPMENT

A. australis (Fig. 4), *A. amphiclitus:* eggs ellipsoidal; linear dimensions 0.40x0.25 mm. Vitelline membrane smooth, 1.3-1.4 μm thick. Inner chorion 10-12 μm thick; densely covered with pillars 7-9 μm high, 4-7 μm diameter, splayed and cusped distally. A cylindrical to distally expanded micropylar projection, height 35 μm, diameter 35 μm, at anterior pole. Lateral surface of micropylar projection with 14-20 mostly distal tuberculate processes approximately twice the thickness of chorionic pillars. Micropylar openings 7, diameter 2.5-3.0 μm, arranged radially on distal surface of micropylar projection. Outermost eggshell layer diffuse, sticky, expanding to height of micropylar projection in live eggs of *A. amphiclitus,* intermittent and approximately 1 μm thick in dried specimens of both species.

A. prasinus (Fig. 3): eggs similar to above, overall dimensions 0.50x0.35 mm.

A guerini (Figs 1, 5): eggs ellipsoidal to slightly oblate. Linear dimensions 0.41x0.25 mm to 0.50x0.34 mm. Vitteline membrane smooth, approximately 1 μm thick. Inner chorion 9 μm thick; covered with cylindrical pillars 4-5 μm high, 3-5 μm in diameter, centred 7-10 μm apart. Micropylar projection a squat cylinder broadened distally; height 25-27 μm, distal diameter 35-37 μm. Distal parts of chorionic pillars not splayed or cusped, or less so than above species. Sides of micropylar projection with tuberculate processes reduced or absent.

Micropylar openings 7, diameter 2.5-3.0 μm, arranged radially on distal surface of micropylar projection. Outermost eggshell layer diffuse; sticky and expanding to height of micropylar projection in live eggs, intermittent and approximately 1 μm thick in dried specimens.

GROUP 2: EGGS WITH ROUNDED AND REDUCED PILLARS

A. collaris, *A.* "c": eggs of similar size and shape to *A. australis*. Chorionic pillars shorter, 4-6 μm high, the majority rounded distally. Pillar spacing as in *A. guerini*. Short tubercules on sides of micropylar projection in *A. collaris*, very short in *A.* "c", micropylar openings 6 in *A. collaris*, 7 in *A.* "c", arranged radially on distal surface of micropylar projection.

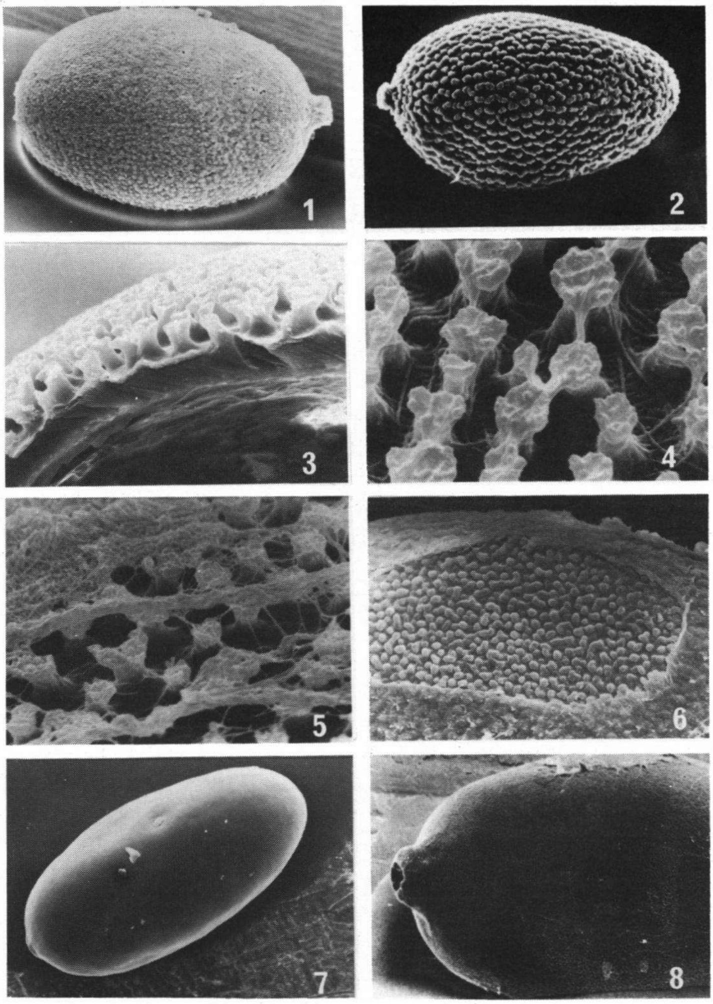
A. bifurcatus (Fig. 2), *A.* "d", *A. ochraceus:* as for *A. collaris* but pillars reduced to rounded knobs and more widely spaced. Approximately 25% of pillars in *A. bifurcatus* and 10% in *A.* "d" conjoined into short ridges. Micropylar projections with short tubercules. Micropylar openings 7 in *A. ochraceus*, 6 in *A. bifurcatus* and *A.* "d", arranged radially on distal surface of micropylar projection. Outermost eggshell layer diffuse, sticky, expanding to height of micropylar projection in live eggs of *A.ochraceus*, intermittent and approximately 1 μm thick in dried specimens of all species of this group.

GROUP 3: EGGS WITH PUSTULATE LAYER, WITHOUT PILLARS

Antipodogomphus acolythus, *A. hodgkini*, *A neophytus* (Fig. 6): eggs ellipsoidal, linear dimensions 0.50x0.25 mm. Faint impressions of follicle cells on outer egg surface in *A. acolythus* and *A. hodgkini*, not so in *A. neophytus*. Underlying chorionic layer densely covered with rounded, pustulate processes giving a verrucose appearance to outer surface of dried specimens in all three species. Micropylar projection a squat cylinder; height 25 μm, diameter 35 μm. Micropylar openings 6 in *A. hodgkini* and *A. neophytus*, 5 in *A. acolythus*, arranged radially on distal surface of micropylar projection.

GROUP 4: EGGSHELL PUNCTATE OR SMOOTH, MICROPYLAR PROJECTION ROUNDED

A. lateralis (Fig. 7): egg cylindrical, linear dimensions 0.60x0.35 mm. Surface punctate, pillars and pustulate projections absent. Micropylar projection smooth, rounded, height approximately 35 μm, basal diameter 65 μm. Micropylar openings 8, arranged radially, indented on distal surface of micropylar projection. Surface of available specimen covered with wisps of dried material apparently identical to gelatinous layer precursor as seen in other *Austrogomphus* species.



Figs. 1-8. Eggshell structure in some Australian Gomphidae: (1) *Austrogomphus guerini*, whole egg prepared after 3-4 days in water (chorionic pillars faintly discernable through collapsed gelatinous layer); — (2) *A. bifurcatus*, whole egg (note reduced pillar structure); — (3) *A. prasinus*, TS eggshell showing pillar structure and placement; — (4) *A. australis*, egg never subjected to wetting. The precursor of the gelatinous layer is present on pillar tips and as inter-pillar strands; — (5) *A. guerini*, egg viewed near equator. Pillars support the dried gelatinous layer above the general egg surface; — (6) *Antipodogomphus neophytus*, egg surface with gelatinous layer partly removed to show underlaying pustulate layer; — (7) *Austrogomphus lateralis*, whole egg; — (8) *Hemigomphus heteroclytus*, anterior part of the egg.

Hemigomphus heteroclytus (Fig. 8): eggs cylindrical, linear dimensions 0.50x0.25 mm. Egg surface smooth. Micropylar projection smooth, rounded, height 10 μm, basal diameter about 60 μm. Micropylar openings 8, arranged radially, exposed on distal surface of micropylar projection. No evidence of gelatinous layer or its precursor.

EGG DEVELOPMENT

Eggs of *A. amphiclitus, A. guerini* and *A. ochraceus* are deep pink when laid, turning yellow-brown after 1-2 days, colour change taking place in the vitelline or inner chorionic layer. The outer chorion comprising the basal layer from which pillars arise, the pillars and gelatinous covering (Figs 3, 5) remain grey or translucent throughout development. The diffuse, gelatinous layer develops to its maximal thickness of approximately 30 μm within a few minutes of oviposition into water. The jelly precursor does not expand in eggs which have previously been dried. Live eggs viewed by light microscopy show chorionic pillars holding the gelatinous layer above the general egg surface, refraction of light at low angles of incidence indicating presence of an air layer. If eggs are incubated in humid air a thin, sticky membrane forms in place of the usual diffuse jelly. Embryonic development proceeds in air as in water but eclosion fails. Transfer of air-incubated eggs into water at or shortly before hatching commences results in normal eclosion giving live first instar and second instar larvae.

DISCUSSION

The pillared structures observed here in *Austrogomphus* eggs are reminiscent of those from *Brachythemis lacustris* and *Tholymis tillarga.* Principal differences are that the outer gelatinous coverings are more tenuous in *Austrogomphus* and that coverings of adjacent eggs do not coalesce. Dimensions of intra-chorionic spaces in *A. australis, A. amphiclitus, A. prasinus* and *A. guerini,* the four species in which these structures appear most highly developed, are similar to those of plastrons known from terrestrial eggs in other insect orders (HINTON, 1981; MARGARITIS, 1985). A plastron function is suggested by the morphology and by the ability of eggs to develop in humid air. Somewhat reduced chorionic pillars in *A. collaris* and *A.* "c", and further reduction and wider spacing in *A. bifurcatus, A.* "d", and *A. ochraceus* suggest reduced aerial developmental capacity in these species. Pustulate structures in *Antipodogomphus* support the outermost eggshell layer more closely above the inner chorion but may also have a plastron capability.

Similarities in plastron structure within genera, and differences between genera, are consistent with current taxonomic placement of the nine *Austrogomphus* and three *Antipodogomphus* species. Observations support the phylogeny suggested by WATSON & O'FARRELL (1985) and do not support FRASER's (1959) placement of *A. amphiclitus, A. prasinus* and, by implication, *A. bifurcatus,* in a separate genus *Austroepigomphus.* The discovery of a somewhat *Hemigomphus*-like egg in *A. lateralis,* must, however, raise doubt as to the generic placement of that species.

ACKNOWLEDGEMENTS

J.A.L. WATSON provided access to specimens in his care, K.L. GARDNER helped in field collecting, R. HEADY assisted with scanning electron microscopy and P.J. GULLAN gave valuable comments on the manuscript.

REFERENCES

FRASER, F.C., 1959. New genera and species of Odonata from Australia in the Dobson Collection. *Aust. Zool.* 12: 352-361.

HINTON, H.E., 1969. Respiratory systems of insect eggshells. *A. Rev. Ent.* 14: 343-368.

HINTON, H.E., 1981. *Biology of insect eggs.* Pergamon Press, Oxford.

MARGARITIS, L.H., 1985. Structure and physiology of eggshells. *In:* G.A. Kerkut & L.I. Gilbert, [Eds], Comprehensive insect physiology, biochemistry and pharmacology, Vol. 1, pp. 153--230, Pergamon Press, Oxford.

MILLER, P.L., 1987. Oviposition behaviour and eggshell structure in some libellulid dragonflies, with particular reference to Brachythemis lacustris (Kirby) and Orthetrum coerulescens (Fabricius) (Anisoptera). *Odonatologica* 16(4): 361-374.

MILLER, P.L. & A.K. MILLER, 1985. Rates of oviposition and some other aspects of reproductive behaviour in Tholymis tillarga (Fabricius) in Kenya (Anisoptera: Libellulidae). *Odonatologica* 14(4): 287-299.

NATION, J.L., 1985. Respiratory systems. *In:* M.S. Blum, [Ed.]. Fundamentals of insect physiology, pp. 185-225. Wiley, New York.

WATSON, J.A.L., 1974. The distributions of the Australian dragonflies (Odonata). *J. Aust. ent. Soc.* 13: 137-149.

WATSON, J.A.L. & A.F. O'FARRELL, 1985. The affinities of the Australian Gomphidae (Odonata). *Aust. J. Zool.* 33: 499-508.