Proc. Arthropod. Embryol. Soc. Jpn. 42, 9–11 (2007)

ISSN 1341–1527

[SHORT COMMUNICATION]

Notes on the Pleuropodia in the Giant Water Bug *Appasus japonicus* (Heteroptera, Belostomatidae)

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Introduction

In giant water bugs of Belostomatidae, which consist of two subfamilies Lethocerinae and Belostomatinae, males take care of the eggs without assistance of females. This behavior is known as 'exclusive paternal care.' In the belostomatine bugs, females deposit their eggs as a form of egg mass on the back (the dorsal surface mainly of the fore-wings) of their mates. Males carry the batch of eggs on their back until the nymphs hatch out in several weeks. The paternal care is very important in the reproduction of belostomatine insects (Tallamy, 2001), and it has been shown that the eggs that fall from male's backs have a reduced rate of successful hatching (*e. g.*, Kraus *et al.*, 1989).

Belostomatine bugs propagate several times [*e. g.*, six times in *Appasus major* (Ohwada, 2002)] in one reproductive season. A unique attribute of these groups due to the requirement for care is that the male backspace is likely to be a limited resource during the periods of the peak egg production by females (Smith, 1979; Kraus *et al.*, 1989). Furthermore, in the reproductive strategy of males, after finishing one propagation cycle, it is necessary to enter into the following propagation cycle promptly. When the egg batch carried by males fully completes hatching, the males must drop the remnants of the hatched egg mass immediately, and begin the following propagation cycle. In fact, in the field, there are almost no instances in which we observe bugs still shouldering egg exuviae.

In regard to the timing of exfoliation and falling off of the egg batch, Ando (1991, 1999) who is an author of this paper has led to the following interesting hypothesis. He has presumed the possibility as detailed below. 1) Pleuropodia (the first abdominal appendages) of belostomatine bugs secrete an enzyme, similar to the hatching one, reported in the grasshopper *Melanoplus differentialis* (Slifer, 1937). 2) This enzyme is squeezed onto the outside of the egg, to dissolve the wax-like material or the mucilaginous cement which connects the egg mass to the male's wing. 3) Consequently, the adherence power between egg mass and the male's wing decreases at this late stage of embryogenesis.

In order to confirm the Ando's "pleuropodian hypothesis," we observed the development of pleuropodia of a belostomatine bug, *Appasus japonicus*.

Materials and Methods

Male and female adults of *Appasus japonicus* (Belostomatidae, Heteroptera) were collected from rice fields and small ponds at Hora, Matsumoto, Nagano Prefecture, and reared in a thermostatic room at 28±0.5℃. Mating and egg oviposition occurred in the thermostatic room, and each male carrying an egg batch was separately reared in the same conditions. After removing the eggs from the male wings, the eggs were fixed with alcoholic Bouin's fluid (saturated alcoholic solution of picric acid : formalin : acetic acid = 15 : 5 : 1) at room temperature for 24 h. From a portion of the eggs, embryos were dissected in Ephrussi-Beadle solution with fine forceps and then fixed. The fixed eggs and embryos were stored in 70% ethyl alcohol.

As for the total observation, eggs fixed and stored in 70% ethyl alcohol were then hydrated through a graded ethyl alcohol series, stained with 0.05% Mayer's acid hemalum (Mayers Hamalaunlosung, Merck) for 2 h, and observed under a microscope.

For histological study, the fixed and stored eggs or embryos were hydrated through a graded ethyl alcohol series, then transferred to 3% hot agar solution in a hole slide glass and then oriented. The egg or embryo specimens were processed into paraffin (Paraplast X-TRA, Fisher HealthCare) sections of 5 μm thickness. To soften the chorion, some eggs were immersed in a mixture containing 70% ethyl alcohol and 50% ammonium mercaptoacetate in the ratio of 9 : 1 for 24 h, prior to the infiltration of paraffin. Sections were stained with Mayer's acid hemalum and eosin (Eosin Yellowish, Nacalai) [in some cases supplementary stained with 0.01% fast green FCF (Tokyo Chemical Industry Co.) alcoholic solution].

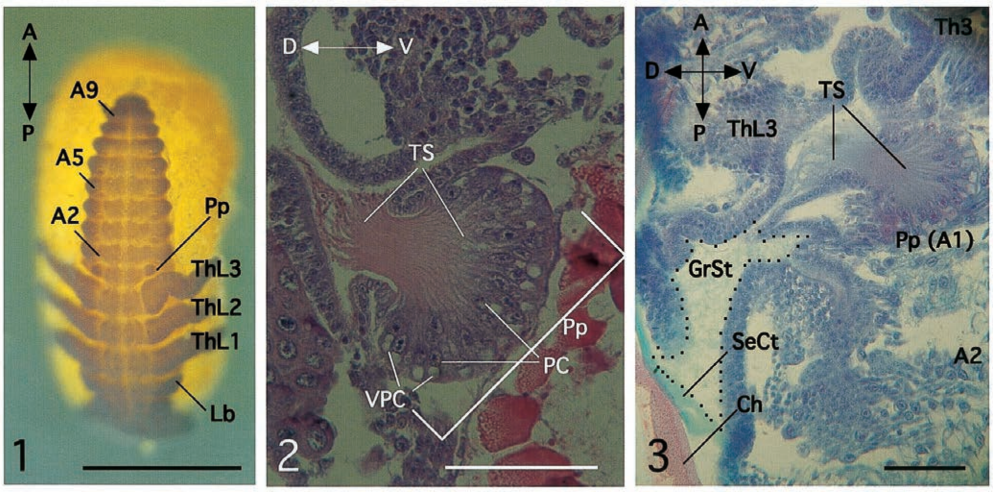
Results and Discussion

The egg period of the belostomatine bug *Appasus japonicus* is eight to nine days at 28±0.5℃. Its embryonic development, being divided into nine stages, then the first instar nymphal stage, according to Tanaka (2001), is described, with special reference to the development of pleuropodium (Table 1). At stage 6, just before the katatrepsis, the appendages of the first abdominal segment or the pleuropodia first appear as a pair of small swellings (Fig. 1). At stage 7, although the pleuropodia invaginate to become undiscernible externally, they have developed, to assume a glandular appearance. The nuclei of the pleuropodial cells are localized at their proximal part, their cytoplasm is vacuolated, and their apical parts extend, to form unique thread-like structures (cf. Fig. 2). The pleuropodial cells are fully grown early in stage 8, as a result of the enlargement of the constituent cells. The thread-like structures protrude over the embryonic body wall (Fig. 2). Later in stage 8, a large quantity of a granular substance appears outside the embryo, in the vicinity of pleuropodia. This substance has a high stainability with fast green FCF solution (Fig. 3). This substance is likely to be secreted from the pleuropodia. At stage 9, the pleuropodial cells are observed to be conspicuously vacuolated. By hatching, the pleuropodia have degenerated, and in the first instar nymphal stage, only their remnants are found. A similar granular secretion is reported in the belostomatine bug *Belostoma flumineum* by Hussey (1926).

In the related species *Appasus major*, Kawano

Table 1 Embryonic stages and first instar nymph of the belostomatine bug *Appasus japonicus*, with special reference to the pleuropodia.

Stage	State of development
Stage 1	Maturation division; fertilization
Stage 2	Egg cleavage
Stage 3	Formation of blastoderm
Stage 4	Formation of embryo; formation of inner-layer
Stage 5	Elongation and segmentation of embryo; appearance of cephalic and thoracic appendages; formation of stomodaeum and proctodaeum
Stage 6	Appearance of pleuropodia; annulation of cephalic and thoracic appendages
Stage 7	Pleuropodia assuming the glandular appearance; revolution (katatrepsis); appearance of compound eyes
Stage 8	Secretion of granular substance by pleuropodium; compound eyes pigmented
Stage 9	Degeneration of pleuropodia; completion of dorsal closure
1st instar nymph	Pleuropodia found in the body surface as remnants



Figs. 1–3 Embryos of the belostomatine bug *Appasus japonicus*.

Fig. 1 Ventral view of a dissected embryo (stage 6) stained with 0.05% Mayer's acid hemalum. Pleuropodia are clearly visible as a pair of small swellings.

Fig. 2 A cross section of the first abdominal segment of a dissected embryo (early stage 8), showing the left pleuropodium.

Fig. 3 A longitudinal section of embryo (late stage 8), showing the left pleuropodium.

A1, 2, 5, 9: first, second, fifth and ninth abdominal segments, A-P: anterior-posterior axis, Ch: chorion, D-V: dorsal-ventral axis, GrSt: granular substance, Lb: labium, PC: pleuropodial cell, Pp: pleuropodium, SeCt: serosal cuticle, Th3: metathorax, ThL1–3: pro-, meso- and metathoracic leg, TS: thread-like structures of pleuropodium, VPC: vacuole of pleuropodial cells. Scales = 1: 500 μm; 2, 3: 50 μm.

(2003) reported that the adherence power of the egg mass to the male back decreases in the later developmental stages. It may be equivalent to the stage 8 to early in stage 9 of *A. japonicus* or the stages approximately when the granular substance is secreted from the pleuropodia in *A. japonicus*, judgeing from the figures of eggs shown in his paper. It is highly probable that the granular substance secreted from the belostomatine pleuropodia is a kind of enzyme related to the decrease in the adherence power between the egg mass and male back. The 'pleuropodian hypothesis' by Ando (1991, 1999) concerning the detachment of the egg mass from the male back in belostomatines is strongly supported.

Acknowledgments: We acknowledge the valuable suggestions and support of Dr. R. Machida, Prof. Y. Kobayashi, Mr. K. Kawano, Prof. K. Hoshikawa, Dr. M. Tanaka, Mr. N. Shimura and Dr. H. Ichiyanagi. We express our thanks to Dr. T. Tsutsumi, Messrs S. Yoshii, T. Sonohara, K. Suzuki, K. Oka, K. Sekiné, K. Miyairi, M. Yoshii and J. Kunimi for their support of collecting materials. The present study was partially supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (17770067) to K.T.

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