



# Mechanism of hemolymph circulation in the pupal leg of tenebrionid beetle *Zophobas atratus*

Toshio Ichikawa\*

Department of Biology, Faculty of Sciences, Kyushu University, Fukuoka 812-8581, Japan

## ARTICLE INFO

### Article history:

Received 30 November 2008  
Received in revised form 2 February 2009  
Accepted 4 February 2009  
Available online 10 February 2009

### Keywords:

Pupa  
Appendages  
Circulation  
Hemolymph  
Pulsatile organ  
Sinus  
Valve

## ABSTRACT

The long legs of insects require adequate hemolymph flow for maintaining their metabolism and functions. The visualization of hemolymph flow in a pupal leg of the tenebrionid beetle *Zophobas atratus* revealed that, in addition to a general circuit across all segments (podomeres) of the leg, there were two shortcut channels running within the femur, which is the largest podomere. A unidirectional hemolymph flow was forced by periodic pumping movements of the abdomen and regulated by a valve that exhibited a metamorphic change from a tongue-shaped to a flap-shaped structure. The results suggest that insects with a simple (open) circulatory system have evolved a sophisticated circulatory mechanism that distribute hemolymph adequately to the individual podomeres, which have diverse morphologies and functions.

© 2009 Elsevier Inc. All rights reserved.

## 1. Introduction

Animals larger than 1 mm generally require blood circulation or some mass flow of body fluid to transport commodities throughout their bodies at adequate rates (Hill et al., 2004). Insects with an open circulatory system have a tubular vessel along the dorsal midline of the body (Jones, 1977; Miller, 1985). The pumping activity of the dorsal vessel can circulate body fluid (hemolymph) in the central body cavity, but it has no effect on hemolymph exchange in long and narrow appendages, such as the antennae, palpi, wings, and limbs. Hence, many orders of insects have evolved special pumping organs for the antennae and wings (Pass, 1998, 2000; Pass et al., 2006). The circulatory mechanism driven by a similar auxiliary pump is often extended to the legs; this assumption is based on the detection of pulsatile organs in Hemiptera (bugs) and Orthoptera (crickets) of the hemimetabolous insect group (Pass, 2000; Hantschk, 1991; Hustert, 1999). However, in most insects, including all holometabolous insects (which account for 88% of all known insect species, Klowden, 2007), specific pumping mechanisms in the legs have not been described (Pass, 1998, 2000), but there has to be a mechanism to ensure circulation in the legs.

Classic anatomical studies have shown that the hemocoelic cavity of an insect leg is usually divided into 2 sinuses that are confluent at the extremity of the leg, and in vivo observations of countercurrent streams in the sinuses of many insect species have suggested that hemolymph enters one (efferent) sinus, passes through all podomeres of the leg to reach the tip of the leg, and then returns to the body cavity

through the other (afferent) sinus (Jones, 1977; Pass, 1998, 2000). This simple view of the circulatory mechanism in the leg is generally accepted and appears in many textbooks (e.g., Wigglesworth, 1972; Hill et al., 2004; Klowden, 2007). However, this circulatory mechanism seems too primitive for the adequate distribution of the hemolymph flow to the individual podomeres, which have diverse morphologies and functions.

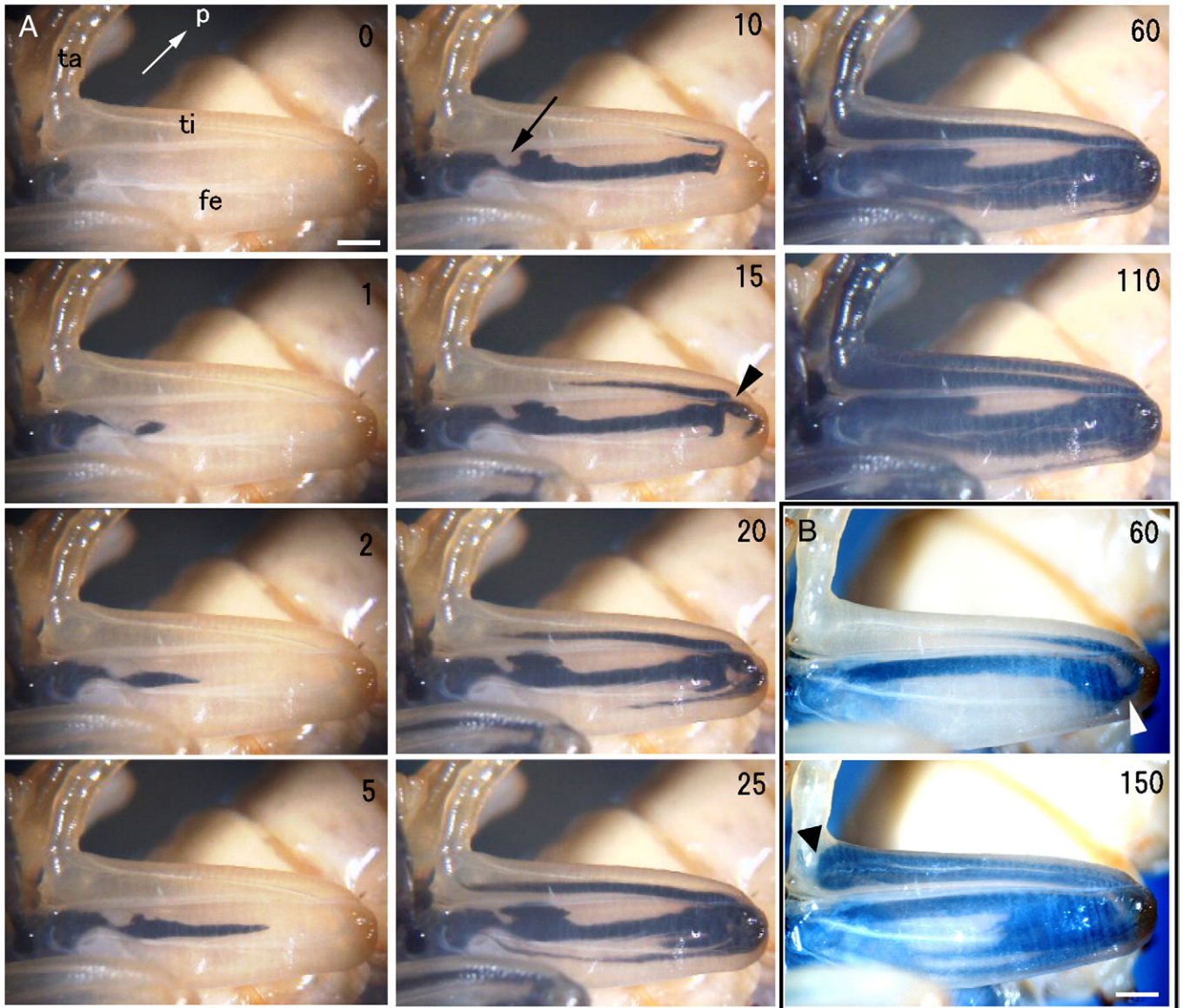
The circulatory systems in the legs of adult insects are often complicated, and active movement of the legs affects hemolymph circulation (Meyer, 1931; Selman, 1965). On the other hand, in the quiescent holometabolous pupae, a developing appendage is usually immobile and the circulatory mechanism in the pupal leg is relatively simple because the imaginal structures are immature. These features make the pupal circulatory system suitable for studying the general organization and dynamics of the circulatory system in insect legs.

The pupae of tenebrionid beetles often exhibit regular patterns of hemolymph pressure pulses that are produced by rhythmic contractions of intersegmental abdominal muscles (Sláma, 1984). The pressure of such pulses in the beetle *Zophobas atratus* reached 20 mmHg, and the complete blockade of the abdominal pulsation during the early pupal period of this insect abolished the normal development of its legs (Ichikawa, 2008). Since the defect in leg development seemed to be due to poor hemolymph circulation in the legs, the mechanism of hemolymph circulation in the leg and the functional role of the abdominal pump in the mechanism were examined.

## 2. Materials and methods

Mealworms *Zophobas atratus* Fab. were purchased as completely grown larvae from a local supplier. The larvae were maintained in a

\* Tel.: +81 92 642 2612; fax: +81 92 642 2315.  
E-mail address: [tichiscb@kyushu-u.org](mailto:tichiscb@kyushu-u.org).



**Fig. 1.** Still images showing the hemolymph flow in the hind legs of 1-day-old (A) and 5-day-old pupae (B). The numbers indicate the number of abdominal pumping motions. fe, femur; ta, tarsus; ti, tibia; p, posterior; arrowheads, splitting point of the hemolymph current; arrow, valve; triangle, turning point of the tibial current. Scale bars, 1 mm.

mixture of peat moss and sawdust and were fed fresh Japanese radishes. Individual larvae were isolated in a plastic cup for pupation. The pupae were maintained at  $26 \pm 1$  °C under a 16 h light/8 h dark photoperiod for an average pupal period of 13 days.

One of the hind legs was usually observed for hemolymph flow because its femur and tibia are almost flat on a plane. After a pupa was immobilized in carbon dioxide, the dorsal region of the thorax of the pupa was fixed to a platform using melted paraffin. In order to visualize hemolymph flow in the hind leg, a glass pipette filled with a solution of Indian ink (diluted by 5 parts of physiological saline for the beetle) or 1% methylene blue was inserted into the coxa, and 5–10  $\mu$ L of the dye was injected using a microinjector (Sutter Instrument, San Rafael, USA). In order to clearly visualize the ventral surface of the femur and tibia, a drop of mineral oil was placed on the surface. Still images or movies of hemolymph flow were recorded on a digital video camera recorder (Sony DCR-HC96; Sony, Tokyo, Japan).

For histological analysis, the hind legs of pupae at various developmental stages or of newly ecdysed adults were fixed in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.0) for 12–18 h, dehydrated through a

graded series of ethanol, and embedded in paraffin. The sections were stained with hematoxylin and eosin.

### 3. Results

#### 3.1. Hemolymph flow in the leg

An insect leg usually consists of 6 podomeres, namely, the coxa, trochanter, femur, tibia, tarsus, and pretarsus (Snodgrass, 1935). The coxa and the trochanter of all pupal legs in *Z. atratus* are short and appear to fuse with the thorax and femur, respectively. The semitransparent cuticles of the pupal legs allow the visualization of hemolymph flow pathways and movement of the valvular device after Indian ink or methylene blue is injected into the coxa. The first pressure pulse pushed the dyed hemolymph into the posterior (efferent) channel through a constricted tract, which served as a one-way valve, and every hemocoelic pressure pulse caused a stepwise movement of the hemolymph in the femur (Fig. 1A, Movie 1). In a young pupa (up to 2 days after pupation), the hemolymph entering the femur reached the femoral-tibial joint by

Download English Version:

<https://daneshyari.com/en/article/1972918>

Download Persian Version:

<https://daneshyari.com/article/1972918>

[Daneshyari.com](https://daneshyari.com)