Journal of Asia-Pacific Entomology 19 (2016) 233–237

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Intra-puparial development and age estimation of forensically important *Hermetia illucens* (L.)

Liangliang Li [a,b], Yu Wang [a,b], Jiangfeng Wang [a,*]

[a] Department of Forensic Medicine, Soochow University, Ganjiang East Road, Suzhou, China
[b] Department of Entomology, College of Agriculture, South China Agricultural University, Wushan Road, Guangzhou, China

ARTICLE INFO

Article history:
Received 16 July 2015
Revised 7 January 2016
Accepted 31 January 2016
Available online xxxx

Keywords:
Black soldier fly
Intra-puparial development
Morphological characteristic
Postmortem interval

ABSTRACT

The black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), is a generalist detritivore commonly present in the advanced decomposed and skeletonized remains stage, and may be useful in the estimation of the postmortem interval (PMI). In order to study the intra-puparial morphological characteristics of *H. illucens* (black soldier flies) under increasing temperatures, flies were obtained from a colony in a successional study conducted in the Panyu District of Guangzhou. After oviposition, eggs were incubated under constant temperatures of 20, 24, 28, 32 (±1) °C with 70% relative humidity and an L12:D12 photoperiod. Sampling began at the onset of the pupal stage (n = 10) and continued daily. Pupae were fixed in Carnoy's solution and maintained in 75% ethanol. Intra-puparial morphological characteristics were photographed and recorded. Some typical intra-puparial morphological characteristics which are valuable for age estimation were documented and the relationships with time under different constant temperatures were listed. Eight phases were described on the basis of the intra-puparial morphological changes, including antennae, appendages, mouthparts, compound eyes, and respiratory horns. The time required for development was inversely correlated with temperature. This article provides fundamental data for forensic entomology in regard to the postmortem interval (PMI) estimation, particularly when the PMI is based on information about the pupa of *H. illucens*.

Introduction

Forensic entomology, which uses insects and other arthropods in forensic investigations, is becoming increasingly important in crime investigations (Amendt et al., 2007). There are many reports showing the successful use of entomological data to estimate the postmortem interval (PMI) (Goff and Odom, 1987; Benecke, 1998; Amendt et al., 2007; Vanin et al., 2011; Vairo et al., 2015). During early decomposition, flies in the families Calliphoridae (blow flies) and Sarcophagidae (flesh flies) predominate and are the most accurate entomological indicators of PMI. Unlike most other species of forensically important Diptera, the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidaeis), frequently predominates remains in the drier, post-decay stage of decomposition (Lord et al., 1994).

The black soldier fly, the most well-known species of Stratiomyidae, is originally a New World species and is now widely distributed from approximately latitudes 46°8′ N to 42°8′ S (Üstüner et al., 2003; McCallan, 1974). The black soldier fly is regarded as an economically important resource insect for managing livestock and poultry manure, kitchen waste and more (St-Hilaire et al., 2007; Liu et al., 2008; Li et al., 2011; Yu et al., 2011). We have identified larvae or pupae of this species in case investigations and in field research and others have reported the presence of the black soldier fly in animal carcasses or human corpses (Turchetto et al., 2001; Tomberlin et al., 2005; Pujol-Luz et al., 2008; Kavitha et al., 2013; Yin et al., 2014). These findings indicate that *H. illucens* is a forensically important species and can be employed to estimate of PMI in the later stages of decomposition.

In forensic investigations, pupae are common entomological evidence at crime scenes and are often the only insect evidence (Goff and Odom, 1987; Benecke, 1998; Vanin et al., 2011). In addition, the pupal stage represents about 50% of the immature developmental phase (Zehner et al., 2006), and thus may serve as an important tool in entomological PMI estimation. The use of pupae has been documented in estimating PMI (Goff and Odom, 1987; Benecke, 1998; Amendt et al., 2000; Vanin et al., 2011) and detecting drugs (Wood et al., 2003). Much research on the metamorphosis of insects has been carried out worldwide, but research on chronology of intra-puparial development is rare (Cepeda-Palacios and Scholl, 2000; Greenberg and Kunich, 2002; Byrd and Castner, 2009; Barros-Cordeiro et al., 2014). In the present study, we investigated the chronology of intra-puparial development of *H. illucens* in different temperatures and propose a feasible method to determine the age of pupae precisely and quickly. Our results

* Corresponding author at: Department of Forensic Medicine, Soochow University, Ganjiang East Road, Suzhou 215000, China. Tel.: +86 181 5111 6801.
E-mail address: wjf701125@aliyun.com (J. Wang).

http://dx.doi.org/10.1016/j.aspen.2016.01.006
1226-8615/

provide a novel database describing the morphological changes that occur during the development of *H. illucens* for forensic practice.

Materials and methods

Source and culturing

Black soldier flies used in this study were obtained from a colony that was established from piglet carcasses in succession studies conducted in the Panyu District of Guangzhou (22°45′ N, 113°14′ E) in late spring of 2012. The flies were reared as described previously (Sheppard et al., 2002). Adults in our colony were housed in fine wire mesh cages (1.5 × 1.5 × 2.0 m) and kept in an insectary at natural temperature and relative humidity.

Eggs were collected in "egg traps" made of three layers of double-faced corrugated cardboard (Booth and Sheppard, 1984) glued together, and cut into 3 × 5-cm blocks. Egg traps were taped to the inside of the rearing boxes without lids (NoA3, Huamei, 25 × 17 × 7 cm) 3–4 cm above a wet diet consisting of 50% wheat bran and 50% peanut bran. Having the diet near the saturation point encourages oviposition in the dry cardboard egg traps. After oviposition, eggs were placed in 10 cm-diameter culture dishes with lids until hatching. The culture dishes were placed into three microenvironment incubators LHP-300 H (Yingmin Co. Ltd., Suzhou, China) with temperatures of 20, 24, 28, and 32 (±1)°C, respectively with 70% relative humidity and an L12:D12 photoperiod. Each incubator contained two boxes of target insects: one box was used for sampling (sampling group) and the other (indicator group) was used as the indicator of emergence time. Neonate larvae in the sampling boxes were placed in rearing boxes, which have wire mesh screen windows (5 × 3 cm) for ventilation. The larvae were placed on the wet diet and the rearing boxes were placed inside of the microenvironment incubators. The boxes were monitored daily.

Sampling

The onset of the pupariation process in larvae is marked by a dark brown color and a folding of the abdomen 45° toward the ventral region. At this point, the pupae were moved to culture dishes containing silt. The pupae selected in the first 6 h were assigned to group A and the pupae in the second 6 h assigned to group B. This labeling system continued until all the larvae had turned into pupae. The pupae were incubated under constant temperatures 20, 24, 28, 32 (±1)°C with 70% relative humidity and L12:D12 photoperiod (same as above). Sampling began with the onset of pupal stage, using a sample size of 10 and a sample interval of 1 d. Group A was sampled first, until there were less than 10 remaining pupae; at this time, sampling began for group B and so forth. The sample interval between two groups (i.e., group A and group B, or group B and group C) was 30 h because the pupation time of the next group is 6 h later than the previous group; this sampling method is named "zeroing of pupation". The sampling was concluded when 50% of the pupae in the indicator group accomplished eclosion. Replications were performed three times under each temperature. The sampled pupae were fixed in Carnoy's solution and maintained in 75% ethanol.

Observations and analyses

The puparium was removed from the pupae via scissors and insect pins on the wax plate. The representative morphological characters of intra-puparial changes with varying times were selected under a Zeiss 2000-C stereomicroscope (Carl Zeiss, Germany). Photographs were taken using a Nikon D700 digital camera (Nikon, Japan). The representative intra-puparial morphological changes and the duration of each stage under different constant temperatures were documented. One-way analysis of variance (ANOVA) was conducted to measure possible differences in the developmental duration of immature stages at different temperatures.

Results

Average developmental duration

The effect of varying temperatures on the average developmental duration of *H. illucens* in the immature stage can be seen in Table 1. Insect development was severely inhibited at 20 °C; therefore no data is reported. The duration of the pre-pupal stage was longer than that of the pupal stage for all temperatures. The time required to reach each stage (egg, larvae, pre-pupae and pupae) was shorter at high temperatures than at lower temperatures. Temperature had a significant effect on the duration of the egg stage ($F = 33.23$, $p = 0.001$), the pupal stage ($F = 7.74$, $p = 0.02$), and total length of the immature stage ($F = 6.09$, $p = 0.03$). The temperature did not significantly affect the duration of the larval stage ($F = 3.11$, $p = 0.118$) or the pre-pupal stage ($F = 0.47$, $p = 0.65$).

Data represent the mean ± SD, one-way ANOVA + LSD test, $P < 0.05$.

Characteristics of H. illucens pupae

The morphology of the pupae of *H. illucens* can be seen in Fig 1. The pupae of the black soldier fly are called pupacoarctata. During development, the cuticle gradually becomes opaque and sclerotized, eventually turning dark brown in color. The pupal body is flat with obvious stomatotheca. The intra-puparial body shortens as the amount of tissue in the anterior part of the puparium decreases. The posterior region of the puparium is folded about 45° toward the ventral region. Each body segment has many bristles.

Intra-puparial morphological characteristics of H. illucens

During the pupal stage, intra-puparial development is characterized by a series of changes (Fig 2) representing different developmental processes, some of which correlated with time. Based on our results, we divided the pupal stage into eight developmental phases. In Phase I (panels A–D), the puparium was easily removed. The entire polypore was covered with a layer of film, and the process of extroversion and distinctness of the head, thorax and abdomen was beginning. The outlines of appendages (legs, wings and compound eyes) and mouthparts were present (panel C), and the respiratory horn appeared and continued to develop (panel D). In Phase II (panels A–D), compound eyes began to develop a yellowish pigment (panel C shows detail. Another key developmental processes observed in Phase II were differentiation and development of the halters (panel D). Phase III (panels A–D) marked the beginning of the appearance of the scutum and scutellum and the development of the antennae (panel C). The compound eyes had pinkish pigmentation (panel D) during this phase. During the course of Phase IV (panels A–D), the eye pigmentation turned red (panel C shows detail). Other features of Phase IV included the appearance of hairs or bristles (tomentum) on the polypide and mouthparts (panels A–B), the darkening and increase in density of bristles on the legs and pigmentation of the wings (panel D). The wings developed veins during Phase V (panels A–D; panel C shows detail), the sulcus of

Table 1
Average developmental duration of *H. illucens* at different temperatures.

Developmental stage (d)	24 °C	28 °C	32 °C
Egg	4.8 ± 0.5^{a}	$2.8. \pm 0.3^{b}$	2.7 ± 0.2^{b}
Larvae	17.0 ± 3.8^{a}	14.0 ± 2.6^{ab}	11.0 ± 2.2^{b}
Pre-pupae	13.0 ± 3.1^{a}	12.0 ± 2.5^{a}	11.0 ± 1.8^{a}
Pupae	11.0 ± 2.5^{a}	7.0 ± 2.0^{ab}	4.5 ± 1.5^{b}
Total	45.8 ± 8.6^{a}	35.8 ± 4.5^{ab}	29.2 ± 3.0^{b}

Data represent the mean ± SD, one-way ANOVA + LSD test, $P < 0.05$.