Arthropod Structure & Development xxx (2018) 1–8

Contents lists available at ScienceDirect

Arthropod Structure & Development

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

Micro-computed tomography of pupal metamorphosis in the solitary bee *Megachile rotundata*

Bryan R. Helm [a, *], Scott Payne [b], Joseph P. Rinehart [c], George D. Yocum [c], Julia H. Bowsher [a], Kendra J. Greenlee [a]

[a] Department of Biological Sciences, North Dakota State University, Fargo, ND 58108-6050, USA
[b] Electron Microscopy Center, North Dakota State University, Fargo, ND 58108-6050, USA
[c] Agricultural Research Service, Insect Genetics and Biochemistry, United States Department of Agriculture, Fargo, ND 58102-2765, USA

ARTICLE INFO

Article history:
Received 22 January 2018
Accepted 9 May 2018
Available online xxx

Keywords:
Development
Metamorphosis
Micro-computed tomography
Megachile rotundata

ABSTRACT

Insect metamorphosis involves a complex change in form and function. In this study, we examined the development of the solitary bee, *Megachile rotundata*, using micro-computed tomography (μCT) and volume analysis. We describe volumetric changes of brain, tracheae, flight muscles, gut, and fat bodies in prepupal, pupal, and adult *M. rotundata*. We observed that individual organ systems have distinct patterns of developmental progression, which vary in their timing and duration. This has important implications for commercial management of this agriculturally relevant pollinator.

1. Introduction and background

An emerging model insect for understanding the effects of environmental conditions on metamorphic development is the alfalfa leafcutting bee, *Megachile rotundata* (Hymenoptera: Megachilidae). *M. rotundata* is a widely-distributed solitary bee pollinator that is commercially-reared and used to enhance production of crops, primarily alfalfa seed, *Medicago sativa* (Pitts-Singer and Cane, 2011). A common problem in managing *M. rotundata* is that populations must be synchronized with flowering of crops in different geographic locations across North America for both effective pollination and population maintenance (Bosch and Kemp, 2005; Pitts-Singer and Bosch, 2010; Pitts-Singer and Cane, 2011).

Synchronization of *M. rotundata* emergence with flowering is achieved by interrupting metamorphosis of developing bees with temperature treatments below which development stops (Pitts-Singer and Cane, 2011; Yocum et al., 2010). However, a number of studies have demonstrated that low temperature-mediated interruption of metamorphosis results in deleterious sub-lethal effects (Bennett et al., 2015; Torson et al., 2017; Yocum et al., 2010). These effects may be shaped by the timing of the low-temperature exposure with respect to metamorphosis progression (Rinehart et al., 2016; Yocum et al., 2010). Chill injury, perturbation of developmental processes, or both are suspected mechanisms for these injuries. While the external developmental changes that occur during metamorphosis in *M. rotundata* have been characterized (Kemp and Bosch, 2000; Yocum et al., 2010), development of internal systems has not been investigated. Better spatial and temporal descriptions of internal development may help identify better timing for low-temperature exposure that minimizes negative effects for this species.

An emerging technology for characterizing both internal and external morphology of arthropods is micro-computed tomography, μCT (Lowe et al., 2013; Metscher, 2009). μCT generates highly resolved 3-dimensional models of internal and external anatomies when performed on insect samples. To this end, μCT has been used successfully to compare morphology among various organisms (Metscher, 2009), including comparisons of physiological systems during insect metamorphosis (Hall et al., 2017; Lowe et al., 2013; Martin-Vega et al., 2017a, 2017b) and adult bees (Greco et al., 2008).

In this study, we used μCT to build a more robust description of internal changes during metamorphosis in the solitary bee, *M. rotundata*. Whole organism μCT imaging was conducted on 6

* Corresponding author.
E-mail address: bryan.r.helm@ndsu.edu (B.R. Helm).

https://doi.org/10.1016/j.asd.2018.05.001

individuals, each representing a sub-stage of metamorphosis. We compare and contrast the changes in the structure of discernible, internal features across these stages. The primary aim was to determine which organ systems were developing when metamorphosis is commonly interrupted by the low-temperature methods described above.

2. Materials and methods

2.1. Study organism

Overwintering prepupal *M. rotundata* were obtained from JWM Leafcutters, Inc. (Nampa, ID) in the spring of 2014. Prepupae were stored in a 6 °C environmental chamber (Conviron, Winnipeg, Manitoba), which maintained a developmentally quiescent state, until the beginning of the study. Six prepupae were removed from storage and placed into a 29 °C environmental chamber (Precision Scientific, Buffalo, New York) to initiate metamorphic development. One individual was sampled for μCT scanning one day following placement into 29 °C (Fig. 1: prepupa). The prepupal stage generally lasts for 1 week before individuals pupate (Kemp and Bosch, 2000). Pupae were sampled 1, 7, 14, and 21 days following pupation (Fig. 1). These time points correspond roughly to previously used reference stages: early pupa, pink eye, red eye, and emergence ready (Yocum et al., 2010). These developmental descriptions are labeled onto the stage sampling and corresponding external development for comparative purposes in Fig. 1. One additional sample was taken of an adult male that had completed metamorphosis to the adult form but not yet emerged. Samples were removed from their brood cells immediately before scans by carefully cutting the "top" of the brood cell open with a safety razor.

2.2. Scanning procedures

Samples were dissected from leaf pods using a safety razor. The cap of the brood cell was first circumscribed with an incision. Then, the cap was removed and the individual was removed carefully with soft, curved forceps. Each sample was placed into a Kapton® tube that was affixed to a glass rod. Samples were then scanned using a GE Phoenix v|tome|x s X-ray computed tomography system equipped with a 180 kV high power nanofocus X-ray tube with a molybdenum target and a high contrast GE DXR250RT flat panel detector (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany). Initial beam parameters were standardized for each sample; however, these beam settings were adjusted to optimize outputs for each individual sample and are provided in Supplemental Table 1. Following scans, samples were removed from the tubing and monitored for survival for 3 days. No bee completed development following the scan. This may have been from x-ray exposure, handling, or rearing conditions outside of the cocoon.

2.3. Analysis of μCT data

We focused our analysis to examine the flight musculature, the gut, the tracheae, brain, and fat bodies, because they were discernible from scans of pupae without fixation or staining (Fig. 2), although not all structures were observed in the prepupa and adult. X-ray images were aligned and optimized using automated protocols within GE Datos|x software (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany), and volumetric data were computed from stacks of x-ray images (Supplemental Fig. 1). Volumetric data were then exported to VGStudio MAX 3.0 analysis software (Volume Graphics Inc., Heidelberg, Germany) for editing and analysis. Data volumes were analyzed using 3D-segmentation and volume analysis tools in VGStudio MAX. Several segmentation tools were applied to remove unwanted components of the CT volume, such as the Kapton® tubing and mounting glass rod. Focal structures were segmented as regions of interest.

Once flight musculature, gut, tracheae, brain, and fat bodies were segmented, measurements of each structure as well as whole body volumes were calculated. Analyzed volumes were then rendered into 3D models, and images were generated of the dorsal, lateral, and ventral perspectives. Volumes of structures were then calculated as a total percentage of body volume and compared among different stages. Because only one sample was used at each stage, statistical analysis was not conducted.

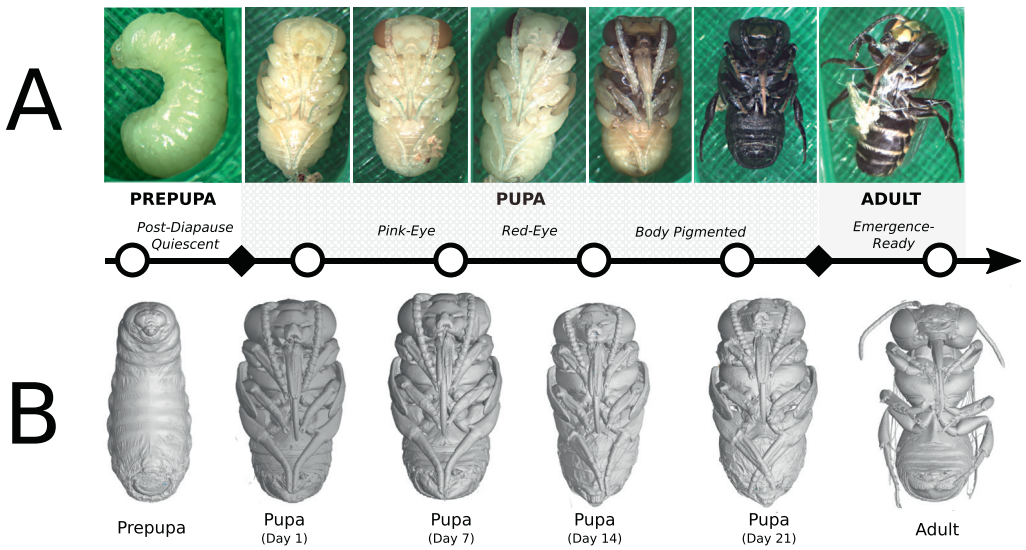

Fig. 1. External stages of metamorphosis in *M. rotundata* (A) used for sampling and their stage descriptions based on prior literature (italicized labels). Individuals were sampled during the early prepupal stage, throughout the pupal stage and in an un-emerged adult (B). The relative timing of pupal and adult eclosion are noted on the timeline (diamonds). Terms used for different stages are labeled with corresponding pupal development times (italics).

Please cite this article in press as: Helm, B.R., et al., Micro-computed tomography of pupal metamorphosis in the solitary bee *Megachile rotundata*, Arthropod Structure & Development (2018), https://doi.org/10.1016/j.asd.2018.05.001