Utilising micro-computed tomography in age estimation during the intra-puparial period of the forensically relevant blowfly, *Chrysomya chloropyga*

Wilmari Uys[1], Calvin Gerald Mole[1], Marise Heyns[2]
[1]University of Cape Town, South Africa; [2]Ulster University, United Kingdom

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Introduction

Age estimation of the immature life stages of blowflies can be useful in determining minimum post-mortem interval ($_{min}$PMI). Changes in morphological features related to the age of blowfly larvae have been extensively investigated; however limited research has been conducted on $_{min}$PMI determination from puparial specimens. Additionally, methods utilised in age estimation usually require the destruction of the specimen. Micro-computed tomography (micro-CT) has emerged as an alternative method for assessing morphological changes in insects. In this study, we evaluated the use of micro-CT to identify age-related morphological changes during the puparial period of the forensically relevant blowfly, *Chrysomya chloropyga*.

Methods

C. chloropyga were reared under controlled conditions at 25°C, 65% relative humidity and a 14:10 photoperiod. Once pre-pupation occurred, pre-pupae were collected and observed at age intervals of every 6 hours for the first 2 days and then every 12 hours until adult emergence. Specimens were stained in a 1% Lugol's iodine solution for 14 days and rinsed in ethanol an hour prior to scanning using a Nikon XT H 225 system. The resulting projections were reconstructed with a voxel size of 9.73 µm in CT-Pro 3D and slice stacks were rendered, reorientated, and visualised using VG Studio Max 2023.1. Development of structures was assessed over time, including changes in the volume of the adult midgut, rectal pouch and indirect flight muscles.

Results

Pre-pupal morphology corresponded significantly with post-feeding larvae (fig. 1a), but as the pupal period progressed, the pupal body and organ systems changed to that of an adult fly. In the head, the lamina started as a bulb shape (fig. 3d) at 36 hours post-pupation and progressed into a horseshoe shape (fig. 3e) by 48 42 hours post-pupation before it started to unfold (fig. 3i) at 84 hours post-pupation, lying parallel to the retinular cells (fig. 3j – l). Equally, the cornea (fig. 3b) was visible 18 hours post-pupation, but the layer of crystalline cones only became apparent 96 hours post-pupation (fig. 3i – l). The larval midgut (fig. 1b) was swiftly replaced by the adult midgut (fig. 1e, 2) which continued to change in shape, with the final portion of the midgut clearly coiled by 60 hours post-pupation. Similarly, the adult hindgut was first observed as a thin tube 30 hours post-pupation (fig. 2c) with the rectal pouch and rectal papillae visible 36 hours post-pupation (fig. 2d). Both the adult midgut and hindgut showed a continued change in shape and size until adult emergence. Volume measurements of the adult midgut showed a decline in volume from 18 hours post-pupation until the end of the intra-puparial period. In contrast, the indirect flight muscles and rectal pouch volumes, measured from 30- and 36 hours post-pupation, respectively, increased greatly until the conclusion of the puparial period (Table 1).

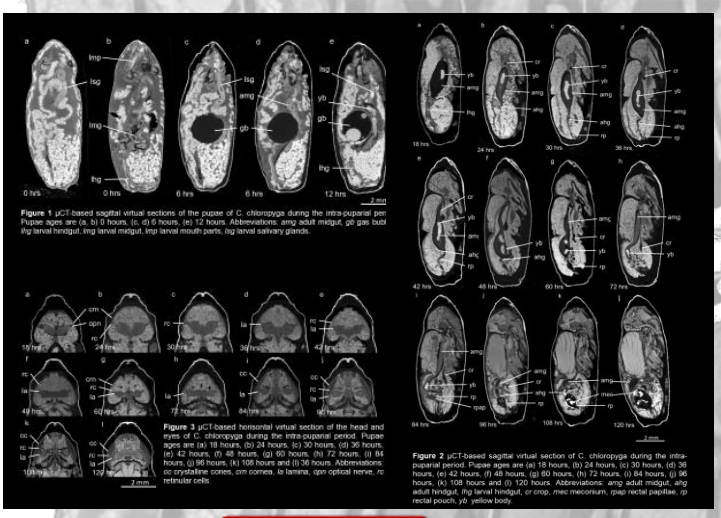

Figure 1 µCT-based sagittal virtual sections of the pupae of *C. chloropyga* during the intra-puparial per Pupae ages are (a, b) 0 hours, (c, d) 6 hours, (e) 12 hours. Abbreviations: *amg* adult midgut, *gb* gas bubl *ihg* larval hindgut, *img* larval midgut, *imp* larval mouth parts, *lsg* larval salivary glands.

Figure 3 µCT-based horisontal virtual section of the head and eyes of *C. chloropyga* during the intra-puparial period. Pupae ages are (a) 18 hours, (b) 24 hours, (c) 30 hours, (d) 36 hours, (e) 42 hours, (f) 48 hours, (g) 60 hours, (h) 72 hours, (i) 84 hours, (j) 96 hours, (k) 108 hours and (l) 36 hours. Abbreviations: *cc* crystalline cones, *crn* cornea, *la* lamina, *opn* optical nerve, *rc* retinular cells

Figure 2 µCT-based sagittal virtual section of *C. chloropyga* during the intra-puparial period. Pupae ages are (a) 18 hours, (b) 24 hours, (c) 30 hours, (d) 36 hours, (e) 42 hours, (f) 48 hours, (g) 60 hours, (h) 72 hours, (i) 84 hours, (j) 96 hours, (k) 108 hours and (l) 120 hours. Abbreviations: *amg* adult midgut, *ahg* adult hindgut, *ihg* larval hindgut, *cr* crop, *mec* meconium, *rpap* rectal papillae, *rp* rectal pouch, *yb* yellow body.

Table 1 Volume measurements of the indirect flight muscles, the adult midgut and the rectal pouch at different ages during the intra-puparial period of the C. chloropyga

Age (hours)	Relative volume of the indirect flight muscle (mm^3): average ± SD	Relative volume of the adult midgut (mm^3): average ± SD	Relative volume of the rectal pouch (mm^3): average ± SD
0	-	-	-
6	-	-	-
12	-	0.2661 ± 0.0835	-
18	-	1.6746 ± 0.4665	-
24	-	1.3103 ± 0.1707	-
30	0.2046 ± 0.0713	1.1816 ± 0.3636	0.0028 ± 0.0009
36	0.2347 ± 0.0641	1.1889 ± 0.1583	0.0056 ± 0.0018
42	0.3164 ± 0.0804	0.9943 ± 0.1126	0.0083 ± 0.0018
48	0.4741 ± 0.1599	0.9710 ± 0.1547	0.0148 ± 0.0059
60	0.7109 ± 0.1696	0.9054 ± 0.1197	0.0418 ± 0.0127
72	1.3749 ± 0.2257	0.6781 ± 0.1198	0.0708 ± 0.0169
84	2.1134 ± 0.4614	0.5567 ± 0.0915	0.0803 ± 0.0196
96	2.8886 ± 0.6446	0.4505 ± 0.0831	0.0960 ± 0.0305
108	3.5063 ± 0.7276	0.2953 ± 0.0903	0.3739 ± 0.2165
120	3.9666 ± 0.9076	0.4352 ± 0.1701	0.6127 ± 0.3891

Conclusion

Numerous age-informative morphological features were identified utilising micro-CT scanning indicating that it can be a useful tool in estimating pupal age. In addition to providing high-resolution qualitative data, quantitative measurement of several key morphological features were also possible. In conclusion, micro-CT scanning can be utilised in identifying age-related morphological features, enabling age-estimation of C. chloropyga pupae without destruction of the specimen.

References available upon request.

Corresponding author: uyswil003@myuct.ac.za

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