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Determining the age of *Lucilia illustris* during the intrapuparial period using two approaches: morphological changes and differential gene expression

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Highlights

- *Lucilia illustris* is a forensically important species for PMI_{min} estimation.
- The development of intra-puparial period was studied using two approaches.
- Age-related intra-puparial morphological changes were photographed and described.
- Expression of target genes exhibit regular changes and temperature dependence.
- Combination of two approaches can generate a more precise $PMI_{min.}$

ABSTRACT *Lucilia illustris* (Meigen, 1826) (Diptera: Calliphoridae) is a cosmopolitan species of fly that has forensic and medical significance. However, there is no relevant study regarding the determination of the age of this species during the intrapuparial period. In this study, we investigated the changes in both morphology and differential gene expression during intrapuparial development, with an aim to estimate the age of *L. illustris* during the intrapuparial stage. The overall intrapuparial morphological changes of *L. illustris* were divided into 12 substages. Structures such as the compound eyes, mouthparts, antennae, thorax, legs, wings, and abdomen, each capable of indicating age during the intrapuparial stage, were observed in detail, and the developmental progression of each of these structures was divided into six to eight stages. We recorded the time range over which each substage or structure appeared. The differential expression of the three genes *15_2*, *actin*, and *tbp* previously identified for predicting the timing of intrapuparial development was measured during *L. illustris* metamorphosis. The expression of these genes was quantified by real-time PCR, and the results revealed that these genes can be used to estimate the age of *L. illustris* during the intrapuparial period, as they exhibit regular changes and temperature dependence. This study provides an important basis for estimating the minimum postmortem interval (PMI_{min}) in forensic entomology according to changes in intrapuparial development and differential gene expression. Furthermore, combination of the two approaches can generate a more precise PMI_{min} than either approach alone.