International Congress Series 1288 (2006) 619–621

www.ics-elsevier.com

Estimating the postmortem interval by determining the age of fly pupae: Are there any molecular tools?

R. Zehner *, S. Mösch, J. Amendt

Institute of Legal Medicine, J W Goethe-University Frankfurt, Germany

Abstract. Forensic entomology, the use of insects in medicolegal investigations, mainly focus on the estimation of the postmortem interval (PMI) by calculating the age of necrophagous specimens. While staging the age of larvae is possible at a quite detailed scale, the age of the pupae is not to specify as easy as the larvae. However, the pupal stage represents about 50% of the immature development time and the pupal age may therefore serve as an important tool in entomological PMI estimation. Our approach was to study gene expression patterns of transcripts, which are differently expressed during pupal development. However, the applied method does not enable a determination of a pupa's age by molecular methods so far.

Keywords: Forensic entomology; dd-PCR; PMI; Fly; Pupa

1. Introduction

When maggots of necrophagous flies infest a human corpse, their stage of development and the determination of their species can be used to estimate the postmortem interval of the corpse. For many species, the larval age can be addressed to specific morphological changes (e.g. length and larval stages) if environmental conditions are known [1]. Because the pupal development represents a metamorphic change without growth, this method cannot be applied in this stage. However, the pupal stage represents about 50% of the immature development; therefore, methods which are suitable for age estimation within this period would be valuable in entomological PMI estimation.

* Corresponding author. Tel.: +49 69 63017571; fax: +49 69 63015882.
E-mail address: zehner@em.uni-frankfurt.de (R. Zehner).

0531-5131/
doi:10.1016/j.ics.2005.12.007

The present study investigates the possibility of using differential display PCR to detect changes in gene expression during the pupal development.

2. Materials and methods

Larvae from the greenbottle fly *Lucilia sericata* were purchased from Biomonde, Hamburg, Germany, and obtained also from a human corpse during autopsy ("wild-type"). They were reared until pupation. The rearing temperature was 25 °C, hatching of the adult insect occurred after about 190 h after pupation. Some of the pupae were used for investigation; the others were reared for complete metamorphosis. Offspring and further descendants were used in repeated investigations.

Total RNA was extracted from a single pupae using Trizol Reagent. The development stage of the pupae was investigated immediately, 48, 96 and 144 h after pupation. Preparations were made in 5 parallels with 10 generations of Biomonde pupae and 3 generations of wild-type pupae. cDNA was synthesized using oligo dTNX Primers (N = any base, X = A or C). PCR was performed with both types of cDNA using following decamers together with the appropriate oligo dTNX Primer: Decamer 1, 5′–CGACGT-CACG; Decamer 2, 5′–TCGCAAGACA; Decamer 3, 5′–AAAGCTGCGG. Fragment detection was performed using PAGE and silver staining. Other oligo dTNX Primers and other reverse Primers, also of other length than decamers, were also tested in a preliminary study but did not provide useful data.

3. Results

Fig. 1 shows dd-PCR patterns of two generations of the same source (Biomonde) which indicates a high reproducibility. In nearly all tests applied so far, the patterns did not show significant alterations in the fragment composition (data of other generations not shown). Significant changes could at least be seen after 144 from 190 h i.e. after ca. 75% of the puparial development time indicated by a fragment of about 230 bp.

Fig. 2 shows dd-PCR patterns of a Biomonde and a wild-type generation generated with the same primers and PCR contitions.

The intensity of the 190-bp fragment of the Biomonde pupae increased with age (arrow). However, this fragment is lacking in wild-type pupae. Beyond this, these two patterns are quite similar, besides minor differences.

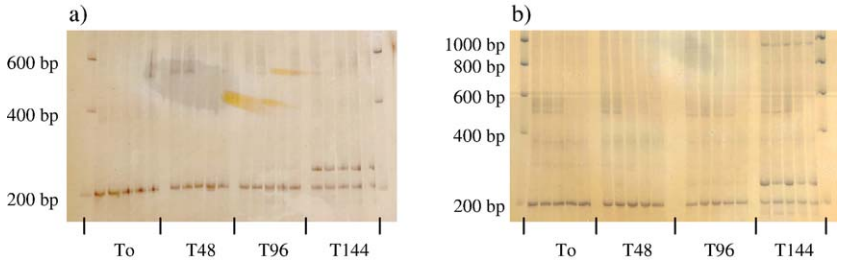

Fig. 1. dd-PCR patterns of one primer combination applied to pupae of generation (a) and (b) from animals of the same source (Biomonde). Each development time (T_0, T_{48}, T_{96}, and T_{144}) was performed in 5 parallels.

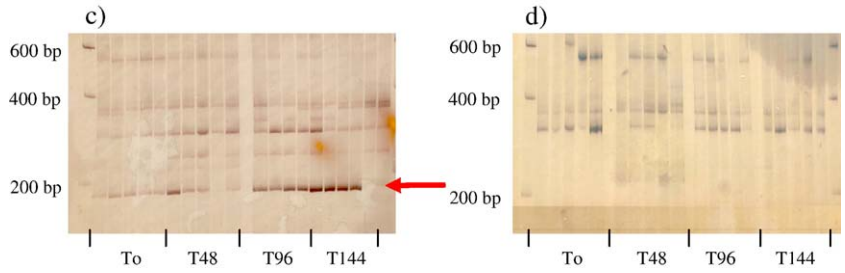

Fig. 2. dd-PCR patterns of one primer combination applied to pupae of generation (c) and (d) from animals of different sources: c = Biomonde, d = "wild-type". Each development time (T_0, T_{48}, T_{96}, and T_{144}) was performed in 5 parallels.

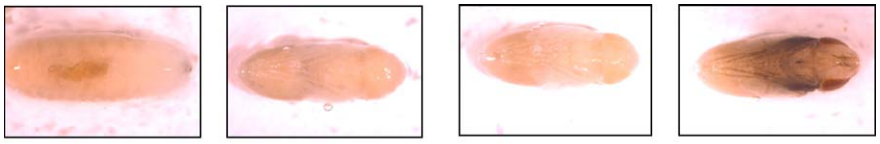

Fig. 3. Ventral view on pupae of different age after removal of the puparium. Note that these pupae are reared at 20 °C resulting in a pupal stage of 280 h, compared to the pupae used in the molecular study.

4. Discussion

In general, the present data demonstrate that it is possible to show an age dependant differential gene expression in pupae of *L. sericata*. However, with the method used, only a small amount of information can be obtained and significant alterations can be demonstrated only in a later phase of the puparial development.

It could be demonstrated that the fragment patterns may rather correspond with the origin of the genetic lineage than with the development stage.

Moreover, only few fragments were detected over all, so only a low amount of information is received. This has been observed also with other primers – of different sequences as well as shorter oligos (latter should lead to an annealing at more targets) and various PCR conditions (data not shown).

The technique applied does not enable a determination of a pupas age by molecular methods. When compared to the morphological alterations during the pupal development (Fig. 3), it is obvious that greater amounts of informative characters are present and can act as more informative tools in age estimation.

Further molecular studies may focus on specific genes which are only expressed in a specific slot during the pupal development or may be analysed quantitatively. For a useable age determination, smaller intervals also have to be investigated.

Reference

[1] J. Amendt, R. Krettek, R. Zehner, Forensic Entomology (review) Naturwissenschaften 91 (2) (2004) 51–65.