Investigating Abdominal Metamorphosis in Dipteran Insects

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INTRODUCTION

What is 'metamorphosis'?

- ➔ It encompasses the complete biological transformation of the organism from an immature form stage to the adult form [1].
- ➔ The **lifecycle trajectory** for the insect orders that undergo full metamorphosis follows **4 distinct stages**: egg, larva, pupa, and adult [1].
- ➔ The most popular model to study metamorphosis is the **fruit fly**, *Drosophila melanogaster*.
- ➔ The process is characterized by **cell replacement**, where polyploid larval epithelial cells (**LECs**) are replaced by diploid progenitor cells (**histoblasts**) [2,3].

Why is it of interest?

- ➔ Such a cell replacement process has only been described in Brachyceran diptera (Fig. 1) [4,5,6].
- ➔ In other insects, the adult abdominal epidermis arises by **LEC transdifferentiation**, where the LECs survive metamorphosis and become adult cells [1].
- ➔ It is **unclear** how the replacement mode of abdominal metamorphosis has evolved in the Diptera.

What was the aim of the investigation?

- ➔ The aim was to ascertain **which mode** of abdominal metamorphosis **can be found in Nematoceran flies** (replacement vs. reprogramming).
- ➔ Species chosen:
  - ◆ *Anopheles stephensi* mosquito (infraorder: Culicomorpha)
  - ◆ An unidentified fungus gnat species (infraorder: Bibionomorpha)

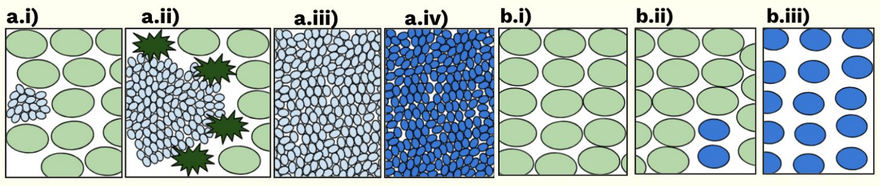

Fig. 1: Two modes of abdominal metamorphosis in the Diptera. (a) histoblast-driven cell replacement, (b) LEC transdifferentiation.

CONCLUSIONS

***A. stephensi* abdominal metamorphosis**

- ➔ **Pupariation** was captured on a stereomicroscope (Leica MZ10 F) equipped with a camera (AmScope MU300-HS) where the pupa shed its larval cuticle (Fig. 3c-f) via a coordinated contraction and relaxation of the abdominal muscles.
- ➔ The analysis of the abdominal epidermis showed that its metamorphosis **differs** from that seen in *D. melanogaster*.
- ➔ No evidence was found to support the presence of imaginal progenitor cells, with the epithelium demonstrating a visible **homogeneous monolayer** beneath the cuticle (Fig. 4a,c).
- ➔ The abdominal metamorphosis was characterized by **cell division** (Fig. 4b) that occurred in the fourth larval instars.

Fungus gnats abdominal metamorphosis

- ➔ The metamorphosis of the unidentified fungus gnat species showed a **greater resemblance** to *D. melanogaster* metamorphosis.
- ➔ I found lateral **small cell populations distinct from the larger LECs** that could be adult progenitor cells.
- ➔ These small cells seemed to **proliferate** (Fig. 5c-e) and **divide** (Fig. 5a) in the larvae, helping explain the decrease in nuclei diameter across the stages (Fig. 6a), with there being a significant statistical difference between every developmental stage studied.
- ➔ The presented evidence for possible adult progenitor cells in fungus gnats (Fig. 5b-e) provides a foundation for the **hypothesis** that **pupal-stage cell replacement evolved within the Neodiptera**.

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METHODS

1. Dissection of the abdomen along axes in Fig. 2
   a. Purpose: to expose the abdominal epithelium
2. Washing tissue with **phosphate-buffered saline (PBS)**
   a. Purpose: to prevent tissue desiccation
3. Placing tissue into 4% **paraformaldehyde (PFA)** for 30 minutes
   a. Purpose: to preserve the tissue
4. Setting it into 0.1% **PBS-Tween** for 30 minutes
   a. Purpose: to permeabilize cells and washing away pigments/lipids
5. Transferring tissue into 4 mM **Hoechst stain** solution for 40 minutes
   a. Purpose: to stain the nuclei so they can be seen with fluorescence-based microscopy
6. Inserting tissue into **Vectashield mounting medium** on a glass slide
   a. Purpose: to protect the tissue from laser-caused damage
7. The prepared samples were looked at using a **confocal microscope** at 405 nm.

Fig. 2: Specimen dissections

a) fungus gnat larva
b) fungus gnat pupa
c) mosquito larva
d) mosquito pupa

RESULTS

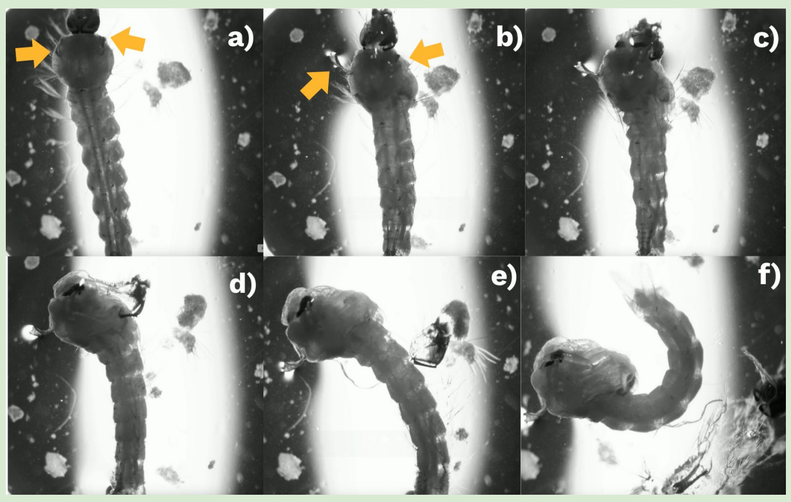

Fig. 3: Live imaging of the pupation of *A. stephensi*. It is possible to identify the two trumpets of the emerging pupa, indicated by the arrows, in (a) and (b).

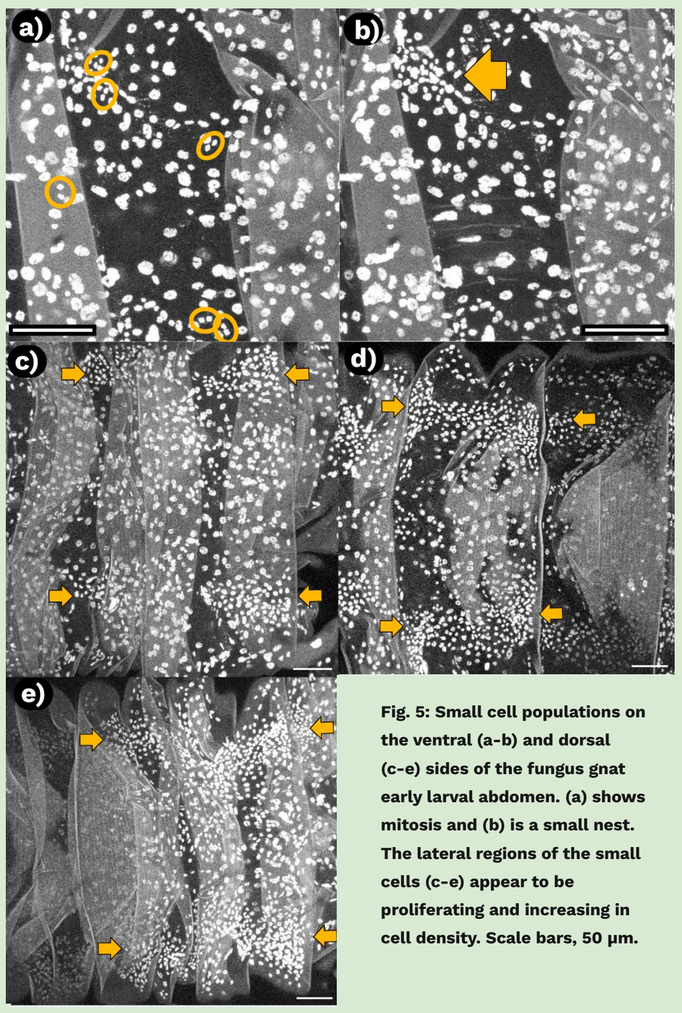

Fig. 5: Small cell populations on the ventral (a-b) and dorsal (c-e) sides of the fungus gnat early larval abdomen. (a) shows mitosis and (b) is a small nest. The lateral regions of the small cells (c-e) appear to be proliferating and increasing in cell density. Scale bars, 50 µm.

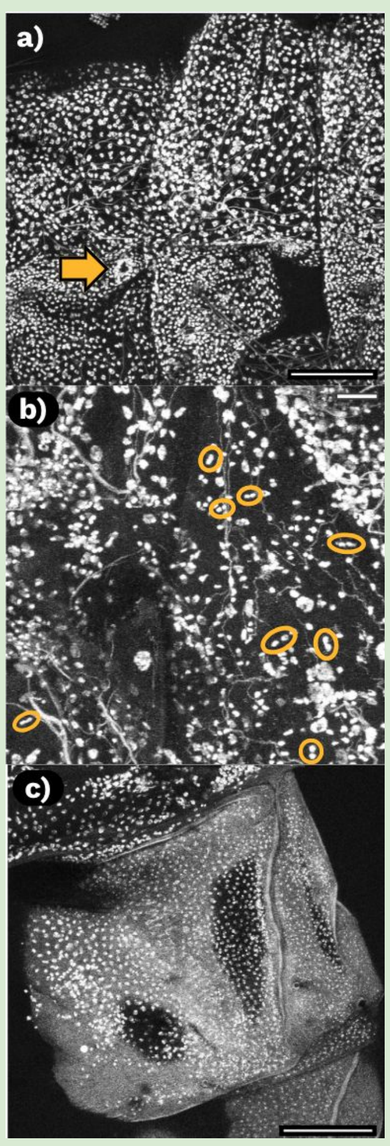

Fig. 4: Mitosis (b) and a homogenous abdominal epithelial cell distribution across development (a, c) in *A. stephensi*. (a,b) late larva, (c) 6-hour old late pupa. In (a) the arrow points to a spiracle. Scale bars, (a,c) 100 µm, (b) 20 µm.

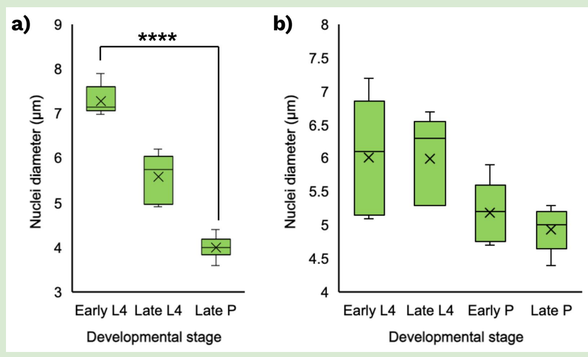

Fig. 6: Change in the nuclei diameters (µm) for the abdominal epithelia of fungus gnats (a) and *A. stephensi* (b).

OUTLOOK

- ➔ **Escargot (Esg) protein localization** would help determine whether the observed small cell populations in the fungus gnat are indeed adult progenitor cells [7].
- ➔ It would be advisable to repeat the fungus gnat study in a **well-characterised laboratory strain** (e.g. *Bradysia coprophila* [8]).
- ➔ It would be interesting to **expand the scope of this study** by examining more **lower fly genera** in the Bibionomorpha to precisely locate the origin of cell replacement-based metamorphosis in the Neodiptera.
- ➔ ***In vivo* imaging** would shed light on the potential cell replacement mechanism in the fungus gnats.

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