Pinpointing termination of diapause in apple maggot flies by reconciling metabolic increase with resumption of cell division and development

Genevieve Ochs, John Fuller, Gregory Ragland and Daniel Hahn, gsochs@gmail.com,
Dept. Entomology & Nematology University of Florida

Background

Apple maggot flies (*Rhagoletis pomonella*) are rapidly speciating via changes in pupal diapause regulation. We are broadly interested in the underlying mechanisms of allochronic reproductive isolation.

Previous studies show an increase in metabolic rate during diapause break precede any visible morphogenesis (Ragland *et al*, 2009).

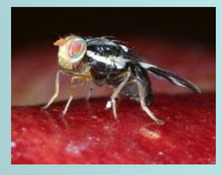
During pupal diapause in *Sarcophaga* cells are arrested in the G0/G1 phase of the cell cycle and cell proliferation is minimal. Upregulation of certain proteins accompany diapause break, including Proliferating Cell Nuclear Antigen (*PCNA*) (Tammriello and Denlinger, 1998).

Our goal is to pinpoint the first signal of diapause break in *R. pomonella* by respirometrically identifying stages of diapause development and screening for PCNA expression.

The Study System

Hawthorns (*Crataegus* sp.)

Apples (*Malus domesticus*)



R. pomonella

R. pomonella, the apple maggot fly, has historically infested hawthorn fruits throughout the United States and Mexico. Recently, a genetically and behaviorally distinct set of flies has developed that attacks domestic apples. These two fruits have slightly different seasonalities and this in turn impacts diapause timing.

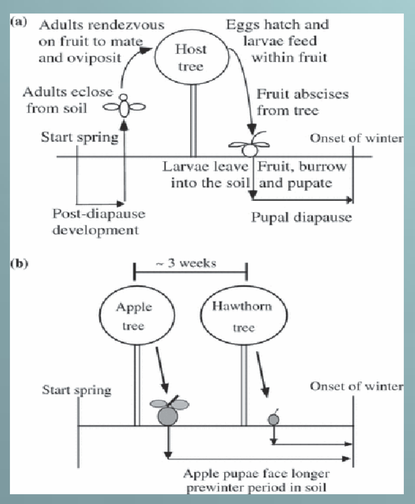


Figure 1 Life cycles of hawthorn and apple infesting *R. pomonella*.

Methods

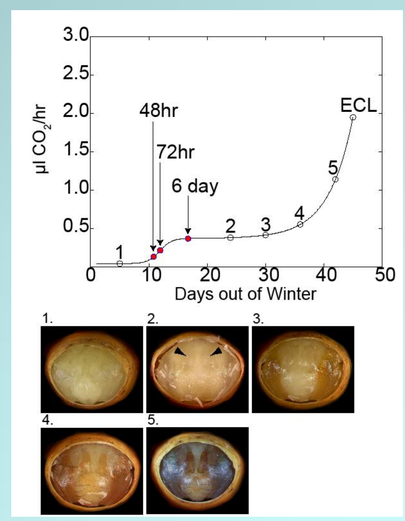


Figure 2. Respirometric/morphological trajectory of diapause break. Red circles denote sampling points post-diapause break.

Previous studies have shown a rapid, pronounced increase in metabolic rate during the transition from diapause to non-diapause (at ~ day 9 in Fig. 2).

After ~25 wks of overwintering (at 4C) we moved pupa to 24C, tracked them respirometrically, and removed brains for PCNA expression: 1) prior to metabolic rate increase (**long-winter diapause**), 2) 10 days after initial increase (**post-break**). (Fig. 2)

We also compared PCNA expression in the following series 1) **48 hrs**, 2) **72 hrs**, 3) **6 days** after the initial metabolic rate increase to 4) diapausing pupae overwintered for only ~5 weeks (**short-winter diapause**). Each sample in Fig. 4 represents 10 brain equivalents.

We performed flow cytometry on a pool of 25 brains from **long-winter diapause** pupae.

Results

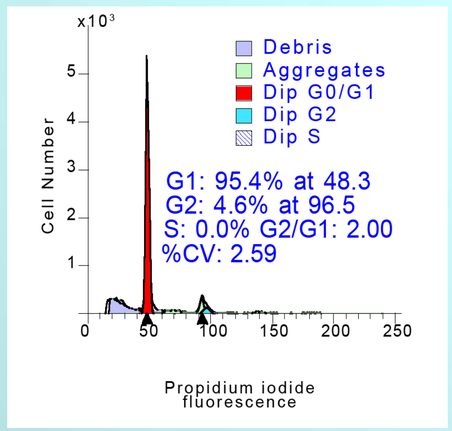


Figure 3 Flow cytometry results showing cell cycle stage of **diapause** pupal brains.

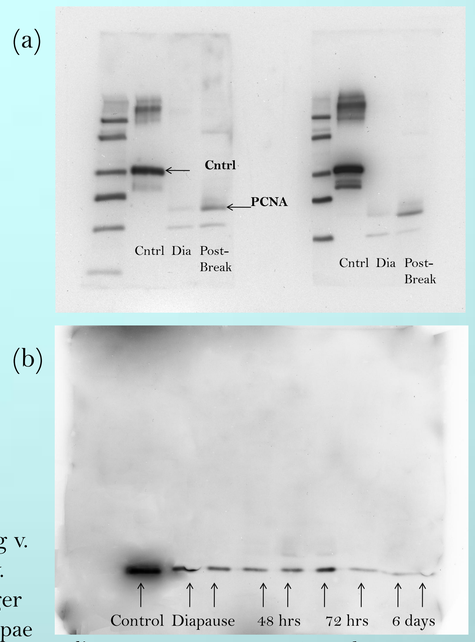


Figure 4 Western blots comparing PCNA in diapausing v. non-diapause pupae. (a) **Long-winter diapause** pupae v. **post-break** pupae. The control used in this blot is a larger recombinant PCNA positive control. (b) The diapause pupae were 5-week overwintered (**short-winter diapause**). The post-diapause pupae were 25-week overwintered. The PCNA positive control used in this study is different than in Fig. 4a (i.e., is not recombinant and runs at the correct molecular weight).

We found 95.4% of cells in diapause pupae are in Go/G1 phase of cell development (Fig 3).

Figure 4a shows little or no PCNA in **long-winter diapause** pupae and appreciable PCNA expression in the pupae **post-break**. These pupae were morphologically in the red eye state of development post-break.

Figure 4b shows comparable PCNA expression in the **short-winter diapause** samples and **48 hrs** and **72 hrs** after the initial metabolic rate increase. Expression is decreased **6 days** past the metabolic rate increase.

Conclusions

The pupa of *R. pomonella* show similar diapause cell cycle patterns as results reported by Tammriello and Denlinger (1998) for pupal diapause in *Sarcophaga* flies with life histories similar to *R. pomonella*. ~95% of pupal cells measured by flow cytometry were arrested in G0/G1. This supports pupal diapause GO/G1 cell cycle arrest in *R. pomonella*.

PCNA is expressed by **48 Hrs** after initial increase in metabolic rate. Microarray studies (unpublished data) also show a two-fold increase in PCNA mRNA expression b/t **long-winter** diapause and 48 hrs post-metabolic increase.

But, protein expression prior to metabolic increase depends on overwintering status (**short-winter** -> PCNA present, **long-winter** -> PCNA minimal/absent)

Because there are appreciable levels of PCNA transcripts until 13 days after diapause initiation in *Sarcophaga* (Hayward *et al*, 2005), PCNA observed in diapause pupae in Figure 4b could be an artifact of previous cell cycling. It is possible that PCNA protein "hangs around" longer because it degrades more slowly than the transcript.

What's next?

Future work will elucidate the relationship between PCNA and diapause-break. Experiments sampling more time points during diapause and post-break and long over-wintered pupa would allow us to pinpoint the exact timing of PCNA expression.

References

Hayward, SAL, SC Pavlides, SP Tammariello, JP Rinehart, and DL Denlinger. "Temporal expression patterns of diapause-associated genes in flesh fly pupae from the onset of diapause through post-diapause quiescence ." *Journal of Insect Physiology* 51.6 (2005): 631-640.

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Tammariello, SP, and DL Denlinger. "G0/G1 cell cycle arrest in the brain of Sarcophaga crassipalpis during pupal diapause and the expression pattern of the cell cycle regulator, proliferating cell nuclear antigen ." *Insect Biochemistry and Molecular Biology* 28.2 (1998): 83-9.