The Embryonic Development of Western Flower Thrips *Frankliniella occidentalis* (Pergande) and Banded Greenhouse Thrips *Hercinothrips femoralis* (O.M. Reuter): Embryogenesis summary

1. Preface

Through the course of my MSc research working with *Frankliniella occidentalis* (refer to Rajput 2007 below), I became fascinated with oviposition and embryonic development as a side project. With encouraging support of my supervisors, Drs. Andrew Keddie and Ken Fry, and Professor Emeritus Dr. Bruce Heming, an expert in insect ontogeny; I felt compelled to explore my curiosity and interests in thrips embryogenesis.

This work follows the great contributions that I have listed below in the references section. To my knowledge, the novel contribution in my approach is that I was able to study the *in vivo* embryonic development of a single egg from oviposition to a hatched 1st instar larva for two thrips species i.e. *Frankliniella occidentalis* and *Hercinothrips femoralis*. At the time of study, this approach was different from previous contributions in that multiple eggs were used and staged accordingly to continue the embryonic development sequence. Below are method details, results, and time-lapse video links for both *Frankliniella occidentalis* and *Hercinothrips femoralis*.

Due to a long publishing time gap, I am proceeding with an alternate channel of communication to share and to honor my supervisors' support and Dr. Heming's inspiration. Thus, it is my pleasure to contribute my empirical data, images, and time-lapse video resources in a collective form of 'laboratory notes' with hope that they will be useful to developmental biologists and other researchers moving forward. I have included links to my thesis and to the time-lapse videos. If you have any questions, comments, or would like a copy of the images, please contact me at: srajput@ualberta.ca.

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2. Methods

2.1 Definitions

- Western flower thrips (WFT)
- Banded greenhouse thrips (BGT)
- Total Development Time (TDT):
  - Expressed as a percent
    - For any significant point in development, the total number of hours (T_i=oviposition and T_f=hatch) divided by the number of elapsed hours post oviposition multiplied by 100.
  - Conditions: 23 +/- 2 degrees Celsius; ~100% relative humidity

2.2 Insect rearing

- For details on WFT rearing, refer to Rajput 2007.

2.3 Egg collection

- An artificial ovipositioning chamber comprising of a finely stretched a piece of parafilm with uniform tension over an acrylic cylinder was constructed. There is a piece of filter paper on the bottom where I could add apple pollen as a nutrient source, two screen vents on the sides and a sealable opening for introducing thrips and for subsequent feedings. Once all of these components are put together, the final component is an inverted petri dish, which contains a reservoir of sterile water. It is within this reservoir where the females deposit their eggs. This system was designed by Dr. Ken Fry and his technician Mr. Rod Werezuk at the Alberta Research Council in Vegreville, Alberta, Canada. At the time of study, previous contributions were mainly describing the use of agar blocks or removing eggs from leaf tissue.
- *Frankliniella occidentalis* oviposition video can be found here: http://hdl.handle.net/10402/era.37571

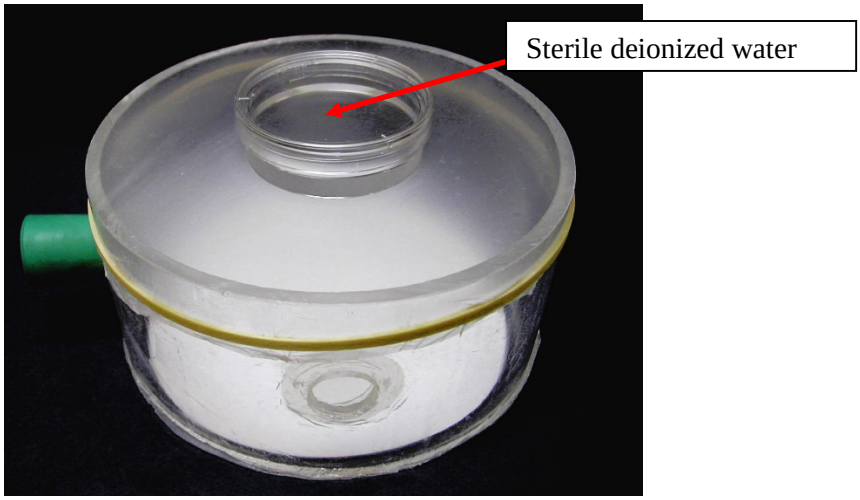

Figure 1. Oviposition chamber. Image taken with a Nikon Coolpix 775 digital camera.

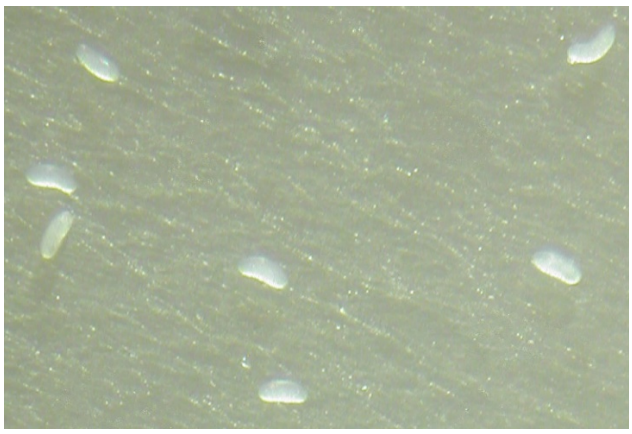

Figure 2. WFT eggs that have been deposited on the surface of the parafilm. Image taken with a Nikon Coolpix 775 digital camera.

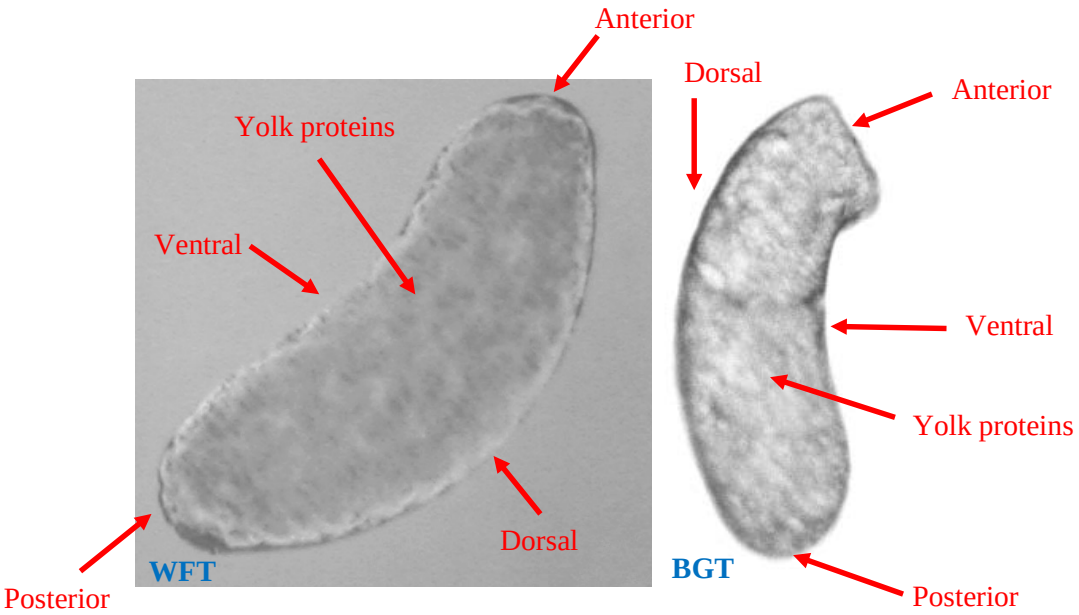

Figure 3. Western flower thrips eggs on the left and a banded greenhouse thrips egg on the right post oviposition. Image taken with a 35 mm Konica FT-1 camera connected to a Reichert-Jung Polyvar microscope equipped for bright-field and differential interference-contrast using Fuji Elite Chrome slide film.

2.4 Slide preparation

- Light microscopy – whole mounting for examining *in vivo* development: Standard pre-cleaned glass microscope slide, 200 µL sterile water, and a 22x40 mm glass coverslip (22x40mm) sealed to the microscope slide using petroleum jelly around coverslip edges. This technique was adapted from Braun and Keddie 1997.
- Conditions: 23 +/- 2 degrees Celsius; ~100% relative humidity

2.5 Video and photo techniques

- Photographs were taken with a 35 mm Konica FT-1 camera connected to a Reichert-Jung Polyvar microscope equipped for bright-field and differential interference-contrast using Fuji Elite Chrome slide film.
- Video recording were made with a Hitachi VK-C350 Solid State Color video camera. The evaluation of video tapes and creation of time-lapse videos was carried out using Apple iMovie.

3. Results

- Below is a summary of developmental milestones. Detailed time observations for *Frankliniella occidentalis* can be found in the Appendix (Section 5).
- Complete time-lapse videos for *Frankliniella occidentalis* and *Hercinothrips femoralis* can be found here:
  - *Frankliniella occidentalis*: http://hdl.handle.net/10402/era.37553
  - *Hercinothrips femoralis*: http://hdl.handle.net/10402/era.37554

3.1 Anatrepsis

- **WFT: starts ~13% and ends ~21% TDT**
- **BGT: starts ~20% and ends ~30% TDT**
- The germ band is pulled lengthwise into the yolk by periodic contraction of the elements within the yolk system collectively referred to as the anatrepsis center (Heming 2003).

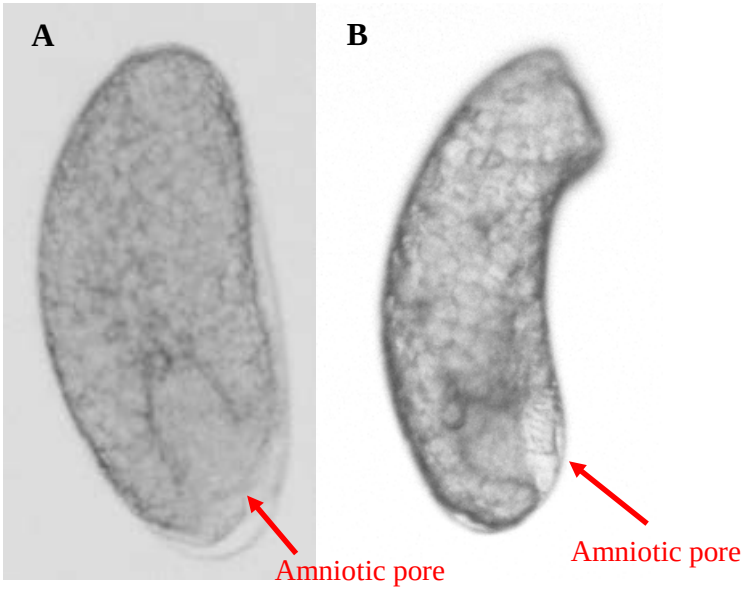

Figure 4. WFT (A) at ~13% TDT and BGT (B) at ~21% TDT.

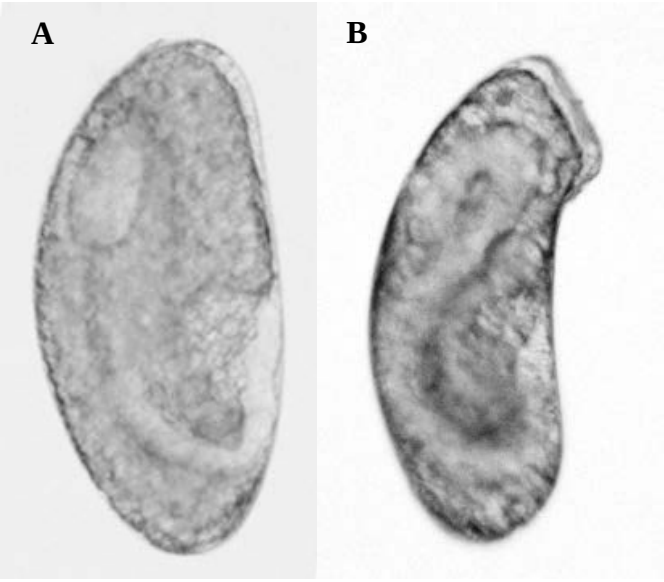

Figure 5. Late anatrepsis. WFT (A) at ~18% TDT and BGT (B) at ~30% TDT.

3.2 Intertrepsis

- **WFT: starts ~21% and ends ~47% TDT**
- **BGT: starts ~30% and ends ~59% TDT**
- Head lobe and abdomen are distinct. Formation of the mouthparts, antennae, thoracic leg appendages, and abdominal segments.

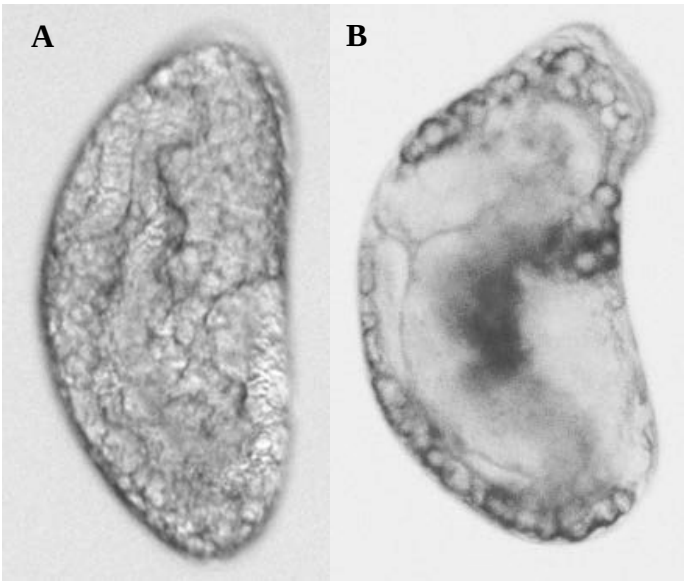

Figure 6. Intertrepsis. WFT (A) at ~22% TDT and BGT (B) at ~53% TDT.

3.3 Katatrepsis

- **WFT: starts ~47% and ends ~49-50% TDT**
  - **Actual time: ~2.5-3.0 hours**
- **BGT: starts ~59% and ends ~60% TDT**
  - **Actual time: ~2.17 hours**
- Amnioserosal fusion and ruptures. Contraction of the serosa leads to the formation of a secondary dorsal organ which pulls the embryo (Heming 2003).

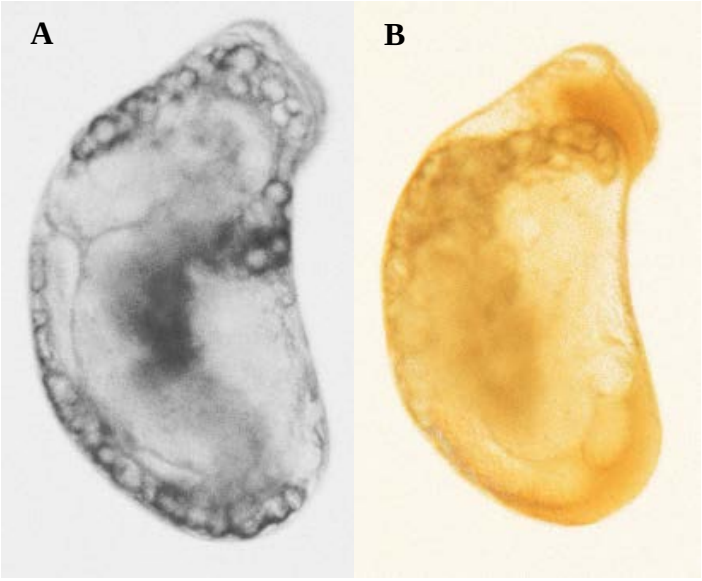

Figure 7. Katatrepsis. BGT (A) at ~53% TDT and BGT (B) at ~59% TDT.

3.4 "Red Eye" Stage

- **WFT: ~66% - 68% TDT**
- **BGT: ~72% TDT**
- Development of pigmentation within the ommatidia.

Figure 8. BGT at 'red eye' stage, ~72% TDT.

3.5 Symbiotic bacteria or other microorganisms in WFT

- **WFT: ~70% TDT**

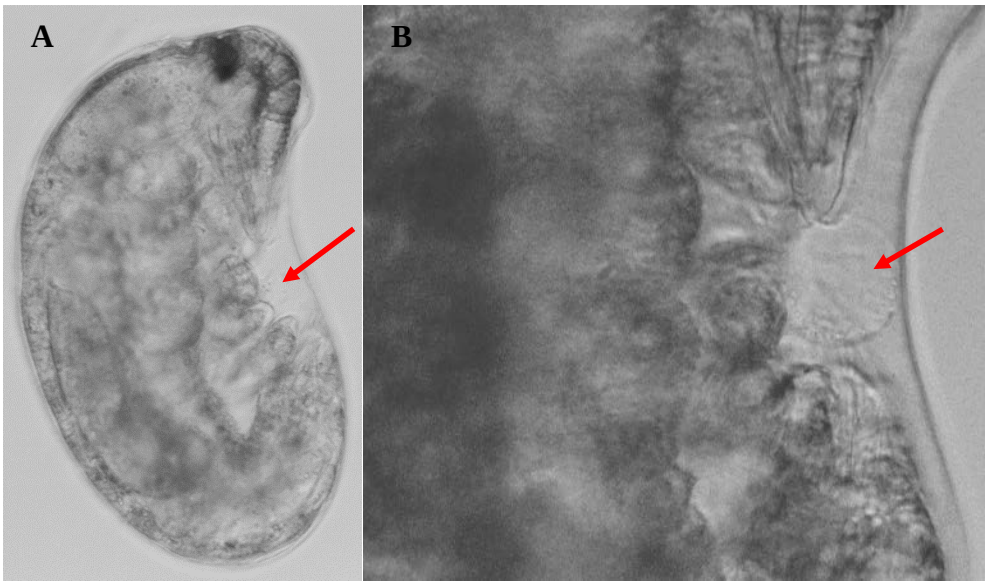

Figure 9. WFT (A) at ~70% TDT. (B) Increased magnification of possible bacteria or other microorganisms in spherical vesicle (arrow).

3.6 Embryonic cuticle 'thread':

- **WFT: ~99% TDT**

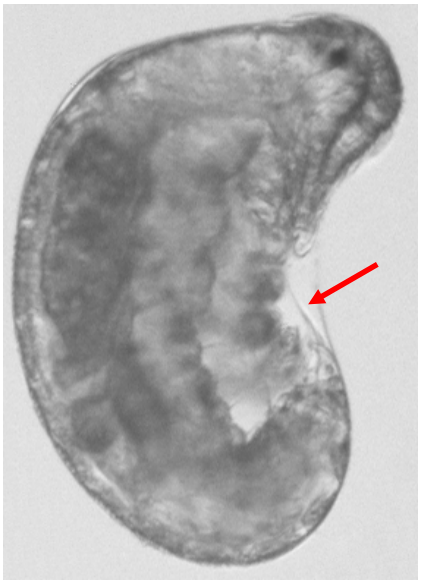

Figure 10. WFT at ~99% TDT. Cuticle thread (arrow).

3.7 Hatch

- **WFT: ~114-137 hours post oviposition (100%TDT)**
- **BGT: ~240 hours post oviposition (100% TDT)**
- Muscular contractions prior to and at the time of hatch:
    - peristaltic movement in the gut (WFT: ~71-85%; BGT: ~82% TDT).
    - dorsal vessel contractions (WFT: ~76-87%; BGT: ~93% TDT).
    - cibarial and salivary pump muscles (infrequent at ~82-83% TDT; frequent minutes *prior to* hatch for WFT and *during* hatch for BGT).

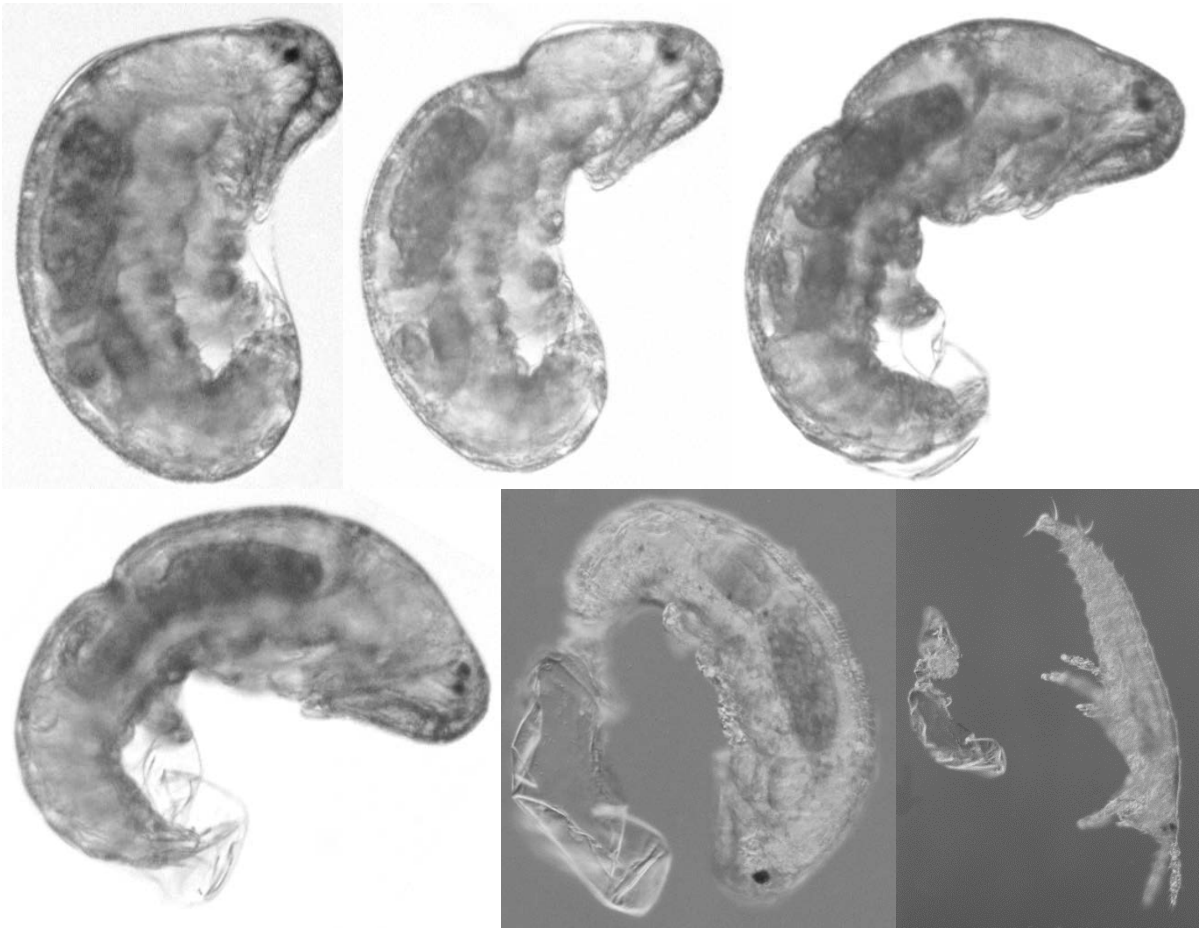

Figure 11. WFT hatch sequence starting from the top left and ending at the bottom right.

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QR link for dissertation:

5. Appendix – Detailed *Frankliniella occidentalis* embryogenesis observations

02.XII.02

14:30: Set up ovipositioning chambers using cohort 32 (about 12 adult females no males). Adult females are seven days old thus have been inseminated.

Initially, adults begin to feed on the apple pollen.

14:35: Added water reservoir = Start time for oviposition event.

15:27: Some females began to search the underside of the parafilm.

15:35: One female started to drink water.

16:22: Between now and the last time point, many females began to roam the underside of the parafilm and drink water.

18:00: No eggs laid yet, 8 females steadily drinking water.

19:00: Between 18:00 and now, three eggs laid!

19:59: Another egg laid.

20:18: Another egg laid.

20:38: Another egg laid.

21:15: Total of six eggs laid so far! Collected one of the eggs at 19:45 for taking pictures. Egg suspended in sterile super water on a glass microscope slide with a 20 x 40 mm coverslip with petroleum jelly on the edges to keep the fluid layer for the egg. Similar preparation when dissecting larvae for midgut tissue (Dr. Keddie's technique).

22:35: The egg looks like a dense aggregation of cells. Cells are spherical and vary in sizes. The operculum is clearly apparent (on the right hand side of image. Anterior = 13:00.

03.XII.02

10:02: (~16-17 hours since being laid). Developing embryo visible and anatrepsis has taken place. *This stage is intertrepsis, confirmed with Dr. Heming.*

10:30: No appendages visible.
*Developing embryo has been left under the polyvar microscope since last day submerged in sterile super water at room temperature (~24 degrees C).

11:00: Dr. Heming suggested that the cells are beginning to differentiate. This is the time to collect the eggs for WFT thrips cell line establishment. *The embryo is beginning to go past pleuripotency.*

Egg orientation: **Posterior (19:00)**
Anterior (13:00)
Ventral (top)
Dorsal (bottom)

Anatrepsis has taken place at the 23:00 position (ventral).

11:30: It appears as though the tail has shifted slightly towards the long body segment.

12:35: I can begin to see the posterior thoracic legs forming.

13:10: It seems like the embryo is rotating laterally because the plane of the ocular focus has changed slightly. This may be the initiation of katatrepsis. More of the thoracic legs are visible.

14:35: Tail has lengthened.

15:55: Thoracic appendages clearly visible. Head is also visible, tail segments have elongated quite significantly.

18:00-19:00: (Oviposition chamber) Many females are ovipositing however, many are piercing the parafilm but not delivering an egg. High rate of ovipositioning.

23:15: Katatrepsis has not taken place yet. Ventral invagination is more pronounced.

23:35: (Oviposition chamber) approximately 100-120 eggs have been deposited and are at various stages in their life history.

00:15: (Oviposition chamber) Most females are feeding, only one female roaming the underside of the parafilm.

04.XII.02

11:05: Katatrepsis has not taken place yet. Developing embryo is still surviving and increasing in size. (~40-41 hours post oviposition).

15:25: Individual segments becoming more apparent especially in the abdomen region. Katatrepsis has not taken place yet (~44.5-45.5 hours post oviposition).

15:53: *Dr. Heming pointed out some additional points: 1. Operculum cap is quite evident. 2. Peachish discoloration due to perhaps cytoplasmic contraction taking place anteriorally just under the operculum cap this may be the serosa developing. This contraction/discoloration was evident first thing in the morning however, was unsure what it was. 3. The last thoracic leg appendage is quite long and it follows the abdomen. 5. The amnion had not ruptured yet, it is a fine line of differentiating contrast when you focus up/down posterior to the antennae. Rupturing is involved in katatrepsis. 6. Pleuropodia also present.*

18:25: Gap between the operculum and the pigmented cytoplasm has increased. Appendages and mouthparts are increasing in size. Surrounding cells are yolk cells.

05.XII.02

12:42: Katatrepsis has taken place prior to this time point, antennae, leg appendages, and eye are all visible. Refer to page 17 of Moritz paper (2nd figure.)

16:15: Apparently there is a point at the posterior of the anus. This may be some sort of extension or perhaps the ovipositor itself. Also there is a space under the (or basal to) the operculum.

16:45: *Dr. Heming indicated that dorsal closure has not completed yet because there is still lots of yolk. In addition, the extension is part of the embryonic cuticle, it allows for the first instar hairs (terminal setae) to develop. WFT have two cuticles: one embryonic then first instar cuticle. The ovipositor develops during late 2nd instar stage.*

18:00 There about 300 eggs in the ovipositioning chamber. Some (~10-15%) are at the red eye stage. No hatchings yet.

18:10 Antennal segments visible.

06.XII.02

09:15 Red eye stage. Four vitellarium visible (characteristic of the species). Dorsal closure has taken place however, there is still yolk. Larval cuticle is being deposited. Larval setae is quite visible within the embryonic cuticle. Hatching spines are also visible under differential interference contrast (DIC-40/IC)-*focus up and down.*

Apparently there are ~3 enclosed vesicles within the chorion (external to the developing embryo) posterior to the abdominal terminal setae. Vesicles appear to contain very small particles with them→Bacteria???
These small particles are also free floating in the dorsal and ventral compartments of the chorion—see slide images. Highest concentration of particular matter is within the vesicles.

Dorsal vessel contractions have also been initiated. Frequency is about every 5-10 seconds. (Measurement made at 12:00).

12:00 Intensity of the contractions is increasing. There is a little bit of larval cuticle which needs to be deposited. Look @18:30 on slide images (roll 6).

20:00 Many contractions in the posterior to dorsal regions of the egg (within the embryo). Egg hasn't hatched yet. Many first instar larvae have hatched in the C2 oviposition chamber and there is a high proportion of eggs that are at the red eye stage. Some of the eggs that are at the red eye stage appear a bit yellowish as opposed to the "normal" whitish.

This may be an indication of age (i.e. very close to hatch). The apple pollen has fungus and the adults are exhibiting decreased activity.

It appears that the bacteria (?) are not only in the vesicles but throughout the spaces of the chorion however, at lower densities than within the vesicles.

A lot of cellular movement taking place throughout the dorsal region of the embryo which start earlier this afternoon.

20:45 Still not hatched.

23:45 Increased (apparently) peristaltic movement.

07.XII.02

13:05 Embryo has filled the entire egg. Dorsal vessel is contracting at a more regular rate. Mouthparts (i.e. stylets) are clearly visible.
The bacterial vesicles are not apparent any longer however the bacteria are quite evident. The cellular morphology are cocci and diplococcus and maybe large yeast cells (later discovered that they are two cells superimposed—maybe they could be cells undergoing mitosis (See roll 9-@16:04)

Transovum transmission? Within the chorion thus extracellular interior.

13:18 **Hatched!** Prior to hatching the ciberial and the epipharengeal (or salivary pump) muscle began to contract. *Pumping action.*
This pumping action may deliver fluid to the abdomen which combines with the peristaltic contractions to allow for the embryo to emerge. (*discussed with Dr. Heming*). *Dr. Heming indicated that with Hercinothrips the peristaltic/abdominal contractions are only responsible for the first instar emergence.*

With respect to the bacteria, after the embryo began to emerge the bacteria and yeasts (?) also came out to potentially inoculate the surrounding substrate.

15:52 Spiracles visible.

17:40 Whole embryo has come out of the egg shell however, it has not shed its embryonic cuticle.

08.XII.02

18:25 First instar larva has fully emerged. Apparently sometime last night the embryonic cuticle was shed and is attached to the operculum, the larva is clearly separated from the embryonic cuticle and the egg as a whole. The concentration of the bacterial cells has decreased substantially as expected due to the nutrient/oxygen deficient system. Slide with first instar emerged larva is preserved.