Population differences in seasonality via diapause regulation among the apple maggot fly Rhagoletis pomonella

Peter Meyers[2], Adam J. Schieferecke[1*] and Gregory Ragland[1]

[1]Department of Entomology, Kansas State University, Manhattan, KS 66506, [2]Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556

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Introduction

- Diapause adaptation is important as insects expand into new geographic regions or experience changes in seasonality.
- Physiological mechanisms controlling diapause have been well studied, but little is currently known about how variation in these mechanisms give rise to diapause adaptation.
- *R. pomonella* is a significant agricultural fruit pest that has rapidly evolved into two genetically distinct populations that infest host plants with different fruiting times. Differences in the timing of diapause allow each population to synchronize with their respective host plant.
- Previous studies suggest that regulatory mechanisms underlying this shift in seasonality may act during the winter independent of any overt cues for diapause termination, such as the typical change from colder to warmer temperatures during the winter-spring transition.

Objective

The objective of this project is to test for potential physiological host race differences before and after a simulated temperature cue using RNAseq.

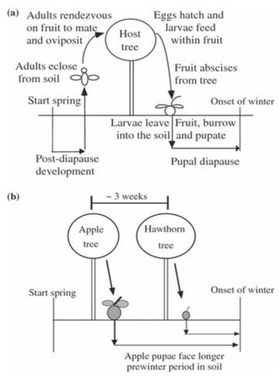


Figure 1 *R. pomonella* life cycle[2]

- Despite ongoing gene flow, apple and hawthorn host races are sympatric but genetically distinct.
- Flies have one generation per year, diapausing as pupae.
- Apple flies emerge and enter the soil as pupae 3-4 weeks earlier than haw flies.

Methods

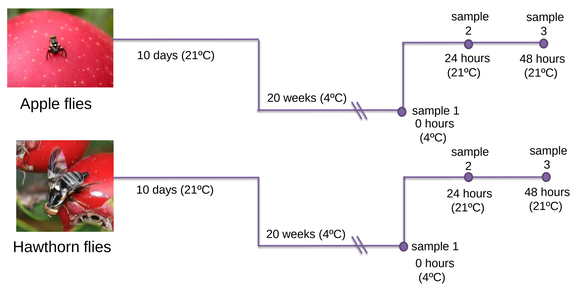


Figure 2 Infested fruits were collected from the field and placed at 21ºC in collecting trays. Wandering larvae emerging from the fruits were maintained at 21ºC for 10 days to allow complete pupal formation. At 10 days, pupae were placed at 4ºC, simulating winter. This study samples diapausing pupae at or after 20 weeks of winter.

- Three time points were sampled: 1) directly out of 4ºC at 20 weeks of winter, 2) 24 hours, and 3) 48 hours after transfer to 21ºC. Using respirometric measurements of metabolic rate, we confirmed that all individuals sampled at the 24 and 48 hour time points had not terminated diapause.[1]
- **Thus, all samples (winter and post winter) remained in diapause.**
- Pupae were decapitated for tissue samples to capture differential regulation in the brain. RNA was extracted from three replicate pools of 10 heads per treatment per population.

- Illumina Truseq libraries were prepared and sequenced on two Illumina HiSeq lanes, 100bp paired-end. Reads were de-multiplexed, adapter trimmed, then *de novo* assembled using Trinity. This yielded an average of 150,10,071 mapped reads per sample.
- Reads were mapped to the *de novo*, Trinity assembly using the STAR aligner. Counts per feature were estimated using RSEM, with 'feature' specified as a single Trinity gene model. Additionally, these gene models were annotated to *D. melanogaster* using Blastx.
- Data were filtered so that only gene models with a minimum of one count per million in at least 50% of the samples were retained.
- Filtered data were analyzed using the following linear model implemented in EdgeR using a negative binomial error distribution and a scaling normalization with effects: time point *T* and host race *R*.

$$y_{ijk} = \mu + T_i + R_j + e_{ijk}$$

- We further tested a model including an interaction term; the ~200 transcripts with significant interaction effects are excluded from the current analysis.
- After fitting the model, linear contrasts were performed for the following comparisons: 1) difference between host races, 2) difference between 'winter' and 24h treatment, and 3) difference between 24h and 48h treatments.

Results

Figure 3 Heat map of full changes for significantly differentially expressed transcripts

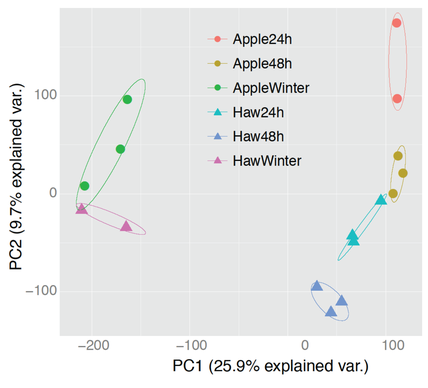
Contrast	N significant Trinity gene models
Haw – Apple (Host Race)	790
24h - Winter	20,711
48h-24h	367

Table 1 Total number of trinity gene models analyzed: 66,235

- There are clear transcriptional differences between host races across all sample points.
- There is a massive transcriptional response to temperature change (~30% of transcriptome).
- The vast majority of changes happen within 24 hours of temperature change.

Figure 4: Principal components analysis of FPKM values for all transcripts

- PC1 represents temporal differences in expression.
- PC2 represents host race differences.
- Most genes differentially expressed between host races are different than genes that are differentially expressed temporally (Figures 3 & 4).



Contrast	enriched functional cat.	nGenes	FDR
24hVsWinter	regulation of transcription	233	6.40E-08
24hVsWinter	zinc-finger	108	5.60E-07
24hVsWinter	ribosome biogenesis	30	3.30E-04
24hVsWinter	cell cycle	52	1.20E-10
24hVsWinter	mitosis	58	1.20E-05
24hVsWinter	imaginal disc development	116	2.70E-03
24hVsWinter	metamorphosis	101	9.60E-03
24hVsWinter	protein folding	43	1.50E-03
48hVs24h	ribosome biogenesis	11	8.50E-08
48hVs24h	imaginal disc development	8	9.50E-01
48hVs24h	helicase	6	1.20E-02
AppleVsHaw	cuticle/chitin biosynthesis	19	4.30E-10
AppleVsHaw	transcription	26	0.007

Table 2 Functional category enrichment analysis implemented in DAVID
Relative to haw pupae, apple pupae show increased expression across all time points consistent with:

- Preparation for molt that occurs at the end of diapause (cuticle formation cat.)
- Increased transcriptional activity (transcription cat.)
- Other genes DE between host races include important developmental regulators such as *wingless* and insulin signaling-related genes (*Tsc1*, *sugarbabe*, and *secreted decoy of InR*)

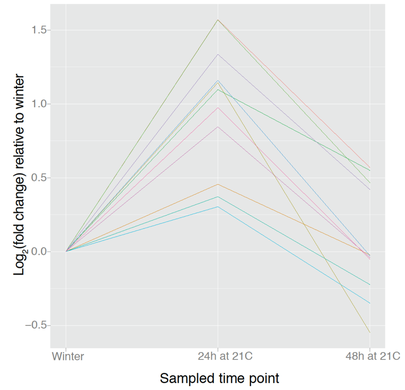


Figure 5 log fold changes of ribosome biogenesis genes relative to expression during winter

- Genes in the ribosome biogenesis pathway are highly upregulated 24h after temperature change but are subsequently downregulated at 48h.
- These findings are consistent with physiological buffering of an environmental response.

- Despite remaining in diapause, there is massive differential expression of developmental genes involved in cell cycling and morphogenesis after the temperature cue. Genes from several of the same functional categories are differentially expressed from the 24h to 48h time points after the temperature cue (e.g., Fig. 5)

Conclusions

- Host race differences in expression are largely independent of a response to temperature change. Thus, differential regulation between host races related to diapause development occurs relatively early in diapause development.
- Because all samples remain in diapause, pronounced expression differences among host races and between sampling points are likely in preparation for diapause as opposed to active cell differentiation and proliferation.
- Expression patterns of genes, such as those in ribosome biogenesis, suggest a homeostatic response suppressing temperature-induced developmental acceleration. This response is consistent with homeostatic buffering of metabolic rate also observed in the system.[3]

Future Directions

- Characterize trajectories of gene expression differences among the host races by upstream sampling and RNAseq of multiple time points during winter
- Ongoing studies of genome-wide SNP variation to provide parallel evidence for loci underlying host race differences in seasonality

Citations

1. G Ragland, J Fuller, JL Feder, and DA Hahn. 2009. Biphasic metabolic rate trajectory of pupal diapause termination and post-diapause development in a tephritid fly. Journal of Insect physiology 55: 344-350
2. HR Dambroski and JL Feder. 2007. Host plant and latitude-related diapause variation in *Rhagoletis pomonella*: a test for multifaceted life history adaptation on different stages of diapause development. Journal of Evolutionary Biology 2101-2112
3. Powell, Hahn, & Ragland unpublished data

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