Adulting 101: Exploring developmental gene expression profiles in Tobacco Hornworm (*Manduca sexta*)

Regan Cross, Marco Lee, Hana Thompson, and Hayden Wainwright

Background

- Holometabolous insects (e.g. moths) have morphologically distinct larvae, pupae, and adults
- These life history stages often have different ecological niches and specializations[1,2] (e.g. larvae specialized for feeding, adults for reproduction)
- Each stage should have physiological adaptations to maximize fitness, reflected in differential gene expression across life stages (e.g. reproductive genes only expressed in adults[3])

Goals

- Explore how tobacco hornworm *Manduca sexta* regulates its physiology through gene expression at different life stages
- Determine which genes change most in expression between larvae and adult using transcriptomic data from different tissues of *M. sexta* at six life stages
- Determine which physiological functions undergo most change during transition to adulthood

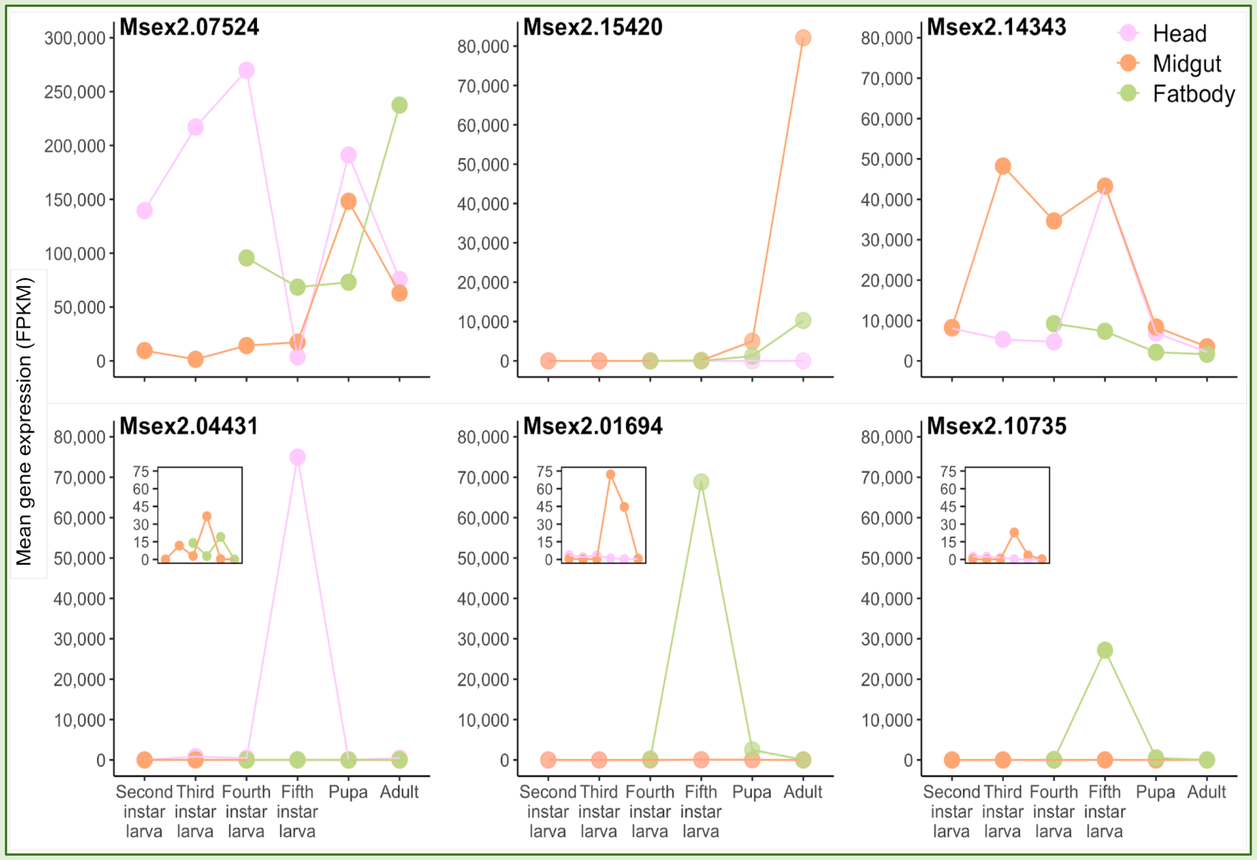

Figure 1. Mean gene expression (FPKM) for 6 genes in the head, midgut and fat body of *M. sexta* at 6 life stages. When lifestage was broken into subgroups, gene expression was averaged across lifestage. Note greater change in gene expression for Msex2.07524 (top left).

Data & Methods

- Data sourced from study examining 67 RNA-seq datasets of 15 544 genes from 8 tissues across 9 life stages of *Manduca sexta*[4]
- Expression levels reported in FPKM (fragments per kilobase per million mapped reads)
- Difference between 5th instar larva and adult was calculated in R
- Functions of top 3 most different genes for each tissue were extracted from NCBI BLAST

Table 1. Top three genes experiencing the greatest change in expression within the head, gut and fat tissue of *Manduca sexta* between the 5th instar and adult life stage. Positive values under Change in Expression Level indicate an increase in expression level in the adult stage while negative values indicate a decrease in expression level. Protein name and function associated with each gene ID were identified from a BLAST analysis.

Gene ID	Protein Name	Function	Tissue Type	Expression Level 5th Instar (FPKM)	Expression Level Adult Stage (FPKM)	Change in Expression Level (FPKM)
Msex2.07524	Ejaculatory bulb-specific protein 3-like	Chemosensation, tissue remodeling, host defense	Head	74964.41	439.17	-7425.24
Msex2.15420	Uncharacterized		Head	3602.61	75574.23	71971.62
			Midgut	17344.096	62948.05	45603.954
			Fat	68477.053	237523.435	169046.382
Msex2.14343	ATP synthase F0 subunit 6 (mitochondrion)	ATP synthesis, oxidative phosphorylation	Head	43171.26	2205.55	-40965.71
			Midgut	43268.05	3490.07	-39777.98
Msex2.04431	Uncharacterized		Midgut	118.14	82120.42	82002.28
Msex2.01694	Basic juvenile hormone-suppressible protein 2-like	Energy storage	Fat	68823.61	26.09	-68797.52
Msex2.10735	Basic juvenile hormone-suppressible protein 1-like	Energy storage	Fat	27176.11	9.57	-27166.54

Conclusion

- Four of the genes experience drastic changes in expression only at the transition from larva to pupa
- The genes that changed most from 5th instar to adult were related to development processes according to NCBI blast
- *M. sexta* larvae are agricultural pests, feeding on solanaceous plants, so studying its genetics could aid in development of specific pesticides
- In the future, the functions of the uncharacterized genes should be evaluated as they seem to play a significant role in the transition from larva to adult

References

[1] Chapman, R. F. 2013. The insects: Structure and function. Simpson SJ, Douglas AE, editors. Cambridge University Press.

[2] Truman JW. 2019. The evolution of insect metamorphosis. *Curr Biol.* 29(23): R1252–R1268.

[3] Zhao J, Sun Y, Xiao LB, Tan YA, Bai LX. 2016. Molecular characterization and expression of vitellogenin gene from *Spodoptera exigua* exposed to cadmium stress. *Gene.* 593(1): 179–184.

[4] Cao, X., & H. Jiang. 2017. An analysis of 67 RNA-seq datasets from various tissues at different stages of a model insect, *Manduca sexta*. *BMC Genomics* 18: 796.