Developing RNAi protocol to target genes in the alfalfa leafcutter bee, *Megachile rotundata*, for gene functionality

Jessica N. Keller¹, Gagandeep Brar¹, Kendra J. Greenlee², Joseph P. Rinehart³, Julia Bowsher¹
North Dakota State University, Fargo, ND¹, Insect Genetics and Biochemistry, USDA-ARS, Fargo, ND²

**Introduction**

- *Megachile rotundata*, alfalfa leafcutter bee is the primary pollinator for alfalfa seed production¹.
- *M. rotundata* goes into diapause as a prepupa or skips diapause to emerge as the parent generation.
- Analysis of gene expression and function could be used to identify the physiological mechanisms behind diapause¹.
- RNA interference (RNAi) is a tool for knocking down the expression of individual genes and has been widely used to study the cellular function of genes².
- We have targeted genes of known function using RNAi which’ll eventually help to discover the function of genes that help *M. rotundata* diapause.

**Objective**

Develop a RNAi protocol by targeting Cinnabar Vermillion, and White in *Megachile rotundata*.

**RNAi mechanism**

[Diagram showing RNAi mechanism]

**Methods**

1. Making double stranded RNA against Cinnabar (dsC), Vermillion (dsV) and White (dsW).
2. Injecting dsRNA: Thorax of *M. rotundata* pupae
   - Control (water)
   - Treatments (dsC, dsV, and dsW)
   - 3ug/ul
3. RNA extraction and cDNA synthesis
4. Running PCR for targeted genes dsC, dsV, dsW and control (water)
5. Gene Expression Analysis using Gel Agrose Electrophoresis

**Results**

*dsRNA expected size: 460 bp*

Agarose gel electrophoresis of dsRNA synthesis against
- 1. dsCinnabar
- 2. dsVermillion
- 3. dsWhite

**Conclusions**

- There will be lower expression in samples where dsRNA genes was injected against dsC, dsV, and dsW as compared to the control (water)
- Samples injected with dsRNA against genes will show light/faint bands on agarose gel as compared to control after running semi-quantitative PCR.
- Phenotypically, there will be changes in eye pigmentation after knocking down the genes Cinnabar, Vermillion and White.

**Future Directions**

- Run qPCR again with increases in concentration of the three dsRNA genes
- Select other genes with known function to use for RNAi protocol to be fully developed
- Expression analysis will be done using qPCR after gene knockdown
- This RNAi protocol will be used for gene knockdown of selected genes that might contribute to *M. rotundata* going into diapause.
- This study will help in understanding the function of different gene in solitary bees

**Acknowledgements**

I would like to thank all the members in the USDA-ARS, Fargo, ND, lab for their help and support, including mentors and other REU students. All the amazing staff and fellow research enthusiasts made this such a memorable experience this summer.

**References**