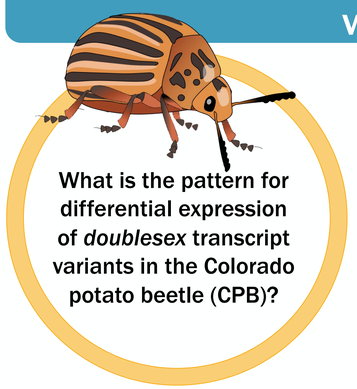
Identification of male molecular markers in the Colorado potato beetle

Kirsten Warcup[1], Alex S. Torson[2]

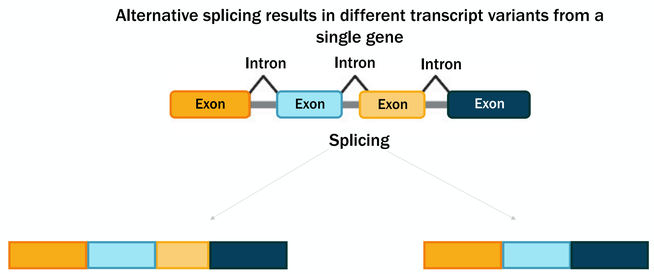
[1]North Dakota State University,
[2]Edward T. Schafer Agricultural Research Center

USDA NDSU NORTH DAKOTA STATE UNIVERSITY

RNA-seq data reveals putative sex-specific *doublesex* transcript variant expression pattern



What is the pattern for differential expression of *doublesex* transcript variants in the Colorado potato beetle (CPB)?



- The *doublesex* (*dsx*) gene is involved in sex determination.
- Data from a diapause RNA-seq study (fig. 1) confirms the presence of five *dsx* transcript variants, three of which appear to follow a sex-specific expression pattern.
- We hypothesize that one transcript is male-specific (*dsx182*), and two transcripts are female-specific (*dsx184* and *dsx185*).

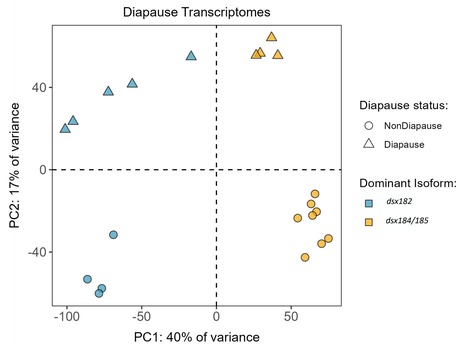


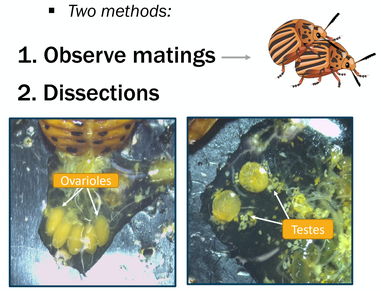
Figure 1. Principal components analysis (PCA) of RNA-seq data from whole CPBs reveals clustering that is associated with diapause and possibly sex-specific transcript expression.

RT-qPCR enables relative quantification of transcript expression

Determine sex

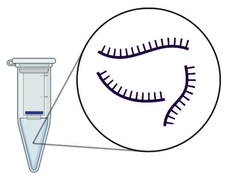
- *Two methods:*

1. Observe matings
2. Dissections



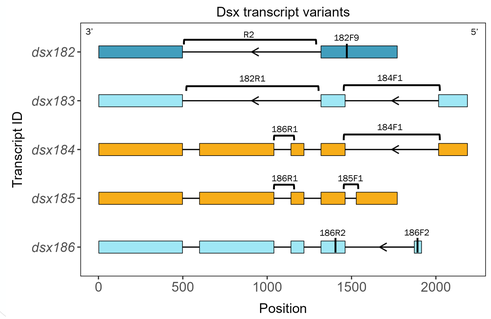
Extract RNA

- *High salt TRIzol extraction*



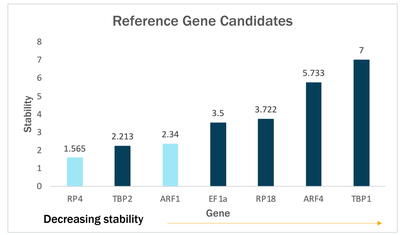
Design qPCR primers

- *Amplicon sizes ranging from 114-163 bp*



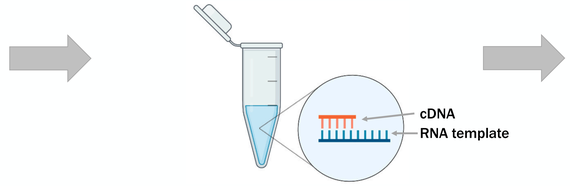
Select reference genes

- *Candidates found in Shi et al. 2013*



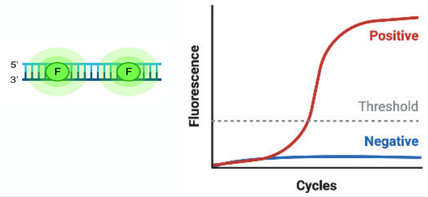
Synthesize cDNA

- *Invitrogen SuperScript IV First Strand Synthesis Kit*



qPCR

- *Roche LightCycler 480*



Dsx182 and *dsx183* are male-specific transcripts

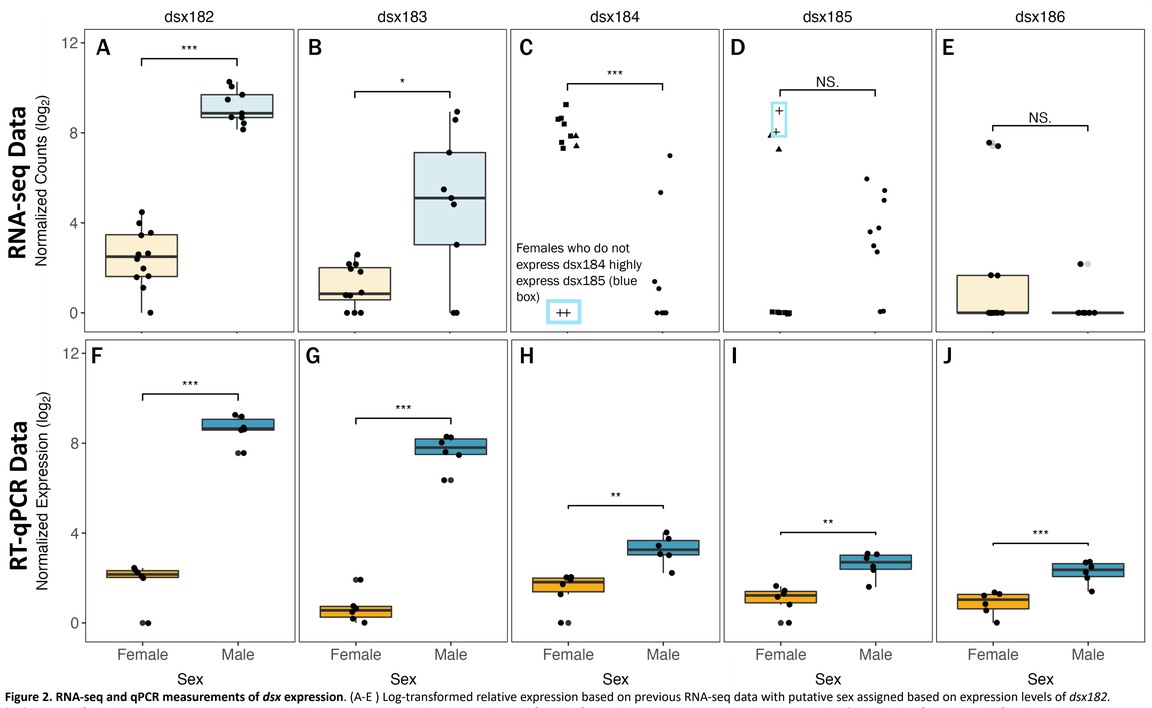


Figure 2. RNA-seq and qPCR measurements of *dsx* expression. (A-E) Log-transformed relative expression based on previous RNA-seq data with putative sex assigned based on expression levels of *dsx182*. (F-J) Log-transformed relative expression measured with qPCR. Comparisons between males and females for each transcript were analyzed using a student's T-test. Significance was defined as p≤0.05.

- The RNA-seq data shows inconsistent high expression of *dsx184* (fig. 2C) and *dsx185* (fig. 2D) in female individuals.
- The high expression in females was not captured in the qPCR data (figures 2H and 2I).
- This suggests there is differential expression across reproductive development.

Investigation of other potential markers for sex

***Dsx182* and *dsx183* are male-specific and can be used as reliable markers for sex**

<u>Future Directions:</u> Investigating other potential markers for sex

- The RNA-seq data reveals five other potential markers for sex (e.g., fig. 3), so finding a female marker should be possible.
- Marker candidates not represented in figure 3 include *Testis-specific STK-3* and *Testicular haploid expressed gene.*

No female-specific transcript?

- The *dsx* gene is not a reliable female marker because it appears to be inconsistently expressed across adult development.
- Sampling older females could potentially capture the high expression of *dsx184* and *dsx185.*
- Based on previous work on *dsx*, we still believe that there must be at least one female-specific transcript.

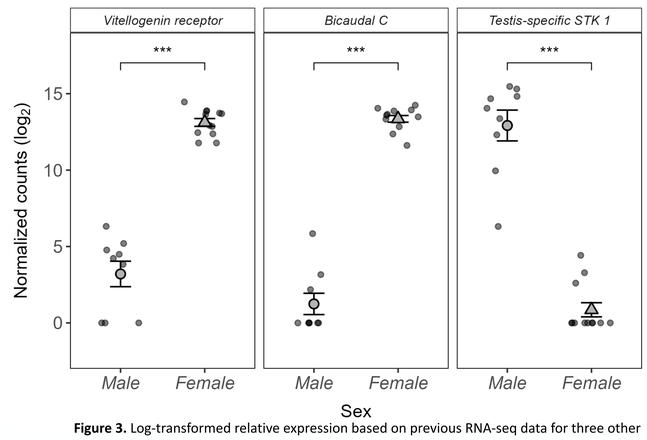


Figure 3. Log-transformed relative expression based on previous RNA-seq data for three other potential markers for sex.

<u>Acknowledgments</u>
Thank you to Dr. Sheri Dorsam, Marnie Larson, Gagan Brar, and Dr. George Yocum for their support and guidance, to Yolanda Chen from the University of Vermont for providing CPB eggs to establish, and to Jackie Lebeuzon for the CPB graphics

<u>References</u>
Shi, X. Q. *et al.,* (2013). Validation of reference genes for expression analysis by quantitative real-time PCR in *Leptinotarsa decemlineata* (Say). *BMC Research Notes*, *6*(1).