

Do Eagles Make Flies Ready for Love?

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How does the expression of *eagle* (*eg*) change with *Drosophila* mating status?

What are the cellular functions of the *eagle* gene product?

- Nuclear hormone receptor
- Transcription factor
- Zinc finger protein
- Serotonin neuroblast fate determining factor

What is known about *eg*'s effect on mating behavior?

QTL analysis identified *eg* as being associated with variations in mating behavior.¹ Mating behaviors could include:

- Courtship occurrence
- Courtship latency
- Copulation occurrence
- Copulation latency



Hypothesis: *eg* is upregulated in mated male flies compared to courting and virgin males

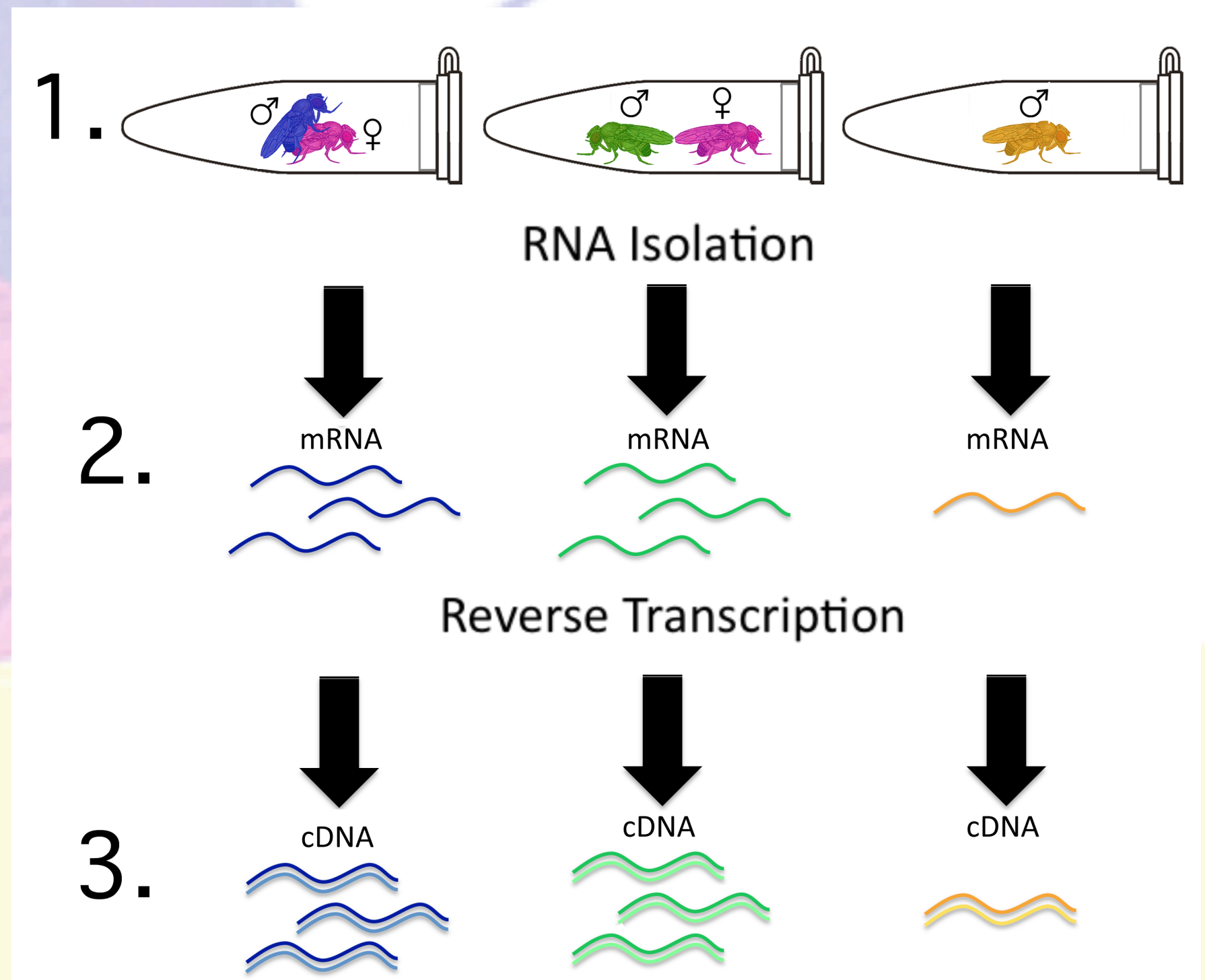
Behavioral Assay, RNA Isolation, and Reverse Transcription

1. 3-day-old virgin male

Drosophila were put in eppendorf tubes with 3-day old virgin females and either **mated** (left), remained **unmated** (center), or remained **virgins** (right).

2. RNA was isolated from 3 flies in each condition (**mated**, **unmated** and **virgin**). The isolated RNA was then treated with DNase I to eliminate contaminant DNA from the samples.

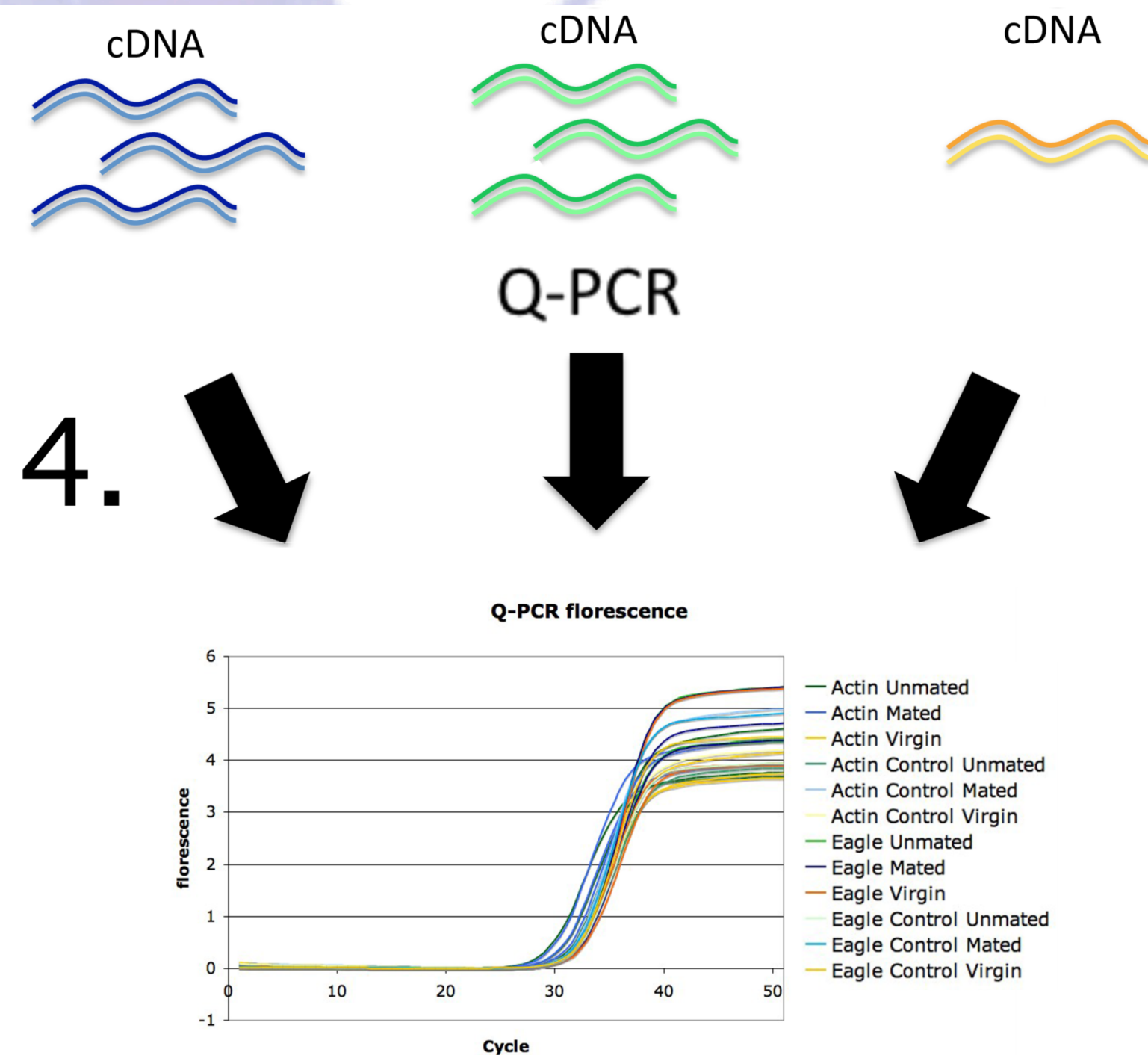
3. RNA isolated in 2. was then reversed transcribed into cDNA.



Hypothesis: *eg* is upregulated in mated male flies compared to courting and virgin males

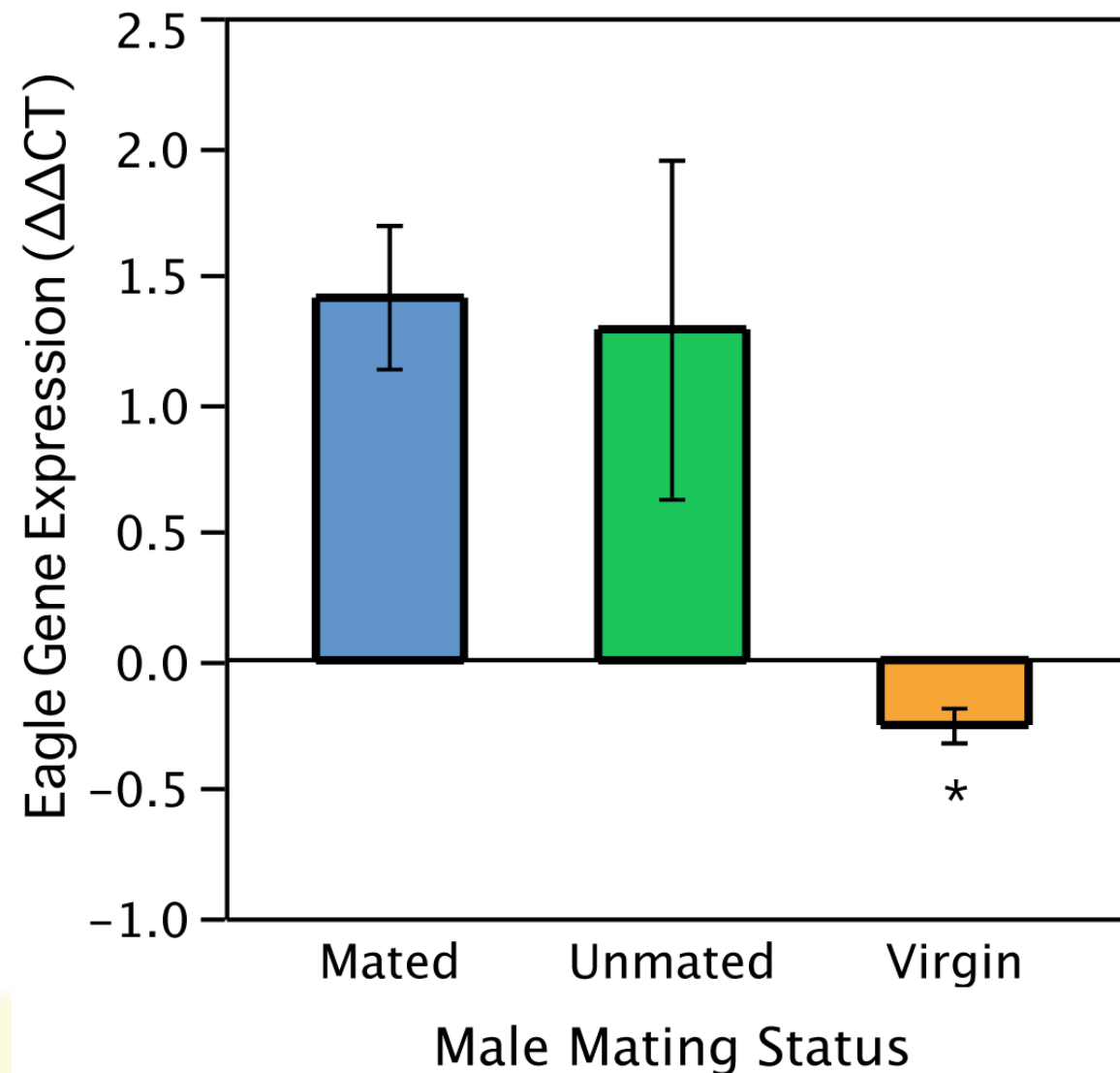
Quantitative PCR

4. cDNA transcripts from 3. were used to perform Q-PCR to determine *eg* expression. Actin isoform B was used to normalize the samples. C_t values were determined from the raw Q-PCR data graph (bottom right) and averaged in each of the 3 different conditions to determine *eg* expression.



Conclusion: *eg* is upregulated in male flies who court or mate with females as compared to isolated virgin males

Analysis of Q-PCR Results



$\Delta\Delta Ct$ values for 3 **mated**, 3 **unmated**, and 3 **virgin** male flies indicated that *eg* is expressed significantly more in males exposed to females as compared to isolated virgin males (ANOVA, $F=11.4$, $df = 1,7$, $P= 0.0118$). Note: * indicates significant difference from other conditions.

Future Directions

- We achieved amplification in Q-PCR with DNase I treated RNA samples, which indicates DNA contamination. Would these same results be observed if our samples hadn't been contaminated?
- We only tested 3-day-old males. Would expression be different in males of different ages or in females?
- Very few of our male flies mated. What conditions could be established to optimize mating?

Acknowledgements & References

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1. Moehring, A.J., Mackay, T. F.C. "The Quantitative Genetic Basis of Male Mating Behavior in *Drosophila Melanogaster*." *Genetics*. 2004; 167 (3): 1249-1263.
2. Benson, D.A., Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. "GenBank." *Nucl Acids Res*. 2005; 1 (33): D34-D38.