

Transcriptomic Response of Housefly to Natural Infection by *Pseudomonas aeruginosa* and *Escherichia coli*

Danial Asgari¹, Richard Meisel¹, Dana Nayduch²

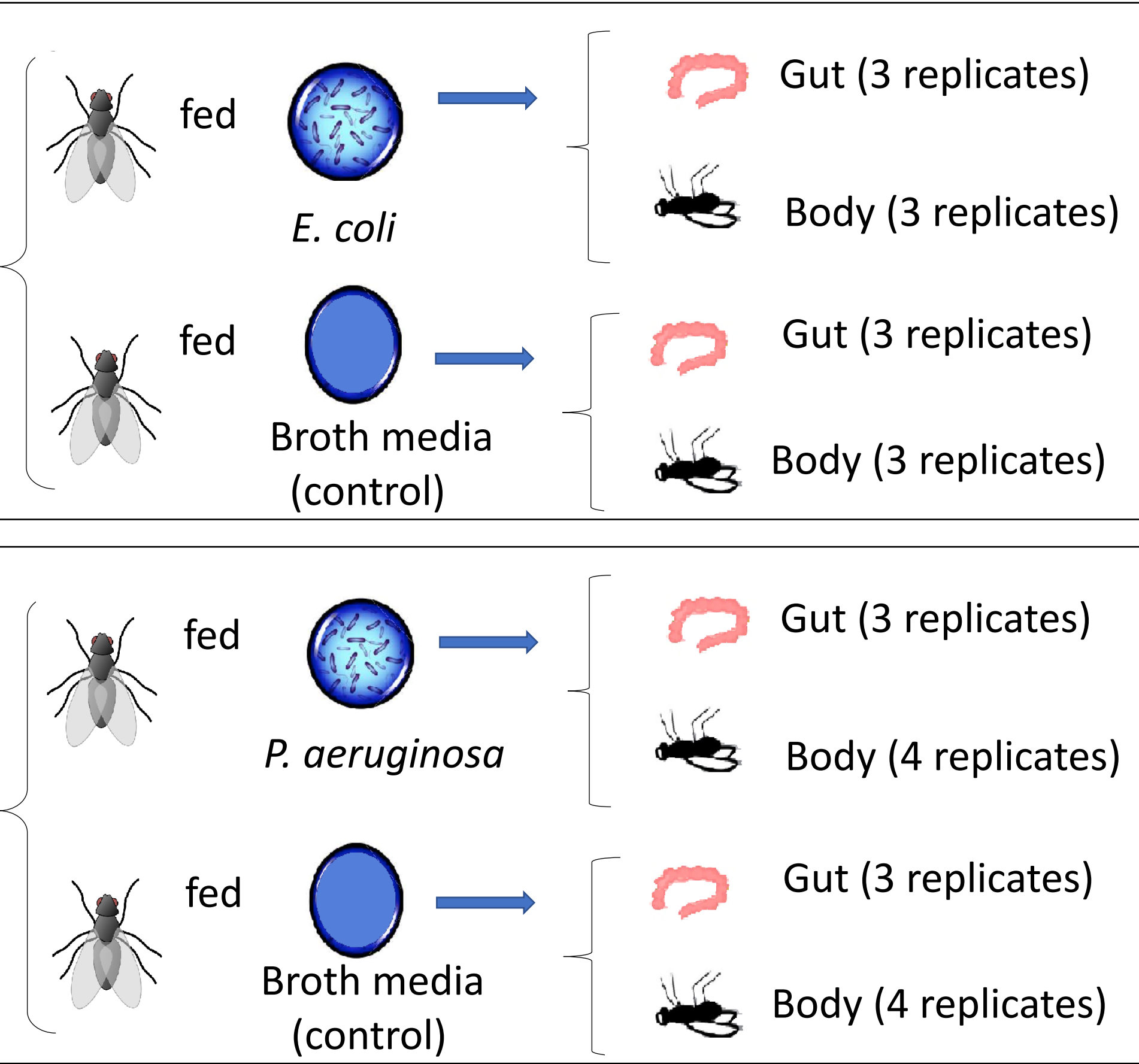
1- Department of Biology and Biochemistry, University of Houston, Houston, TX, USA

2- USDA-ARS, Center for Grain and Animal Health Research, Arthropod-Borne Animal Diseases Research Unit, Manhattan, KS, USA

Introduction

Living in septic habitats presents challenges that should be addressed by evolution of an efficient immune system. Study of organisms adapted to septic habitats provide us the opportunity to evaluate the influence of life history on evolution of defensive strategies. This helps us understand how natural selection shapes defensive strategies to increase the fitness of the organisms in such environments. Particularly, choosing an induced or a constitutive defensive strategy can have significant effect on the fitness of the organism. Here we use house fly (*Musca domestica*), a species adapted to microbe-rich environments for our model organism to examine the influence of ecological habitat on evolution of defensive strategies.

RNA-seq Experiment:



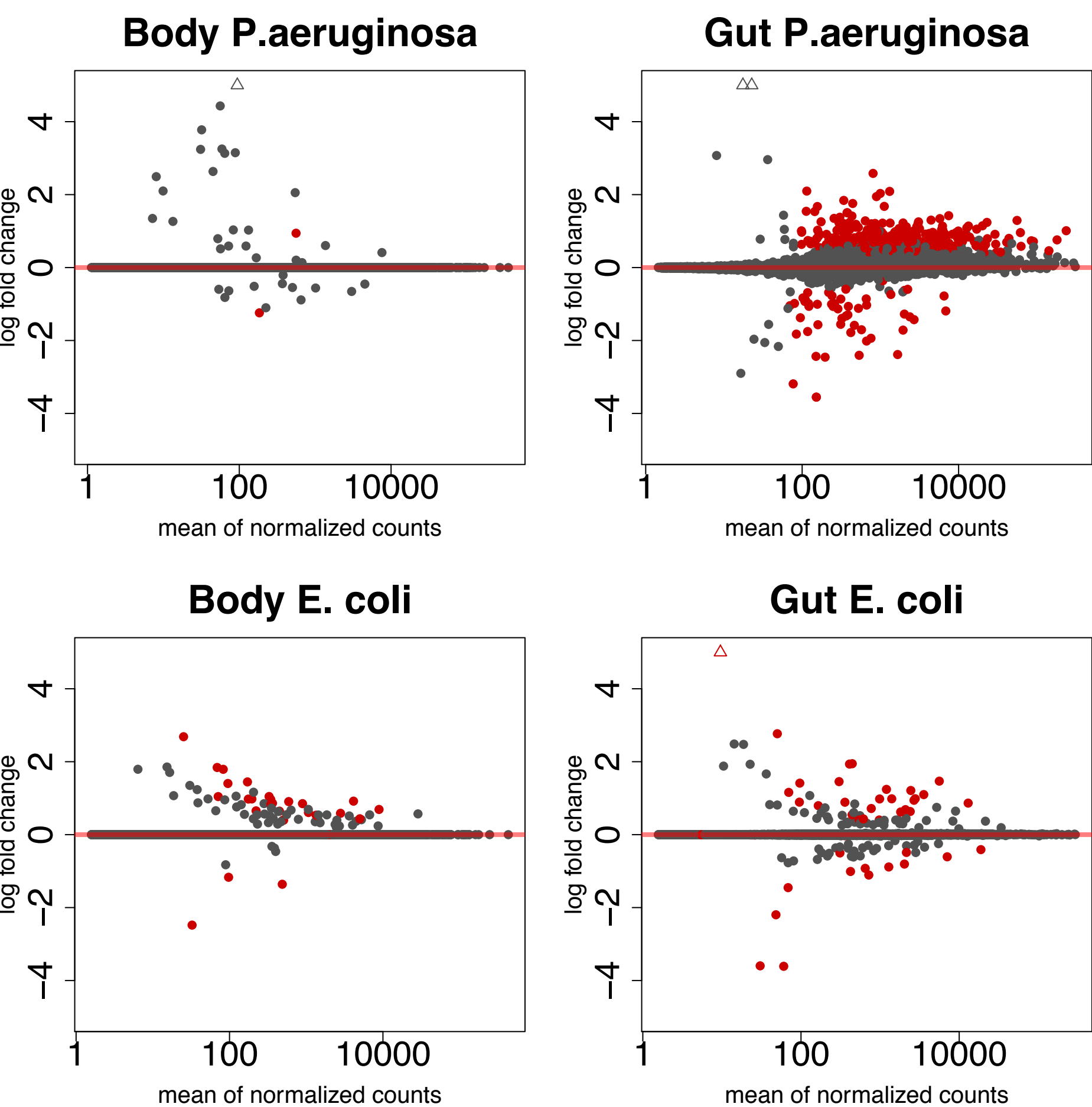
Data analysis

- Count matrix produced by Kallisto (0.44.0)
- Differential expression performed by DESeq2 (R 3.6.1)
- Model: $E = B0 + B1 \times T$

$B0$ = Expression level of control replicates
 $B1$ = indicator variable 0 (control) 1 (infected)

Results:

Expression of more genes in the gut are affected by infection with *P. aeruginosa* compared to *E. coli*.



Infection with *E. coli* does not have a transcriptional effect on house flies.

- In accordance with the previous study (Fleming et al 2014) antimicrobial peptides (AMPs) were not induced by infection with *E. coli*. We also did not detect the induction of other immune effectors such as lysozymes.

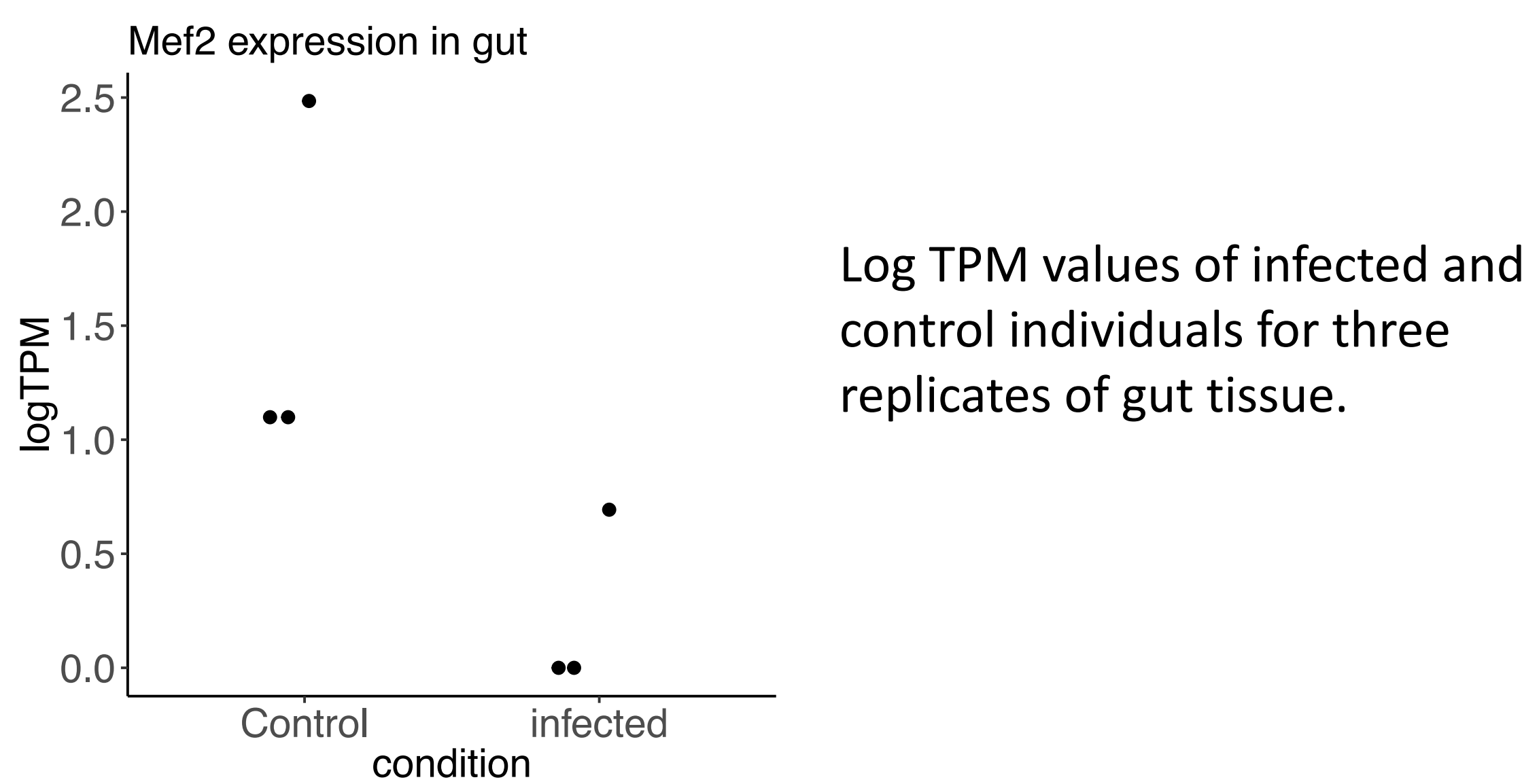
- Only a small number of differentially expressed (DE) genes identified in gut or body

- Gene ontology enrichment analysis does not show enrichment of DE genes for a biological function or pathway.

Infection of flies with *P. aeruginosa* results in suppression of immunity and activation of metabolism

- Perhaps due to difference in methodology (qPCR vs RNA-seq) unlike the previous study (Joyner et al 2013), DE of AMPs were not observed.

- Myocyte enhancer factor 2 (*Mef2*) is down regulated in the gut. This gene is reported to be important for induction of AMPs in fruit fly (Clark et al 2013).



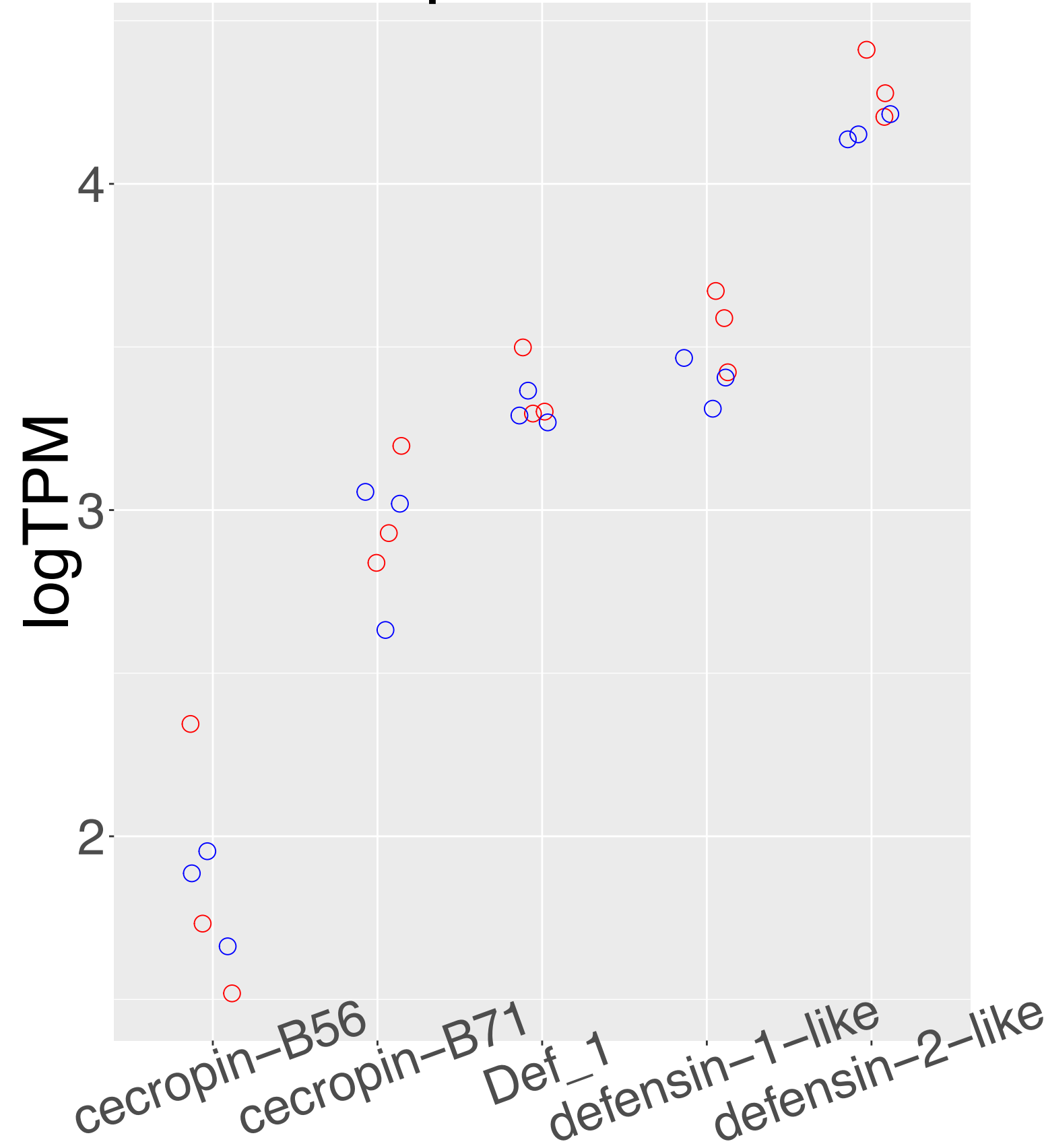
- Metabolism and ion transportation is increased upon infection with *P. aeruginosa*.

GO Term	Adjusted p-value	Number of genes
catalytic activity	0.002889	69
anion transmembrane transporter activity	0.003714	11
active ion transmembrane transporter activity	0.004023	10
organic anion transmembrane transporter activity	0.004294	9
secondary active transmembrane transporter activity	0.005069	9
active transmembrane transporter activity	0.005088	12
transmembrane transporter activity	0.005631	23
transporter activity	0.005771	23
ion transmembrane transporter activity	0.023723	17

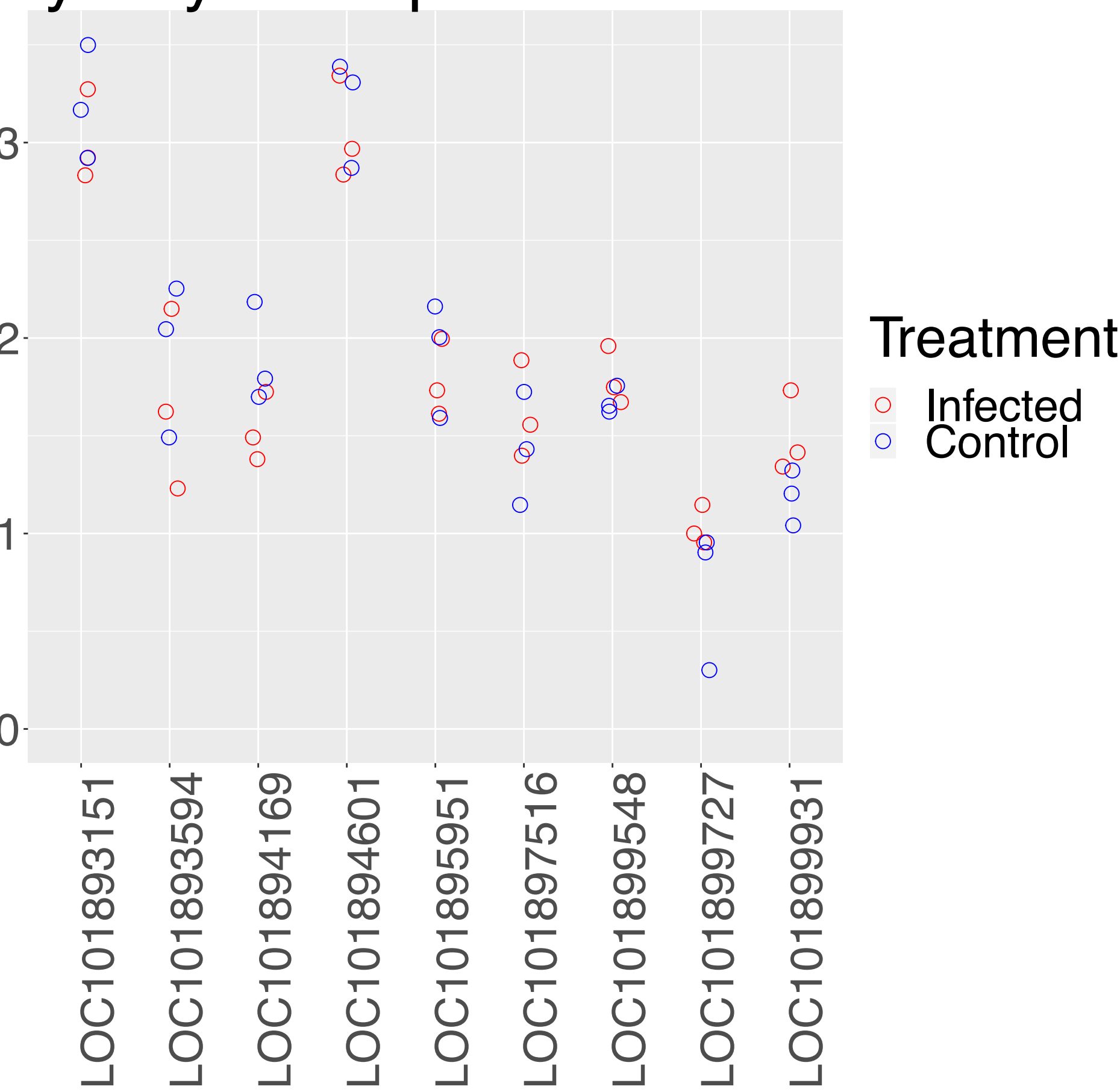
- Upregulation of metabolism might suggest mobilization of resource to provide energy for transcription and translation of activated genes upon infection (cost of defense).

Five AMPs and nine lysozymes are constitutively expressed in house fly gut

AMP Expression in Gut



Lysozyme Expression in Gut



In gut we have constitutive expression (overlapping TPM value for control and infected replicates) of two *cecropins* (cecropin-B56 (LOC109613656) and cecropin-B71 (LOC105262171)), one newly annotated *defensin* (Def-1) and two previously annotated *defensins* (defensin-1-like and defensin-2-like). Many lysozymes are also constitutively expressed in the gut.

Conclusions:

- Housefly does not change its transcriptome upon infection with *E. coli*. This entails not inducing any AMPs or other effectors of immune system (e.g. lysozymes).
- We have identified constitutive transcription of 5 AMP and 9 lysozyme genes in the gut of house flies.
- P. aeruginosa* might be able to suppress the AMP production of house flies. This might happen through down regulation of *MEF2*.
- Up regulation of metabolic genes upon infection with *P. aeruginosa* might be an indicator of the cost of defense.
- We hypothesize constitutive expression of AMPs in the gut protect the flies from infection even if induction is impaired. This offers a huge advantage to house flies which makes living in septic environments possible.

References:

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