Proteomic characterization of inbreeding-related cold sensitivity in *Drosophila melanogaster*

Cornelis J Vermeulen[1,2*], Kamilla S Pedersen[3*], Hans C Beck[4], Jørgen Petersen[5], Kristina Kirilova Gagalova[6], Volker Loeschcke[1]

[1] Ecology and Genetics, Dept. of Biological Sciences, Aarhus University; [2] Evolutionary Genetics, CEES, University of Groningen; [3] Dept. of Genetics and Biotechnology, Faculty of Agricultural Sciences, Aarhus University; [4] Center for Clinical Proteomics, Dept of Clinical Biochemistry and Pharmacology, Odense University Hospital; [5] Dept. of Biochemistry and Molecular Biology, University of Southern Denmark; [6] Dept. of Pharmacy and BioTechnology, University of Bologna; * these authors contributed equally to this work

2013 PLoS ONE 8(5) e62680

Introduction

- Inbreeding depression is caused by the expression of rare recessive deleterious alleles.
- This study aims to identify specific loci contributing to inbreeding depression, and to characterize their environmental sensitivity.
  - Our model: Flies (*Drosophila melanogaster*) of the highly inbred L10 line are killed by mild cold shock
  - This conditional lethality is an aspect of inbreeding depression
  - We have characterised this phenomenon at the proteomic level

Material and Methods

- Flies were sampled after cold shock, but before mortality occurs in the cold-sensitive inbred line
- The inbred control line controls for general effects of increased homozygosity
- Separation by 2-dimensional gel electrophoresis
- Protein identification by mass spectrometry
- RT-qPCR of genes coding for selected proteins

Table 1: experimental setup

Line	Cold shock	Control temp.
Conditional lethal line	dead	OK
Inbred control line	OK	OK
Outbred control line	OK	OK

Results

PCA graph of protein expression

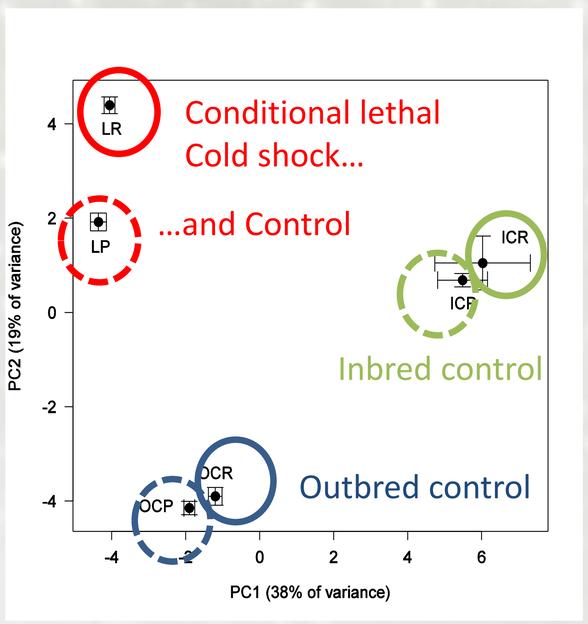
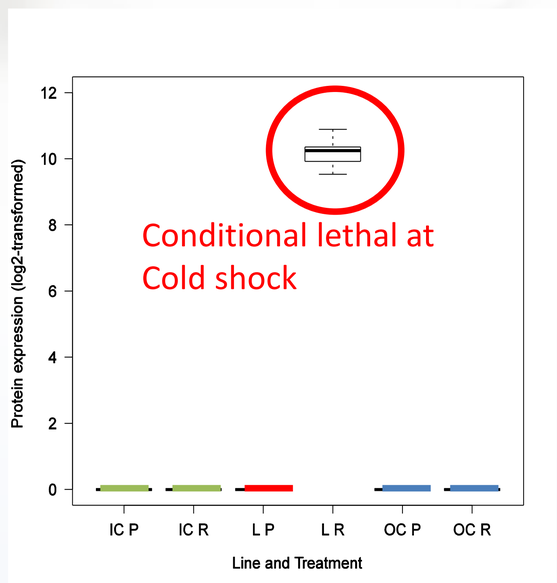


Figure 1: Principle Component Analysis of protein expression readily separates all lines. The effect of cold shock is clear in the conditional lethal line.

Example of expression profile of a single protein

Figure 2 :Some expression patterns are spectacular. This is the expression profile of CG18067. Note that it is only expressed in conditional lethal flies at cold shock. Variation in mRNA expression is much smaller, indicating (post) translational regulation.



GO enrichment of differentially expressed proteins

Gene Ontology enrichment suggests overrepresentation of proteins involved in **hexose and pyruvate metabolism** and proteins associated with **lipid droplets**

Conclusion

- There is a clear proteomic signature of inbreeding depression in thermal resistance in the conditional lethal line
- We obtained promising leads for follow up research into the physiology and genetics underlying this inbreeding depression phenotype
- Results suggests disruption of hexose metabolism and lipid storage / trafficking, which are known physiological mechanisms of cold resistance.
- Inbreeding depression in this case might thus stem from the expression of recessive deleterious alleles in cold resistance pathways.