Environmental Heat Stress Induces Epigenetic Inheritance of Robustness in *Artemia* Model

Parisa Norouzitallab, Kartik Baruah, Gilbert Van Stappen, Karel De Schamphelaere, Patrick Sorgeloos & Peter Bossier
Crustacean aquaculture

- Highly demanded
- High market value
Finding solution

Finding solution to the problem of the disease is a primary goal
Parental manipulation to improve phenotypes in progenies

F0 manipulation

Selection
- Intense pressure
- Across generations
- Long period of time
- Major loss of the miss-fit
- Labor intensive

Epigenetics
- Mild pressure
- One generation
- Short period of time
- Minor loss of miss-fit
- Labor intensive
DNA molecule can record the environment

Epigenetic is the study of heritable changes in gene expression and function that cannot be explained by changes in DNA sequence.
Constraints for using shrimp as model organism

- Long culture period of approximately 1 year for one generation
- Labour intensive
Selection of the model organism
Artemia as model organism for our studies

- Short generation time – 3 generations in about 6 months
- Genome is sequenced
- Easy and cheap to maintain and manipulate
- Produce large numbers of offspring
Artemia as model organism for epigenetic studies

**Bisexuality**

Wild population
maternal/paternal
heredity

**Parthenogenesis**

NO MALE

Clonal population

Artemia as model organism for epigenetic studies
Cyst (dormant egg) production – Common garden experiments
Common garden experiments – axenic / gnotobiotic

Chemical removal of the embryo outer shells
Sodium hydroxide hypochlorite solution
Abiotic stressor and transgenerational inheritance of robustness
Background information

Induction of heat shock protein 70 (HSP70)

Protection against subsequent stressors
✓ abiotic - lethal heat shock
✓ biotic - pathogenic *Vibrio campbellii*

Heat shock protein (HSP) 70 against abiotic stressors

Synthesized constitutively in the cells (heat shock cognate 70)

Role in house keeping

Induced after exposure to stressors

Danger signal
Parthenogenetic Artemia

(clonal population)

Dally non-lethal heat shocks (T)

Iso-thermic (C)

TF1

TF2

TF3

ISO

THERMIC

CF1

CF2

CF3

I

S

O

T

H

E

R

M

I

C
Common garden test - verifying stress resistance phenotypes

- F1
- F2
- F3

20 instar II nauplii in 6 replicates

- 42°C for 15 min
- Or
- *Vibrio campbellii* $10^7$ cells/ml
- Or
- Zinc
Thermo-tolerance test

TF1 > CF1

TF2 > CF2

TF3 > CF3
V. campbellii resistance

![Graphs showing the proportion of surviving animals over time for different conditions](image)

- **TF1 > CF1**
- **TF2 > CF2**
- **TF3 > CF3**
Zn in O (P) and F3 generation

\[ \text{LC}_{50} = 13.4 \ (6.6 - 15.7) \ \text{mg/l} \]

Heat shock (F3)

Non-heat shock (O)

Non-heat shock (F3)
Phenotypic trait - HSP70 production

![Graph showing HSP70 production levels for different treatments.]

- **TP**: Treatment 0
- **CP**: Control 0
- **TF1**: Treatment 1
- **CF1**: Control 1
- **TF2**: Treatment 2
- **CF2**: Control 2
- **TF3**: Treatment 3
- **CF3**: Control 3

**Hsp70 (ng/µg of protein)**

- **T-F0**: Treatment 0
- **C-F0**: Control 0
- **T-F1**: Treatment 1
- **C-F1**: Control 1
- **T-F2**: Treatment 2
- **C-F2**: Control 2
- **T-F3**: Treatment 3
- **C-F3**: Control 3

The graph illustrates the HSP70 production levels across different treatments and controls, with arrows indicating increased levels in specific treatments compared to controls.
Histone acetylation

Transcriptionally active

HAT

HDAC

Transcriptionally repressed
Epigenetic mark - histone H3 acetylation

Histone H3 acetylation (ng/µg of histone)

Treatment

T-F0  C-F0  T-F1  C-F1  T-F2  C-F2  T-F3  C-F3

* indicates statistically significant differences.

T-F0 vs. C-F0, T-F1 vs. C-F1, T-F2 vs. C-F2, T-F3 vs. C-F3.
Epigenetic mark - histone H4 acetylation

Histone H4 total acetylation (ng/µg of histone)

T-F0 | C-F0 | T-F1 | C-F1 | T-F2 | C-F2 | T-F3 | C-F3
---|---|---|---|---|---|---|---
1.5 | 0.3 | 1.2 | 0.6 | 0.9 | 0.9 | 0.9 | 0.9
DNA methylation

- Addition of methyl group to 5′ cytosine on CpG sites
- Reduced chance of transcription

Silenced gene
Epigenetic mark – global DNA methylation

![Graph showing %mdc/dG for different generations.](image)
Conclusions

- Environmental heat stress on parental generation induces epigenetic inheritance of resistance against disease, lethal heat shock and Zn.
- These transgenerational epigenetic inheritance of traits were associated with production of HSP70 and sustained hyper-acetylation of histones H3 and H4.
- Abiotic environmental stimuli in parental generation can result in transgenerational modification of phenotypes.
Future perspectives

Artemia franciscana

Navigation
- BLAST - SEARCH - WIKI - DOWNLOAD - WORKBENCH - WATCHLIST - HELP

Browse
The brighter the color, the higher the gene-density in that region. Click on a region to go to that location in the browser. Only contigs larger than 10Kb are displayed here, the complete list of contigs is available in the dropdown menu from the genome browser.

Annotation
- Direct To Gene: [Input Field] Go!
Acknowledgement

Promoter
Prof. Dr. ir. Peter Bossier

Funding
BOF PhD Grant, UGent
Belspo, AquaStress Project
Cu in Wild population

- Heat shock (W)
- Non-heat shock (W)

LC$_{50}$ = 0.18 (0.15 - 0.26) mg/l
LC$_{50}$ = 0.26 (0.18 - 0.3) mg/l

axenic
Zn in Wild population

![Graph showing mean survival over log[con] (mg/l) with LC50 values for heat shock and non-heat shock conditions.]

- LC50 = 10.6 (6.6 - 15.7) mg/l
- LC50 = 4.6 (4.3 - 7.6) mg/l

Heat shock (W)
Non-heat shock (W)