

The identification of genes from the oyster *Crassostrea gigas* that are differentially expressed between progeny exhibiting opposed susceptibility to summer mortality



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Summer mortality of *Crassostrea gigas*

- It affects adults and especially **juveniles**
 - associated with high **temperatures**, **reproductive** period
 - **pathogens** (bacteria, Herpes virus-like)
 - pollutants
- ↪ **BUT** their single implication in numerous events of summer mortality appeared uncommon
- complex interaction between host, pathogen and environment
- ↪ one main goal for physiologists = identify and understand **physiological dysfunction** leading to summer mortality

MOREST program

- **progenies** obtained by divergent selection on survival
 - ↪ two groups: **R** "Resistant" & **S** "Sensitive", depending on their in situ summer survival (Dégremont et al, 2003)
- one relevant approach = analyzing **phenotype differences** between Resistant & Sensitive progenies

Aim of the present study

Molecular comparison of *C. gigas* Resistant and Sensitive progenies

↳ (1) Identify **differentially regulated genes** between R and S progenies in conditions leading to summer mortality (i.e. spat stage, reproductive period, high temperature) using suppressive soustractive hybridization technology

↳ (2) **Screening the identified genes** with different R and S samples of different generation, age, location... to sort the best candidates to study summer survival using macroarray technology

FIRST STEP: identification of differentially-regulated genes between R & S progenies

Experimental design

3 Resistant & 3 Sensitive G2 progenies

Spat rearing
(Bouin nursery)

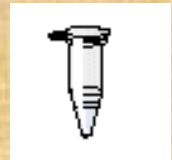
as soon as
first oysters
died (July)...

10 individuals collected/progeny
dissection of gonad
(no dying oyster collected)



Mortality R < 5% S = 75%
Vibrio splendidus isolated
in dying S oysters

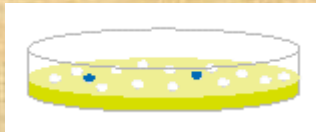
RNA extraction
for SSH



SSH experiment

mRNA "R" — mRNA "S"

Forward & reverse
subtractions were realized

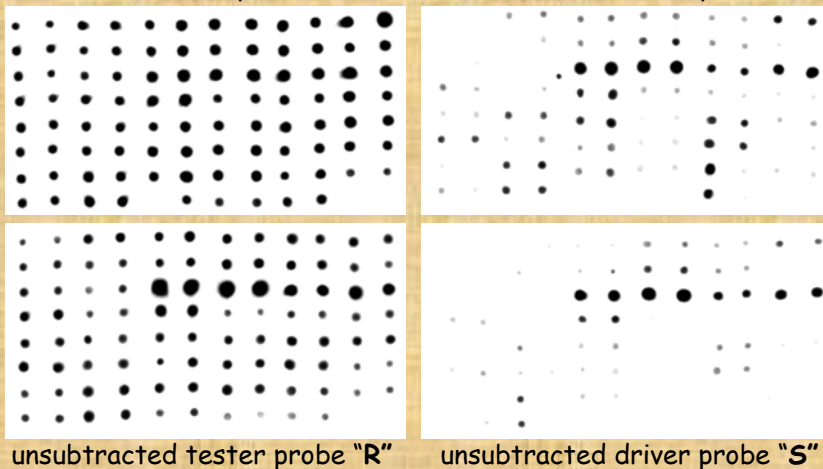


Subtracted library = **376 clones**



forward-subtracted probe "R-S"

reverse-subtracted probe "S-R"



150 differentially expressed clones
(40%), **induced** in R compared to S,
after blots with 4 probes
(tester & drivers of the forward & reverse subtractions)

↪ higher expression in R progeny confirmed by Real time PCR analysis on 8 selected mRNAs (mean additional expression in R \approx 2)

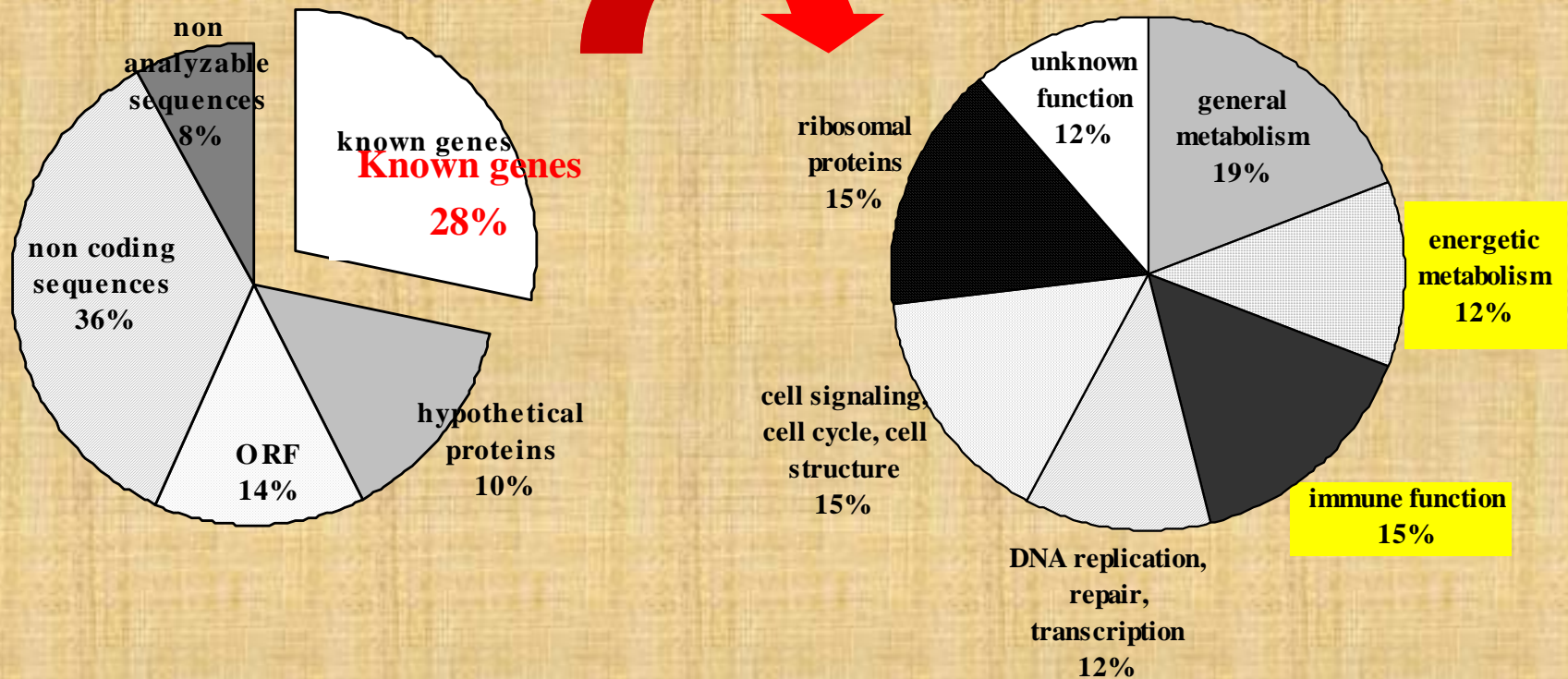
Differentially expressed sequences

150 clones differentially expressed were sequenced and analyzed

mean size = 452bp

55 singletons, 20 contigs

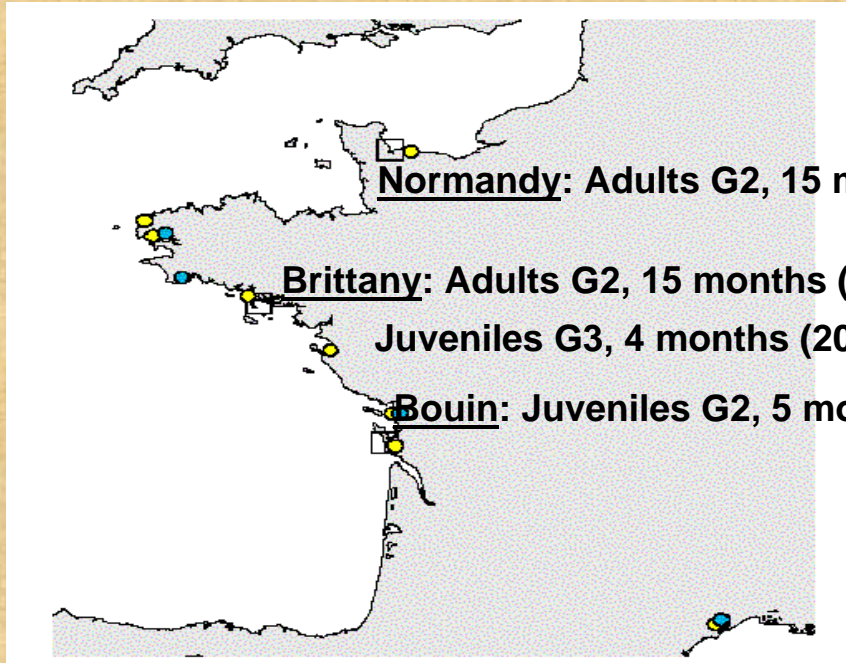
redundancy 20%



Up to now, energetic and/or immunological dysfunction were commonly supposed in summer mortality phenomena

SECOND STEP: Screening the SSH clones with different R and S samples using macroarray

In situ design



Mortality rate

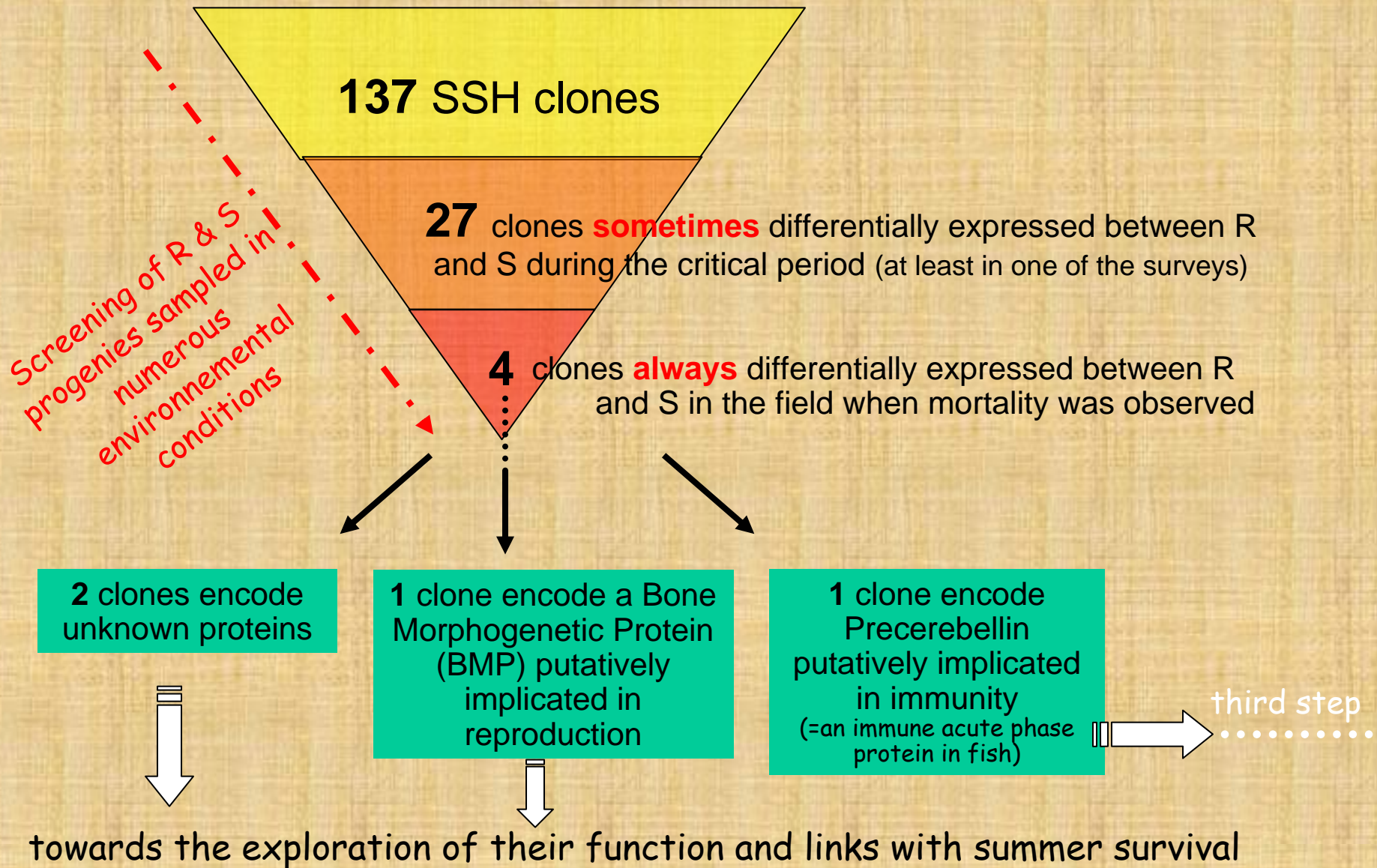
Normandy: Adults G2, 15 months (2003) ➤ R=5% S=8%

Brittany: Adults G2, 15 months (2003) ➤ R=5% S=14%

Juveniles G3, 4 months (2003) ➤ R=38% S=80%

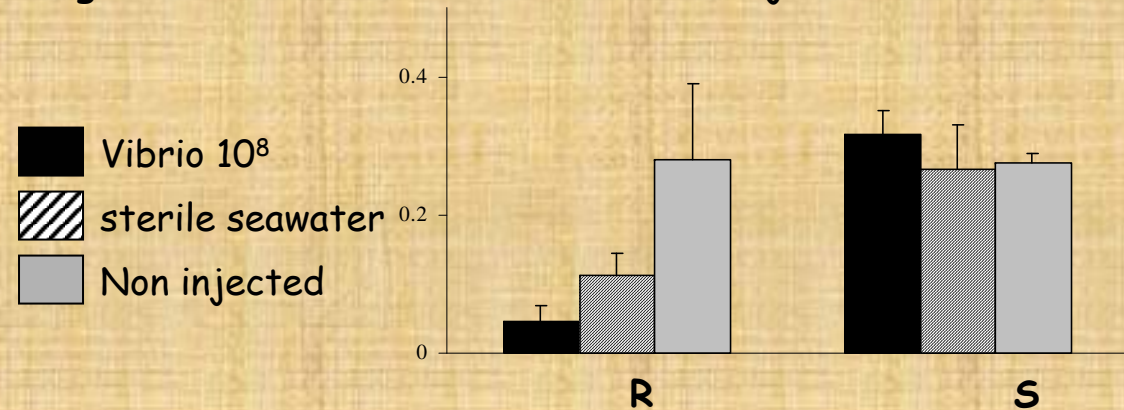
Bouin: Juveniles G2, 5 months (2002) ➤ R=4% S=75%

Only 4 SSH clones were always differentially expressed between R and S in the field



THIRD STEP: functional analysis of candidate genes (in progress...)

Precerebellin mRNA level in gonad after seawater and Vibrio injection into R & S oysters



precerebellin : strong differential response in the gonad between R and S after exposure to a stress: seawater and much more bacteria injection

Hypothesis: a greater capability to rapidly recruit phagocytes towards infected muscle for tissue regeneration and defense in R compared to S oysters (might correspond to the observed decrease of precerebellin mRNA level in R gonad)

However, R & S progeny suffered a high mortality rate in response to bacterial injection (~ 75%)

Conclusion & perspectives

❖ this work = the first step towards elucidating the physiological and genetic basis of summer survival of R and S selected progeny

↳ towards high throughput technology (microarray) to identify the temporal steps leading to summer mortality and to sort main effects of the environmental factors

from the genes suggested to operate differentially between S and R progeny, our data suggest 4 clones as possible starting points for further research

↳ functional exploration (spatio-temporal expression,...)

↳ mapping analysis to look for QTLs associated to summer survival