Genetic solutions to mitigate disease impact on shellfish species: Vivaldi main achievements

Jean-Baptiste Lamy & Sylvie Lapègue (WP3 coordinator)

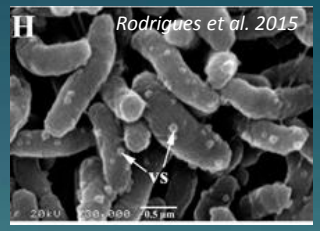
27/11/2019

VIVALDI

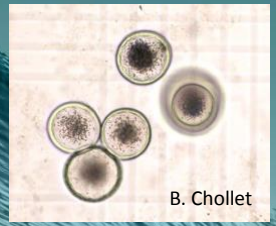
Preventing and mitigating farmed bivalve diseases

Our best friends…

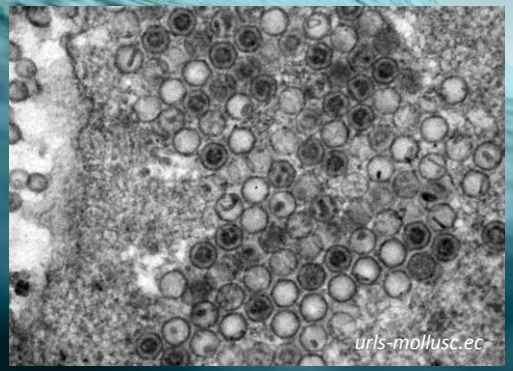
Vibrio tapetis LP2



Perkinsus Hypnospores



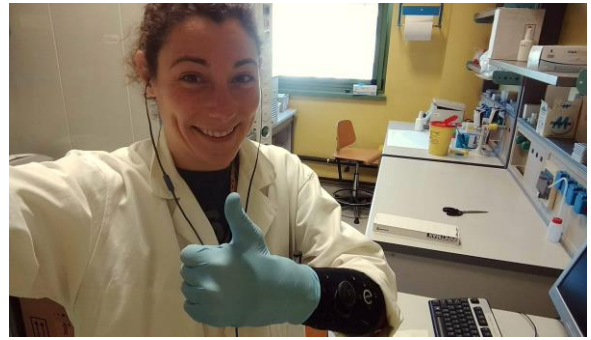
Ostreid herpesvirus



Ruditapes philipinarum

Crassostrea gigas

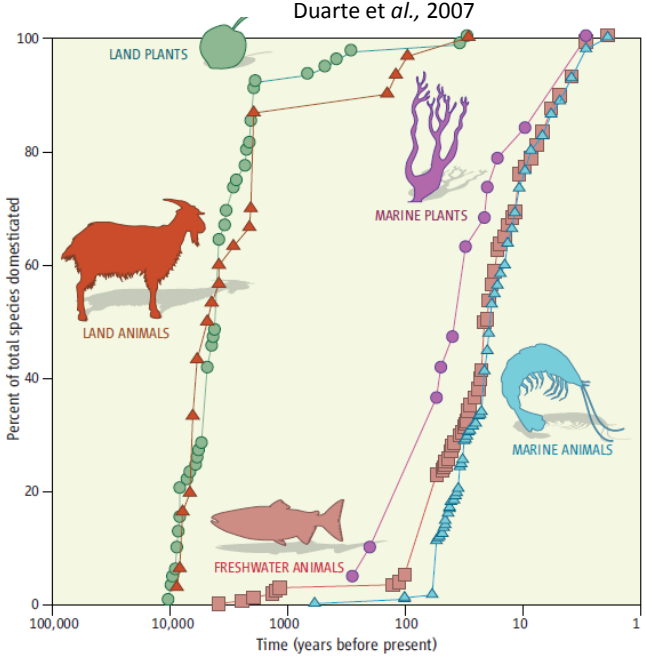
Morgan Smits (UNIPD & LEMAR) will present a brilliant talk about it.



Non edible and dying oyster

The sad story of all domesticated species

Duarte et *al.*, 2007



Introduce a new species when the economic burden is too heavy (with new pathogens and aliens species)

Livestock intensification

↓

Diseases outbreaks

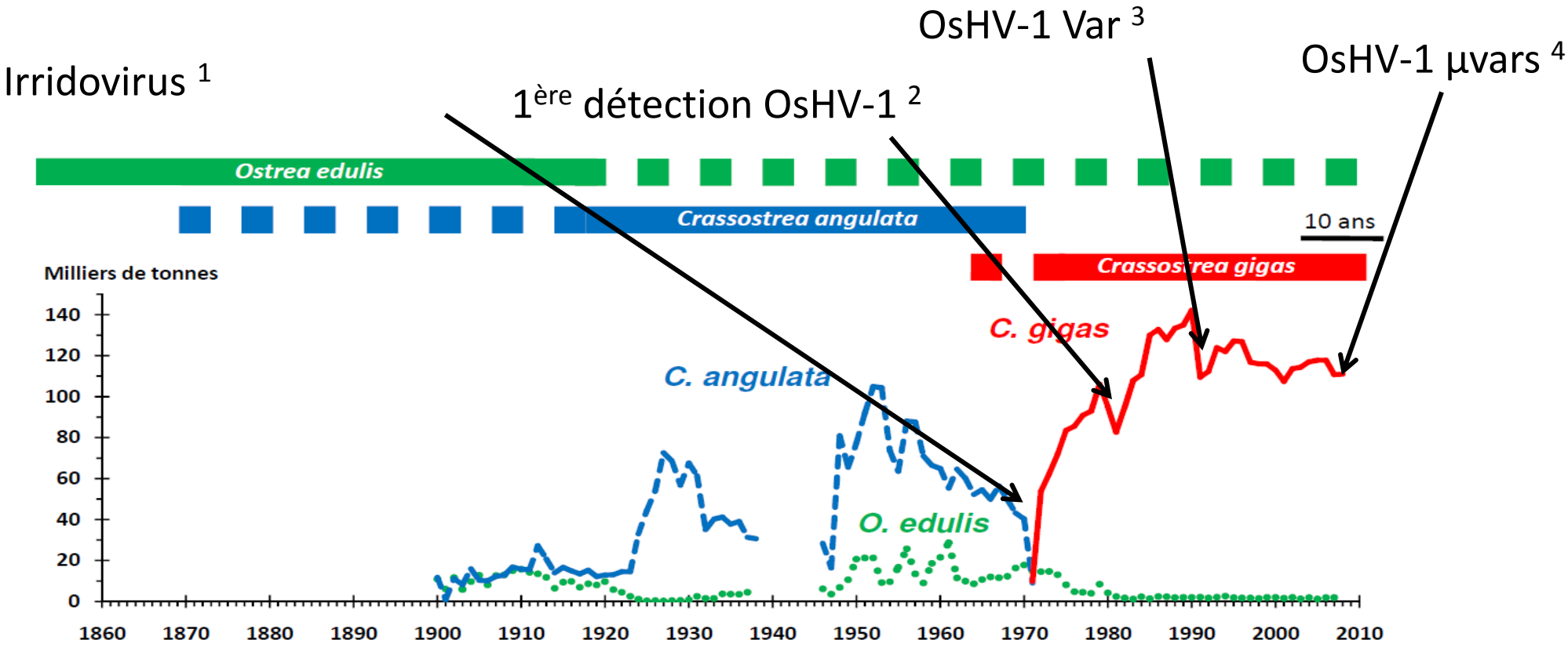
↓

More intensification

Encyclopédie Larousse

Modified from Mineur et *al.*, 2014

Oyster French livestock intensification…



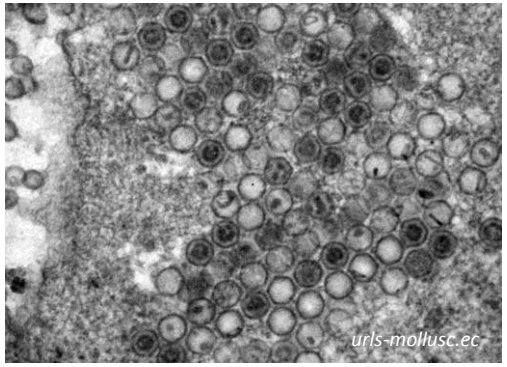
[1] Comps et *al*., 1988 ; [2] Renault et *al*., 1994 ; [3] Arzul et *al*., 2001 ; [4] Martenot et *al*., 2011

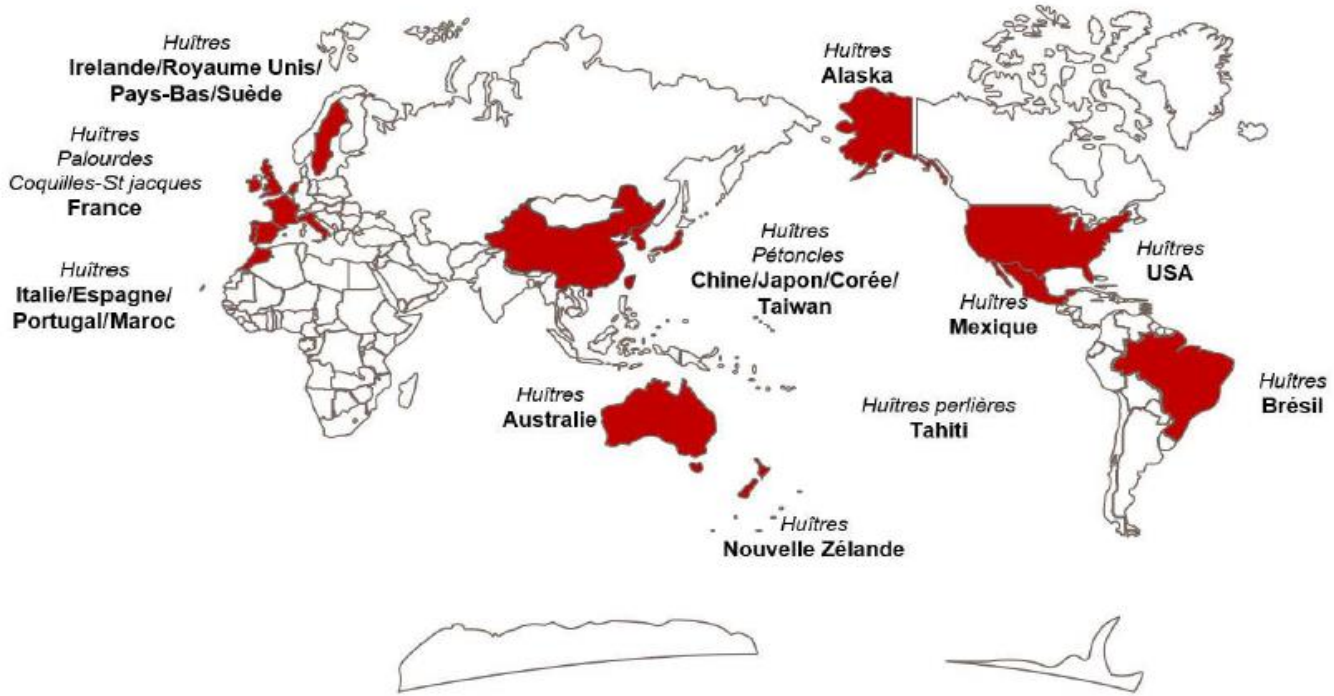
OsHV1 is a world-wide pathogens…

OsHV1 is now dispersed worldwide in all oyster producing areas

Crassostrea gigas

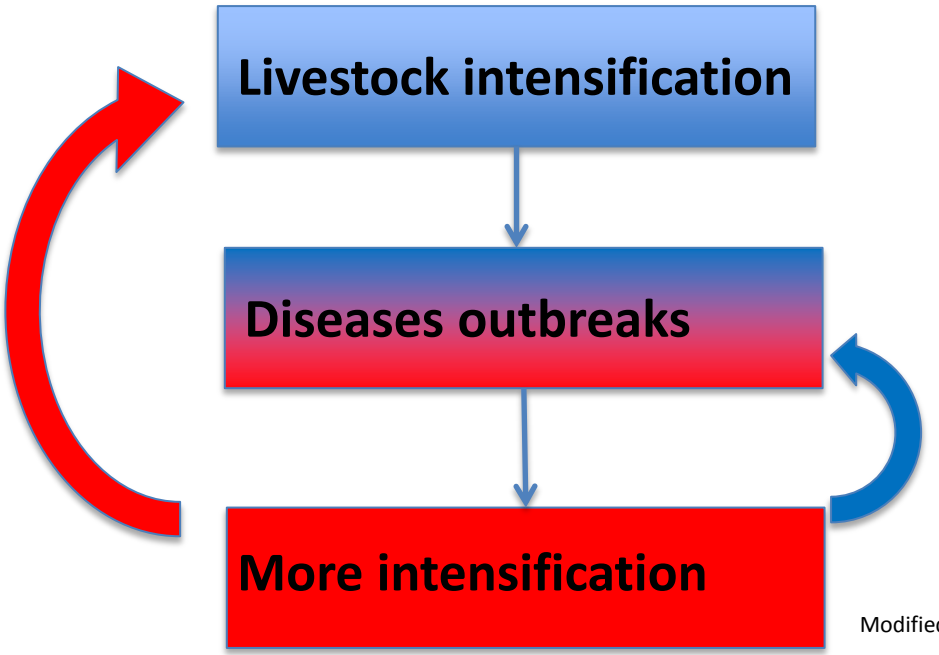
Ostreid herpesvirus





Segarra *et al.*, 2010 ; Keeling *et al.*, 2014 ; [3] Burge et *al.*, 2006 ; [4] Hwang et al., 2013 ; Martenot et al., 2013;, de Kantzow et *al;*, 2017

The sad story of all domesticated species



Introduce a new species (with new pathogens and aliens species)

Mitigation

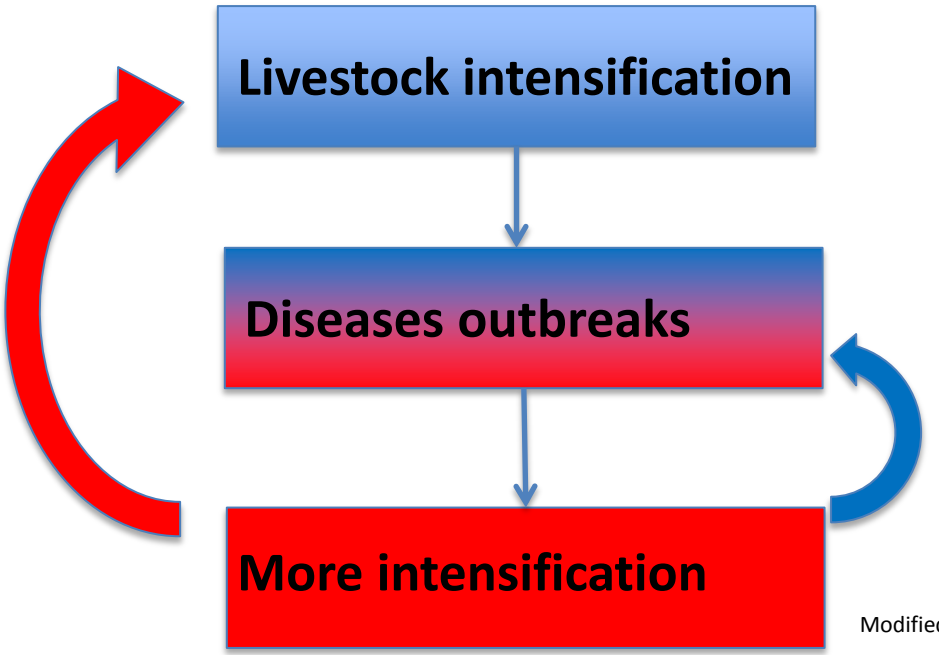
1. **Biosecurity measures**
2. **Zootechnicals practices**
3. **Genetic resistance** (avoi
tolerance)

Modified from Mineur et *al.*, 2014

To initiate genetic selection programs, we need a good protocol (trade-off inbreeding and gains) and phenotypic variations on the targeted traits.

The sad story of all domesticated species

Introduce a new species (with new pathogens and aliens species)



Mitigation

1. Biosecurity measures
2. Zootechnicals practices
3. Genetic resistance (avoi
tolerance)

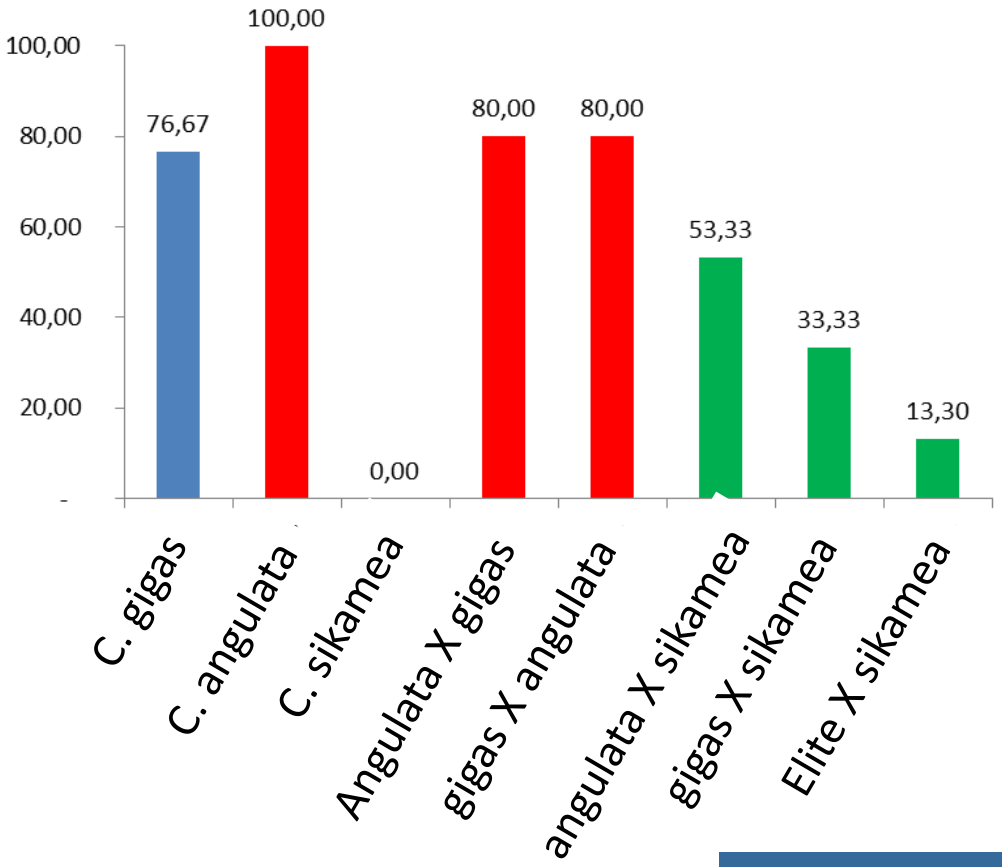
Modified from Mineur et *al.*, 2014

Florian Enez (Sysaaf) will expose some results about how to implement and manage breeding programs in shellfish

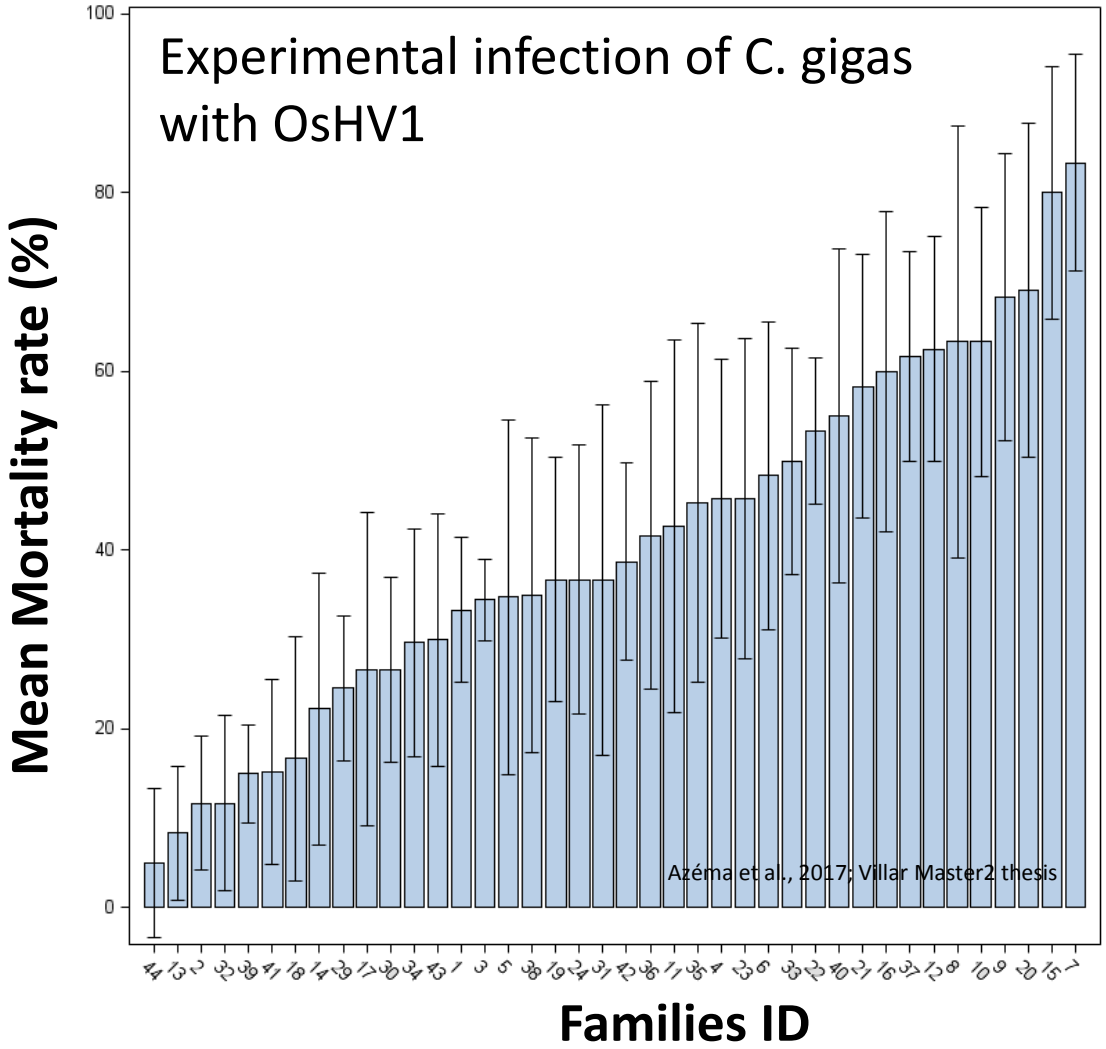
We are lucky for OsHV1 resistance

Vivaldi project Abdellah Benabdelmouna

Experimental infection of various oyster species and hybrids with OsHV1



Experimental infection of C. gigas with OsHV1



Plenty genetic variance

Resistance against OsHV1 infection is under tight genetic control with various phenotypic expression.

Across various experimental designs and estimation procedures $h^2_{ns} \sim 0.6$

Dégremont et al., 2011; Dégremont et al., 2015; Azéma et al., 2017

Correlation between field and laboratory $r_{pearson} \sim 0.7$

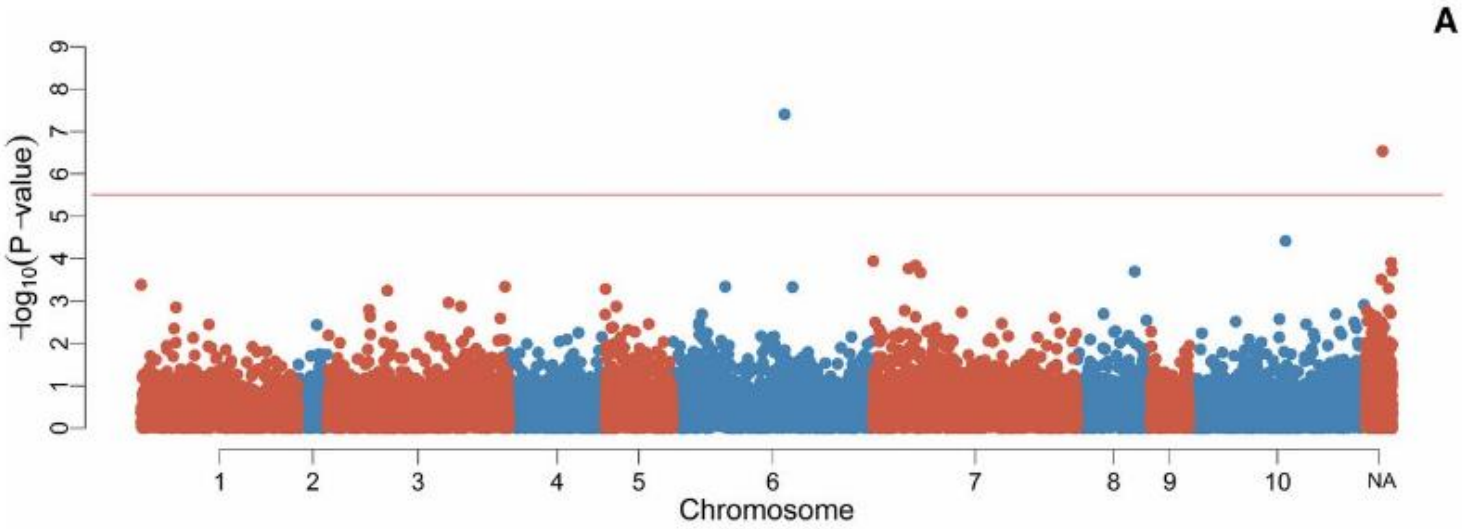
Dégremont et al., 2015

Such trait is likely to be a good candidate for genomic selection (improve genetic gains while controlling inbreeding rate).

Genomic determinism of OsHV1

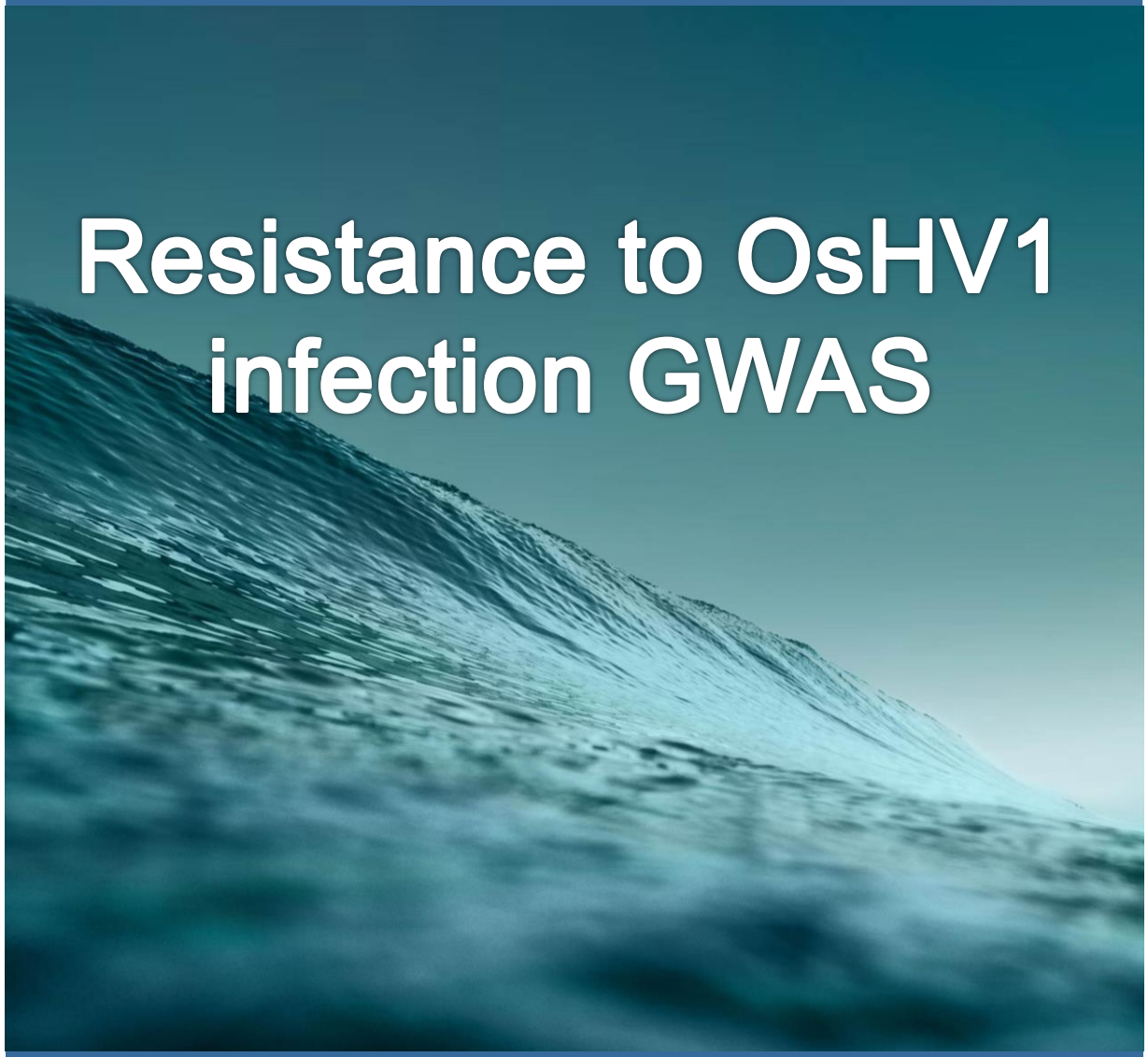
Such heritable traits seem highly polygenic :

- Sauvage et al 2010, found various QTL segregation in 3 families
- Small evidence of QTL in association studies despite decent experimental design
- size.



They found quite modest evidence about two QTL for OsHV1 resistance in Cawthron Insitute's populations from Nelson and Guernsey Sea Farms populations.

Gutierrez et al., 2018; Gutierrez et al., 2019


Resistance to OsHV1
infection GWAS

Hypothesis

1. Previous and recent studies have shown that resistance to OsHV1 is likely polygenic character (with very small effects).

Luqman Alsam (Nofima)

Ifremer

Nofima

Hypothesis

1. Previous studies have shown that resistance to OsHV1 is a polygenic character (with small effects).

2. Test the possibility of genomic selection on this traits (even if not QTL are found).

Binyam Dagnachew (Nofima) will give an talk on this later and illustrate some pitfalls to avoid in shellfish.

Hypothesis

1. Previous studies have shown that resistance to OsHV1 is a polygenic character (with small effects).

2. Test the possibility of genomic selection on this trait even if not QTL are found.

3. Make an independent validation using a natural population-based experiment during a disease outbreak.

Mathias Wegner (AWI) has some shiny results on this part

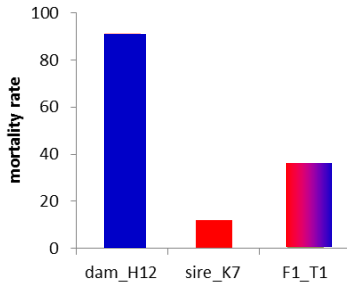
Experimental design

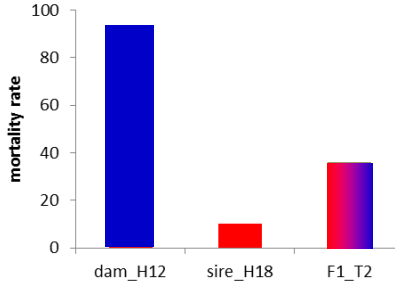
Resistant X *Susceptible*

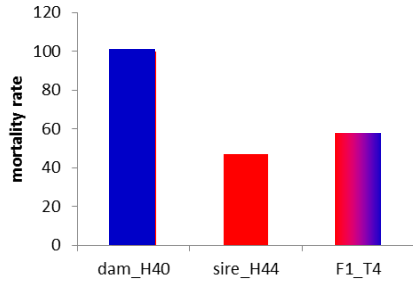
F1

Grand-parents are from 7th generation of selection and control lines extensively tested in both field and lab.

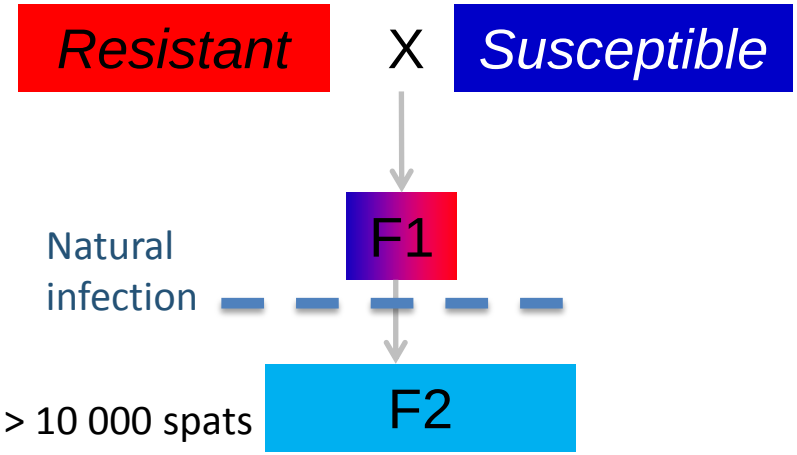
Mean mortality rate of the family of each Grand-Parents and Parents (across field and lab experiments). The Parents are close to average of grands-parents mean (as theoretically expected)

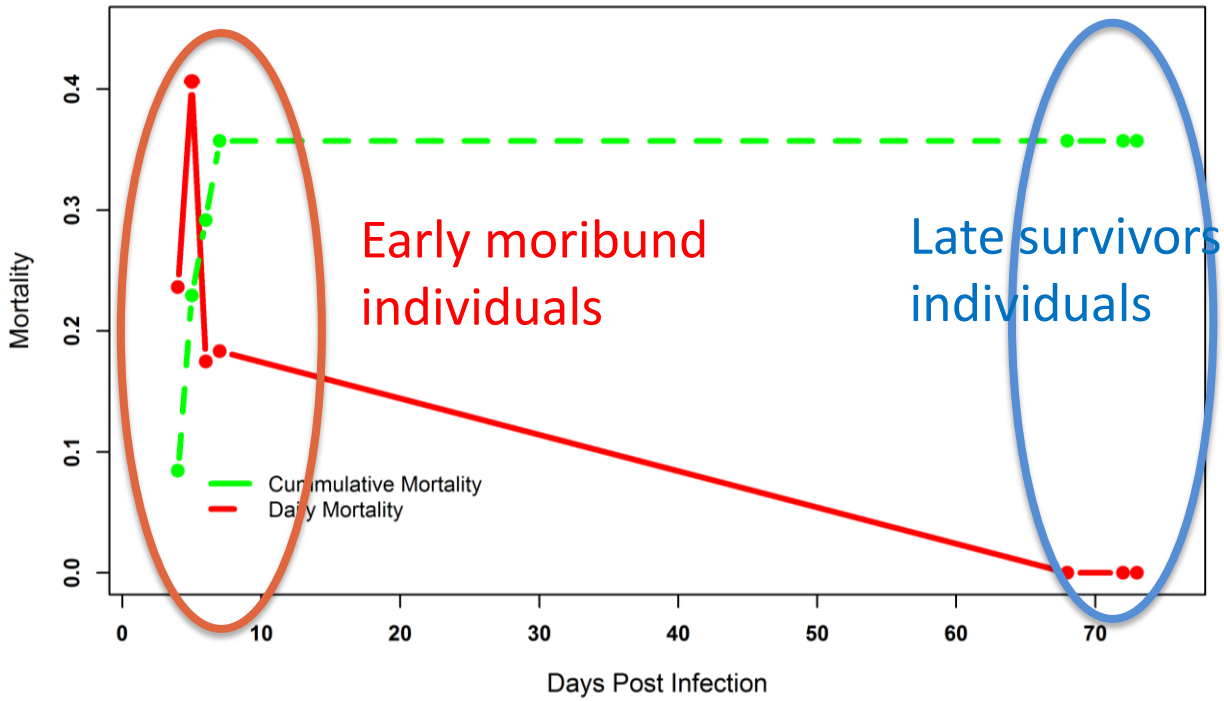






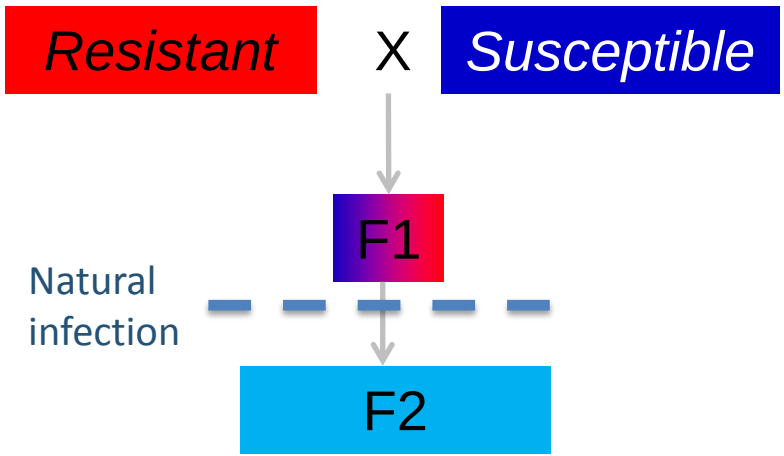
Experimental design and Mortality cinetics



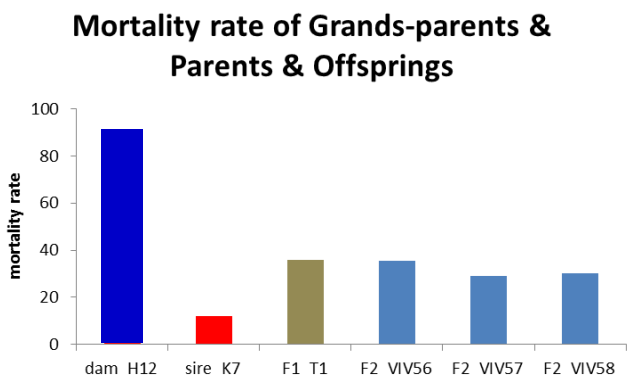


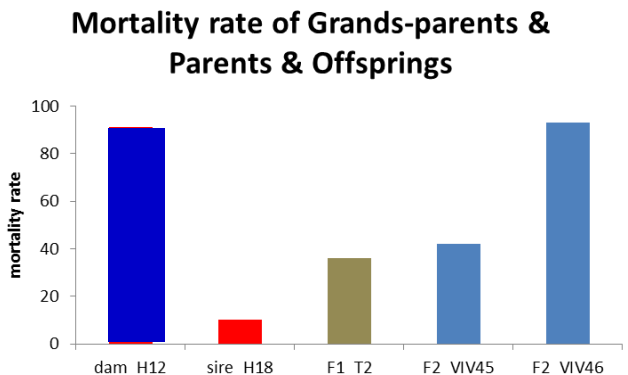
As expected during OsHV1 outbreaks, the mortality observed was massive and rapid as soon as the sea water is above 16 ° C. Two extremes phenotypes were sampled in each family.

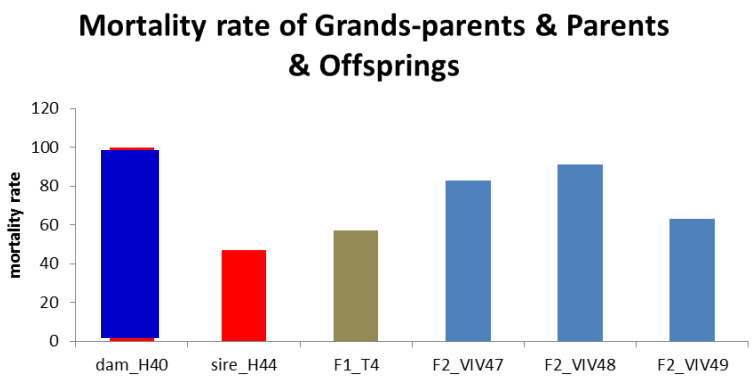
Experimental design and Phenotyping (mortality)



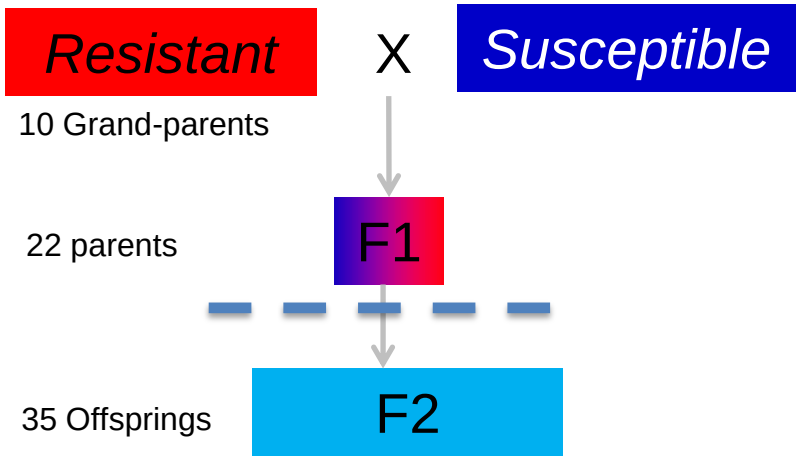
The mortality rate was variable across the F2 families, within the range of the grand-parents' mean mortality rate.



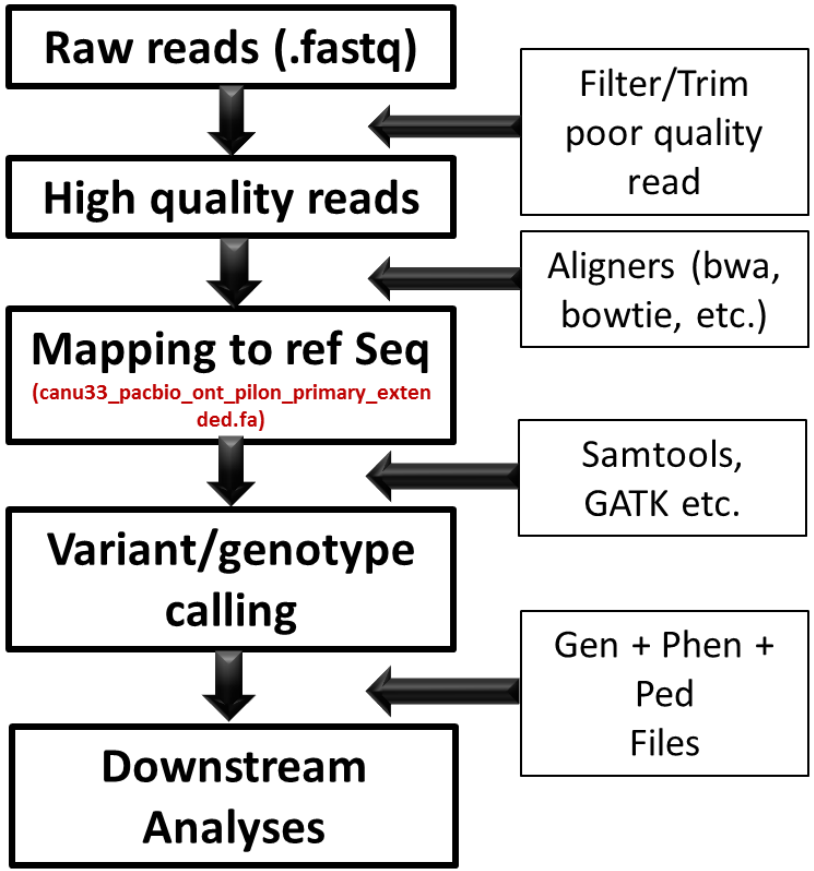




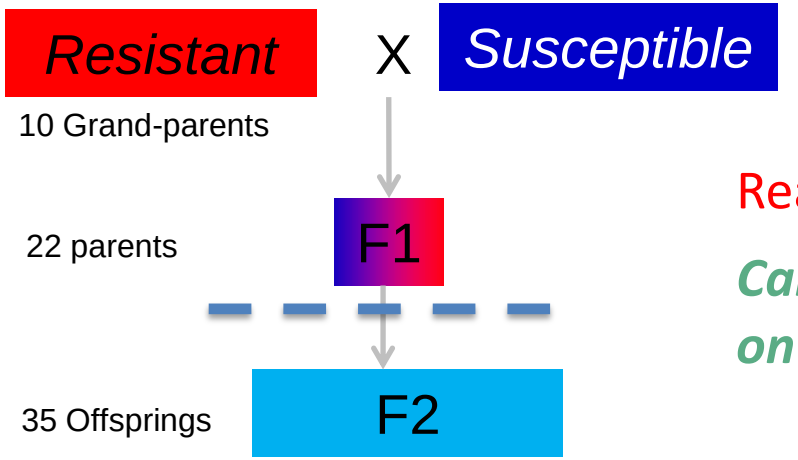
Experimental design and Whole Genome Sequencing



We use a custom oyster reference genome (from PacBio and ONT sequencing)

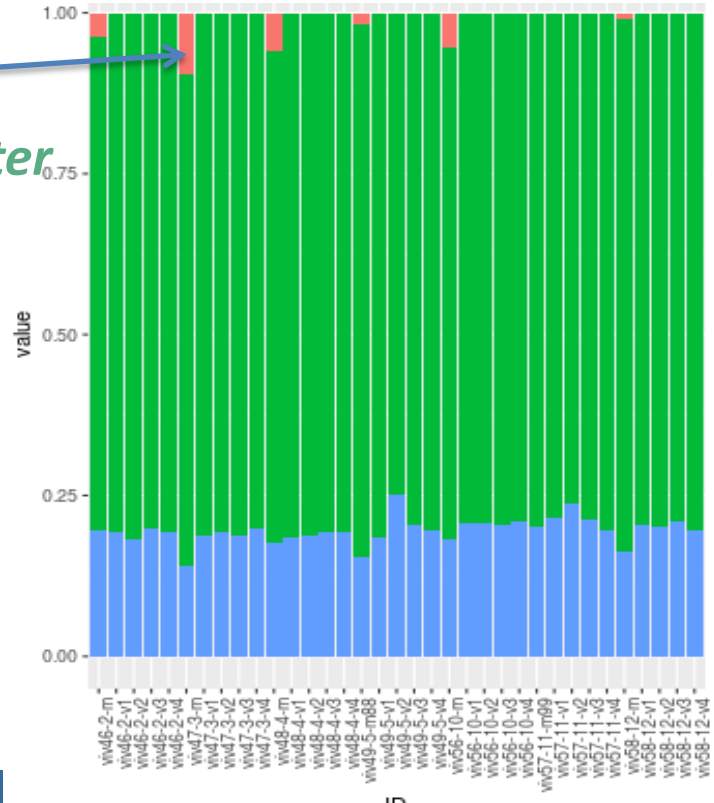


Experimental design and Whole Genome Sequencing

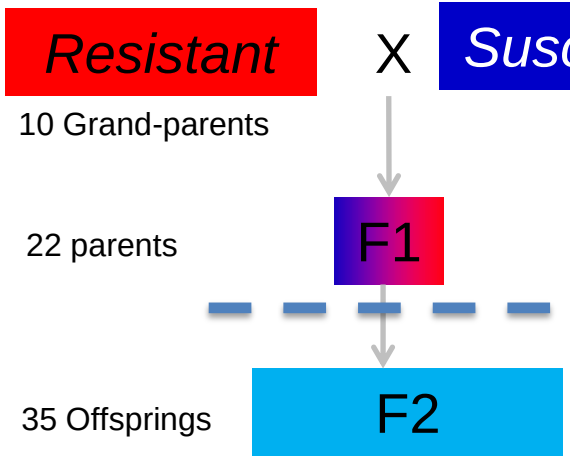


Reads from OsHV1 (~1%)

Camille Pelletier has a poster on that part



Experimental design and Whole Genome Sequencing

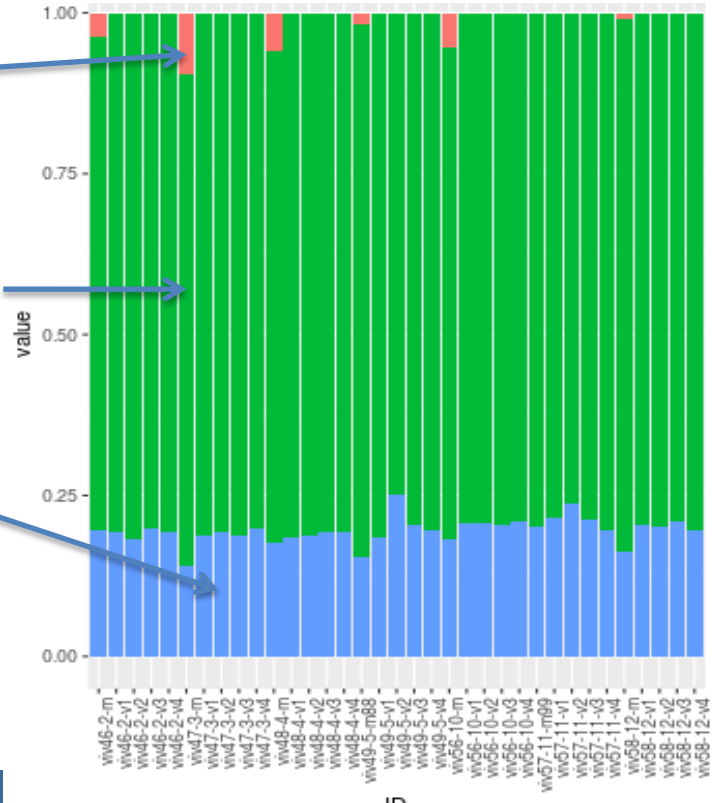


Reads were aligned on oyster genome reference and SNP called.

Reads from OsHV1 (~1%)

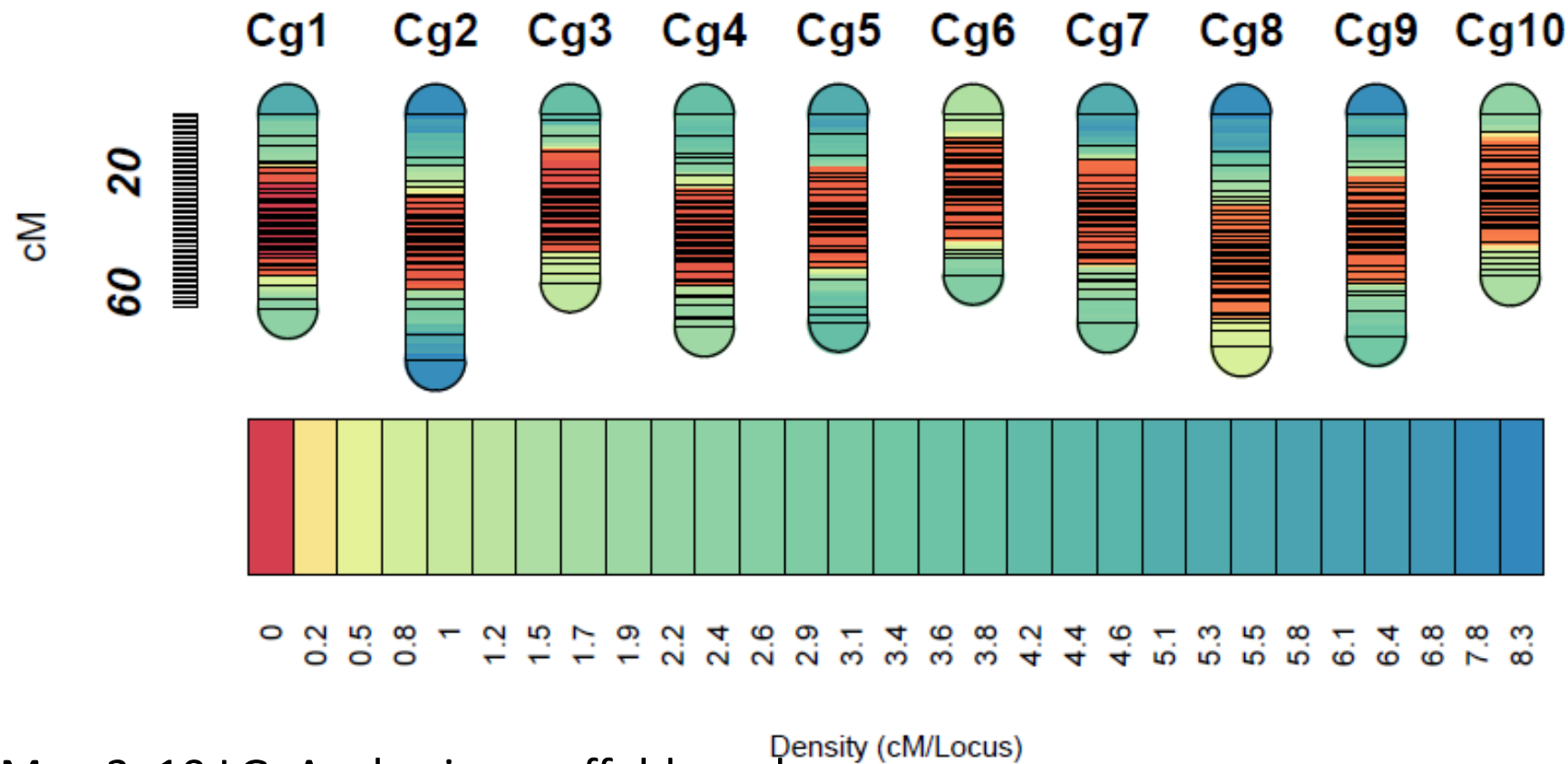
Reads from C. gigas (~80%)

Reads from Unknowns (~19%)



Moribunds oyster are full of OsHV1 DNA copies.
The mean coverage is 12 X of GP and P and 5 X for the offsprings as expected

High-density genetic maps



LepMap 3, 10 LG, Anchoring scaffold on chromosomes

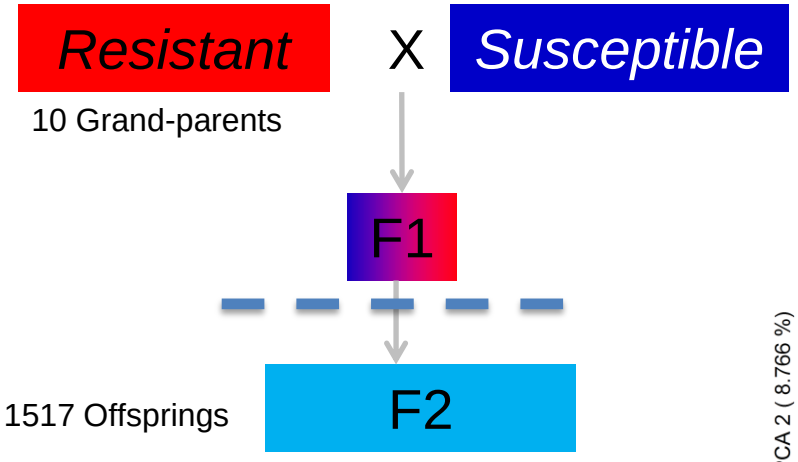
High density genetic maps

LG	N	AverageMap	MaleMap	FemaleMap
Cg01	243,721	64.88	76.67	76.25
Cg02	158,759	82.16	81.63	102.19
Cg03	211,038	56.10	66.41	72.15
Cg04	167,746	70.94	83.70	75.91
Cg05	104,755	69.21	68.22	68.45
Cg06	89,547	53.61	60.08	67.35
Cg07	93,939	69.59	86.82	56.15
Cg08	28,708	77.54	76.04	93.45
Cg09	44,915	73.97	93.55	67.10
Cg10	42,568	53.99	59.89	77.60
Total	**1,185,696**	**671.99**	**753.01**	**756.6**

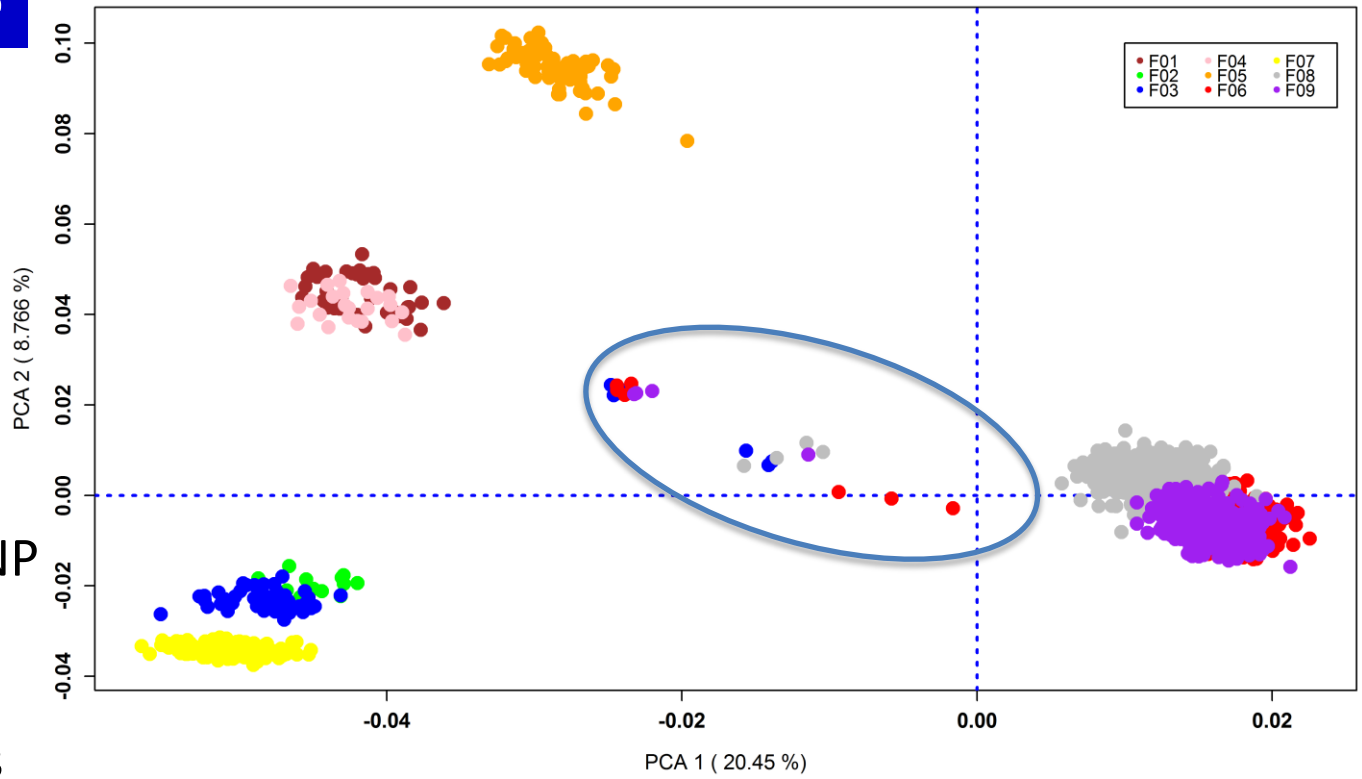
- Linkage maps were constructed using Lep-MAP v 3.0 (Rastas *et al.* 2017)
- 10 linkage groups equal to the number of chromosomes of this species
- ~1.18 million markers in 10 linkage groups
- Average map length 672 cM.

Across all contigs we observed a poor correlation between physical order and the genetical order (r ~ 0.23). More work is needed to compare the collinearity with previous genetic maps.

Experimental design and 56K Genotyping (Gutierrez array)



After filtration (MAF > 0.01, CR >0.90 HWE < 1×10^{-5})



40 625 => ~ 15 000 informative SNP
Much of the individuals are well clustered by family as expected.
All individuals in-between clusters (contaminations) were removed

GWAS and Variance Component estimations

- Linear mixed animal model (DEAD/ALIVE—binary phenotype)

$$\boldsymbol{y} = \mu + \boldsymbol{Zu} + \boldsymbol{e}$$

$$\boldsymbol{y} = \mu + \boldsymbol{pca}^{\boldsymbol{top10}} + \boldsymbol{Ma} + \boldsymbol{Zu} + \boldsymbol{e}$$

M = marker genotypes as covariate
a = allele susbtition effect

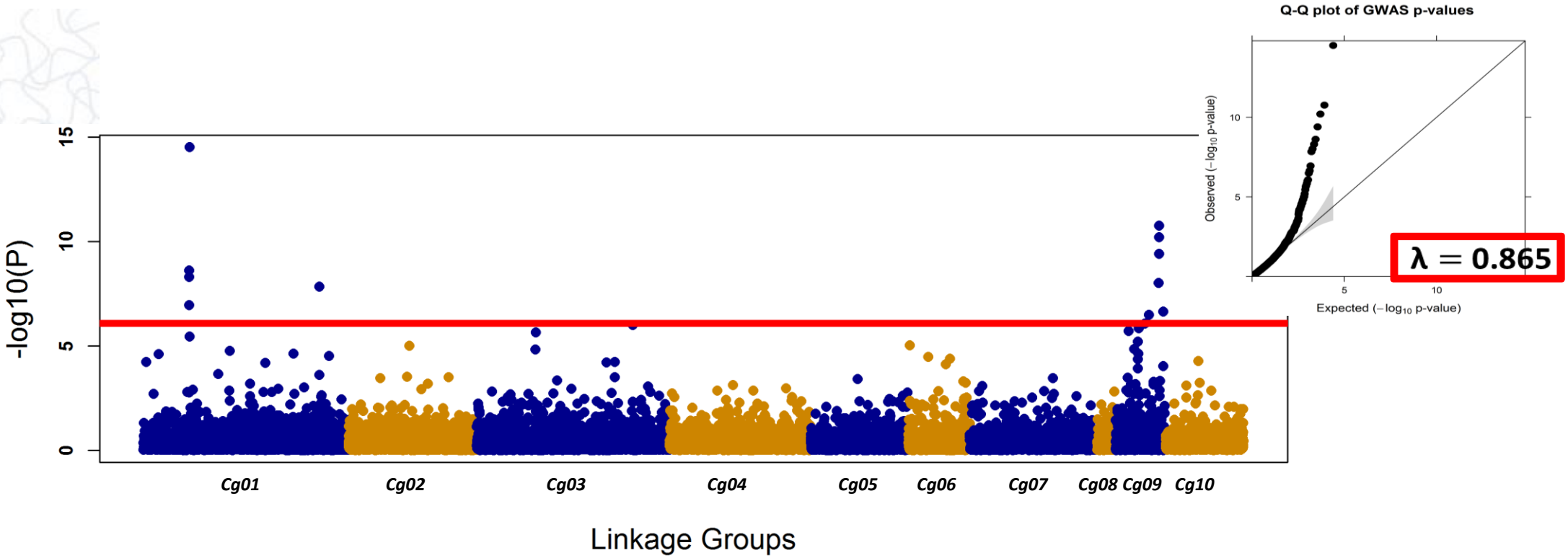
- GCTA v2, was used for GWAS and estimation of variance components
- *GWAS Results are presented as qqplots and mahanttan plots*

Variances component and Heritability Estimates

- Vg(Pedigree) = 0.06 (0.04) Vg(GRM) = 0.21 (0.03)
- Ve(Pedigree) = 0.21 (0.02) Ve(GRM) = 0.12 (0.005)

Model	Liability Scale	Observed Scale
Pedigree	0.77 (0.30)	0.23 (0.14)
Genomic	0.97 (0.05)	0.63 (0.03)

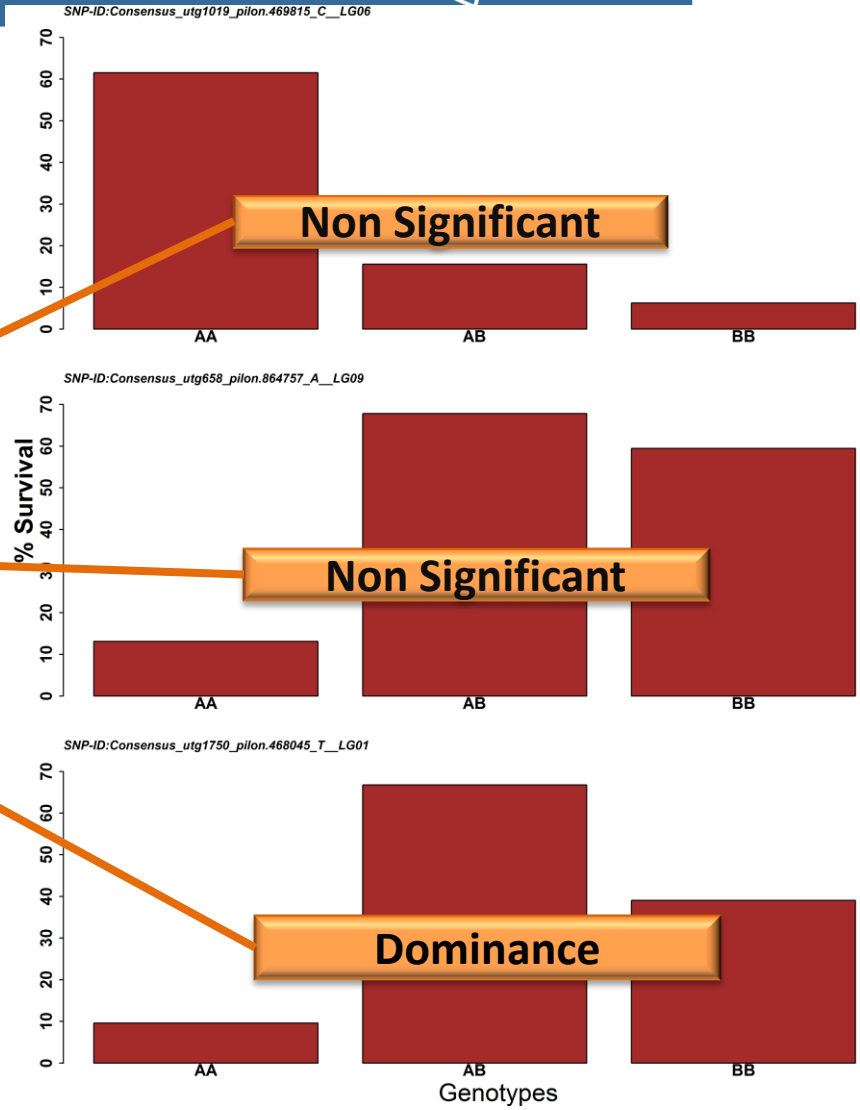
Manhattan Plot of P value across the genome



Survival distribution across genotypes and effects

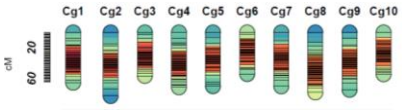
Markers	Additive*	D. D. (P-value)
SNP at LG06	0.107	-0.059 (0.080)
SNP at LG09	0.094	0.113 (0.021)
SNP at LG01	0.171	0.154 (<0.001)

*P < 0.001

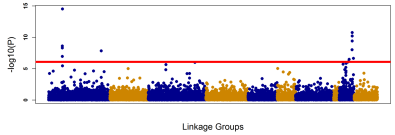


Take home messages about Genomic Resistance to OsHV1

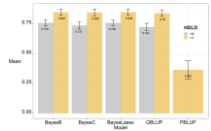
- Re-sequencing resulted in discovery of **1.68 million quality SNP markers**
- **~1.18 million markers in 10 linkage groups,** which are equal to the number of chromosomes for this species



- Estimated heritability for resistance against OsHV1 was 0.23 and 0.62 (observed scale) with pedigree vs. genomic information respectively
- Two significant and one putative QTL were detected for resistance against OsHV1



- This experimental population could be used as the proof of concept of Genomic prediction using genomic information



More general thoughs and perspectives

Given previous GWAS and our results, we need to explore alternative formulations for genetic architecture of the trait (epistasis.

Our high-density genetic map reveals errors in our home-made long-reads assembly (PacBio-ONT).

The efficiency of genotyping array (informative SNP at the end) is still quite disappointing (1/2 of usable SNPs) and they are not evenly spaced in the oyster genome.

A scientific proof of concept is not necessarily viable from an economical point of view

Preventing and mitigating farmed bivalve diseases

This project has received funding from the European Union's Horizon 2020 Research and innovation programme under grant agreement N° 678589

Questions, remarks...

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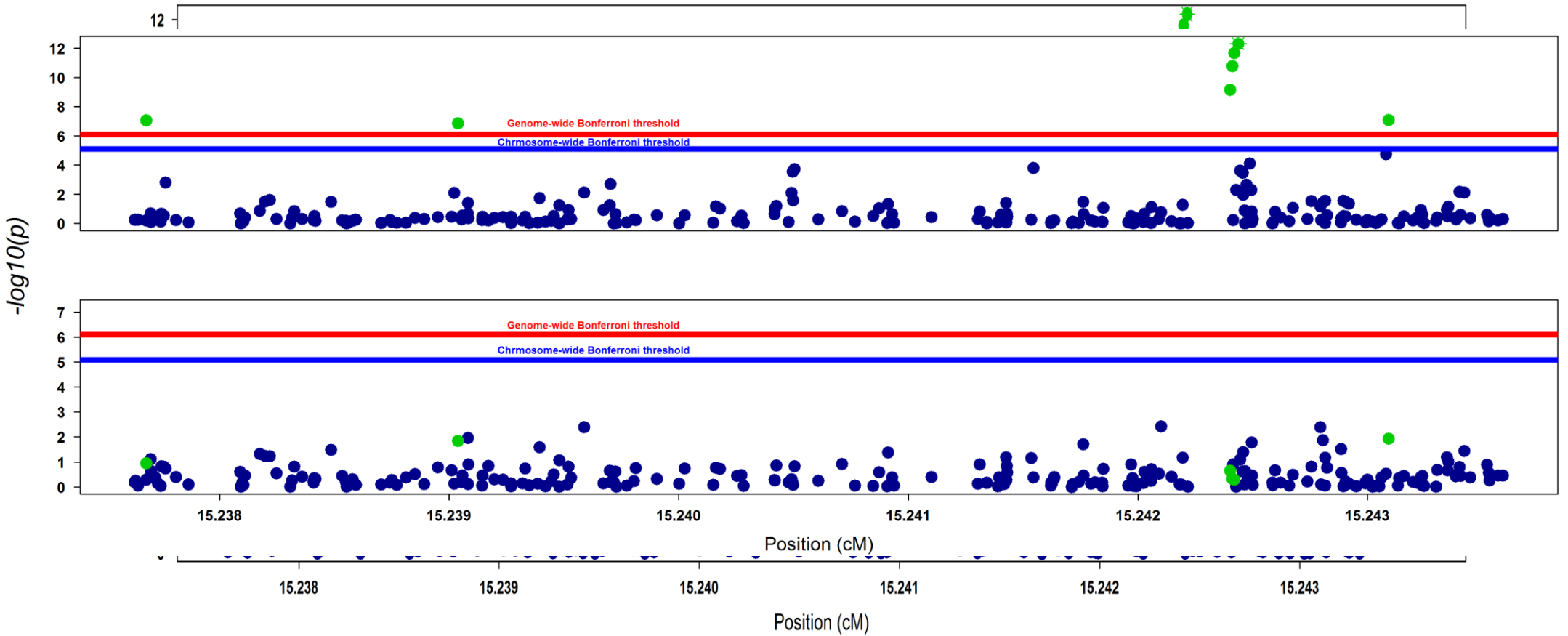
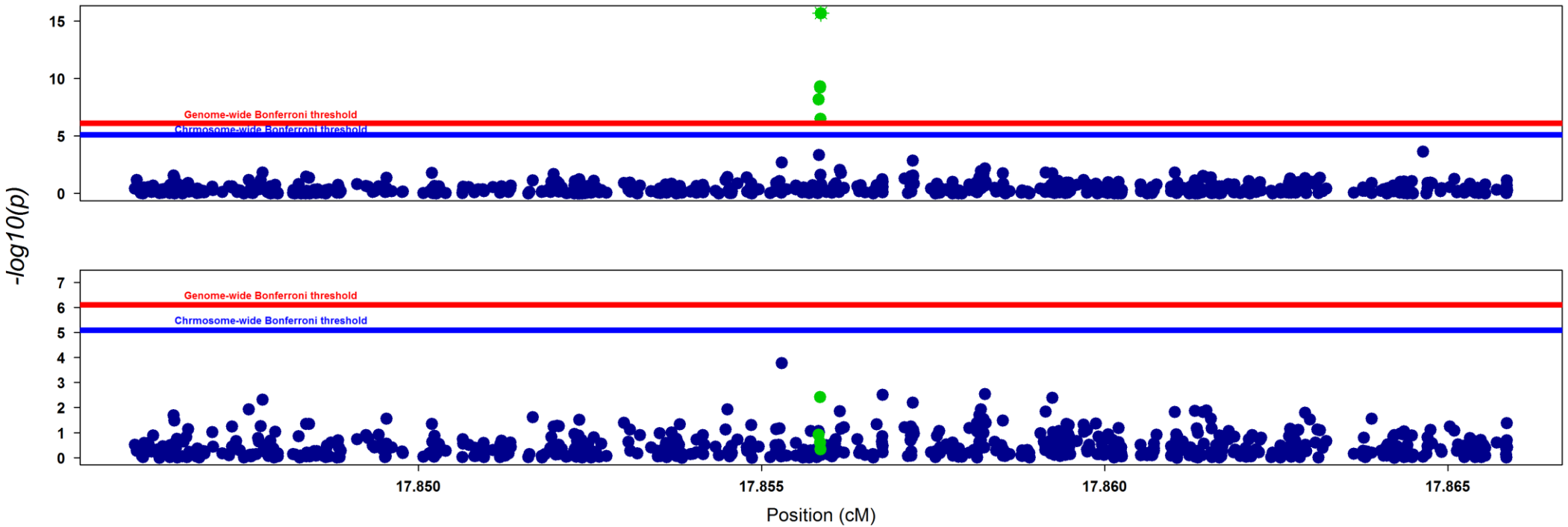
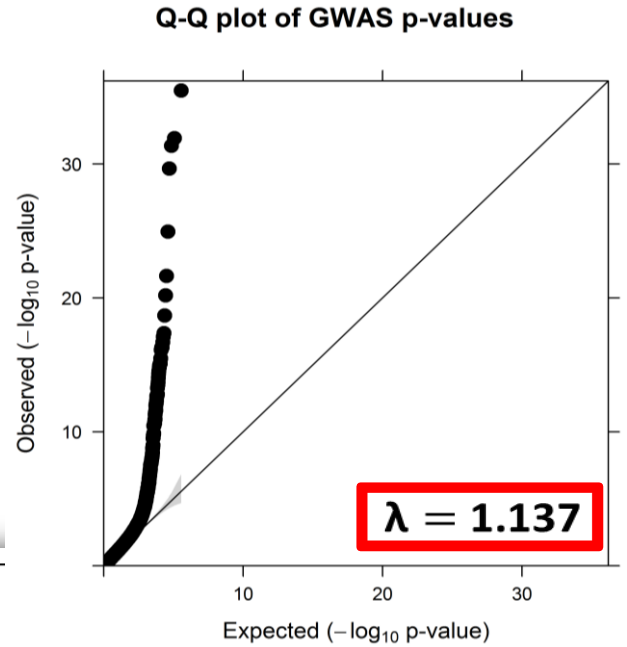
LG09

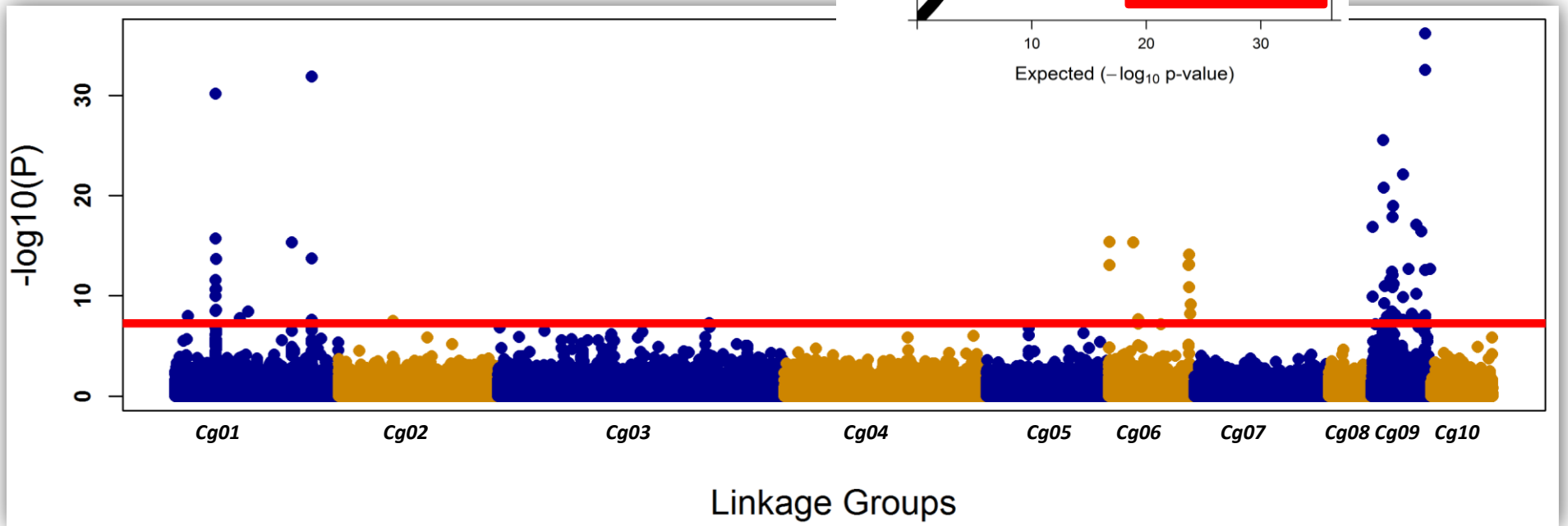

12
12
10
8
6
4
2
0
Genome-wide Bonferroni threshold
Chrmosome-wide Bonferroni threshold
-log10(p)
7
6
5
4
3
2
1
0
Genome-wide Bonferroni threshold
Chrmosome-wide Bonferroni threshold
15.238
15.239
15.240
15.241
15.242
15.243
Position (cM)
15.238
15.239
15.240
15.241
15.242
15.243
Position (cM)

LG01

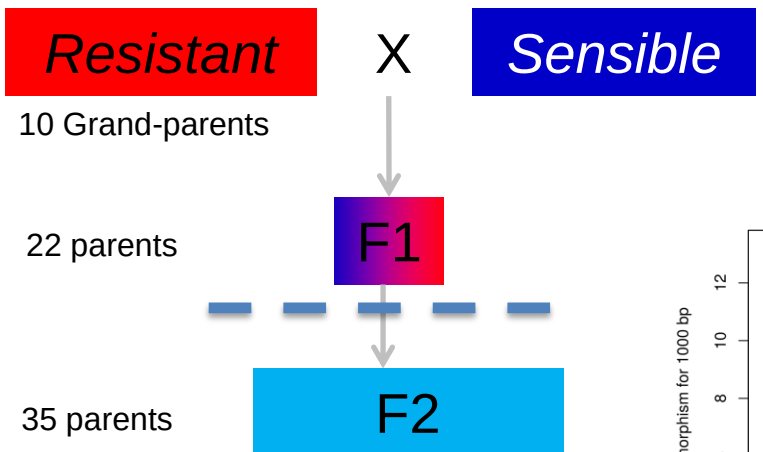

15
10
5
0
Genome-wide Bonferroni threshold
Chrmosome-wide Bonferroni threshold
7
6
5
4
3
2
1
0
Genome-wide Bonferroni threshold
Chrmosome-wide Bonferroni threshold
-log10(p)
17.850
17.855
17.860
17.865
Position (cM)

Manhattan Plot

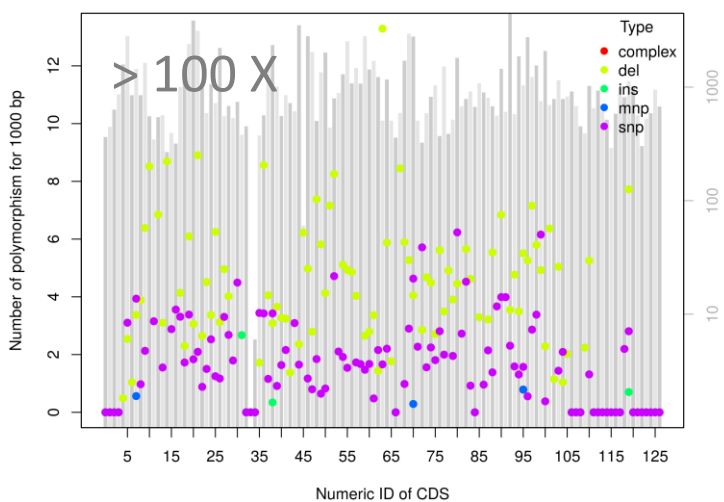




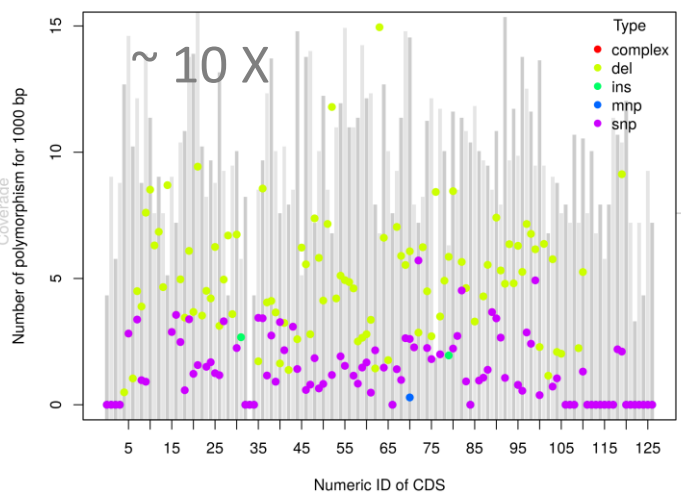
Experimental design and Whole Genome Sequencing



Moribund from VIV58



Survivors from VIV58



Some survivors (with active growth) harbours a non negligible amount of viral DNA.

Camille Pelletier explore DNA exchange between Host and Virus in a poster

LABOGENA
DNA Experts

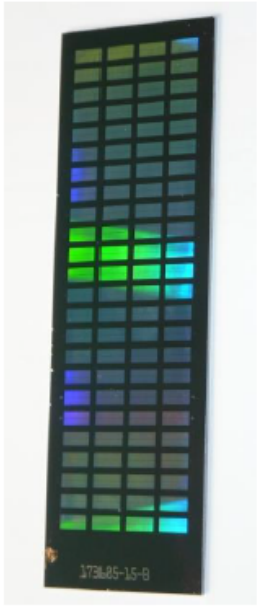
LABOGENA: genomic solutions for fish selective breeding

CONTACT

An efficient SNP panel for Pacific Oyster

LABOGENA
DNA Experts

- 384 markers on new high throughput Illumina XT chemistry
- Including OsHV-1 resistance markers
- Assignments made with AccurAssign Labogena software
  - Taking into account mating plans
  - Using both likelihood and exclusion
  - Ranking of parents



96samples DNA chip Illumina

ng from
2020
amme
'8589

An efficient SNP panel for Pacific Oyster

LABOGENA DNA Experts

	marinove	Novostrea Bretagne	Satmar Naturellement...	France Naissain
Assigned to 1 couple	**466**	**514**	**577**	**408**
Assigned to many couples	12	0	0	0
Not assigned	12	47	23	6
Inexploitable	31	35	12	152
Useful assignment rate	89%	86%	94%	72%

Results from GenOyster Project, obtained with GigADN Project markers

SYSAAF Ifremer FranceAgriMer

An efficient SNP panel for Pacific Oyster

LABOGENA DNA Experts

WAGENINGEN UNIVERSITY & RESEARCH

Assigned to 1 couple	522	1098
Assigned to many couples	2	3
Not assigned	49	55
Inexploitable	225	53
Useful assignment rate	65%	91%

Results from Vivaldi Project, obtained with GigADN Project markers

eceived funding from
ion's Horizon 2020
ovation programme
ement N° 678589

CONTACT

More than 13 000 oysters analyzed already !

A new SNP panel for Manilla Clams

LABOGENA
DNA Experts

- Two DNA extraction protocols validated from gills or coat
- 250 markers on high throughput Illumina XT chemistry

VIVALDI
Preventing and mi… bivalve diseases

Satmar
Naturellement…

	Marenne s 1	Marenne s 2
Assigned to one couple	**69**	**78**	**604**
Assigned to many couples	0	2	3
Not assigned	9	15	339
Inexploitable	1	1	70
Usefull asisgnment rate	87%	81%	64%

96samples DNA chip Illumina

Sampling problems did not allow us to get correct results for Padova samples and extrapalial fluids

CONTACT