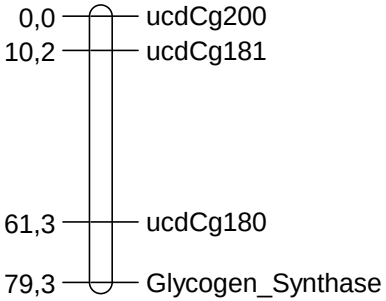
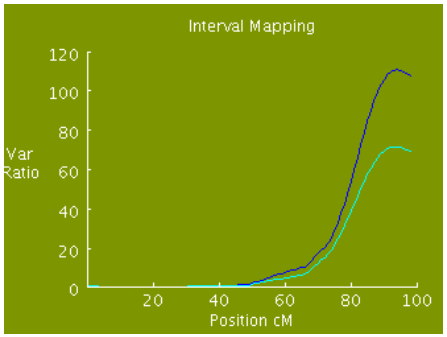
# Mapping QTL for Resistance to Summer Mortality in the Pacific Oyster *Crassostrea gigas*

**Christopher Sauvage[1,*], Serge Heurtebise[1], DJ De Koning[2], Chris S. Haley[2], Pierre Boudry[1,3] and Sylvie Lapègue[1]**




1-Ifremer, Avenue Mus de Loup, 17390, La Tremblade, France
2-Roslin Institute, Midlothian EH25 9PS, Roslin, United Kingdom
3- Ifremer Technopole de Brest Iroise, Plouzané, France

# The Pacific oyster culture – a quick overview

- Most cultured species – 4.4Mt for a value > to 3 $billions (FAO, 2005)
- Large geographic and environmental repartition (except artic zones)
- Deliver social benefits

# The mortality phenomenon

Significative mortalities were recorded since the mid 70's (>30%)

Affects spat of oyters (<1yr old) ➔ Economical loss for farmers!

PATHOGENS
Vibrio, Herpes…

ENVIRONMENT
Nutrients, T°C…

GENETICS

Multi-disciplinary research project : MOREST (2001-2006)

# The ‘MOREST’ project

GENETICS

Quantitative studies

Genetic basis of resistance to SM

After 3 generations of Selection, Resistant and Sensitive stocks of oysters to summer mortality

Heritability of Spat Survival during summer:

$h^2=0.70\pm0.35^{***}$

(Degrémont et al., 2005)

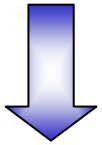
High variance in Spat Survival rates

(Ernande et al., 2004)

⇒ Opens the way to selection: MAS

# Our objectives in the EU project ‘Aquafirst’

1. Produce F1 R/S hybrid & F2 biological material
2. To challenge F2 families during the summer
3. Development of molecular markers



4. QTL detection in the Pacific oyster

# A 3 Generation Experimental Design

2001-04

Divergent Selection of
sensitive and resistant
stocks of oysters
to summer mortality

20 F0 Individuals

2004

10 F1 Individuals

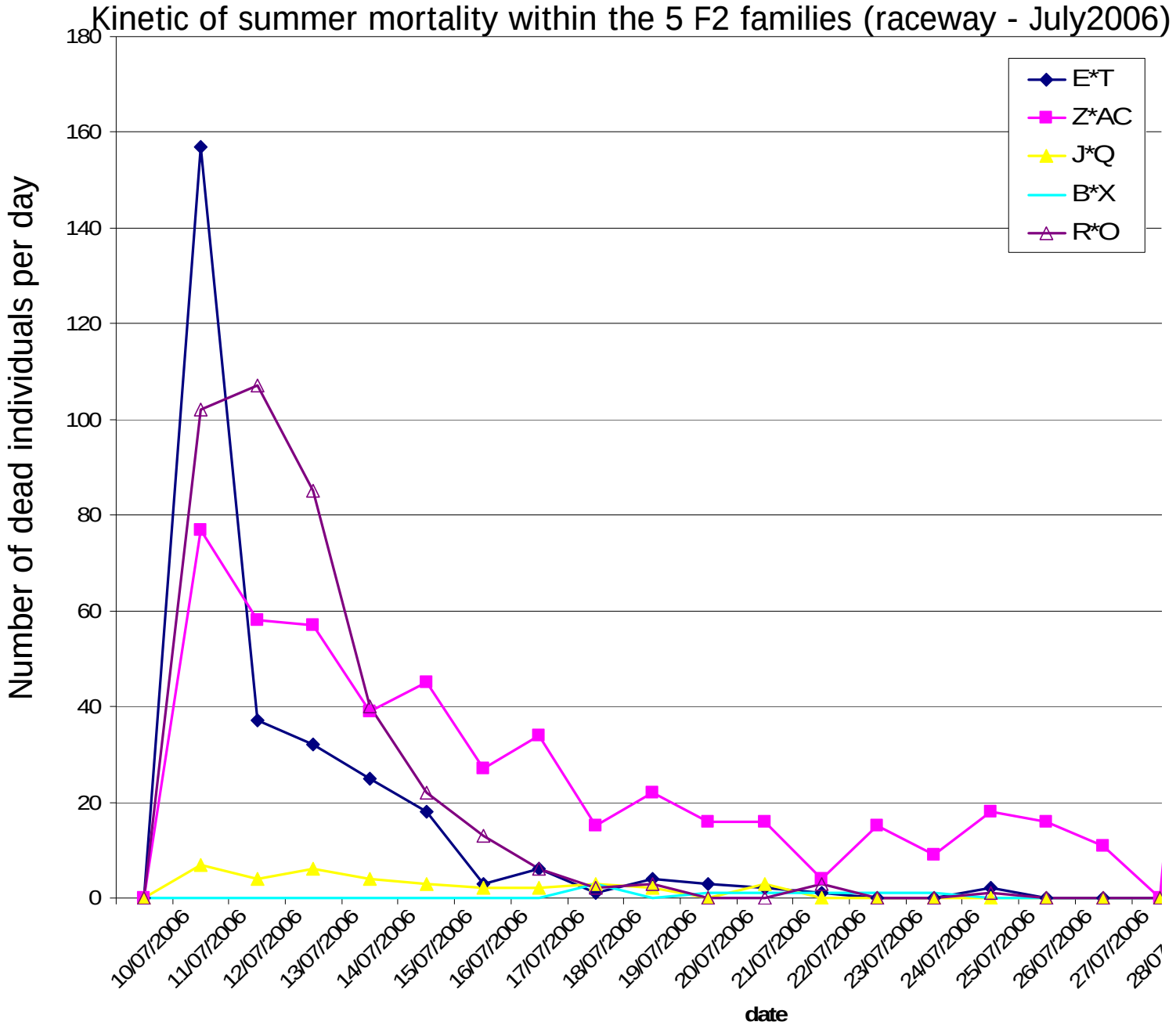
2006

5 F2 families

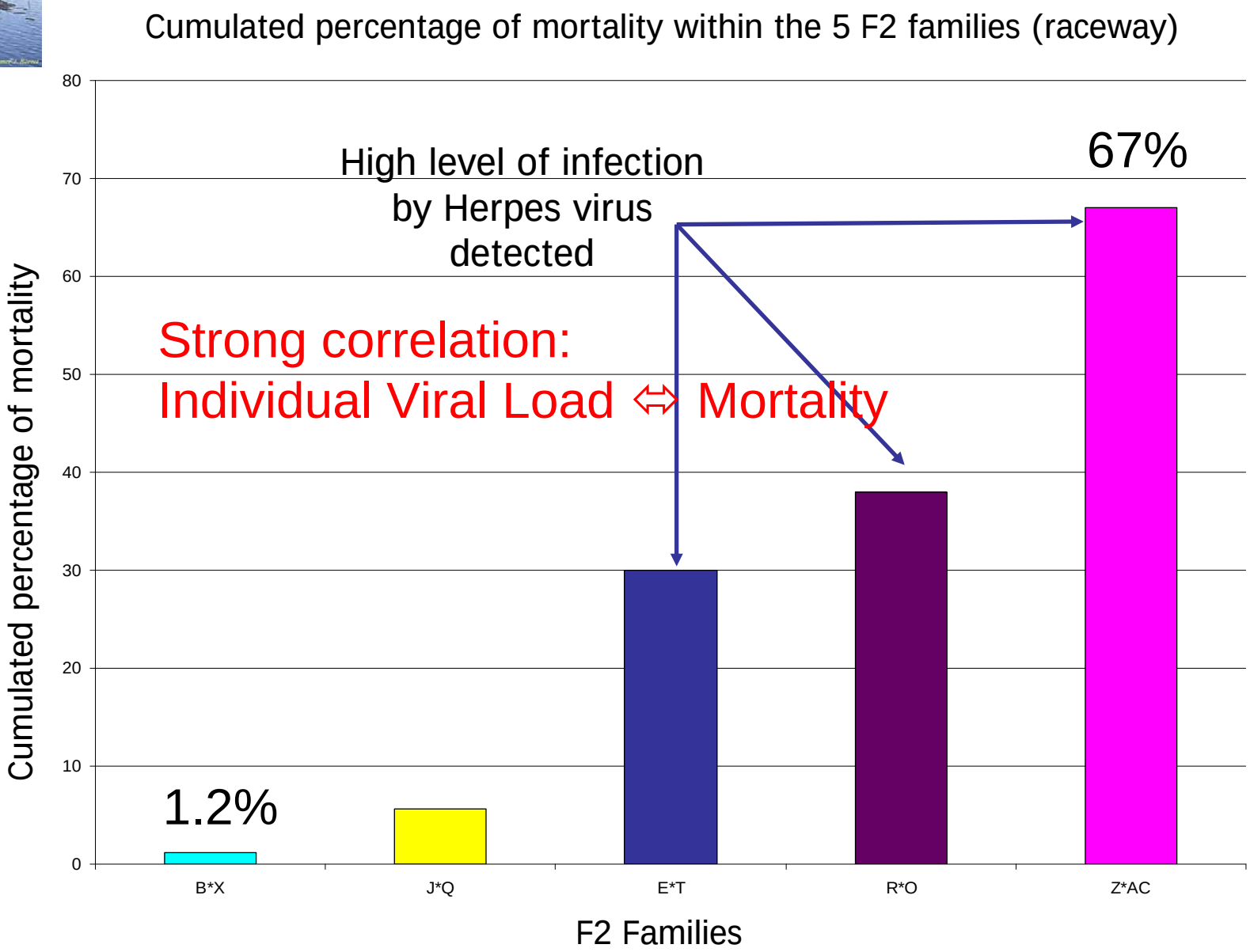
# Phenotyping - Mortality

6 months old oysters

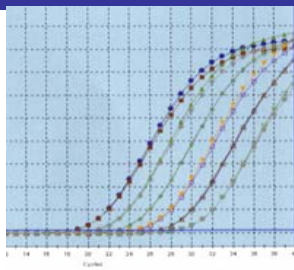
Daily monitoring and Sampling



# Phenotyping - Mortality



# Phenotyping – Individual quantification of OsHV1 load



OSHV1, the Ostreid Herpes Virus type I is known to be involved in mass mortalities events (*Renault et al., 2000*)

Individual viral load was quantified by a newly developed Q-PCR assay (*Pépin et al., submitted*)

Phenotyping was performed on two traits: viral load and survival

# SNP Development

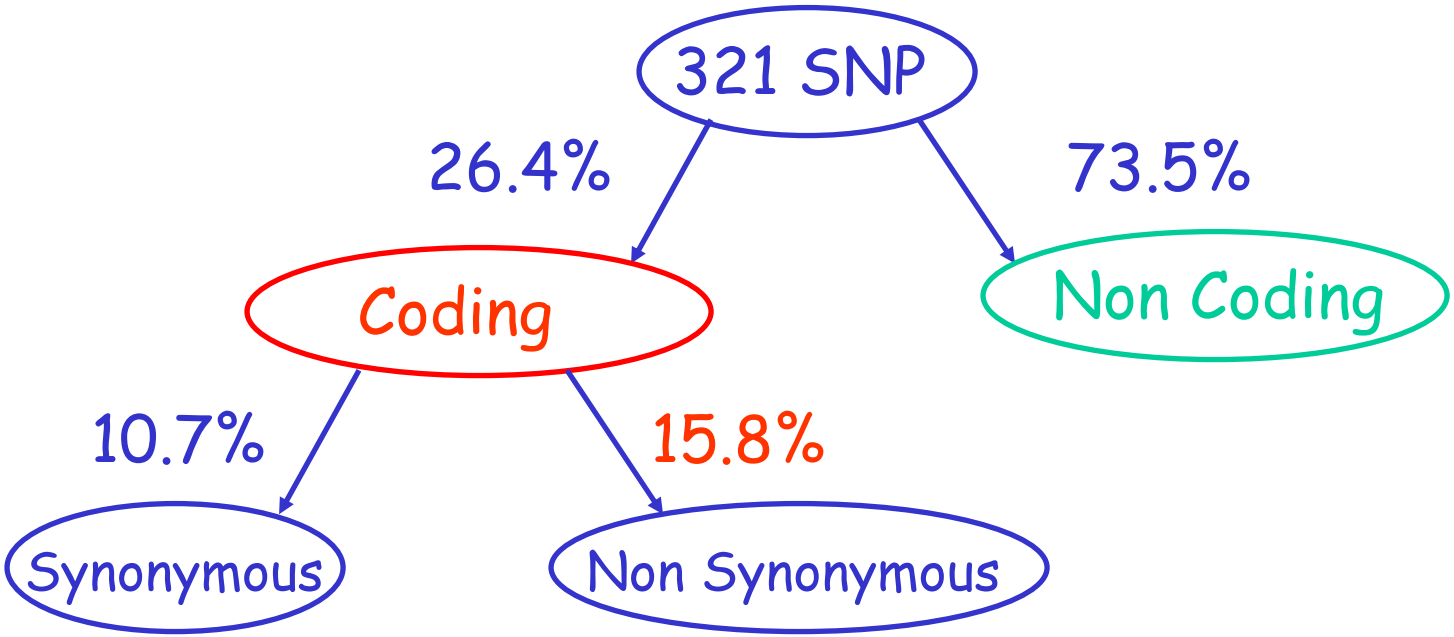
- Direct sequencing of partial sequence in a set of 61 EST of known function in the F0 individuals

- Marker characterization

    - Position
    - C / NC
    - Ts / Tv
    - S / NS

- Informativeness in the F0 & F1 individuals

# SNP Development




- In each EST, 1 SNP was selected according to its informativeness in the F0 & F1 individuals
- Genotyping was performed in the F2 by Good-assay in the MPI

# Molecular Resources

Microsatellites Markers

- 46 from Hubert & Hedgecock (2004)
- 3 from Yu & Li (2007)

*In silico* SSR derived form EST

- 18 from Sauvage et al. (2008, submitted)

SNP markers

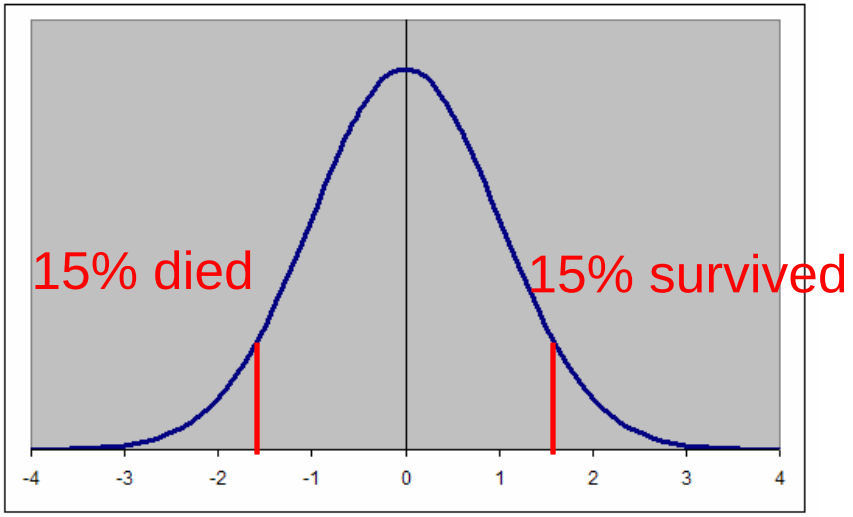
- 47 from Sauvage et al. (2007)

Selective genotyping was performed

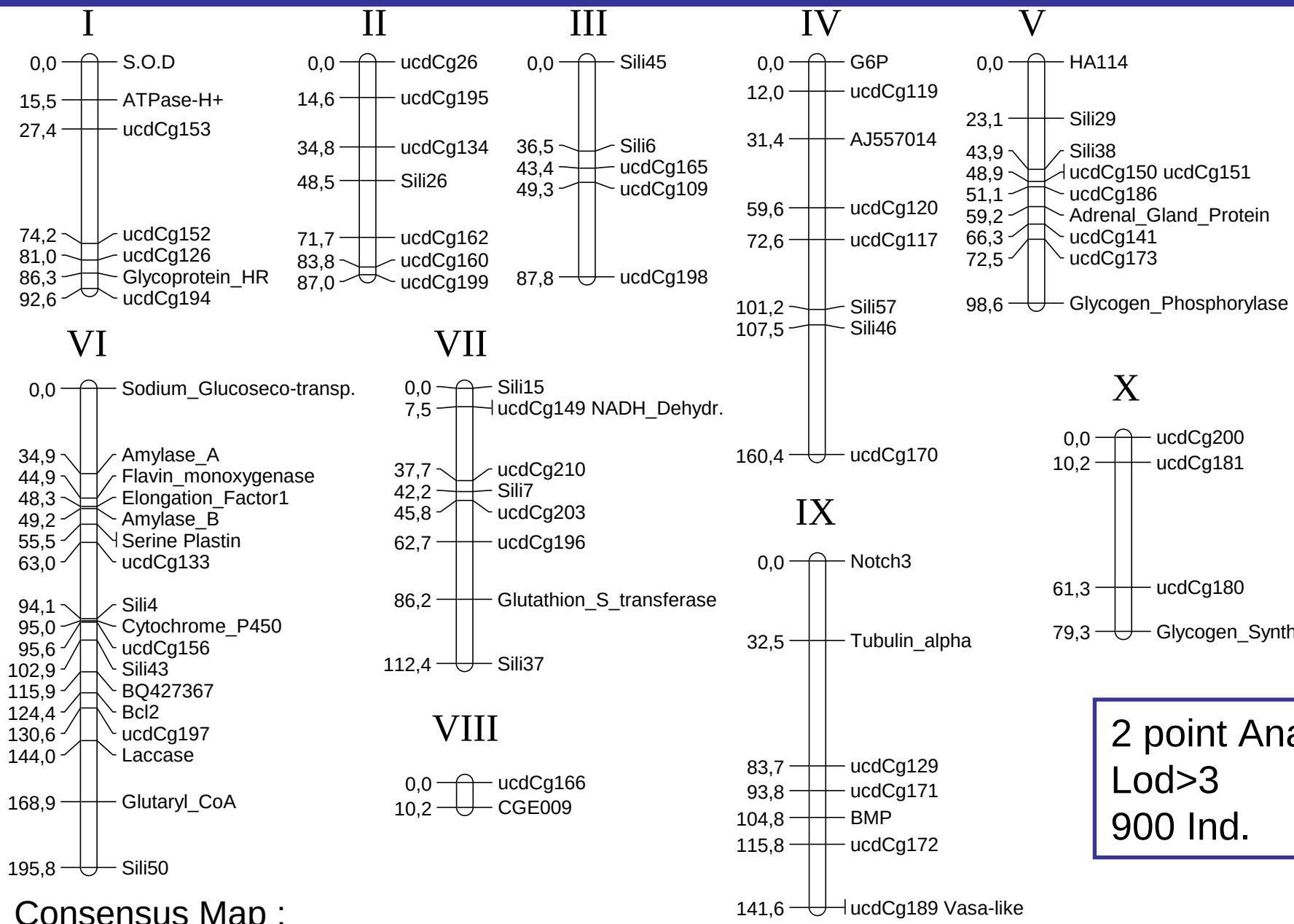
3 F2 segregating families

900 individuals

30 % of the distribution



# Linkage Mapping – Crimap (*Green, 1990*)

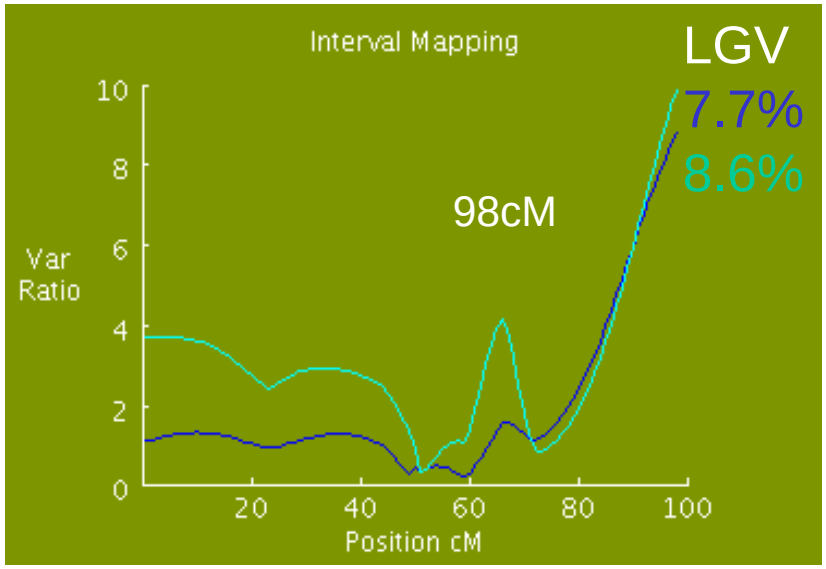


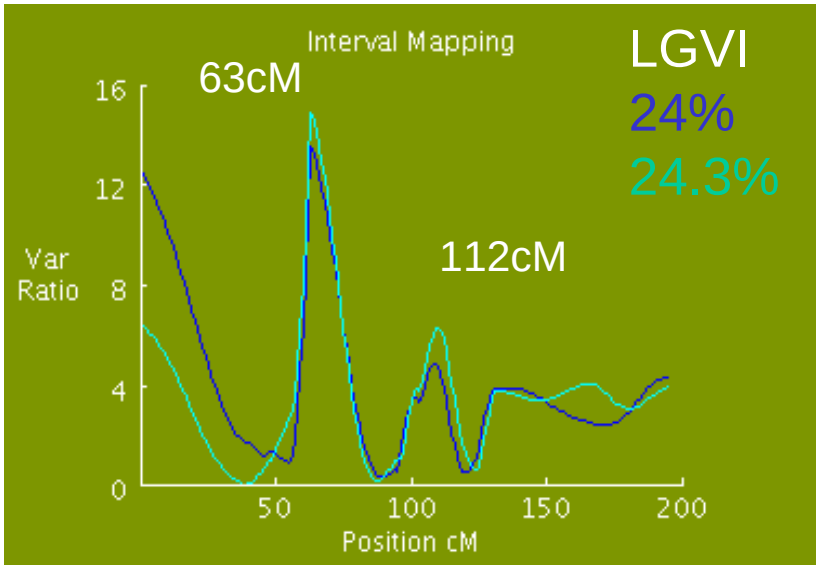

2 point Analysis
Lod>3
900 Ind.

Consensus Map :

10 LG **/** Length 1218cM **/** 82 Markers **/** average spacing of 13.5 cM/ Coverage=93%

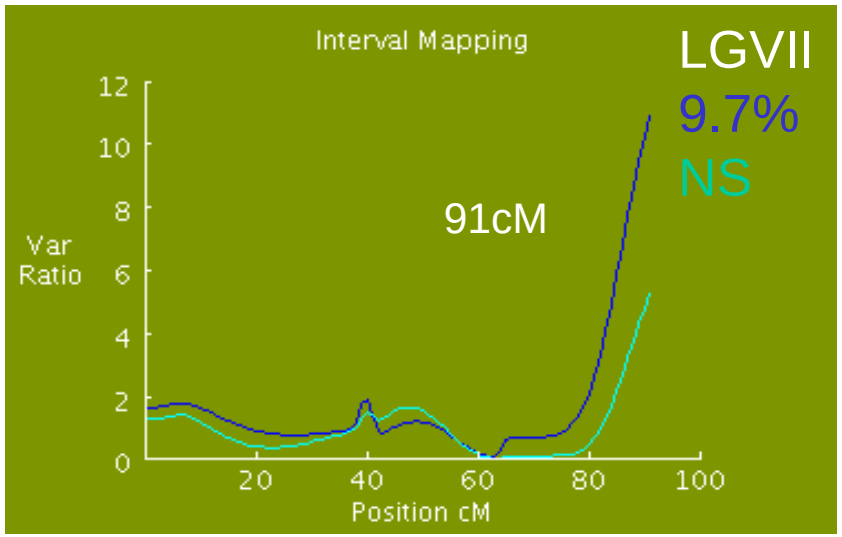
# QTL detection – ‘Consensus’ F2 analysis

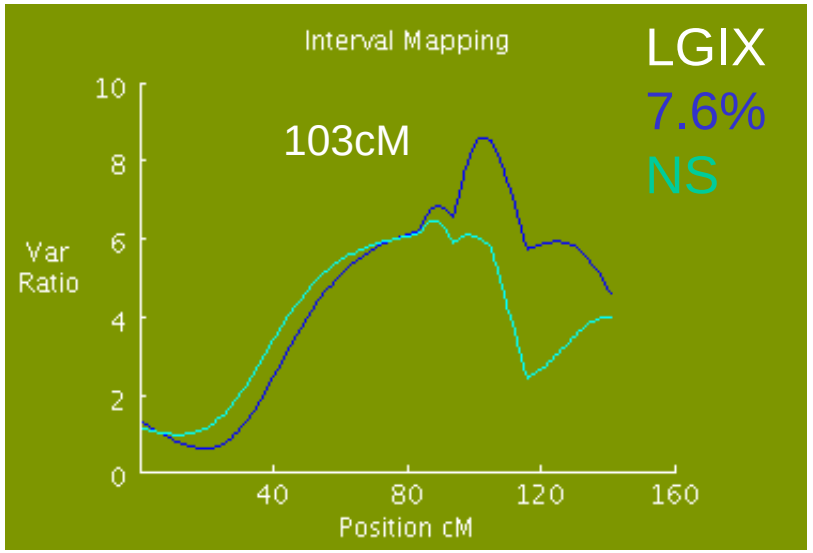


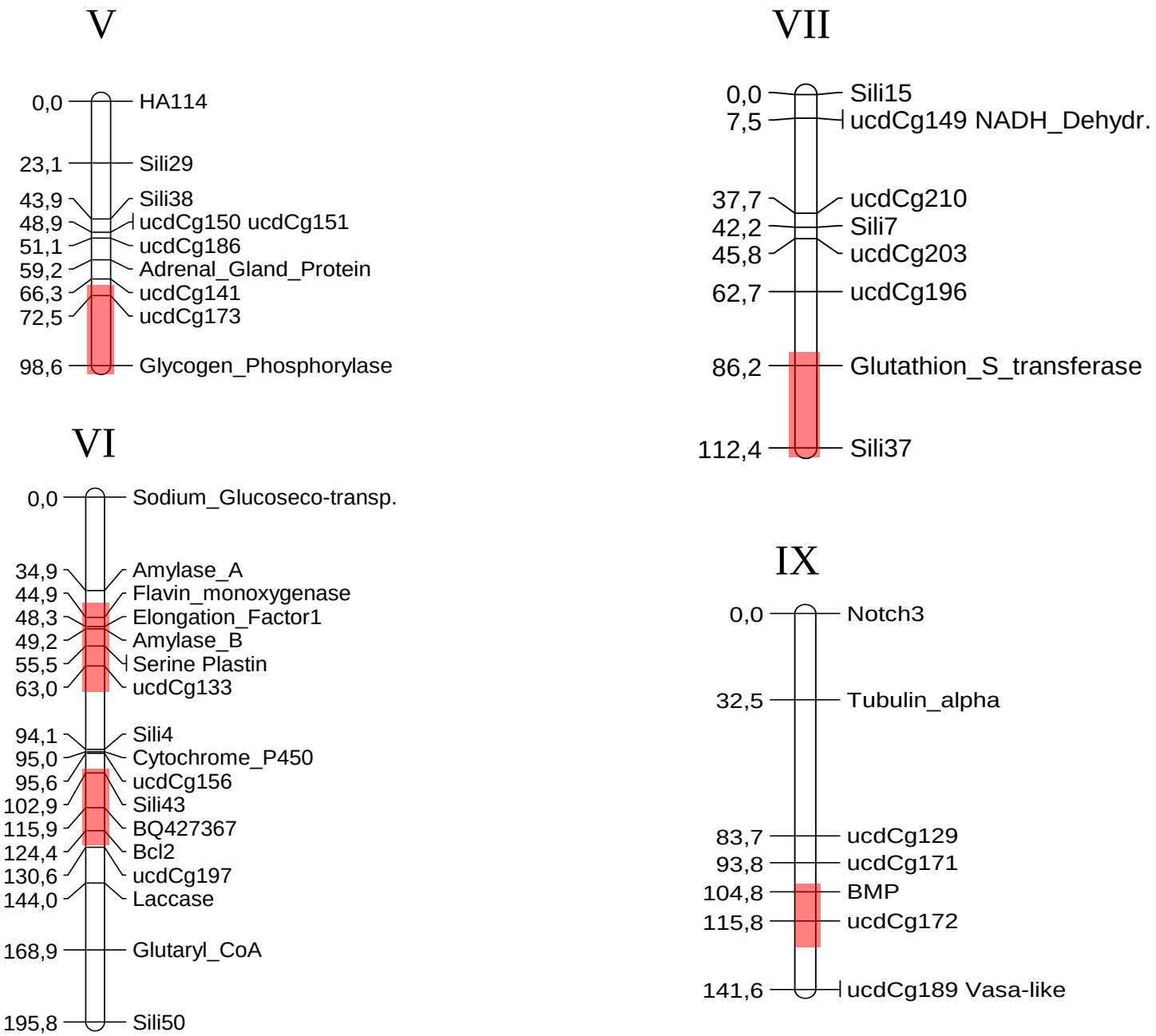

Mortality

Viral Load



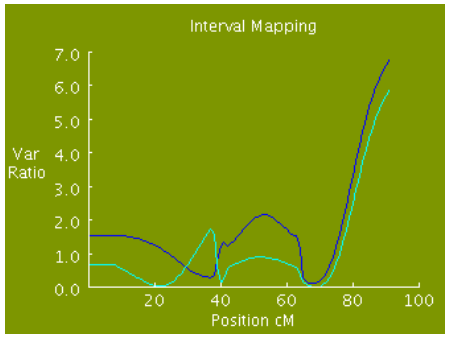



# QTL detection – ‘Consensus’ F2 analysis

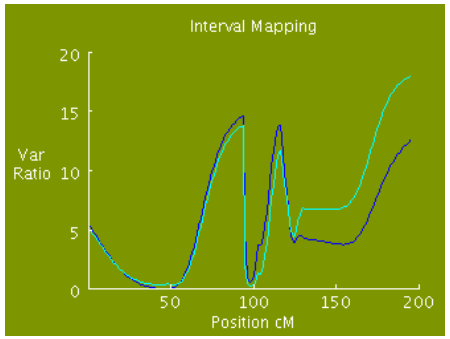


# QTL detection – F2 Single full-Sib analysis

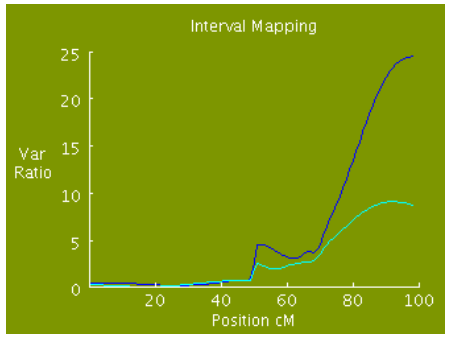
Family1 LGV



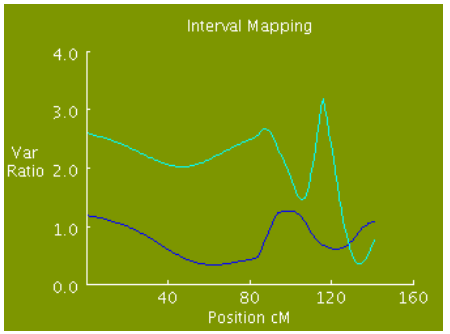

LGVI



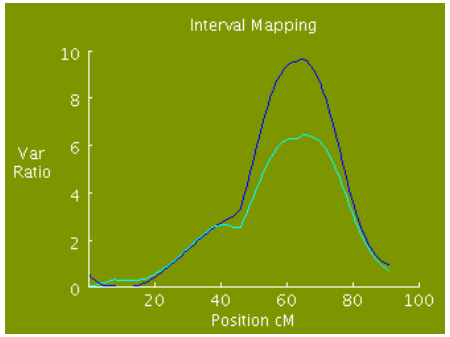

LGVII

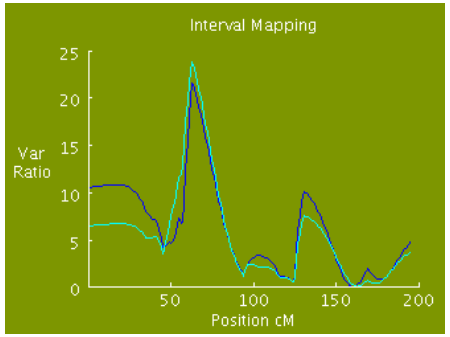


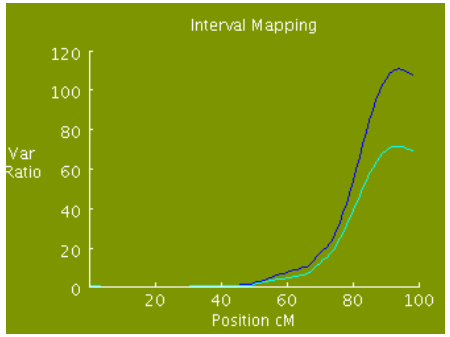

LGIX

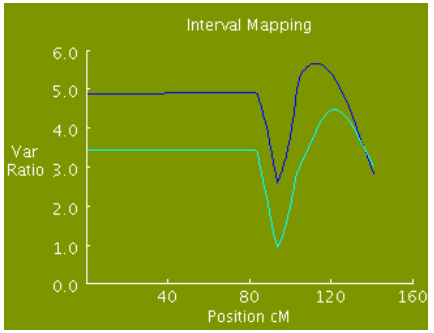



Family 2

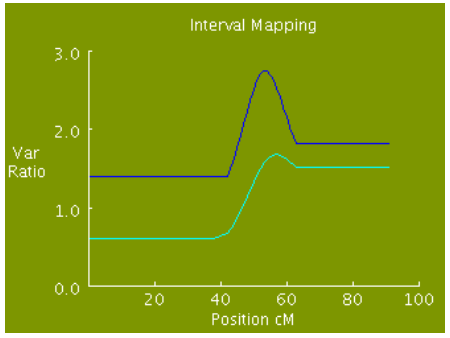


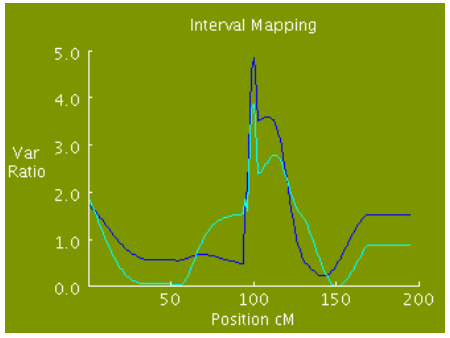



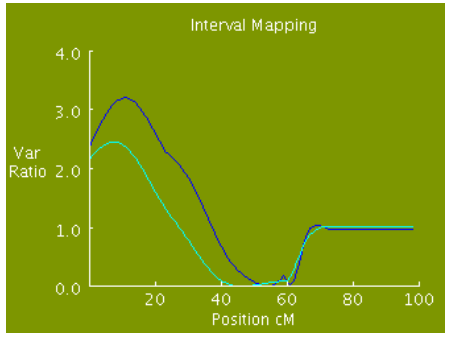



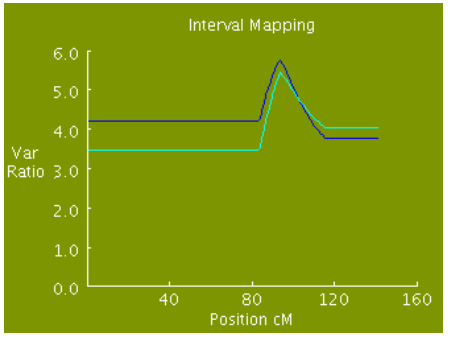




Family 3



# QTL detection – F2 Single full-Sib analysis

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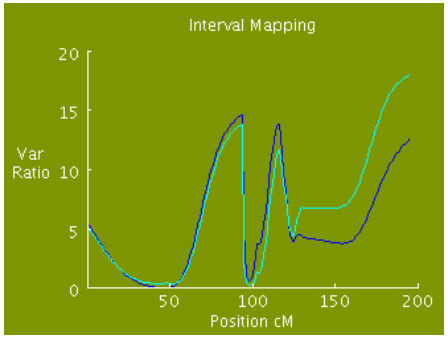
LGVI

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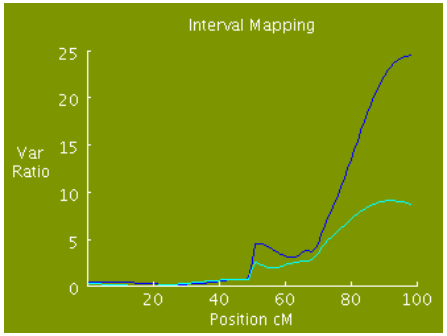
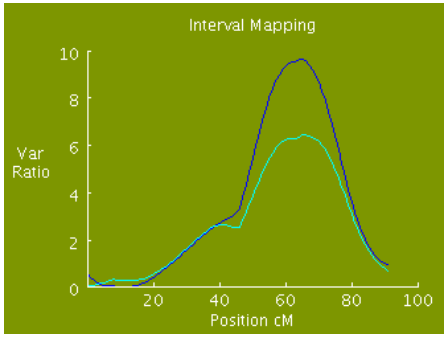
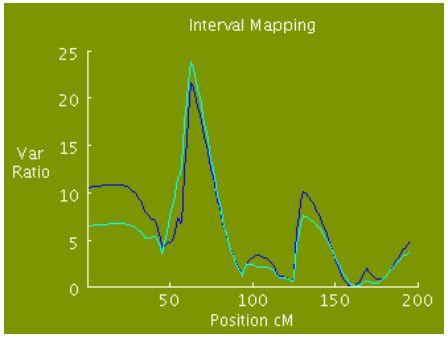


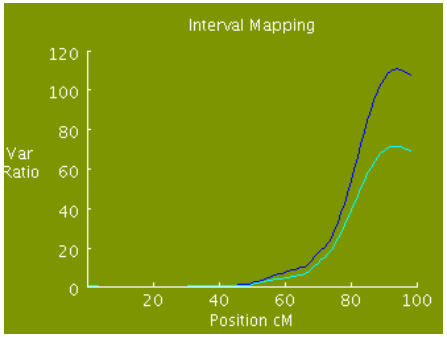

LGIX




Family 2







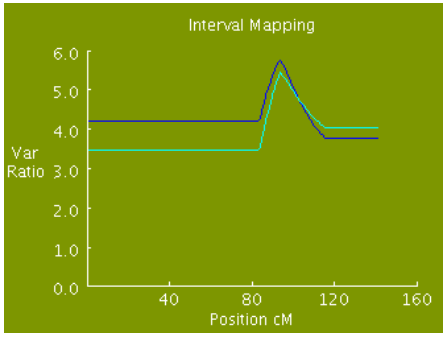
Family 3









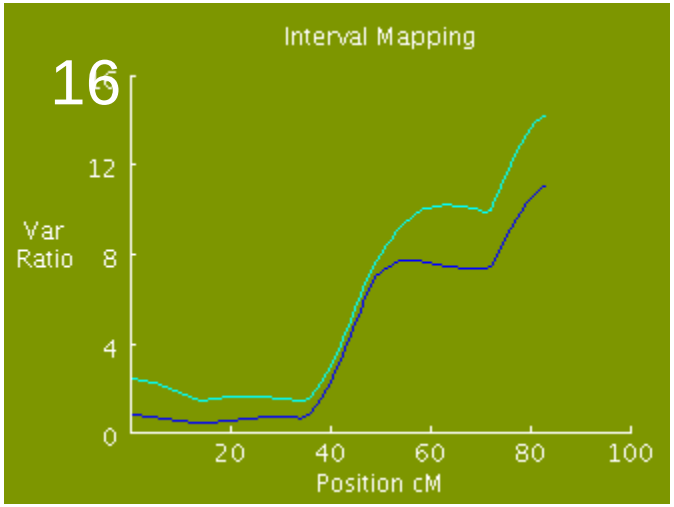



# Fitting QTL effect as a genetic background

To investigate the strengh of the QTL

- One QTL a time (e.g. fixing QTL of LGV)
- Several QTL a time (e.g. fixing QTL effect of LG V and IX)
- All QTL at the same time (e.g fixing QTL effect of the three other LG)

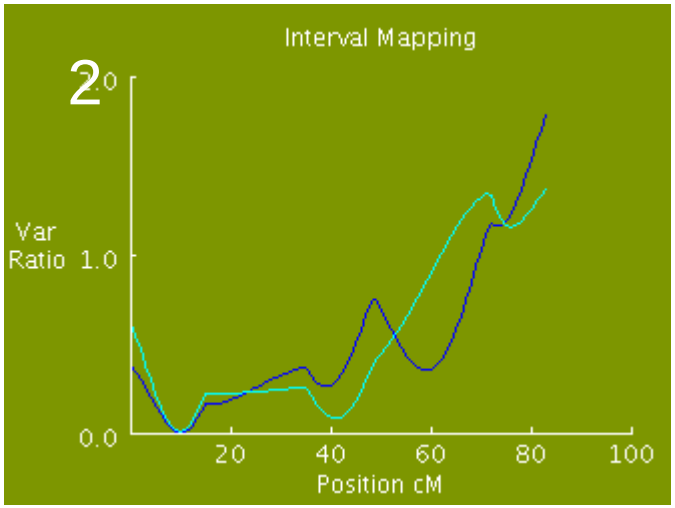
F2 Analysis - LGIV




Lod mortality: 4.666

Lod viral load: 5.939

F2 Analysis in LGIV with all other QTL effect fixed




Lod mortality: 0.771

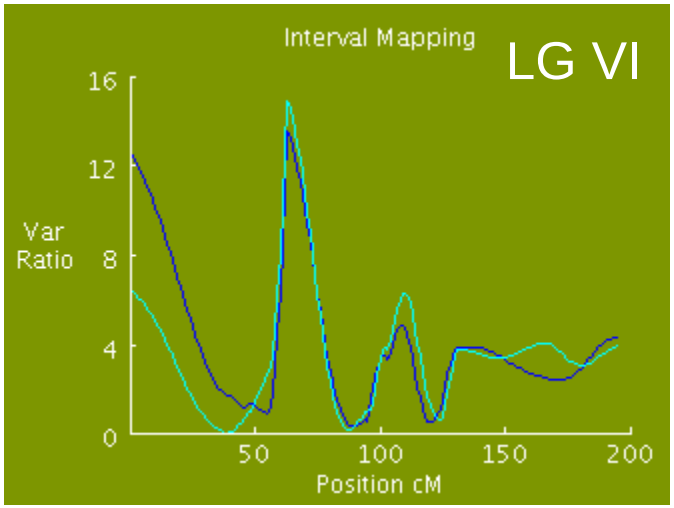
Lod viral load: 0.595

« False positive QTL »

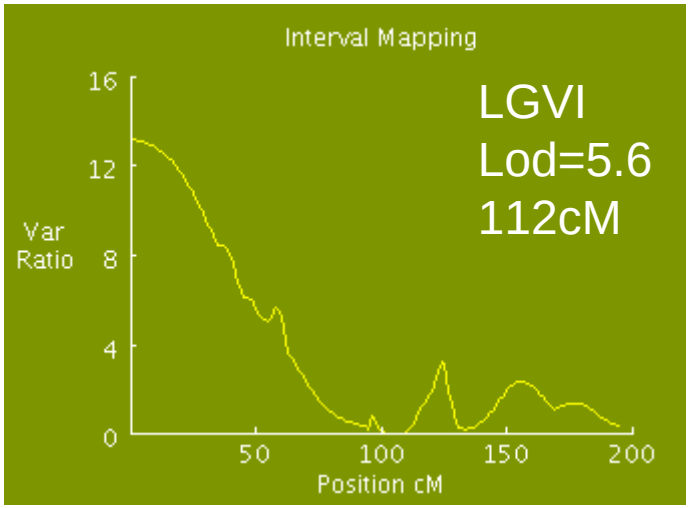
# Fitting one of the phenotype as a cofactor

To investigate the variance in trait

- Fixing Viral load phenotype as cofactor




QTL detection




Not all the variance in the mortality trait is explained by the viral trait

# Sum up of the Results

- First Linkage map that includes type I markers (SNP) in *C.gigas*

- QTL detection was Successful !!!

  - 5 QTLs regions (LG V, VI, VII and IX)
  - robustness of the QTL
  - (too?) large part of the Variance (49%)
  - Differential QTL segregation among F2 families

  - However, relatively large 95% IC in QTLs position (≈40cM)
  - The variance explained appears over inflated
    1. Selective Genotyping
    2. Bias introduced by segregation distorsion

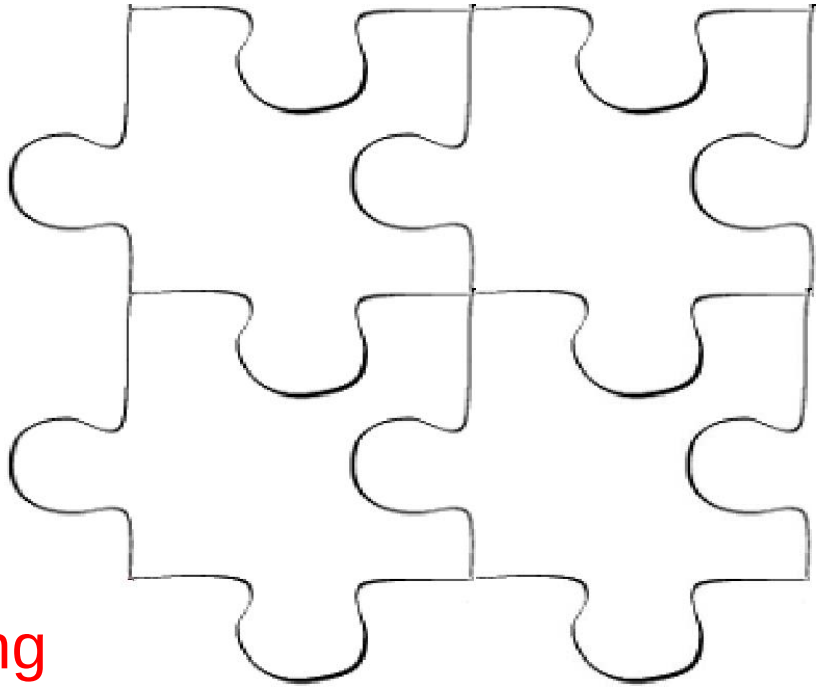
# Next steps in the hunt for QTLs…

1. Investigate the genetic architecture of the two traits

2. Add more markers to get a fine scale map

# Perspectives …

Genetic Basis ($h^2$) of Resistance to SM
(Degrémont, 2005)



Biological Phenomenon of Mortality

Linkage Mapping
&
QTL detection

Differentially Expressed Genes (R vs S)
(Fleury, 2008, On Line)

# Acknowledgement


- Aquafirst EU Project
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