

Markers for resistance to white spot syndrome virus in black tiger shrimp (*P. monodon*) and correspondence to cSNPs in white shrimp (*L. vannamei*)

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White spot syndrome virus disease

- Worldwide problem limiting shrimp aquaculture
- Causes 100% mortality in 7-10 days
- Many epizootic outbreaks in India
- 60% wild caught gravid females infected
- No effective prevention, vaccination or treatment
- Few genetic resources

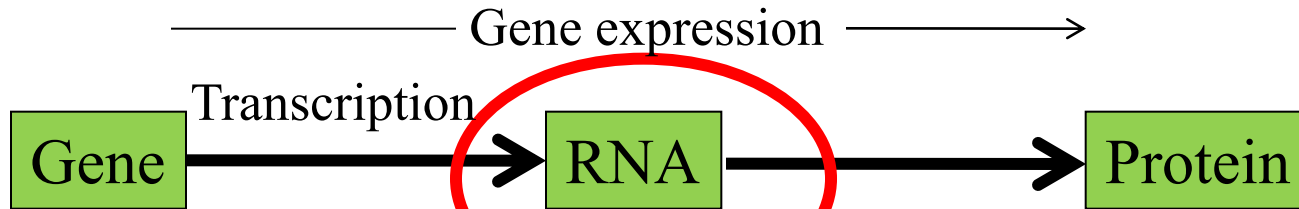
Genes affecting WSSV resistance in shrimp?

- Many genes of small & large effect (quantitative trait)
- Shrimp show a limited adaptive immune response (eg. lack immunoglobulin, T-cell receptor and MHC loci)
- Innate immune system likely to be important

Aim

- Find gene markers in *P. monodon* that can be used to
 - Breed shrimp with high resistance to WSSV
- Improve knowledge of genes affecting resistance to disease in shrimp

Latest sequencing technology to find variation in the genome



Code (sequence)
Copy number (level of expression)

TCCACTTCAAAGGGAAAACAC CACA
TCCACTTCAAAGGGAAAACAC CACAACCTAAAACCA
TCCACTTCAAAGGGAAAACAG CACAACCTAAAACCA
TCCACTTCAAAGGGAAAACAG CACAACCTAAAACCAT
TCCACTTCAAAGGGAAAACAC CACAACCTAAAACCAT
TCCACTTCAAAGGGAAAACAG CACAACCTAAAACCAT
TCCACTTCAAAGGGAAAACAG CACAACCTAAAACCAT
TCCACTTCAAAGGGAAAACAG CACAACCTAAAACCAAT
TCCACTTCAAAGGGAAAACAG CACAACCTAAAACCAT
TCCACTTCAAAGGGAAAACAG CACAACCTAAAACCAT

Single nucleotide
polymorphism
(SNP)

Mapping SNPs associated with WSSV resistance

- Expressed genome of *P. monodon* sequenced
- High density gene linkage map for *P. monodon* (~4000 markers, 44 linkage groups)
- Seven families (~200 full-sibs per family) sexed & challenge tested with WSSV (intramuscular injection)
- 1024 progeny genotyped (most susceptible and resistant 40 percentiles of progeny in terms of hrs survival)

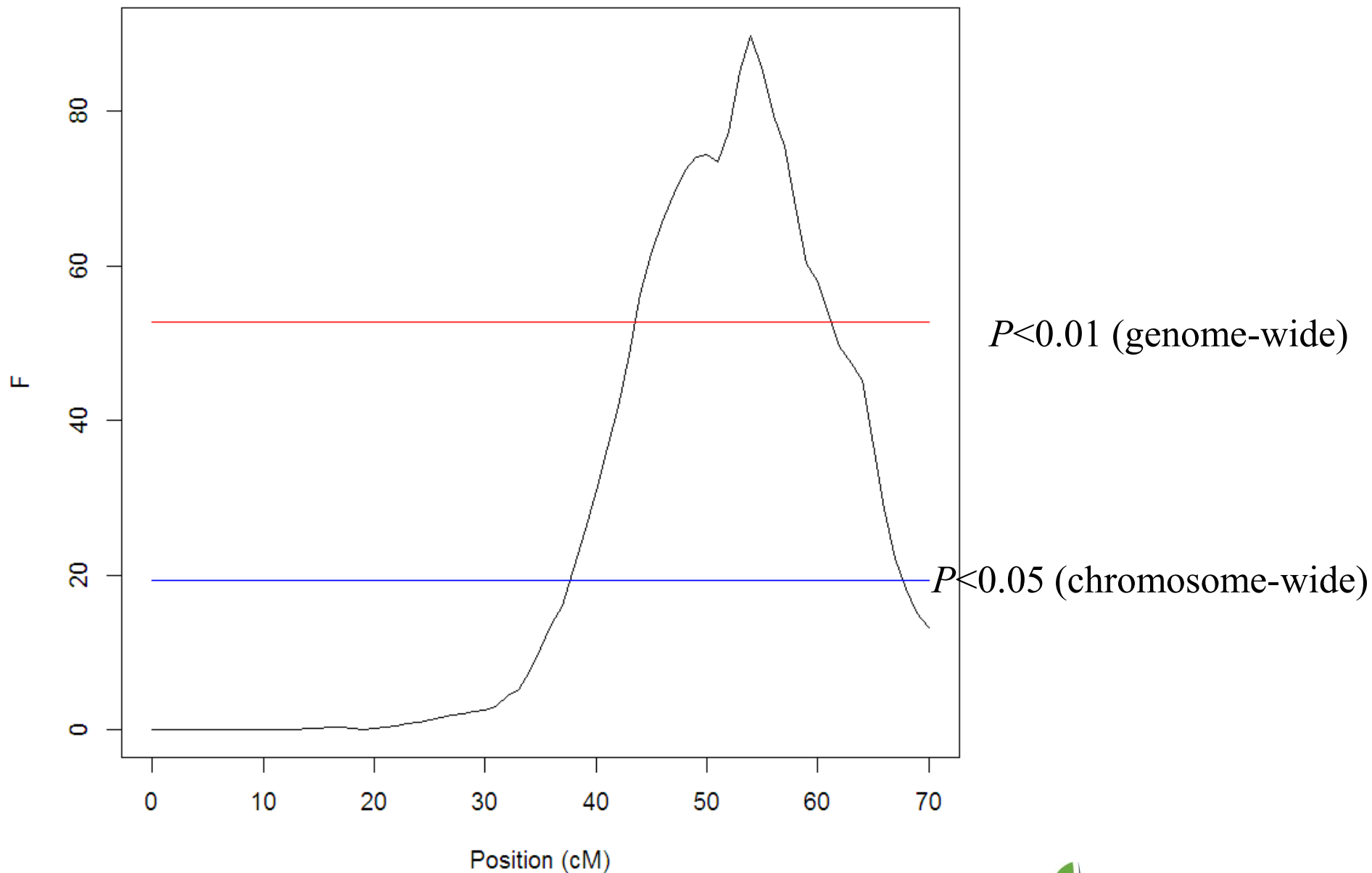
Analysis- WSSV resistance

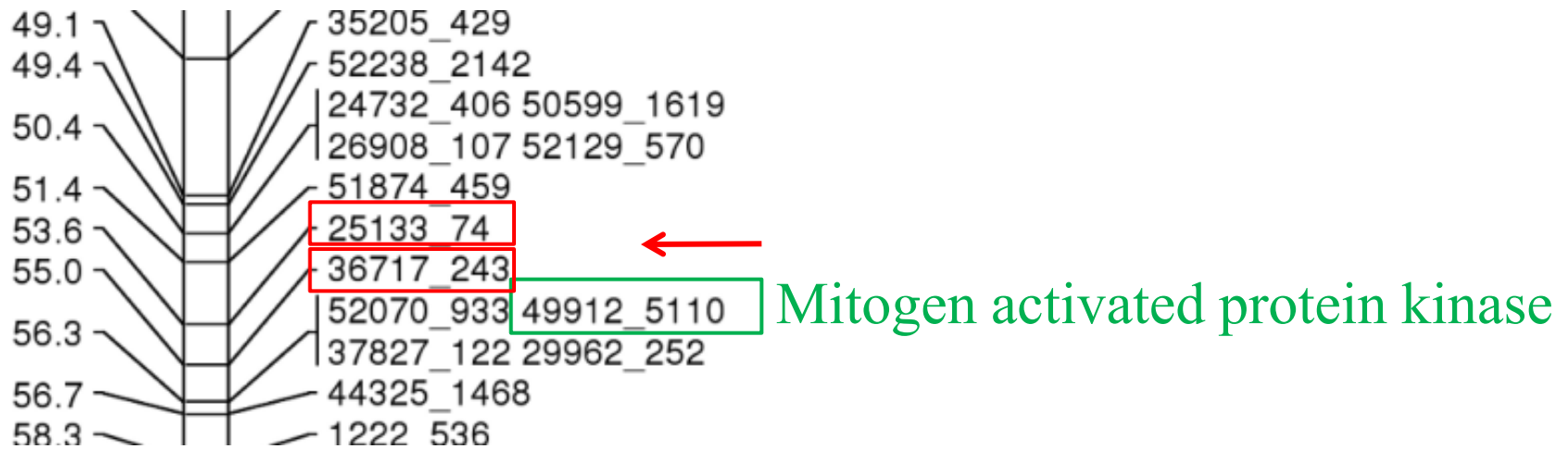
- Trait- Continuous (hrs survival)
- LA (GridQTL) & GWAS (GenABEL & PLINK)
- Threshold for calling association a QTL
 - Bonferroni correction → Benjamini Hochberg q-value ~ 0.98

Overview of findings

- Significant associations with WSSV resistance found on several linkage groups
 - Many map near genes with putative innate immune function
- Sex determining region identified

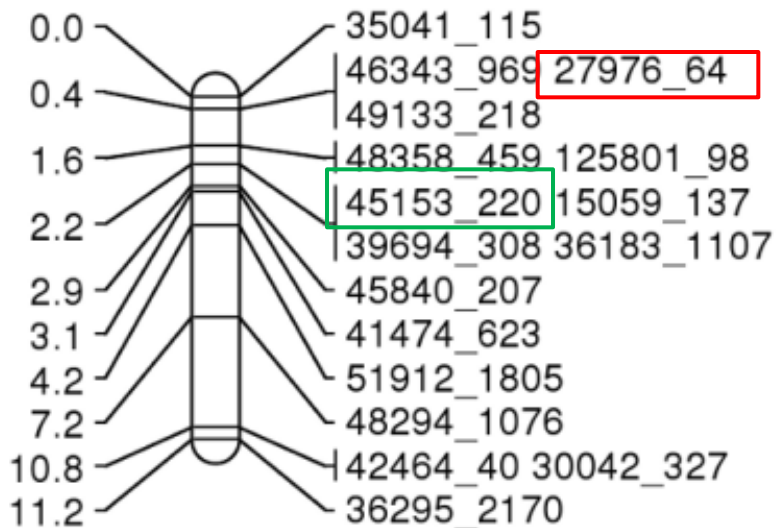
WSSV resistance on LG 17





LG 17

- MAP kinases → regulate interleukin-1 expression (role in host defence & immune response). Direct cellular response to stimuli (including viral infections)

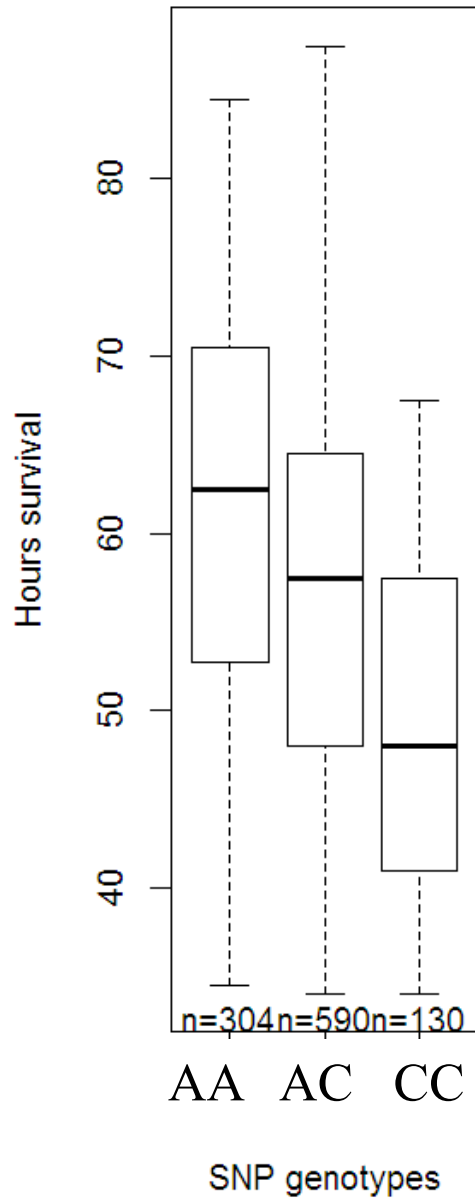


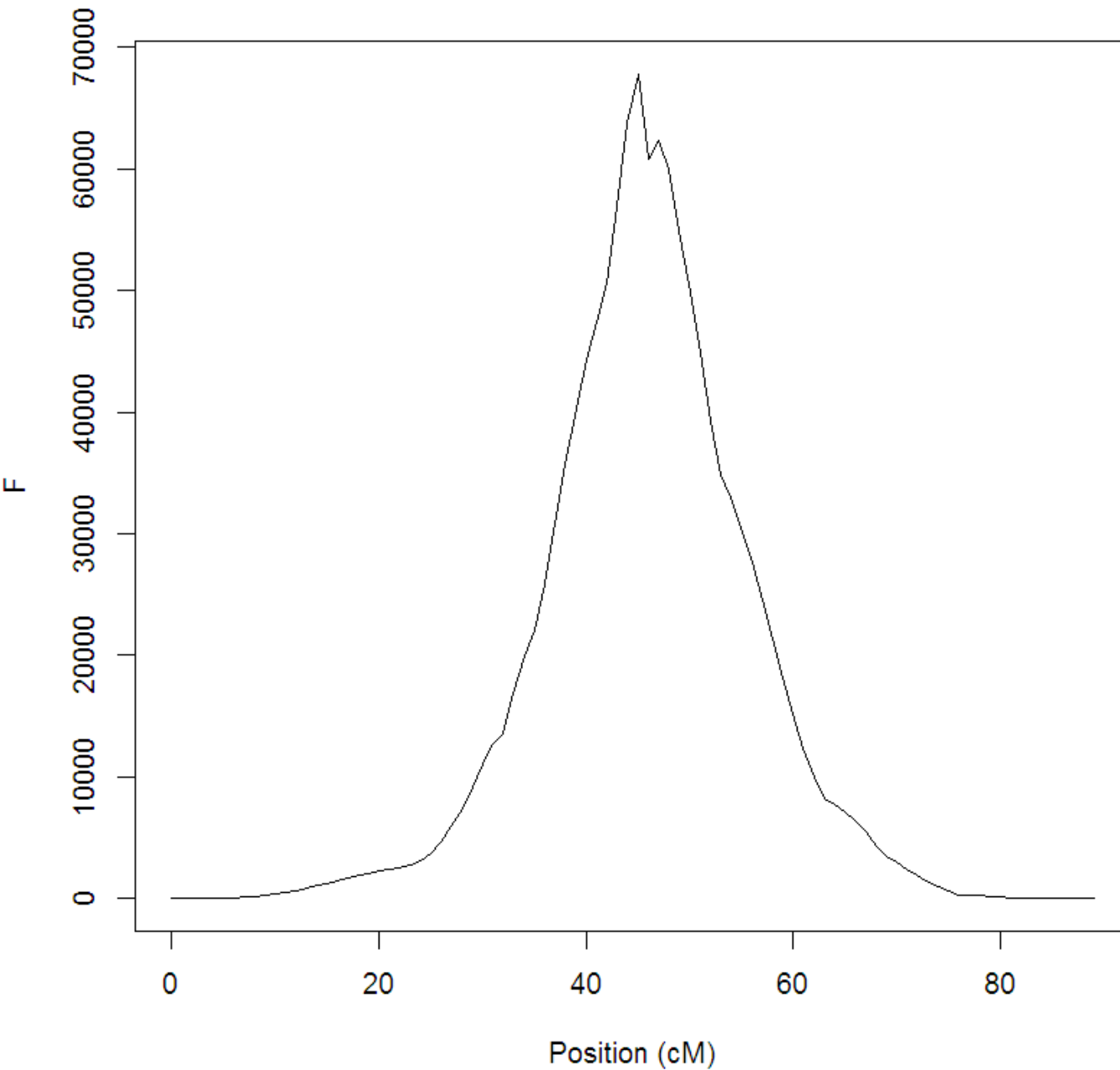
C-type lectin protein

LG 43

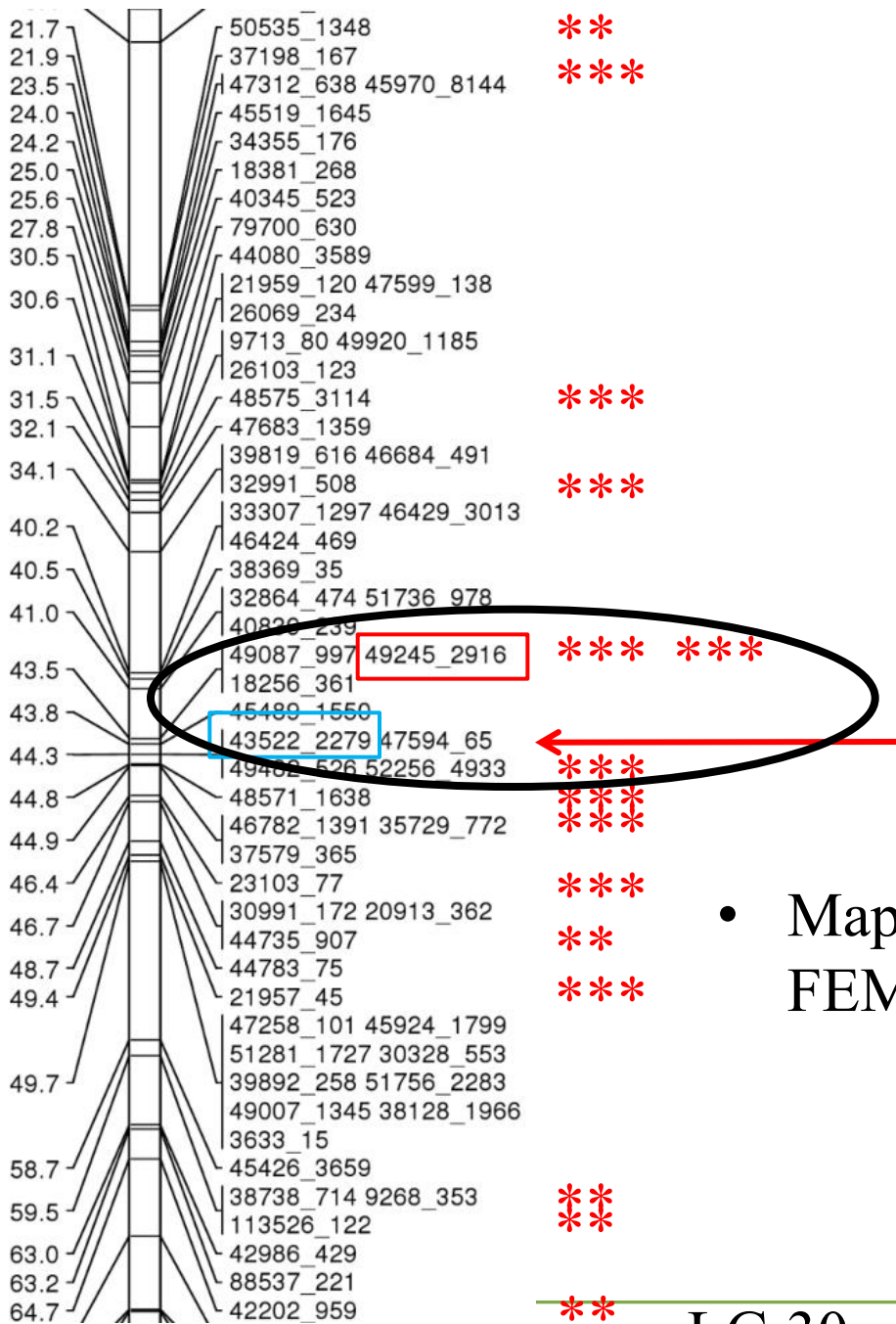
- Lectins → immune recognition & micro-organism phagocytosis in crustaceans
- *P. monodon* surviving more than 84hrs post-WSSV infection have higher haemocyte expression of c-type lectin
- Lectin more highly expressed in hepatopancreas of resistant *L. vannamei* and resistant *P. japonicas* than more susceptible shrimp

SNP 18472_352 across families





GridQTL
positions locus
for sex at 45 cM
on LG 30



- 15 SNP loci (all on LG30) significantly associated with gender ($P < 0.01$)
- Three SNPs of highest significance map to a small (0.8cM long) region of this LG

FEM-1 homolog

- Mapping to this 0.8cM long stretch is FEM-1

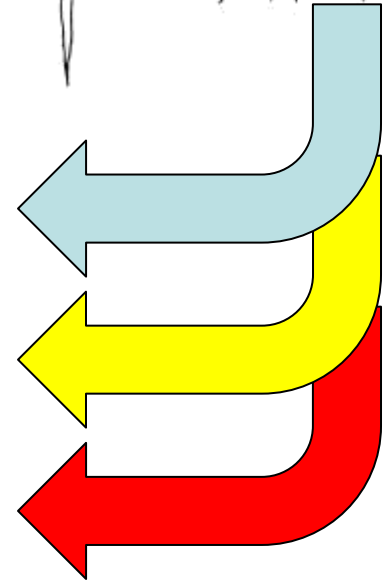
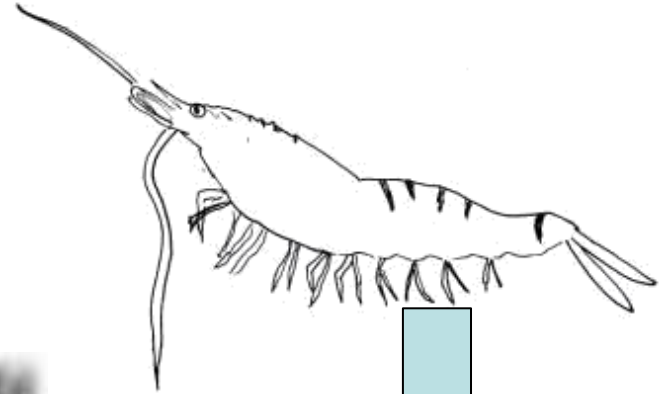
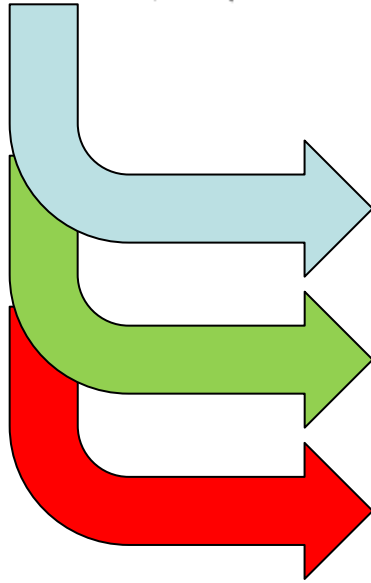
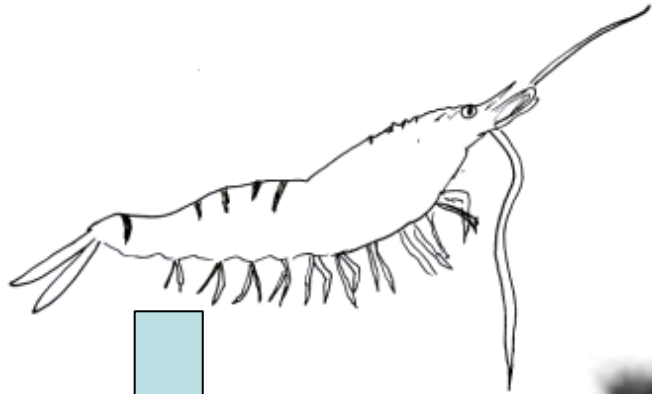
LG 30

Feminization-1 (Fem-1)

- Known signal transducing regulator affecting sex determination in nematodes
- FEM-1, FEM-2 and FEM-3 form a complex which promotes proteolysis of the male-repressing transcription factor TRA-1

Strong candidate for effect on sex-determination!

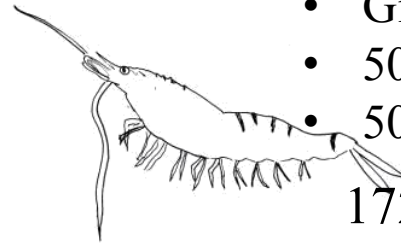
Do the same genes affecting WSSV resistance in monodon affect WSSV resistance in vannamei?



Choice of *L. vannamei* SNPs for genotyping

Expressed genome naïve Columbian *L. vannamei*

- Gill, pleopod & muscle
- 50 Pacific (selected 3 gens for WSSV resistance)
- 50 Atlantic (3 gens GR & TSV resistance) lines



172 homologous *vannamei* contigs identified, 2805 SNPs

Filter SNPs

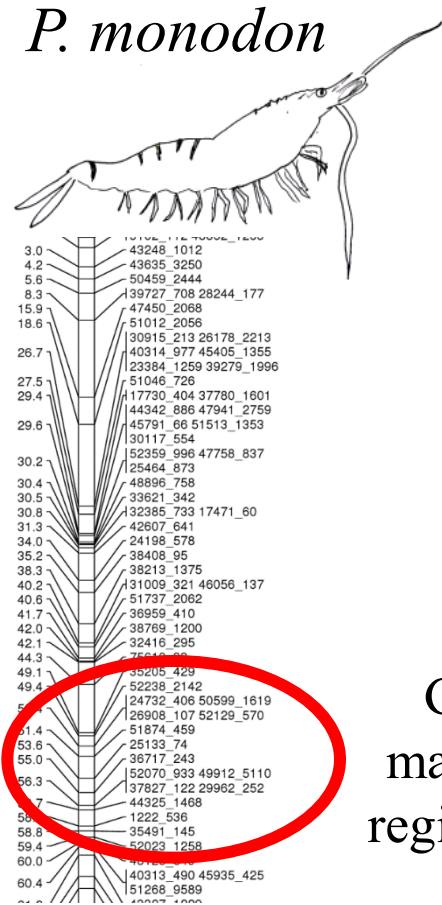
- 100 bases each side
- >5 X coverage
- MAF >0.2

168 *vannamei* contigs containing 1990 SNPs

SNPs found in 10 *vannamei* contigs with homology to *monodon* contigs of significance (+22 genes with putative immune or sex-determining function mapping to QTL regions)

BLAST

Gene contigs mapping to QTL regions (370 total)



Plan for *L. vannamei* QTL scan

- Challenge ~30 families with WSSV
- Record hrs survival and sex
- Genotype upper and lower percentiles (hrs survival) for ~ 50 SNPs

Summary

- Scanned *P. monodon* genome & found gene markers associated with WSSV resistance & sex determination
 - Tests for genomic or marker assisted selection to increase disease resistance in selective breeding programs
 - Possibility to produce an all female crop
 - Leads about genes and pathways involved in affecting these traits in shrimp
 - Follow up work in *L. vannamei* underway



Collaboration between the Central Institutes for Freshwater and Brackishwater Aquaculture in India (CIFA & CIBA), AFGC, Ceniacqua Columbia, SyAqua Thailand and Nofima

**Funders:
Norwegian Research Council
Department of Biotechnology India
SyAqua & Ceniacqua**