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QTL for resistance to summer mortality and OsHV-1 load in the Pacific oyster (*Crassostrea gigas*)

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Abstract:

Summer mortality is a phenomenon severely affecting the aquaculture production of the Pacific oyster (*Crassostrea gigas*). Although its causal factors are complex, resistance to mortality has been described as a highly heritable trait, and several pathogens including the virus Ostreid Herpes virus type 1 (OsHV-1) have been associated with this phenomenon. A QTL analysis for survival of summer mortality and OsHV-1 load, estimated using real-time PCR, was performed using five F₂ full-sib families resulting from a divergent selection experiment for resistance to summer mortality. A consensus linkage map was built using 29 SNPs and 51 microsatellite markers. Five significant QTL were identified and assigned to linkage groups V, VI, VII and IX. Analysis of single full-sib families revealed differential QTL segregation between families. QTL for the two-recorded traits presented very similar locations, highlighting the interest of further study of their respective genetic controls. These QTL show substantial genetic variation in resistance to summer mortality, and present new opportunities for selection for resistance to OsHV-1.

Keywords: *Crassostrea gigas*; herpes virus; Pacific oyster; QTL mapping; summer mortality

1. Introduction

The Pacific oyster, *Crassostrea gigas*, is one of the major aquacultured species with an annual worldwide production of 4.5 millions tons (FAO, 2006). However, several countries have encountered massive mortalities during the reproductive period (Gouletquer *et al.*, 1998, Burge *et al.*, 2006). This syndrome known as 'summer mortality' was described in Japan since the 1940s (Koganezawa, 1974) and it has been reported in many other countries (USA, Australia). In France, the Pacific oyster production has experienced periodic mass mortalities for at least 20 years (Renault *et al.*, 1994; Soletchnik *et al.*, 1999).

To better understand this phenomenon and propose solutions to reduce its impact on oyster production, a multidisciplinary project was conducted in France including studies on genetics, physiology, immunology and pathology (Samain and McCombie, 2007). Among pathogens, the Ostreid Herpes virus 1 (OsHV-1) has notably been detected during summer mortality outbreaks in France (Renault *et al.*, 1994) and in the USA (Burge *et al.*, 2007). A large additive genetic component was shown for survival from summer mortality of spat by divergent selection (estimated heritability = 0.70 ± 0.28 ; Dégremont *et al.*, 2005) and this result was confirmed by a divergent selection experiment (realized heritability ranging from 0.64 to 0.82 according to sites, Boudry *et al.*, 2008) However, even if the interest of developing a selective breeding program to improve this trait was clear (Boudry *et al.*, 2008), two major challenges can be identified. Firstly, the trait itself (i.e. dead or alive after the summer period), although easy to record on large number of individuals or families, is highly dependant on field testing conditions and varies both spatially (between sites) and temporally (timing of placement on the field - Dégremont *et al.*, 2005). Tight temporal monitoring of oysters is more difficult in tidal areas than in land-based tanks (Sauvage *et al.*, 2009b). Secondly, the identification of the causal factors leading to mortality is essential to better understand and define the selected trait and therefore optimize its genetic improvement. This identification requires close individual monitoring of dying and surviving individuals to describe their physiological and immunological status, in relation to the eventual detection of pathogens. In this context, the identification of markers or, even better, genes associated with the genetic basis of resistance to summer mortality outbreaks are likely to contribute to the development of genetically improved oyster strains.

In recent years, genomic resources and tools have greatly increased notably in the Pacific oyster, with the development of more than 50.000 expressed sequenced tags (EST; Tanguy *et al.*, 2008), the development of type II molecular markers (Yu and Li, 2008 ; Sauvage *et al.*, 2009a) and the development of type I markers (Sauvage *et al.*, 2007). Moreover, two linkage maps and a centromere map have been published in *C. gigas* (Li and Guo, 2004; Hubert and Hedgecock, 2004; Hubert *et al.*, 2009). To date, no detailed QTL mapping study has been published using these maps. Microsatellites are particularly polymorphic in marine bivalves and are relatively easy to score (Sauvage *et al.*, 2009a) and SNP markers are the most abundant molecular markers in most genomes (Brookes, 1999).

QTL have great potential for use in marker-assisted selection (MAS) for phenotypic traits such resistance to mortality and provide the basis for cloning the genes that underlies the genetic architecture of the traits. Clarification of the genetic bases of complex traits has broad implications for fundamental research, but will also be of practical benefit to aquaculture. This approach has been applied in many livestock species and has been introduced recently in aquacultured species such as in the rainbow trout, *Oncorhynchus mykiss* (Ozaki *et al.*, 2001), the Atlantic salmon, *Salmo salar* (Houston *et al.*, 2008) or in the Eastern oyster, *Crassostrea virginica* (Yu et Guo, 2006) to improve traits of economical interest such as the resistance to a disease. The aim of the present study was to identify QTLs for two traits in the Pacific oyster: mortality during a summer mortality outbreak and OsHV-1 load using real time PCR (Pépin *et al.*, 2008; Sauvage *et al.*, 2009b) in F₂ full-sibs families resulting from a divergent selection experiment (Boudry and Dégremont, 2008).

2. Materials and methods

Biological material: The QTL population was created using divergently selected lines of oysters from the MOREST program (French multi-disciplinary project on summer mortalities in the Pacific oyster, Samain and McCombie, 2008). Briefly, in G₀, two groups of half-sib families (“R” and “S”) were selected on the basis of average summer survival of their progenies on the field (Dégremont *et al.*, 2005). The two groups were used as progenitors to generate successive generations (G₁, G₂ and G₃), following a divergent selective breeding design where inbreeding was avoided. For the QTL families, F₀ individuals were selected and sampled from the G₃ generation of the MOREST program. From the F₁, five full-sib F₂ families were produced (named 1 to 5).

Phenotyping: Monitoring of survival and OsHV-1 load is described in detail in Sauvage *et al.* (2009b). On the 5th of July 2006 (*i.e.* prior to mortality outbreak), 96 individuals per family were sampled and checked by real time-PCR to evaluate the viral prevalence of OsHV-1. For each family, one thousand randomly sampled individuals were placed in bags and daily checked and sampled from day 138 (8th of July 2006) to day 224 (October 15th 2006). During the experiment, dead oysters (*i.e.* presenting permanently open valves) were collected daily and immediately frozen at -20°C for subsequent DNA extraction. The monitoring ended after 86 days, when no mortality was observed after several weeks and the environmental conditions were unlikely to lead to further mortality. At the end of the experiment (day 224), 150 oysters per family that survived to the summer mortality period were sampled.

OsHV-1 DNA detection and quantification was performed on frozen material by real time-PCR (Pépin *et al.*, 2008) that enables both detection and quantification of viral DNA (as absolute number of copies or relative amount when normalized to DNA input or additional normalizing genes). The specificity of the PCR products was systematically controlled with the melting temperature (T_m) value from dissociation curve using a Mx3000P Q-PCR machine (Stratagene). The quantification of the samples was based on a standard five-fold dilution curve derived from a stock solution of OsHV-1 genomic DNA extracted from purified virus particles (Le Deuff and Renault, 1999).

Molecular markers and selective genotyping: In three F₂ families, 300 individuals from the extremes of the phenotypic distribution (150 dead and 150 surviving juveniles) were genotyped for type I markers whereas a subset of 96 (chosen randomly among the 150 individuals) were genotyped for type II markers. In total 900 individuals were genotyped. Because of the higher level of polymorphism, microsatellites were genotyped on the subset of 96 individuals reducing the costs and the genotyping effort. Total DNA was extracted using the Qiagen Qiaamp[®] tissue mini kit (Robledo *et al.*, 2000) according to the manufacturer's protocol, from mantle tissue. DNA qualities were visually checked to remove and replace samples showing degraded DNA.

The 20 F₀ individuals were genotyped for a total of 114 previously described microsatellites markers (Huvet *et al.*, 2000; Li *et al.*, 2003; Yamtich *et al.*, 2005; Yu and Li, 2007; Sauvage *et al.*, 2009a) using a 3130 Applied Biosystems automatic sequencer (Applied Biosystems).

SNP markers were developed as described in Sauvage *et al.* (2007) and were genotyped using the MALDI-TOF mass spectrometry (Matrix-assisted laser desorption/ionization-time of flight, Griffin and Smith, 2000) on the Max Planck Institute genotyping platform in Berlin (Germany). Briefly, primers were designed using Primer 3 (Rozen and Skaletsky, 2000) in ESTs of interest related to summer mortality, identified through a SSH (Suppression Subtractive Hybridization approach, Fleury *et al.*, 2009) or were selected from existing database such as GigasBase (<http://www.ifremer.fr/GigasBase>) and Genbank. In a second step, one informative locus per EST was chosen to be genotyped in the F₀, F₁ and F₂ individuals.

Data analyses: Phenotypes were either binary for survival (1, died; 0, survived) or quantitative for OsHV-1 load (number of viral DNA copies/mg of fresh tissue). The normality of the quantitative data was investigated using a Shapiro-Wilk test and quantitative phenotype data were Log₁₀ transformed before analysis.. A Spearman correlation test was

used to evaluate the correlation between the two studied traits. A Chi² test was used to evaluate the Mendelian segregation distortion in the F₂ families.

Linkage between markers was evaluated using the 'twopoint' option in Crimap (Green *et al.*, 1990). Linkage was considered a significant when LOD (Log of the Odd Ratio) scores, i.e. the likelihood of a linkage between two markers, was superior to 3, allowing to assemble markers in linkage groups (LG). Genetic distances (cM, Kosambi) were calculated using 'build' option and their most probable order was estimated with 'flipsn' option. Finally, 'chrompic' option was used to highlight putative genotyping errors. A consensus map was built and the genome length (G_e) was estimated by the formula $N(N - 1) X/K$, where N is the number of markers, X is the maximum interval between two adjacent markers, and K is the number of marker pairs at an LOD score of 3.0 (Chakravarti *et al.*, 1991). The observed genome length (G_o) was calculated according to Cervera *et al.* (2001). The genome coverage was determined by dividing G_o/G_e.

Linkage groups were named according to the published map by Hubert and Hedgecock (2004) to allow direct comparison. Mapchart (Voorrips, 2002) was used to provide a graphical representation of the maps.

QTL analysis was carried out with QTL Express (<http://qtl.cap.ed.ac.uk/>; Seaton *et al.*, 2002), in the three families, using a large single full-sib analysis module with the following parameters (model (pat+mat), 1cM intervals, two traits). The chromosome-wide thresholds were calculated for each linkage group, using 1,000 permutations and the genome-wide threshold (Lander and Kruglyak 1995) was calculated by applying a Bonferroni correction to account for the analysis of multiple linkage groups (Knott *et al.*, 1998). Confidence intervals for the QTL position were calculated for each linkage group where a putative QTL was detected using the bootstrapping approach with 1000 replicates to define 95% confidence for each QTL position. The full-sib family analysis is a general model that can be used when large full-sib families are available to infer segregation of QTL from both parents. It makes no assumption that the QTL alleles are fixed in the original grandparental lines, but can benefit from the increased probability of parental QTL heterozygosity if the grandparental lines differ in their QTL allele frequencies. The QTL effects were interpreted as mean, paternal and maternal components. This allows for differences in QTL genotypes between the parents as well as potential parent-of-origin effects like genomic imprinting. The variation due to a QTL is a function of its effects and the allele frequencies (see Falconer and MacKay, 1996). The genetic variance is summarized as: $V_g = 2pq[a + d(q-p)]^2 + (2pqd)^2$, where a is the additive effect, d is the dominance effect and p is the frequency of increasing allele (with q = 1-p). In F₂ design, we assume that p = q = 0.5. So, V_g becomes: $0.5a^2 + (0.5d)^2$. The explained part of variance (EPV) of the QTL loci was deduced from the ratio V_g/V_p, where V_p is estimated from the total phenotypic variance of the data. In our case, the EPV of QTL linked to the mortality trait was not estimated due to the binary nature of the trait. Moreover, due to the selective genotyping strategy, unbiased values for QTL effects were roughly estimated as proposed by Darvasi and Soller (1992).

Results

Phenotyping : The detailed description of mortality and OsHV-1 load is described in Sauvage *et al.* (2009b). Prior to the monitoring period, no significant mortality was recorded during the larval and spat stages of life. Mortality began three days after the start of the experiment (day 141) and has affected families of oysters in varying degrees. The F₂ families 4 and 5 exhibited a very low mortality rate of 1.2% and 5%, respectively, whereas cumulative mortality was estimated as 29.8, 39.3 and 67% in the families 1, 2 and 3. As a result, families 4 and 5 were discarded from the QTL analysis because we hypothesized that QTL linked to summer mortality and viral load trait were not segregating in these two families. In the other families, mortality occurred very abruptly and on a very large scale. More than 60% of dead individuals were sampled during a single week.

Viral load measured by RT-PCR: Estimated number of copies of OsHV-1 DNA/mg of fresh tissue in the set of 96 randomly chosen individuals sampled on the 5th of July was low (i.e. always below 1.10^3 copies). Individual viral quantification was significantly different ($P < 0.001$) between dead and surviving individuals. In dead individuals, the viral load showed a high

variance both within and between families ($P < 0.001$ – Table 1) where the highest level of infection was detected in family 3 ($Z = 27.25$, $P < 0.001$). The lowest level of infection was observed in family 2 with a mean value of 2.02×10^6 copies. Moreover, the correlation between the level of mortality that occurred in the three families and the average level of infection by OsHV-1 appeared to be highly significant (Spearman's $\rho = 0.338$; $P < 0.001$). In surviving oysters, viral load was significantly different to the viral load detected in dead oysters ($P < 0.01$) while ranging from 0 to 2.3×10^3 copies in the family 1, from 0 to 1.7×10^3 copies in the family 2 and finally from 0 to 2.8×10^2 copies in the family 3.

Genotyping: A total of 114 previously published microsatellites markers were tested in the F₀. Only 43% (49/114) of microsatellites were genotyped in the F₁ and F₂ generation, the remaining being either monomorphic or giving complex banding patterns in the F₀ individuals. For type I markers, the estimated overall rate of success was 41% (61/149). This result may probably be due to the design of primers on splicing sites with the amplification of large insert during PCR. Within the three F₂ families, 77% (47/61) of the markers gave reliable results. A total number of 114 markers (67 microsatellites and 47 SNP) were combined to build a consensus linkage map.

Segregation of markers and linkage mapping: χ^2 tests revealed segregation distortion in the data ($P > 0.05$). Observed allelic proportions were not in Hardy-Weinberg equilibrium for 40% and 65% of type I and type II markers, respectively. This is partly a result of the selective mortality that occurred during the larvae stage of life. For each molecular marker, genotype data exhibiting the lowest level of distortion within each family were kept to build the linkage map. The consensus sex-averaged linkage map showed 10 LG (Fig. 1), in agreement with the haploid number of chromosomes of the species, and spanned 1016.5cM with an average spacing of 12.7cM. Irresolvable mapping problems were encountered with 18 of the 47 SNP markers with 12 SNP markers showing mapping distances exceeding 50 cM while 6 SNP and 16 of the 67 microsatellites showed multiple significant linkages with markers belonging to different linkage groups. Finally 29 SNPs and 51 microsatellites were successfully mapped. The consensus linkage map comprises a total of 80 markers of which 79 segregated in all three F₂ families (only one marker was not segregating in the three families). The estimated genome coverage was 73.6%.

QTL detection: QTL showing significant F values were detected on LGV, LGVI, LG VII and LG IX (Table 2 – Fig. 2). In family 1, two different QTL on LGVI linked to resistance to the summer mortality trait were detected at 94cM (65-110) and 117cM (89-125) and one single QTL on LG VII at 92 cM (80-100). QTL linked to viral load trait were detected roughly at the same position in the two LG (LGVI: 94 cM (70-115) and 118 (92-120); LG VII: 98 cM (82-100)). In terms of effects, for the mortality trait, paternal effects ranged from -0.237 ± 0.034 to 0.008 ± 0.003 and ranged from -0.108 ± 0.013 to 0.008 ± 0.001 for the viral load trait. Maternal effects ranged from -0.176 ± 0.035 to -0.089 ± 0.053 for the mortality trait and ranged from -0.155 ± 0.018 to -0.049 ± 0.034 for the viral load trait.

In the family 2, significant QTL linked to both traits were detected in LG V, VI and VII. In LGV, QTL linked to the two traits were positioned at 61 and 62cM and showed the same 95% confidence interval (42-80). In LG VI, two QTL were detected at 63cM (45-70) and 145cM (128-155) for the mortality trait and at 65cM (48-72) and 142cM (128-143) for the viral load trait. In this family, a significant QTL was detected in LG VII but only for the mortality trait. Its relative position was 94cM (89-98). Paternal and maternal effects were estimated to range respectively from -0.03 ± 0.021 to 0.122 ± 0.034 and from -0.173 ± 0.04 to 0.194 ± 0.035 , for the mortality trait. For the viral load trait, paternal and maternal effects respectively ranged from -0.183 ± 0.125 to 0.138 ± 0.063 and from -0.469 ± 0.231 to -0.165 ± 0.099 .

Finally, in the family 3 significant QTL loci were detected in LGIX and were positioned at 94cM (80-93) and 95cM (80-99) for the mortality and viral load traits. The paternal effects associated were estimated to -0.091 ± 0.043 and -0.441 ± 0.202 for the mortality and viral load traits whereas maternal effects for both traits were estimated to -0.118 ± 0.038 and -0.253 ± 0.179 . More detailed maternal, paternal and main components effects are given in Table 2.

The total explained proportion of variance (EPV) for both traits was estimated to 0.412 and 0.378 for the mortality and viral load traits, respectively. Detailed EPV for each QTL locus are reported in Table 2.

3. Discussion

This study is the first to report the detection of QTL affecting resistance to summer mortality and viral load in the Pacific oyster *C. gigas*. The first study of marker – phenotype association in *ostreidae* was reported in the American cupped oyster, *Crassostrea virginica* (Yu and Guo, 2006). The studied phenotype was the resistance to two pathogens (MSX and *Perkinsus marinus*) that affect farming yields analysed by differences in alleles frequencies prior to and after infection. The present experiment, designed with a high frequency of sampling of dead oysters reared under identical environmental conditions throughout the life of the individual and especially during the mortality event, allowed (i) the individual viral quantification, as a measure of the viral infection using RT-PCR and (ii) to perform a QTL detection using quantitative data. It should be noticed that the same approach was also applied in the European flat oyster *Ostrea edulis*, where several QTL linked to the resistance to a pathogen (*Bonamia ostreae*), were mapped using survival data (Lallias *et al.*, 2009).

In our study, the analyses were based on a large single full-sib analysis and the results were interpreted as paternal and maternal components. These results supports evidence that QTL show parent-of-origin effects, which may be due to imprinting from the parental lines in the phenotypic expression of the progenies. However, these models could also reflect different QTL genotypes in the F_1 parents.

Significant evidence for the segregation of 5 QTL was found on 4 linkage groups in the 3 studied F_2 families. However, QTL positions were estimated with a moderate degree of accuracy in the average 95% confidence interval. The relatively low number of markers that are mapped in *C. gigas* compared to other organisms is the major limitation of the present study, which is balanced by the effect of the QTL and their potential use in marker-assisted selection programs with the difference in survival rates between families. Another limitation in accuracy is the limited amount of recombination that can be observed within one family of 150 or 94 genotyped offspring. In our study, the high level of significance of the 5 QTL regions for both phenotypic traits appears very promising for future applications. The results strongly suggest that major-effect QTL may be involved in the resistance to summer mortality in *C. gigas*. Our results are congruent with previous ones published in the European flat oyster (Lallias *et al.*, 2009) that report the identification of a small number of large effect QTL associated to a disease resistance trait (i.e. susceptibility to the parasite *Bonamia ostreae*). However, the QTL effect reported in the present study are over-estimated as a result of the selective genotyping that was performed and the so-called Beavis effect (for a review, see Xu, 2003). Moreover, our results show a high level of heterozygosity for the QTL, which is relatively unusual for phenotypic traits such as mortality, partly due to the selection of families that showed a wide range in survival. In the aquaculture context, the intense culture (e.g. density, stress) of the animals may tend to exacerbate the effect of the viruses, which corroborates the large effect of the QTL. These observations are also congruent with previous ones reporting large QTL effect, notably in the Atlantic salmon, where QTL related to the resistance to IPN (Infectious Pancreatic Necrosis) were reported (Houston *et al.*, 2008).

Using several families in our study was very useful to detect different segregating QTLs as highlighted by the results of the analysis of single full sib families. Lines of oysters are not fully homozygous and are not fixed for the traits of interest, which means that the high variance in the viral prevalence and survival rate between the 5 full-sib oysters may be inferred by their parental origin. The low level of mortality exhibited in the families 4 and 5 supports this result. Consequently, these two families were not included in our QTL analysis, hypothesizing that QTL linked to the resistance to summer mortality were not segregating in

these families, even if they may be the more promising families for further breeding. Each group of oysters may be composed of individuals with different degrees of tolerance to the infection and may be differentially affected. This means that marker – QTL association and the associated linkage disequilibrium were generated at the family level and not at a higher level allowing us to detect several different QTLs.

Our study shows very similar location of QTLs linked the two studied traits. We can therefore hypothesize that the genetic architecture underlying these two traits are similar. However, we need to get a better understanding of the involvement of OsHV-1 in the summer mortality phenomenon to support this hypothesis. Additionally, the close relationship between viral load and mortality suggests a direct relationship between individual OsHV-1 load and mortality during our experiment (Sauvage *et al.*, 2009b). However, this hypothesis should be interpreted with caution as no other pathogen (such as *Vibrio* bacteria) associated to the ‘summer mortality’ phenomenon was looked for.

Detection of OsHV-1 using PCR in some of these lines was performed at the larval and adult stages in successive generations under hatchery conditions (Barbosa-Solomieu *et al.*, 2005). No significant differences were observed between “R” and “S” lines for survival at larval stage (Dégremont, 2003) neither for OsHV-1 prevalence (Barbosa-Solomieu *et al.*, 2005). The susceptibility of these lines to OsHV-1 remains to be investigated because of the difficulties associated with experimental transmission of OsHV-1 after the larval stage (Le Deuff and Renault, 1999).

Although selective breeding is still limited for the Pacific oyster (for review see Boudry, 2009), the future application of QTL identification will be through implementation in breeding programs to initiate a marker-assisted selection (MAS). In many farmed species, there is still a contrast between the large number of papers reporting the identification of QTL and the limited number of cases where they have been applied. QTL must be validated in further studies before MAS is implemented because accurate estimates of QTL magnitude, position and effect are needed to optimize their use (Spelman and van Arendonk, 1997). However, in the Pacific oyster, before the implementation of the present results in MAS, the role of epistasis and genomic background in the genetic architecture of both traits should be assessed (Danzmann *et al.*, 1999; Perry *et al.*, 2003). Moreover, the identification of ‘outlier QTL loci’ in natural population may be of high interest in order to validate the QTL loci detected through QTL mapping experiment (Rogers and Bernatchez, 2005). As previously described, the relatively low number of markers that are mapped in *C. gigas* compared to other organisms is the major limitation of the present study. The development and the mapping of a higher number of genetic markers will increase the accuracy of the genetic map obtained, the power of detection of the QTL and the accuracy of the estimation of the QTL effects and their confidence interval. The recent increase of genomic resources in this species such as dedicated EST databases (Fleury *et al.*, 2009), cDNA microarrays (Jenny *et al.*, 2007), BAC libraries (Cunningham *et al.*, 2006), and the current international efforts to sequence the whole genome of this species (Hedgecock *et al.*, 2005; Zhang *et al.*, 2008) will allow the development of large numbers of microsatellite and SNP markers, the integration of linkage and physical maps. Moreover, with an increased density of markers, QTL will be detected with a higher degree of accuracy and allow to narrow their confidence interval. Thus, the sequencing of BAC clones corresponding to the QTL loci will provide a list of candidate genes relying under the variance of the two studied traits. Moreover, further co-location of QTLs and candidate genes differentially expressed between sensitive and resistant lines (Fleury *et al.*, in press) should contribute to better understand the complex genetic architecture between genes underlying the resistance to summer mortality in the Pacific oyster.

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Tables

Table 1. Descriptive statistics of the viral load measured in the dead and survived individuals and expressed as a number of viral DNA copies number/mg of fresh tissue

		Family	Cumulative percentage of mortality	N ^a	Average viral load ^b (copies/mg)	Standard error
Dead individuals	1		28	150	3.99×10^6	2.23×10^6
	2		38	150	2.04×10^6	3.86×10^6
	3		49	150	1.14×10^7	4.66×10^7
Surviving individuals	1		-	150	1.02×10^2	2.48×10^2
	2		-	150	3.34×10^2	1.47×10^2
	3		-	150	1.86×10^2	2.54×10^2

^a N - Number of individuals analysed by Q-PCR

^b OsHV-1 viral load is expressed as a number of particles per mg of fresh tissue

Table 2. Linkage groups containing QTLs significant for mortality and viral load for the three F2 families and the associated main, paternal and maternal effects

F2 Family	LG ^a	Trait ^b	Position ^c	CI ^d 95%	F ^e	Mean ^f	Standard error	Paternal effect	Standard error	Maternal effect	Standard error	PVE ^g (%)
1	VI	Mortality	94/117	65-110 /89-125	14.62***	0,568 (0,523)	0,031	0,008	0,003	-0,176	0,035	-
		Viral load	94/118	70-115 / 92-120	17.93***	0,558 (0,541)	0,128	0,008	0,001	-0,155	0,018	9,93
	VII	Mortality	92	80-100	24.5***	0,654 (0,578)	0,034	-0,237	0,034	-0,089	0,053	-
		Viral load	98	82-100	9.1***	0,612 (0,589)	0,022	-0,108	0,013	-0,049	0,034	3,55
2	V	Mortality	61	42-80	110.82***	0,492 (0,412)	0,022	0,37	0,025	0,09	0,043	-
		Viral load	62	42-80	71.65***	0,638 (0,586)	0,109	-0,183	0,125	-0,469	0,231	7,71
	VI	Mortality	63/145	45-70 / 128-155	21.63***	0,356 (0,299)	0,034	0,122	0,034	0,194	0,035	-
		Viral load	65/142	48-72 / 128-143	23.86***	0,456 (0,413)	0,154	-0,138	0,063	-0,11	0,164	9,35
	VII	Mortality	94	89 -98	9.68***	0,535 (0,497)	0,029	-0,03	0,021	-0,173	0,04	-
		Viral load	-	-	6.44	0,58 (0,532)	0,017	-0,156	0,143	-0,165	0,099	4,37
3	IX	Mortality	94	80-93	5.76***	0,497 (0,451)	0,028	-0,091	0,043	-0,118	0,038	-

Viral load	95	80-99	5.44***	0,563 (0,512)	0,134	-0,441	0,202	-0,253	0,179	2,97
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^a Linkage group nomenclature from Hubert and Hedgecock (2004)

^b Phenotypes

^c Average position of the QTL

^d Confidence interval estimated by bootstrapping

^e *F*-test with chromosomewide criterion of significance (***) $P < 0.001$) using permutation test with re-sampling in QTL express with 1000 permutations followed by a Bonferroni correction based on the number and size of linkage groups.

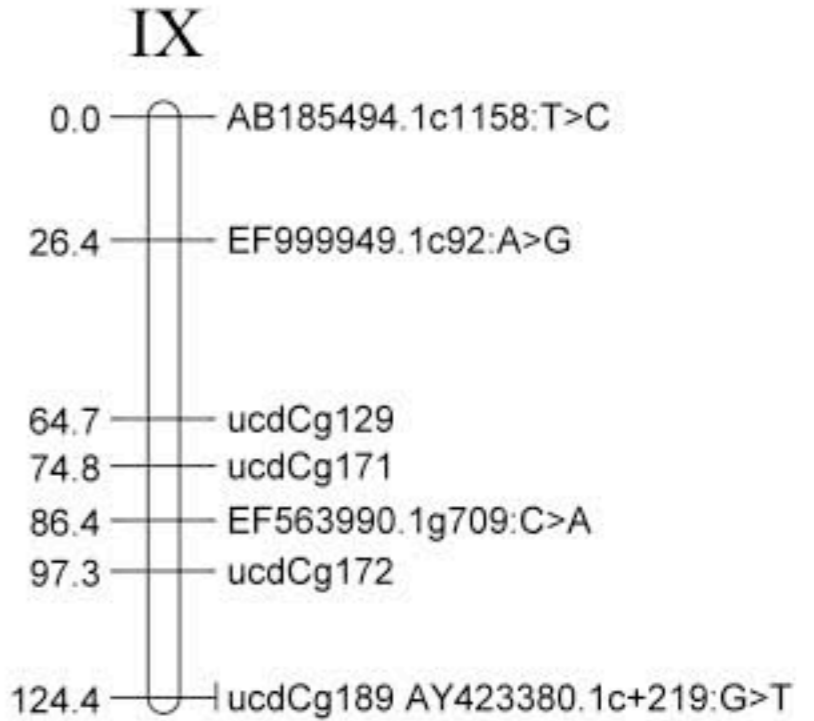
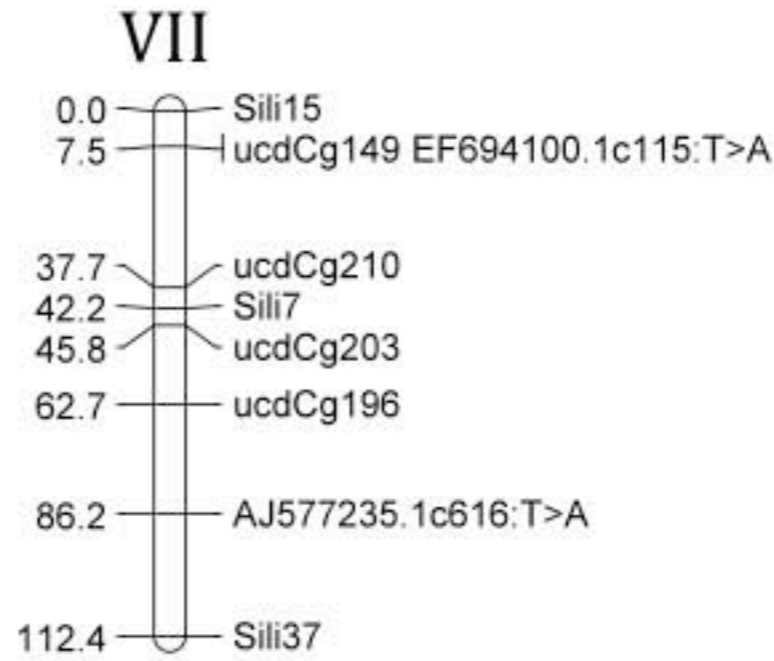
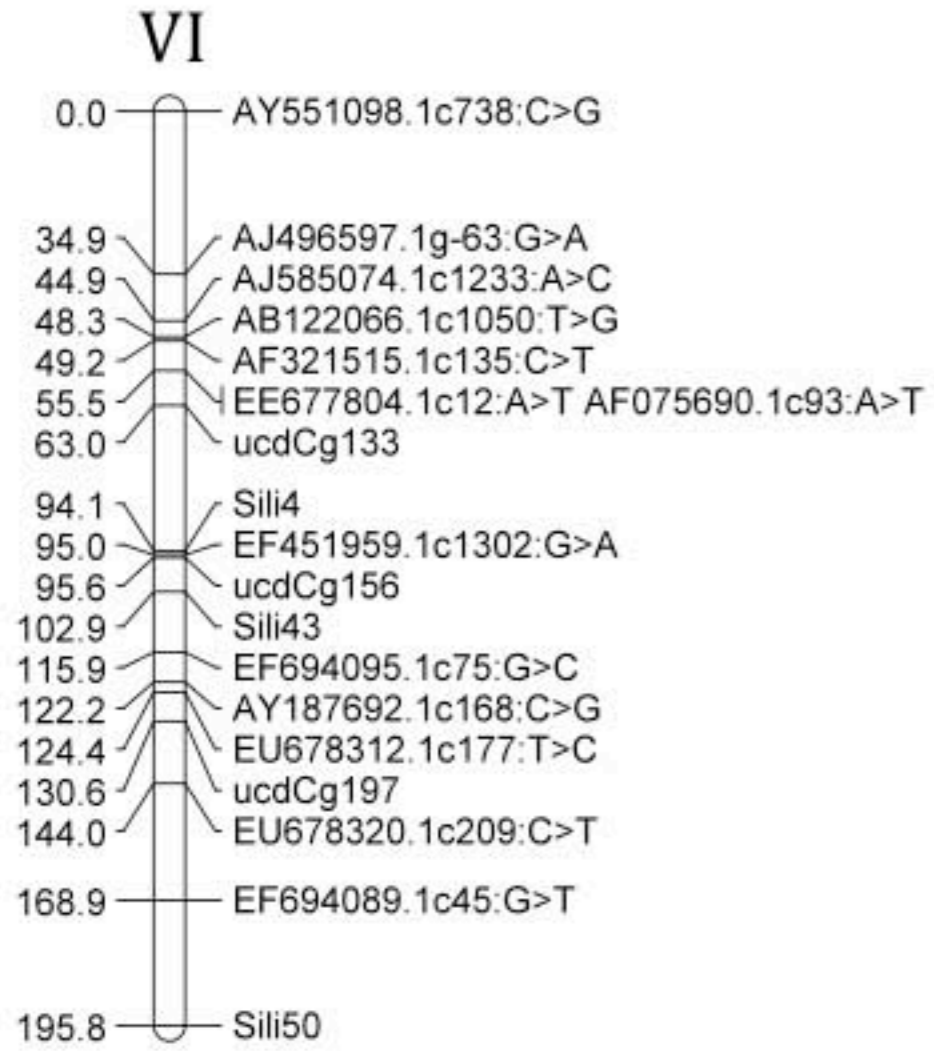
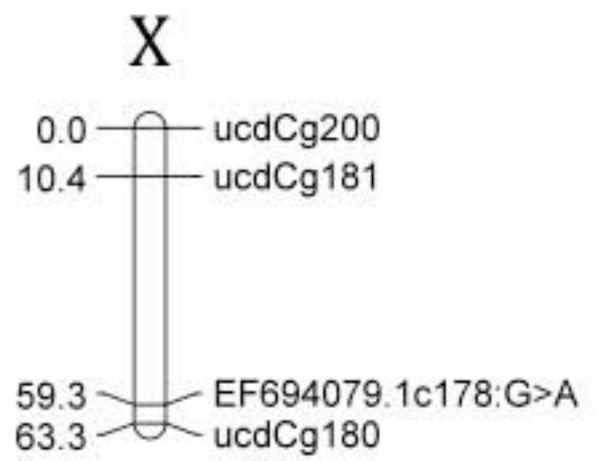
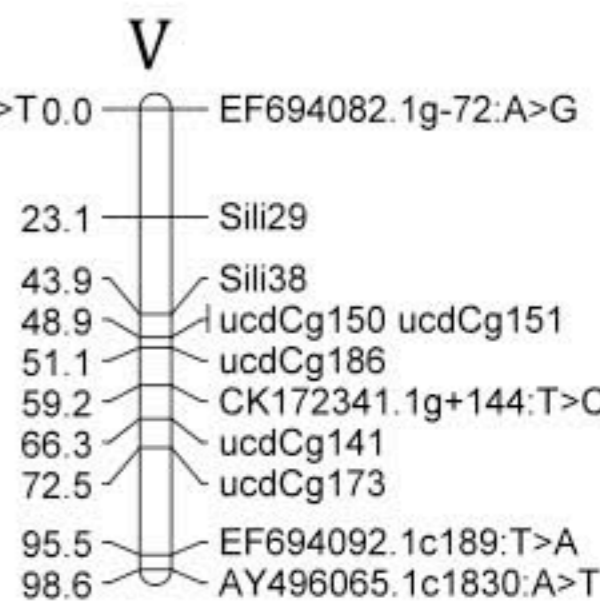
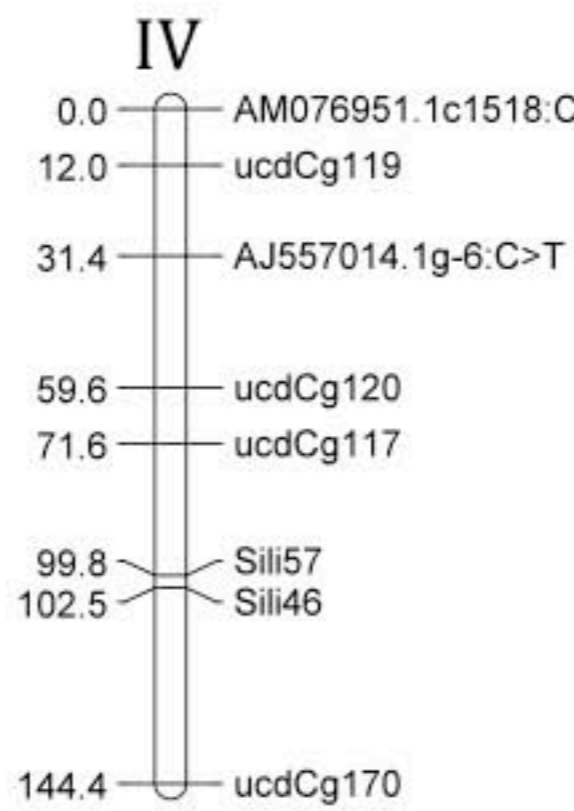
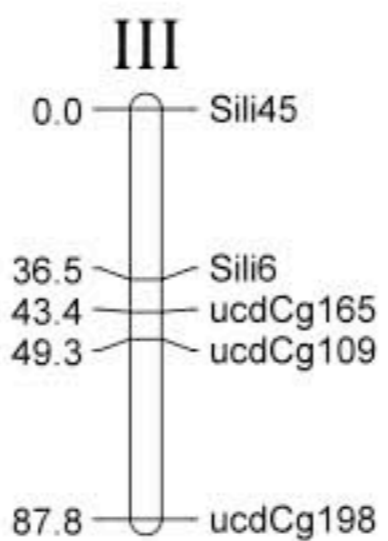
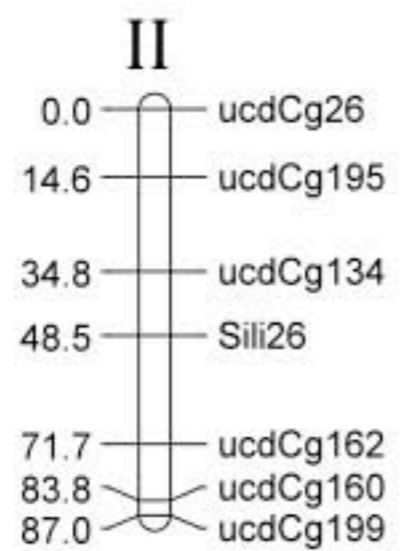
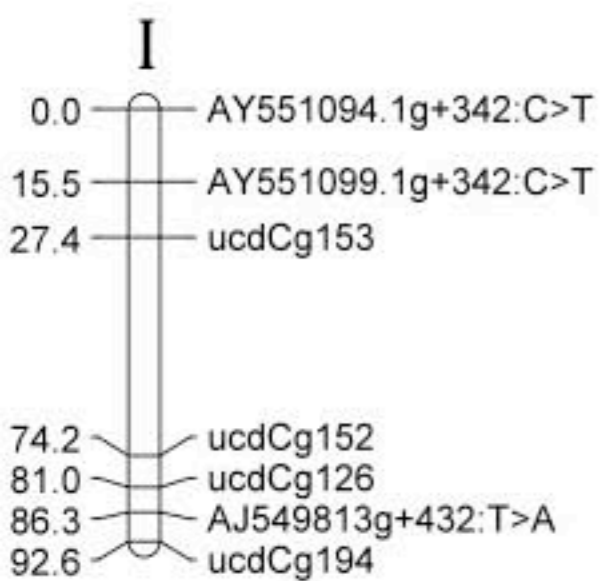
^f Mean effect of the QTL. Unbiased estimation of the QTL effect are figured between brackets

^g Part of the Variance Explained by the QTL

Figures

Figure 1.- The consensus linkage map, based on the analysis of the three F2 families, is composed of 10 LG, includes 80 markers and spans 1016.5cM. Linkage groups were named as their homolog in the map published by Hubert and Hedgecock (2004).

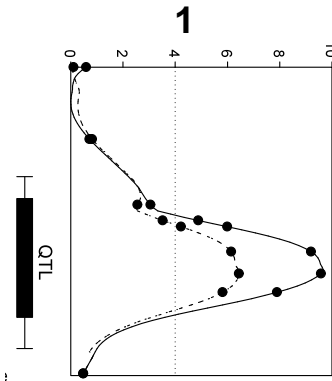
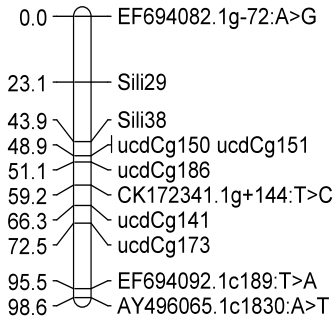
Figure 2.- QTL graphs with their respective 95% confidence interval in linkage groups V, VI, VII and IX. Solid lines represent QTL F ratio values for the mortality trait whereas dot lines represent F ratio values for the OsHV-1 viral load trait. Constant dot lines represent the level of significance calculated at the chromosome-wide significance threshold.



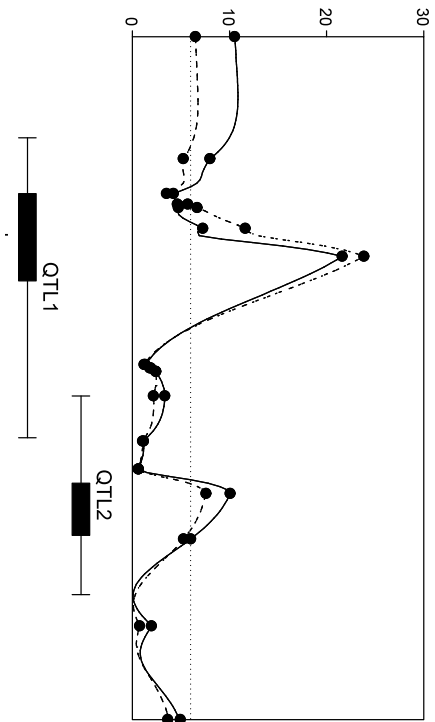
Linkage Group

F2 Families

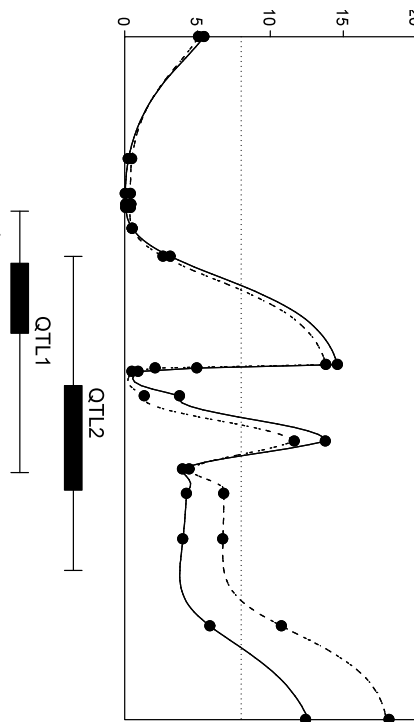
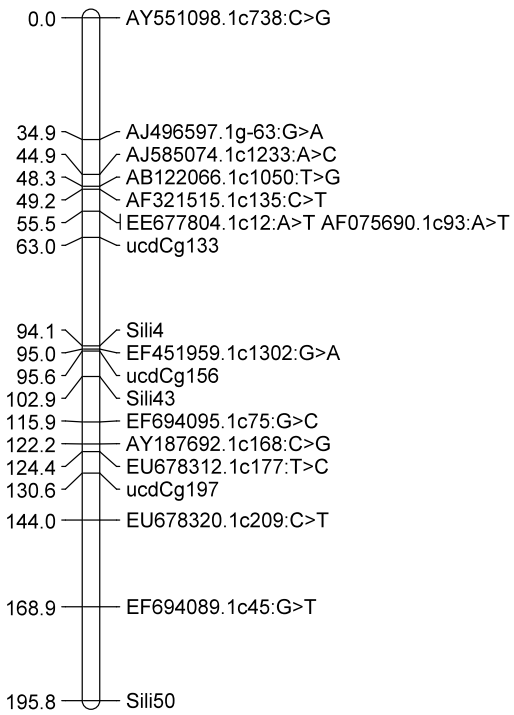
LG V



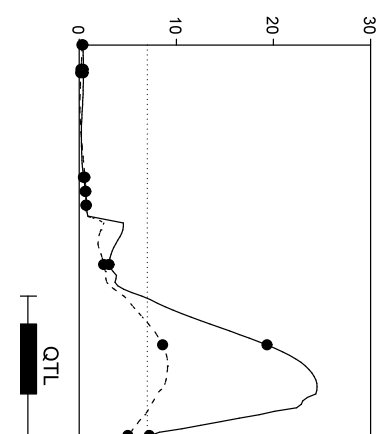
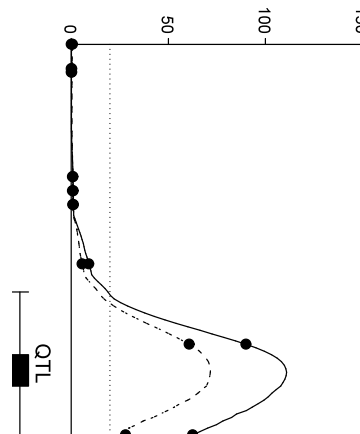
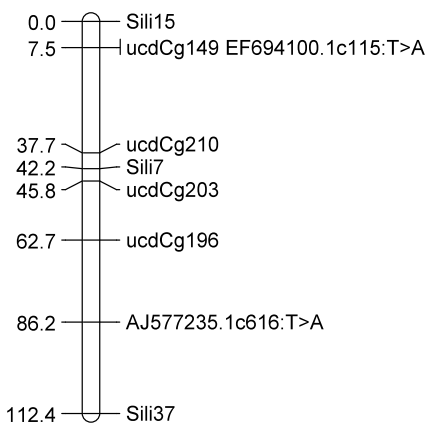
2



LG VI

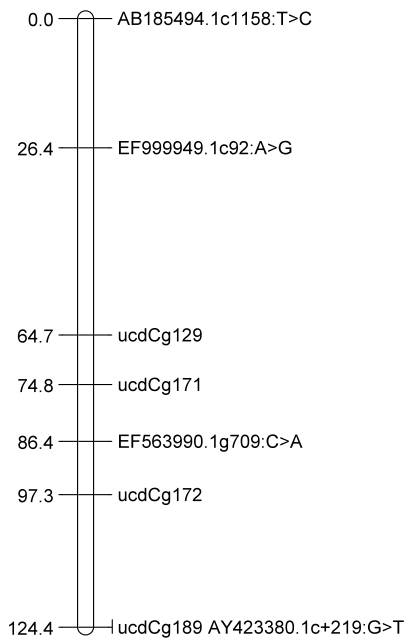


LG VII



Linkage Group

LGIX



F2 Families

3

