Genomic selection for resistance to one pathogenic strain of *Vibrio splendidus* in blue mussel *Mytilus edulis*

Munusamy Ajithkumar[1+], Jonathan D'Ambrosio[2], Marie-Agnès Travers[3], Romain Morvezen[2*], Lionel Degremont[1*]

[1]Ifremer ASIM, avenue Mus de Loup, 17390 La Tremblade, France
[2]SYSAAF, Station LPGP/INRAE, Campus de Beaulieu, Rennes 35042, France
[3]IHPE, Université de Montpellier, CNRS, Ifremer, Univ Perpignan Via Domitia, Montpellier, France

* Romain Morvezen and Lionel Dégremont contributed equally to this work and should both be considered as last co-authors

[+]Corresponding author: Munusamy Ajithkumar (ajithkumar.munusamy@ifremer.fr)

Highlights

- Moderate heritability was observed for resistance to *Vibrio splendidus*
- Genomic selection has better prediction accuracy than pedigree-based selection
- Resistance to *V. splendidus* is a polygenic trait

Abstract

The blue mussel is one of the major aquaculture species worldwide. In France, this species faces a significant threat from infectious disease outbreaks in both mussel farms and the natural environment over the past decade. Diseases caused by various pathogens, particularly *Vibrio* spp., have posed a significant challenge to the mussel industry. Genetic improvement of disease resistance can be an effective approach to overcoming this issue. In this work, we tested genomic selection (GS) in the blue mussel (*Mytilus edulis*) to understand the genetic basis of resistance to one pathogenic strain of *Vibrio splendidus* (strain 14/053 2T1) and to predict the accuracy of selection using both pedigree and genomic information. Additionally, we performed a genome-wide association study (GWAS) to identify putative QTLs underlying disease resistance. We conducted an experimental infection involving 2,160 mussels sampled from 24 half-sib families containing each two full-sib families which were injected with *V. splendidus*. Dead and survivors mussels were all sampled, and among them, 348 dead and 348 surviving mussels were genotyped using a recently published multi-species medium-density 60K SNP array. From potentially 23.5K SNPs for *M. edulis* present on the array, we identified 3,404 high-quality SNPs, out of which 2,204 SNPs were successfully mapped onto the recently published reference genome. Heritability for resistance to *V. splendidus* was moderate ranging from 0.22 to 0.31 for a pedigree-based model and from 0.28 to 0.36 for a genomic-based model. GWAS revealed the polygenic architecture of the resistance trait in the blue mussel. The GS models studied showed overall better performance than the pedigree-based model in terms of accuracy of breeding values prediction. This work provides insights into the genetic basis of resistance to *V. splendidus* and exemplifies the potential of genomic selection in family-based breeding programs in *M. edulis*.

Keywords: Mussels, Mortality, Breeding program, Prediction accuracy, GWAS, Linkage disequilibrium, *Vibrio*

1. Introduction

Aquaculture is a rapidly growing food production industry globally, supplying over 50% of aquatic protein sources and having a lower carbon footprint compared to terrestrial animals (Norman et al., 2019). Mussels are one of the major aquaculture species worldwide, with France being the second European producers, with around 65,000 tons in 2021 (FAO, 2023). Two species as well as their hybrids are cultivated in France: the blue mussel *Mytilus edulis,* and the Mediterranean mussel *Mytilus galloprovincialis*. Production is distributed along the English Channel to the southwest coastline of France, and Mediterranean shores (FAO, 2023; Prou and Goulletquer, 2002). This species has been widely cultured due to its strong environmental adaptability, high nutritious value, and consumer preference (Prou and Goulletquer, 2002; Suplicy, 2020). French mussel production entirely relies on wild spat collection, mainly in Pays de Loire and in Nouvelle-Aquitaine regions (Prou and Goulletquer, 2002). Consequently, the French cultivated mussels are not genetically selected through selective breeding programs.

Recurrent mass mortality outbreaks of bivalves reduce production, cause economic losses, and negatively impact the ecosystem of natural bivalve populations as well as terrestrial food web (Bódis et al., 2014; Soon and Ransangan, 2019). Mass mortality of various cultured mussels have been reported worldwide such as in blue mussels (Capelle et al., 2021; Lupo et al., 2021), Mediterranean mussels (Avdelas et

al., 2021; Lupo et al., 2021), green-lipped mussels (Ericson et al., 2023), and pheasantshell mussels (Putnam et al., 2023), and their occurrence seems to increase in the context of global warming. Since 2014, French mussel farms have been vulnerable to abnormal mussel mortality (AMM) with a mortality rate varying from 30 to 100% depending on sites, years or seasons (Normand et al., 2022; Polsenaere et al., 2017). Peak of mortality outbreaks generally occurs during spring (Charles et al., 2020a; Degremont et al., 2019). Various studies are being conducted to identify the cause(s) of AMM outbreaks in France and to propose solutions for reducing mass mortalities in mussel farms and wild stocks. Until now, the etiology of AMM outbreaks remains unclear, but it could be linked to environmental pollutions, seawater characteristics, mussel characteristics, culture practices, and climate change (Lupo et al., 2021; Polsenaere et al., 2017). Pathogens could also be involved in mortality outbreaks, with the pathogenic bacteria *Vibrio splendidus* being found in large abundance in moribund mussels during AMM outbreaks (Bechemin et al., 2015; Ben Cheikh et al., 2016) . *V. splendidus* is a complex species comprising multiple strains, ranging from highly virulent to relatively innocuous (Ben Cheikh et al., 2016). Some virulent strains have been shown to be highly pathogenic to blue mussels, causing high mortality rates up to 90% within a week in experimental challenges (Ben Cheikh et al., 2017; Ben Cheikh et al., 2016; Oden et al., 2016). The virulence of these strains can vary based on factors such as mussel physiology, environmental conditions, and seasonal changes (Charles et al., 2020b). While *V. splendidus* is not the direct cause of AMM, its consistent association with mortality outbreaks suggests it may play a contributory role under specific conditions. Recent studies on bivalve immune responses often lack a validated understanding of immune effectors or pathways,

reflecting their reliance on innate rather than adaptive immunity, limiting the efficacy of vaccination strategies (Allam and Raftos, 2015; Rey-Campos et al., 2019).

Selective breeding could be a useful approach to enhance the innate immune responses in bivalves (Dégremont et al., 2015; Hollenbeck and Johnston, 2018). Understanding genetic basis of disease resistance is critical for its improvement through selective breeding. The potential for genetic improvement through mass selection is well documented in many bivalve species during the past decades, particularly due to their short generation intervals and their high reproductive capacity allowing the possibility of applying high selection pressures (Gjedrem and Rye, 2018; Tan et al., 2020). Mass selection has been carried for growth traits in Chilean blue mussel *Mytilus chilensis* (Toro et al., 2004a; Toro et al., 2004b), and ploidy status for Mediterranean mussel *M. galloprovincialis* (Ajithkumar et al., 2024a). Lately, a mass selection scheme implemented for resistance to AMM outbreaks in the blue mussel *M. edulis* resulted in a 34–48% increase in survival after one generation of selection (Degremont et al., 2019). Although mass selection is effective, it may quickly lead to inbreeding if genetic diversity is not properly monitored (Hu et al., 2022). However, as an alternative strategy to individual selection, family based selective breeding programs have been initiated to estimate breeding values by combining phenotypic information and pedigree. These programs have targeted various traits across different mussel species, such as growth in *M. edulis* (Mallet et al., 1986), *M. galloprovincialis* (Díaz-Puente et al., 2020; Nguyen et al., 2014; Pino-Querido et al., 2015), *M. chilensis* (Alcapán et al., 2007; Guiñez et al., 2017), *Hyriopsis cumingii* (Bai et al., 2017; Jin et al., 2012), *Perna calaniculus* (Camara and Symonds, 2014); shell nacre color in *H. cumingii* (Bai et al., 2017); toxin accumulation and mantle color in *M. galloprovincialis* (Pino-Querido et al., 2015); and survival in *M. edulis* (Mallet et al., 1986).

Accurate estimations of breeding values are essential for developing a breeding program and predicting the responses of traits of interest to selection. Recent developments of high throughput genotyping technology now enable the implementation of genomic selection (GS) (Boudry et al., 2021). GS is particularly suitable for traits that are expensive or difficult to measure (e.g. resistance to diseases, meat quantity) because less phenotypic data is needed to obtain similar accuracies from estimated breeding values (EBV) resulting from pedigree-based selection (Regan et al., 2021; Yáñez et al., 2023). GS can improve the genetic gain by capturing both within and between family genetic variation components (Boudry et al., 2021). Next-generation sequencing (NGS) and genotyping-by-sequencing (GBS) tools have been developed for oyster, clam, abalone and scallop (Jiao et al., 2014; McCarty et al., 2022; Nie et al., 2017; Ren et al., 2016; Wang et al., 2016; Yang et al., 2022). However, they do not provide the same set of markers from one population to another (e.g. between training population and breeding population) and are dependent on DNA quality, which limits their potential to develop repeatable genomic analyses. Alternatively, SNP arrays have been developed in some commercially important bivalve species such as the silver-lipped pearl oyster *Pinctada maxima*, with an Illumina ~3k iSelect custom array (Jones et al., 2013a), the Pacific oyster (*Crassostrea gigas)*, with a 190K SNP array (Qi et al., 2017), the medium density bi-species (Pacific oyster *C. gigas* and European flat oyster *Ostrea edulis*) 57K SNP array (Gutierrez et al., 2017), the Eastern Oyster (*C. virginica*), with a high density 566K and 66K SNP array (Guo et al., 2023), or medium density multi-species (*M. edulis*, *M. galloprovincialis*, *M. trossulus*, and *M. chilensis*) 60K SNP array (Nascimento-Schulze et al., 2023). SNP arrays have been used for various applications in aquaculture species, including identification of genetic architecture of traits, genomic selection (GS), characterization of genetic resources,

pedigree monitoring, sex-determination and inbreeding management, but have rarely been used in bivalves (Gutierrez et al., 2020; Jourdan et al., 2023).

In aquaculture, the salmon industry has been leading the way in GS for several years (Ajasa et al., 2024; Correa et al., 2017; Odegård et al., 2014; Robledo et al., 2018; Tsai et al., 2015). To date, more and more aquaculture species are following this trend such as rainbow trout, European sea bass, sea bream, Nile tilapia, Channel catfish or whiteleg shrimp (see for review (Boudry et al., 2021; Houston et al., 2020; Song et al., 2022; Yáñez et al., 2023)). The recent development of genotyping tools in bivalves has so far resulted in a relatively limited number of studies on the potential of genomic selection. GS has been investigated in the Portuguese oyster for morphometric traits, edibility traits and disease traits (Vu et al., 2021), in the American oyster for low salinity tolerance (McCarty et al., 2022) and in the silver-lipped oyster for pearl quality traits (Zenger et al., 2019) and growth traits has been studied in the triangle sail mussel (Wang et al., 2022), European flat oyster (Penaloza et al., 2022) and Pacific oyster (Gutierrez et al., 2018; Jourdan et al., 2023). A few studies have been conducted in oysters showing the increase in accuracy of GS over pedigree-based approaches notably for difficult to measure traits, such as disease resistance (Gutierrez et al., 2018; Gutierrez et al., 2020; Jourdan et al., 2023). To date, trials with low-density panels to reduce genomic evaluation costs have been conducted in several aquaculture species, indicating that developing cost-effective strategies for genomic selection will be pivotal in shaping modern aquaculture breeding programs (Penaloza et al., 2022).

The aim of our study was to assess the potential of genomic selection for resistance to one pathogenic strain of *V. splendidus* in *M. edulis*. Using a multi-species Axiom Affymetrix 60K SNP array (Nascimento-Schulze et al., 2023), we first

characterized the genetic structure and linkage disequilibrium of the blue mussel population. We then estimated genetic parameters for resistance to *V. splendidus* and performed GWAS to investigate its genetic architecture. Finally, we compared the accuracies of genomic selection and pedigree-based selection to provide recommendations for optimizing selective breeding programs.

2. Material and Methods

2.1 Family production

The 48 families of *M. edulis* used in this study are detailly described in (Ajithkumar et al., 2024b). Briefly, three wild mussel populations (OLE-PON, WIM, and YEU_001) were sampled and transferred to the Ifremer hatchery in La Tremblade in the fall of 2016. Each mussel population was cleaned and placed in separate tanks containing unheated UV-treated, and filtered seawater (400 L per hour). To favor gametogenesis, mussels were fed a cultured phytoplankton diet (*Isochrysis galbana*, *Tetraselmis suecica*, and *Skeletonema costatum*). Two sets of crosses were performed in January 2017 (set 1) and in February 2017 (set 2). For each population, 100 mussels were individually placed in 400 mL beakers, and spawning was triggered by alternating cold (10°C) and warm seawater (20°C). Depending on the ripeness of the mussels and the sex ratio, 4 males for OLE-PON, and 11 males for YEU_001 were used in set 1, while 9 males were used for WIM in set 2. Within population, each male was mated with two females, producing in total 24 half-sib families, each containing two full-sib families. Each family was grown separately in 30 L tanks filled with filtered and UV-treated seawater at 20°C until the pediveliger stage. Then, downwelling system were used until mussels reached 1 cm. At that size, they were transferred to

our nursery in Bouin in April and May 2017 for set 1 and set 2, respectively. For each family, 1000 spat were maintained in 15 L SEAPA© baskets, and all families were raised in a 20 m³ concrete raceway until the start of the experiment, which occurred in July 2018. More detailed on the larval and nursery culture are provided in (Ajithkumar et al., 2024b).

2.2 Experimental infection and phenotyping

Detailed step-by-step protocol of the experimental infection is given in Ajithkumar et al. (2024b). Briefly, two experimental infections (EI_1 and EI_2) were conducted in July 2018, each using 24 families randomly sampled among the 48 families (mean individual total weight of approximately 5 g). Additionally, a third experimental infection (EI_3) was performed, involving 12 families from each of the first two experiments, to increase the phenotype and genotype sample size. To investigate their resistance to *V. splendidus,* a highly pathogenic strain (strain 14/053 2T1) isolated during AMM outbreak in 2014 was injected in 30 mussels per family. First, mussels were anesthetized using $MgCl_2$ (50 g per L), and 50 µL of bacterial solution (10^9 bacteria/mL) was injected into the muscle. Then, ten injected mussels per family, for all the 24 families of one set were hold in one 120 L tank containing UV-filtered seawater. Three replicate tanks were used and, in each tank, water recirculation was maintained using a TECO®pump (Ravenna, Italy), which also maintained the seawater temperature at 17°C. Dead mussels were counted and sampled daily up to 72 h post-injection. The adductor muscle/gills of the dead mussels during the experiment and the surviving mussels at the end of the experiment were collected using scalpels disinfected with 70% ethanol and stored in 1.5 ml sterile tubes at room temperature. Individuals for genotyping were randomly sampled from the

challenge experiments (EI_1, EI_2, and EI_3), including both dead and alive mussels, with 13 to 16 individuals sampled from each of the 48 families (Table 1).

2.3 Genotyping and quality control

Among the 2160 individuals from the 48 families, a total of 768 were sent for DNA extraction and genotyping to the Gentyane INRAE Platform (Clermont-Ferrand, France) using the multi species medium-density 60K SNP-array, Axiom_Myt_v1_r1 (Thermo Fisher Scientific, Waltham, Massachusetts, USA), which comprises 23,252 markers for *M. edulis* (Nascimento-Schulze et al., 2023). Among the 768 individuals genotyped, 348 were from the dead group, 348 were from the alive group, and the remaining 72 were their parents (48 dams and 24 sires). Quality controls on the 60K SNPs from the SNP array and genotyped individuals were performed as described in D'Ambrosio et al. (2019). Firstly, genotypes of all individuals were analyzed using the Axiom Analysis Suite software (AxAS; v.4.0.3.3) with the default best practice workflow suggested by the manufacturer, with few threshold modifications, which includes individual quality control (QC) and SNP quality control analysis (DQC ≥ 0.20; QC call rate ≥ 85; percent of passing samples ≥ 98; average call rate for passing samples ≥ 92%; call rate cutoff ≥ 95; FLD ≥ 2.6). Consequently, 7,476 polymorphic SNPs were retained for further analysis. Subsequently, final quality control was performed using PLINK v1.9 software (Chang et al., 2015). Two individuals with an identity-by-descent value over 0.90 were considered as duplicated and both individuals were removed from the analysis. Only SNPs with a minor allele frequency (MAF) higher than 0.01 and those passing the Hardy-Weinberg equilibrium test (p-value < 0.0000001) in the genotyped mussels were retained. After the quality control, data comprised of a total of 766 genotyped individuals for 3,406 SNPs.

2.4 Parentage assignment

Parentage assignment was performed in the R package APIS (Griot et al., 2020) with a mismatch number set to 5%. The best 1471 SNPs (Supplementary Table 1), selected with call rate greater than 90% and MAF value greater than 0.1 were used. Parentage assignment allowed the reconstruction of the pedigree of 647 offspring with assignment rates reaching 93.2% of the mussels having both parents assigned, while the remaining 47 mussels potentially from outside the cross-mating design, which were excluded from following analyses.

2.5 Genetic structure of the population

To evaluate potential genetic sub-structuring of populations and any associated biases, a principal component analysis (PCA) was performed using PLINK 1.9 (Chang et al., 2015) and the genetic structure was visualized using in RStudio (Team, 2024). Three individuals were identified as outliers beyond the population structure and were subsequently excluded from further analysis. Genetic differentiation between populations was measured through pairwise fixation index (F_{ST}) estimates using PLINK 1.9 (Chang et al., 2015).

2.6 SNP mapping, genome coverage and linkage disequilibrium estimation

All markers of the array along with their flanking regions were blasted using a BLASTn® procedure on the reference genome (*Mytilus edulis* genome assembly, xbMytEdul2, GenBank accession number: GCA_963676595.2). To map SNPs,

considering the high polymorphism in the mussel genome, four mismatches were allowed over a length of around 71 base pairs. Only SNPs mapping to a unique position on the reference genome were retained for the subsequent stage of quality control as mentioned in previous section. Out of the 3406 SNPs, only 2204 matched our mapping criteria and were successfully positioned on the reference genome (Supplementary Tables 1 & 2).

The pairwise linkage disequilibrium (LD) analysis was performed between all SNPs and adjacent markers for each linkage group and population to determine LD decay within the genome of *M. edulis* using Plink 1.9 (Chang et al., 2015).

2.7 Estimation of genetic parameters

2.7.1 PBLUP

Estimated breeding values, variance components, and heritability were calculated using the BLUPF90 software package (Misztal et al., 2014) through two different approaches: a linear mixed model with AIREMLF90 (Misztal et al., 2014) for assessing the trait on the observed scale, and a Gibbs analyses with THRGIBBS1F90 (Tsuruta and Misztal, 2006) for evaluating it on the underlying scale, based on pedigree-based relationship.

$$\mathrm{Y}_i = \mathrm{X}_i\beta_i + Z_i\mu_i + e_i$$

where Y_i is the binary mortality outcome at the end of the experiment (1 = dead, 2 = alive) of mussel, β_i is the vector of fixed effects, including set of crosses (set 1, set 2), population origins (OLE-PON, WIM, YEU_001), and replication of the experimental infection (EI_1, EI_2 and EI_3). μ_i is the vector of additive genetic effect of the animal,

following a normal distribution $\mu \sim N\ (0, A\sigma_a^2)$, where A is the pedigree relationship matrix, and σ_a^2 is a matrix of additive genetic variance. e_i is the vector of random residuals, assumed to be distributed as $e \sim N\ (0, I\sigma_e^2)$, where I is an identity matrix and σ_e^2 is a matrix of the residual variance. X_i and Z_i are known incidence matrices relating observations to the fixed and random effects mentioned above.

The EBV were estimated using BLUPF90 package and the variance components using AIREMLF90 and THRGIBBS1F90 programs. With the threshold model, the variance components were estimated using a Gibbs sampler with 100,000 iterations, 10,000 of burn-in and one sample was kept every 10 iterations for posterior analysis. Variance components were estimated using the average information restricted maximum likelihood algorithm (Gilmour et al., 1995).

Heritability (h^2) was estimated as: $h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}$

2.7.2 GBLUP

The GBLUP model uses the same approach as the PBLUP model, but with μ replaced by g and A replaced by G. Here, g is the vector of additive genomic effects, and G is the genomic relationship matrix. The matrix G was computed as described by VanRaden (2008).

$$G = \frac{ZZ'}{\sum_i^m 2p_i(1-p_i)}$$

where Z is a matrix of centered genotypes $(0 - 2p\ =$ homozygous, $1 - 2p\ =$ heterozygous, $2 - 2p\ =$ homozygous), p_i is the frequency of the reference allele for the i^{th} marker, and m is the total number of markers.

Heritability (h^2) was estimated as: $h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$

2.7.3 ssGBLUP

The single-step GBLUP (ssGBLUP) model enhances the PBLUP and GBLUP model by fitting the H matrix, which integrates both genomic and pedigree data (Aguilar et al., 2010). The inverse of the H matrix was constructed as follows:

$$H^{-1} = A^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & (0.95G + 0.05A_{22})^{-1} - A_{22}^{-1} \end{bmatrix}$$

where G is as described above and A_{22} is the pedigree-based relationship matrix for genotyped animals.

Heritability (h^2) was estimated as: $h^2 = \frac{\sigma_h^2}{\sigma_h^2 + \sigma_e^2}$

2.8 Genome wide association study

To identify SNPs associated with resistance to *V. splendidus*, a genome wide association study (GWAS) was performed using a mixed linear model association through ssGBLUP analysis. The postGSF90 module (Misztal et al., 2014) from the BLUPF90 package was used to estimate the effects of the SNPs ($\hat{a}_i$) based on the genomic breeding values $\hat{g}_i$ predicted for the genotyped animals. The SNP effects were estimated according to the following equation:

$$\hat{a}_i = dZ'[ZdZ']^{-1}\hat{g}_i$$

where d is the vector of weights associated with the SNP effects and Z is the incidence matrix relating SNP effects to genomic breeding values.

A linear mixed model was applied to assess resistance to *V. splendidus* on the observed scale, incorporating the genotype of an individual SNP as a fixed effect. The p-values for each SNP were computed using the POSTGSF90 module.

For the GWAS, a Bonferroni correction with $\alpha = 5\%$ was used to determine the genome-wide significance threshold $[-log10(\alpha/n)]$, where $n = 2{,}204$ (total number of SNPs genome-wide) and the chromosome-wide suggestive threshold $[-log10(\alpha/m)]$, where $m = 157$ (average number of SNPs per chromosome). Only the SNPs with a $-log\ P(value)$ over the chromosome wide threshold were considered to detect QTL associated with the resistance. Genome-wide significant threshold used in this study was considered to $-log\ P(value) = 4.64$, while chromosome-wide significant threshold was opted to $-log\ P(value) = 3.49$.

For each QTL, the additive effect (a) of the top SNP was used to estimate the proportion of genetic variance explained by this peak SNP using:

$$\%V_g = \frac{2\mathrm{p}(1-\mathrm{p})a^2}{\sigma_g^2} * 100$$

with σ_g^2 the total genetic variance estimated using the linear mixed model with PROGSF90 and p the minor allele frequency of the target SNP.

2.9 Prediction accuracy

Prediction accuracy for the BLUP, GBLUP, and ssGBLUP models was assessed using the 'leave-one-out' method. In this approach, each observation is systematically excluded one at a time. The model is then trained on the remaining data, and the (G)EBV for the excluded individual is predicted by masking its phenotype.

The accuracy (r) of prediction was computed as the correlation between the (G)EBVs and the corrected phenotype ($\hat{y}$) of the mussel divided by the square root of the heritability, using the formula:

$$r = \frac{[(\mathrm{G})\mathrm{EBV}, \hat{y}]}{\sqrt{h^2}}$$

The heritability value (h^2) used in this analysis was calculated using the variance components (σ_a^2 and σ_e^2) from the ssGBLUP model.

2.9.1 Evaluation of the effect of SNP density and training population size on genomic predictions

SNP panels of varying densities were assessed by selecting subsets from the full QC-filtered SNP panel for each dataset. Panels of the following densities were tested: 500 SNPs, 1,000 SNPs, 1,500 SNPs, annotated SNPs (~2,200), and all high-quality SNPs (~3,400). SNPs for each panel were selected randomly within each chromosome, with the number of SNPs chosen from each chromosome being proportional to the total number of high-quality SNPs per chromosome. Different training population sizes were evaluated by randomly selecting subsets from the population. Training population of 100, 300, 500, and all individuals were tested using annotated SNPs panel information. The analysis performed only with the ssGBLUP model, which is known for its effectiveness in genomic selection. To mitigate biases, we generated five different SNP panels for each SNP density randomly, and similarly five subsets randomly selected for each training population to address size-based selection biases.

3. Results

3.1 *Vibrio* challenge

The cumulative mortality rate 72 hours post-injection was 47%. At endpoint, mortality rates were 63% for EI_1, 41% for EI_2, and 37% for EI_3. Among mussel populations, the WIM population (54%) showed higher susceptibility to *V. splendidus* compared to the YEU_001 (45%) and OLE-PON (37%) populations. Mortality rates varied significantly among families upon exposure to *V. splendidus*, ranging from 17% to 83%. The mean mortality rates for all families are depicted in Figure 1.

3.2 Population structure

Figure 2 illustrates the results of the principal component analysis (PCA), revealing the population structure of the mussel population. The first two PCA axes collectively account for over 15% of the total genetic variation. The populations were generally homogeneous, with the exception of two families whose offspring showed greater isolation from others. F_{ST} analysis revealed low genetic differentiation between populations. The mean genetic distances between populations are shown in Table 2, with F_{ST} values ranging from 0.02 to 0.03, suggesting genetic similarity across all three populations (Figure 3). Overall, the absence of significant genetic differentiation between populations provides favorable conditions to merge data from all the populations for performing genomic selection analysis.

3.3 SNP mapping and genome coverage

In fact, 2,204 SNPs were positioned on the reference genome, resulting a loss of 1,202 SNPs. The positions of markers on the chromosomes is illustrated in Figure

4. The average SNP density per megabase (Mb) ranges from 0.57 to 2.37, varying among chromosomes and within chromosome (Supplementary Table 2). Approximately, only 9% of all 1 Mb segments contain more than 5 SNPs. SNP density exhibits non-uniformity throughout the genome, with each chromosome demonstrating varying densities. The lower marker density results in greater mean average distances between adjacent SNPs, ranged from 421 kb to 1739 kb depending on the chromosome.

3.4 Linkage disequilibrium analysis

Figure 5 illustrates that linkage disequilibrium (LD) decreases sharply as the distance between pairs of SNPs increases, with the most rapid decline occurring within the first 100 kb. Beyond this range, LD continues to decline and becomes more variable. The OLE-PON population consistently shows higher LD throughout the genome compared to other populations. On average, the LD values (r^2) for SNPs less than 15 kb apart are 0.12 for OLE-PON, 0.10 for WIM, and 0.06 for YEU. Linkage disequilibrium values are generally low between adjacent SNPs for all the chromosomes, where distances between adjacent SNPs are larger.

3.5 Heritability

The estimates of heritability using the linear and Gibbs sampling models are summarized in Table 3. Pedigree-based heritability estimates for resistance to *V. splendidus* in *M. edulis* ranged from 0.22 to 0.31. Genomic heritability was slightly higher, varying between 0.33 and 0.36. The ssGBLUP based estimated heritability ranging from 0.28 to 0.33, which combines genomic and pedigree information

3.6 Genetic architecture

GWAS for resistance to *V. splendidus* identified only one significant SNP surpassing the genome-wide threshold on chr 2, and seven significant SNPs surpassing the suggestive chromosome-wide threshold on chr 2, chr 4, chr 7, chr 9, chr 12, and chr 13 (Figure 6 and Table 4). However, none of these markers explained more than 1.06% of genetic variance (Figure 7 and Table 4).

3.7 Prediction accuracy

Accuracy with all data are 0.36, 0.43, 0.43 for BLUP, GBLUP and ssGBLUP, respectively. Genomic selection (GBLUP and ssGBLUP) is better than BLUP by 19%. Overall, prediction accuracy for GS increased with the density of markers (Figure 8). Incorporating genomic information generally enhanced accuracy compared to pedigree-based estimation, except with 500 SNPs where PBLUP exhibited higher accuracy than GBLUP (Figure 8). With maximum training population and SNP subsets, genomic evaluation improved accuracy by 17%, 19%, 25%, and 19% for 1,000, 1,500, annotated (2,204), and all SNPs (3,400), respectively, compared to PBLUP. When comparing GBLUP and ssGBLUP models, the prediction accuracy was consistently favored the ssGBLUP model, except when using annotated SNPs in the GBLUP model (Figure 8). In evaluating the size of the training population, accuracy ranged from 0.50 to 0.36 in BLUP, and from 0.47 to 0.45 in ssGBLUP with sizes from 100 to all individuals, respectively (Figure 9).

4. Discussion

In our study, we aimed to demonstrate the feasibility of genomic selection in a mussel breeding program in France. We used a recently developed multi species medium-density 60K SNP-array (Nascimento-Schulze et al., 2023) to perform genomic analysis.

4.1 Genotyping quality and genome covering by selected SNPs

To the best of our knowledge, our study is the first to use the multi species medium-density 60K SNP-array (Nascimento-Schulze et al., 2023) to estimate genetic parameters in blue mussel (*M. edulis*). Following the AxAS software's best-practice workflow with minor adjustments to thresholds, we identified 7,476 poly high-quality SNPs from 23,252 initially screened SNPs across 768 individuals. The necessity for stringent filtering of genotyping data is highlighted by the prevalence of poor-quality markers. After quality control using plink, we retained 3,406 SNPs, representing only 15% of the total SNPs designed for *M. edulis*. This reduction may be attributed to the polymorphic nature of mussel species or limited number of individuals used to construct the SNP array design (Gerdol et al., 2020; Nascimento-Schulze et al., 2023).

The *Mytilus* genus exhibits a complex evolutionary history characterized by extensive gene flow among congeneric species, and its genome is known for its complexity and high degree of polymorphism (Gerdol et al., 2020; Smietanka et al., 2014). The array used in the present study was developed using a whole-genome low coverage approach. Out of 23,253 poly high SNPs identified in *M. edulis*, only 16,213 (70%) were annotated on the recently published reference genome of *M. edulis*. Assembly errors in the reference genome may rise from several factors, such as

exceptionally high genetic polymorphism levels, non-Mendelian segregation of marker loci in paired crosses, and a significant occurrence of null alleles in genetic markers (Hedgecock et al., 2015). While a moderate proportion of our selected markers (3,406 out of 23,252) aligned well with the latest reference genome (2,204 SNPs; 65%), we observed a sparse distribution of SNPs across the linkage map. This limited coverage and sparse SNP distribution could potentially lead to the omission of QTLs in specific regions, suggesting the necessity for developing an optimized SNP array to address these challenges effectively. The bi-species Axiom Affymetrix 57K SNP array has been used in Pacific oysters, where applying the AxAS software's best practice workflow led to a notable reduction in the number of informative SNPs. Specifically, Gutierrez et al. (2018) reported 23,000 informative SNPs from 820 individuals, Vendrami et al. (2019) identified 21,499 SNPs from 232 individuals, and Jourdan et al. (2023) obtained 14,500 SNPs from 2,420 individuals. This reduction is largely attributed to the complex genetic structure of molluscs, stemming from the highly polymorphic nature of their genomes (Jiao et al., 2021; Song et al., 2021), and is further influenced by the genetic relationship between the training population used for array design and the breeding candidates in selective program (Houston et al., 2020). However, recent studies on bivalves have demonstrated that a moderate number of high-quality markers (1,000 - 3,000) could suffice for accurate predictions (Gutierrez et al., 2018; Kriaridou et al., 2020; Penaloza et al., 2022).

4.2 Linkage disequilibrium

Linkage disequilibrium (LD) at the genome level plays a crucial role in the efficacy of breeding programs, influencing genetic variance and the accuracy of

association analyses (Goddard and Hayes, 2009; Siol et al., 2017). In our study, values for r^2 ranged between 0.07 and 0.09 for SNPs within a distance of 10 kb and from 0.03 to 0.08 within 50 kb across the studied populations. However, LD levels decreased to less than 0.05 at 100 kb in two populations. Overall, LD between adjacent markers within each population was predominantly less than 0.1 within 2 kb, indicating a rapid decline in LD within the blue mussel genome. This swift decay suggests a historically large effective population size and high recombination rate, reflecting substantial genetic diversity within the population (Ellegren and Galtier, 2016). Moreover, LD values are population-specific, and influenced by evolutionary factors such as natural selection, mutation, genetic drift, line origin and migration, as well as molecular forces including historical recombination events, and breeding history such as historical effective population sizes, intensity and direction of artificial selection, population admixture, and mating patterns (Du et al., 2007). Our findings confirm the low LD in *M. edulis* populations, consistent with previous studies on bivalves (Jones et al., 2013b; Jourdan et al., 2023; Vera et al., 2022).

4.3 Population structure

F_{ST} is widely applied to evaluate genetic differentiation between/among populations (Hu et al., 2022). The low F_{ST} values ($F_{ST} < 0.03$) observed in our study suggest minimal genetic differentiation among mussel populations, indicating a lack of significant genetic structure. This phenomenon may be attributed to similar selection pressure and limited gene flow among the mussel populations, irrespective of geographic location. Similar findings have been reported in other studies, such as pairwise F_{ST} (< 0.02) among wild edible cockle using SNPs information (Vera et al.,

2022) and among wild populations of Pacific oyster using allozymes and microsatellites markers (Appleyard and Ward, 2006).

PCA provided robust evidence supporting the classification of mussels into the same groups, consistent with the low F_{ST} values observed. The PCA did not reveal population genetic stratification except in two families in WIM population, suggesting that the observed genetic variation is homogeneous and indicative of genetic proximity among populations. The two families whose offspring showed greater isolation from others in the WIM population may be due to due to the peculiar characteristics of the parents, which drive the first axis of the PCA.

4.4 Heritability

Our study presents the first report of heritability estimates for resistance to *V. splendidus* experimental infection in *M. edulis* based on genome-wide SNPs. We observed moderate heritability for *V. splendidus* resistance (0.22–0.36), which are higher compared to our previous study using the same population. This increase may be attributed to the inclusion of a third experimental infection in this study, despite the overall lower mortality rate (Ajithkumar et al., 2024b). Disease resistance to pathogens in bivalves seems to be a heritable trait, with moderate to high heritability in oysters, clams, and abalone, ranging from 0.21 to 0.63 (Brokordt et al., 2017; Dégremont et al., 2015; Smits et al., 2020). Studies on oysters have shown varying levels of heritability (h^2: 0.09-0.54) against different *Vibrio spp*. pathogens at different life stages (Azema et al., 2017; Dietrich et al., 2022; Nordio et al., 2021; Zhai et al., 2021). Comparing heritability estimates among methods, both GBLUP and ssGBLUP consistently showed higher heritability compared to pedigree-based methods. This difference is

likely due to the genomic relationship matrix constructed based on genome-wide SNPs information can capture both within and between-family genetic variance, whereas traditional pedigree selection only captures genetic variance between families (Boudry et al., 2021). To date, numerous studies across aquaculture species have similarly demonstrated that GBLUP methods provide higher estimated heritability and greater accuracy compared to PBLUP (Gutierrez et al., 2018; Tsai et al., 2015). These results underscore the presence of genetic variation for resistance to *V. splendidus* in our mussel populations, and highlight significant opportunities for enhancing disease resistance through selective breeding programs, whether using pedigree-based or genomic selection strategies.

4.5 Genome wide association study

QTL detection in our populations posed challenges due to limited number of markers and individuals. Given the data in the current study do suggest a polygenic nature of resistance to *V. splendidus*, utilizing all markers to calculate genomic breeding values for resistance may be the most effective approach. Our association analyses suggest that resistance against *V. splendidus* exhibits a polygenic architecture without major QTLs. Similar findings have been reported for bacterial disease resistance in various aquaculture species including, Atlantic salmon (Correa et al., 2015), Coho salmon (Barría et al., 2018), Gilthead Sea Bream (Palaiokostas et al., 2016), European seabass (Oikonomou et al., 2022), and Pacific Oyster (Yang et al., 2022). For instance, a study on catfish identified four QTLs associated with columnaris resistance using a high-density SNP array (Geng et al., 2015), highlighting the importance of high-density SNP array for GWAS studies. Our study used 2,204 SNPs, which may not provide sufficient coverage given the rapid LD decay, potentially

leading to the omission of important QTLs. This underscores the need for increased SNP coverage to ensure robust association analyses (Jones et al., 2013b). Additionally, a larger number of individuals (> 1,000) would be beneficial for enhancing overall QTL detection (Barría et al., 2018). Future studies could benefit from using a greater number of markers and phenotypes, as well as by creating resistant and susceptible lines in the F2 generation. These approaches can exploit more genetic variation and assist in identifying potential QTLs (Geng et al., 2015).

4.6 Prediction accuracy

The accuracy of genomic selection is affected by several factors, including the relationship between training and validation animals, sample size in the reference population, marker density, effective population size, LD structure, underlying trait architecture and heritability of trait (Yáñez et al., 2023). Therefore, the lower range of the prediction accuracies estimated here may reflect the underlying trait architecture or marker density. The choice of genomic selection model for breeding programs requires a prior understanding of the genetic architecture of the selected trait(s). In the current study on *M. edulis* populations, the genetic contribution to the observed variation in resistance to *V. splendidus* was largely polygenic in nature. For the improvement of polygenic traits, GBLUP is the most reliable model and typically provides the highest prediction accuracy for highly polygenic traits, while the Bayesian models are preferable for traits controlled by few large effect loci in genomic selection (Legarra et al., 2015; Yáñez et al., 2023)

Genomic selection improves accuracy of up to 19% compared to pedigree selection. A key consideration for the commercial implementation of genomic selection

in shellfish aquaculture is the high cost of genotyping. Reference population size and marker density are two key factors for effectively reducing the cost of genomic selection (Song et al., 2022). Applying a low density SNP panel is one way to increase economic viability of genomic selection (Kriaridou et al., 2020). The prediction accuracies for genomic models in our study ranged from 0.32 to 0.48 for resistance to *V. splendidus* (with SNP densities ranging from 500 to ~3400), whereas the accuracy of PBLUP was 0.36. This result is slightly lower than the ranges reported for disease-related traits in other bivalve species. For instance, genomic selection prediction accuracies from GBLUP models for resistance to Ostreid herpesvirus (OsHV-1-lvar) ranged from 0.68 to 0.76 in the Pacific oyster (Gutierrez et al., 2020). Prediction accuracies for growth-related traits using the GBLUP model in other bivalves are relatively similar, e.g., 0.52-0.73 in the Pacific oyster (Gutierrez et al., 2018; Jourdan et al., 2023), 0.67-0.79 in the Portuguese oyster (Vu et al., 2021), and > 0.83 in European flat oyster (Penaloza et al., 2022). Other reports on genomic prediction accuracies for disease-related traits in finfish aquaculture species show the prediction accuracies as low as 0.21, reviewed in Houston et al. (2020) and 0.25 - 0.48 for growth related-traits in the Zhikong scallop (Wang et al., 2018). However, this result highlight that genomic selection is a useful approach to increase resistance to *V. splendidus* in our blue mussel populations.

Overall, our results showed that genomic methods predict better accuracy (25 - 33%) for resistance to *V. splendidus* using ~2000 SNPs in a family-based design compared to pedigree-based estimation. This indicates that substantial improvements in the rate of genetic gain can be achieved through genomics-based selection techniques. It also increases the possibility of a low-density genomic selection approach for *Vibrio* resistance in mussel breeding, as low-density genotyping can be substantially cheaper than high-density SNP arrays. Furthermore, studies on disease

resistance in the Pacific oyster, growth traits in the European flat oyster, and heat tolerance in the Pacific abalone have shown that low-density SNP panels of around 1000-2000 SNPs can achieve EBV accuracies similar to those obtained with medium-density arrays (Gutierrez et al., 2020; Kriaridou et al., 2020; Liu et al., 2022; Penaloza et al., 2022). Similar findings in multiple aquatic species have shown that low-density panels can achieve higher accuracies than the pedigree-based approach, making them a feasible alternative for identifying candidates with the highest genetic merit for complex traits such as growth and disease resistance (Kriaridou et al., 2020).

Although the mussel genome is 1.4 Gb in size, our study suggests that a relatively low number of genetic markers can still achieve high prediction accuracy, with a rapid LD decay observed across all populations. Additionally, both the training and validation datasets comprised closely related animals (half-sibs/full-sibs). These individuals will share large genomic segments, which can be capture by few markers. However, as the genetic distance between the training and validation sets increases, genomic prediction accuracy is likely to decrease (Palaiokostas et al., 2019). Therefore, regular mating among close relatives of breeding candidates is required to maintain prediction accuracy (Gutierrez et al., 2020). Moreover, additional populations with different effective population sizes, genetic backgrounds, and degrees of relatedness should be assessed to obtain estimates expected in practical breeding programs.

Although our results highlight the possibility of reducing the genotyping costs associated with genomic prediction approaches, caution should be exercised regarding the smallest marker density. Our study found that using only 500 SNPs in the GBLUP model resulted in an estimated decrease in the accuracy of genomic breeding values (GEBVs) for resistance to *V. splendidus* by 11% compared to PBLUP.

It's important to note that when both pedigree information and genotypes are available, using ssGBLUP is preferable, as it demonstrates superior accuracy compared to PBLUP. Furthermore, our findings emphasize that annotated SNPs on the *M. edulis* genome provided more information about the studied population and led to higher prediction accuracy than using all SNPs in either the GBLUP or ssGBLUP model. This difference could be due to even distribution of phenotypes among genotyped individuals (50% mortality), or unannotated markers may introduce noise, thereby affecting the accuracy of GEBV estimation. Further investigations using more SNPs, and larger reference population hold potential for genomic selection to further increase the prediction accuracy for host resistance to *V. splendidus* in farmed mussel populations.

5. Conclusions

Our study estimated moderate heritability for resistance to *V. splendidus* in blue mussel populations using both pedigree and genomic data from a challenge experiment. GWAS analysis suggests that the trait is polygenic, indicating that genomic selection is more effective than marker-assisted selection. We found that genomic selection can improve accuracy by up to 19% compared to pedigree-based selection. Additionally, our results show the potential for reducing the number of markers, which could make genomic selection more cost-effective. Overall, selective breeding appears to be a promising approach to enhance resistance to *V. splendidus* in blue mussels, and genomic selection could significantly increase genetic gains.

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Author contributions

Munusamy Ajithkumar: Data curation, Formal analysis, Software, Visualization, Writing - original draft, and Writing - review & editing. Jonathan D’Ambrosio: Software, Writing - review & editing. Marie-Agnès Travers: Methodology, Writing - review & editing. Romain Morvezen: Supervision, Software, Writing - review & editing. Lionel Dégremont: Conceptualization, Funding acquisition, Methodology, Supervision; Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available upon reasonable request

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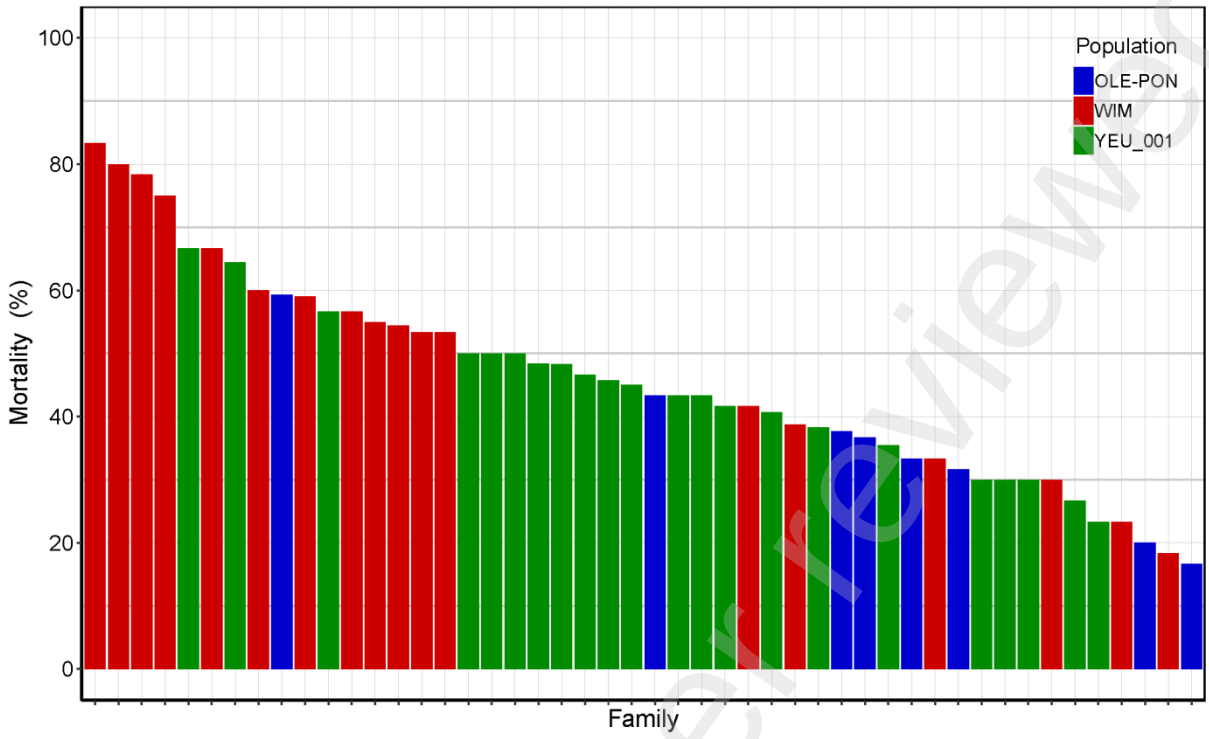

Figure 1: Final cumulative mortality 72 hours post-injection for each family. Each bar represents a family and each color represent a population.

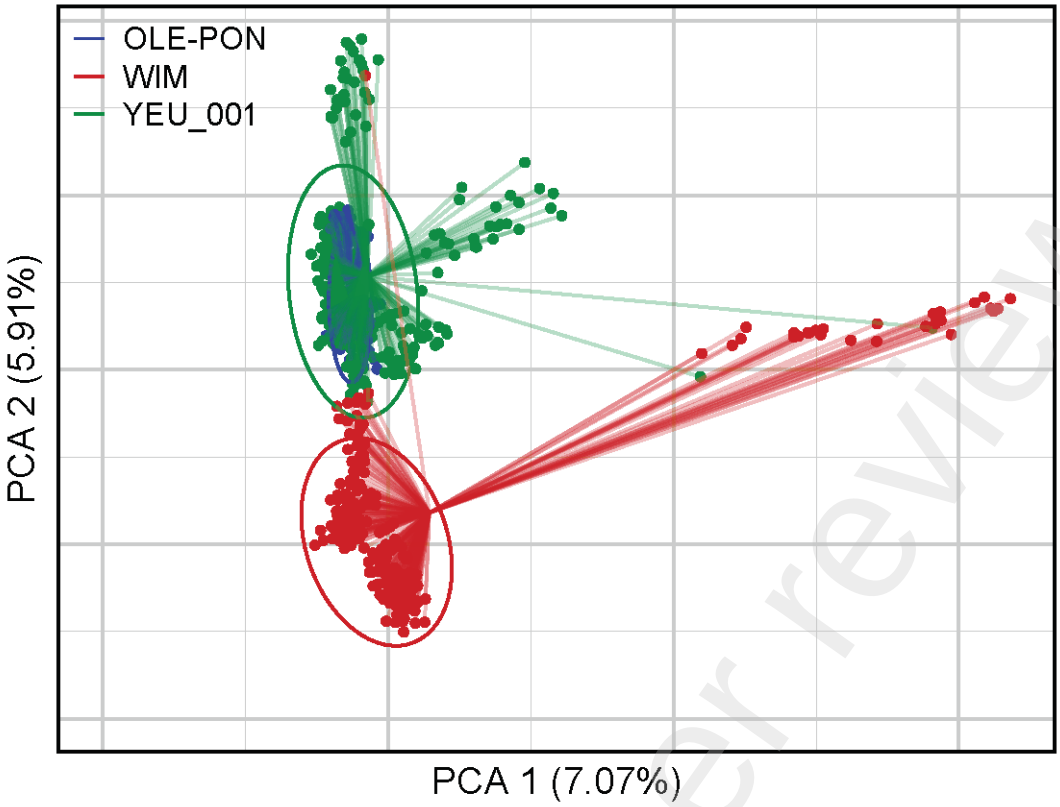

Figure 2: First two axes and associated variances of the principal component analysis (PCA) of the genetic diversity among the three populations of *Mytilus edulis*. The ellipses are constructed with axes defined as 1.5 times the standard deviation of the projections of individual coordinates on the axes. PCA was performed with 644 individuals and 3096 SNPs.

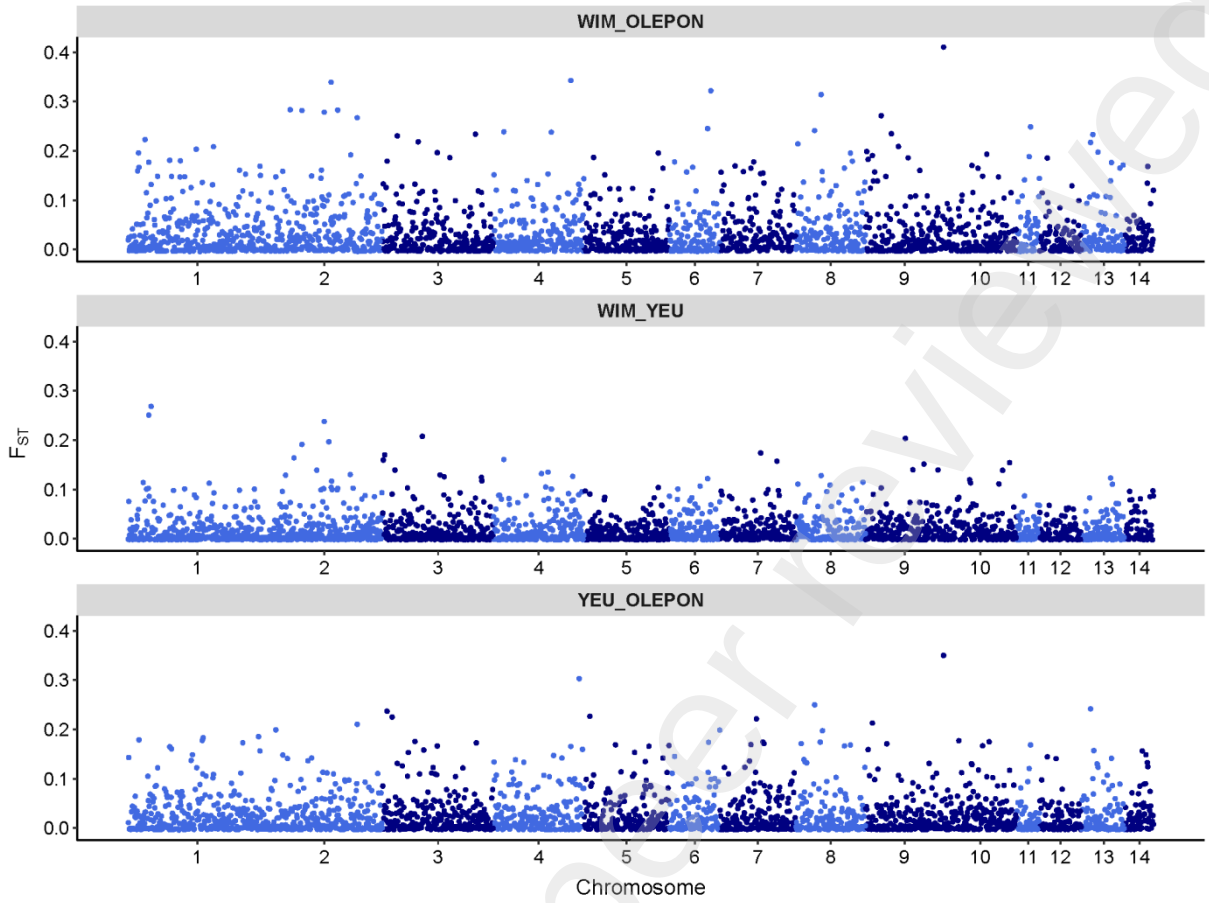

Figure 3: Genomic distribution of fixation index (F_{ST}) values as a function of chromosome position in the mussel genome for different studied population

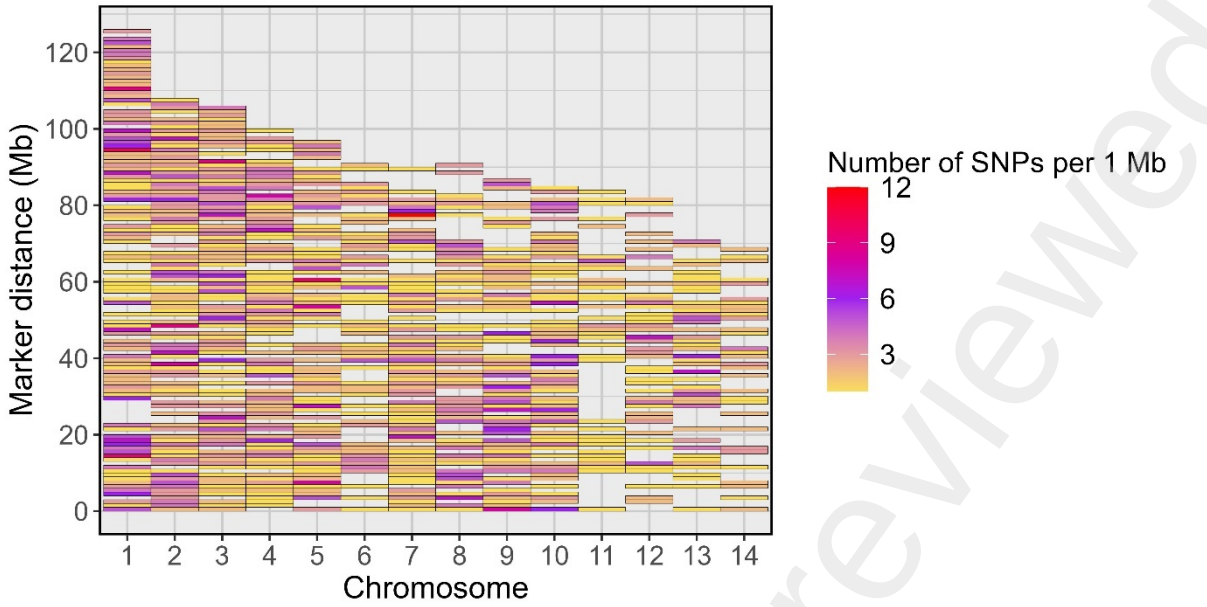

Figure 4: Identification of high-quality SNPs and their distribution across the 14 chromosomes of *M. edulis*. The gradient colors from yellow to red denote the increase of SNP density within 1 Mb interval.

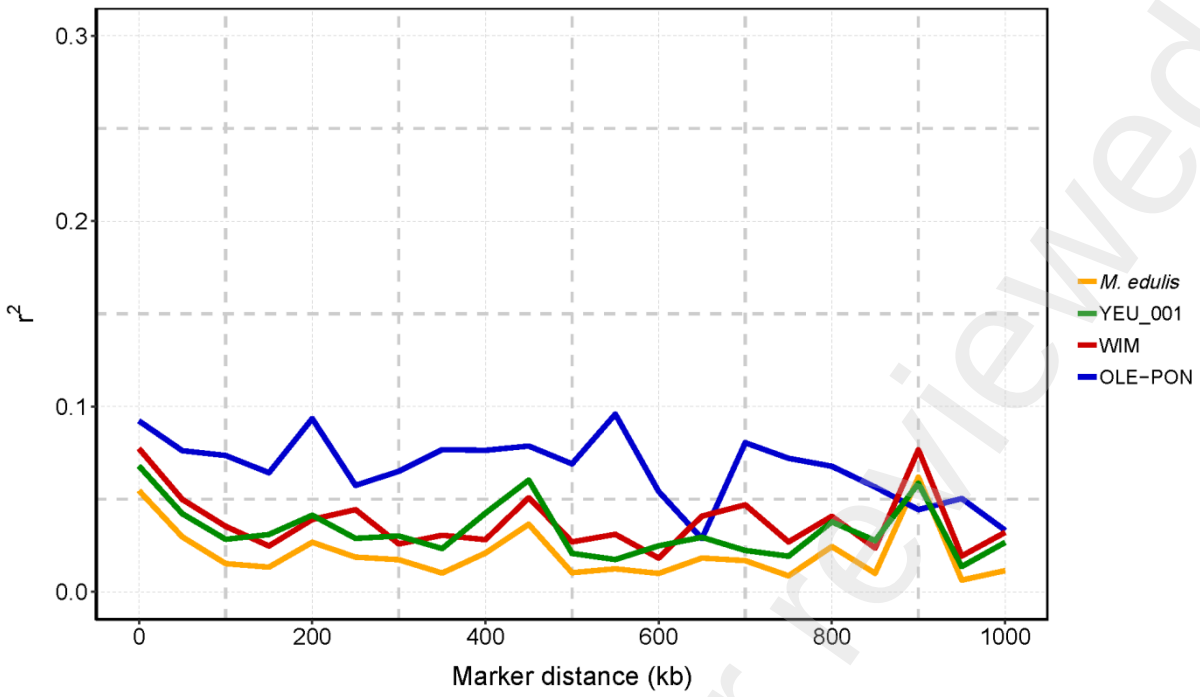

Figure 5: Linkage disequilibrium (r^2) decay with physical distance between markers in each population and overall challenged to *V. splendidus*. The X-axis is the physical location, and the Y-axis is the linkage disequilibrium value (r2).

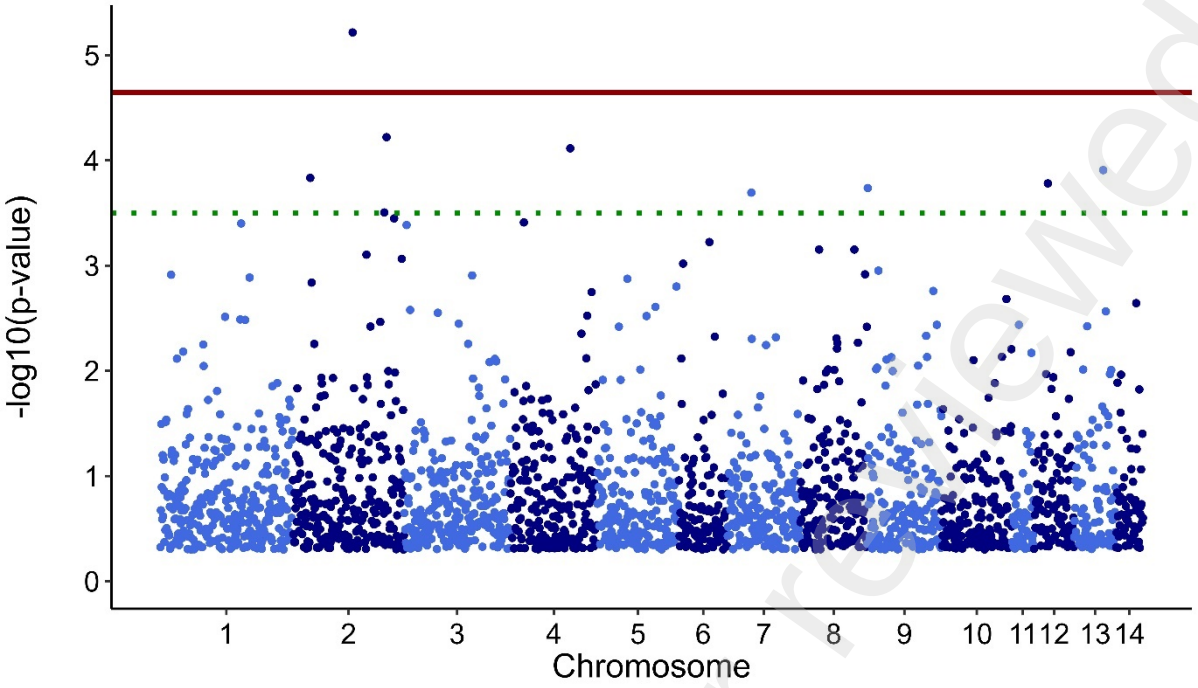

Figure 6: Manhattan plot of GWAS with p-values distributed across different chromosomes. Horizontal red line represents the 5% genome-wide significance threshold and the green line is the 5% chromosome-wide significance threshold calculated with the Bonferroni correction.

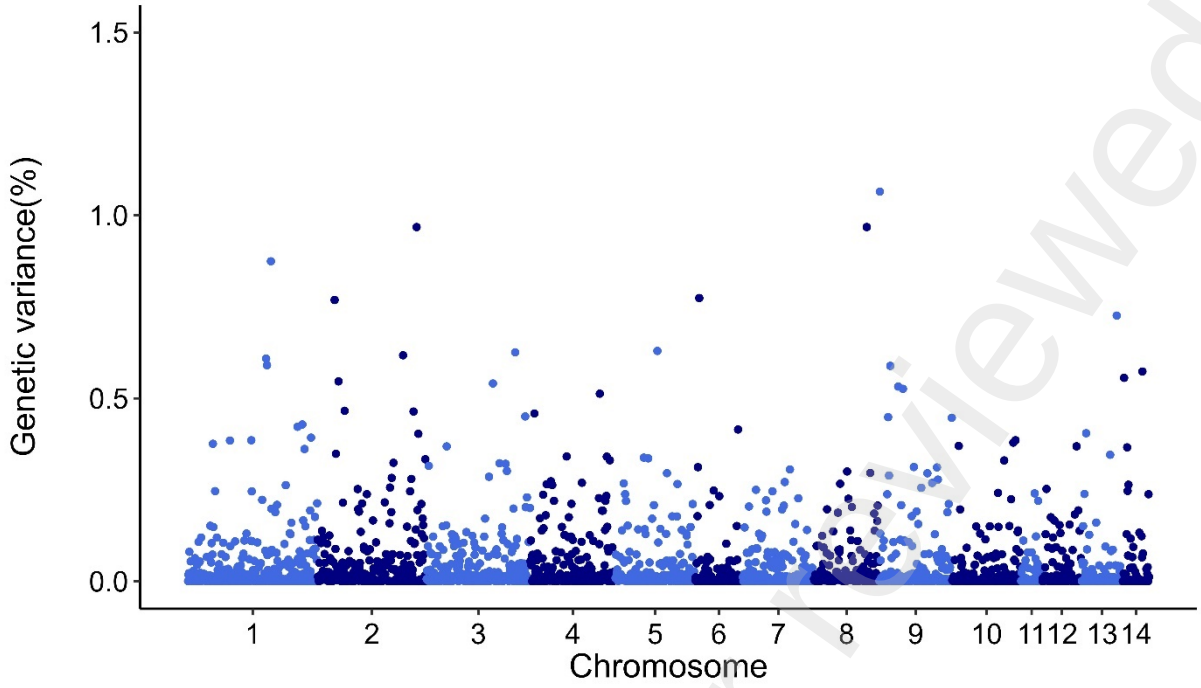

Figure 7: Manhattan plot of genetic variance explained by each SNP for resistance to *V. splendidus* in *M. edulis* using ssGBLUP approach. In X axis SNP per chromosome and Y axis percentage of genetic variance explained per each SNP.

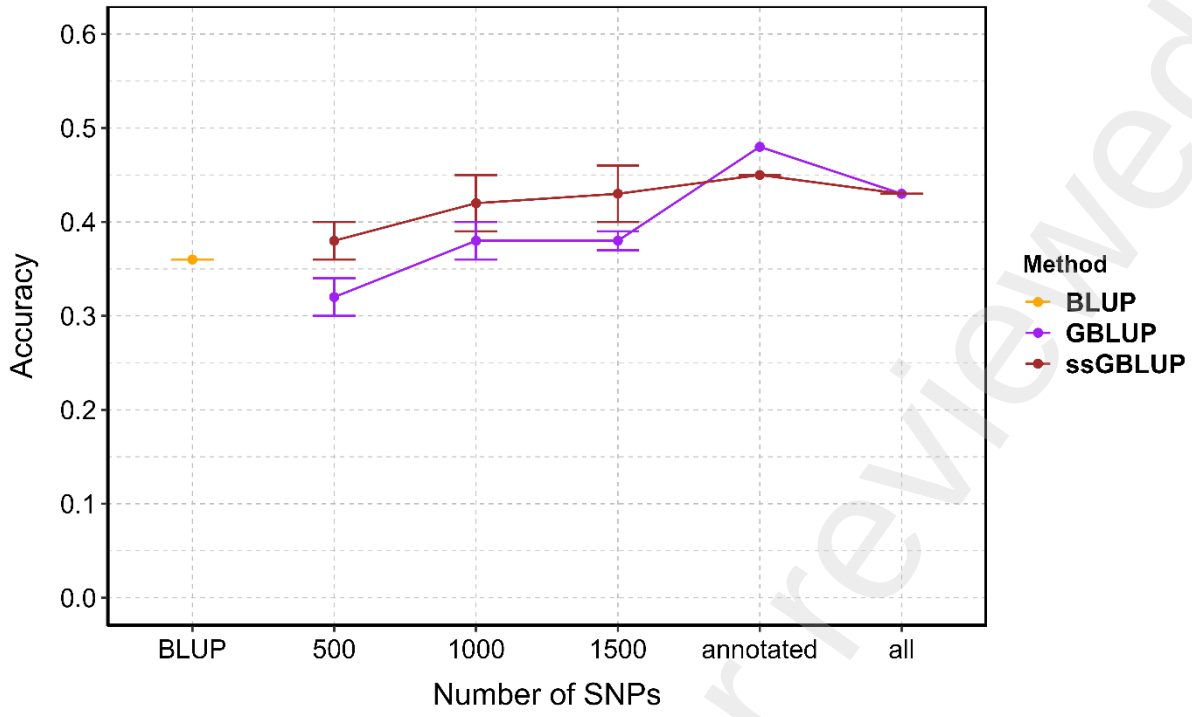

Figure 8: The estimated prediction accuracy of *Vibrio splendidus* resistance *in Mytilus edulis* using PBLUP, GBLUP and ssGBLUP across different marker densities. Each point is the average of 5 replicates. Error bars represent the standard error of the mean of 5 replicates. PBLUP - Pedigree based breeding values using all phenotyped animals, respectively. GBLUP - Genomic breeding values from only genotype animals, and ssGBLUP - Genomic breeding values from all genotyped and phenotyped animals obtained with a combined relationship matrix (H).

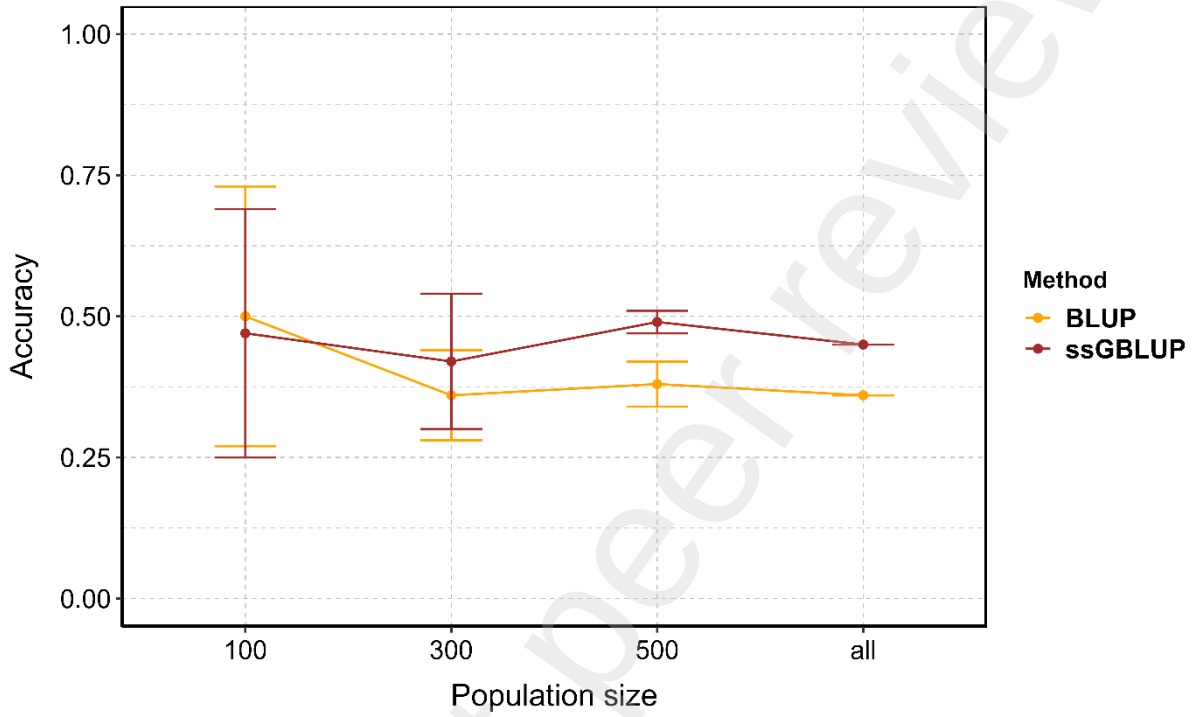

Figure 9: The estimated prediction accuracy of *Vibrio splendidus* resistance in *Mytilus edulis* using different training population size and fixed SNP density (annotated SNPs, ~2,400). Each point is the average of 5 replicates. Error bars represent the standard error of the mean of 5 replicates.

Table 1: Summary of the experimental infection using the pathogenic strain 14/053 2T1 of *Vibrio splendidus* in *Mytilus edulis*

	Phenotyped	**Genotyped**
Number of families	48	48
Total number of mussels	2160	768
Mean number of mussels/family	45	14.5 (Min:13; Max:16)
Mean mortality	47.3%	50%

Table 2: Pairwise FST between populations of *Mytilus edulis*

	WIM	YEU_001
OLE-PON	0.03	0.03
WIM		0.02

Table 3: Variance components and genetics parameters for *V. splendidus* resistance in *M. edulis*

Method	**Model**	**Relationship matrix**	**h^2 (± se)**
PBLUP	Linear	A	0.22 (0.06)
	Gibbs sampling	A	0.31 (0.05)
GBLUP	Linear	G	0.33 (0.11)
	Gibbs sampling	G	0.36 (0.05)
ssGBLUP	Linear	H	0.28 (0.08)
	Gibbs sampling	H	0.33 (0.05)

σ_a^2: Additive genetic variance; σ_e^2: Residual variance; σ_p^2: Phenotypic variance; A: Pedigree based relationship matrix; G: Genomic based relationship matrix; H: genomic and pedigree combined relationship matrix; Linear: Linear mixed model; h^2: heritability. The h^2 for linear model on observed scale transferred into underlying scale using the formulae from Dempster and linear (1950).

Table 4: The significant SNPs detected in GWAS analysis (ssGBLUP) ranked with respect to level of significance. Position = Physical position of SNP on the chromosome; A1 & A2 = Minor & major alleles, respectively; MAF = Minor allele frequency; P =Significance value; varG = percentage of genetic variance explained by SNP

SNP_ID	Chromosome	Position	A1	A2	MAF	P-value	VarG (%)
AX-604335979	2	61,614,609	A	C	0.07	5.21	0.10
AX-603804982	2	91,444,473	A	T	0.04	4.22	0.04
AX-604452846	4	74,391,047	C	T	0.04	4.11	0.04
AX-603077107	13	50,001,059	A	T	0.11	3.9	0.04
AX-604514378	2	12,942,241	T	C	0.25	3.83	0.77
AX-604131039	12	33,942,361	G	A	0.06	3.79	0.09
AX-604710899	9	3,56,838	A	T	0.32	3.74	1.06
AX-604289929	7	30,079,060	C	T	0.09	3.64	0.22