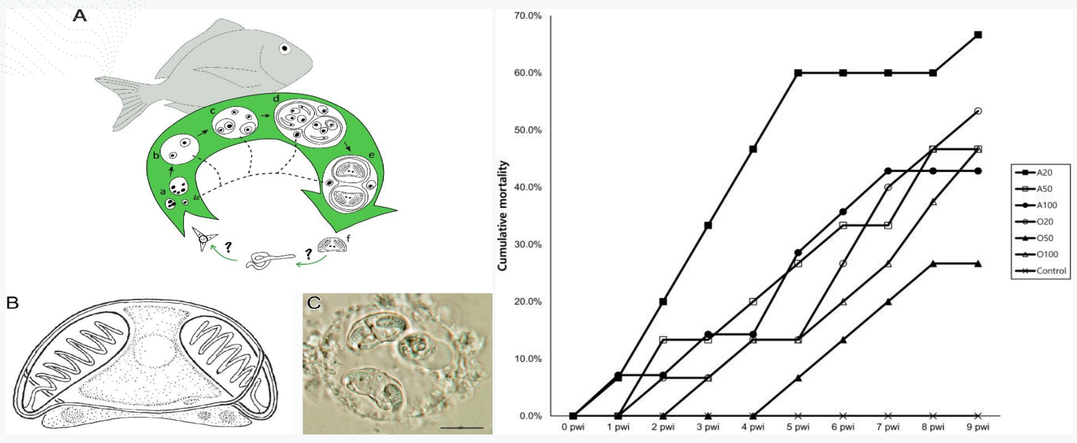
APPLICATION OF GENOME-WIDE ASSOCIATION STUDY AND GENOMIC PREDICTION FOR ENHANCING *Enteromyxum leei* RESISTANCE IN OLIVE FLOUNDER, *Paralichthys olivaceus*

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Introduction

Enteromyxum leei (*E. leei*) is an intestinal myxozoan parasite of fish. This parasite leads to loss of appetite, poor food conversion rate and decreased growth such as emaciation and cachexia in fish. Therefore, it causes high mortalities and significant financial losses within South Korean aquaculture industries. In this study, we focused to identify the resistance to *E. leei*-associated single nucleotide polymorphisms (SNPs) using GWAS in olive flounder. Additionally, genomic prediction analysis was conducted to evaluate the potential of genomic selection for improving disease resistance in olive flounder.



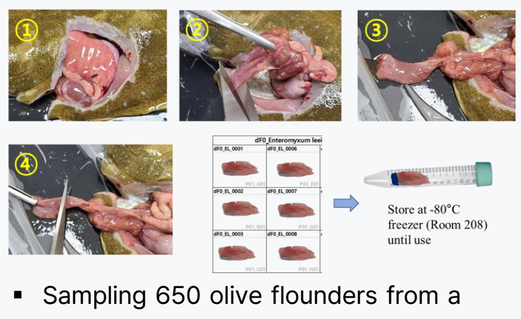
(A) Life cycle of *E. leei*. In the intestinal epithelium of the fish, proliferative (a-c) and sporogonic (d-f) development occurs. (B) Spore of *E. leei* (after Diamant et al. 1994). (C) Disporus sporoblast in a fresh smear of posterior intestine. Scale bar = 10 µm.

Reference: Atienza, I.E. (2013).

Cumulative mortality of fish experimentally oral or anal inoculated with *E. leei* and control group. The seven groups include (a) control, (b-d) anal 20, 50, and 100 µL of inoculum/fish (A20, A50, and A100), (e-g) oral 20, 50, and 100 µL of inoculum/fish (O20, O50, and O100).

Reference: Shin, S. P., Jin, C. N., Sohn, H., & Lee, J. (2022).

Materials & Methods



- Sampling 650 olive flounders from a natural *E. leei* outbreak farm
- Measurement of traits and collection of intestinal tissues



- Genotyping of animals
- Affymetrix® Axiom® myDesign™ Genotyping Arrays

Traits:

1. Infection status
2. rCF
3. Absolute copies
4. Spore per cell
5. Abs log
6. Spc log

- Extraction gDNA
- qPCR for the *E. leei* 28S rDNA
- Absolute copies, spores per cell, infectious status, etc.

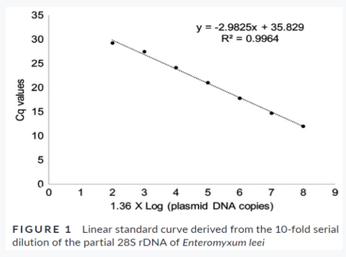


FIGURE 1 Linear standard curve derived from the 10-fold serial dilution of the partial 28S rDNA of *Enteromyxum leei*

Reference: Sohn, H, Jin, CN, Kang, BJ, Shin, SP, Lee, J (2021)

GWAS

- LMM-based GWAS using 61,024 SNPs
- Wald test with Bonferroni threshold (8.19×10^{-7})
- Manhattan and QQ plot visualization

Predictive ability

- Bayesian C model using BGLR package
- 5-fold cross-validation with 10 replicates
- Prediction ability estimated by correlation between phenotype and gEBV

Intestinal tissues from 650 naturally infected olive flounders were collected, and *E. leei* infection intensity was quantified using real-time PCR. After quality control, 627 individuals and 61,024 SNPs were retained for analysis. GWAS was conducted to identify SNPs associated with disease resistance traits, and genomic prediction was evaluated using GBLUP and Bayesian C models with cross-validation.

Results

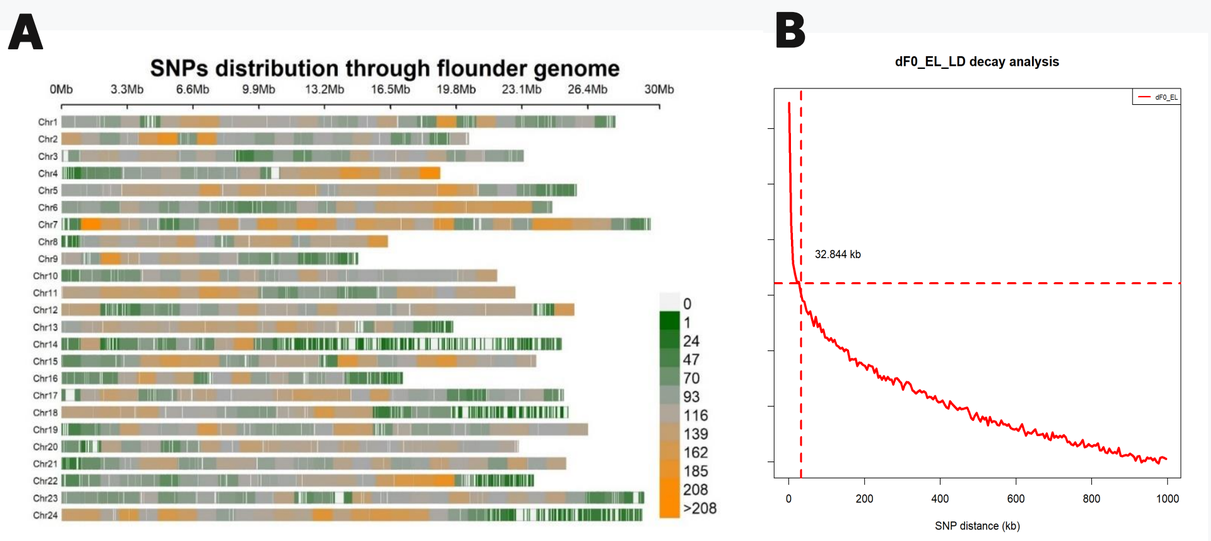


Figure 1. Visualization of population structure. (A) SNP density plot. Given that the assembled olive flounder reference genome is 588.22 Mb, and one SNP represents an average distance of 32.844 kb, a minimum of 17910 SNPs would be required for full genome coverage. The average marker density of the SNP panel was 103.74 SNPs/Mb. **(B) Genome-wide linkage disequilibrium (LD) decay plots.** Fifty percent LD decay was observed when the distance between adjacent SNPs was 32.844 kb.

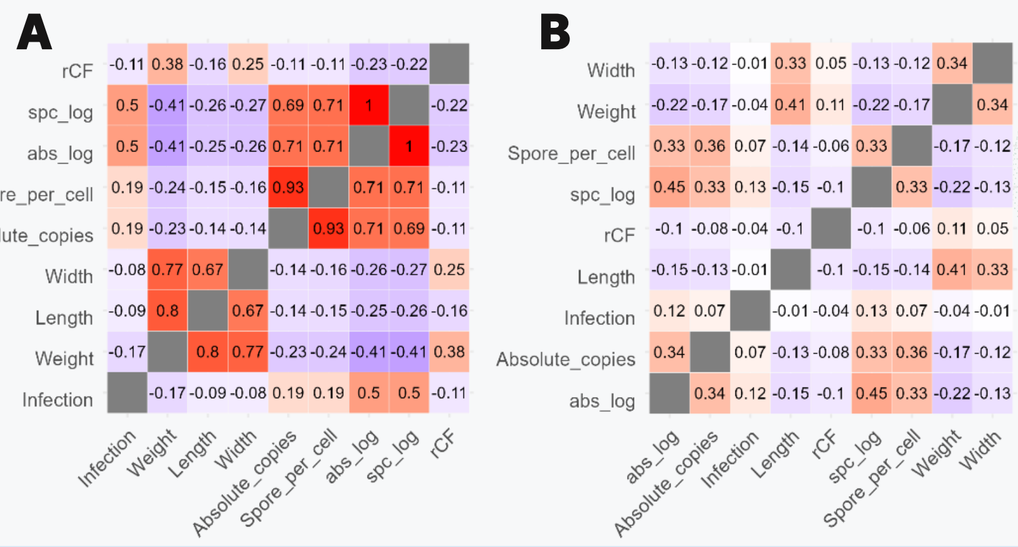


Figure 2. Heat map of the (A) phenotype correlation and (B) genotype correlation of flounder traits. The absolute copies, spores per cell, Abs_log, and Spc_log showed moderate genetic correlations and high phenotypic correlations. The infection status showed weak or negative correlations with all other traits, both genetically and phenotypically.

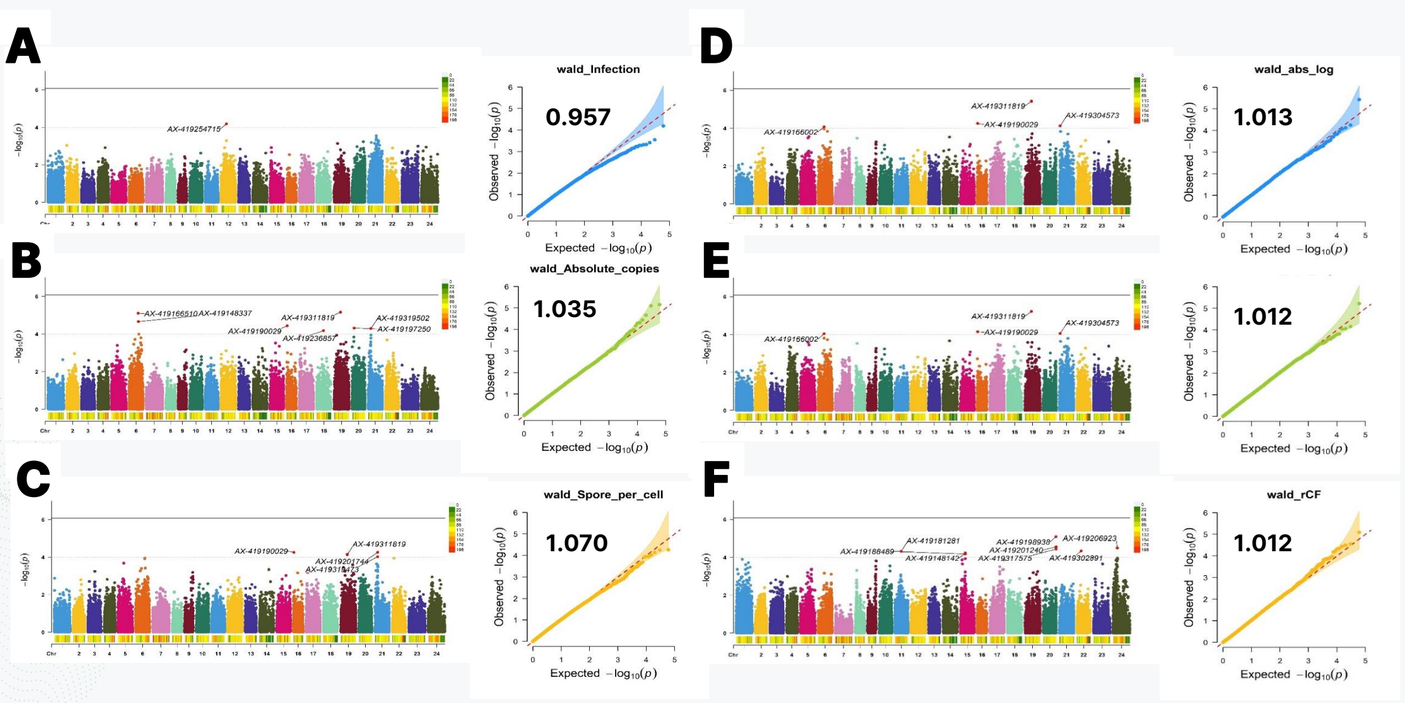


Figure 3. Manhattan plots and QQ plots of (A) infection, (B) absolute copies, (C) spore per cell, (D) Abs_log, (E) Spc_log, and (F) relative condition factor (rCF). The Bonferroni and suggestive thresholds are marked with bold black lines and dash lines, respectively. SNPs above the suggestive cutoff were labeled and marked in red color

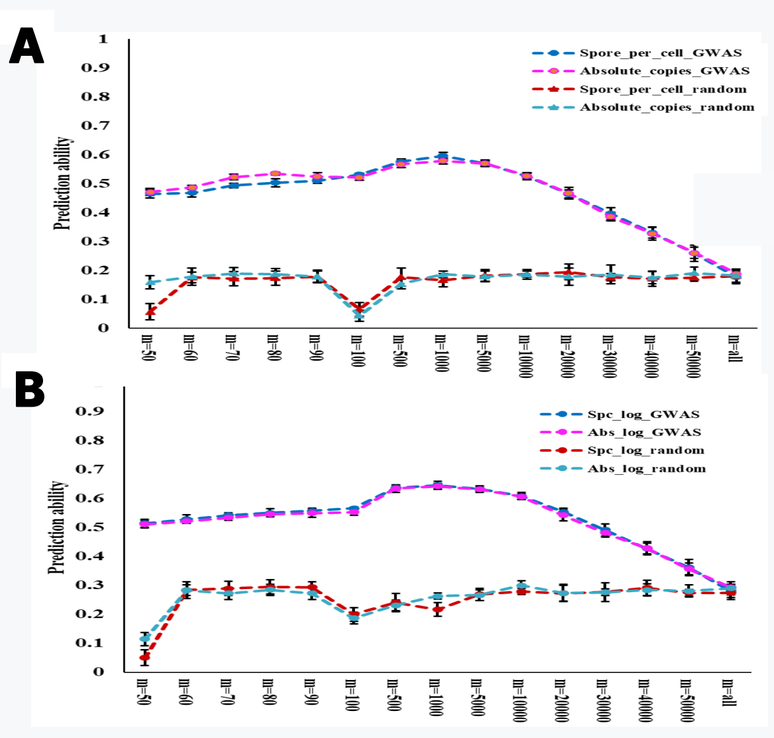


Figure 4. Prediction ability of (A) absolute copies and spore per cell, (B) Abs_log and Spc_log by the Bayesian C method using randomly selected or GWAS-based SNPs

GWAS identified 20 SNPs suggestively associated with disease-related traits ($p < 1 \times 10^{-4}$), although none reached genome-wide significance. Several SNPs were located near candidate genes involved in protein catabolism and signal transduction. Moderate heritability estimates suggested a genetic contribution to *E. leei* resistance. Genomic prediction accuracy improved when GWAS-selected SNPs were used, with the highest accuracies observed for log-transformed traits (0.660 for Spc_log and 0.657 for Abs_log) using the Bayesian C model. In addition, GWAS-associated SNPs showed higher predictive ability than randomly selected markers. GO enrichment analysis indicated that candidate genes were mainly related to protein catabolic processes, immune regulation, and PI3K/AKT signal transduction pathways.

Conclusion

This study demonstrated the applicability of GWAS and genomic prediction for improving resistance to *Enteromyxum leei* in olive flounder. Although no genome-wide significant SNPs were identified, several suggestive markers and candidate genes associated with disease resistance were detected. Moderate heritability and improved prediction accuracy using GWAS-selected SNPs indicate that genomic selection is a promising strategy for developing *E. leei*-resistant broodstock.

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