Implementation of genomic selection on production and quality traits and linkage disequilibrium in Crassostrea gigas

Antoine Jourdan, Pierre Boudry, Florian Enez, Pierrick Haffray, F Chenier, E Vetois, François Allal, Pascale Le Roy, Florence Phocas, Jérôme Bugeon, et al.

567. Implementation of genomic selection on production and quality traits and linkage disequilibrium in *Crassostrea gigas*

A. Jourdan[1*], P. Boudry[2], F. Enez[1], P. Haffray[1], F. Chenier[3], E. Vetois[4], F. Allal[5], P. Leroy[6], F. Phocas[7], J. Bugeon[8], L. Degremont[9] and R. Morvezen[1]

[1]SYSAAF (French Poultry and Aquaculture Breeders Technical Centre), 35042 Rennes, France; [2]Ifremer, Department of Biological resources and Environment, 29280 Plouzané, France; [3]FRANCE NAISSAIN, 85230 Bouin, France; [4]SATMAR, 50760 Gatteville, France; [5]MARBEC, Université Montpellier, CNRS, Ifremer, IRD, Palavas-les-Flots, France; [6]INRAE, Physiology, Environment and Genetics for Animals and Livestock Systems, 35590 Saint Gilles, France; [7]Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy-en-Josas, France; [8]INRAE, LPGP, Bâtiment 16A, Allée Henri Fabre,Campus de Beaulieu, Rennes, 35042, France; [9]Ifremer, RBE, ASIM, Avenue de Mus de Loup – Ronce les Bains, 17390 La Tremblade, France; ajourdan@ifremer.fr

Abstract

The recent progress and cost reduction of genotyping technologies allows for the implementation of genomic selection (GS) on more and more cultivated species. In this study, we explore the genomic determinism of commercial traits and test the effectiveness and the possible cost reduction of GS in breeding programs of the cupped oyster *Crassostrea gigas*. Two populations of more than 1000 individuals have been phenotyped and genotyped for 40,000 SNP (Guitierez *et al.*, 2017). Heritability was estimated to be moderate for commercial traits of interest (between 0.19 and 0.34). The accuracy of the genomic prediction models outperformed the classical selection on pedigree by 22 to 55%. A limited linkage disequilibrium (LD) level (less than 0.1) was observed. These results suggest that the use of GS in oyster breeding can improve the selection of breeding candidates to enhance commercial traits but need a specific account and exploration of the very low LD.

Introduction

Growth, morphology, and color are essential characteristics for the oyster farming industry and have been the focus of breeding programs. As for other aquaculture species (salmon, sea bass, sea bream), the recent development of genomic tools in the cupped oyster (57K SNP genotyping chip) opens up the possibility of integrating genetic markers into breeding programs by through genomic selection (GS) (Hollenbeck and Johnston, 2018; Boudry *et al.*,2021). According to Lillehammer *et al.* (2013) and Guitierez *et al.* (2018), a few hundred to a few thousand markers could be enough to significantly increase the quality of predictions compared to the standard method of selection on relatives but this requires a sufficiently high LD. In this study we propose to estimate the genetic parameters and to test the efficiency of GS on two commercial breeding programs, on traits of interest, by comparing the accuracy of GS with a standard, pedigree-based approach and evaluating the usefulness of the available genotyping tool, by estimating SNP quality and LD between them.

Materials & methods

Biological material. The two populations in this study were derived from the breeding Company Vendée Naissain (Bouin, France) and SATMAR (Gattevile, France). They were reared on sea shore in Normandy. The first population consists of 1,261 offspring generated from 130 parents (64 males/69 females). The second population comprises 1,136 individuals including the 104 parents (59 males/45 females).

Phenotyping. All individuals were phenotyped for the same traits in both populations: total weight before opening, length, width and height of the shell, upper and the lower valve and wet meat weight. Meat yield was estimated using residue of linear regression between meat weight and total weight. A photo of all

R.F. Veerkamp and Y. de Haas (eds)

DOI: 10.3920/978-90-8686-940-4_567, © A. Jourdan *et al.* 2022

the individuals was taken using a Canon EOS 2000D camera. Images were analysed with an automatic image analysis pipeline using the FIJI software to define surface measurements of the two valves and measurements of the mean external colour of the upper valve estimated in the CIE LAB space.

Genotyping. All individuals of the two populations, including parents and offspring, were genotyped on a bi-species Axiom Affimetrix 57K chip with 40,625 markers for the cupped oyster. A quality analysis carried out on the AxAS software made it possible to retain 14,469 quality markers.

Genetic parameter estimation. Genetic parameters and estimated breeding values (EBV) were estimated with BLUPF90 software suite under animal model. EBV were estimated using pedigree or genomic BLUP (Best Linear Unbiased Prediction). For each, the prediction accuracy was estimated in 40-replicated five-fold crossvalidations (training population 80%, validation population 20%). Prediction accuracy was calculated as the correlation between the predicted EBVs and the actual phenotypes of the validation population, divided by the square root of the heritability estimated. Prediction accuracy values were compared between the pedigree and genomic approaches.

Linkage disequilibrium (LD). The 14,469 good quality markers were blasted on the new reference genome (cgigas_uk_roslin_v1, GCA_902806645.1) in order to map the SNPs. Approximately 12,581 SNPs were well positioned on the 10 linkage groups. To estimate LD, we used the squared correlation based on genotypic allele counts (number of non-reference alleles at each locus) using the PLINK v1.9 software (Chang *et al.*, 2015). Pairwise LD between all SNPs in a 75-Mb long window were derived for each chromosome. The mean r^2 values was calculated for every 50 kb and covering up to 1000 kb.

Results

All of the traits measured have coefficient of variation between 13 and 53%. The two populations were phenotyped at three years post fertilization. The heritability estimated are limited to moderate ranging from 0.04 to 0.34 for color traits and between 0.19 and 0.27 for biometric traits. The gain in prediction accuracy of GBLUP compared to pedigree BLUP on classically measured and selected growth traits is presented in Figure 1. This figure shows GS is superior to selection on pedigree in most cases, with however median levels of gain in accuracy which vary according to the trait studied with values between 22.6 and 55%. The mapping of the 13,000 markers on the most recent assembly (cgigas_uk_roslin_v1, GCA_902806645.1) of the cupped oyster genome shows an important coverage heterogeneity. For example, only 159 markers belonging to chromosome 2 (73 Mb) were genotyped, while more than 2,000 were genotyped for chromosome 10 (58 Mb). The LD is represented by the r^2 as a function of the physical distance over the assembly of the oyster genome. We can observe in Figure 2 that we have a low level of DL with a slow decrease as a function of the distance. LD level is very low even over a short distance between markers (less than 0.1).

Discussion

In our study, we show moderate levels of heritability for production and quality traits. This level of heritability seems lower than when compared to other recent publications in Pacific or Portuguese oysters in Asia (Kong *et al.*, 2015). Difference in rearing methods (suspended *vs* bag) and practices (immersed vs on shore) may have a significant impact on expression of additive genetic components of oyster growth (Sheridan, 1997). Under this hypothesis, estimates of the heritability are moderate but adapted for setting up breeding programs in Europe as they are estimated in the environment of rearing. The medium density chip allows to investigate application of GS in Pacific oysters in other traits than OHsV1 genetic resistance (Guittierez *et al.,* 2017). The results obtained from cross-validation on genomic prediction are similar to

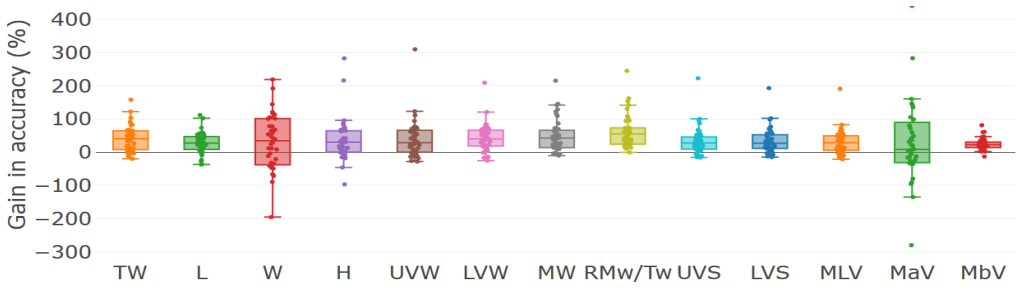

Figure 1. Boxplot of prediction accuracy gain (%) between GBLUP and BLUP for all the 40 simulations and all traits. TW = total weight; L = length; W = width; H = height; UVW = upper valve weight; LVW = lower valve weight; MW = wet meat weight; RMw/Tw = residual meat weight/total weight; UVS = upper valve surface; LVS = lower valve surface; MLV = mean upper valve L value; MaV = mean upper valve a value; MbV = mean upper valve b value.

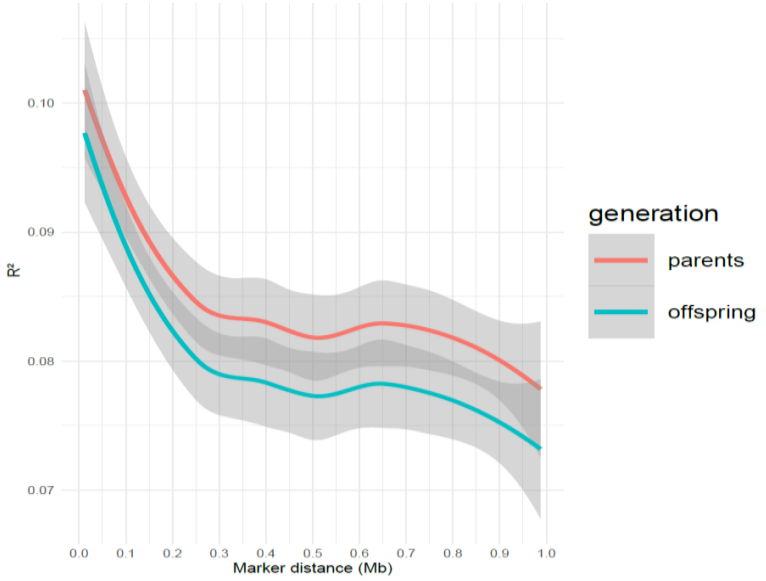

Figure 2. Decay of linkage disequilibrium (LD) with physical distance between markers for the first hatchery population.

those obtained on other aquaculture species with a clear improvement in the accuracy of the prediction. This therefore reinforces the interest of using genomic tools in the breeding programs of the cupped oyster.

One important result is very limited LD observed in two distinct populations. Indeed, to make GS more cost-effective, it is necessary to reduce the number of markers to be genotyped or to go through other methods of GS (such as GWAS) with fewer individuals to genotype. To achieve this, it is essential to have a sufficiently high level of LD in the population. Some parameters can influence the level of LD such as the number of generations for which they were raised in isolation or reduce the effective size (Ne) which would result into increase the levels of relatedness between individuals and therefore a greater extent of LD. Our LD levels are consistent with recent reports describing low levels of LD in wild populations of *C.gigas*

(Guittierez *et al.*, 2017). This result is questioning about the possibility to identify QTLs with repeated and conserved effects across generations with the actual genomic tools developed.

Acknowledgements

The data presented here were obtained in the Quality-Huitre project which received funding from the European Maritime and Fisheries Fund (EMFF) research grant number PFEA470018FA1000011. and by National Association for Research Technology (ANRT) with CIFRE CONVENTION no. 2019/1511. I would like to add a special thanks to Mr. J-B Lamy who accompanied me at the begining of this study.

References

Boudry, P., Allal, F., Aslam, M. L., Bargelloni, L., Bean, T. P., *et al.* (2021). Current status and potential of genomic selection to improve selective breeding in the main aquaculture species of International Council for the Exploration of the Sea (ICES) member countries. *Aquac Rep*, *20*, 100700. https://doi.org/10.1016/j.aqrep.2021.100700

Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., *et al.* (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*, *4*(1), s13742-015. https://doi.org/10.1186/s13742-015-0047-8

Gutierrez, A. P., Turner, F., Gharbi, K., Talbot, R., Lowe, N. R., *et al.* (2017). Development of a medium density combined-species SNP array for Pacific and European oysters (Crassostrea gigas and Ostrea edulis). *G3-Genes Genom Genet*, *7*(7), 2209-2218. https://doi.org/10.1534/g3.117.041780

Gutierrez, A. P., Matika, O., Bean, T. P., & Houston, R. D. (2018). Genomic selection for growth traits in Pacific oyster (Crassostrea gigas): potential of low-density marker panels for breeding value prediction. *Front genet*, *9*, 391. https://doi.org/10.3389/fgene.2018.00391

Gutierrez, A. P., Bean, T. P., Hooper, C., Stenton, C. A., Sanders, M. B., *et al.* (2018). A genome-wide association study for host resistance to ostreid herpesvirus in Pacific oysters (Crassostrea gigas). *G3-Genes Genom Genet*, *8*(4), 1273-1280. https://doi.org/10.1534/g3.118.200113

Hollenbeck, C. M., & Johnston, I. A. (2018). Genomic tools and selective breeding in molluscs. *Front genet*, *9*, 253. https://doi.org/10.3389/fgene.2018.00253

Kong, N., Li, Q., Yu, H., & Kong, L. F. (2015). Heritability estimates for growth-related traits in the Pacific oyster (Crassostrea gigas) using a molecular pedigree. Aquac Res, *46*(2), 499-508. https://doi.org/10.1111/are.12205

Lillehammer, M., Meuwissen, T. H., & Sonesson, A. K. (2013). A low-marker density implementation of genomic selection in aquaculture using within-family genomic breeding values. *Genet Sel Evol*, *45*(1), 1-8. https://doi.org/10.1186/1297-9686-45-39

Sheridan, A. K. (1997). Genetic improvement of oyster production – a critique. *Aquaculture*, *153*(3-4), 165-179. https://doi.org/10.1016/S0044-8486(97)00024-0