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SELECTION FOR *Piscirickettsia salmonis* (SRS) RESISTANCE IN ATLANTIC SALMON (*Salmo salar*) USING GENOTYPING BY SEQUENCING (GBS)

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SUMMARY

The genetic variance for SRS resistance in Atlantic salmon was estimated based on the challenge test performed in VESO (Norway). Data was obtained from 1881 juveniles tested at average weight of 38.7 grams. The juveniles belong to full and half sib mating structure comprising 100 full sib and 100 and paternal half-sibs families. The challenge test, which lasted for 47 days, had a cumulative mortality of 82%. Two statistical models were used to estimate genetic parameters: test survival model (TS) and day of death (DD). Estimated heritabilities for the models differ from 0.23 (TS) to 0.41 (DD). A tissue sample was taken from each juvenile for DNA extraction prior to Genotyping-by-Sequencing (GBS) using *Pst*I for the restriction digest. Subsequent filtering of GBS SNPs resulted in 22.917 SNPs (~23k) derived from the diploid region of the genome for further analysis. Using the Kinship using GBS with Depth adjustment (KGD) method to estimate a genomic relationship matrix (GRM) allowed a Genomic Best Linear Unbiased Prediction (GBLUP) evaluation of breeding value for SRS resistance. The results suggest that by using GBS with GBLUP in genotyped but non-challenged half and full sib candidates, both the accuracy and genetic gain, would increase 21-22% compared with conventional pedigree based BLUP methodology.

INTRODUCTION

Piscirickettsia salmonis (SRS) is caused by the intracellular Gram-negative bacterium, *Piscirickettsia salmonis*, first identified in Chile and later in Canada and several European countries (Corbeil and Crane 2005). SRS has been reported to infect a wide range of Salmonidae pink salmon (*Oncorhynchus gorbuscha*), chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*) (Corbeil and Crane 2005). Although SRS has wide geographic range, it has caused larger outbreaks in South America than in Europe.

SRS is epizootic in Chile and losses due to SRS are significant and have severely hit the Chilean Atlantic salmon and Coho salmon industry (Cvitanich *et al*. 1991). Mortality rates have been reported to be 30-90% among Coho salmon (Corbeil and Crane 2005). Treatments with antibiotic and vaccination have provided some advantage, but do not give control of the disease.

In recent years there has been an increased focus on genetic improvement programs to select more robust and resistant individuals towards diseases. To date a number of studies have been conducted to determine additive genetic variation for disease resistance for both bacterial and virus diseases in Atlantic Salmon (Ødegård *et al*. 2011; Gjedrem *et al*. 2012). For the last ten years these studies have been supported by extensive genomic research including the use of genomic selection (GS). Studies in Atlantic salmon breeding have shown that genetic gain and accuracy can be improved substantially with GS, even with sparse SNPs (4K) (Sonesson and Meuwissen 2009; Villanueva *et al*. 2011; Ødegård *et al.* 2014). Most of the genotyping in salmon breeding has used SNP-chips, however, more recently high throughput, low cost GBS genotyping and analysis methods have been developed (Elshire *et al*. 2011; Dodds *et al.* 2015). These methods offer several advantages albeit at the expense of more complicated bioinformatics analysis.

The aim of this study was to estimate genetic variance of salmon towards SRS resistance. GBS together with KGD analysis were utilized for SNP filtering and later GBLUP was used to estimate

breeding values for both challenged and non-challenged test groups, which were compared with conventional pedigree based BLUP methodology.

MATERIALS AND METHODS

Animals for both challenged and non-challenged test groups were chosen from Stofnfiskur breeding population and hatched in November 2012, year class 2012-2 (YC12-2). A nested mating design was used in which, one male was used to fertilize eggs from two females, creating groups of full-sibs and paternal half-sibs. In total, 100 females were mated with 50 males. Each family was reared in a one cubic meter tank until the fish were tagged with a PITtag. The average tagging weight was 15.8 (SD = 6.6) g. After tagging the fish were pooled and reared in a single communal tank for 6 months. The challenge group was then moved to VESO in Norway. In total, 2.400 fish (20 fish per family) were transported in bags from Stofnfiskur to VESO Vikan by plane. The fish were kept in two separate tanks until challenge testing. After 4 weeks of acclimatization at 12°C in freshwater, the fish were acclimatized to 15°C freshwater for one week before the challenge. The challenge weight was 38.7 (SD = 9.7) g. Out of 2.400 fish, 400 were used as challenge carriers (shedders) and marked by adipose fin. The cohabitation challenge was performed in one tank by injecting the shedder fish with *Piscirickettsia salmonis* and adding these fish directly to the same tank as the tested fish. Mortality was observed throughout a 47-day period after challenge.

In January 2016, 2.846 fish from were selected from YC12-2 as a non-challenged test group and future breeding candidates in the Stofnfiskurs breeding nucleus. Fin clips were taken from both challenged and non-challenged test groups and stored in 96 % ethanol for DNA analyzing. The tissue samples were sent to AgResearch, New Zealand, for DNA extraction and GBS using *Pst*I and the protocol and subsequent processing was as described in Dodds *et al*. (2015) except that 190 bar-coded samples were sequenced per lane.

GBLUP and BLUP were fitted in mixed linear models using DMU 6, software package for animal breeding (Madsen and Jensen 2013). Two models were used for the analysis. Model one was Test survival (TS) where the individuals are scored 0 if it dies within challenge test time and 1 otherwise. The second model was Day of death (DD) where individuals were scored at the day of death in the challenge ranging from day 1- 47 and individuals which survive the challenge test were considered censored. Non-challenged individuals were given missing values. The model is as follows: $\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Za} + \boldsymbol{e}$, where $\boldsymbol{y}$ is the vector of the survivals score either as 0/1 or day of death, and $\boldsymbol{b}$ is a vector of fixed effects, which included sex and rearing tanks. The vector $\boldsymbol{a}$ is a vector of random additive genetic effects of individual animals. KGD method was used to estimate a GRM in GBLUP and pedigree information was used in BLUP.

RESULTS AND DISCUSSION

The mean survival at day 47 of the SRS disease challenge test was 18% and ranged from 0 to 60% (Figure 1). A total of 29.671putative SNPs were identified using GBS methodology. After filtering by using the KGD method, the 22.917 remaining SNPs were used to create the GRM for GBLUP evaluation.

Running the TS and DD models in DMU 6 gave in both cases higher estimate of heritability and accuracy of the estimated value compared to conventional pedigree based BLUP methodology (see Table 1).

Table 1. The estimates of genetic parameters. BLUP refers to pedigree based BLUP methodology and GBLUP refers to genomic based BLUP methodology

BLUP methods	Models	h^2	Accuracy in challenged group	Accuracy in non-challenged group	Increased accuracy
BLUP	DD	0.35	0.71	0.56	
GBLUP	DD	0.41	0.82	0.68	21%
BLUP	TS	0.23	0.69	0.54	
GBLUP	TS	0.26	0.76	0.66	22%

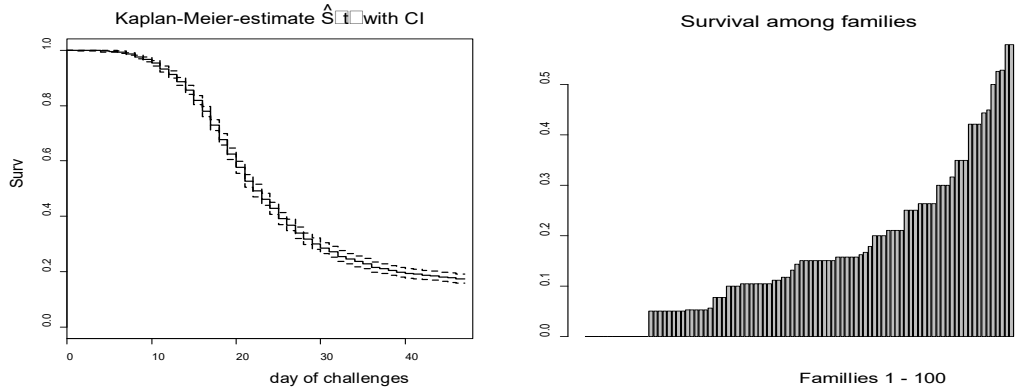

Figure 1. On the left is the Kaplan–Meier mortality curves for 47 days of challenge, on the right is the variation among 100 families tested

This study shows a substantial increase in accuracy by applying GBS with GBLUP where the KGD method is applied to create the GRM. This is in line with other studies in salmon breeding. Ødegård *et al*. (2014) showed that a considerable improvement can be gained even from sparse SNPs (4k) but increased accuracy starts to converge rapidly from 22k to 220k, confirming the 23k SNPs from this present study would be sufficient to utilize the full potential of GBLUP.

Estimated heritabilities indicate that there is moderate additive genetic variance of SRS resistance. Moreover, heritability of DD model was higher than estimated in the TS model for both BLUP and GBLUP (Table 1). However, the estimates from the two models give different results in heritability. It should be noted that traits are defined very differently in these two models. These heritability estimates are similar to Yáñez *et al*. (2013). In both models GBLUP gives an increased accuracy and heritability compared to pedigree based BLUP methodology. Where GRM is created with SNPs, such as in GBLUP, random deviations from relationships caused by Mendelian sampling terms can be quantified more accurately.

In salmon, breeding for SRS disease resistance is difficult because breeding companies don't use infected challenged fish for breeding. Instead non-challenged sibs are used as breeding candidates (sib testing). Such evaluation has many drawbacks in relation to the amount of genetic progress that can be realized within a breeding program when depending only on pedigree information to predict breeding values by using conventional BLUP. When the predicted breeding values are not based on an individual's own performance, selected accuracy would be lower. Moreover, variation of

Mendelian sampling effects within a family cannot be used to select superior animals within the best family.

Using genomic information, such as GBLUP, increases the importance of the Mendelian sampling term, or the within family variance, and reduces the importance of family compared to traditional BLUP valuation. Thus, breeding programs for traits with low heritability and relatively few records per trait measured, such as carcass and disease resistance, are those which can benefit from GS.

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