Unravelling Genetic Co-variation For Resistance And Tolerance Against *Piscirickettsia salmonis* Infection In Atlantic Salmon (*Salmo salar*)

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Introduction

Disease resistance can be defined as the ability of the host to limit the infection (Råberg *et al* 2007). A considerable number of studies have shown genetic variation for resistance against different infectious diseases in salmonids (Yáñez and Martinez In Press). In these studies, resistance has typically been measured as the survival of infected individuals using data from field outbreaks or experimental challenges. On the other hand, tolerance can de defined as the ability to limit the disease severity induced by a given infection (Råberg *et al*, 2007). As a measure of tolerance, the performance difference in a given production trait between infected and uninfected hosts can be used (Carr *et al* 2006). Until now, there are no studies aiming to determine whether salmonids may also show genetic variation for tolerance. For breeding purposes it is essential to make the distinction between these two traits, because artificial selection for resistance and/or tolerance can have different consequences in both the host-pathogen co-evolution and the success of the breeding programme depending on the correlation between these two traits. In this work we investigate whether there is genetic (co) variation for resistance and tolerance against a pathogen, which significantly affect Chilean salmon industry (*Piscirickettsia salmonis*) using survival and productive performance data obtained from an experimental challenge in a commercial population of Atlantic salmon (*Salmo salar*).

Material and methods

Fish. Survival data were obtained from a total of 2461 pre-smolt individuals of Atlantic salmon belonging to 29 full-sibs families. Each individual was PIT-tagged in order to keep pedigree known during the challenge test.

Challenge Test. The challenge was carried out at the Piscicultura Experimental Chiloé of the Centro de Estudios Acuícolas of the Universidad de Chile, located in Castro, Chile. We used an isolate of *P. salmonis*, which had been proven to be virulent in a *in vivo* assay. The bacterium was cultured in CHSE-214 cell line until 90% cytopathic effect. Infection was conducted by means of intraperitoneal injection reaching a 0.1 ml of a titre ($0.2 \times 10^{4.8}$

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TCID50/ml) of *P. salmonis*. Inoculated fish of each family were divided into two tanks of fresh water, thus each tank had a half of infected individuals from each family.

Records, trait definitions and models. The average daily gain of weight (ADG) has been calculated in all fish seven weeks before the inoculation and the same trait has been measured in the all the infected individuals during the challenge test. Mortalities were daily recorded until day 52 post-inoculation. Necropsy examination was performed in order to confirm Piscicketssiosis as the primary cause of death. Tolerance (TOL) has been defined as the performance difference in terms of ADG between an infected host and the same uninfected host, assuming that the curve for ADG was similar for all families across the period of the study. This trait was analyzed using the following linear model:

$$Y_{ijk} = \mu + t_i + f_j + e_{ijk}$$

Where, Y_{ijk} is the phenotype for the fish k, in full-sib family j, in tank i; μ is the fixed effect of the overall mean; t_i is the fixed effect of tank i; f_j is the random additive genetic effect of the full-sib family j; e_{ijk} is the random residual for fish k. On the other hand, Resistance (RES) has been assessed as the challenge-test survival, which was scored as a binary trait: 0 if the fish died during the test period and 1 if the fish survived at the end of the experiment. This trait was analyzed using the following threshold (logit) model:

$$Pr(Y_{ijk}=1) = \Phi(\mu + t_i + f_j)$$

Where, $\Phi(\cdot)$ is the cumulative standard normal distribution, and the other parameters as defined above. For estimation of (co)variance components we used the ASREML software (Gilmour *et al* 2002).

Heritabilities and correlations. Heritabilities were estimated as follow:

$$h^2 = \frac{2\sigma_f^2}{\sigma_f^2 + \sigma_e^2}$$

Where, σ_f^2 is the additive genetic variance of the full sib-family and σ_e^2 is the residual variance (being $\pi^2/3$ in the threshold model). Phenotypic and genetic correlations among RES and TOL were estimated fitting a bivariate binomial-normal analysis using the same parameters defined above.

Results and discussion

The total cumulative mortality reached 35% at the end of the test period. Figure 1 shows the distribution of mortalities across the challenge test. Cumulative mortality rates ranged from 10.5% to 70% between different families, showing a great variation in susceptibility for Piscirickettsiosis (Figure 2). The inoculation of *P. salmonis* significantly affected the ADG of infected fish ($P<0.05$). Significant additive genetic variation was detected for RES and TOL and heritabilites are shown in Table 1. Similar results have been found by studies carried out for resistance against other bacterial diseases in farmed salmon populations (Yáñez and Martinez In Press). Nevertheless, this is the first study that demonstrates the existence of additive genetic variation for tolerance to an infectious disease in fish.

Phenotypic and genetic correlations between RES and TOL were significant and slightly to moderately negative, respectively (Table 1). These findings indicate that the there is a trade off between resistance and tolerance for Piscirickettsiosis in this population of Atlantic salmon. The same situation has been previously demonstrated in plants and mice (Strauss and Agrawal 1999, Råberg *et al* 2007). This trade off can be explained on the basis of that resistance and tolerance may represent alternative strategies to deal with antagonists (Strauss and Agrawal 1999). For instance, families of fish with higher survival rates (more resistant) they may redirect energy towards establishing a more efficient immune response (in order to limit the pathogen replication) having an effect a detriment in productive performance. On the other hand, families that maintain their productive performance (more tolerant) does not respond well against the infection, probably because they does not redirect energy towards limit the infection, and so they die faster than resistant individuals. It has been demonstrated that resistance and tolerance have contrasting effects on the epidemiology of infectious diseases (Roy and Kirchner 2000; Miller *et al* 2006;). In this case, resistant fish will have a negative effect on the prevalence of infection in the population because they tend to elude or limit the infection. On the other hand, tolerant fish should have a neutral or positive effect because they favour the replication of the bacteria. Hence, artificial selection for more resistant individuals could lead to counter adaptations in the agent, which selects for improved resistance in the host and so on (Råberg *et al* 2007). However, selection for tolerance will not act negatively on the fitness of the pathogen and so it cannot intensify antagonistic co-evolution in the same way as is expected of resistance (Råberg *et al.* 2007). This opens the question about which trait is most appropriate when establishing a sustainable control of an infectious disease in the long term. Thus, more studies are needed to clarify the epidemiological effects of improving both traits in a commercial population of fish.

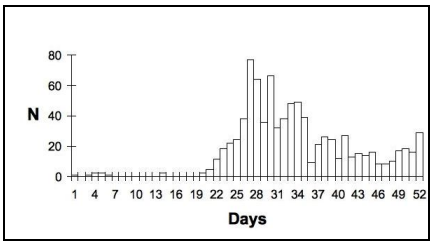

Figure 1: Distribution of mortalities during the challenge with *Piscirickettsia salmonis* in the total of individuals (N = Number of individuals).

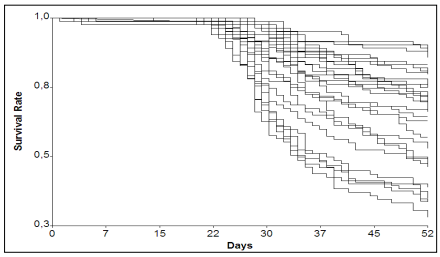

Figure 2: Kaplan-Meier curves of the survival function for the 52 days of the test period for each of the 29 families.

Table 1: Heritabilities (diagonal), Genetic (above diagonal) and phenotypic (below diagonal) correlations estimated for resistance (RES) and tolerance (TOL) to Piscirickettsiosis. The standard error is in parenthesis.

Trait	RES	TOL
RES	0.41 (0.04)	-0.61 (0.15)
TOL	-0.21 (0.01)	0.12 (0.04)

Conclusion

We have shown that significant genetic variation for both tolerance and resistance against Piscirickettsiosis exists in Atlantic salmon. These results indicate that selective breeding for these traits can be successfully applied in the studied population. However, the negative genetic correlation estimated between resistance and tolerance must be taken into account when both traits are included in the breeding objective. The determination of molecular basis of tolerance and resistance to Piscirickettsiosis will be crucial for implementing breeding programmes assisted by molecular information in order to improve these two traits simultaneously. Finally, more studies aiming to determine the epidemiological considerations of improving tolerance and resistance are needed in order establish the guidelines of a long-term control of infectious diseases in farmed salmon populations.

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