



OPEN

Genome-scale comparative analysis for host resistance against sea lice between Atlantic salmon and rainbow trout

Pablo Cáceres¹, Agustín Barría², Kris A. Christensen³, Liane N. Bassini⁴, Katharina Correa¹, Baltasar García^{1,5}, Jean P. Lhorente⁶ & José M. Yáñez^{1,7,8}✉

Sea lice (*Caligus rogercresseyi*) is an ectoparasite which causes major production losses in the salmon aquaculture industry worldwide. Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) are two of the most susceptible salmonid species to sea lice infestation. The objectives of this study were to: (1) identify genomic regions associated with resistance to *Caligus rogercresseyi* in Atlantic salmon and rainbow trout by performing single-step Genome-Wide Association studies (ssGWAS), and (2) identify candidate genes related to trait variation based on exploring orthologous genes within the associated regions across species. A total of 2626 Atlantic salmon and 2643 rainbow trout were challenged and genotyped with 50 K and 57 K SNP panels, respectively. We ran two independent ssGWAS for sea lice resistance on each species and identified 7 and 13 regions explaining more than 1% of the genetic variance for the trait, with the most important regions explaining 3% and 2.7% for Atlantic salmon and rainbow trout, respectively. We identified genes associated with immune response, cytoskeleton function, and cell migration when focusing on important genomic regions for each species. Moreover, we found 15 common orthogroups which were present in more than one associated genomic region, within- or between-species; however, only one orthogroup showed a clear potential biological relevance in the response against sea lice. For instance, *dual-specificity protein phosphatase 10-like (dusp10)* and *dual-specificity protein phosphatase 8 (dusp8)* were found in genomic regions associated with lice density in Atlantic salmon and rainbow trout, respectively. *Dusp10* and *dusp8* are modulators of the MAPK pathway and might be involved in the differences of the inflammation response between lice resistant and susceptible fish from both species. Our results provide further knowledge on candidate genes related to sea lice resistance and may help establish better control for sea lice in fish populations.

Sea lice is currently the most harmful parasite for salmon farming worldwide¹. Economic losses due to different sea lice species are mainly associated with the reduction of feed conversion rate, growth, indirect mortalities and loss of product value. Furthermore, it has been estimated that the global costs for sea lice control have reached \$480 million (USD) annually². The two most important sea lice species which generate a considerable negative impact in salmon farming are *Lepeophtheirus salmonis* and *Caligus rogercresseyi*³.

Caligus rogercresseyi, first described in 2000 by Boxshall and Bravo⁴, is the main sea lice species affecting salmon aquaculture in Chile⁵. *C. rogercresseyi* primarily affects Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), while coho salmon (*Oncorhynchus kisutch*) has an innate lower susceptibility to the parasite⁶. The consequences of the infestation by sea lice include skin lesions, osmotic imbalance and greater susceptibility to bacterial and viral infections through the suppression of the immune response by the damage

¹Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Av. Santa Rosa 11735, La Pintana, 8820808 Santiago, Chile. ²The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh Easter Bush, Midlothian EH25 9RG, UK. ³Fisheries and Oceans Canada, 4160 Marine Drive, West Vancouver, BC, Canada. ⁴Escuela de Medicina Veterinaria, Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile. ⁵School of Agricultural and Veterinary Sciences, UNESP-Sao Paulo State University, Jaboticabal 14884900, Brazil. ⁶Aquainnovo, Cardonal S/N, Puerto Montt, Chile. ⁷Núcleo Milenio INVASAL, Concepción, Chile. ⁸Center for Research and Innovation in Aquaculture (CRIA), Universidad de Chile, Santiago, Santiago, Chile. ✉email: jmayanez@uchile.cl

Species	Mean BW (g)	BW SD (g)	Min BW(g)	Max BW (g)	LC means	LC SD	Min LC	Max LC
<i>S. salar</i>	278.1	90.3	104.0	569.0	5.9	6.61	0	106
<i>O. mykiss</i>	173.1	31.4	86.0	265	6.1	4.22	0	28

Table 1. Summary statistics for body weight (BW) and lice count (LC) in Atlantic salmon and rainbow trout. SD standard deviation.

generated in the skin of the host⁷. The parasite life cycle is comprised of eight stages of development⁸: two states of nauplii, one copepod state, four chalimus states and the adult state. The stages of nauplii (I and II) and copepods (infectious stage) are planktonic. The four stages of chalimus (I–IV) are sessile while the adult is a mobile stage⁹.

Recent studies have estimated low to moderate genetic variation for resistance to sea lice in Atlantic salmon populations, with heritabilities ranging from 0.12 to 0.32 and from 0.13 to 0.33 when resistance was defined as the number of parasites fixed in all the fins^{7,9,10}, or as the logarithm of the parasite density^{11,12}, respectively. A recent study reported a heritability value of 0.09 for sea lice resistance in a rainbow trout breeding population¹³. These results indicate that it is feasible to improve resistance to sea lice in Atlantic salmon and rainbow trout populations by utilizing selective breeding^{9,10,14}.

Comparative genomic approaches¹⁵ allow the identification of genomic similarities between different species, including conserved genes and motifs, traces of genome duplication and gene functions¹⁶. Traditionally, these analyses are focused on orthologous genes, which are homologous genes present in different species resulting from direct transmission from a common ancestor¹⁶. To date, comparative genomic studies between salmonids have mainly focused on finding evolutionary similarities in the genetic basis of body and sex related traits, including growth, development and sexual differentiation^{17–19}.

A recent study compared results from genome-wide association studies (GWAS) on three salmonid species and identified functional candidate genes involved in resistance to the infection caused by *Piscirickettsia salmonis*, an intracellular bacterium²⁰. To date, no studies have aimed at comparing genomic regions associated with resistance to sea lice in salmonid species.

The main objectives of the present study were to: (1) identify genomic regions associated with resistance to *Caligus rogercresseyi* in Atlantic salmon and rainbow trout through GWAS, and (2) identify functional candidate genes potentially related to trait variation through a comparative genomics approach, based on exploring orthologous genes within the associated regions between species.

Results and discussion

The comparative genomics analysis presented here allowed us to identify groups of orthologous genes and several candidate genes among adjacent single nucleotide polymorphisms (SNP) that explained more than 1% of the genetic variance for resistance to *C. rogercresseyi*. This is the first study aimed at comparing the genomic basis of sea lice resistance in both Atlantic salmon and rainbow trout.

Sea lice challenge test. There was no difference between the average number of sea lice found on Atlantic salmon or rainbow trout in the experimental challenge (Table 1). An average of 5.9 ± 6.6 and 6.1 ± 4.2 parasites per fish was estimated for Atlantic salmon and rainbow trout, respectively. In terms of the maximum number of parasites, this value varied from 106 parasites in Atlantic salmon to 28 in rainbow trout and, for both species, some animals did not present any parasites. The average weight at the end of the experimental challenge was 278.1 ± 90.3 g (ranging from 104 to 569 g) and 173.1 ± 31.4 g (ranging from 86 to 265 g), for Atlantic salmon and rainbow trout, respectively. Although the average number of parasites was not significantly different between the two species, the difference in the average final weight at the end of each challenge could explain the difference in the maximum number of parasites found (~4 times more parasites in Atlantic salmon). The number of parasites counted in each species after the challenge is below the range determined in previous studies. For instance, Ødegård et al.¹¹ obtained an average of 20.96 ± 19.68 , while Robledo et al.²¹ reported an average of 38 ± 16 sea lice. The lower number of parasites in comparison to these studies is most likely related to differences in the area of counting (whole body surface versus only fins), parasite species (*Lepeophtheirus salmonis* versus *C. rogercresseyi*) and the time of sampling after infestation (8–15 versus 6 days).

To measure resistance to *C. rogercresseyi*, lice count values were transformed into lice density on the log scale (LogLD), which allows for correction of the number of parasites based on the body weight of each fish¹¹. The empirical LogLD distribution for both species is shown in Fig. 1. The range of LogLD for Atlantic salmon was greater than for rainbow trout, varying from -4.60 to 1.02 and from -4.06 to 0.02 , respectively. The average LogLD was -2.14 ± 0.9 and -1.66 ± 0.67 for Atlantic salmon and rainbow trout, respectively, which are similar to those reported in a previous study in a different Atlantic salmon population (between -1.66 ± 0.73 and -2.55 ± 0.58)¹¹.

Genotyping and genomic heritabilities. A total of 2040 (77.6%) animals, and 45,117 (96.7%) SNPs passed the genotyping quality control for Atlantic salmon. In the case of rainbow trout, 2466 (93.3%) fish and 27,146 (67.4%) SNPs remained for subsequent analyses. In both species, significant genetic variation for resistance to *C. rogercresseyi* was estimated by using genomic information, with heritability values of 0.19 ± 0.03 and 0.08 ± 0.01 for Atlantic salmon and rainbow trout, respectively (Table 2).

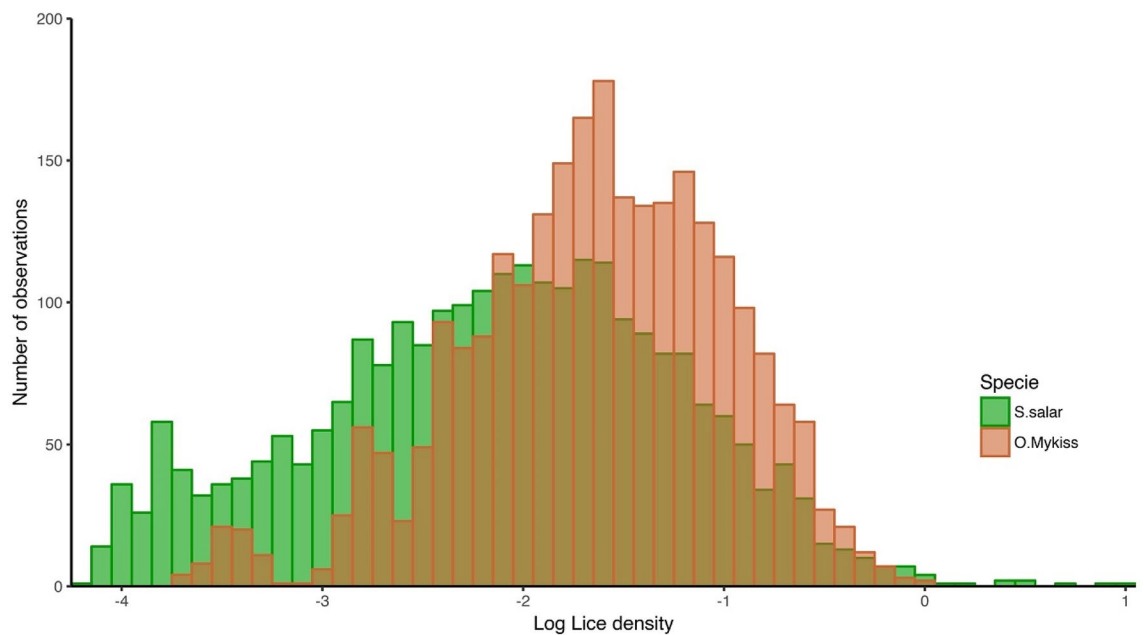


Figure 1. Histogram for log lice density (LogLD) for *S. salar* (green) and *O. mykiss* (orange).

Specie	Additive variance	Residual variance	h^2
<i>S. salar</i>	0.09 ± 0.01	0.41 ± 0.01	0.19 ± 0.03
<i>O. mykiss</i>	0.02 ± 0.005	0.27 ± 0.008	0.08 ± 0.01

Table 2. Summary of heritability (h^2) for the LogLD trait in both species.

In previous studies in Atlantic salmon populations, Tsai et al.¹², estimated genomic heritability values for resistance against *L. salmonis* of 0.22 ± 0.08 and 0.33 ± 0.08 , while Ødegård et al.¹¹, found heritability values of 0.14 ± 0.03 and 0.13 ± 0.03 . Similarly, Yañez et al.¹⁰ and Correa et al.⁷ estimated heritability values ranging from 0.10 to 0.12 when defining resistance as the total number of parasites found on all fins using pedigree and genomic information, respectively, and Lhorente et al.⁹ estimated heritability values of 0.22 ± 0.06 in Atlantic salmon for total count of sessile sea lice per fish, corrected by body weight in the statistical analysis.

GWAS. In Atlantic salmon, we found genomic regions explaining more than 1% of the genetic variance for sea lice resistance in five different chromosomes (Fig. 2). Two of these chromosomes (*Ssa3* and *Ssa9*) showed two QTL peaks associated with the trait. Only four SNPs located in *Ssa3*, *Ssa11*, *Ssa14* and *Ssa23* were shown to be significantly associated with the trait at a chromosome-wide level. However, none of these QTLs explained more than 1% of the genetic variance of the trait (Supplementary Figure S1). In general, these regions explained a low percentage of the total genetic variation with a maximum of 3% explained by a single locus. The two SNP windows in *Ssa3* explained 1% and 1.4% of the genetic variance while those found in *Ssa9* explained 1.7% and 3%. Other QTLs found in *Ssa6*, *Ssa20*, and *Ssa25* explained 1.9%, 1.05% and 1.33% of the genetic variance, respectively. Supplementary Table S1 shows the variance explained by each window of SNP in both species.

In the case of rainbow trout, the wssGWAS for LogLD identified 13 regions located in different chromosomes that exceeded 1% of the total genetic variance for the trait (Fig. 3). Similar to Atlantic salmon, these windows explained a low percentage of the total variance with a minimum of 1% and a maximum of 2.7%, for QTLs located in *Omy17* and *Omy15*, respectively. In addition, three SNPs located in *Omy3*, *Omy6*, *Omy9* showed chromosome-wide significant association with the resistance trait in rainbow trout (Supplementary Figure S2).

Our results suggest that resistance against *C. rogercresseyi* is mainly under polygenic control (i.e., influenced by several genes with small effects) in both species. These results are in agreement with previous studies on sea lice resistance, where a similar genetic architecture was suggested for resistance against *L. salmonis* and *C. rogercresseyi* resistance^{7,12,22}. Recently Robledo et al.²³ described and characterized three QTLs related to sea lice resistance in Atlantic salmon by using GWAS and RNA-sequencing approaches. Since sea lice resistance is a polygenic trait, the acceleration of genetic improvement will most likely be best accomplished by employing genomic selection instead of marker-assisted selection or pedigree-based genetic evaluations. For instance, Correa et al.¹² and Tsai et al.¹⁴ described an increase of over 22% in the accuracy of estimated breeding values (EBVs) using genomic selection over the use of pedigree-based models in Atlantic salmon²⁴.

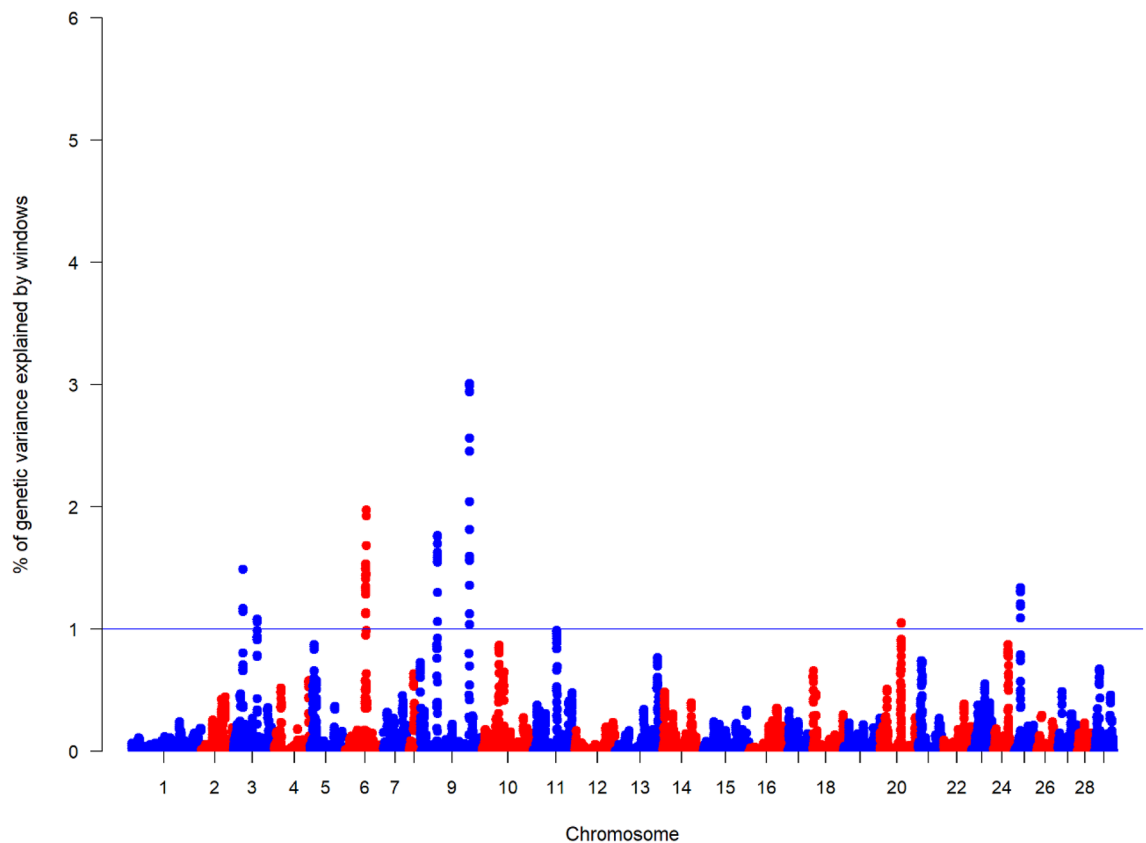


Figure 2. Weighted single-step GWAS (wssGWAS) results for Log lice density (LogLD) in Atlantic salmon. The Manhattan plot represents the genetic variance explained by windows of 20 SNP and the blue horizontal line indicates 1% threshold.

Candidate genes. The exploration of the genes within the windows that explained over 1% of the genetic variance for LogLD showed several potential candidate genes that were classified into three groups: related to the immune response, cytoskeleton or metalloproteases. The genes are listed in Tables 3 and 4 for Atlantic salmon and rainbow trout, respectively.

A recent study on gene expression with *C. rogercresseyi* infestation in susceptible and resistant Atlantic salmon indicated that several components of the immune system (inflammatory response, cytokine production, TNF and NF-kappa B signaling and complement activation) and tissue repair are upregulated during infestation²¹. In salmonids, the main response of the immune system to parasites is mediated by T-Helper 1 and T-Helper 2 cells²⁵. Thus, genes related to the immune response, either by promoting leukocyte growth or favoring migration or activation are strong candidate genes. For instance, in Atlantic salmon we found, *T-cell activation Rho GTPase-activating protein (tagap)* in Ssa6, which participates in the activation and recruitment of T cells by cytokines²⁶, and *tenascin R (tnr)* in Ssa3, which is an extracellular matrix protein, present in bone marrow, thymus, spleen and lymph nodes²⁷. The latter has been described as having an adhesion function favoring the mobility of lymphocytes and lymphoblasts^{27,28}. In rainbow trout, we found candidate genes with similar functions, such as *T-box 21 (tbx21)*, also known as *T-bet (T-box expressed in T cells)*, found in *Omy16*. This gene belongs to the *sub-Tbr1 family*²⁹, and generates type 1 immunity and participates in the maturation and migration of T-helper 1 (Th1) cells, which in turn produce *interferon-gamma (IFN-γ)*. Studies have described *T-bet* expression in NK cells (natural killer), dendritic cells and T CD8+ cells^{30,31}.

Forkhead box protein N1-like (foxn1) present on Ssa09 of Atlantic salmon is part of a family of genes widely studied in humans, which are related to various functions including cell growth, lymph node development, and T cell differentiation³². It has been proposed that *foxn1* has a role in the activation of fibroblast growth factor receptors³².

Meanwhile, in rainbow trout on *Omy21*, *serine/threonine-protein phosphatase 2A 56 kDa* was identified, which is described as having participated in cell growth and signaling³³. Robledo et al.²¹ recently found that in Atlantic salmon, this protein showed the most significant change in the expression differences between healthy skin and skin where sea lice were attached²¹. In Atlantic salmon, we identified *Tripartite motif-containing protein 45 (trim45)* on Ssa25 which belongs to a large family of proteins present in diverse organisms that can function as a ligase and can modify ubiquitin and proteins stimulated by *interferon of 15 kDa (isg15)*³⁴.

Several metalloproteases were found in genomic regions associated with resistance in both species, but for the interest of this study, we focused on GEM-interacting protein which interacts with *rab27a* or its effector in leukocytes. *Rab* is a large family of GTPases responsible for vesicle cellular transport³⁵. Deficiencies of this

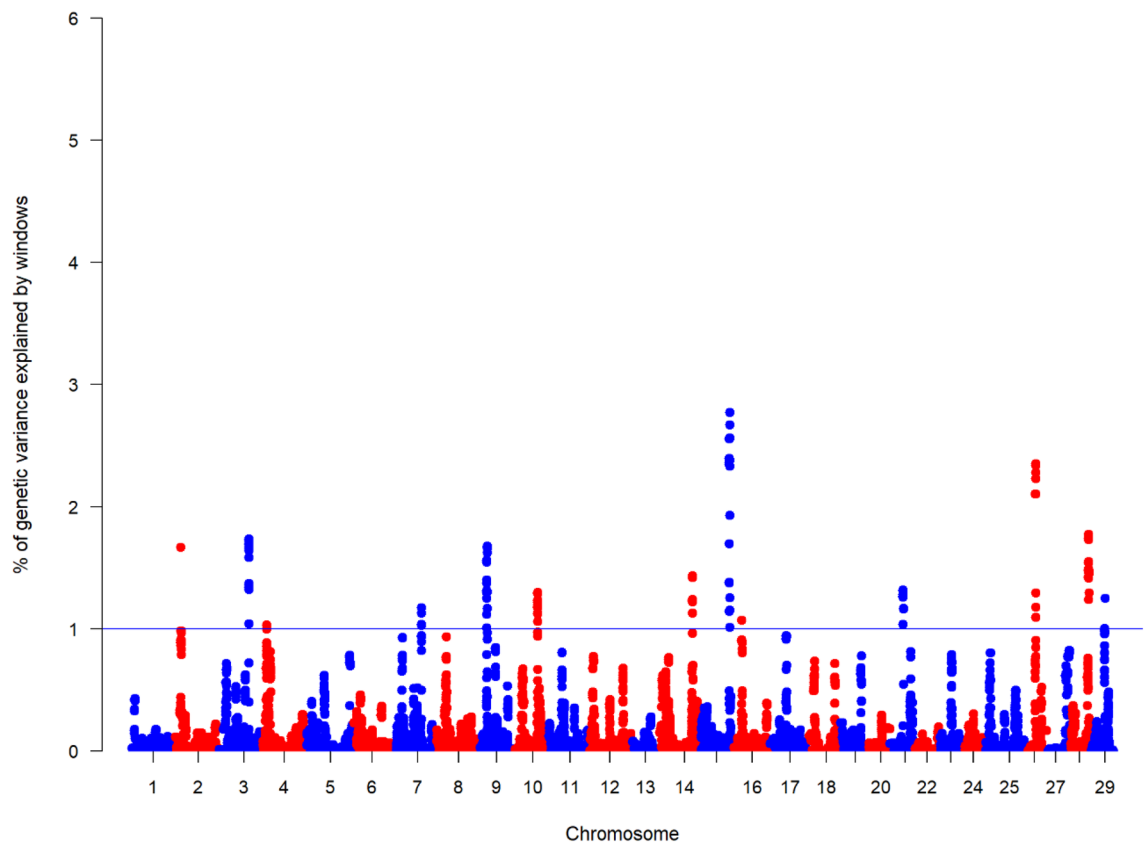


Figure 3. Weighted single-step GWAS (wssGWAS) results for Log lice density (LogLD) in rainbow trout. Manhattan plot represents the genetic variance explained by windows of 20 SNP and the blue horizontal line indicates 1% significance threshold.

Protein name	Gene ID ^a	Location (BP) ^b	Chr ^c	Function
Tenascin R	<i>tnr</i>	22,113,558–22,312,716	3	Immune response
T-cell activation Rho GTPase-activating protein	<i>tagap</i>	47,344,667–47,350,357	6	Immune response
Forkhead box protein N1-like isoform X1	<i>LOC106612922</i>	111,631,268–111,668,978	9	Immune response
Immunoglobulin superfamily member 11-like	<i>igsf11</i>	47,453,435–47,570,948	20	Immune response
Tripartite motif-containing 45	<i>trim45</i>	31,699,662–31,705,320	25	Immune response
pre-B-cell leukemia transcription factor 1-like	<i>LOC106607421</i>	43,977,204–44,037,235	6	Immune response
Bromodomain-containing protein 4-like isoform	<i>LOC106600922</i>	53,482,098–53,526,053	3	Immune response
PAPPALYSIN-2	<i>pappa2</i>	21,972,287–22,050,408	3	Metalloprotease
Carboxypeptidase D	<i>cdpa</i>	111,778,091–111,828,613	9	Metalloprotease
GEM-interacting protein-like isoform X3	<i>LOC106600913</i>	53,672,635–53,709,985	3	Metalloprotease
Epidermal growth factor	<i>egf</i>	55,552,926–55,584,698	9	Cytoskeleton
Procollagen galactosyltransferase 1	<i>LOC106607427</i>	43,820,342–43,851,337	6	Cytoskeleton
Heat shock protein HSP 90-beta	<i>hs90b</i>	48,362,362–48,374,359	6	Cytoskeleton
Collagen alpha-1(XXVIII) chain-like	<i>col28a1</i>	13,872,240–13,923,778	25	Cytoskeleton
Gap junction alpha-4 protein-like	<i>LOC106611294</i>	39,373,024–39,373,931	9	Cytoskeleton
Pleckstrin homology domain-containing family H member 1-like	<i>LOC106611291</i>	39,101,628–39,165,084	9	Cytoskeleton
Serine/threonine-protein kinase OSR1-like	<i>LOC106600551</i>	44,726,186–44,781,350	3	Cytoskeleton
Rho-related GTP-binding protein RhoB-like	<i>LOC106607565</i>	46,401,032–46,403,034	6	Cytoskeleton
Pleckstrin homology and RhoGEF domain containing G1	<i>plekhg1</i>	64,024,693–64,178,709	6	Cytoskeleton

Table 3. Candidate genes for sea lice resistance in Atlantic salmon. ^aGene identification (Gene ID) from the NCBI database GenBank assembly accession: GCA_000233375.4. ^bLocation in base pair (bp). ^cChromosome (Chr).

Protein name	Gene ID ¹	Location (BP) ^b	Chr ^c	Function
Nuclear factor of activated T-cells, cytoplasmic 1-like	LOC110518416	35,501,191–35,592,262	3	Immune response
Thymocyte selection-associated family member 2	<i>themist2</i>	33,704,133–33,726,370	4	Immune response
C–C motif chemokine receptor 10	<i>ccr10</i>	21,961,454–21,965,777	16	Immune response
T-box 21	<i>tbx21</i>	15,560,804–15,578,932	16	Immune response
Leucine-rich repeat-containing protein 15-like	LOC110539182	11,715,000–11,717,281	2	Immune response
Toll-like receptor 13	LOC110490289	48,117,536–48,120,927	15	Immune response
Adhesion G protein-coupled receptor L3	LOC110531730	14,279,162–14,524,070	9	Immune response
Inhibin Beta A Chain	<i>inhba</i>	36,734,061–36,745,506	28	Immune response
Interferon gamma 2	<i>ifng2</i>	153,268–183,367	Unplaced scaffold	Immune response
Heat shock protein family A (Hsp70) member 8	<i>hspa8</i>	17,691,060–17,715,579	10	Immune response
Fibroblast growth factor	<i>fgf13</i>	26,660,561–26,715,195	29	Cytoskeleton
Fibroblast growth factor	<i>fgf11</i>	38,417,625–38,489,806	10	Cytoskeleton
Alpha-actinin-3	<i>3a, 3b</i>	43,817,039–43,841,681	10	Cytoskeleton
Dipeptidyl peptidase 3	<i>ddp3</i>	33,026,452–33,044,218	29	Cytoskeleton
ELMO/CED-12 domain-containing 1	<i>elmod1</i>	16,419,827–16,441,415	10	Cytoskeleton
Cysteine-rich protein 2-like	LOC106611283	38,639,636–38,675,313	9	Cytoskeleton
Coagulation factor IX-like	LOC110534144	43,984,179–43,991,091	10	Cytoskeleton
Lysyl oxidase homolog 1 isoform X2	LOC110526332	59,312,382–59,352,343	26	Cytoskeleton
Putative ferric-chelate reductase 1	LOC110490455	53,972,784–53,985,982	15	Metalloprotease
AFG3 Like Matrix AAA Peptidase Subunit 2	<i>afg3l2</i>	35,488,672–35,515,514	28	Metalloprotease
Afg3-Like Protein 1	LOC110506600	19,946,590–19,959,716	26	Metalloprotease

Table 4. Candidate genes for sea lice resistance in rainbow trout. ^aGene identification (Gene ID) from the NCBI database GenBank assembly accession: GCA_002163495.1. ^bLocation in base pair (bp). ^cChromosome (Chr).

molecule is correlated with immune deficiencies due to the malfunction of cytotoxic activity of T-lymphocytes, natural killer cells and neutrophils³⁶.

Considering the importance of cell growth and movement in response to sea lice infestation, the cytoskeleton may play a considerable role in sea lice resistance as well. For Atlantic salmon, genes related to the cytoskeleton, such as *epidermal growth factor (egf)* in *Ssa9*, were identified. This gene is part of a superfamily of receptors with tyrosine kinase activity that have been described in a variety of organs with growth promoter functions, cellular differentiation³⁸ and could participate in tissue repair by promoting cell growth²⁹. In rainbow trout, fibroblast growth factors (*fgf11*, *fgf13*) located in *Omy10* and *Omy29* respectively, are involved in angiogenesis and pro-inflammatory responses, and were identified as important genes in sea lice resistance in previous transcriptomic studies by Skugor et al. (2009) and Robledo et al. (2018) in Atlantic salmon^{21,39}. In addition, *ELMO/CED-12 domain-containing prot 1* was identified in *Omy10* in rainbow trout. This protein participates in phagocytosis of apoptotic cells, and in mammals, it also has a role in cell migration⁴⁰. Other cytoskeleton related candidate genes include: *Procollagen galactosyltransferase 1* present in *Ssa6*, *collagen alpha-1 (XXVIII) chain-like* on *Ssa25* and *pleckstrin homology domain-containing family H member 1-like* on *Ssa9*⁴¹. The top ten SNPs that explained the highest variance for sea lice resistance are located on *Ssa9* in Atlantic salmon, representing the most important QTL in this species. This QTL is harboring the *breast carcinoma-amplified sequence 3 (bcas3)* gene, which in Atlantic salmon codes for a cell migration factor associated with microtubules that favor cellular mobility⁴². Cell migration is generally induced in response to chemotactic signals, which induces changes in the cytoskeleton and extracellular matrix⁴³. We also found, the *tripartite motif-containing protein 16-like* on *Omy15*, which is part of the *trim* superfamily and has functions related to cell differentiation, apoptosis, regulation of transcription and signaling pathways³⁴. This gene is similar to *Tripartite motif-containing protein 45* present on *Ssa25*. In this region, we also found a locus that codes for *interferon-γ 2 (ifng2)*, which is a cytokine that participates in type 1 immune responses and that favors the presentation of antigens and activation of macrophages⁴⁴. On this same chromosome (*Omy15*), we also identified *putative ferric-chelate reductase 1 (frs1)*, which functions in the fixation of iron in teleosts⁴⁵. Robledo et al.²¹ identified *heme-binding protein 2 (hebp2)* as a gene involved in Atlantic salmon sea lice resistance, which has an iron-binding function. Different authors^{46,47} have stated that decreasing the availability of iron can be part of a nutritional defense mechanism against sea lice infestation.

Comparative genomics. The comparative genomic analyses performed show regions of synteny between chromosomes associated with sea lice resistance in Atlantic salmon and rainbow trout (Fig. 4). Thus, there are homologous regions which are associated with the trait and share similarities between chromosomes from both species. However, there were no obvious shared regions associated to sea lice resistance within species (i.e. homeologous regions). The examined populations shared homeologous regions harboring genes controlling resistance, which might suggest similar genomic regions involved in the regulation of resistance in the two species. For example, *Ssa3* (Atlantic salmon) shares extensive homology with *Omy28* (rainbow trout) and *Ssa25*

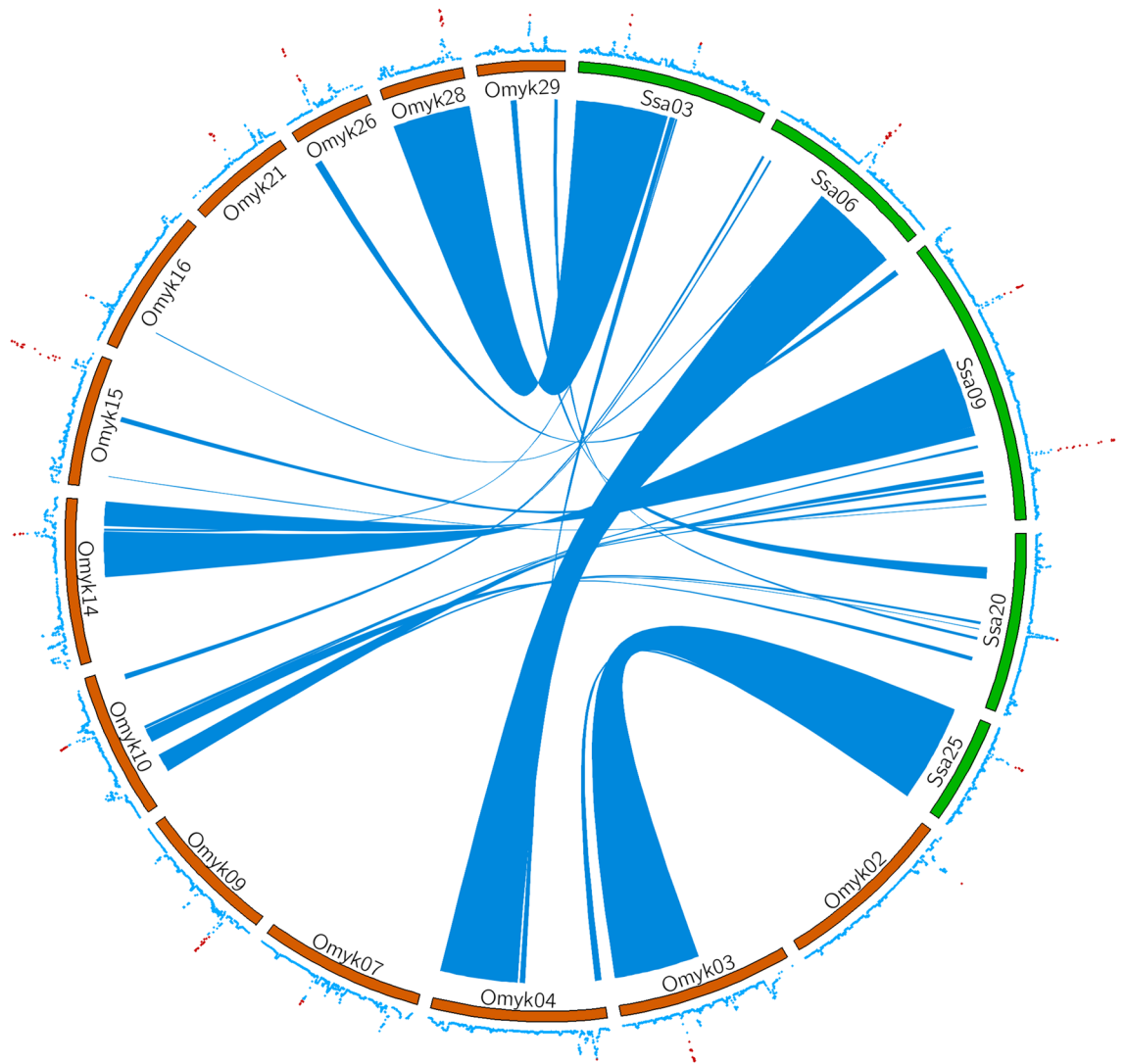


Figure 4. A circos plot for genomic regions explaining more than 1% of the genetic variance for sea lice resistance in Atlantic salmon and rainbow trout. The inner ribbons mark syntenic regions between Atlantic salmon (green and labeled *Ssa*) and rainbow trout (orange and labeled *Omyk*) chromosomes. Genetic variance explained by 20 SNP windows obtained from the wssGWAS analysis are plotted on the outer ring, with the most important windows plotted in red (windows explaining $\geq 1\%$ of the genetic variance).

with *Omy3*. In addition, when performing the search for orthologue genes (Supplementary Table S2), we found uncharacterized proteins in both species, which shared functionality identified by genomic ontology.

We determined 15 orthogroups that were present in QTLs for sea lice resistance and were shared both within and between species (Supplementary Table S2). These orthogroups were classified according to gene ontology annotations⁴⁸. One of the most interesting groups is orthogroup 12 which contained *lysophosphatidic acid receptor 2-like (lpa2)* in Atlantic salmon, and a *G-protein coupled receptor 12-like* in rainbow trout. This orthogroup shares the same GO categories (GO: 0004930, GO: 0007186, GO: 0016021, GO: 0070915, GO: 0007165, GO: 0016020) related to the receptor signaling pathway associated with protein G. The activation of LPA₂ participates in multiple biological processes, such as cytoskeleton modification via actin fiber formation⁴⁹ and have a role in the activation of *related adhesion focal tyrosine kinase (raftk)*⁵⁰, which in turn participates like a stimulating factor for monocytes and macrophages⁵¹. In orthogroup 13, we identified *dual-specificity protein phosphatase 10-like (dusp10)* in Atlantic salmon and *dual-specificity protein phosphatase 8 (dusp8)* in rainbow trout. These genes might have a similar function in both species, which is most likely related to modulating p38⁵² within the MAPK cascade⁵³, a pathway of pro-inflammatory regulators. It has been previously shown that the lice resistant individuals have an up regulated production of pro inflammatory genes than most susceptible fish⁵⁴. The other orthogroups found here did not show a clear relationship with sea lice resistance (Supplementary Table S2).

Conclusion

The GWAS performed here for Atlantic salmon and rainbow trout made it possible to compare the genetic basis of sea lice resistance in both species. We present novel information about resistance to sea lice in both species. Our results suggest that resistance might be mediated by genes controlling leukocyte response and the cytoskeleton, which promote cell mobility and repair of the wound. The analysis of orthologous proteins provided few characterized proteins. Therefore, further investigations are needed to better annotate genes and generate advances in the elucidation of the genetics behind resistance to *Caligus rogercresseyi* and other important traits in salmonids. We found uncharacterized common genes classified under similar mechanisms by GO terms that could explain resistance in both species. These results suggest that similar mechanisms may regulate sea lice resistance in Atlantic salmon and rainbow trout. Our results provide further knowledge to help establish better control and treatment measures for one of the most important parasitic diseases affecting Atlantic salmon and rainbow trout aquaculture.

Material and methods

Experimental animals. All experiments were performed under relevant guidelines and regulations and were approved by the Institutional Committee for Animal Care and Use of the University of Chile (Certificate N 17,041-VET-UCH).

Sea lice challenge in rainbow trout. The fish for this study belong to a rainbow trout breeding population established in 1998 by Aguas Claras S.A., at Quetroleufu, IX Region, Chile, and currently owned by EFFIGEN S.A. (Puerto Montt, Chile). The population of this study were from the year-class 2011, which has undergone three generations of selection growth, carcass quality and others traits of interest. The details of the population management and breeding program were described by Yoshida et al.⁵⁵. For this study, a total of 2588 PIT (Passive Integrated Transponder) -tagged rainbow trout, originated from 105 maternal full-sib families from the 2012 year-class, were used. For the challenge, the fish were separated into three different tanks so that each family was equally represented in each tank. The *C. rogercresseyi* infestation was conducted with a total of 105,600 copepodites, i.e., an infestation pressure of ~40 copepods/fish, which were produced in vitro from ovigerous females. The infestation consisted of depositing the copepodites in each one of the three test tanks, stopping the water flow and keeping the room in darkness for 6 h. On the sixth day after infestation, parasite counting on all fins was performed and caudal fins were sampled for genetic analysis. All fish were euthanized and fins were examined for parasite count using a stereoscopic magnifying glass. Body weight was also recorded for each animal at the end of the challenge.

Sea lice challenge in Atlantic salmon. A total of 2559 Atlantic salmon smolts belonging to 118 maternal full-sib families from the 2010 year-class of Salmenes Chaicas S.A. (Puerto Montt, Chile), were challenged with *C. rogercresseyi*. The fish were PIT-tagged, acclimated and distributed into three tanks as described in previous studies^{9,10}. Infestation with the parasite was carried out using 13–24 copepods per fish, stopping the water flow for 6 h after the infestation. The challenge lasted 6 days, then the fish were euthanized and the sea lice were counted on all of the fins. A sample of the caudal fin was taken for genetic analysis and the body weight of each fish was measured at the end of the challenge.

Genotyping. Genomic DNA was extracted from the caudal fin of each challenged fish using the DNeasy Blood & Kit tissue kit (Qiagen), following the manufacturer's instructions. The 2628 Atlantic salmon samples were genotyped using a custom Affymetrix® 50 K Axiom® myDesign™ Genotyping Array designed by AquaIn-novo and the University of Chile⁵⁶, while the 2643 rainbow trout samples were genotyped with a 57 K SNP array developed by the United State Department of Agriculture (USDA)⁵⁷. Quality control of the genotypes was carried out in PLINK v1.90b3.34. SNPs with a call rate ≤ 0.95, a Minor Allele Frequency (MAF) < 0.05 and those that were not in Hardy–Weinberg equilibrium ($p < 1 \times 10^{-6}$) were discarded. Individuals were filtered if they had a call rate ≤ 0.95. All the SNPs and fish that passed quality control, were used for downstream analysis.

Genomic association analysis. Resistance to *C. rogercresseyi* was defined as follows, according to Øde-gård et al.¹¹:

$$\text{LogLD} = \log_e \left(\frac{\text{LC} + 1}{\sqrt[3]{\text{BW}^2}} \right)$$

where LD is the *C. rogercresseyi* density defined as the lice count (LC) on each fish at the end of the experimental challenge plus a unity, divided by the cube root of the squared body weight of the fish on the same day (BW), which is an approximation of the surface of the skin of each fish. The logarithm of LD was used as it has an approximately normal distribution.

A weighted single-step genomic association study (wssGWAS)⁵⁸ was used to identify associations between SNPs and resistance to *C. rogercresseyi* in both species, using the BLUPF90 family of programs⁵⁹. This approach uses a combination of both genomic and pedigree matrixes. Genotype and pedigree information were used to generate the **H** kinship matrix⁶⁰, as defined in the following equation:

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

where A^{-1} is the inverse of the relationship matrix for all the animals, constructed from the pedigree, A_{22}^{-1} is the inverse of the pedigree relationship matrix for the genotyped animals, and G^{-1} is the inverse of the genomic relationship matrix for the genotyped animals. The SNPs were weighted with equal value and assigned the constant 1 to perform the single-step GWAS method. For the weighted single-step GWAS method, the markers were assigned to weights estimated by the previous method. The association analysis for both species were performed using the following mixed linear model $y = Xb + Za + e$, where y is the vector of phenotypic values (LogLD); b is the vector of fixed effects (tank); a is the vector of random animal effects, considering the structure of covariance between individuals established by matrix H , and e is the vector of random residuals; X and Z are the incidence matrices for fixed and random animal effects, respectively.

To identify the regions of the genome associated with resistance, we generated windows of 20 adjacent SNPs. Thereafter, if a window explained more than 1% of the genetic variance, it was considered associated with the trait. We also estimated the p-values for individual SNP-trait associations by using BLUPF90 software⁶¹.

Genome comparison. The rainbow trout (GCF_002163495.1)⁶² and Atlantic salmon (GCF_000233375.1)⁶³ genomes were downloaded from the NCBI database and the subset for chromosomes associated with sea lice resistance was aligned with software Samtools⁶⁴. Synteny between the chromosomes was identified by aligning sequences using the program Symap v3.4⁶⁵. Circos⁶⁶ was used to visualize the relationships between genomic regions associated to sea lice resistance in rainbow trout and Atlantic salmon chromosomes.

Candidate genes. The 71 pb flanking sequences surrounding SNPs associated with sea lice resistance were aligned to the most recent reference genomes of Atlantic salmon and rainbow trout using BLASTn⁶⁷. Sequences covering 1 Mb, flanking the associated SNPs (0.5 Mb downstream and 0.5 Mb upstream), were saved in the FASTA format. BLASTx was then used to identify coding sequences for proteins in these 1 Mb associated windows. Blast2GO⁶⁸ was used in parallel with the FASTA file to identify proteins and classify them by function. For both species, the reference genome of *Danio rerio* (GenBank Assembly Accession: GCA_000002035.4) was used to annotate proteins that were not characterized in the rainbow trout or Atlantic salmon reference genomes. To identify orthologous proteins/genes between species, the OrthoFinder⁶⁹ program was used with the FASTA sequences obtained with BLASTx.

Ethics approval and consent to participate. All the experimental challenges were approved by the Institutional Committee for Animal Care and Use of the University of Chile (Certificate N 17,041-VET-UCH). We also confirm that the study was carried out in compliance with the ARRIVE guidelines.

Data availability

Atlantic salmon and rainbow trout phenotype and genotype data are available at https://figshare.com/articles/Comparative_genomic_of_O_mykiss_and_S_salar_for_resistance_to_Sea_lice/7676147 and https://figshare.com/projects/Comparative_genomic_of_O_mykiss_and_S_salar_for_resistance_to_Sea_lice/70904, respectively.

Received: 13 May 2019; Accepted: 10 June 2021

Published online: 24 June 2021

References

- Costello, M. J. How sea lice from salmon farms may cause wild salmonid declines in Europe and North America and be a threat to fishes elsewhere. *Proc. R. Soc. B Biol. Sci.* **276**, 3385–3394 (2009).
- Abolofia, J., Asche, F. & Wilen, J. E. The cost of lice: Quantifying the impacts of parasitic sea lice on farmed salmon. *Mar. Resour. Econ.* **32**, 329–349 (2017).
- Barrett, L. T., Oppedal, F., Robinson, N. & Dempster, T. Prevention not cure: A review of methods to avoid sea lice infestations in salmon aquaculture. *Rev. Aquac.* **12**, 2527–2543 (2020).
- Boxshall, A. & Bravo, S. On the identity of the common Caligus (Copepoda: Siphonostomatoida: Caligidae) from salmonid netpen systems in southern Chile. *Contrib. Zool.* **69**, 137–146 (2000).
- Lhorente, J. P., Araneda, M., Neira, R. & Yáñez, J. M. Advances in genetic improvement for salmon and trout aquaculture: The Chilean situation and prospects. *Rev. Aquac.* **11**, 340–353 (2019).
- Hamilton-West, C. *et al.* Epidemiological description of the sea lice (*Caligus rogercresseyi*) situation in southern Chile in August 2007. *Prev. Vet. Med.* **104**, 341–345 (2012).
- Correa, K. *et al.* Genome wide association study for resistance to *Caligus rogercresseyi* in Atlantic salmon (*Salmo salar* L.) using a 50K SNP genotyping array. *Aquaculture* **472**, 61–65 (2017).
- Gonzalez, L. & Juan, C. Life cycle of *Caligus rogercresseyi*, (Copepoda: Caligidae) parasite of Chilean reared salmonids. *Aquaculture* **220**, 101–117 (2003).
- Lhorente, J. P. *et al.* Quantitative genetic basis for resistance to *Caligus rogercresseyi* sea lice in a breeding population of Atlantic salmon (*Salmo salar*). *Aquaculture* **324–325**, 55–59 (2012).
- Yáñez, J. M. *et al.* Genetic co-variation between resistance against both *Caligus rogercresseyi* and *Piscirickettsia salmonis*, and body weight in Atlantic salmon (*Salmo salar*). *Aquaculture* **433**, 295–298 (2014).
- Ødegård, J. *et al.* Genomic prediction in an admixed population of Atlantic salmon (*Salmo salar*). *Front. Genet.* **5**, 1–8 (2014).
- Tsai, H. Y. *et al.* Genomic prediction of host resistance to sea lice in farmed Atlantic salmon populations. *Genet. Sel. Evol.* **48**, 1–11 (2016).
- Bassini, N. *et al.* Genetic parameters for resistance to *Caligus rogercresseyi*, *Piscirickettsia salmonis* and body weight in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **472**, 127–127 (2017).

14. Correa, K., Bangera, R., Figueroa, R., Lhorente, J. P. & Yáñez, J. M. The use of genomic information increases the accuracy of breeding value predictions for sea louse (*Caligus rogercresseyi*) resistance in Atlantic salmon (*Salmo salar*). *Genet. Sel. Evol.* **49**, 1–5 (2017).
15. Hardisson, R. C. Comparative genomics. *Brenner's Encycl. Genet. Second Ed.* **1**, 102–105 (2013).
16. Fang, G., Bhardwaj, N., Robilotto, R. & Gerstein, M. B. Getting started in gene orthology and functional analysis. *PLoS Comput. Biol.* **6**, 20 (2010).
17. Reid, D. P., Szanto, A., Glebe, B., Danzmann, R. G. & Ferguson, M. M. QTL for body weight and condition factor in Atlantic salmon (*Salmo salar*): Comparative analysis with rainbow trout (*Oncorhynchus mykiss*) and Arctic charr (*Salvelinus alpinus*). *Heredity (Edinb.)* **94**, 166–172 (2005).
18. Moghadam, H. K., Ferguson, M. M. & Danzmann, R. G. Evolution of Hox clusters in salmonidae: A comparative analysis between atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). *J. Mol. Evol.* **61**, 636–649 (2005).
19. Woram, R. A. Comparative genome analysis of the primary sex-determining locus in salmonid fishes. *Genome Res.* **13**, 272–280 (2003).
20. Yáñez, J. M. *et al.* Comparative genomic analysis of three salmonid species identifies functional candidate genes involved in resistance to the intracellular bacterium *Piscirickettsia salmonis*. *Front. Genet.* **10**, 665 (2019).
21. Robledo, D., Gutiérrez, A. P., Barria, A., Yáñez, J. M. & Houston, R. D. Gene expression response to sea lice in Atlantic salmon skin: RNA sequencing comparison between resistant and susceptible animals. *Front. Genet.* **9**, 1–10 (2018).
22. Rochus, C. M. *et al.* Genome-wide association analysis of salmon lice (*Lepeophtheirus salmonis*) resistance in a North American Atlantic salmon population. *Aquac. Res.* **49**, 1329–1338 (2018).
23. Robledo, D. *et al.* Discovery and functional annotation of quantitative trait loci affecting resistance to Sea lice in Atlantic salmon. *Front. Genet.* **10**, 1–10 (2019).
24. Vallejo, R. L. *et al.* Genomic selection models double the accuracy of predicted breeding values for bacterial cold water disease resistance compared to a traditional pedigree-based model in rainbow trout aquaculture. *Genet. Sel. Evol.* **49**, 1–13 (2017).
25. Wiegertjes, G. F., Wentzel, A. S., Spaink, H. P., Elks, P. M. & Fink, I. R. Polarization of immune responses in fish: The 'macrophages first' point of view. *Mol. Immunol.* **69**, 146–156 (2016).
26. Tamehiro, N. *et al.* T-cell activation RhoGTPase-activating protein plays an important role in T H 17-cell differentiation. *Immunol. Cell Biol.* **95**, 729–735 (2017).
27. Ruegg, C. R., Chiquet-Ehrismann, R. & Alkan, S. S. Tenascin, an extracellular matrix protein, exerts immunomodulatory activities. *Proc. Natl. Acad. Sci.* **86**, 7437–7441 (1989).
28. Clark, R. A., Erickson, H. P. & Springer, T. A. Tenascin supports lymphocyte rolling. *J. Cell Biol.* **137**, 755–765 (1997).
29. Fast, M. D. Fish immune responses to parasitic copepod (namely sea lice) infection. *Dev. Comp. Immunol.* **43**, 300–312 (2014).
30. Berry, G. *et al.* Distinct effects of T-bet in TH1 lineage commitment and IFN- γ production in CD4 and CD8 T cells. *Science* **295**, 338–342 (2002).
31. Townsend, M. J. *et al.* T-bet regulates the terminal maturation and homeostasis of NK and Va14i NKT cells. *Immunity* **20**, 477–494 (2004).
32. Coffey, P. J. & Burgering, B. M. T. Forkhead-box transcription factors and their role in the immune system. *Nat. Rev. Immunol.* **4**, 889–899 (2004).
33. Veerle, J. & Jozef, G. Protein phosphatase 2A: A high regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *Biochem. J.* **353**, 417–439 (2001).
34. McNab, F. W., Rajsbaum, R., Stoye, J. P. & O'Garra, A. Tripartite-motif proteins and innate immune regulation. *Curr. Opin. Immunol.* **23**, 46–56 (2011).
35. Stenmark, H. Rab GTPases as coordinators of vesicle traffic. *Nat. Rev. Mol. Cell Biol.* **10**, 513–525 (2009).
36. Johnson, J. L., Monfregola, J., Napolitano, G., Kiosses, W. B. & Catz, S. D. Vesicular trafficking through cortical actin during exocytosis is regulated by the Rab27a effector JFC1/Slp1 and the RhoA-GTPase-activating protein Gem-interacting protein. *Mol. Biol. Cell* **23**, 1902–1916 (2012).
37. Valenzuela-Muñoz, V., Boltaña, S. & Gallardo-Escárate, C. Comparative immunity of *Salmo salar* and *Oncorhynchus kisutch* during infestation with the sea louse *Caligus rogercresseyi*: An enrichment transcriptome analysis. *Fish Shellfish Immunol.* **59**, 276–287 (2016).
38. Chen, J. *et al.* Expression and function of the epidermal growth factor receptor in physiology and disease. *Physiol. Rev.* **96**, 1025–1069 (2016).
39. Skugor, S., Glover, K. A., Nilsen, F. & Krasnov, A. Local and systemic gene expression responses of Atlantic salmon (*Salmo salar* L.) to infection with the salmon louse (*Lepeophtheirus salmonis*). *BMC Genom.* **9**, 1–18 (2008).
40. Brzostowski, J. A., Fey, P., Yan, J., Isik, N. & Jin, T. The Elmo family forms an ancient group of actin-regulating proteins. *Commun. Integr. Biol.* **2**, 337–340 (2009).
41. Lemmon, M. A., Ferguson, K. M. & Abrams, C. S. Pleckstrin homology domains and the cytoskeleton. *FEBS Lett.* **513**, 71–76 (2002).
42. Wang, T., Holland, J. W., Martin, S. A. M. & Secombes, C. J. Sequence and expression analysis of two T helper master transcription factors, T-bet and GATA3, in rainbow trout *Oncorhynchus mykiss* and analysis of their expression during bacterial and parasitic infection. *Fish Shellf. Immunol.* **29**, 705–715 (2010).
43. Lamalice, L., Le Boeuf, F. & Huot, J. Endothelial cell migration during angiogenesis. *Circ. Res.* **100**, 782–794 (2007).
44. Zou, J. *et al.* Identification and bioactivities of IFN- γ in rainbow trout *Oncorhynchus mykiss*: The first Th1-type cytokine characterized functionally in fish. *J. Immunol.* **175**, 2484–2494 (2005).
45. Bury, N. & Grosell, M. Iron acquisition by teleost fish. *Comp. Biochem. Physiol. C* **135**, 76490 (2003).
46. Jones, S. R. M., Fast, M. D., Johnson, S. C. & Groman, D. B. Differential rejection of salmon lice by pink and chum salmon: Disease consequences and expression of proinflammatory genes. *Dis. Aquat. Organ.* **75**, 229–238 (2007).
47. Valenzuela-Muñoz, V. & Gallardo-Escárate, C. Iron metabolism modulation in Atlantic salmon infested with the sea lice *Lepeophtheirus salmonis* and *Caligus rogercresseyi*: A matter of nutritional immunity?. *Fish Shellf. Immunol.* **60**, 97–102 (2017).
48. Bateman, A. *et al.* UniProt: The universal protein knowledgebase. *Nucleic Acids Res.* **45**, D158–D169 (2017).
49. Moolenaar, W. H., Kranenburg, O., Postma, F. R. & Zondag, G. C. M. Lysophosphatidic acid: G-protein signalling and cellular responses. *Curr. Opin. Cell Biol.* **9**, 168–173 (1997).
50. Park, S. Y., Schinkmann, K. A. & Avraham, S. RAFTK/Pyk2 mediates LPA-induced PC12 cell migration. *Cell. Signal.* **18**, 1063–1071 (2006).
51. Hatch, W. C. *et al.* The related adhesion focal tyrosine kinase (RAFTK) is tyrosine phosphorylated and participates in colony-stimulating factor-1/macrophage colony-stimulating factor signaling in monocyte-macrophages. *Blood* **91**, 3967–3973 (1998).
52. Keyse, S. M. Dual-specificity MAP kinase phosphatases (MKPs) and cancer. *Cancer Metastasis Rev.* **27**, 253–261 (2008).
53. Owens, D. M. & Keyse, S. M. Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases. *Oncogene* **26**, 3203–3213 (2007).
54. Núñez-Acuña, G. *et al.* Transcriptome immunomodulation of in-feed additives in Atlantic salmon *Salmo salar* infested with sea lice *Caligus rogercresseyi*. *Fish Shellf. Immunol.* **47**, 450–460 (2015).
55. Yoshida, G. M. *et al.* Genomic prediction accuracy for resistance against *Piscirickettsia salmonis* in farmed rainbow trout. *G3 Genes Genomes Genet.* **8**, 719–726 (2018).

56. Yáñez, J. M. *et al.* Genomewide single nucleotide polymorphism discovery in Atlantic salmon (*Salmo salar*): validation in wild and farmed American and European populations. *Mol Ecol Resour* **16**, 1002–1011 (2016).
57. Palti, Y. *et al.* The development and characterization of a 57K single nucleotide polymorphism array for rainbow trout. *Mol. Ecol. Resour.* **15**, 662–672 (2015).
58. Legarra, A., Christensen, O. F. & Aguilar, I. M. Single step, a general approach for genomic selection. *Livest. Sci.* <https://doi.org/10.1016/j.livsci.2014.04.029> (2014).
59. Misztal, I., Aggrey, S. E. & Muir, W. M. Experiences with a single-step genome evaluation. *Poult. Sci.* **92**, 2530–2534 (2013).
60. Aguilar, I. *et al.* Hot topic: A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score1. *J. Dairy Sci.* **93**, 743–752 (2010).
61. Misztal, I. *et al.* Manual for BLUPF90 family of programs. *Univ. Georg. Athens, USA* 125 (2018).
62. Pearse, D. E. *et al.* Sex-dependent dominance maintains migration supergene in rainbow trout. *bioRxiv* <https://doi.org/10.1101/504621> (2018).
63. Lien, S. *et al.* The Atlantic salmon genome provides insights into rediploidization. *Nature* **533**, 200–205 (2016).
64. Li, H. *et al.* The sequence alignment/map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).
65. Soderlund, C., Bomhoff, M. & Nelson, W. M. SyMAP v3.4: A turnkey synteny system with application to plant genomes. *Nucleic Acids Res.* **39**, 20 (2011).
66. Connors, J. *et al.* Circos: An information aesthetic for comparative genomics. *Genome Res.* **19**, 1639–1645 (2009).
67. Madden, T. The BLAST sequence analysis tool. In *The NCBI Handbook* (eds McEntyre, J. & Ostell, J.) (National Center for Biotechnology Information, 2002).
68. Conesa, A. *et al.* Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* **21**, 3674–3676 (2005).
69. Emms, D. M. & Kelly, S. OrthoFinder: Solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. *Genome Biol.* **16**, 1–14 (2015).

Acknowledgements

We would like to thank to EFFIGEN S.A. (Puerto Montt, Chile) by providing phenotypic and genotypic data from the rainbow trout population used here.

Author contributions

P.C. assessed the analyses and wrote the initial version of the manuscript. A.B. contributed with results interpretation discussion and writing. K.C. contributed to genome comparison analysis, writing, and discussion. L.N.B. and K.C. managed samples, performed DNA extraction and performed the quality control of genotypes. B.G. contributed with writing, revision and discussion of the results. J.P.L. contributed to the study design. J.M.Y. conceived the study, contributed to results interpretation with a discussion. All authors approved the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-021-92425-3>.

Correspondence and requests for materials should be addressed to J.M.Y.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2021