istituto zooprofilattico sperimentale
del Piemonte, Liguria e Valle d'Aosta

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Genetic resistance to infectious disease in trout

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The production of rainbow trout has grown exponentially since the 1950s, especially in Europe and more recently in Chile.
Chile is currently the largest producer. Major producing countries include Norway, Denmark, France, Italy, Spain, Greece, USA, Germany, Turkey, Iran and the UK.

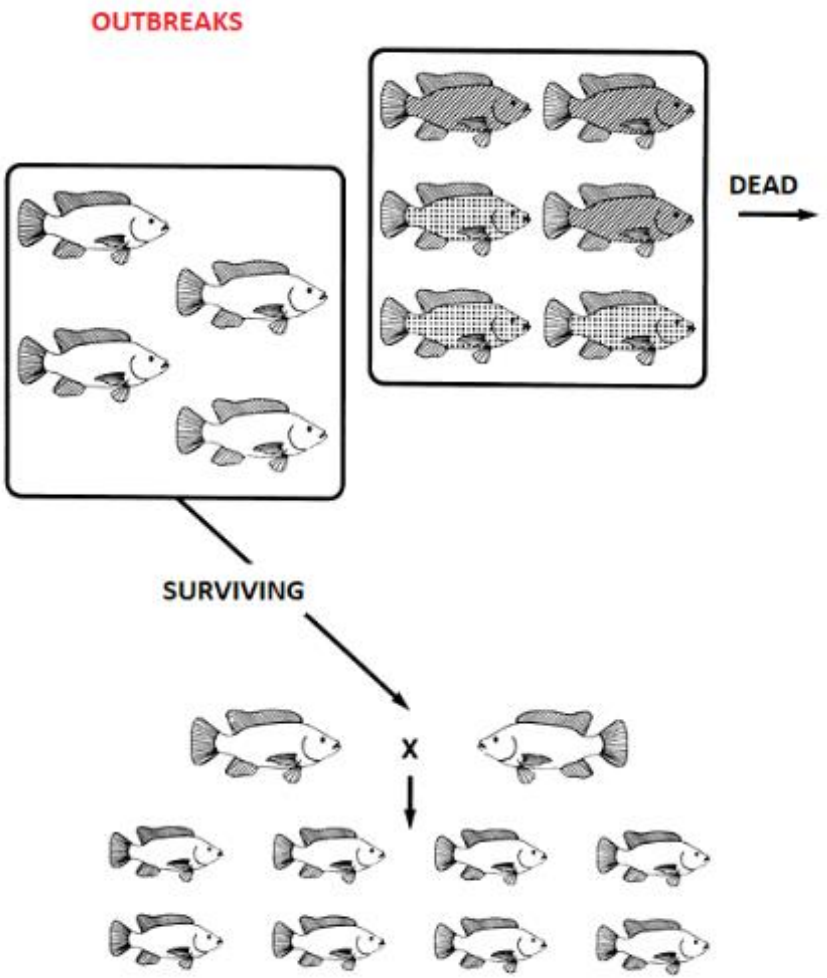
DISEASE	AGENT	TYPE	SYNDROME	MEASURES
Furunculosis	Aeromonas salmonicida	Bacterium	Inflammation of intestine; reddening of fins; boils on body; pectoral fins infected; tissues die back	Antibiotic mixed with food, e.g. oxytetracycline
Similar to furunculosis	Aeromonas liquefaciens	Bacterium	Smaller lesions on body that become open sores; fins become reddened and tissues break down	Same treatment as furunculosis
Vibriosis	Vibrio anguillarum	Bacterium	Loss of appetite; fins and areas around vent and mouth become reddened; sometimes bleeding around mouth and gills; potential high mortality	Same as furunculosis, plus vaccine for greater protection
Bacterial kidney disease (BKD)	Corynebacterium	Bacterium	Whitish lesions in the kidney; bleeding from kidneys and liver; some fish may lose appetite and swim close to surface; appear dark in colour	Same as furunculosis
Bacterial gill disease	Myxobacterium	Bacterium	Loss of appetite; swelling and reddening of gills; eventually gill filaments mass together and become paler with a secretion blocking gill function in later stage	Bathing in bacteriocide and regular filtering of water supply to remove particles in water
Infective Pancreatic Necrosis	IPN	Virus	Erratic swimming, eventually to bottom of tank where death occurs	No treatment available; eradicate disease by removal of infected stock
Infective Haematopoietic Necrosis	IHN	Virus	Erratic swimming eventually floating upside down whilst breathing rapidly after which death occurs; eyes bulge; bleeding from base of pectoral fins, dorsal fin and vent	As above
Viral Haemorrhagic Septicaemia	VHS	Virus	Bulging eyes and, in some cases, bleeding eyes; pale gills; swollen abdomen; lethargy	As above
White spot	Ichthyophthirius multifilis	Protozoan	White patches on body; becoming lethargic; attempt to remove parasites by rubbing on side of tank	Formalin bath for surface parasites; copper sulphate for parasites below surface; prevented by fast-flowing water
Whirling disease (Myxosomiasis)	Myxosoma cerebralis	Protozoan	Darkening of skin; swimming in spinning fashion; deformities around gills and tail fin; death eventually occurs	No treatment; fish must be kept out of infected water; water treated with calcium cyanamide
Hexamitosis Octomitis	Hexamita truttae	Protozoan	Lethargic, sinking to bottom of tank where death occurs; some fish make sudden random movements	Feed calomel with food
Costiasis	Costia necatrix	Protozoan	Blue-grey slime on skin which contains parasite	Formalin bath
Fluke	Gyrodactylus sp.	Trematode	Parasites attached to caudal and anal fins; body and fins erode, leaving lesions that are attacked by Saprolegnia	Formalin bath
Trematodal parasite	Diplostomum spathaceum	Trematode	Eye lens cloudy; loss of condition	No treatment available. Water supply kept clear of snail hosts

Gram negative bacterial diseases
Red mouth-disease *Yersinia ruckeri*

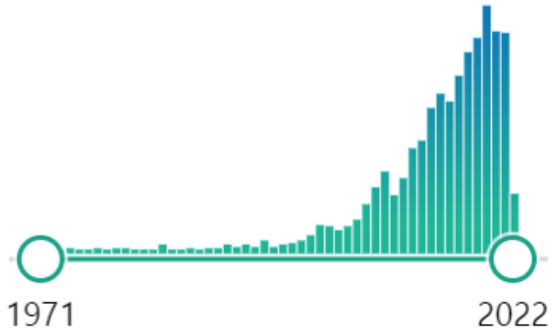
Gram positive bacterial diseases
Streptococcosis

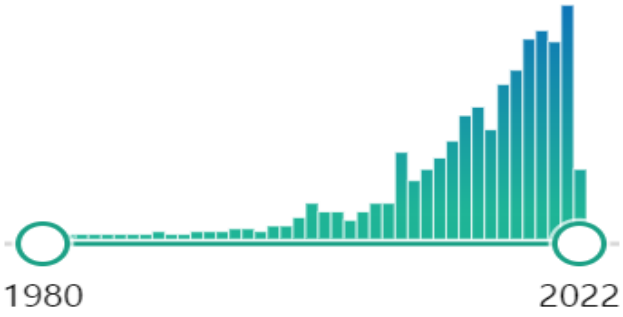
https://www.fao.org/fishery/en/culturedspecies/oncorhynchus_mykiss/en

1. **Economic impact (incidence, mortality)**
2. **Vaccine availability**
3. **Treatments availability**
4. **Zoonotic potential**


OUTBREAKS
DEAD
SURVIVING
X

In the late 1990s, genetic-based methods began to appear alongside the phenotypic approach and a variety of genetic markers for aquaculture species were developed.





animals

MDPI

Review

What Can Genetics Do for the Control of Infectious Diseases in Aquaculture?

Simona Sciuto [1], Licia Colli [2], Andrea Fabris [3], Paolo Pastorino [1,*], Nadia Stoppani [1], Giovanna Esposito [1], Marino Prearo [1], Giuseppe Esposito [1], Paolo Ajmone-Marsan [2], Pier Luigi Acutis [1] and Silvia Colussi [1]

AFLP	Amplified Fragment Length Polymorphisms	Restriction enzymes are used for genom the restriction fragments is selected for complementary to the ligation adaptor sequence, and a few nucleotides inside The markers are a cost-effective alternat economic resources are limited.
RAPD	Random Amplified Polymorphic DNA	The genome is amplified using several arbitrary short primers (10–12 nucleotides). AFLP markers are usually preferred to RAPD because of their greater reproducibility.
RFLP	Restriction Fragment Length Polymorphic DNA	Genomic DNA is digested by restriction enzymes; the fragments separate on agarose gel and create different patterns. The markers are poorly polymorphic, however, which is a major drawback.
SSR/STR/VNTR	Microsatellite Repeats	Specific sequences of DNA containing tandem repeats. The number of repeats differs for alleles at a specific locus; a specific set of primers is used in simplex or multiplex PCR for loci amplification. These markers are commonly used because of their high polymorphic information content and their wide distribution throughout the genome.
ESTs	Expressed Sequence Tags	ESTs derived from c-DNA libraries, constructed using mRNA expressed in tissues. They are useful tools for marker development in species where the full genome is not yet available.
SNP	Single Nucleotide Polymorphism	DNA sequence variations at a single nucleotide level are used as genetic markers. They are the most frequent polymorphism in any organism, adaptable to automation, and reveal hidden polymorphisms not detected by other methods.
ddRAD	Double-Digest Restriction-Site-Associated DNA Sequencing	This method is based on the enzymatic digestion of the whole genomic DNA and the creation of multiplexed libraries, with consequent binding to specific adapters (reduced representation libraries) which are more laborious and less accurate than SNP analysis.

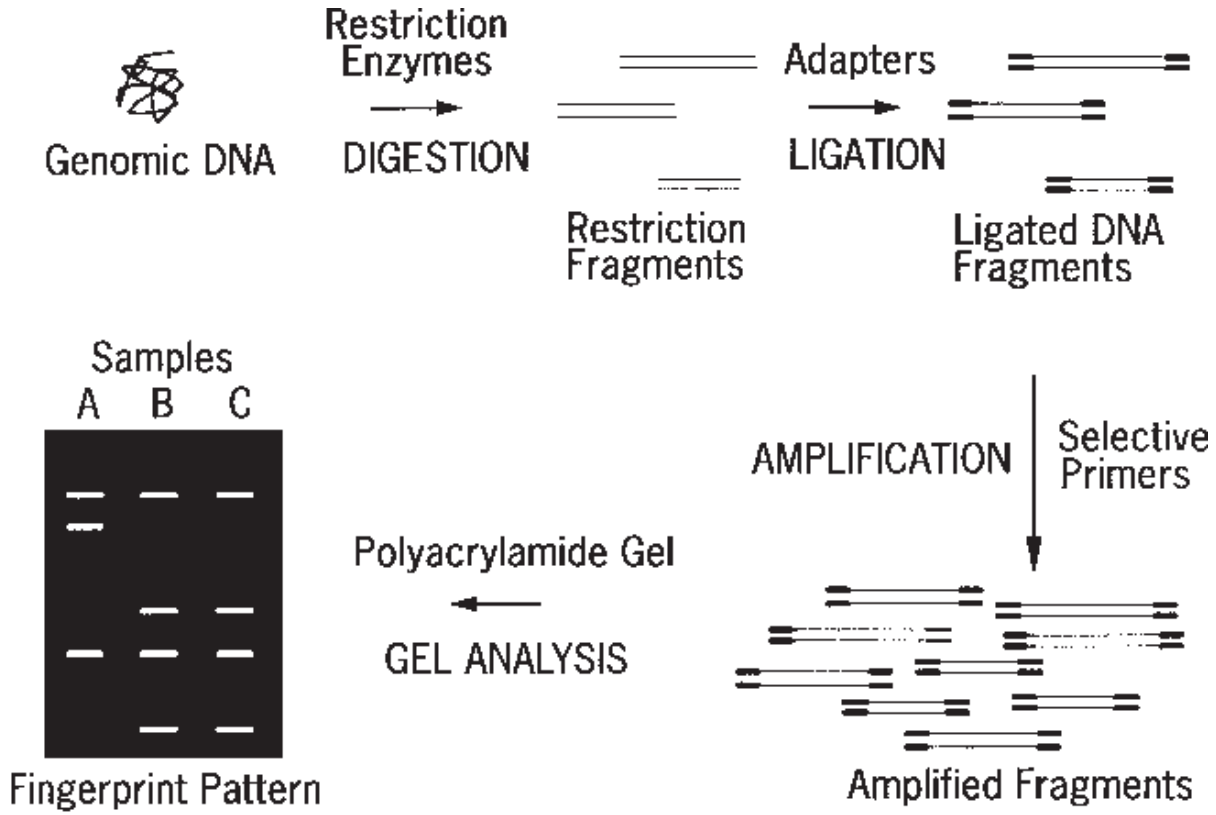


Figure 1 A schematic displaying the four basic steps of AFLP: diges

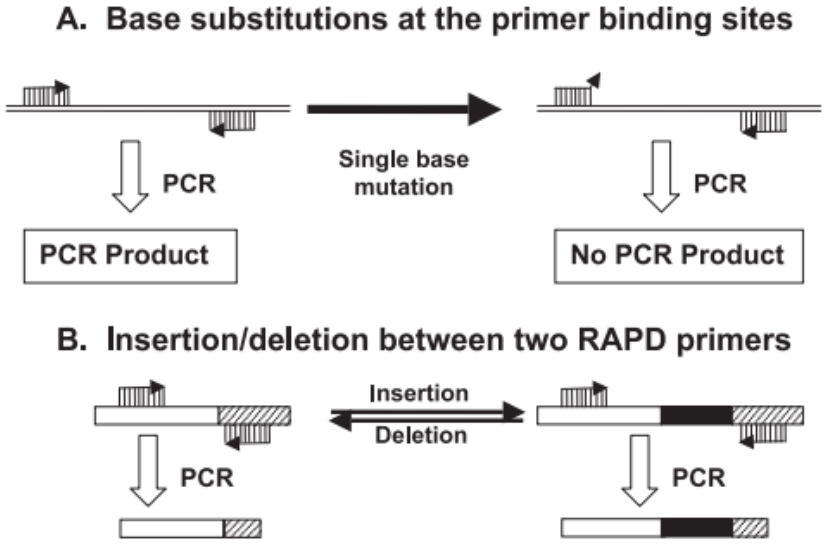
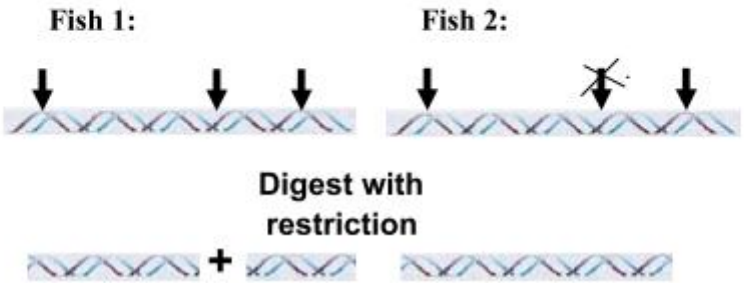
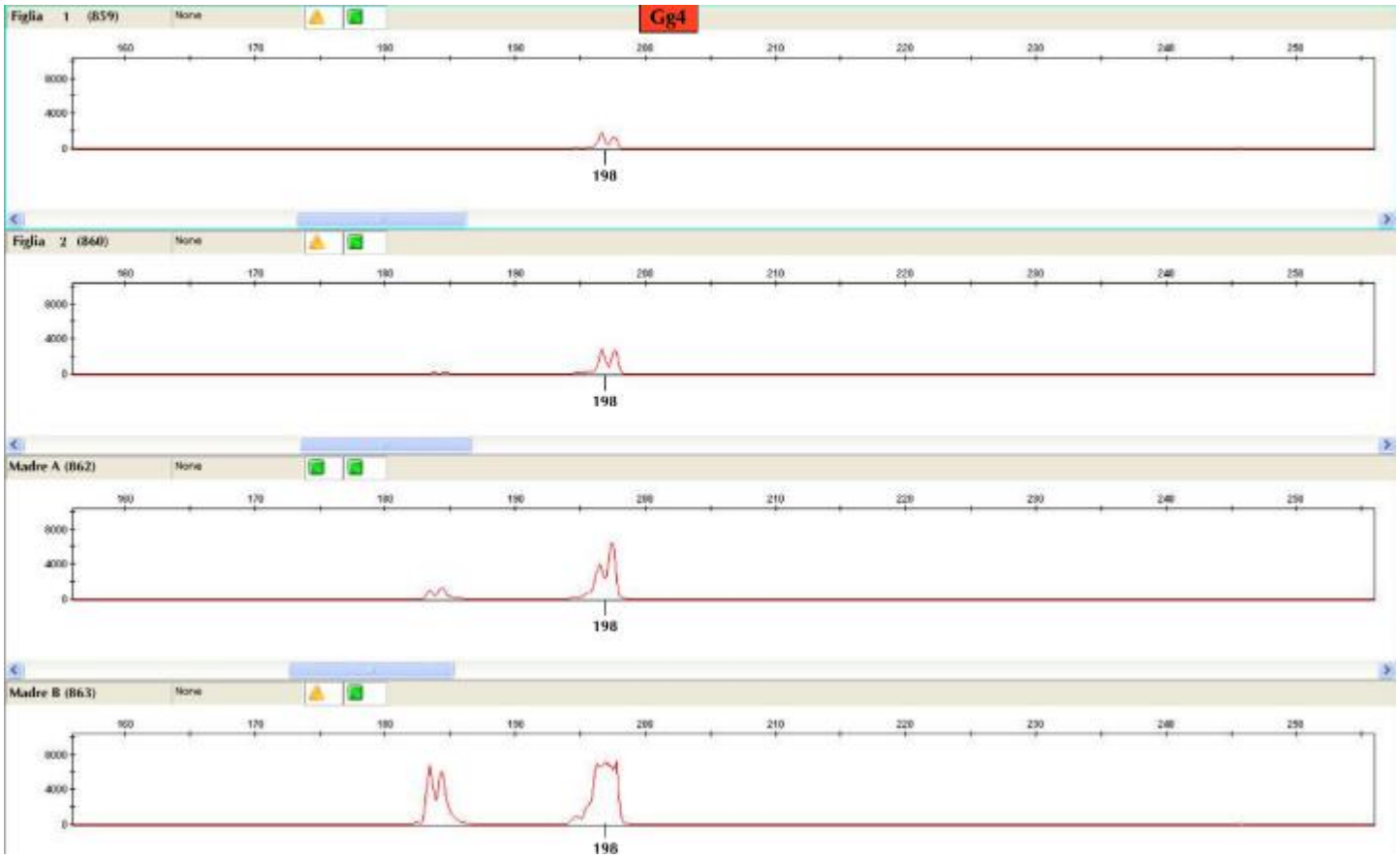


Fig. 3. Molecular basis of RAPD polymorphism. (A) Base substitutions in the primer binding sites, especially at the 3′ end of the primer binding sites may lead to decrease (as shown) or increase of the number of RAPD bands. (B) Insertion or deletion between two primers may lead to increase or decrease of fragment sizes.

Z.J. Liu, J.F. Cordes / Aquaculture 238 (2004) 1–37

A. Base substitutions at the restriction sites




Figlia 1 (859)
None
Gg4
198
Figlia 2 (860)
None
198
Madre A (862)
None
198
Madre B (863)
None
198

WHAT COULD WE DO WILL ALL THESE MARKERS?

We could apply Marker-assisted selection (MAS) for disease resistance, by selecting for genetic variants associated with resistance through the use of **nearby genetic markers.**

WHAT DOES IT MEAN?

It means to use genetic markers as flags for the target genes
Selection targets region, termed a quantitative trait locus (QTL), that may comprise several genes. MAS has been applied to viral, bacterial, and parasitic diseases in trout

Table 1. Genetic selection in aquaculture species.

Genetic Approach	Species	Pathogen/Disease	Reference
Marker-assisted Selection	Rainbow trout	*Rhabdovirus*	[24]
	Rainbow trout	*Aeromonassalmonicida*	[25]
	Rainbow trout	*Vibrioanguillarum*	[26]
	Rainbow trout	*Flavobacterium psychrophilum*	[27]
	Rainbow trout	Viral hemorrhagic septicemia virus	[27]
	Rainbow trout	*Flavobacterium columnare*	[28]
	Rainbow trout	*Flavobacterium psychrophilum*	[29]

OPEN ACCESS Freely available online

PLOS ONE

Resistance to a Rhabdovirus (VHSV) in Rainbow Trout: Identification of a Major QTL Related to Innate Mechanisms

Eloi R. Verrier[1,2,3], Michel Dorson[2], Stéphane Mauger[1¤], Corinne Torhy[2], Céline Ciobotaru[1], Caroline Hervet[1], Nicolas Dechamp[1], Carine Genet[1], Pierre Boudinot[2], Edwige Quillet[1]*

1 INRA, UMR 1313 Génétique Animale et Biologie Intégrative, Jouy-en-Josas, France, **2** INRA, UR892 Virologie et Immunologie Moléculaires, Jouy-en-Josas, France, **3** AgroParisTech, Paris, France

ELSEVIER

Aquaculture

Volume 241, Issues 1–4, 26 November 2004, Pages 93-115

Genetic markers associated with resistance to infectious hematopoietic necrosis in rainbow and steelhead trout (*Oncorhynchus mykiss*) backcrosses

M. Fernanda Rodriguez [a, b, ⁂], Scott LaPatra [c], Scott Williams [c], Thomas Famula [a], Bernie May [a]

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istituto zooprofilattico sperimentale

del Piemonte, Liguria e Valle d'Aosta

frontiers in Genetics

ORIGINAL RESEARCH

A Major QTL for Resistance to *Vibrio anguillarum* in Rainbow Trout

Asma M. Karami[1]*, Jørgen Ødegård[2], Moonika H. Marana[1], Shaozhi Zuo[1], Rzgar Jaafar[1], Heidi Mathiessen[1], Louise von Gersdorff Jørgensen[1], Per W. Kania[1], Inger Dalsgaard[3], Torben Nielsen[4] and Kurt Buchmann[1]

[1] Laboratory of Aquatic Pathobiology, Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [2] AquaGen, Trondheim, Norway, [3] Institute of Aquatic Resources, Technical University of Denmark, Kongens Lyngby, Denmark, [4] Aquaculture Ova ApS, Jelling, Denmark

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DOI: 10.1111/jfd.13264

ORIGINAL ARTICLE

Journal of Fish Diseases — WILEY

Quantitative trait loci (QTL) associated with resistance of rainbow trout *Oncorhynchus mykiss* against the parasitic ciliate *Ichthyophthirius multifiliis*

R Jaafar[1] | J Ødegård[2] | H Mathiessen[1] | A M Karami[1] | M H Marana[1] | L von Gersdorff Jørgensen[1] | S Zuo[1] | T Nielsen[3] | P W Kania[1] | K Buchmann[1]

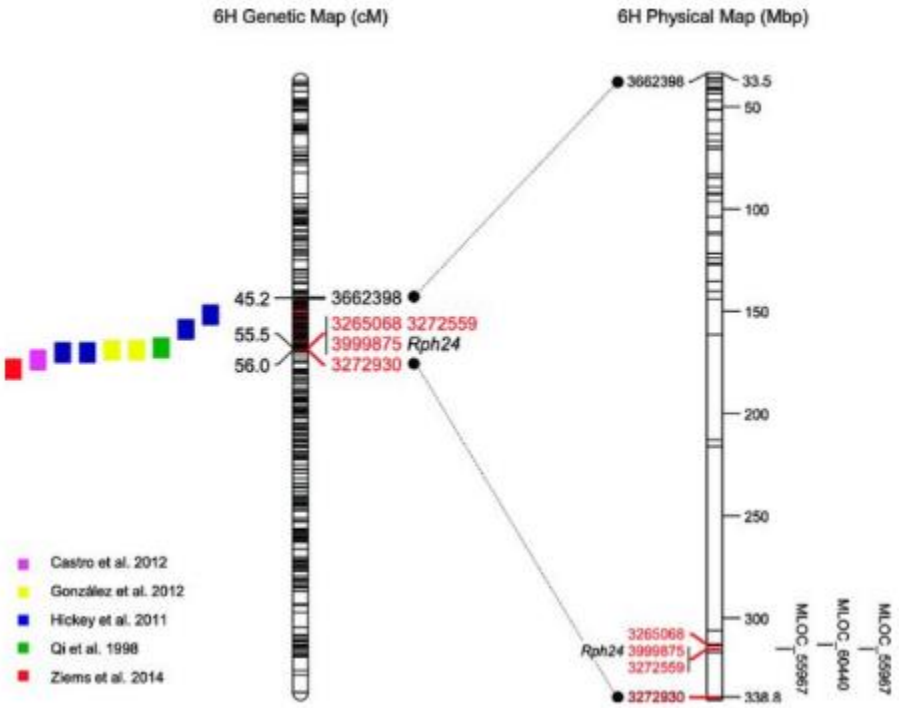
Genet Sel Evol (2018) 50:60
https://doi.org/10.1186/s12711-018-0431-9

GSE Genetics Selection Evolution

RESEARCH ARTICLE — Open Access

Quantitative trait loci for resistance to *Flavobacterium psychrophilum* in rainbow trout: effect of the mode of infection and evidence of epistatic interactions

Clémence Fraslin[1,2], Nicolas Dechamp[1], Maria Bernard[2], Francine Krieg[1], Caroline Hervet[1,5], René Guyomard[1], Diane Esquerré[3], Johanna Barbier[4], Claire Kuchly[1], Eric Duchaud[1], Pierre Boudinot[3], Tatiana Rochat[1], Jean-François Bernardet[3] and Edwige Quillet[1*]


6H Genetic Map (cM)
6H Physical Map (Mbp)
45.2
55.5
56.0
3662398
3265068 3272559
3999875 Rph24
3272930
Castro et al. 2012
González et al. 2012
Hickey et al. 2011
Qi et al. 1998
Ziems et al. 2014
3662398
33.5
50
100
150
200
250
300
338.8
3265068
Rph24 3999875
3272559
3272930
MLOC_55967
MLOC_60440
MLOC_55967

The example of IPN in salmon

Infectious pancreatic necrosis (IPN) is a viral disease that was one of the primary concerns for salmon farming, particularly around the turn of the 21st century, with frequent outbreaks causing high levels of mortality (up to 90%) in stocks both in freshwater hatcheries and following transfer to sea cages. Resistance to IPN was shown to be moderately to highly heritable[217], and breeding companies began to implement family-based selection. In parallel, teams from the UK and Norway identified a single major quantitative trait locus on chromosome 26 that could explain 80–100% of genetic variation in resistance to IPN virus in seawater field trials[218] and experimental freshwater trials[219–221]. High-throughput sequencing subsequently enabled the development of SNP-based genetic tests to predict IPN resistance of salmon without the need for regular disease challenge experiments[222,223]. The practical outcome of these experiments was extensive use of marker-assisted selection for the favourable allele in all major salmon breeding programmes, assisted by the fact that the resistance allele is dominant[220,223]. The results were striking, with a sustained decrease in the incidence of IPN outbreaks to near zero[72] (see the figure). Follow-up functional studies highlighted marked differences in gene expression response to infection between resistant and susceptible salmon fry[224] and suggested that epithelial cadherin may be part of the mechanism underlying the quantitative trait locus[223]. However, the exact causative mutations and the nature of their effect remain at least partly elusive.

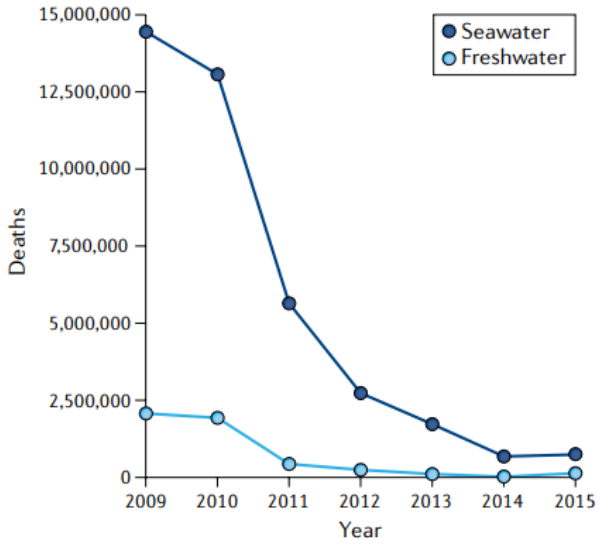


Figure adapted from REF.[72], Elsevier.

Application of the results in Norwegian breeding programmes resulting in a significant disease reduction

Mol Genet Genomics (2001) 265: 23–31
DOI 10.1007/s004380000392

ORIGINAL PAPER

A. Ozaki · T. Sakamoto · S. Khoo · K. Nakamura
M. R. M. Coimbra · T. Akutsu · N. Okamoto

Quantitative trait loci (QTLs) associated with resistance/susceptibility to infectious pancreatic necrosis virus (IPNV) in rainbow trout (*Oncorhynchus mykiss*)

Received: 14 June 2000 / Accepted: 11 October 2000 / Published online: 14 December 2000

DOI: 10.1534/genetics.107.082974

Major Quantitative Trait Loci Affect Resistance to Infectious Pancreatic Necrosis in Atlantic Salmon (*Salmo salar*)

Ross D. Houston,*,[1] Chris S. Haley,* Alastair Hamilton,[†] Derrick R. Guy,[†] Alan E. Tinch,[†] John B. Taggart,[‡] Brendan J. McAndrew[‡] and Stephen C. Bishop*

**Division of Genetics and Genomics, Roslin Institute and Royal (Dick) School of Veterinary Studies, Roslin BioCentre, Midlothian EH25 9PS, United Kingdom, [†]Landcatch Natural Selection, Alloa, Clackmannanshire FK10 3LP, United Kingdom and [‡]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, United Kingdom*

Manuscript received October 5, 2007
Accepted for publication December 14, 2007

ABSTRACT

Infectious pancreatic necrosis (IPN) is a viral disease currently presenting a major problem in the production of Atlantic salmon (*Salmon salar*). IPN can cause significant mortality to salmon fry within freshwater hatcheries and to smolts following transfer to seawater, although challenged populations show clear genetic variation in resistance. To determine whether this genetic variation includes loci of major effect, a genomewide quantitative trait loci (QTL) scan was performed within 10 full-sib families that had received a natural seawater IPN challenge. To utilize the large difference between Atlantic salmon male and female recombination rates, a two-stage mapping strategy was employed. Initially, a sire-based QTL analysis was used to detect linkage groups with significant effects on IPN resistance, using two to three microsatellite markers per linkage group. A dam-based analysis with additional markers was then used to confirm and position any detected QTL. Two genomewide significant QTL and one suggestive QTL were detected in the genome scan. The most significant QTL was mapped to linkage group 21 and was significant at the genomewide level in both the sire and the dam-based analyses. The identified QTL can be applied in marker-assisted selection programs to improve the resistance of salmon to IPN and reduce disease-related mortality.

ANIMAL GENETICS
Immunogenetics, Molecular Genetics and Functional Genomics

Effect of a major QTL affecting IPN resistance on production traits in Atlantic salmon

A. A. Gheyas, C. S. Haley, D. R. Guy, A. Hamilton, A. E. Tinch, J. C. Mota-Velasco, J. A. Woolliams

First published: 10 November 2010 | https://doi.org/10.1111/j.1365-2052.2010.02051.x | Citations: 14

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E-mail: agheyas@swim-back.com or a.gheyas@yahoo.com

Summary

This study investigated the effect of a major QTL for resistance to IPN in salmon on performance and production traits. The traits studied were related to growth, fillet and gutted yields, and fat content. Two different analyses were performed: (1) regression of

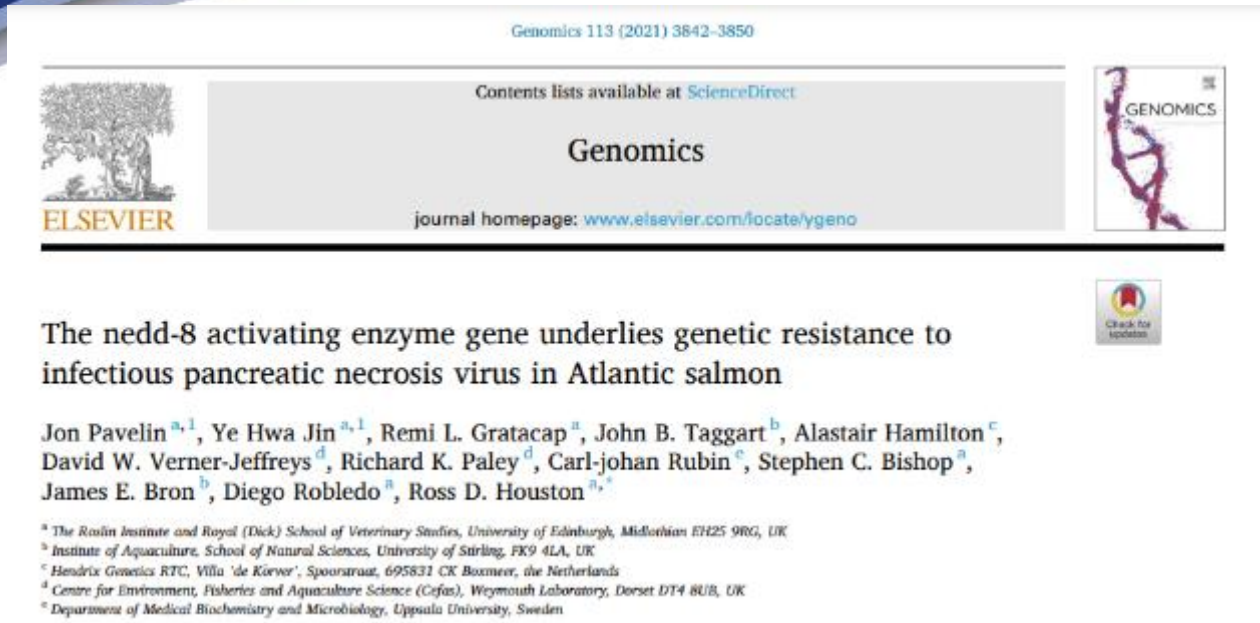
When selection is based on candidate or causative genes, it is termed gene-assisted selection (GAS). Studying IPN resistance in salmon, Moen et al. identified the epithelial cadherin gene as the causative locus of resistance to this disease

GENETICS | INVESTIGATION

Epithelial Cadherin Determines Resistance to Infectious Pancreatic Necrosis Virus in Atlantic Salmon

Thomas Moen,*,† Jacob Torgersen,* Nina Santi,* William S. Davidson,‡ Matthew Baranski,* Jørgen Ødegård,* Sissel Kjøglum,* Bente Velle,§ Matthew Kent,§ Krzysztof P. Lubieniecki,‡ Eivind Isdal,** and Sigbjørn Lien†
*AquaGen, 7462 Trondheim, Norway, †Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, V5A 1S6 Canada, ‡Nofima, NO-9291 Tromsø, Norway, §Centre for Integrative Genetics and Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, 1432 Ås, Norway, and **Vaxinova, 5008 Bergen, Norway

Moen suggested that viral entry into the host may be prevented simply by certain conformation of the surface molecules



Genomics 113 (2021) 3842–3850

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

The nedd-8 activating enzyme gene underlies genetic resistance to infectious pancreatic necrosis virus in Atlantic salmon

Jon Pavelin [a,1], Ye Hwa Jin [a,1], Remi L. Gratacap [a], John B. Taggart [b], Alastair Hamilton [c], David W. Verner-Jeffreys [d], Richard K. Paley [d], Carl-johan Rubin [e], Stephen C. Bishop [a], James E. Bron [b], Diego Robledo [a], Ross D. Houston [a,*]

[a] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian EH25 9RG, UK
[b] Institute of Aquaculture, School of Natural Sciences, University of Stirling, FK9 4LA, UK
[c] Hendrix Genetics RTC, Villa 'de Körver', Spoorstraat, 695831 CK Boxmeer, the Netherlands
[d] Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, Dorset DT4 8UB, UK
[e] Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden

NEDD8 activating enzyme (NAE) has been identified as an essential regulator of the NEDD8 conjugation pathway, which **controls the degradation of many proteins with important roles in cell-cycle progression, DNA damage, and stress responses**.

frontiers in Genetics

ORIGINAL RESEARCH
published: 26 November 2021
doi: 10.3389/fgene.2021.630165

Identification of a New Infectious Pancreatic Necrosis Virus (IPNV) Variant in Atlantic Salmon (*Salmo salar* L.) that can Cause High Mortality Even in Genetically Resistant Fish

*Borghild Hillestad, Stein Johannessen, Geir Olav Melingen and Hooman K. Moghadam**

Benchmark Genetics Norway AS, Bergen, Norway

Abstract: Infectious diseases place an economic burden on aquaculture and a limitation to its growth. An innovative approach to mitigate their impact on production is breeding for disease resistance: selection for domestication, family-based selection, marker-assisted selection, and more recently, genomic selection. Advances in genetics and genomics approaches to the control of infectious diseases are key to increasing aquaculture efficiency, profitability, and sustainability and to reducing its environmental footprint. Interaction and co-evolution between a host and pathogen can, however, turn breeding to boost infectious disease resistance into a potential driver of pathogenic change. Parallel molecular characterization of the pathogen and its virulence and antimicrobial resistance genes is therefore essential to understand pathogen evolution over time in response to host immunity, and to apply appropriate mitigation strategies.

Aquaculture Reports 23 (2022) 101078

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Validation of two QTL associated with lower *Ichthyophthirius multifiliis* infection and delayed-time-to-death in rainbow trout

Kurt Buchmann [a,*], Torben Nielsen [b], Heidi Mathiessen [a], Moonika H. Marana [a], Yajiao Duan [a], Louise V.G. Jørgensen [a], Shaozhi Zuo [a], Asma M. Karami [a], Per W. Kania [a]

[a] *Laboratory of Aquatic Pathobiology, Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C., Denmark*
[b] *Aquasearch ova ApS, Jelling, Denmark*

ARTICLE INFO

Keywords:
Fish
Breeding
QTL
Disease
Parasite

ABSTRACT

Single nucleotide polymorphisms (SNPs) on rainbow trout chromosomes Omy16 and Omy17 are associated with a lower parasitic load and delayed-time-to-death following exposure to the parasitic ciliate *Ichthyophthirius multifiliis* causing white spot disease (WSD). We have evaluated the application of two quantitative trait loci (QTL) represented by two of these SNPs for practical breeding purposes. Homozygous males served as parent fish securing offspring with at least one allele (heterozygous and homozygous fish) associated with higher resistance (QTL fish). We measured the infection levels and time to morbidity/mortality in QTL fish and in non-QTL fish (male parent fish negative for the SNPs) following exposure to infective theronts. We conducted hexaplicate challenge trials (common garden experimental set-up in each tank) and recorded the development of trophonts (white spots) in the fish epidermis and associated morbidity in all six fish tanks and in both fish groups. QTL fish showed a significantly lower infection and delayed development of WSD morbidity. Analyses of SNP locations on the trout chromosomes Omy 16 and Omy 17 may in the future indicate genes associated with higher natural protection. Evidence points at immune factors, physiological functions, mucus production and regulatory elements (lncRNA and pseudogenes).

Elevated natural resistance towards infection could rely on a lower mucous cell density in the skin and/or a lower production of mucus or host molecules excreted through the skin openings.

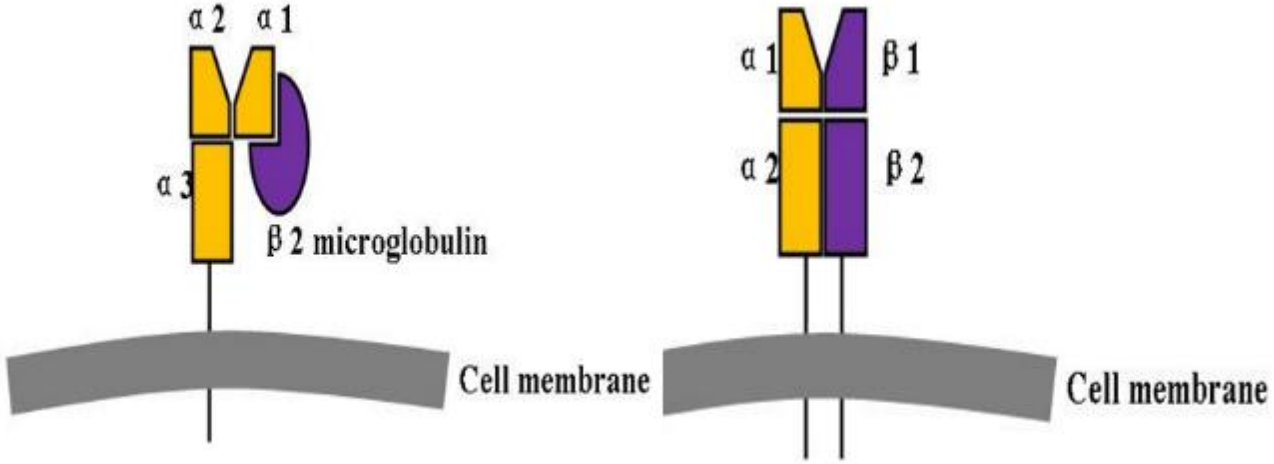
When the rainbow trout genome was scrutinized and searched for genes associated with the SNPs Affx-88916021 and Affx-88911623, it was evident that such a search is challenged by the incomplete annotation of Omy 16 and 17.

Candidate genes

The candidate gene theory states that a significant proportion of the phenotypic variant of a trait can be ascribed to polymorphisms within genes known to be involved in the physiological regulation of the trait

Candidate Gene

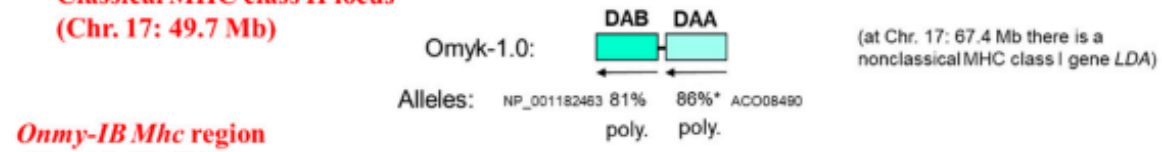
In salmonids, genes of the major histocompatibility complex (MHC) have been well characterized and reported to be associated with resistance to various diseases.



MHC class I displays peptide fragments of proteins from within the cell to cytotoxic T cells. This pathway is often called cytosolic or endogenous pathway.

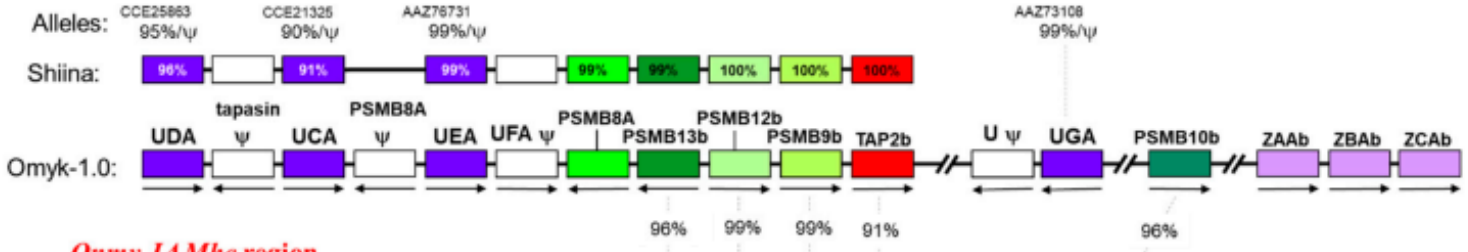
The highly polymorphic MHC class II molecules can present exogenous antigenic peptides including those derived from pathogens to CD4+ T lymphocytes in the acquired immune system.

Classical MHC class II locus (Chr. 17: 49.7 Mb)

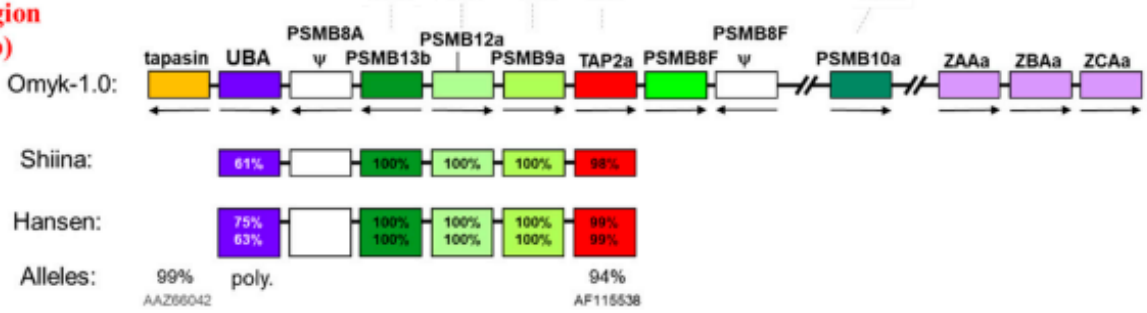


(at Chr. 17: 67.4 Mb there is a nonclassical MHC class I gene *LDA*)

***Onmy-IB Mhc* region (Chr. 14: 28.6 Mb)**



***Onmy-IA Mhc* region (Chr. 18: 33.1 Mb)**



- In rainbow trout we described the MHC class II B-1 domain gene as a candidate for resistance to lactococcosis

- Johnson et al. found a suggestive association between MHC I and resistance to BCWD in rainbow trout.

- Combinations of MHC I and II were found to significantly influence disease resistance to infectious salmon anemia, furunculosis, and infectious hematopoietic necrosis virus in Atlantic salmon

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Association of a specific major histocompatibility complex class IIβ single nucleotide polymorphism with resistance to lactococcosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum)

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ORIGINAL ARTICLE

WILEY

Buccal swab: A tissue sampling method for refinement of experimental procedures involving rainbow trout

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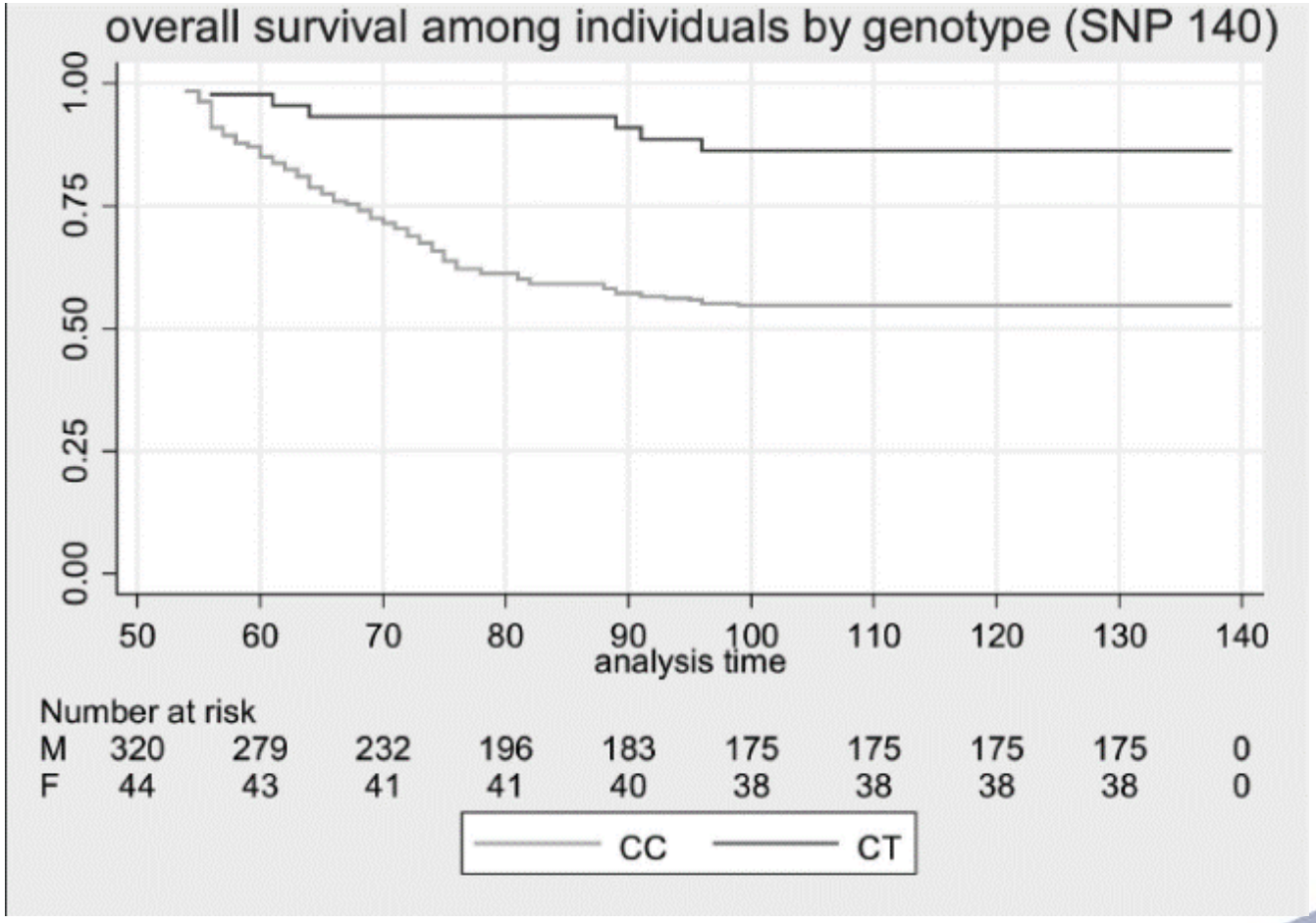
Summary
Buccal swabbing is a minimally invasive method to obtain DNA and biological material from humans and animals, including fish. Reports on buccal swabbing in fish are few and only for a limited number of species. Rainbow trout (*Oncorhynchus mykiss*) is an important animal model and because the yield of DNA may vary among and within different species in individuals of different sizes, it was selected as useful to optimize the buccal DNA collection in this species. Different storage methods were evaluated, aimed at DNA preservation by limiting DNA degradation and bacterial growth, using commonly available and inexpensive reagents. DNA quality was also tested by amplification of a single-copy nuclear gene and a mitochondrial gene. The results suggest that ethanol is the best storage choice for buccal swab sampling in fish genetic studies, as well as suitable for small-bodied rainbow trout.

- 400 trout naturally exposed
- 323 females and 77 males


overall survival among individuals by genotype (SNP 140)
1.00
0.75
0.50
0.25
0.00
50
60
70
80
90
100
110
120
130
140
analysis time
Number at risk
M 320 279 232 196 183 175 175 175 175 0
F 44 43 41 41 40 38 38 38 38 0
CC
CT

			Casi		Controlli		P-value
SNPs	AA	H-W	N°	Freq	N°	Freq	
126 a>g	42 I>M	>0.05	72	0.31	118	0.33	0.72
130 t>c	44 F>H	<**0.05**	18	0.07	23	0.05	NA
131 a>t	44 F>Y	<**0.05**	109	0.55	179	0.58	NA
132 t>g	44 F>L	<**0.05**	21	0.09	41	0.10	NA
135 a>t	45 I>I	<**0.05**	11	0.05	32	0.08	NA
136 g>a	46 D>N	<**0.05**	11	0.05	32	0.08	NA
137 a>c	46 D>A	<**0.05**	11	0.05	32	0.08	NA
140 c>t	47 S>F	>0.05	6	0.02	38	0.09	**0.00**
143 a>t	48 Y>F	>0.05	37	0.15	68	0.16	0.50
145 g>t	49 V>F	>0.05	36	0.14	68	0.16	0.43
154 a>c	52 K>Q	>0.05	60	0.25	105	0.27	0.76
155 a>t	52 K>M	>0.05	5	0.02	11	0.03	0.78
158 t>c	53 V>A	<**0.05**	80	0.38	128	0.37	NA
163 t>c-g	55 Y>D 55 Y>H	<**0.05**	(c) 47 (g) 63	f (c) 0.19 f (g) 0.26	(c) 57 (g) 119	f (c) 0.15 f (g) 0.33	NA
166 a>g	56 I>V	<**0.05**	24	0.11	35	0.08	NA
194 a>t	65 Y>F	<**0.05**	80	0.39	144	0.45	NA
210 a>g	70 E>E	>0.05	39	0.16	66	0.16	0.99
211 c>t	71 H>Y	<**0.05**	16	0.07	20	0.05	NA
217 g>c	73 V>L	<**0.05**	85	0.41	129	0.38	NA
235 t>c	79 W>R	>0.05	8	0.03	11	0.02	0.86
236 g>t	79 W>L	>0.05	15	0.06	14	0.03	0.17
253 a>t	85 I>F	>0.05	14	0.05	38	0.09	**0.03**
255 c>g	85 I>M	>0.05	25	0.08	37	0.09	0.88
272 c>t-g	91 A>V 91 A>G	<**0.05**	(t) 39 (g) 55	f (t) 0.15 f (g) 0.25	(t) 65 (g) 86	f (t) 0.16 f (g) 0.21	NA
274 c>g	92 Q>E	<**0.05**	117	0.61	179	0.62	NA
283 a>c	95 S>R	<**0.05**	123	0.63	164	0.58	NA
286 t>g	96 Y>D	<**0.05**	42	0.19	75	0.20	NA
287 a>t	96 Y>V	<**0.05**	109	0.57	177	0.61	NA
295 c>a	99 H>N	<**0.05**	18	0.08	15	0.04	NA
296 a>c	99 H>P	>0.05	62	0.27	107	0.29	0.55
301 g>a	101 A>T	<**0.05**	14	0.06	13	0.06	NA
305 a>c	102 D>A	>0.05	82	0.37	109	0.29	**0.02**
307 a>c	103 I>L	<**0.05**	66	0.28	124	0.35	NA
308 t>a	103 I>N	>0.05	46	0.19	56	0.13	0.29
309 c>t	103 I>I	<**0.05**	18	0.07	6	0.02	NA
310 g>c-t	104 D>H 104 D>Y	<**0.05**	(c) 109 (t) 41	f (c) 0.55 f (t) 0.17	(c) 169 (t) 74	f (c) 0.75 f (t) 0.20	NA
311 a>c	104 D>A	<**0.05**	19	0.08	4	0.01	NA

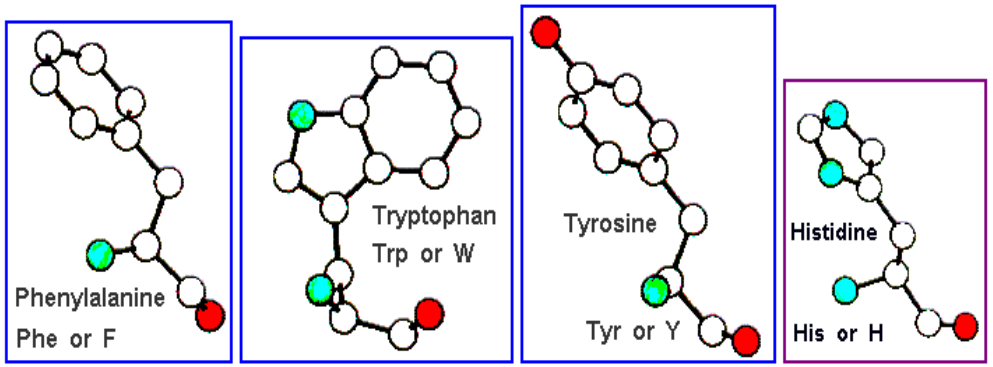
Haplotype number	Freq	SNP 126	SNP 140	SNP 143	SNP 145	SNP 154	SNP 210	SNP 253	SNP 255	SNP 296	SNP 305	SNP 308
4	0.07	0	0	0	0	0	0	0	0	0	1	1
5	0.12	0	0	0	0	0	0	0	0	1	0	0
8	0.07	0	0	0	0	0	0	1	1	0	0	0
11	0.08	0	0	0	0	0	1	0	0	1	0	0
13	0.07	0	0	0	0	1	0	0	0	0	0	0
20	0.08	0	0	1	1	0	0	0	0	0	0	0
25	0.06	0	1	1	1	0	0	0	0	0	0	0
26	0.06	1	0	0	0	0	0	0	0	0	0	0
27	0.06	1	0	0	0	0	0	0	0	1	0	0
32	0.14	1	0	0	0	1	0	0	0	0	1	0

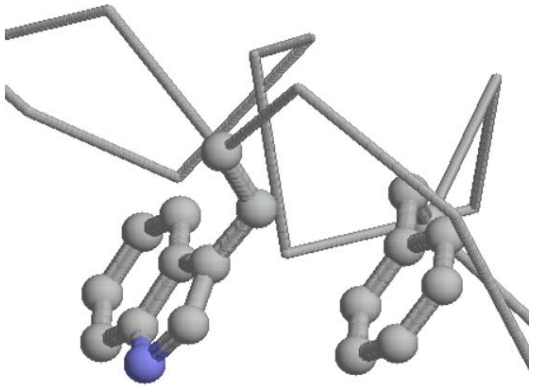
	Casi		Controlli		P-value
Aplotipo	**N°**	**Freq**	**N°**	**Freq**	
4	21	0.09	26	0.06	0.25
5	29	0.13	45	0.11	0.49
8	14	0.05	35	0.08	0.06
11	19	0.07	39	0.09	0.30
13	12	0.05	32	0.08	0.25
20	26	0.10	30	0.07	0.20
25	5	0.02	38	0.09	**0.00**
26	17	0.07	23	0.05	0.59
27	13	0.04	34	0.08	0.06
32	34	0.13	63	0.15	0.36

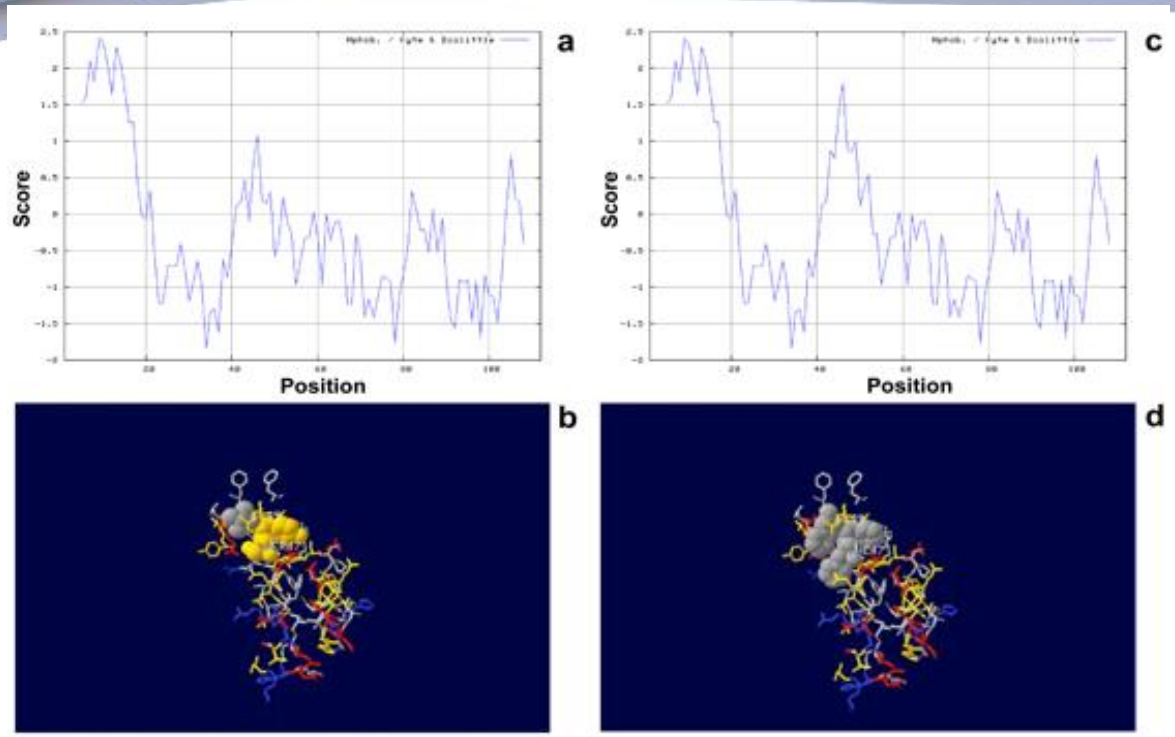
Leader β-1 domain

Glamann	M	S	M	P	I	A	F	Y	I	C	L	T	L	L	W	S	I	F	S	G	T	D	G	Y	F	H	Q		27
Glamann	S	V	T	Q	C	R	Y	S	S	K	D	L	H	G	I	E	F	I	D	S	Y	V	F	N	K	V	E		54
Haplotype 25																				F	F	F							
Glamann	Y	I	R	F	N	S	T	V	G	R	Y	V	G	Y	T	E	H	G	V	K	N	A	E	A	W	N	S		81
Haplotype 25																													
Glamann	D	A	G	I	L	G	Q	E	Q	A	Q	L	E	S	Y	C	K	H	N	A	D	I	D	Y	S	A	I		108
Haplotype 25																													
Glamann	L	D	K	T		112																							
Haplotype 25																													

Aromatic amino acids:







Phe is characterized by an high affinity for poli-proline binding sites described in surface proteins of Gram positive bacteria, such as *S. agalactiae*

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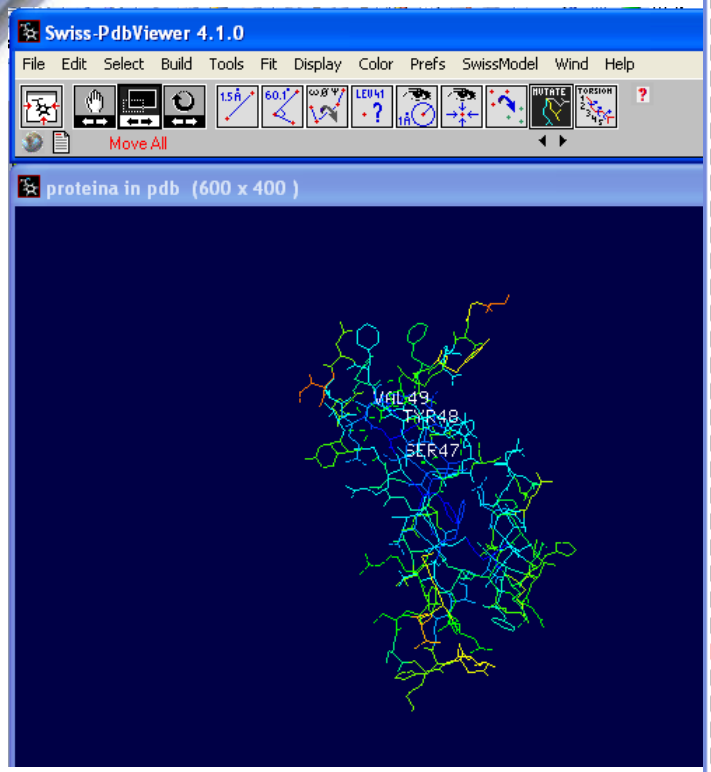
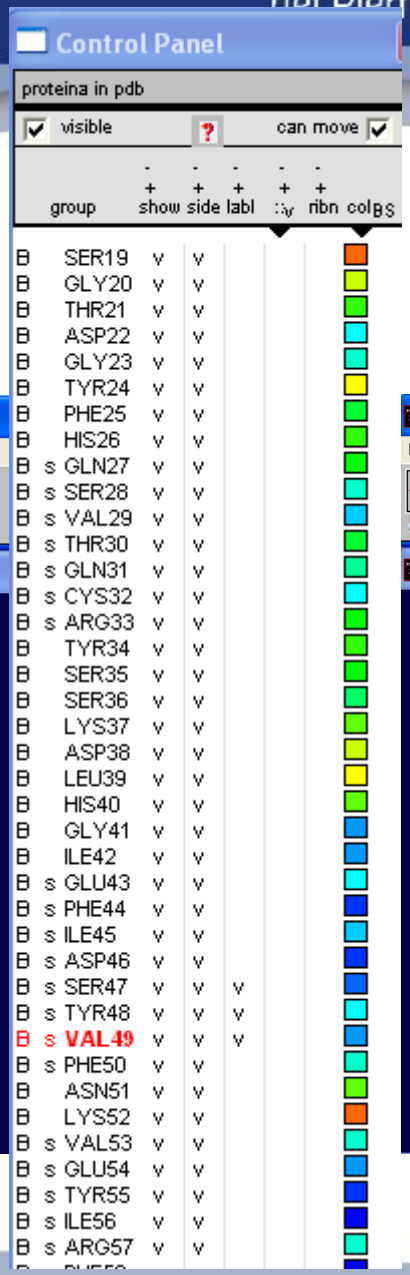
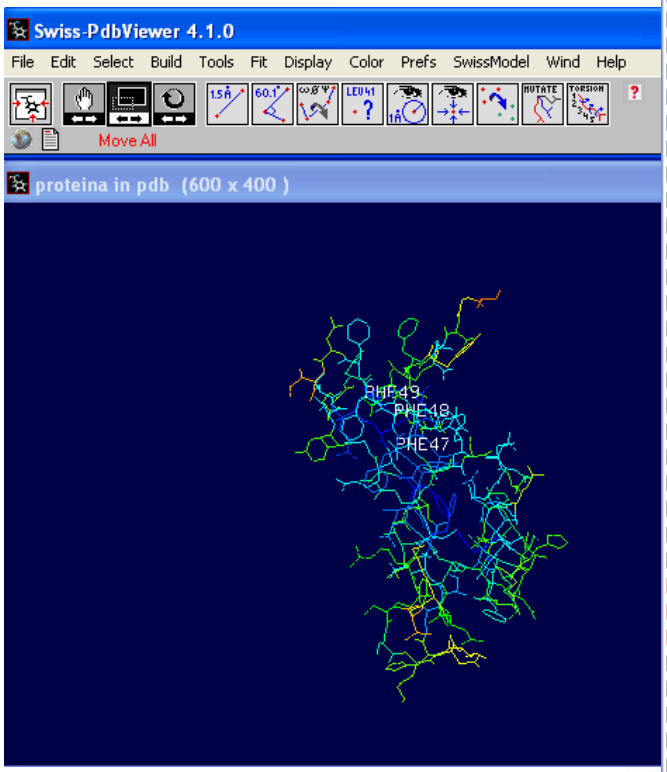
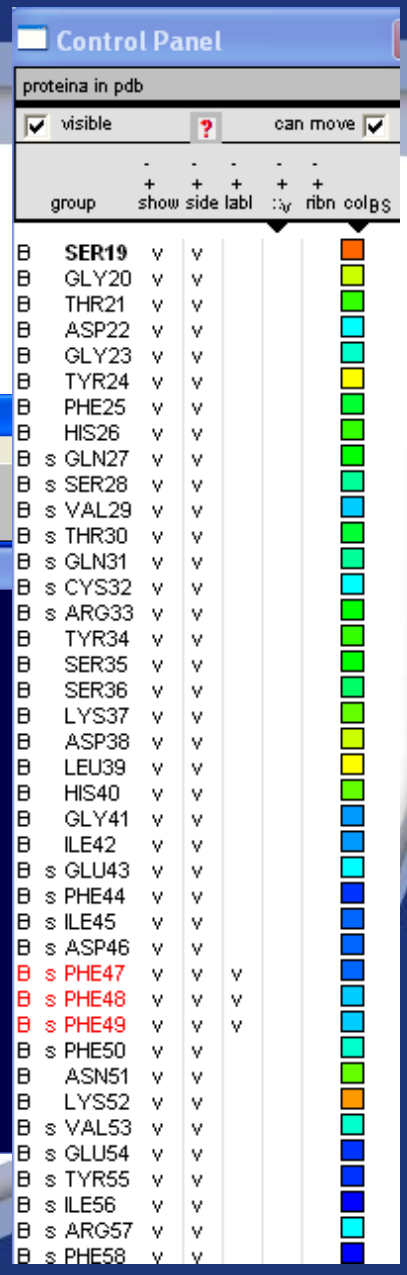
Solvent accessibility









LIMITS OF MAS AND GAS

MAS and GAS are efficient in selecting traits controlled by a few genes or in which few variants explain a substantial portion of the trait's genetic variance.

When the trait is highly multigenic, a different approach can be taken that uses the information provided by myriad markers spread along the genome (genomic selection)

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