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Identification of genetic markers associated with *Gyrodactylus salaris* resistance in Atlantic salmon *Salmo salar*

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ABSTRACT: *Gyrodactylus salaris* Malmberg, 1957 is a freshwater monogenean ectoparasite of salmonids, first recorded in Norway in 1975 and responsible for extensive epizootics in wild Atlantic salmon *Salmo salar* L. The susceptibility of different populations of Atlantic salmon to *G. salaris* infection differs markedly, with fish from the Baltic being characterised as relatively resistant whereas those from Norway or Scotland are known to be (extremely) susceptible. Resistance to *Gyrodactylus* infection in salmonids has been found to be heritable and a polygenic mechanism of control has been hypothesised. The current study utilises a 'Quantitative trait loci' (QTL) screening approach in order to identify molecular markers linked to QTL influencing *G. salaris* resistance in B1 backcrosses of Baltic and Scottish salmon. Infection patterns in these fish exhibited 3 distinct types; susceptible (exponential parasite growth), responding (parasite load builds before dropping) and resistant (parasite load never increases). B1 backcross fish were screened at 39 microsatellite markers and single marker-trait associations were examined using general linear modelling. We identified 10 genomic regions associated with heterogeneity in both innate and acquired resistance, explaining up to 27.3% of the total variation in parasite loads. We found that both innate and acquired parasite resistance in Atlantic salmon are under polygenic control, and that salmon would be well suited to a selection programme designed to quickly increase resistance to *G. salaris* in wild or farmed stocks.

KEY WORDS: *Gyrodactylus salaris* · Atlantic salmon · Resistance · Linkage mapping · Quantitative trait loci

INTRODUCTION

Gyrodactylus salaris Malmberg, 1957 is a freshwater monogenean ectoparasite of salmonids, first recorded in Norway in 1975 (Johnsen 1978), and responsible for extensive epizootics of wild Atlantic salmon *Salmo salar* L. By 1994, 38 rivers had become infected (Soleng & Bakke 1995), rising to 45 in 2004 (Mo 2004, Mo & Norheim 2005). The average density of salmon parr in infected rivers has fallen by around 86% (NOU 1999) with few, if any, salmon parr reaching the smolting stage in rivers where the parasite has occurred for some time (Johnsen & Jensen 1991). This has led to an annual loss of 250 to 500 t of salmon (NOU 1999) and a total cost exceeding 500 million US dollars (Bakke et al. 2004).

Recent years have seen research into genetic methods of *Gyrodactylus salaris* control based on the heterogeneity in susceptibility of different salmon stocks to *G. salaris* that has been noted in laboratory and wild studies (e.g. Bakke et al. 1990, 2002, Bakke & MacKenzie 1993, Jansen & Bakke 1993a,b, Rintamäki-Kinnunen & Valtonen 1996, Cable et al. 2000, Dalgaard et al. 2003, 2004, Lindenstrøm et al. 2006).

Atlantic salmon consist of 3 groups, genetically distinct and geographically separated, situated in the

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Western Atlantic Ocean, the Eastern Atlantic Ocean and the Baltic (Stahl 1987, Bakke et al. 1990). Within these groups the species comprise multiple, genetically differentiated and, to a large extent, reproductively isolated river populations (Stahl & Hindar 1988). There is virtually no migration of fish from the Baltic into the Eastern Atlantic, or vice versa (Christensen & Larsson 1979). Population level genetic heterogeneity in resistance within and between salmon populations may thus be important in determining whether an epizootic takes place in a particular river (Pickering 1987), and for the future development of stocks resistant to the parasite (Bakke et al. 1999).

Most species of freshwater fish seem to be more susceptible to attack from parasites to which they have not been previously exposed (Dobson & May 1987, Bakke et al. 1990). Heterogeneity in susceptibility of different salmon stocks, and of individuals of the same stock, to *Gyrodactylus salaris* has been noted in a number of studies, with fish from the Baltic being less susceptible than those of the Eastern Atlantic (Bakke et al. 1990, 2002, Bakke & MacKenzie 1993, Jansen & Bakke 1993a,b, Rintamäki-Kinnunen & Valtonen 1996, Cable et al. 2000, Dalgaard et al. 2003, 2004, Lindenstrøm et al. 2006). The presence of the parasite in the Baltic since the last glacial period has exerted heavy selection pressure on local populations and has resulted in these populations developing an heritable resistance to infection (Bakke et al. 2002, Dalgaard et al. 2003), although even within the Baltic differences in population susceptibility have also been observed (Bakke et al. 2004). It is this heritable resistance to infection that has been proposed as a method for the control of *G. salaris*.

Identification of resistant individuals and of the genes involved in the host response to infection can be incorporated into selective breeding programme to produce stocks which are less susceptible to parasite attack (Jones et al. 2002). This natural resistance may then be commercially exploited (without the need for genetic engineering) using modern molecular genetic techniques to identify molecular markers for resistance. These markers may then be used to increase the efficiency of selective breeding programmes by an order of magnitude compared to traditional breeding programmes (Jones et al. 2002), something already achieved with resistant crop plants produced using this type of marker-assisted selection (e.g. Williams et al. 2001).

In this study the underlying genetic basis for individual and population level differences in resistance was examined using 'Quantitative trait loci' (QTL) analysis. Linkage maps were used to systematically search for chromosomal regions with significant statistical associations with parasite resistance, within salmon families created using hybrid male parents produced from stocks divergent in their ability to resist *Gyrodactylus salaris*.

MATERIALS AND METHODS

Line crosses. The fish used in this investigation were B1 backcross hybrids produced from the crossing of Baltic salmon from the river Neva, which have been shown to be relatively resistant to *Gyrodactylus salaris* infection (Bakke et al. 1990, Cable et al. 2000), and Scottish salmon from the river Conon, which have been shown to be extremely susceptible (Bakke & MacKenzie 1993, Dalgaard et al. 2003). To obtain the experimental crosses, 2 Conon females were initially crossed with 2 Neva males to obtain a F1 hybrid generation. The following year a mature (precocious) hybrid F1 male was selected at random from each of 2 of the F1 families and crossed to a Neva female (Family 1) and a Conon female (Family 2), producing the 2 experimental B1 backcross families. Eggs and fish were maintained at the FRS Marine Laboratory Hatchery at Aultbea, Ross-Shire, until the fish were sent by airfreight to the National Veterinary Institute, Oslo, Norway, where the parasite challenge experiments were performed.

***Gyrodactylus salaris* exposure and rearing conditions.** Fish were retained in family groups for ~30 d to allow acclimatisation. At this stage the 1+ experimental fish were on average 85.1 mm (±10.7 mm SD) and 10.3 g (±3.7 g). We then infected 50 fish from each family with *G. salaris* before individually isolating them in 10 × 15 × 20 cm mesh-bottomed plastic boxes, floating in four 100 × 100 × 50 cm fibre-glass tanks each containing 400 l of water; or in individual net-covered plastic buckets with isolated water flows. Initial infection of all 100 experimental fish was carried out overnight in aerated 40 × 40 × 100 cm tanks. Groups of ~35 fish were retained in ~10 l of water; *G. salaris* were added to the tanks on fins removed from heavily infected salmon.

Evaluation of *Gyrodactylus salaris* resistance/susceptibility. After 18 h (average), the start-infection was registered for every fish before they were moved to their respective experimental tanks. The infections were thereafter monitored at weekly intervals until Day 36, counting all parasites on the fins and body of the fish. Fish were anaesthetised in a 0.04% solution of chlorbutanol before the parasites were counted under a stereomicroscope in half the concentration of the anaesthetic. Chlorbutanol solutions were renewed after each fish counted. At the end of the experimental period all fish were killed with an overdose of anaesthetic and the sex determined by examination of

gonadal tissue. Samples of adipose fin tissue were retained in ethanol for molecular analysis.

Genotyping and linkage-mapping. Each fish (including parents and grandparents) was screened at 39 microsatellite loci. The choice of which microsatellites to use was complicated by the unavailability at the time of a comprehensive linkage-map for the Atlantic salmon. The markers were thus chosen to cover all linkage groups in the partial linkage-map of Gilbey et al. (2004), and as many as possible from a provisional draft of the comprehensive linkage-map being produced by the SALMAP EU-project (B. Hoyheim pers. comm.; updated version now available at http://grasp.mbb.sfu.ca/). Recombination in male Atlantic salmon is significantly reduced compared to females and shows the largest sex-specific recombination rate differences so far found in any vertebrate species (e.g. 3.92:1 female:male, Gilbey et al. 2004; see Wright et al. 1983, Johnson et al. 1987, Moen et al. 2004 for details of salmonid recombination). As it was the male fish that were informative in this study, and because of the very low levels of recombination within linkage groups of these fish, a single marker was chosen from each linkage-group identified with the available maps. Markers were chosen such that, where possible, they covered all of the 15 linkage-groups identified by Gilbey et al. (2004) and the 30 contained in the early SALMAP draft (Table 1).

Table 1. *Salmo salar.* Microsatellites used in analysis. Linkage-groups identified from linkage-map of Gilbey et al. (2004) and linkage-map of SALMAP. TD: Touchdown-PCR was used (see 'Materials and methods'). unl.: unlinked. nu: not used in linkage study

Locus	Linkage-groups Gilbey	Linkage-groups SALMAP	Primers	Annealing *T* (°C)	$MgCl_2$ conc. (mM)	GenBank Acc. No.
Ssa202	1	1	F-CTTGGAATATCTAGAATATGGC			
			R-TTCATGTGTTAATGTTGCGTG	58	1.5	U43695
Ssa171	2	4	F-TTATTATCCAAAGGGGTCAAAA			
			R-GAGGTCGCTGGGGTTTACTAT	58	1.5	U43693
Ssosl311	3	2	F-TAGATAATGGAGGAACTGCATTCT			
			R-CATGCTTCATAAGAAAAAGATTGT	55	1.5	Z48597
Ssa42	3	nu	F-TCTGGCTGTAAATCAGAATATA			
			R-CAGCACATTTAAGTAACTTGAA	52	1.5	AF019174
Ssa48	4	10/22	F-AGTCTGTCTCCAGCCTCTTCTC			
			R-ATGGCTTGTGGACTGTGAAGTA	56	1.5	AF019176
Ssa11	5	nu	F-ATGACCAACAAATCAATGTGAC			
			R-GGATTCTCTGAATGTGTTCGTC	TD	1.5	U58894
Ssa14[a]	6	17	F-CCTTTTGACAGATTTAGGATTTC			
			R-CAAACCAAACATACCTAAAGCC	57	1.5	
Ssa164	7	13	F-TGTGTTTAGGATACATGCCATG			
			R-ACAGCATTCCCCATACATACAG	54	1.5	AF019152
SSsp3016	8	9	F-GGGCAGGCTAGGACAGGGCTAAGTC			
			R-AGTAAGCCAGGGCAATAGCCTGCTTG	62	1.5	AF372820
SSsp2201	9	5	F-TTTAGATGGTGGGATACTGGGAGGC			
			R-CGGGAGCCCCATAACCTTACTAATAAC	58	1.5	AY081817
Ssosl444	10	3	F-CCCACAAGGACAAAGTAATCCTCA			
			R-CACGATTCTATTTCTCCCATCCAA	TD	1.5	Z49997
CmrSS1.22	11	nu	F-TCCTCGGCCAGCTGGTTCTTTA			
			R-TGTGTACGCATGGATAGTCTC	60	1.5	AF020848
Ssa197	12	8	F-GGGTGGAGTAGGGAGGCTTG			
			R-TGGCAGGGATTTGACATAAC	58	1.5	U43694
Ssa213	13	19	F-ATGCTGACTACCCAGATCGAAC			
			R-CTGACACCAAAGAGGAAGAACA	TD	1.5	AF019163
Ssa9	14	25	F-GTCAGCCAAACTCCATTGTGAG			
			R-GTGACAACGATTCTGACGACAA	56	1.5	AF019197
SSsp2210	15	6	F-AAGTATTCATGCACACACATTCACTGC			
			R-CAAGACCCTTTTTCCAATGGGATTC	58	1.5	AY081818
Ssa420	nu	5	F-AGCTCTGGTGGGTACATCATGTG			
			R-GATCTATGCCCACAAACAGACAGG	58	2.0	AJ402737
Ssa64	nu	6	F-GCTGCTCAGCTTTGTCCTCAAG			
			R-TCCTCCTTCTCCAGAATCACCAGG	TD	1.5	AF019183
Ssa107	nu	nu	F-GAGAATATGGTCTGCCGAGAGC			
			R-ATGTACGGAGTCCACCCTC	58	1.5	AY402542
Ssa417	nu	11	F-CAGACAGGTCCAGACAAGCAC			
			R-CGTCGTTGCTCCTAAACGTTA	TD	1.5	AJ402734
Ssa85	nu	18	F-TGTCAGACAGATAGCACTGCGG			
			R-GCTCCTCACTTAATCAGATTTC	TD	1.5	U43692

(Table continued on next page)

Table 1. (continued)

Locus	Linkage groups Linkage	SALMAP	Primers	Annealing *T* (°C)	$MgCl_2$ conc. (mM)	GenBank Acc. No.
Ssa20	unl.	20	F-TCAGGGGTTTGAATTACATTAG			
			R-ATTGTATGGTCCATGCATGCTG	TD	1.5	AF019162
Ssa79	nu	21	F-CACTCACTGCCAGGCTCTACTGGT			
			R-TCACTACAATGCCAATGCCCA	58	2.5	AF420543
Ssa13	nu	23	F-GCTAAATTGCACCTTGTGATTC			
			R-GTTAGCTAGAGTCTAGACCCGTG	57	2.0	U58903
Ssa405	nu	28	F-TGAGTGGGAATGGACCAGACAG			
			R-ACCATTGTGCCATCTCGTGTCTA	59	2.0	AJ402722
Ssa68	nu	22	F-ACTCTGCTAAAACCAGCCTTCCA			
			R-TCTACAGCAAACAACCCGAGACA	58	2.0	AF420538
Ssa77	nu	6/2	F-GCCATCACCTCACTGTGTGGA			
			R-ACCCTGTTACAAACTGGGCGCA	58	2.0	AF420553
uF43	unl.	nu	F-AGCGGCATAACGTGCTGTGT			
			R-GAGTCACTCAAAGTGAGGCC	TD	1.5	U37494
Ssleen82	unl.	nu	F-CATGGAGAATCCCACTTTCTTA			
			R-CAGGGAGTGATATGGGACATAA	58	1.5	U86706
SSsp1605	unl.	18	F-CGCAATGGAAGTCAGTGGACTGG			
			R-CTGATTTAGCTTTTTAGTGCCCAATGC	58	1.5	AY081812
SSsp2215	unl.	7	F-ACTAGCCAGGTGTCCTGCCGGTC			
			R-AGGGTCAGTCAGTCACACCATGCAC	58	1.5	AY081810
SSsp2216	unl.	7	F-GGCCCAGACAGATAAACAAACACGC			
			R-GCCAACAGCAGCATCATCACCCAG	58	1.5	AY081811
Rsa172	unl.	nu	F-AACAGTCGCCCTGATGCTATCTCA			
			R-GGCCATGCTACAGAAGCAGAAAGA	58	1.5	AF271582
Hae050	unl.	nu	F-TTTATCGCGACCCTTATACCCTC			
			R-ATTCTTAGCCCTGGGTCTCTGC	58	1.5	AF271499
Ssa138	unl.	nu	F-GCATGTTTCAGTGCCTTATC			
			R-CTGACTATATCCCAGCCATG	TD	1.5	U58902
Ssa65	unl.	32	F-GTGGGACATCCCAAGAGAAAG			
			R-ATCCCAGAACACAGGGTAGAG	TD	1.5	AF019184
Hae029	unl.	nu	F-AGTCAGCTGGGTCTTGTCTGAGTC			
			R-ACATGCAAGACCTCCAGGACGC	57	2.5	AF271486
Hae065	unl.	nu	F-TGGTGTAATCATGCTGCAACATC			
			R-CTGAGGAGCAAGACGGAGGTTAGT	56	1.5	AF271510
Hae085	unl.	nu	F-TAAAGTCCTGGGTACCTGGATGG			
			R-CCTGCTCACAGACACTGTGATGTT	54	1.5	AF271522

[a]*Ssa14* from McConnell et al. (1995)

Total genomic DNA was isolated from the adipose tissue samples using chloroform/ethanol extraction (Mullenbach et al. 1989). Microsatellites obtained from the literature were screened following published methods regarding primers, PCR conditions and annealing temperatures. Where possible, primers for the microsatellites obtained directly from the GenBank database were those detailed in the GenBank records. Where the record did not describe primers, they were designed using Primers for the Mac© (Apple Pi) (Table 1). All primers were obtained from Life Technologies, Paisley, Scotland. PCR was performed in a 12.5 µl reaction volume containing 5 to 100 ng DNA, 75 mM Tris-HCl pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% v/v Tween, 1.5 mM $MgCl_2$, 200 µM each dNTP and 0.25 U ABgene *Taq* DNA polymerase (ABgene). PCR cycling conditions consisted of a modified 'touchdown' sequence (Don et al. 1991) consisting of a denaturing step of 95°C for 2 min followed by a series of cycles consisting of 95°C for 30 s, annealing temperature for 30 s and 72°C for 30 s. Annealing temperatures were from 65 to 56°C, decreasing in 0.5°C steps, followed by 20 cycles at 56°C. PCR was completed with an extension step of 72°C for 10 min. PCR products were visualised and sized using 6% denaturing polyacrylamide electrophoresis followed by silver staining (after Boulikas & Hancock 1981).

Linkage arrangements of all markers were examined using 'Mapmaker/EXP 3.0b' (Lander et al. 1987) using a minimum LOD (log of odds ratio) score of 3.0. Linkage was examined separately in both families.

Statistical analysis. Tests for single marker-trait associations were performed using general linear modelling (GLM). The traits examined were parasite counts at each sampling point, maximum parasite numbers, and the ratio of maximum to final parasite numbers (to give insight into whether parasite numbers have risen or fallen) — a total of 7 traits. Before analysis, parasite counts were normalised using log transformations.

For each trait, at each marker, a general linear model containing family, sex and developmental status (i.e. parr or smolt, determined from length frequency histograms) as covariates, was first fitted. This was then compared to a model in which the separate influences of marker alleles inherited from dam and sire were also included. Those marker-trait combinations which showed a significant change in the amount of variance explained when the influence of the marker alleles was incorporated were taken as suggestive of marker-QTL linkages. The 2 models were

Part model: $Y_{ijk} = c + f_i + s_j + d_k + e_{ijk}$, and
Full model: $Y_{ijklm} = c + f_i + s_j + d_k + f_i(M1_l) + f_i(M2_m) + e_{ijklm}$

where: Y_{ijk} = the phenotypic trait measurement of the ith individual of the jth sex of the kth developmental status (and possessing the lth and mth marker alleles in the full model); c is the constant; f_i is the proportion of Y attributable to family; s_j is the proportion of Y attributable to the sex of the fish; d_k is the proportion of Y attributable to developmental status (parr or smolt); $f_i(M1_l)$ is the proportion of Y attributable to the marker allele inherited from the sire nested within family; $f_i(M2_m)$ is the proportion of Y attributable to the marker allele inherited from dam nested within family; e is the random residual associated with each individual. The marker alleles were included as nested variables within families in order to take into account epistatic interactions which could differ with family backgrounds. The part model allowed estimation of the influence of family, sex and developmental status on the trait in question. The full model included the influence of any QTL linked to the markers, and a comparison of the 2 models allows determination of the magnitude of this influence. The significance of the difference between the 2 models was examined using an F-test, with the results of the 5 parasite counts being combined using Fisher's technique (Sokal & Rohlf 1995). Markers which showed significant associations during the individual marker-trait analysis were then combined in a new GLM which allowed the total amount of variance in parasite numbers associated with the genetic markers identified during the current study to be determined.

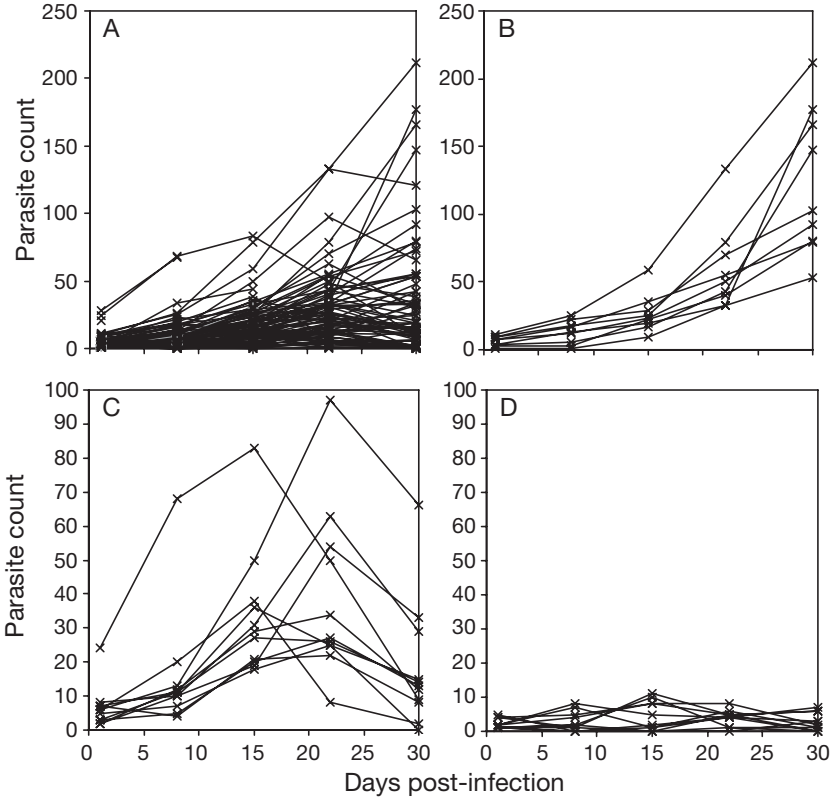

Fig. 1. *Salmo salar* infected with *Gyrodactylus salaris*. (A) All fish, showing continuous range of infection profiles; (B) selected 'susceptible' fish, showing exponential-like parasite growth; (C) selected 'responding' fish, showing increase in parasite load to maxima before parasite numbers fall; (D) selected 'resistant' fish, in which parasite load increases little above initial infection numbers

RESULTS

Fish size (length, weight or condition factor) did not significantly influence parasite numbers at any sampling point, including the initial infection, $p \geq 0.090$. Initial infection levels were an average of 5.0 ± 4.6 SD parasites fish^{-1}. Previously, such initial infection levels have been found to produce representative patterns of resistance/susceptibility (T. A. Mo unpubl. data). We observed 3 general types of infection profiles: 'susceptible' (Fig. 1B), in which the parasite load increases in an exponential manner; 'responding' (Fig. 1C), in which the parasite load increases to some maximum before falling; and 'resistant' (Fig. 1D), in which the parasite load never builds much above initial levels. Attempts to separate all fish into these 3 groups using multivariate statistics were unsuccessful due to the continuous range of infection profiles observed (Fig. 1A). There were no significant differences in parasite counts between the families at any sampling point ($p \geq 0.060$).

Mapmaker analysis of all markers revealed 11 new linkage arrangements allowing linkage-groups from Gilbey et al. (2004) to be related to those of the SALMAP linkage-map (Table 2). These

Table 2. *Salmo salar*. Microsatellite linkage-groups identified during current study. Linkage-groups identified from linkage-map of Gilbey et al. (2004) and linkage-map of SALMAP. unl.: unlinked; ns: non-significant linkage, np: not possible due to parental homozygosity

Microsatellites	Recombination rates		Linkage-group	
	Family 1	Family 2	Gilbey	SALMAP
Ssosl311 + Ssa42	0.0612	0.0200	3	2
SSsp2201 + Ssa420	0.0	0.0	9	5
SSsp2216 + Rsa172	0.1739	0.2917^{ns}	unl.	7
Ssa213 + Ssa417	0.0465	0.0526	13	11
Ssa85 + SSsp1605	0.2292	0.2000	unl.	18
Ssa405 + Ssleen82	0.0426	0.5200^{ns}	unl.	28
Ssa107 + Hae065	0.0200	0.0	unl.	
Ssa197 + Hae029	0.4618^{ns}	0.1633	12	8
Ssa68 + uF43	0.4694^{ns}	0.0667	unl.	22
CmrSS1.22 + Ssa13	np	0.1111	11	23
Ssa64 + Ssa77	np	0.0		6

include 4 restricted to a single family (where it is possible to make comparisons between families): *SSsp2216 + Rsa172*; *Ssa405 + Ssleen82*; *Ssa197 + Hae029* and *Ssa68 + uF43*. These family dissimilarities probably reflect individual male differences in the degree of chromosome homologies being reflected in recombination rate heterogeneity.

There were 10 significant marker-trait associations when the *F*-test significance values of the difference between the part and full models for each count point were combined (Table 3). Of these, 7 were related to parasite counts at the different sampling points, 2 with maximum numbers and 3 with the ratio of final to maximum numbers. The final combined GLMs were run using these significant markers. Parasite counts at the different sampling points were examined using a model which contained *Hae029*, *Ssa171*, *Ssa405*, *Ssa42*, *Ssa68* and *Ssa85*; maximum numbers with a model containing *Hae029* and *Ssa420*; and the ratio of maximum to final numbers with a model containing *Ssa171*, *Ssa138* and *Uf43*.

The amount of variance explained by the putative QTL linked to individual and combined markers was determined by the difference between the R^2 values of the part and full models (Table 4). Individual markers explained up to 12.3% of the variation in parasite numbers, up to 9.5% of the maximum parasite load and up to 10.5% of the ratio of maximum to final numbers, while the combined GLMs explained from 10.0 to 27.3% of parasite numbers and 19.3 and 24.2% of the maximum and ratio of maximum to final numbers respectively.

DISCUSSION

We have identified multiple genetic markers linked to QTL influencing *Gyrodactylus salaris* resistance in Atlantic salmon. These markers are located on different linkage-groups and suggest polygenic control of *G. salaris* resistance in Atlantic salmon.

The 3 responses to infection seen in the B1 families established from resistant and susceptible populations have been reported by other investigators. The fish classed as 'susceptible' showed a more or less continuous increase of parasite numbers in an exponential-like growth, similar to that seen with pure Conon and other Scottish and Norwegian fish not previously exposed to the parasite (e.g. Bakke & Mackenzie 1993, Bakke et al. 2002, Dalgaard et al. 2003). These have no apparent inherent resistance. Fish classed as 'responding' acquired significant parasite numbers until some maximum was reached, after which the parasite load decreased. This pattern is similar to that seen in the pure parental line from the Neva, which is known to be highly resistant. As the parasite load on Neva fish rises, it has been suggested that an acquired host response starts to become effective (e.g. Bakke et al. 1990, Cable et al. 2000) and parasite age-specific mortality and developmental times for parasite embryos increase. As a consequence, no parasites survive to give birth more than once and the population level falls (Cable et al. 2000). In contrast, there were also 'resistant' fish, in which parasite numbers remained more or less at the initial infection level. This response has also been

Table 3. *Salmo salar*. General linear model results. Values are significance (p) of difference in amount of variance explained by the 2 models at 5 count points; values in **bold** are significant at $p < 0.05$ level. Combined probability (Cp) is combined significance values for the 5 parasite count points using Fisher's method (Sokal & Rohlf 1995)

Micro-satellite	Parasite counts (d post-infection) 1	8	15	22	30	Cp	Max. no.	Max.: final no.
Hae029	0.710	0.986	**0.034**	**0.015**	**0.026**	**0.0101**	**0.010**	0.380
Ssa171	0.578	0.503	0.286	0.074	**0.005**	**0.0227**	0.159	**0.011**
Ssa405	0.051	0.292	0.275	0.312	**0.042**	**0.0326**	0.339	0.234
Ssa42	0.725	0.054	**0.036**	0.502	0.060	**0.0280**	0.178	0.526
Ssa68	0.240	0.169	**0.027**	0.112	0.794	**0.0475**	0.278	0.150
Ssa85	**0.020**	**0.041**	0.181	0.375	0.791	**0.0287**	0.304	0.850
SSsp2215	0.137	**0.004**	0.149	0.138	0.155	**0.0031**	0.082	0.390
Ssa138	0.981	0.454	0.298	0.141	0.234	0.3684	0.583	**0.032**
Ssa420	0.123	0.114	0.673	0.282	0.406	0.1890	**0.026**	0.747
Uf43	0.225	0.771	0.910	0.949	0.194	0.7182	0.856	**0.042**

Table 4. *Salmo salar*. Proportion of variance explained (% total variance in parasite numbers) by QTL linked to microsatellite markers in *S. salar*. Combined: combined general linear models that include all markers associated with variance in parasite numbers at each sample point/trait during individual analysis

Micro-satellite	Parasite counts (d post-innfection) 1	8	15	22	30	Parasite counts (mean ± SD)	Max. no.	Max.: final no.
Hae029	0	0	3.0	8.9	5.1	3.4 ± 3.8	9.5	0
Ssa171	0	0	0	5.2	12.0	3.4 ± 5.3	2.1	10.5
Ssa405	5.1	1.4	0	1.2	6.1	2.8 ± 2.7	0.1	0.4
Ssa42	0	6.9	5.4	0	4.9	3.4 ± 3.2	2.9	0
Ssa68	0	4.4	6.4	4.7	0	3.1 ± 2.9	0.5	2.2
Ssa85	6.8	6.7	0	0.3	0	2.8 ± 3.6	0.3	0
SSsp2215	1.3	12.3	0	3.5	0.3	3.5 ± 5.1	4.7	0
Ssa138	0	5.5	0	2.3	0	1.6 ± 2.4	0	3.4
Ssa420	1.7	3.7	0	1.4	0	1.4 ± 1.5	8.5	0
Uf43	0	4.6	0	0	0.1	1.0 ± 2.0	0	5.3
Combined	10.0	22.1	21.9	21.1	27.3	20.5 ± 6.4	19.3	24.2

observed in fish from the Neva (Bakke et al. 1990) and when other species of salmonids and non-salmonids have been infected with *Gyrodactylus salaris* (Bakke 1991). This type of response is suggestive of an innate resistance and has been attributed to host factors such as the alternative complement pathway, acute phase reactants and macrophages which bind to monogeneans such as *G. salaris* and elicit severe damage to the parasite (Buchmann & Lindenstrøm 2002). Excess mucus production and a highly proliferative epithelium also interfere with infection (Jones 2001, Buchmann & Lindenstrøm 2002, Dalgaard et al. 2003) by making passage through the mucus energetically unfavourable for infective stages and by the removal of parasites via sloughing of mucus and/or cells (Jones 2001). This mucus also contains immunoglobulin, complement, C-reactive proteins, lectins, lysozyme and haemolysins, all substances with biostatic or biocidal activity (Yano 1996, Jones 2001). It must be noted, however, that Lindenstrøm et al. (2006) suggest that excess mucus production may actually contribute to parasite population increase in susceptible fish, as the parasites obtain nourishment from such mucus.

The actual responses of the fish screened showed a continuous range of patterns of infestation (Fig. 1), as expected in this type of cross if resistance to infection is under polygenic control. This view is supported by the QTL analysis, which identified a total of 10 markers for linkage-groups with QTL influencing infection. Single marker-trait associations were found to explain up to 12.3% of the variation in parasite numbers at single sample points. However, the amount explained by each marker varied with the ontogeny of the infection. *Ssa85*, *Ssa77* and *SSsp2216* were associated with infection in the early stages, suggesting involvement in innate resistance. In contrast, *Ssa171*, *Ssosl311*, *Ssa42*, *Ssa68* and *Hae029* were associated with infection in the mid and/or later stages, suggesting linkage to QTL involved in acquired resistance. Finally, QTL linked to the 2 remaining markers, *Ssa420* and *SSsp2215*, seem to influence infection throughout the experiment.

A number of studies have previously suggested a polygenic control of Atlantic salmon resistance to infection with *Gyrodactylus salaris* (e.g. Bakke et al. 1990, 1999, 2002). Indeed, if variations in a number of immunological pathways and host physiologies are involved, which seems likely because of the influence of both innate and acquired resistance, this is unsurprising. No other ectoparasite resistance, QTL mapping studies have been performed with Atlantic salmon, but resistance to the myxozoan endoparasite *Ceratomyxa shasta* Noble has been examined in rainbow trout *Oncorhynchus mykiss* Walbaum. Ibarra et al. (1992, 1994) showed that inheritance of resistance was polygenic and involved significant additive, dominant and digenic interaction effects and that the nature of the different genetic components differed during the ontogeny of infection, as in the current study. Nichols et al. (2003), in a QTL mapping study similar to the current analysis, identified up to 8 unlinked markers associated with putative QTL influencing this trait, even though the trait was binary (i.e. live/dead fish).

The QTL associated in the present study with *Gyrodactylus salaris* resistance in salmon show an exponential distribution in their effects. However, QTL studies identify large effects before small ones, potentially making such a distribution artifactual. Also, estimates of QTL effect can be upwardly biased, especially for loci with large effects (Melchinger et al. 1998, Goring et al. 2001), so the true effect of a QTL may be smaller than the experimental estimate. Finally, estimates of numbers and effects of QTL are really estimates of numbers and effects of chromosomal regions; each region might contain hundreds or thousands of genes. As such, estimated single locus large effects might actually be due to additive small effects of many loci. This latter qualification is especially relevant to the present study, as the markers used here represent entire linkage groups, which may even be complete chromosomes (Gilbey et al. 2004). However, further fine-scale mapping is required to determine the true number and strengths of the factors involved.

Despite these qualifications, ranking of QTL according to their effects at the different time points reveals

significant exponential distributions in ranked marker/trait influences at each point. Exponential regression equations (in the form $y = ae^{-bx}$) were fitted to ranked marker/trait influences effects at 1, 8, 22 and 30 d post-fertilisation (those sample times for which sufficient loci were identified as influencing resistance

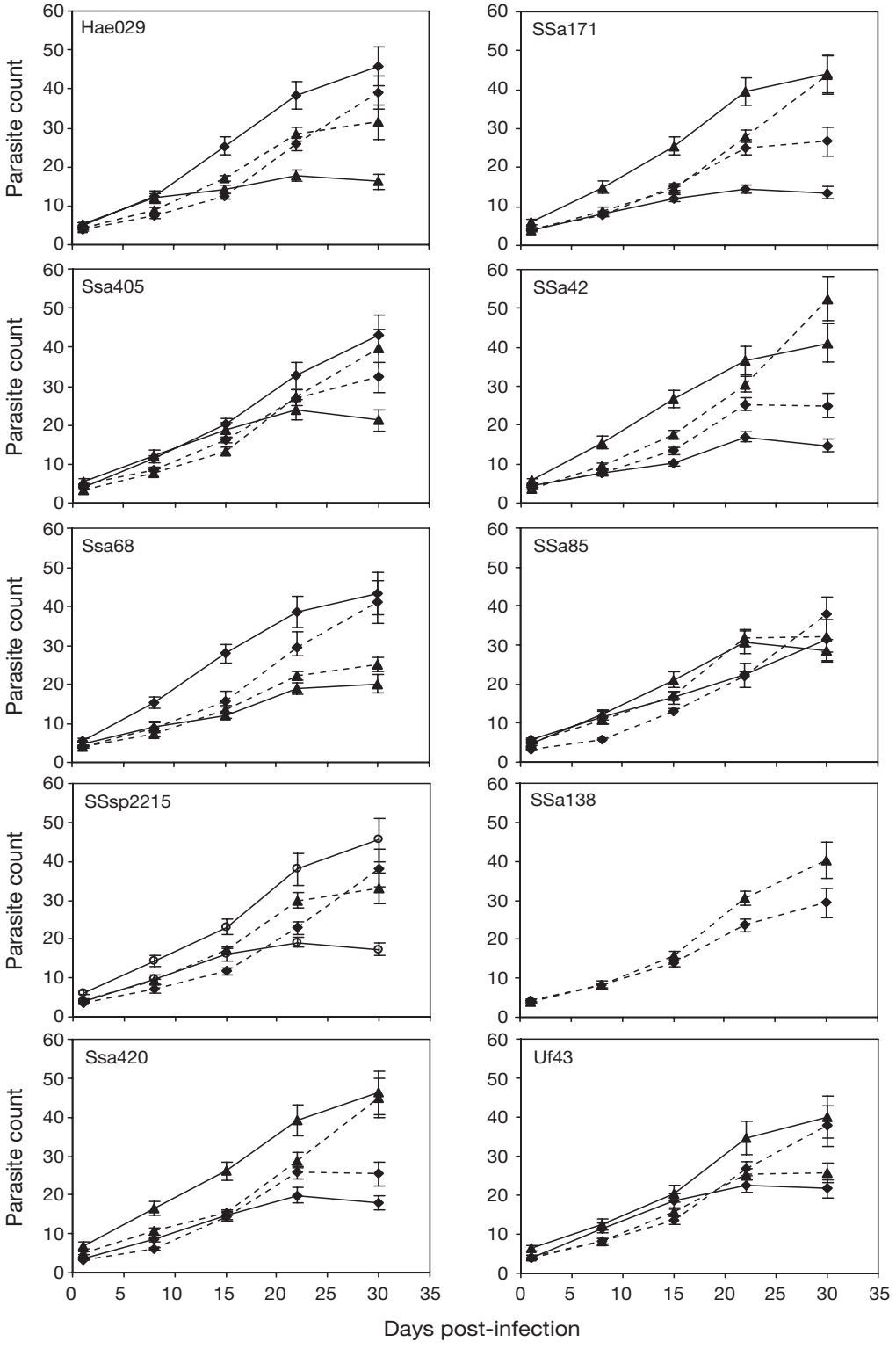

Fig. 2. *Salmo salar* infected with *Gyrodactylus salaris*. Mean ± 95% CI parasite infection patterns associated with alternate paternally inherited marker alleles (and maternally inherited in Locus *SSsp2215*). Continuous lines: Family 1 (Neva female parent); dashed lines: Family 2 (Conon female parent). (◆) alleles inherited from Neva groundparental line; (▲) alleles inhherited from Conon grandparental line; (O) in *SSsp2215* relate to alternate maternally inherited alleles in Family 1

to fit such a regression). At all sample times the regressions are significant ($p \leq 0.037$) with R^2 values ≥ 0.960. The distribution of QTL effects found in the present study are thus in agreement with the exponentially declining effects model for QTL (see Orr 1998, Hayes & Goddard 2001, Barton & Keightley 2002, and references therein).

The distribution of QTL effects has implications for breeding programmes. Single genes of major effects are most amenable to marker-assisted selection (MAS) (Lande & Thompson 1990). Markers or alleles can be readily identified and MAS used to incorporate resistance alleles at major QTL into the selected line. Inclusion of such major gene resistance alleles could thus be achieved in a few generations, quickly boosting a population's resistance to the parasite. This boost may be enough to allow the fish to coexist with *Gyrodactylus salaris* in the wild, when natural selection will then be able to select for other QTL with smaller effects, that are much harder to identify and to incorporate into breeding programmes.

Marker-assisted selection of native broodstock to influence a phenotypic trait relies on the availability of heritable variation within the population under selection. The results of the present study suggest that in both the source populations utilised as broodstock such variation exists. Fig. 2 details the parasite counts associated with alternate alleles inherited from the informative hybrid male parents at the markers found to be linked to QTL that significantly influence resistance. The largest differences in parasite numbers associated with the inheritance of alternate alleles is consistently seen when these alleles are expressed in Family 1, in which the hybrid males were backcrossed to a Neva female. These differences are less pronounced, but still significant, in Family 2, in which the hybrid males were backcrossed to a Conon female. These differences suggest that some kind of epistatic interaction is occurring; however, the significance of the interactions between male and female genotypes is hard to quantify. Attempts to examine interaction terms in GLM or ANOVA models were unsuccessful due to the small numbers of fish in each group when family/female/male alleles were separated. It would be interesting to examine these effects in larger experimental groups.

Genotyping of the pure-strain grandparents of the experimental generation allowed identification of the origin of the alleles inherited from the hybrid F1 males. It was expected, *a priori*, that the more resistant fish might be associated with the possession of alleles originating from the resistant Neva grandparental line. However, while this pattern was observed with some of the markers (e.g. *Ssa171*) this was found not to be the case for many of the markers identified. For example, in Family 1 at the *Hae029* marker, fish inheriting the grandparental Conon allele had significantly lower parasite numbers than fish with the grandparental Neva allele, even in a Neva female background. It is a QTL originating from the Conon line that is conferring increased resistance in these fish. Further evidence for the possession of markers linked to QTL conferring differential resistance within the pure strains is seen in Family 1 with marker *SSsp2215*. In this case the hybrid male was homozygous at *SSsp2215*, but significant differences in resistance were found to be associated with alternate versions of the marker alleles inherited from the pure Neva female parent (the only time this occurred with female alleles). These results suggest that fish from both the Neva and Conon lines are polymorphic with respect to QTL influencing resistance, and as such both strains may be susceptible to breeding schemes designed to enhance resistance using native fish only.

This is the first study to evaluate the genetic basis of resistance to *Gyrodactylus salaris* using molecular markers. We found multiple genome regions involved in resistance that explained up to 17.6% of the variation in parasite numbers infecting the fish. The relatively high levels of variation explained by the putative QTL linked to the microsatellite markers, together with the polymorphic nature of resistance alleles within stock types, suggest that a future 'marker-assisted selection' breeding programme has the potential to greatly improve the resistance of both wild and farmed fish to *G. salaris*.

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