Genome-wide association testing reveals quantitative trait loci for fillet texture and fat content in Atlantic salmon

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Abstract

Within the aquaculture industry downgrading losses due to poor quality represent large costs to the producers, and an interest in implementing quality parameters in breeding goals has therefore emerged. Through genome-wide association studies (GWAS) one can identify genetic variation affecting quality parameters, which can in turn facilitate efficient implementation in breeding schemes by use of marker-assisted selection.

This study aimed to identify genetic variation affecting fillet fat content and fillet firmness in farmed Norwegian Atlantic salmon using 5650 genome-wide distributed SNPs. Phenotypic records from instrumental analyses of fillet fat content and fillet firmness were retrieved from fish provided by a commercial Norwegian breeding company and implemented in GWAS. Further, genome partitioning by estimation of variance components for individual chromosomes based on SNPs was conducted for the purpose of validating results from GWAS.

Combined the results from GWAS and genome partitioning suggested that genetic variation affecting fillet fat content is present on Atlantic salmon chromosomes 9 and 10, and that genetic variation affecting fillet firmness is present on Atlantic salmon chromosomes 3 and 11.

Keywords

Atlantic salmon, Genome-wide association study, Fillet quality, Fat content, Fillet firmness.

1. Introduction

Fillet quality traits such as fat content and fillet firmness are of great economical importance to the aquacultural industry, as downgrading losses due to poor quality represents large costs to the producers. Fillet softness or gaping alone constitutes approximately 40% of downgrading losses for Atlantic salmon (Mitchie, 2001).

The first breeding program for Atlantic salmon was established in Norway 1971 and during forty years traits relating to growth rate, age at sexual maturation, disease resistance, fat distribution and flesh colour have been included in breeding goals (Gjedrem, 2000; Powell, et al., 2008; Quinton, et al., 2005). The main focus of the breeding programs has been on increasing growth rate, which may in turn have affected other production traits (Johnston, et al., 2006; Vieira, 2007). For instance, selection for increased harvest weight is likely to have increased the fat content in farmed fish since heavier fish also tend to have a higher fat content (Powell, et al., 2008; Quinton, et al., 2005). Although desired fillet fat content differs relative to processing technique, and preferences vary

between consumer groups, a high content of fat generally makes processing of the fillet more difficult (Rye, 1996). It is believed that a fat percentage exceeding 18% may reduce the firmness of the fish fillet (Gjedrem, 1997; Mørkøre, 2008). Reported heritability estimates for fat percentage in Atlantic salmon have ranged between 0.19 and 0.28 (Powell, et al., 2008; Quinton, et al., 2005; Vieira, et al., 2007), while for texture a Larsson *et al.* (2012) reported a heritability of 0.16. In Coho salmon Neira et al. (2004) reported a heritability estimate of 0.17-0.26 for fat percentage and 0.06 for fillet firmness as measured by an instrumental texture analyzer.

Other properties of the fish muscle besides fat distribution may be affecting the firmness of the fish fillet. The fillet consist of muscle fibres held together by connective tissue, and two factors that have been suggested to influence its firmness are muscle collagen characteristics and muscle cellularity (Johnston, et al., 2000; Li, et al., 2005). After slaughter disintegration of muscle fibres and connective tissue by proteolysis will soften the fillet, and a tendency for higher activity of protease inhibitors have been found in families with firmer fillets (Bahuaud, et al., 2010).

While genetic parameters of Atlantic salmon quality traits have been the focus of a large number of studies, the genetic architecture of these traits remains uncertain. Through genome-wide association studies (GWAS) one can identify genetic variation affecting phenotypic traits of interest, and identification of such polymorphisms can in turn facilitate efficient implementation in breeding schemes by use of marker-assisted selection. The development of an Atlantic salmon SNP array (Gidskehaug, et al., 2011) facilitates identification of genetic variation affecting traits of economic importance in this species through GWAS. The power of GWAS depends upon sufficient linkage disequilibrium (LD) between the genetic markers and the genetic variation affecting the trait in question, where LD is a measure describing the level of allelic association between genomic loci. Long-ranging LD has previously been reported for farmed Atlantic salmon, which may be due to admixture in a 'synthetic' breeding population with contributions from several genetically distinct strains (Moen, et al., 2007). This long-ranging LD is an advantage in genome wide scans and increases the power to detect genetic variation affecting a phenotypic trait even with moderate numbers of genetic markers.

Besides GWAS, an emerging method for assessing the genetic architecture of a phenotypic trait is genome partitioning. Following this approach, the genetic markers are portioned into genomic segments and the variance explained by each segment is estimated. It has been shown that such an approach can be applied to identify chromosomes harbouring genetic variation affecting a trait of interest, and can be considered an alternative to traditional linkage mapping (Pimentel Eda, et al., 2011; Visscher, et al., 2007; Yang, et al., 2011). This approach is less likely to be affected by spurious associations caused by high LD between unlinked loci than GWAS.

The aim of this study was to identify genetic variation affecting the quality parameters fillet fat content and fillet firmness in farmed Norwegian Atlantic salmon using 5650 SNPs integrated in a dense linkage map for Atlantic salmon (Lien, et al., 2011).

Formatert: Engelsk (USA)

Feltkode endret

2. Materials and methods

2.1 Genotyping and data filtering

Genomic DNA was extracted from fin clips from approximately 3500 Atlantic salmon (offspring and parents) provided by the Norwegian breeding company Aqua Gen. Full-sib families were generated from sires that were sampled from four separate subpopulations that hatched in the years 1998-2001, and dams that were sampled from two separate subpopulations that hatched in the years

2000-2001. The fish were genotyped according to the manufacturers instructions using an Atlantic salmon iSelect SNP array developed by Center for Integrative Genetics (CIGENE; www.cigene.no) in Norway. Genotype data were analyzed using Genome Studio (Illumina Inc., San Diego, US) and the R-package beadarrayMSV (http://cran.r-project.org/). After quality filtering the genotyped material, genotypes for 3297 individual fish remained for further analyses. Genotyping, SNP filtering and construction of linkage maps are described in detail by Lien *et al.* (2011).

2.2 Sampling of fish and phenotypic measurements

Instrumental analyses of fillet fat content and fillet firmness were conducted on Atlantic salmon provided by the Norwegian breeding company Aqua Gen (described above). Full-sib families were reared in separate tanks until individual tagging, and fish were vaccinated when they were about five grams. After tagging all fish were held in the same environment until they reached an age of two years. All fish were starved for two weeks before slaughter at a commercial slaughter house. Fillets were packed in individual plastic bags and stored at 4°C. A more detailed description of the rearing and handling of the fish can be found in Bahuaud *et al*. (2010).

Fillet fat distribution (FD) was determined for 2428 of the genotyped fish by analyses of near-infra-red reflections (NIR) in fish fillets using the QMonitor system (Q Vision, Asker, Norway). The fish were sampled from 252 full-sib families, with an average of 9.6 offspring per family. Fat content across each fillet was mapped to a depth of 2cm and average values were found for each fillet.

Measurements of fillet firmness were conducted with a Texture Analyzer TA-XT2 (Stable Micro Systems LTD., Godalming, UK) equipped with a 5-kg load cell and a flat-ended cylindrical probe (Delrin black cylinder, 10-mm diameter, Stable Micro System P/10). Test speed of 1mm s^{-1} and penetration depth of 60% of fillet height were applied, the latter to ensure reaching the penetration point. Textural evaluations were performed at 2 days *post*-mortem (T2D) for 771 of the genotyped fish and at 5 days (T5D) *post*-mortem for 777 of the genotyped fish, where the aim of using two time-points was to capture differences in both *pre-rigor* and *post-rigor* fillet softening. The fish evaluated for fillet firmness were sampled from 109 full-sib families, with an average of 7 offspring per family. The two measures used for fillet firmness was force required for puncturing the fillet surface, or area of the force-time curve (ARFT), and force applied at fillet puncture (FA). Fillet height and weight were also recorded.

2.3 Linkage disequilibrium

Linkage disequilibrium measured by r^2 was calculated for all adjacent SNP pairs with the PLINK software package (Purcell, 2007). The distribution of LD measured by r^2 between adjacent markers for the 5650 genome-wide distributed SNPs used in this study was found and is presented in Additional file 1. Mean and median values of r^2 between adjacent SNPs were found to be 0.16 and 0.04, respectively.

2.4 Statistical models

2.4.1 Genome-wide association studies

GWAS were performed for each of the traits FD, T2D-FA, T2D-ARFT, T2D-FA and T5D-ARFT. The statistical model used was:

$$P_i = Xg_i + Ya_i + Zm_k + e_{ijk}$$

Where P is phenotypic value of individual i, g is fixed effects for individual i, a is random effect of individual i where co-variance structure between individuals is determined from pedigree relationships, m is random effect of genetic marker k, and e is an error term. In the model used for fat distribution fixed effects were gender and population of origin of each sire and in the models used for fillet firmness fixed effects were fillet height, fillet weight and population of origin of each sire. Population of origin of individual dams were not found to give significant effects on any of these traits. Estimation of SNP effects were conducted with the ASREML software (Gilmour, 2000).

2.4.2 Heritability and variance component estimates

For all five quality traits the models used for GWAS were also used to estimate heritability (h^2) and the variance explained by a genomic relationship matrix (V_G). Here h^2 is an estimate of the component of the phenotypic variance that is explained by pedigree relationships, while V_G is an estimate of the component of the phenotypic variance that is explained by a genomic relationship matrix calculated from all genome-wide distributed SNPs.

For the V_G estimate a_I was modelled by the genomic relationship matrix found from the procedure described by Yang et al. (2010)instead of a co-variance structure determined by pedigree relationships. This approach was also used to estimate variance components for individual chromosomes. Estimation of heritabilities and variance components were conducted with the ASREML software (Gilmour, 2000).

2.5 Test score for each SNP and correction for multiple testing

The Wald F-statistic and denominator degrees of freedom were estimated with the ASREML software (Gilmour, 2000). To correct for multiple testing significance thresholds were corrected with the effective number of independent tests ($Meff_G$) by the SimpleM procedure (Gao, et al., 2008). $Meff_G$ was found to be 2240 for the 5650 genotyped SNPs, corresponding to adjusted p-values of 2.2E-5 for α of 0.05 (0.05/2240=2.2E-5) and 4.4E-6 for α of 0.01 (0.01/2240=4.4E-6).

3. Results

GWAS were performed with genotypes from 5650 SNPs for the fat content trait FD and the four fillet firmness traits T2D-FA, T2D-ARFT, T2D-FA and T5D-ARFT. Results are presented in Figure 1 and the five highest-scoring SNPs for each trait are given in Table 1. For the three traits FD, T2D-ARFT and T5D-ARFT SNPs giving significant test scores were found after correcting for multiple testing by the SimpleM procedure (Gao, et al., 2008). For FD significant associations were detected on chromosome 10, for T2D-ARFT a significant association was detected on chromosome 3 and for T5D-ARFT a significant association was detected on chromosome 11. The highest test score in the study were found for FD on chromosome 10, and for this trait altogether four closely linked SNPs reached significant test scores. None of the traits gave significant test scores on the same chromosome. However, SNPs on chromosome 10 were among the five highest scoring SNPs for both FD and T5D-ARFT, SNPs on chromosomes 3 were among the five highest scoring SNPs for both T2D-ARFT, T2D-FA, T5D-ARFT and T5D-FA, and SNPs on chromosomes 19 were among the five highest scoring SNPs for both T2D-ARFT and T5D-ARFT. Correlations in test-scores from GWAS between the five traits FD, T2D-FA, T2D-ARFT, T2D-FA and T5D-ARFT are given in Table 2.

For each of the five traits FD, T2D-FA, T2D-ARFT, T2D-FA and T5D-ARFT h^2 and V_G were estimated (Table 3) by the same statistical model as used for GWAS. While h^2 is an estimate of the component of the phenotypic variance that is explained by pedigree relationships, V_G is an estimate of the component of the phenotypic variance that is explained by a genomic relationship matrix calculated from all 5650 genome-wide distributed SNPs. Genomic relationship matrices were estimated by the

method presented in (Yang, et al., 2010). For FD, T2D-ARFT and T5D-ARFT the estimates for h^2 and V_G were very similar, while for T2D-FA and T2D-FA V_G was notably smaller than h^2.
Chromosomal relationship matrices were also found from SNPs from each of the 29 Atlantic salmon chromosomes, and the variance explained in each of the five quality traits by the chromosomal relationship matrices were estimated (Figure 2). For the three traits FD, T2D-ARFT and T5D-ARFT the chromosome giving the largest variance component also contained one of the five highest scoring SNPs for the trait in question from GWAS (Table 1). The largest variance component estimate was found for chromosome 3 on the fillet firmness trait T5D-ARFT. This chromosome gave the second largest estimate for T2D-ARFT for which chromosome 19 gave a slightly higher estimate. For FD the highest estimate was found for chromosome 9 and the second largest was found for chromosome 10. For the two traits T2D-FA and T5D-FA there was less concordance between SNPs giving high test scores and chromosomes giving large variance components.

4. Discussion

Identification of polymorphisms affecting quality traits in Atlantic salmon could facilitate more effective breeding through marker-assisted selection, and thereby reduce downgrading losses for the aquaculture industry. Here GWAS were conducted for the fat content trait FD and the four fillet firmness traits T2D-ARFT, T2D-FA, T5D-ARFT and T5D-FA. The highest test score from GWAS was found for a SNP on chromosome 10 associated with FD. The result was supported by three additional significant associations for FD on the same chromosome. For fillet firmness significant test scores were found on chromosome 3 for T2D-ARFT and on chromosome 11 for T5D-ARFT. Correlations in test-scores from GWAS were higher between the traits T2D-ARFT and T2D-FA and between T5D-ARFT and T5D-FA compared with other pairs (Table 2). There were no correlations between the fat distribution trait and any of the fillet firmness traits.
GWAS for all five traits were conducted with mixed-models where co-variance relationships between individuals were modelled from pedigree information. It was demonstrated by MacLeod *et al.* (2010) that including effect of individual based on pedigree relationship in the mixed model reduces the number of false positives in genome-wide scans. To further account for population structure the population of origin for the sire of each individual fish were also included in the model. It has previously been shown that population structure can inflate test-statistics from GWAS even after inclusion of a pedigree-based relationship matrix (Patterson, et al., 2006; Price, et al., 2006).
Significance thresholds were corrected for multiple-testing with the SimpleM procedure by determination of the effective number of independent hypotheses being tested ($Meff_G$). While being less conservative than a Bonferroni correction, the SimpleM procedure is approximately equivalent to determination of significance thresholds by a permutation procedure (Gao, et al., 2008).
Further, a genomic relationship matrix based on all 5650 genome-wide distributed SNPs was determined and the proportion of variance explained by this matrix on the quality traits FD, T2D-ARFT, T2D-FA, T5D-ARFT and T5D-FA were estimated. The proportion of variance explained by the genomic relationship matrix were very close to the heritability estimates for the three traits FD, T2D-ARFT and T5D-ARFT, but less so for the traits T2D-FA and T5D-FA (Table 3).
It is traditionally assumed that a high concordance between h^2 and V_G would be due to the existence of LD between the genotyped SNPs and all major polymorphisms affecting these traits. From the distribution of LD between adjacent SNPs for the 5650 genome-wide distributed SNPs used in this

study (Additional file 1), with a mean r^2 value of 0.16 and a median r^2 value of 0.04, one would not expect much of the genetic variation in these traits to be picked up by the SNPs. A likely explanation as to why a quite high estimates of V_G relative to h^2 estimates are found here could be that although these SNPs are not in LD with all major polymorphisms affecting these traits, they give good estimations of the relatedness between fish in the relatively small and homogeneous population used in this study, and thereby accurately model the genetic variance in this population (Clark, et al., 2012; Habier, et al., 2010).

Estimation of variance components based on SNPs was also conducted for the purpose of validating the genetic variation associated with the quality traits identified by GWAS. Detection of polymorphisms affecting fat content or fillet firmness was attempted by genome partitioning and estimation of variance components for individual chromosomes. This approach has previously been shown suited for identification of chromosomes harbouring polymorphisms affecting a phenotypic trait of interest, and was therefore chosen as an alternative to traditional linkage mapping (Pimentel Eda, et al., 2011; Visscher, et al., 2007; Yang, et al., 2011). The genome partitioning approach is considered less affected by spurious associations than GWAS and was here used to validate GWAS results. By genome partitioning the largest variance component was found for chromosome 3 for the fillet firmness trait T5D-ARFT. By this approach chromosome 3 explained more than 12 percent of the observed variation in this trait. A SNP on chromosome 3 was also among the five highest scoring SNPs forT2D-FA, T5D-ARFT and T5D-FA in the GWAS. For T2D-ARFT the largest variance components was found for chromosomes 3 and 19, which were also the two chromosomes giving the highest test scores for this trait in the GWAS. The largest variance components for FD was found for chromosomes 9 and 10, which were the two chromosomes giving the highest test scores for this trait in the GWAS.The variance explained by chromosome segments is expected to be proportional to the segment length (Yang, et al., 2011). However, since the genome sequence for Atlantic salmon is not yet complete and chromosome lengths remain to be determined, correction for chromosome length was not conducted in the estimation of chromosomal variance components here.

The power of both GWAS and genome partitioning to detect genetic variation affecting Atlantic salmon quality traits is influenced by the number of samples, sampling methods and procedure chosen for phenotypic evaluations. In this study phenotypic evaluations for FD were available from over 2400 Atlantic salmon reared and slaughtered under the same conditions and slaughtered at the same age, reducing the influences of environmental factors to a minimum. FD was determined by analyses of near-infrared reflections (NIR) in fish fillets using the QMonitor system (Q Vision, Asker, Norway). NIR methods have been shown to give accurate measures of fat content in Atlantic salmon fillets (Wold, 1996). For the fillet firmness traits T2D-ARFT, T2D-FA, T5D-ARFT and T5D-FA phenotypic evaluations from approximately 770 fish were available. An instrumental texture analyzer with a cylindrical probe was used for evaluation of fillet firmness, which allowed for the large number of samples to be processed under similar conditions. Use of a cylindrical probe has been reported to give better predictions of sensory firmness evaluations compared with using blades or spherical probes (Casas, 2006; Mørkøre, 2003). Conducting the first measurement of fillet firmness at 2 days *post-mortem* ensured that all the fish were in the *post-rigor* state before the textural evaluation, while performing the second measurement at 5 days *post-mortem* sufficed enough time between the first and the second evaluation to capture differences in *post-rigor* fillet softening. The fillet firmness measures used in this study was force required for puncturing the fillet surface, or area of the force-time curve, and force applied at fillet puncture. These measures has been reported to be strongly correlated with sensory evaluations (Mørkøre, 2003).

Although this is the first study in which a high density SNP array is used to dissect the genetic architecture of Atlantic salmon quality traits, several reports have been published on the existence of a genetic component to fat content and fillet firmness in salmonids. Bahuaud *et al.* (2010) have previously reported significant differences in fillet firmness between the same families of farmed Norwegian Atlantic salmon as used in this study. In Scottish Atlantic salmon differences in texture between wild and farmed Atlantic salmon reared and slaughtered under the same conditions have been reported (Johnston, et al., 2006), where firmer fillets where observed in the wild than in the farmed fish. Evidence of genetic components to fillet firmness have also been found for other salmonid species, namely for rainbow trout (Salem, 2005), Artic charr (Gines, 2004) and Coho salmon (Neira, et al., 2004). Reported heritability estimates for fat percentage in Atlantic salmon have ranged between 0.19 and 0.28 (Powell, et al., 2008; Quinton, et al., 2005; Vieira, et al., 2007).
Since fillet texture traits can only be measured after slaughter, selective breeding for fillet firmness would at present have to be based on family selection. An alternative to family selection is marker-assisted selection or genomic selection, where information from genetic markers highly associated with a phenotypic trait on the population level are used in selective breeding instead of using phenotypic information for the particular individual.
The results presented here suggest that genetic variation affecting fillet fat content might be present on Atlantic salmon chromosomes 9 and 10, and that genetic variation affecting fillet firmness might be present on Atlantic salmon chromosomes 3 and 11. To identify causal polymorphisms in the regions identified here and further dissolve the underlying mechanisms behind these observations, one will need both a more complete genome sequence for this species and a more detailed map of the variation this genome contains. An international collaboration to complete the sequencing and assembly of the Atlantic salmon genome has been formed, and a high-quality assembly is expected to be made publically available in the near future (Davidson, et al., 2010). This will provide a very valuable resource for genomic studies of this species, facilitating large scale detection of genetic variation as well as genome annotation.

5. Conclusions

Through GWAS genetic variation affecting important quality parameters in commercial aquaculture strains may be identified. Identification of such polymorphisms can in turn facilitate efficient implementation in breeding schemes by use of marker-assisted selection.
Results presented here suggest that genetic variation affecting fillet fat content might be present on Atlantic salmon chromosomes 9 and 10, and that genetic variation affecting fillet firmness might be present on Atlantic salmon chromosomes 3 and 11. Completion of a high-quality genome assembly for this species is expected in the near future and will hopefully provide the necessary resource to identify causal polymorphisms in the regions identified here, and to further dissolve the underlying genetic mechanisms affecting Atlantic salmon quality parameters.

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Tables

Table 1: For genome-wide association studies for the traits FD, T2D-FA, T2D-ARFT, T2D-FA and T5D-ARFT the five highest-scoring SNPs for each trait, or all SNPs with a significant test-score, are given. Chromosome (Chr), female linkage map position (Map_F), male linkage map position (Map_M) and P-value. SNPs giving a test-score significant at α of 0.05 after correction for multiple testing (p-value <2.23E-05) are indicated in italic.

Trait	Chr	Map_F	Map_M	P-value	SNP
FD	*10*	*52.7*	*2.1*	*2.11E-06*	rs159401888
FD	*10*	*86.6*	*8.9*	*4.79E-06*	rs119097032
FD	*10*	*52.8*	*2.1*	*5.24E-06*	rs159401463
FD	*10*	*48.9*	*1.7*	*2.23E-05*	rs159405132
FD	9	75.5	5	3.23E-05	rs159404202
T2D-ARFT	*3*	*106.9*	*57.6*	*8.28E-06*	rs159405272
T2D-ARFT	3	108.7	57.6	9.81E-05	rs159405064
T2D-ARFT	19	57.5	4.1	1.23E-04	rs159401885
T2D-ARFT	19	56.5	3.9	2.02E-04	rs119097501
T2D-ARFT	3	111.3	57.7	2.36E-04	rs159407529
T2D-FA	18	2.2	1	2.96E-04	rs159402543
T2D-FA	3	100.1	57.2	3.19E-04	rs159403725
T2D-FA	18	2.1	0.7	4.14E-04	rs159402542
T2D-FA	17	69.3	11.3	6.30E-04	rs159406302
T2D-FA	4	13	2.1	9.14E-04	rs159405926
T5D-ARFT	*11*	*49.4*	*1.6*	*9.05E-06*	rs159401981
T5D-ARFT	10	73.8	4.8	5.50E-05	rs159406678
T5D-ARFT	3	111.3	57.7	8.01E-05	rs159407529
T5D-ARFT	19	23.3	3.1	8.17E-05	rs159403285
T5D-ARFT	29	31.6	0.1	8.42E-05	rs159403171
T5D-FA	3	106.9	57.6	1.27E-04	rs159405272
T5D-FA	3	111.3	57.7	1.87E-04	rs159407529
T5D-FA	28	53.7	22.8	4.23E-04	rs159406653
T5D-FA	13	82.5	5.7	5.96E-04	rs159406058
T5D-FA	3	11.4	54.9	7.81E-04	rs159404245

Table 2: Correlations in GWAS test-scores between the five traits FD, T2D-FA, T2D-ARFT, T2D-FA and T5D-ARFT are given.

	FD	T2D-ARFT	T2D-FA	T5D-ARFT
T2D-ARFT	0.01			
T2D-FA	0	0.41		
T5D-ARFT	0	0.24	0.21	
T5D-FA	0.02	0.19	0.26	0.54

Table 3: Heritabilities (h^2) and the variance explained by the genomic relationship matrix (V_G) for each of the five traits FD, T2D-FA, T2D-ARFT, T2D-FA and T5D-ARFT are given.

	h^2	V_G
FD	0.28	0.26
T2D-ARFT	0.18	0.18
T2D-FA	0.22	0.14

T5D-ARFT	0.20	0.18
T5D-FA	0.19	0.16

Figures

Figure 1: Results from genome-wide association studies with 5650 genome-wide SNPs are presented as –log(p) values for the traits FD, T2D-ARFT, T2D-FA, T5D-ARFT and T5D-FA. Adjusted p-value thresholds of 2.23E-5 for α of 0.05 and 4.46E-6 for α of 0.01 after corrections for multiple testing are indicated by dotted horizontal lines.

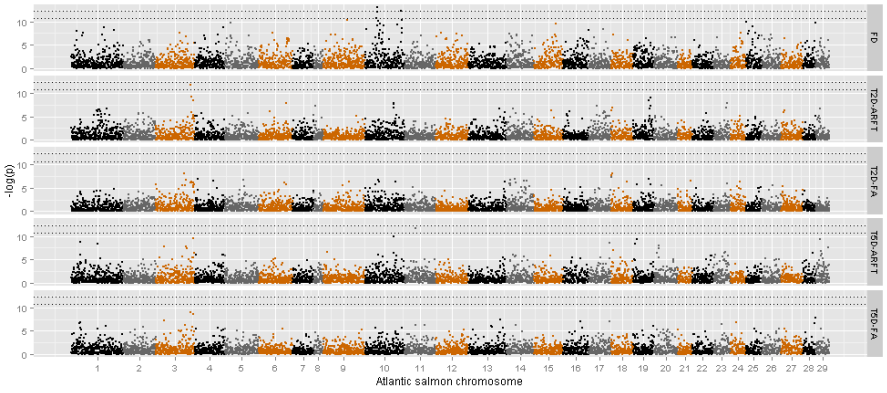
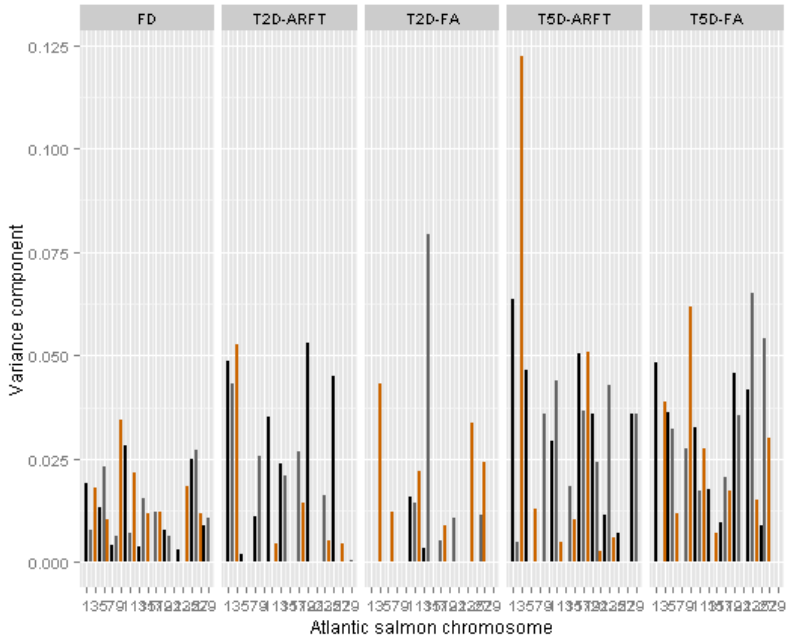
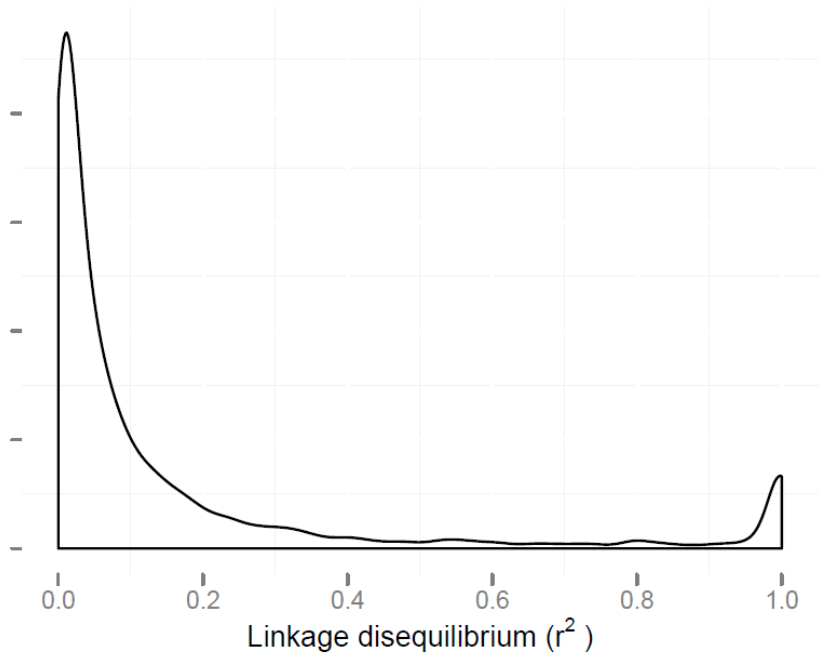


Figure 2: Variance component estimates for the 29 Atlantic salmon chromosomes for each of the traits FD, T2D-ARFT, T2D-FA, T5D-ARFT and T5D-FA.



Additional file 1

A1: Distribution of linkage disequilibrium (r^2) between adjacent SNPs for the 5650 genome-wide distributed SNPs used in this study.


0.0
0.2
0.4
0.6
0.8
1.0
Linkage disequilibrium (r^2)