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Research Article

Comparative mapping reveals quantitative trait loci that affect spawning time in coho salmon (*Oncorhynchus kisutch*)

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Abstract

Spawning time in salmonids is a sex-limited quantitative trait that can be modified by selection. In rainbow trout (*Oncorhynchus mykiss*), various quantitative trait loci (QTL) that affect the expression of this trait have been discovered. In this study, we describe four microsatellite loci associated with two possible spawning time QTL regions in coho salmon (*Oncorhynchus kisutch*). The four loci were identified in females from two populations (early and late spawners) produced by divergent selection from the same base population. Three of the loci (*OmyFGT34TUF*, *One2ASC* and *One19ASC*) that were strongly associated with spawning time in coho salmon ($p < 0.0002$) were previously associated with QTL for the same trait in rainbow trout; a fourth loci (*Oki10*) with a suggestive association ($p = 0.00035$) mapped 10 cM from locus *OmyFGT34TUF* in rainbow trout. The changes in allelic frequency observed after three generations of selection were greater than expected because of genetic drift. This work shows that comparing information from closely-related species is a valid strategy for identifying QTLs for marker-assisted selection in species whose genomes are poorly characterized or lack a saturated genetic map.

Key words: coho salmon, QTL, spawning time.

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Introduction

Spawning time in salmonids is an important sex-limited life-history trait that determines fertilization and progeny emergence dates and also affects the probability of survival and growth rate of small fry (Quinn *et al.*, 2002). An increase in the reproductive period in salmon farming allows better management of fish production (to account for seasonal variations) and increases the period during which eggs are available on the market (Gall and Neira, 2004).

Spawning time for rainbow trout (*Oncorhynchus mykiss*) shows highly additive genetic variation ($0.53 \leq h^2 \leq 0.65$) (Siitonen and Gall, 1989; Su *et al.*, 1999). Current evidence supports a polygenic inheritance for spawning time with several quantitative trait loci (QTL) affecting this trait (Sakamoto *et al.*, 1999; Fishback *et al.*, 2000; O'Malley *et al.*, 2003; Leder *et al.*, 2006).

In coho salmon (*Oncorhynchus kisutch*), it is possible to shift the spawning time of cultivated populations (Quinn *et al.*, 2002). Estimates of heritability in cultivated populations from Chile range from 0.24 ± 0.07 (Gall and Neira, 2004) to 0.40 ± 0.06 (Neira *et al.*, 2006) and this trait responds to early spawning selection. Traditional selection works well, with the phenotypic response to selection for early spawning fluctuating between -2.74 ± 0.7 and -3.23 ± 1.3 days per generation (Neira *et al.*, 2006). However, phenotypic selection is still inefficient as it is impossible to impose selection directly on males. Furthermore, this phenotype is expressed during the reproductive age, at the end of the salmon's life. In this context, marker-assisted selection could increase the response to early or late spawning time selection in the short term.

Despite its importance as a Chilean farmed species, coho salmon remains poorly characterized from a genetic standpoint. In contrast to rainbow trout and Atlantic salmon (*Salmo salar*) (Davidson *et al.*, 2010; Palti *et al.*, 2011), the lack of a dense genetic map for this species has delayed the search for QTLs related to commercial and life-history traits (Araneda, 2005). Although a map has been published for coho salmon, it is based on an analysis of 48 F_2 individuals and has low resolution, with only 133 co-dominant markers spanning 429.7 cM in the female map (McClelland and Naish, 2008). This map has allowed the QTL mapping of minor effects for growth rate, length, weight and hatch-

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ing time (McClelland and Naish, 2010; O'Malley *et al.*, 2010). Nevertheless, it is possible to use a comparative approach to discover new QTL by using genetic markers linked to QTL in closely-related species. This approach has been used to identify QTL associated with temperature tolerance in Arctic char (*Salvelinus alpinus*) (Somorjai *et al.*, 2003a) based on previously-identified QTL from rainbow trout (Jackson *et al.*, 1998; Perry *et al.*, 2001).

The main aim of this study was to use a comparative approach to identify microsatellite loci associated with potential QTL that affect spawning time (SPT-QTL) in coho salmon; the microsatellites used are reportedly linked to this trait in rainbow trout. If the microsatellite markers linked to SPT-QTL in rainbow trout are conserved in coho salmon then we would expect to find strong allelic heterogeneity between populations under divergent selection for spawning time. Such heterogeneity would indicate an association between these microsatellite loci and SPT-QTL in coho salmon. The identification of loci linked to SPT-QTL should allow marker-assisted selection for spawning time in coho salmon.

Materials and Methods

Experimental population and phenotypic evaluation

The fish used in this study were reared in the Coho Breed Improvement Program facilities (Centro de Mejoramiento Genético) located in Coyhaique in southern Chile (S 45° 34.422' W 72° 04.436' W). The program started with two-year classes in 1992 and 1993, both of which were closed populations managed under a two-year reproductive cycle. The populations consisted of 30-35 males that were mated with 100-120 females in each cycle followed by selection for harvest weight and early spawning using an animal model (Winkler *et al.*, 1999). In 1995, a divergent selection experiment was initiated using two sets of fish as breeders: those that spawned during the first third of the spawning season (40 females and 13 males, N_e = 39.2; early spawning population) and those that spawned during the last third of the spawning season (40 females and 12 males, N_e = 36.9; late spawning population). The effective size was held essentially constant for the next three generations ($N_e \approx 40$) by mating 12-14 males with 40 females (Araneda *et al.*, 2009). Both populations were selected for three generations and spawning time was recorded as the number of days starting from December 31st to the date of spawning for every season (Gall and Neira, 2004). In 2001, blood samples for DNA extraction were obtained from 20 females from the early spawning population and 20 females from the late spawning population. Additionally, DNA samples from 40 base population females were obtained from our sample bank. The average difference in spawning time between early and late populations in 2001 was 85 days (Araneda *et al.*, 2009).

Microsatellite loci and PCR conditions

Nine microsatellite loci were used to screen for associations with spawning time (Table 1). Six and three microsatellite loci were previously identified as linked and unlinked with spawning time QTL (SPT-QTL) in rainbow

Table 1 - Primer sequences, annealing temperatures and SPT-QTL linkage evidence for nine microsatellite loci used in the QTL screening of female coho salmon selected from early and late spawning populations.

Locus	Primer sequences (5'-3')	Tm (°C)	References	Rainbow trout linkage group*	SPT-QTL linkage status**
Ogo1UW	F: GATCTGGGCCTAAGGGAAAC R: ACTAGCGGTTGGAGAACCC	59	Olsen *et al.* (1998)	RT3	Linked
Oki10	F: GGAGTGCTGGACAGATTGG R: CAGCTTTTTACAAATCCTCCTG	60-54†	Smith *et al.* (1998)	RT19	Linked
One2ASC	F: GGTGCCAAGGTTCAGTTTATGTT R: CAGGAATTTACAGGACCCAGGTT	62	Scribner *et al.* (1996)	RT24	Linked
*One*μ6	F: CAGAGTGGCCTAGATGCTTTAAT R:CCACACACCAAATCCTACCCTTA	60	Scribner *et al.* (1996)	RT4	Unlinked
One19ASC	F: CTGGAAAGCACAGAGAGAGCCTT R: TCCAACAGTCTAACAGTCTAACCA	57	Scribner *et al.* (1996)	RT24	Linked
OmyFGT22TUF	F: AGTGAACTCCAGTGTTCCGG R: CTATGACGCGGCAGGAAC	70-60†	Sakamoto (1996)	RT25	Unlinked
OmyFGT34TUF	F: ACAGTAAGATGTGGGGGCTG R: TAAATTGACTGAGCAGCTGCC	64-58†	Sakamoto (1996)	RT19	Linked
Ots4BML	F: GACCCAGAGCACAGCACAA R: GGAGGACACATTTCAGCAG	58	Banks *et al.* (1999)	RT24	Linked
OmyPuPuPyDU	F: ATGCAGCGGATGTAGGGGGA R: TTAAGTGAAAAGACGTAACTTACC	58	Morris *et al.* (1996)	RT24	Unlinked

†Includes a "touchdown" profile of 8 cycles of –1.0 per cycle, prior to final annealing temperature. *According to Guyomard *et al.* (2006). **According to Sakamoto *et al.* (1999) and O'Malley *et al.* (2003).

trout, respectively. *Oki10* has never been used for SPT-QTL mapping; however, it was considered to be QTL-linked because it is located in the rainbow trout RT19 linkage group, between *One3ASC* and *OmyFGT34TUF*. *Oki10* is 10.3 cM from *OmyFGT34TUF* (Guyomard *et al.*, 2006) and 14.2 cM from a SPT-QTL closely linked to *OmyFGT34TUF* (Sakamoto *et al.*, 1999; O'Malley *et al.*, 2003). For all descriptions of rainbow trout linkage groups we used the nomenclature proposed by Guyomard *et al.* (2006).

The forward primers used to amplify each locus were dye labeled and PCR amplicons were run on an automated sequencer (Model ABI377, Applied Biosystems) with GeneScan-500 ROX as the size standard. The thermal profile was 94 °C for 2 min, followed by 30 cycles at 94 °C for 30 s, 57 °C to 70 °C for 1 min (see Table 1 for the specific annealing temperature of each primer pair), 72 °C for 1 min, and a final 5 min extension step at 72 °C. For some primer sets, we used a touchdown protocol to improve the PCR fragment resolution (Table 1). PCR was done in a total volume of 15 µL containing 1.5 µL of 10x PCR buffer, 4.0 µM of each dNTP, 0.4 µM of primer, 1.8 mM $MgCl_2$, 0.5 units of *Taq* DNA polymerase (Invitrogen) and 40 ng of DNA from each individual. DNA was extracted from blood samples using a phenol/chloroform protocol (Medrano *et al.*, 1990) and quantified spectrophotometrically (Hewlett Packard model 8452A spectrophotometer).

Association analysis

Marker-trait associations were assessed using three statistical methods: (1) First, we applied the L_D statistic, a multiple comparison approach based on contingency tables between microsatellite alleles and populations (Araneda *et al.*, 2009; Colihueque *et al.*, 2010). This procedure tests the null hypothesis that two populations are homogeneous with respect to the probability distribution of microsatellite alleles; the alternative hypothesis is that at least one allele is excessively associated with a particular population (Choulakian and Mahdi, 2000). For every locus, the highest value of L_D across alleles was compared to the chi-squared value, with one degree of freedom of 13.8 being equivalent to an LOD score of 3.0 [$Z \approx \chi^2/2 \log(10)$], which corresponded to an α level of approximately 0.0002. (2) Second, we used an χ^2 Monte-Carlo bootstrapping algorithm with 10,000 iterations to test allelic heterogeneity between populations (Zaykin and Pudovkin, 1993). (3) Finally, to assess genetic drift, we used a 99% confidence interval (CI) for allelic frequency variance for a locus with two alleles in which:

$$P\left[\left[\frac{(d.f.)\hat{S}^2}{\chi^2_{d.f.,1-\alpha/2}}\right] \le \sigma^2 \le \left[\frac{(d.f.)\hat{S}^2}{\chi^2_{d.f.,\alpha/2}}\right]\right] = 1-\alpha$$

using $\hat{S} = \sqrt{pq/2n_e}$ as an estimate of genetic drift, where p is the frequency of the most frequent allele, q is the pooled frequency of all other alleles, $\alpha = 0.01$ and $N_e = 37$ (the lowest value in our populations), so that d.f. was $2N_e - 1 = 73$. We also estimated the prediction of change by drift for the allele frequency with the highest L_D value for every locus after three generations as: $p_3 = p_0 + 3\hat{S}$, where p_0 is the allele frequency in the base population (1995) and p_3 is the frequency of the same allele in 2001 (after three generations of selection).

Results

Table 2 shows a reduction in the number of alleles from 1995 to 2001 in nearly all of the loci sampled. This table also shows the range of allele sizes and the frequency of the most frequent allele across the three populations that were used to assess drift. The complete allele distributions and frequencies are shown in Tables S1 to S3 and the as-

Table 2 - Allelic characteristics of nine microsatellite loci in coho salmon females from base, early and late populations.

Locus	Base (1995)				Early (2001)				Late (2001)			
	No. of alleles	Size range (bp)	Most frequent allele	p	No. of alleles	Size range (bp)	Most frequent allele	p	No. of alleles	Size range (bp)	Most frequent allele	p
Ogo1UW	3	114-144	122	0.5500	2	114-122	114	0.6250	2	114-122	114	0.5750
Oki10	18	113-243	150	0.1500	11	125-243	153	0.2750	9	125-231	231	0.2750
One2ASC	11	185-264	210	0.3250	9	185-256	202	0.2250	8	185-248	242	0.3750
Oneµ6	11	269-325	273	0.2051	10	259-311	269	0.2750	6	269-299	275	0.2750
One19ASC	7	222-240	234	0.3125	6	222-240	222	0.3500	5	226-236	232	0.5000
OmyFGT22TUF	13	211-262	227	0.2125	8	219-262	223	0.3250	11	207-262	229/211	0.1750
OmyFGT34TUF	14	135-210	153	0.1842	11	139-210	185	0.2500	8	135-182	143	0.4000
Ots4BML	4	134-140	138	0.4000	4	134-140	134	0.4000	3	134-140	140	0.5750
OmyPuPuPyDU	9	385-430	385	0.2895	7	385-430	408	0.2500	9	134-424	408	0.3000

p: frequency of the most common allele.

sessment of genetic drift is shown in Table S4 (all in Supplementary Material).

Six loci showed allelic heterogeneity among fish belonging to early and late spawning populations, which suggested that these loci could be associated with spawning time. Subsequent association analyses indicated that three loci (*One2ASC*, *One19ASC* and *OmyFGT34TUF*) were strongly associated with spawning time ($p < 0.0002$) and a fourth locus, *Oki10,* was close to the limit of significance (Table 3). All four microsatellite loci that were possibly associated with spawning time showed differences in the allelic distribution of early and late spawning females compared to females from the base population (Figure 1). In particular, for *OmyFGT34TUF*, alleles 139 and 143 occurred at a high frequency in late spawning females (32.5% and 40%, respectively), but were infrequent in early spawning females (5% and 2.5%, respectively). On the same locus, allele 185 also occurred at a high frequency (25%) and was found exclusively in early spawning females (Figure 1). Locus *One2ASC* had significantly higher allele 214 and 242 frequencies (30% and 37.5%, respectively) in late spawning compared to early spawning females (0% and 5%, respectively), and locus *One19ASC* had a high frequency (50%) of allele 232 in late spawning females compared to a frequency of only 10% in early spawning females (Figure 1). In early spawners, the latter locus also showed a high proportion (35%) of an exclusive allele (222). Finally, locus *Oki10* contained two alleles (223 and 231) exclusive to the late spawning group that both had high frequencies (20% and 27.5%, respectively), while allele 129 (frequency of 20%) was observed exclusively in the early spawning group (Figure 1).

The genetic drift effect estimated by using the most frequent allele in 1995 showed an average change in allele frequency of 5% due to drift per generation, with an upper confidence interval (99%CI) limit of 7.3% (Table 4). The estimate of change due to drift, based on the allele frequency with the highest L_D value, showed a drift effect that was always inferior to the change in gene frequency observed in 2001 for the three loci associated with spawning time (*One2ASC*, *One19ASC* and *OmyFGT34TUF*) and for *Oki10*. Thus, the frequency change expected due to drift was always inferior to the change observed after three generations of selection. For the other five loci, the change observed after selection was in the range of drift prediction (Table 4).

Discussion

Association analyses are always suspect because of the higher rate of false positives produced by spurious associations between phenotypes and non-causative marker loci. Such spurious associations can be produced by population subdivisions or genetic drift (Pritchard and Rosenberg, 1999). The reduction in the number of alleles from 1995 to 2001 was possibly a by-product of divergent selection for spawning time instead of a consequence of genetic drift. Our results indicated the association of three microsatellite loci (*One2ASC*, *One19ASC* and *OmyFGT34TUF*) with spawning time in coho salmon while a fourth locus (*Oki10*) had a suggestive association. In four loci the changes in allele frequencies were higher than expected by drift, which is consistent with a marker locus under co-selection with the QTL region. A similar pattern of co-selected markers linked to QTL has been shown for ethanol drinking in mice (Belknap *et al.*, 1997) and such co-selection is proof of a true QTL (Abiola *et al.*, 2003). As additional evidence, it should be noted that three of these loci were previously linked with QTL for the same trait in rainbow trout linkage groups RT24 and RT19 (Sakamoto *et al.*, 1999; O'Malley *et al.*, 2003). We have thus identified

Table 3 - Association analysis between eight microsatellite loci and spawning time in coho salmon females selected for early and late spawning time.

Locus	χ^2	p[†]	L_D	Allele with highest L_D value	p	LOD score[††]
Ogo1UW	0.208	0.8223	0.208	114	0.648077	0.05
Oki10	50.288	0.0000*	12.751	231	0.000355	2.77
One2ASC	45.162	0.0000*	14.118	214	0.000170**	3.07
Oneμ6	34.107	0.0001*	8.889	279	0.002869	1.93
One19ASC	32.410	0.0000*	16.970	222	0.000038**	3.68
OmyFGT22TUF	29.649	0.0000*	7.679	211	0.005611	1.67
OmyFGT34TUF	56.088	0.0000*	16.807	143	0.000041**	3.65
Ots4BML	9.589	0.0109*	7.314	138	0.006841	0.89
OmyPuPuPyDU	12.188	0.2115	5.164	385	0.023051	1.59

[†]Estimated using a bootstrapping algorithm with the program CHIRXC (Zaykin and Pudovkin, 1993).
[††]Approximate value estimated as L_D value/4.6052.
*Indicates allelic heterogeneity.
**Indicates association with spawning date ($p < 0.0002$ or LOD score > 3.0).

four SSR loci that are potentially useful in marker-assisted selection for early or late spawning time in coho salmon.

Our findings, along with previous evidence from QTL mapping in rainbow trout, support the presence of two QTL regions that affect spawning time in coho salmon. The proposed position of both QTL is based on assumed synteny between the chromosomes of coho salmon and rainbow trout; this assumption suggests that these QTL

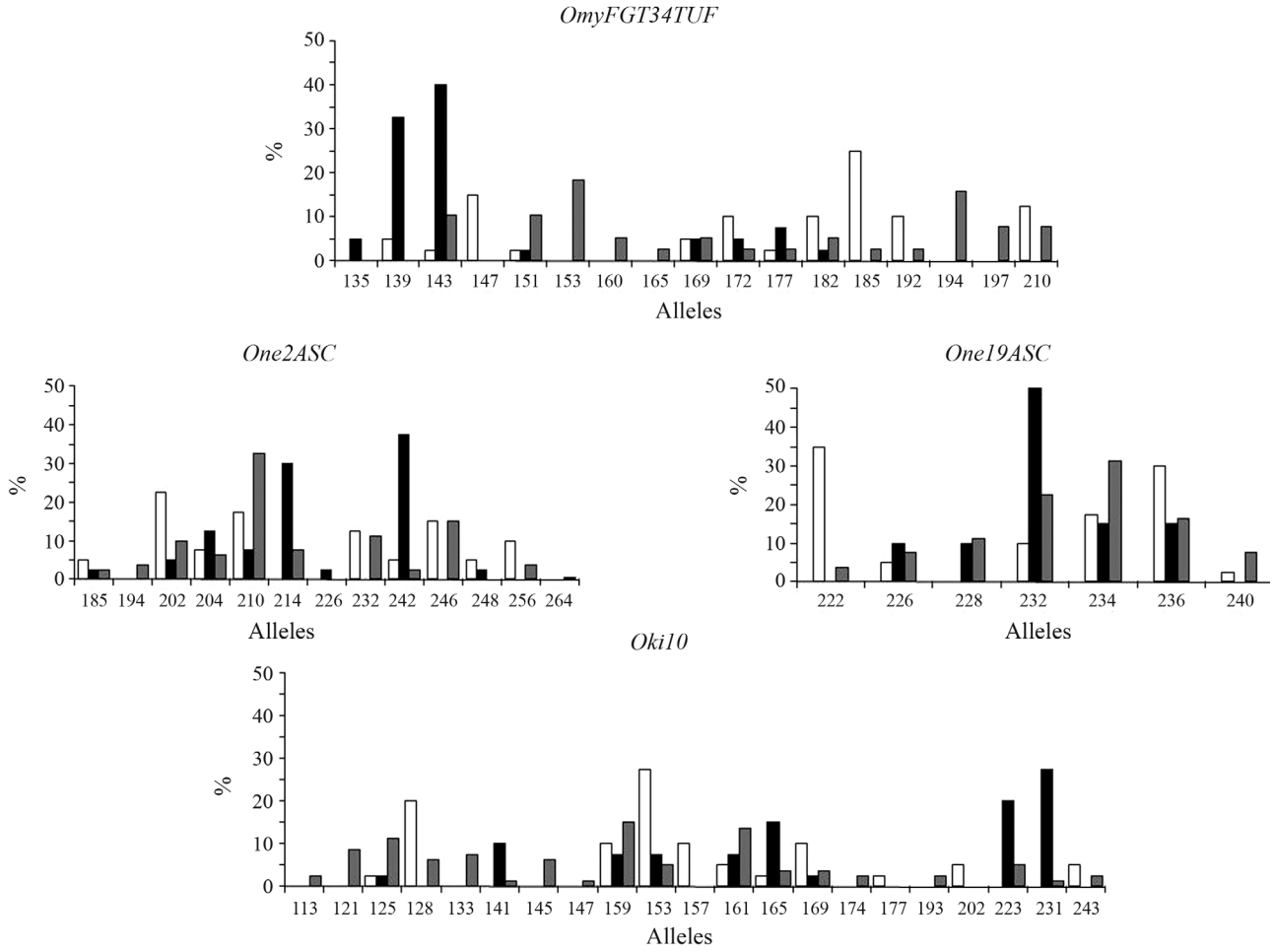

Figure 1 - Distribution of alleles in the four microsatellite loci of coho salmon showing the association with spawning date. White bars correspond to allelic frequencies for the early spawning population, black bars indicate allelic frequencies for the late spawning population and grey bars indicate allelic frequencies for the base population.

Table 4 - Change due to genetic drift predicted in alleles with the highest L_D value relative to the initial frequency (base population) across all nine loci.

Locus	Estimated drift in 1995	Drift 99%CI 1995	Allele with highest L_D value	Frequency in base population in 1995	Maximum frequency observed in 2001	Maximum frequency expected by drift
Ogo1UW	0.05783	[0.0046-0.0731]	114	0.4375	0.6525 E	0.6105
Oki10	0.04151	[0.0033-0.0525]	231	0.0125	0.2750 L	0.0512
One2ASC	0.05445	[0.0043-0.0689]	214	0.0750	0.3000 L	0.1669
Oneμ6	0.04694	[0.0037-0.0594]	279	0.1026	0.2000 L	0.2084
One19ASC	0.05388	[0.0043-0.0682]	222	0.0375	0.3500 E	0.1038
OmyFGT22TUF	0.04755	[0.0038-0.0601]	211	0.0375	0.1750 L	0.1038
OmyFGT34TUF	0.04506	[0.0036-0.0570]	143	0.1053	0.4000 L	0.2123
Ots4BML	0.05695	[0.0045-0.0720]	138	0.4000	0.2250 E	0.5708
OmyPuPuPyDU	0.05272	[0.0042-0.0667]	385	0.2895	0.2250 E	0.4407

E: Maximum frequency observed in the early population.
L: Maximum frequency observed in the late population.

were present in ancestral genomes from which these species originated.

We hypothesize that one of these QTL is located close to the region bearing the loci *One19ASC* and *One2ASC* in a coho salmon linkage group syntenic with RT24 of rainbow trout. The RT24 linkage group of rainbow trout contains *Ots4BLM* and *OmyPuPuPyDU*, but these loci are located 23.5-24.5 cM from the pair *One19ASC*/*One2ASC* and, in agreement with our association analysis, they have never been linked with SPT-QTL (Sakamoto *et al.*, 1999; O'Malley *et al.*, 2003). The second QTL must be located in a linkage group syntenic to the rainbow trout linkage group RT19, in a region between *Oki10* and *OmyFGT34TUF*, possibly near the latter locus. We expect that these putative SPT-QTL positions will be confirmed by formal linkage studies using these marker loci when coho salmon have a saturated genetic map.

Chromosome segment conservation among salmon species is being increasingly documented through the construction of genetic maps for salmonids and comparative genomic studies (Danzmann *et al.*, 2005, 2008; Timusk *et al.*, 2011). In addition, comparative QTL mapping is actively being undertaken for salmon species belonging to different genera. This approximation has been used to identify QTL for upper temperature tolerance among rainbow trout and Arctic char (Somorjai *et al.*, 2003b), as well as for body weight and Fulton's condition factor among *Oncorhynchus*, *Salvelinus* and *Salmo* (Reid *et al.*, 2005). Further evidence of synteny and conservation of the different priming sites for these microsatellites markers lies in the feasibility of using heterologous primers to amplify microsatellite loci across all salmon species (Araneda *et al.*, 2008; Danzmann *et al.*, 2008). Currently, all evidence obtained from comparative QTL mapping indicates that chromosome regions that affect the quantitative variation of several fitness-related traits in salmon, *e.g.*, body weight, growth rate, spawning time and temperature tolerance, must have been present before the separation of lineages that gave rise to the modern salmonid species (O'Malley *et al.*, 2003; Somorjai *et al.*, 2003b; Reid *et al.*, 2005).

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Supplementary Material

The following online material is available for this article:

Table S1 – Allele frequencies in the base population

Table S2 – Allele frequencies in the early spawning population

Table S3 – Allele frequencies in the late spawning population

Table S4 – Assessment of the influence of genetic drift

This material is available as part of the online article from http://www.scielo.br/gmb.

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Table S1 – Allele frequencies in the base population (1995).

One2ASC			*One*μ *6*			*One19ASC*			*Ogo1UW*			*OmyFGT22TUF*	
Allelles	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**
185	0.0250	1	269	0.0769	1	222	0.0375	1	114	0.43750	1	211	0.0375
194	0.0375	2	273	0.2051	2	226	0.0750	2	122	0.55000	2	219	0.0250
202	0.1000	3	275	0.1154	3	228	0.1125	3	144	0.01250	3	223	0.2125
204	0.0625	4	279	0.1026	4	232	0.2250	4			4	225	0.0375
210	0.3250	5	283	0.0769	5	234	0.3125	5			5	227	0.2125
214	0.0750	6	287	0.0897	6	236	0.1625	6			6	229	0.0375
232	0.1125	7	291	0.0513	7	240	0.0750	7			7	231	0.1000
242	0.0250	8	299	0.0769	8			8			8	234	0.0250
246	0.1500	9	305	0.0897	9			9			9	238	0.0750
256	0.0375	0	308	0.0769	0			0			0	240	0.0500
264	0.0500	1	325	0.0385	1			1			1	246	0.0375
		2			2			2			2	254	0.1000
		3			3			3			3	262	0.0500
12			11			7			3			13	

Oki10			*OmyFGT34TUF*			*Ots4UW*			*OmyPuPuPyDU*	
Allelles	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**
113	0.0250	1	143	0.1053	1	134	0.1875	1	385	0.2895
121	0.0875	2	151	0.1053	2	136	0.0500	2	388	0.0789
125	0.1125	3	153	0.1842	3	138	0.4000	3	393	0.0921
129	0.0625	4	160	0.0526	4	140	0.3625	4	399	0.0526
133	0.0750	5	165	0.0263	5			5	405	0.1316
141	0.0125	6	169	0.0526	6			6	408	0.1842
145	0.0625	7	172	0.0263	7			7	410	0.0395
147	0.0125	8	177	0.0263	8			8	421	0.1053
150	0.1500	9	182	0.0526	9			9	430	0.0263
153	0.0500	0	185	0.0263	0			0		
161	0.1375	1	192	0.0263	1			1		
165	0.0375	2	194	0.1579	2			2		
169	0.0375	3	197	0.0789	3			3		
174	0.0250	4	210	0.0789	4			4		
193	0.0250	5			5			5		
223	0.0500	6			6			6		
231	0.0125	7			7			7		
243	0.0250	8			8			8		

Table S2 – Allele frequencies in the early spawning population (2001).

One2ASC			*One μ 6*			*One19ASC*			*Ogo1UW*			*OmyFGT22TUF*	
Allelles	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**
185	0.0500	1	259	0.1000	1	222	0.3500	1	114	0.62500	1	219	0.2250
202	0.2250	2	269	0.2750	2	226	0.0500	2	122	0.37500	2	223	0.3250
204	0.0750	3	273	0.1500	3	232	0.1000	3			3	227	0.0500
210	0.1750	4	275	0.1250	4	234	0.1750	4			4	229	0.0500
232	0.1250	5	283	0.0750	5	236	0.3000	5			5	231	0.2000
242	0.0500	6	287	0.0250	6	240	0.0250	6			6	240	0.0500
246	0.1500	7	291	0.1250	7			7			7	246	0.0750
248	0.0500	8	299	0.0250	8			8			8	262	0.0250
256	0.1000	9	305	0.0500	9			9			9		
		0	311	0.0500	0			0			0		
		1			1			1			1		
9		2	10		2	6		2	2		2	8	

Oki10			*OmyFGT34TUF*			*Ots4BML*			*OmyPuPuPyDU*	
Allelles	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**
125	0.0250	1	139	0.0500	1	134	0.4000	1	385	0.2250
129	0.2000	2	143	0.0250	2	136	0.0250	2	388	0.1000
150	0.1000	3	147	0.1500	3	138	0.2250	3	393	0.1750
153	0.2750	4	151	0.0250	4	140	0.3500	4	408	0.2500
157	0.1000	5	169	0.0500	5			5	410	0.0250
161	0.0500	6	172	0.1000	6			6	421	0.1500
165	0.0250	7	177	0.0250	7			7	430	0.0750
169	0.1000	8	182	0.1000	8			8		
177	0.0250	9	185	0.2500	9			9		
202	0.0500	0	192	0.1000	0			0		
243	0.0500	1	210	0.1250	1			1		
		2			2			2		
11		3	11		3	4		3	7	

Table S3 – Allele frequencies in the late spawning population (2001).

One2ASC			*One μ 6*			*One19ASC*			*Ogo1UW*			*OmyFGT22TUF*	
Allelles	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**
185	0.0250	1	269	0.2250	1	226	0.1000	1	114	0.5750	1	207	0.0500
202	0.0500	2	275	0.2750	2	228	0.1000	2	122	0.4250	2	211	0.1750
204	0.1250	3	279	0.2000	3	232	0.5000	3			3	219	0.0250
210	0.0750	4	287	0.1500	4	234	0.1500	4			4	223	0.1500
214	0.3000	5	291	0.0500	5	236	0.1500	5			5	227	0.1250
226	0.0250	6	299	0.1000	6			6			6	229	0.1750
242	0.3750	7			7			7			7	231	0.0750
248	0.0250	8			8			8			8	240	0.0500
		9			9			9			9	246	0.0250
		0			0			0			0	256	0.1000
		1			1			1			1	262	0.0500
		3			3			3			3		
8			6			5			2			11	

Oki10			*OmyFGT34TUF*			*Ots4UW*			*OmyPuPuPyDU*	
Allelles	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**		**Allelles**	**Frequencies**
125	0.0250	1	135	0.0500	1	134	0.4000	1	134	0.0250
141	0.1000	2	139	0.3250	2	138	0.0250	2	140	0.0250
150	0.0750	3	143	0.4000	3	140	0.5750	3	385	0.0500
153	0.0750	4	151	0.0250	4			4	388	0.1750
161	0.0750	5	169	0.0500	5			5	393	0.1250
165	0.1500	6	172	0.0500	6			6	405	0.0250
169	0.0250	7	177	0.0750	7			7	408	0.3000
223	0.2000	8	182	0.0250	8			8	421	0.1000
231	0.2750	9			9			9	424	0.0250
									430	0.1500
		1			1			1		
9		2	8		2	3		2	10	

Table S4 – Assessment of the effect of genetic drift.

The approximate genetic drift was estimated in a simple way by using the formula for a locus with two alleles S = pq/sqrt(2Ne), where Ne = 37 was applied because this was the lowest value in our populations and the harmonic means across three generations must be close to this value. The confidence intervals (Lower lim and Upper lim) for S (drift) were estimated from the CI of the variance.

Drift was estimated using as p the frequency from the most frequent allele in 1995 and q = 1- p.

Locus	p	*S* = drift	S^2	Lower Lim	Drift	Upper Lim
Ogo1UW	0.5500	0.05783	0.0033446	0.0046	≤ *S* ≤	0.0731
Oki10	0.1500	0.04151	0.0017230	0.0033	≤ S ≤	0.0525
One2ASC	0.3250	0.05445	0.0029645	0.0043	≤ S ≤	0.0689
One µ 6	0.2051	0.04694	0.0022032	0.0037	≤ S ≤	0.0594
One19ASC	0.3125	0.05388	0.0029033	0.0043	≤ S ≤	0.0682
OmyFGT22TUF	0.2125	0.04755	0.0022614	0.0038	≤ S ≤	0.0601
OmyFGT34TUF	0.1842	0.04506	0.0020307	0.0036	≤ S ≤	0.0570
Ots4BML	0.4000	0.05695	0.0032432	0.0045	≤ S ≤	0.0720
OmyPuPuPyDU	0.2895	0.05272	0.0027796	0.0042	≤ S ≤	0.0667
Mean		0.05052	0.0025521	0.0040	≤ S ≤	0.0639

gl = 2Ne-1 = 73

X^2 (0.995) = 45.629

X^2 (0.005) = 107.862

Drift was estimated using as p the frequency in 1995 from the allele with the highest LD value of the two 2001 populations.

Locus	Allele	Initial frequency in 1995	Frequencies observed after 3 generations			Change by drift after 3 generations		Dif O-E Max †
			drift = S	2001_Early	2001_Late	Min Freq.	Max Freq.	
Ogo1UW	114	0.4375	0.0577	0.6250	0.5750	0.2645	0.6105	0.0145
Oki10	231	0.0125	0.0129	0.0000	0.2750	-0.0262	0.0512	**0.2238**
One2ASC	214	0.0750	0.0306	0.0000	0.3000	-0.0169	0.1669	**0.1331**
One µ 6	279	0.1026	0.0353	0.0000	0.2000	-0.0032	0.2084	-0.0084
One19ASC	222	0.0375	0.0221	0.3500	0.0000	-0.0288	0.1038	**0.2462**
OmyFGT22TUF	211	0.0375	0.0221	0.0000	0.1750	-0.0288	0.1038	0.0712
OmyFGT34TUF	143	0.1053	0.0357	0.0250	0.4000	-0.0017	0.2123	**0.1877**
Ots4BML	138	0.4000	0.0569	0.2250	0.0250	0.2292	0.5708	-0.3458
OmyPuPuPyDU	385	0.2895	0.0527	0.2250	0.0500	0.1313	0.4477	-0.2227

† Maximum differences between observed and expected (by drift) alleles frequencies.

Locus	Allele	Allele with highest LD	Most freq. allele
Ogo1UW	114	0.0145	0.0140
Oki10	231	**0.2238**	**0.1380**
One2ASC	214	**0.1331**	**0.0617**
One µ 6	279	-0.0084	-0.0434
One19ASC	222	0.2462	**0.1509**
OmyFGT22TUF	211	0.0712	-0.0052
OmyFGT34TUF	143	0.1877	**0.1595**
Ots4BML	138	-0.3458	-0.3458
OmyPuPuPyDU	385	-0.2227	-0.2227