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Rapid evolution of osmoregulatory function by modification of gene transcription in steelhead trout

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Abstract Populations experiencing sudden environmental change must be capable of rapidly evolving to survive. Here we explore changes in gene transcription as a mechanism for rapid adaptation at four osmoregulatory genes (*CFTR I*, *NaK ATPase1αa*, *NaK ATPase1αb* and *GHRII*) in anadromous steelhead trout versus a derived land-locked population after 14 generations. Transcription was measured before and after a 24-h saltwater challenge in pure and reciprocal hybrid offspring of fish from both populations reared in a common environment for two generations. Significant differences between the landlocked and migratory populations were observed, particularly in fresh water at the *NaK ATPase1αa* and *GHRII* genes, indicating rapid evolutionary change, possibly associated with reduced energy expenditure in the landlocked lake system. Phenotypic divergence analysis (Q_{ST}) shows that the observed transcriptional differences deviate from neutral expectations. Some reciprocal crosses exhibited anomalous transcription consistent with sex-linked epistatic or genetic imprinting effects. Our results highlight unpredictable phenotypic outcomes of hybridization among locally adapted populations and the need to exercise caution when interbreeding populations for conservation purposes.

Keywords Gene transcription · Epistasis · Non-additive genetic variance · Reciprocal crosses · Rapid evolution

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Introduction

Increasing awareness of global ecological degradation and human anthropogenic impacts, combined with the need for better natural resource management, has directed more attention to conservation biology, and in particular to studies concerning population viability in rapidly changing conditions. Indeed, a growing body of evidence documents examples of rapid evolution in a variety of taxa and ecosystems (e.g. Hendry and Kinnison 1999). Rapid trait divergence is thought to be correlated with changing environmental factors, for example: precipitation and Galapagos finches (Grant and Grant 2002), host/food networks and soapberry bugs (Carroll et al. 2001), spawning habitat and salmon (Hendry et al. 2000), predator–prey interactions in guppies (Reznick et al. 1997), and invasion of a novel environment in sticklebacks (Barrett et al. 2008). The conceptual link among all of those studies is rapid adaptive phenotypic change in natural populations.

Empirical examples of rapid evolutionary change refute the once commonly accepted idea that fitness-related traits are expected to have low additive genetic variance (Mousseau and Roff 1987) and hence be incapable of rapid evolutionary change. Fitness-related traits can, in fact, evolve as fast as neutral traits (Houle 1992; Kinnison and Hendry 2001; Merila and Sheldon 2000). This apparent contradiction of quantitative genetics theory and experiment

is resolved through non-additive genetic models which provide a basis for preserving genetic variation and ongoing capacity for rapid evolution in traits associated with fitness (Cheverud and Routman 1996; Goodnight 1988; Merila and Sheldon 1999). The molecular genetic mechanisms behind rapid evolution and non-additive genetic contributions to evolutionary change are generally poorly understood; however one exception is gene transcription. Transcription is a polygenic, heritable trait (Roberge et al. 2007; Roelofs et al. 2006) and it harbors substantial genetic variation that can contribute to phenotypic evolution (Gilad et al. 2006; Oleksiak et al. 2002). The phenotypic plasticity (Ghalambor et al. 2007), stochasticity (Raser and O'Shea 2004) and significant non-additive genetic components of transcription (Gibson et al. 2004; Hedgecock et al. 2007) provides further buffering of genetic variation against loss by selection. Given the expectation for a role of transcription modification in the evolutionary response to environmental perturbation and the reduced costs and technical difficulty of transcription quantification, transcription has become the focus of a number of evolutionary population studies. Such studies are designed to test for local adaptation in natural populations (Giger et al. 2008; Jeukens et al. 2009; Larsen et al. 2007, 2008; Nilsen et al. 2007), rapid adaptive changes in captive populations (Normandeau et al. 2009; Roberge et al. 2006, 2008), and ecotypic divergence in the wild (Roberge et al. 2007).

An important, but little studied, genetic outcome of local adaptation and rapid divergence is the change in the non-additive genetic variance component in populations experiencing strong selection pressures (Carroll et al. 2001, 2003). For example, introgression between wild and farmed Atlantic salmon (*Salmo salar*) results in remarkable non-additive variation in gene transcription, where farmed escapees that interbreed with wild fish may produce offspring with unpredictable phenotypes that would likely reduce their viability (Normandeau et al. 2009; Roberge et al. 2008). However, the genetic architecture of transcription upon introgression of naturally diverging wild populations has not yet been explored.

The ecological and demographic properties of salmonids provide an excellent natural system to test for rapid evolution of gene transcription and the genetic architecture of transcriptional divergence. Salmonid populations, naturally or as a result of human impact, tend to have low effective population size and undergo frequent bottlenecks (e.g., Heath et al. 2002; Koskinen et al. 2002; Shrimpton and Heath 2003; Thrower et al. 2004a), hence, they are expected to have relatively low additive genetic variation. Yet, they exhibit considerable genetic variation in transcriptional traits within and among populations (e.g., Derome et al. 2006; Roberge et al. 2006) and have a high capacity for rapid evolution (e.g., Heath et al. 2003; Hendry et al. 1998, 2000; Kinnison et al. 1998; Koskinen et al. 2002).

Here we document evolutionary change at four osmoregulatory genes in steelhead trout (*Oncorhynchus mykiss*) introduced into a freshwater lake 80 years (14 generations) ago and the genetic architecture of these genes upon hybridization with the ancestral population. We targeted genes that play central roles in the osmoregulatory changes associated with the parr-smolt transformation (in preparation for saltwater migration) in salmonids (i.e., *CFTR I*, *NaK ATPase1αa*, *NaK ATPase1αb* and *GHRII*; see "Materials and methods"). All are known to respond to short-term salt water challenge, and three of them are known to require high energetic input for expression (*NaK ATPase1αa*, *NaK ATPase1αb* and *GHRII*). Here we test two predictions; (1) the transplanted freshwater fish will exhibit a reduced transcriptional response to the saltwater challenge due to evolutionary loss of function, and (2) the freshwater expression of the osmoregulatory genes will be reduced in the landlocked fish due to selection favoring lower energetic costs in the lake habitat. Our results support the second prediction, and we propose that energy constraints may play a role in the transcriptional evolution of osmoregulatory genes in the landlocked population. Also, since we found that gene transcription had a substantial non-additive component, the potential for hybridization among salmon populations to result in unpredictable and possibly maladaptive transcriptional profiles is high, and it should be considered in the planning of future conservation and management action.

Materials and methods

The study system

In 1926, wild steelhead trout (anadromous form of *O. mykiss*) from Sashin Creek (Alaska, USA) were introduced into fishless Sashin Lake, upstream of Sashin Creek (Fig. 1). The lake is isolated from the lower stream by impassable waterfalls that prevent upstream migration (Fig. 1). A large resident population of rainbow trout was established in the lake, with a low number of founding individuals (3–8 founding females; Thrower et al. 2004a). After approximately 14 generations (80 years), substantial phenotypic differentiation with high heritability was documented between the two populations for life history traits such as size, growth and smoltification (Thrower et al. 2004b). High heritability in morphological and developmental traits also suggested the populations were capable of responding to selection, despite the small founding population (Thrower et al. 2004b).

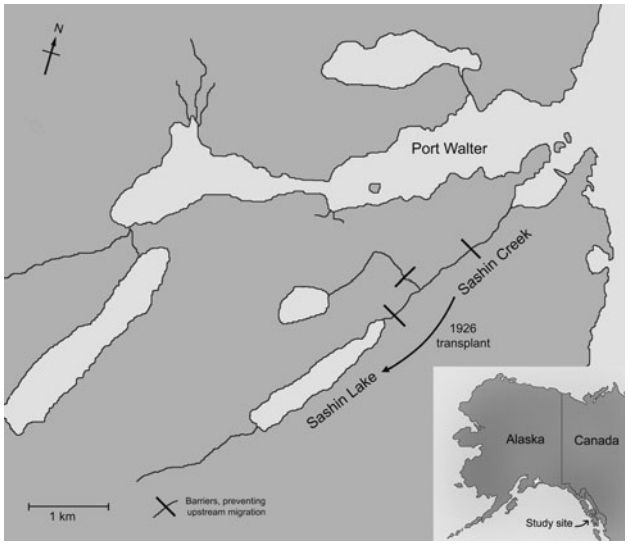

Fig. 1 Map of Alaska (USA) showing the source of anadromous steelhead trout (Sashin Creek) and the site of introduction of the resident population in Sashin Lake 1926 (adapted from Thrower et al. 2004a). Impassable barriers to upstream migration are marked with *black bars* across rivers

Breeding and rearing

Fish from both the anadromous and introduced (resident) lake populations (wild-caught fish in 1996; Thrower et al. 2004b) were bred and reared in a common hatchery environment for two generations. In May 2004, sexually mature fish (Table 1) from two pure lines were bred to generate four cross-types: pure resident (R × R; 8 families), pure anadromous (A × A; 10 families), female resident by male anadromous (R × A; 8 families), and female anadromous by male resident (A × R; 10 families). The R × R dams were significantly smaller than A × A and A × R dams, while no other groups differed (Table 1). The difference in the size of the females used could lead to maternal effects affecting both offspring size and possibly gene transcription; however, the effects associated with maternal size generally become indistinguishable by the time of our sampling, at the age of 2 years (Thrower et al. 2004b; Heath and Blouw 1998). Offspring from the various families within each cross-type were mixed and reared in a common hatchery environment in multiple identical tanks, thus minimizing the likelihood of tank or family effects. The two generations of common rearing environment likely minimized or eliminated source-related environmental and maternal effects (Roff 1997).

Saltwater challenge and sampling

The experiment included 2-year-old fish at the parr-smolt transformation stage. Parr-smolt transformation is the process by which the morphology, physiology, and behavior of salmonids change for saltwater acclimation prior to ocean migration (McCormick and Saunders 1987). Smolting fish were identified by their characteristic silver coloration and loss of parr marks (Thrower et al. 2004b). Both smolt and non-smolt offspring from all cross types were randomly selected from pooled families. The fish were sampled prior to, and after, exposure to 30 ppt salt water for 24 h. A 24 h saltwater challenge (at 30 ppt salt) is a standard protocol for the physiological measurement of saltwater tolerance in anadromous salmonids (e.g., Blackburn and Clarke 1987). Fish were humanly euthanized by a blow to the head, and gill tissue was immediately removed and preserved in RNA preservation medium (3.5 M Ammonium Sulfate; 15 mM EDTA; 15 mM Sodium Citrate; pH: 5.2,) at −20°C for later RNA extraction. All fish were individually measured for wet body mass (g). Eight fish of the smolt phenotype and seven of the non-smolts from each cross and treatment were assayed in this study.

Genes assayed

We targeted four osmoregulatory genes (*CFTR I*, *NaK ATPase1αa*, *NaK ATPase1αb* and *GHRII*) whose functions are relatively well characterized and are known to play key roles in saltwater acclimation. Other assayed genes included; Elongation factor 1a (*EF1a*) as the reference for normalization of quantification, *β-actin* and immunoglobulin M heavy chain (*IgM*) as "control" genes to assess neutral expectations of change between the two populations (since neither gene is expected to be under strong directional selection in either environment). Both *β-actin* and *IgM* have been shown to exhibit variable transcription under stress in Pacific salmonids (Ching et al. 2009).

Cystic fibrosis transmembrane receptor (CFTR I) is a chloride channel, located apically in the gills in Atlantic salmon (*S. salar*), and is important for saltwater adaptation (Singer et al. 2002). Transcription of *CFTR I* gene is upregulated during saltwater exposure and expression varies among strains (Singer et al. 2002). Landlocked Atlantic salmon have been shown to have reduced levels of *CFTR I* expression (Nilsen et al. 2007).

NaK ATPase1αa and NaK ATPase1αb are the two isoforms of the active subunit (*1α*) of the sodium potassium ATPase pump (Blanco and Mercer 1998). Saltwater exposure downregulates *NaK ATPase1αa* expression, while upregulating *NaK ATPase1αb* in *O. mykiss*, 24 h after exposure (Richards et al. 2003). Protein and immunohistochemistry studies further supports these two subunits having different functions in fresh and salt water (McCormick et al. 2009). The protein is expressed in gills and kidney, is highly ATP-dependent, and the protein activity is correlated with smoltification and saltwater tolerance in Atlantic salmon (Kiilerich et al. 2007). Nilsen

Table 1 Mean body mass (g) with one standard error in parentheses, for parental and offspring experimental fish by cross-type

Cross-type	*N*	Dam mass	Sire mass	*N*	Smolt mass	*N*	Non smolt mass
A × A	10	3,250[a] (862)	2,810 (660)	16	104 (21.7)	14	64.1 (22.0)
A × R	10	3,380[a] (798)	2,490 (449)	16	101 (14.5)	14	46.8 (16.0)
R × A	8	2,640[ab] (774)	2,840 (714)	16	105 (21.7)	14	68.4 (14.0)
R × R	8	2,130[b] (791)	2,820 (693)	16	110 (24.9)	14	56.5 (22.4)

Parental fish weight is given for dam and sire separately. Freshwater and saltwater challenged fish are pooled for each cross-type in the offspring. Significant differences among parental crosses are indicated with different letters for Dams (ANOVA, Tukey multiple comparison test, $P < 0.05$). Differences were not significant among sire groups

et al. (2007) showed seasonal expression in *NaK ATPase1α* subunits (a and b) is elevated in anadromous Atlantic salmon (*S. salar*) compared to landlocked populations.

Growth hormone receptor II (GHRII): Growth hormone (GH) has wide range of functions in teleost fish, and it known to influence somatic growth, lipid metabolism and saltwater acclimation (Bjornsson 1997; McCormick 2001, Deane and Woo 2009; Kiilerich et al. 2007). GHRII acts to modulate tissue-specific activity of GH (Norbeck et al. 2007). The role of GHRII as the receptor of growth hormone has been verified by protein–protein interaction experiments (Reindl et al. 2009). GHRII is upregulated during saltwater exposure (Poppinga et al. 2007), differentially expressed among anadromous and landlocked strains of Atlantic salmon (*S. salar*), and exhibits seasonal elevations that are associated with smolting and growth in Atlantic salmon (Nilsen et al. 2008).

RNA extraction and cDNA synthesis

Gill tissue was homogenized in 1.0 mL TRIZOL with a glass mortar and pestle. Total RNA was isolated by acid guanidium thiocyanite, phenol chloroform extraction using TRIZOL reagent (Invitrogen) following Chomczynski and Sacchi (1987). A subset of the total RNA extracts was evaluated for quality and quantity using a bioanalyzer (Agilent Technologies). RNA concentrations and RNA integrity number (RIN) values ranged from 0.2 to 1.2 μg/μL and 5.7 to 9.4, respectively [mean RIN = 7.7 ± 1.2 SD]. For cDNA synthesis, 0.5 μL total RNA was reverse transcribed using reverse transcriptase (Invitrogen SuperScript II), 0.5 μg Oligo (dT), 50 ng random hexamers, 10 mM dNTP with total RNA, incubated for 5 min at 65°C and chilled on ice. Subsequently, 5× RT buffer (Invitrogen), 40 units of RNaseOUT (Invitrogen) and 0.1 mM dithiothreitol (DTT) was added to the reaction and incubated 2 min at 42°C. Finally, 100 units of reverse transcriptase was added and the reaction was incubated at; 42°C for 10 min., 25°C for 10 min. and 42°C for 20 min. The enzyme was inactivated at 70°C for 15 min. The resulting cDNA was washed with 70% ethanol twice and resuspended in 10 mM TRIS (pH 8.0) prior to quantitative real time PCR.

Quantitative real-time PCR (qRT-PCR)

Assayed genes were quantified in eight smolt and seven non-smolt offspring from each cross-type in fresh water and after 24 h in salt water (except for *IgM* which was assayed only for freshwater transcription). Salmon have a tetraploid ancestry, and many of their genes have two isoforms with similar DNA sequences. We therefore designed our probes and primers in regions where the isoform sequences are most dissimilar, and that lie across intron–exon boundaries (Table 2). All assays were developed for this study, except *β-actin* and *IgM* which are described in Ching et al. (2009). The *CFTR I* gene of *O. mykiss* had not been characterized, and thus we amplified and sequenced it using degenerate primers designed from *Salmo salar CFTR I & II*. Sequence information for the other genes for *O. mykiss* was obtained from GenBank cDNA sequences (Table 2). Quantitative real-time PCR analyses were performed in triplicates for low expression genes (*CFTRI* and *GHRII*) and in duplicates for the others (*EF1a*, *NaK ATPase 1αa*, *NaK ATPase 1αb*, *β-actin*, and *IgM*). qRT-PCR critical threshold (Ct) values were obtained using ABI's 7500 System SDS software and assayed genes were quantified using the efficiency-corrected method (Pfaffl 2001) and were normalized to the Elongation Factor 1a (*EF1a*). qRT-PCR efficiencies are presented in Table 2.

Quantifying response to saltwater challenge

We report plasticity of gene transcription as the response of the various cross-types to a 24 h saltwater challenge (30 ppt). The same number of fish that were sampled in fresh water were subjected to a 24-h saltwater challenge and RNA was extracted post-challenge. Here we report transcriptional response (saltwater minus freshwater gene transcription) to the 24 h saltwater challenge, rather than gene expression in saltwater, since transcription response is a more functional measure of evolutionary "loss of function" in the landlocked population. We calculated response by subtracting the average transcription value in fresh water from the individual fish transcription values after the 24 h saltwater challenge.

Table 2 Quantitative real time PCR details for selected genes in steelhead trout

Gene	PCR efficiency (%)	Product length (bp)	Species (GenBank accession) used for assay development	TAQMAN MGB Probe, forward and reverse primer (nM)
CFTR I	92	112	*S. salar* (AF161070, AF155237)	TAA AAC TGG CGG TGC TC (150)
				CGA TAG GAC ACA GGT GCA GTG A (350)
				TGG AGA TGT CCA CCA GAA TAC ATA TT (350)
GHRII	83	85	*O. mykiss* (AY861675, AY751531)	CTG GGC GAC CAC CCT (250)
				ACC CTG AGC TCT TCA AGA AAG GTA (900)
				CAG TAC AGC TCT GGC CTC AGG T (900)
NaK ATPase 1αb	88	69	*O. mykiss* (AY319390)	CCT ACT ACT GAC AAA AAG A (200)
				CAG GAG GTT GGG TGG AAC AG (900)
				GAC ATT GAG TGA TCC TGG GGA TA (900)
NaK ATPase 1αa	93	99	*O. mykiss* (AY319391)	TAT TGA GAC GAA GAG GCC (200)
				CCC AGG AGG TTG GGT GTA CC (450)
				TGC ATT ACA AGG CAA TAC TGC A (450)
β-actin	90	64	See reference: Ching et al. (2009)	CAC AGC TTC TCC TTG ATG T (250)
				ACG GCC GAG AGG GAA ATC (900)
				CAA AGT CCA GCG CCA CGT A (900)
IgM heavy chain	93	69	See reference: Ching et al. (2009)	ACCTTGGTAAAGAAAGC (250)
				CGCTGTAGATCACTTGGAAAACC (900)
				TCTCCTCCAGTCTCCCTCTTGT (900)
EF1a	84	80	*O. mykiss* (AF498320)	TGC GTG ACA TGA GGC (100)
				AAT ACC CTC CTC TTG GTC GTT TC (450)
				CTT GTC GAC GGC CTT GAT G (450)

PCR efficiency, final product length, and primer-probe sequence information (with concentration in parentheses) is provided. Intron–exon junctions are underlined. *EF1a* was used as endogenous control

Statistical analysis

Pure-type cross analysis: First, we used *t*-tests to test for significant differences in freshwater transcription and response to salt water (relative to EF1a) for each gene between pure-type crosses (i.e., R × R and A × A). Since we performed multiple tests, we calculated global *P*-values and false discovery rates (FDR) by permutating the data 1,000 times. We calculated the global *P*-value as the ratio of the number of permutations with greater significance than the actual *t*-test divided by the total number of permutations, and FDR as the random expectation of the number of significant comparisons divided by the observed number of comparisons. For the random expectation of the number of significant comparisons we used the average number of significant comparisons per permutation. For *t*-tests and permutations, we used R software version 2.10.1 (R Development Core Team 2009). We also tested whether the observed transcriptional response to the 24 h saltwater challenge was significantly different from zero for each gene using *t*-test (SYSTAT v7.0.1, SPSS Inc., Evanston, Illinois). Unless otherwise noted, all other statistical analyses were performed using SYSTAT v7.0.1 (SPSS Inc., Evanston, Illinois).

Reciprocal cross analysis

We compared freshwater transcription and saltwater challenge response in each reciprocal cross with the pure-type crosses using two-sample *t* tests. Non-additive genetic effects are identified as significant deviations of reciprocal cross trait values from the midpoint of the pure-type cross trait values. We did not include *IgM* and *β-actin* in the reciprocal cross analysis, since those genes were solely included to characterize transcriptional evolution (drift) associated with pure-type crosses at genes not under osmoregulatory selection pressure.

Body size effects

Body size can influence the transcription of genes involved in osmoregulation, since smolting is sensitive to body size variation (McCormick and Saunders 1987; McCormick 2001). Since A × R non-smolts were significantly smaller than other cross-types (Table 1), we tested for an effect of individual body mass on variation in transcriptional traits among the four cross-types using an analysis of covariance (ANCOVA), with body mass as the covariate.

Q_{ST} calculation

We estimated phenotypic divergence (Q_{ST}) using the formula: $Q_{ST} = \sigma^2_{GB}/(\sigma^2_{GB} + 2\sigma^2_{GW})$, where σ^2_{GB} and σ^2_{GW} are among-population and average within-population components of genetic variance respectively (Whitlock 2008). Variance components (σ^2_{GB} and σ^2_{GW}) were estimated using ANOVA, and 95% confidence intervals (CI) were estimated by bootstrapping the data 30 times. The average within-population genetic variance (σ^2_{GW}) also includes environmental variance and thus may be overestimated, which may lead to an under-estimation of the true Q_{ST} (Whitlock 2008). No differential selection is expected in the two populations at *β-actin* or *IgM*, thus transcriptional differentiation at those genes should reflect primarily neutral (drift) divergence.

Results

Pure-type cross analysis

CFTR I and *NaK ATPase1αa* showed significant up-regulation and down-regulation respectively in A × A smolts in response to salt water (results not shown), consistent with previously published results (Richards et al. 2003; Singer et al. 2002). Up-regulation of *GHRII* and down-regulation of *NaK ATPase1αa* in response to salt water was significant in RxR non-smolts, as expected. All other comparisons of pre- and post-challenge transcription levels were not significant. Contrary to Richards et al. (2003), *NaK ATPase1αb* transcriptional response to the saltwater challenge was not significant, although we did observe a non-significant up-regulation trend.

Differences between pure lines (A × A vs. R × R) were significant in 5 comparisons (Figs. 2, 3), while neither *IgM* nor *β-actin* expression were significantly different between pure-type crosses ($P > 0.20$; results not shown). Multiple test analyses showed that our significance estimates are highly meaningful with FDR = 0.183 and a global *P*-value of 0.005. In all comparisons, *GHRII* and *NaK ATPase1αa* were differentially expressed in the two pure cross-types. Comparisons among pure cross-types were not significant for *CFTR I* and *NaK ATPase1αb* in either the smolt or non-smolt trials, both in fresh water and in response to the saltwater challenge (Figs. 2, 3).

The transcription of genes which are associated with high energy demand (i.e. *GHRII*, *NaK ATPase1αa* and *NaK ATPase1αb*) were consistently lower in the R × R fish in the fresh water ($t = 0$, Fig. 2). However, differences in the transcriptional response to salt water were not as consistent: in some cases the R × R crosses showed a greater change in response to the saltwater challenge, in others, a lower change (Fig. 3).

Reciprocal cross analysis

We measured gene transcription in reciprocal crosses (A × R and R × A) to assess additive versus non-additive genetic variance contribution to the expression of the selected genes. Dominance and epistatic effects would be evident by reciprocal cross transcription values that depart equally from the midpoint between the two pure-type crosses, while reciprocal cross transcription at the midpoint would indicate primarily additive genetic variance. Two sample *t*-tests identified significant departures from additive genetic variance expectation in reciprocal crosses in 5 of 16 cases (Figs. 2, 3). In all cases, the departures from additivity were characterized by a single reciprocal cross exhibiting overdominance, while the other did not deviate from additivity (Figs. 2, 3). *NaK ATPase1αa* and *NaK ATPase1αb* did not significantly deviate from additivity. Interestingly, *CFTR I* transcription shows significant non-additivity (non-smolts in fresh water and in response to salt water; Figs. 2, 3), despite no significant difference in transcription between the pure lines.

Body size effects

Individual body mass was not a significant factor for most transcriptional traits among cross-types (ANCOVA). Mass was marginally significant ($P = 0.04$) for freshwater transcription expression of *NaK ATPase1αb* in non-smolts. Out of 22 comparisons, this one significant effect may be due to chance alone, and post-hoc Bonferonni correction renders it non-significant.

Q_{ST} calculation

Q_{ST} estimates for transcription varied considerably. The Q_{ST} values for *β-actin* and *IgM*, which are expected to be under little or no selection in this system, average 0.33 (95% CI = ±0.07; Fig. 4). *CFTR* I showed generally low Q_{ST} values, indicative of no strong selection. Overall, most Q_{ST} estimates lie in a plateau between 0.2 and 0.4, whereas traits with elevated Q_{ST} estimates are generally in agreement with significant pure-type cross differences as determined by *t*-test (Fig. 4).

Discussion

In Pacific salmon (*Oncorhynchus* spp.), both genetic and environmental mechanisms have been proposed as contributing to the observed diversity in life history (e.g.,

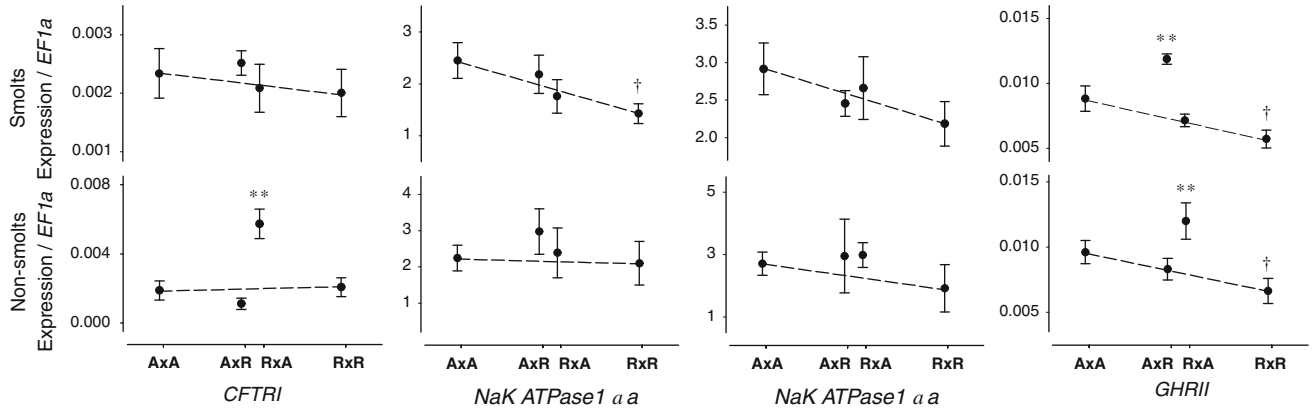

Fig. 2 Mean gene transcription (±1 standard error of the mean, SEM) normalized to *EF1a* for four osmoregulatory genes in steelhead trout (*Oncorhynchus mykiss*) from two divergent populations (anadromous—"A"; and land-locked, or resident—"R") and their reciprocal crosses in fresh water. Relative transcription is shown as the comparison between pure (A × A and R × R) and reciprocal crosses (R × A and A × R). Significant differences among pure types are indicated with †(*t*-test, $P < 0.05$). Deviation of reciprocal crosses from additive expectation are estimated using *t*-test and indicated with *($P < 0.05$) and **$P < 0.001$). Smolt and non-smolt phenotypes are presented in *upper* and *lower panels* respectively

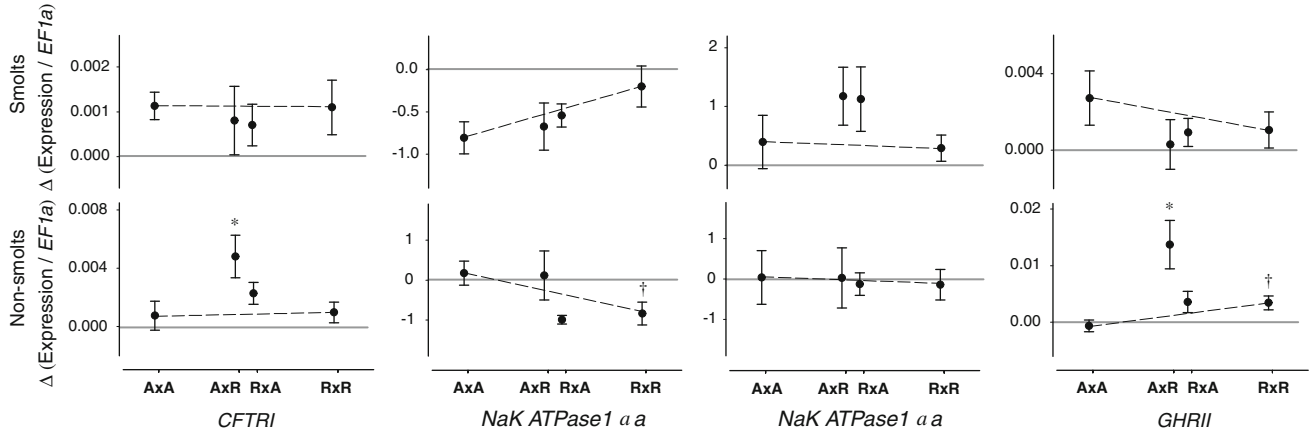

Fig. 3 Mean gene transcription response (±1 SEM) normalized to *EF1a* at four osmoregulatory genes in steelhead trout (*Oncorhynchus mykiss*) from two divergent populations (anadromous—"A"; and land-locked, or resident—"R") and their reciprocal crosses as a response to 24-h saltwater challenge. The difference between relative transcription ($t = 24$–$t = 0$) is shown as the comparison between pure (A × A and R × R) and reciprocal crosses (R × A and A × R). Significant differences among pure types are indicated with †(*t*-test, $P < 0.05$). Deviation of reciprocal crosses from additive expectation are estimated using *t*-test and indicated with *($P < 0.05$) and **($P < 0.001$). The "no response" line is indicated with a *thick gray bar*. Smolt and non-smolt phenotypes are presented in *upper* and *lower panels* respectively

Heath et al. 2008). In the Alaskan steelhead trout, we demonstrate both rapid evolution in transcriptional traits as well as plasticity of transcription as a response to a saltwater challenge (i.e., differences in transcription between the freshwater and saltwater environments). Thus, gene transcription provides a single mechanism for both the rapid evolution of adaptive life history characters as well as the well known physiological plasticity associated with gene expression.

Ecological dissimilarities between the two habitats (ionic and energetic) are ideally suited to promote rapid transcriptional evolution at genes associated with osmoregulation and seaward migration during the parr-smolt transformation (Barrett et al. 2008; Leonard and McCormick 2001). Generally our results in the freshwater environment are in accordance with our prediction of reduced energetic expenditure for osmoregulation in the resident freshwater population. For example, the two ATP dependent isoforms of the sodium–potassium pump (*NaK ATPase1αa*, *NaK ATPase1αb*), which are highly energy dependent and expressed at high levels in fish (Tseng and Hwang 2008), were expressed at lower levels in the land-locked population relative to the ancestral population. On the other hand, *GHRII* is not ATP dependent, but is

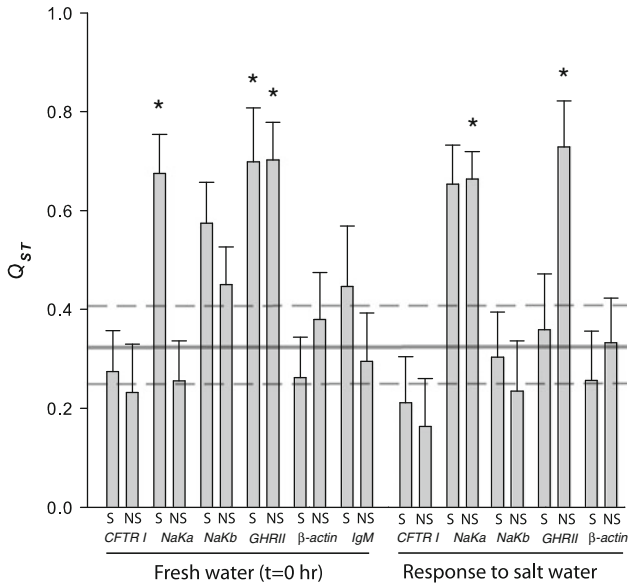

Fig. 4 Q_{ST} estimations and 95% CI of investigated traits. Significant differences between pure cross types are also included in the figure and denoted with *($P < 0.05$). Abbreviations, *S* smolt, *NS* non-smolt, NaKa: *NaK ATPase 1αa*, NaKb: *NaK ATPase 1αb*. Mean and 95% CI for putatively neutral response (*IgM* and *β-actin*) are marked with a *line* and *dashed lines*, respectively

associated with high energy demanding physiological processes such as smoltification, osmoregulation and growth (Kiilerich et al. 2007; Nilsen et al. 2008; Norbeck et al. 2007). *GHRII* shows downregulation in the resident smolts and non-smolts in the fresh water, consistent with our first prediction.

Overall, the landlocked population shows an evolutionary shift towards a lower energy consumption state, which is consistent with the low productivity of northern lakes and the high energetic costs of osmoregulation (Tseng and Hwang 2008). Similarly, juvenile anadromous Arctic charr exhibit lower rates of growth than their resident freshwater counterparts, despite having higher feeding rates, and the authors suggested that the anadromous fish had higher metabolic costs associated with their saltwater environment (Morinville and Rasmussen 2003). Thus, we suggest that gene transcription at selected loci adaptively evolved in the landlocked population due to energetic constraints. However, the energetic cost of osmoregulation is still under debate (Boeuf and Payan 2001; Tseng and Hwang 2009), and the direct measurement of O_2 consumption, or a microarray based approach to investigate energy related metabolic pathways could be implemented to confirm our conclusions.

Several previous studies have confirmed that the expression of the osmoregulatory genes used in this study do respond to abrupt salinity changes (e.g., Singer et al. 2002; Richards et al. 2003; Poppinga et al. 2007). These genes are also known to change during the seawater preparatory period of anadromous salmon (Nilsen et al. 2007). Despite the documented association between gene expression and saltwater acclimation, experiments demonstrating the direct role of variation in gene transcription in osmoregulation have yet to be done.

The resident freshwater population is known to have experienced hard selection for traits that are correlated with seaward migration (i.e., over the waterfalls; Thrower et al. 2004a, b). In osmoregulatory gene expression, we predicted the resident fish would exhibit a loss of response to saltwater exposure if selection favoured a saltwater intolerant state. However, we did not observe a pattern of transcriptional response that was consistent with the hypothesis of an evolutionary loss of saltwater response in the resident fish. Two examples of the predicted loss of response to the saltwater challenge in the resident fish was for the *NaK ATPase1αa* and the *GHRII* genes in smolts, which showed virtually no change in expression for the R × R, while the A × A showed a negative and positive response, respectively, to the saltwater challenge. Curiously, we observed significant transcriptional differences in the saltwater response in non-smolts for both genes, but the direction of the difference was contrary to our predictions; the R × R non-smolts exhibited a greater transcription response than the A × A non-smolts. However, the transcriptional response to short-term saltwater stress is not well characterized in non-smolt salmonids, hence it is difficult to interpret the functional significance of our non-smolt results. A more exploratory approach (such as microarray analyses) would perhaps identify additional genes that have responded to the environmentally-based selection between the isolated populations in this study.

The inheritance of transcription is known to be more complex than simple additive genetic variance models can account for since transcription includes substantial non-additive genetic effects (Gibson et al. 2004; Hedgecock et al. 2007; Roberge et al. 2008). The non-additive genetic component of variance in *CFTR I* and *GHRII* transcription reported here is likely an important factor in the maintenance of genetic variation and evolutionary potential in small and isolated salmon populations. On the other hand, non-additive genetic variance may result in the disruption of co-adapted genotypes and may lead to extreme phenotypes and generally reduced fitness (Tymchuk et al. 2007). The non-additive effects we identified at *CFTR I* and *GHRII* are curious, since the reciprocal crosses differ substantially. Classically, reciprocal cross divergence is explained by sex-linkage or extra-nuclear inheritance, although sex-linked epistatic effects or maternal imprinting are also possible explanations (Falconer and Mackay 1996; Tuiskula-Haavisto and Vilkki 2007). There is no evidence for sex-linkage or extra-nuclear inheritance of the genes assayed here, thus sex-linked epistatic effects or genetic

imprinting are more likely explanations. However, genetic imprinting has not yet been reported in lower vertebrates (Xie et al. 2009).

The non-additive response in *CFTR I* expression is particularly notable, since there was no significant difference in transcription between the pure-type crosses. This suggests that stabilizing selection for transcription may be acting at *CFTR I*, but the disrupted genomic background generated in reciprocal crosses affected the transcription control, likely resulting in the observed anomalous gene expression response. Such unexpected gene expression patterns in hybrid offspring highlight the need for caution when crossing individuals from putatively locally adapted populations for conservation or management purposes (Roberge et al. 2008; Tymchuk et al. 2007).

This study presents empirical evidence of rapid transcriptional evolution in a recently colonized population of steelhead trout. Transcriptional variation can not only mediate the evolution of physiological traits (such as osmoregulatory function), but it is also recognized as a primary mechanism for phenotypic plasticity associated with physiological acclimation. Transcriptional modification thus plays a role in the rapid adaptation and acclimation processes necessary for local adaptation in a changing environment. Our results also show that interbreeding locally adapted populations may increase the overall phenotypic variation but, in a cautionary conservation note, it can give rise to anomalous gene transcription responses in genes closely related to survival and performance (Tymchuk et al. 2007).

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