Comparison of Migratory and Resident Populations of Brown Trout Reveals Candidate Genes for Migration Tendency

Alexandre Lemopoulos[1,2,*], Silva Uusi-Heikkilä[2,3], Ari Huusko[4], Anti Vasemägi[2,5,6,†], and Anssi Vainikka[1,†]

[1]Department of Environmental and Biological Sciences, University of Eastern Finland, Joensuu, Finland

[2]Department of Biology, University of Turku, Finland

[3]Department of Biological and Environmental Science, University of Jyväskylä, Finland

[4]Aquatic population dynamics, Natural Resources Institute Finland (Luke), Paltamo, Finland

[5]Department of Aquaculture, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia

[6]Department of Aquatic Resources, Institute of Freshwater Research, Swedish University of Agricultural Sciences, Drottningholm, Sweden

[†]These authors shared senior authorship.

*Corresponding author: E-mail: alexlem@uef.fi.

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Abstract

Candidate genes associated with migration have been identified in multiple taxa: including salmonids, many of whom perform migrations requiring a series of physiological changes associated with the freshwater–saltwater transition. We screened over 5,500 SNPs for signatures of selection related to migratory behavior of brown trout *Salmo trutta* by focusing on ten differentially migrating freshwater populations from two watersheds (the Koutajoki and the Oulujoki). We found eight outlier SNPs potentially associated with migratory versus resident life history using multiple ($\geq$3) outlier detection approaches. Comparison of three migratory versus resident population pairs in the Koutajoki watershed revealed seven outlier SNPs, of which three mapped close to genes *ZNF665-like*, *GRM4-like*, and *PCDH8-like* that have been previously associated with migration and smoltification in salmonids. Two outlier SNPs mapped to genes involved in mucus secretion (*ST3GAL1-like*) and osmoregulation (*C14orf37-like*). The last two strongly supported outlier SNPs mapped to thermally induced genes (*FNTA1-like*, *FAM134C-like*). Within the Oulujoki, the only consistent outlier SNP mapped close to a gene (*EZH2*) that is associated with compensatory growth in fasted trout. Our results suggest that a relatively small yet common set of genes responsible for physiological functions associated with resident and migratory life histories is evolutionarily conserved.

Key words: salmonids, smoltification, RADseq, migratory behavior, life-history strategy.

Introduction

Diverse migration patterns are almost ubiquitous within the animal kingdom. Migrations have evolved to maximize fitness in heterogeneous environments (Gross et al. 1988; Dingle and Drake 2007; Dingle 2014). Although these migrations are proximately induced by a combination of environmental factors, changes in physiology, morphology, and/or behavior (Chapman et al. 2011; Chapman, Hulthén, et al. 2012), multiple studies have documented the genetic underpinnings of migration propensity (e.g., Pulido 2007; Zhu et al. 2008). Nonetheless, comparative studies across a wide range of organisms are needed to understand the mechanisms of evolution leading to adaptive migrations (Liedvogel et al. 2011).

Salmonids can adopt migratory, partially migratory or resident life history strategies, with feeding migrations directed to sea (anadromy), lakes (adfluvial), or larger river sections (potamodromy), and spawning migrations back to natal freshwater stream habitats (Chapman, Skov, et al. 2012; Dodson et al. 2013). Whether migratory salmonids originate from marine or freshwater ancestors have been under a long debate (McDowall 2002). Nor it is known whether migration, as a behavioral trait, drives evolution of physiology required for migration or vice versa. Ancestral diadromy might have induced the evolution of physiological adaptations that could drive migratory behavior even in currently landlocked species and populations (Piironen et al. 2013). Salmonid migrations

are proximately influenced by individual condition and a number of environmental factors (e.g., Olsson et al. 2006; Wysujack et al. 2009; Vainikka et al. 2012). Dichotomy between migratory and resident life histories has a significant heritable component in the *Oncorhynchus* (e.g., Hecht et al. 2012; Hu et al. 2014), *Salvelinus* (Thériault et al. 2007), and *Salmo* (Näslund 1993; Palm and Ryman 1999) genera.

Genome scans and environmental association analyses have been increasingly used to detect signature of selection (e.g., Berg et al. 2015; Babin et al. 2017). These methods detect markers that deviate from neutrality (i.e., are outliers) and are associated with particular environmental variables (for a review, see Ahrens et al. 2018). Recent studies investigating the molecular mechanisms of migratory behavior in salmonids have identified a long list of candidate loci (e.g., Moore et al. 2017; Veale and Russello 2017). Migration tendency has also been linked with differential gene expression (McKinney et al. 2015) and multiple quantitative trait loci (QTL; Nichols et al. 2008; Hecht et al. 2012). However, only a small proportion of the identified QTL regions and genes appear to be shared among populations and species. One particular genomic region (*Omy5*) has been highlighted by several studies in the rainbow trout/steelhead *Oncorhynchus mykiss* complex as the most influential genetic component for migration propensity (Hecht et al. 2012; Pearse et al. 2014; Leitwein et al. 2017). However, this association has not been observed across all *O. mykiss* populations (Hale et al. 2013). Thus, there is little evidence for a master locus for a life-history switch, whereas ample evidence exists for family, population, and species-specific genetic effects on phenotypic migration decisions (e.g., Narum *et al.*, 2011; Nichols *et al.*, 2016). This suggests that the molecular mechanisms behind migratory-resident life-history variation may vary between species and even populations or that only a subset of relevant causative genes has been identified and characterized.

Brown trout *Salmo trutta* is one of the most diverse species in terms of migratory behavior (Olsson et al. 2006). Brown trout shows a wide variety of migration strategies from strictly anadromous to complex and plastic feeding and spawning migrations in freshwater (Hindar et al. 1991; Charles et al. 2005; Aarestrup et al. 2017). However, it is not clear to what extent the migratory behavior is driven by genetic effects and to what extent it is plastic and relying on environmental clues. Migration-related life-history variation in brown trout have been extensively characterized from different perspectives (e.g., Boel et al. 2014; Jones et al. 2015). Classic studies have also shown that propensity for migration in brown trout must have a genetic component (Näslund 1993; Palm and Ryman 1999).

In this study, we screened single nucleotide polymorphisms (SNPs) to explore and characterize signatures of selection associated with migration and residency. We focused on multiple migratory versus resident population pairs from river systems in two distinct watersheds (see Lemopoulos et al. 2017). We predicted that comparison of populations pairs would reveal putative signs of selection related to migratory behavior and adaptation to different environments. We further predicted that comparison of the two distant watersheds could potentially reveal parallel genomic signatures associated with migration tendency, whereas watershed-specific signs might be linked with adaptation to particular environmental conditions.

Materials and Methods

Sampling

Koutajoki Watershed

Six naturally connected brown trout populations were studied within the Koutajoki (K) watershed in North-East Finland (66°17′N 29°53′E [WGS84], fig. 1*b*); three migratory: Kuusinkijoki (KM1), Oulankajoki (KM2), and Kitkajoki (KM3) and three resident: (Juumajoki, KR1, flowing into Kuusinkijoki; Maaninkajoki, KR2, flowing into Oulankajoki; and Pesospuro, KR3, flowing into Kitkajoki). These represent a subset of populations that were previously studied by Lemopoulos et al. (2017).

In short, three resident populations (KR1–KR3) in headwater streams were sampled by electrofishing in 2015 (Reid et al. 2009; Luhta et al. 2012). The length of the sampled sections varied between streams from 300 to 800 m. Based on the stream-specific length frequency distributions and scale readings the sampled fish belonged to the age groups of 0–3, including mainly parr but also some mature fish. Although the residency of individuals could not be confirmed through telemetry, residency was inferred from the observed reproductive isolation (Lemopoulos et al. 2017).

The landlocked adfluvial populations (M1–M3) were sampled by capturing upstream-migrating adults during their spawning migration from the feeding lake Pyaozero (659 km^2) in 2014. The river Oulankajoki samples (scales, see Saraniemi et al. 2008) were from taken at the Kiutaköngäs Falls on the river. These natural waterfalls form a partial migration obstacle, and local fisheries managers have caught trout annually since 1965 at the falls and transferred them upstream. The fish from the other two rivers were sampled using a fyke net further downstream in River Olanga in Russia, and the river of origin was inferred from radiotelemetry data. Right after capture, 149 individuals were equipped with external (100 individuals) or internal (49 fish were surgically implanted) radiotags. The fish that were tracked by automated recording stations and manual tracking of the radiotag signals into the rivers Kitkajoki and Kuusinkijoki at the spawning time were used as representative individuals of these river-specific populations. Based on scale readings, the sampled fish from the main stems were 6–8 years old, being on their first or second spawning run (Huusko et al. 2017). All captured brown trout were anaesthetized with clove oil (40 $mg \cdot l^{-1}$ in 1:9 water:

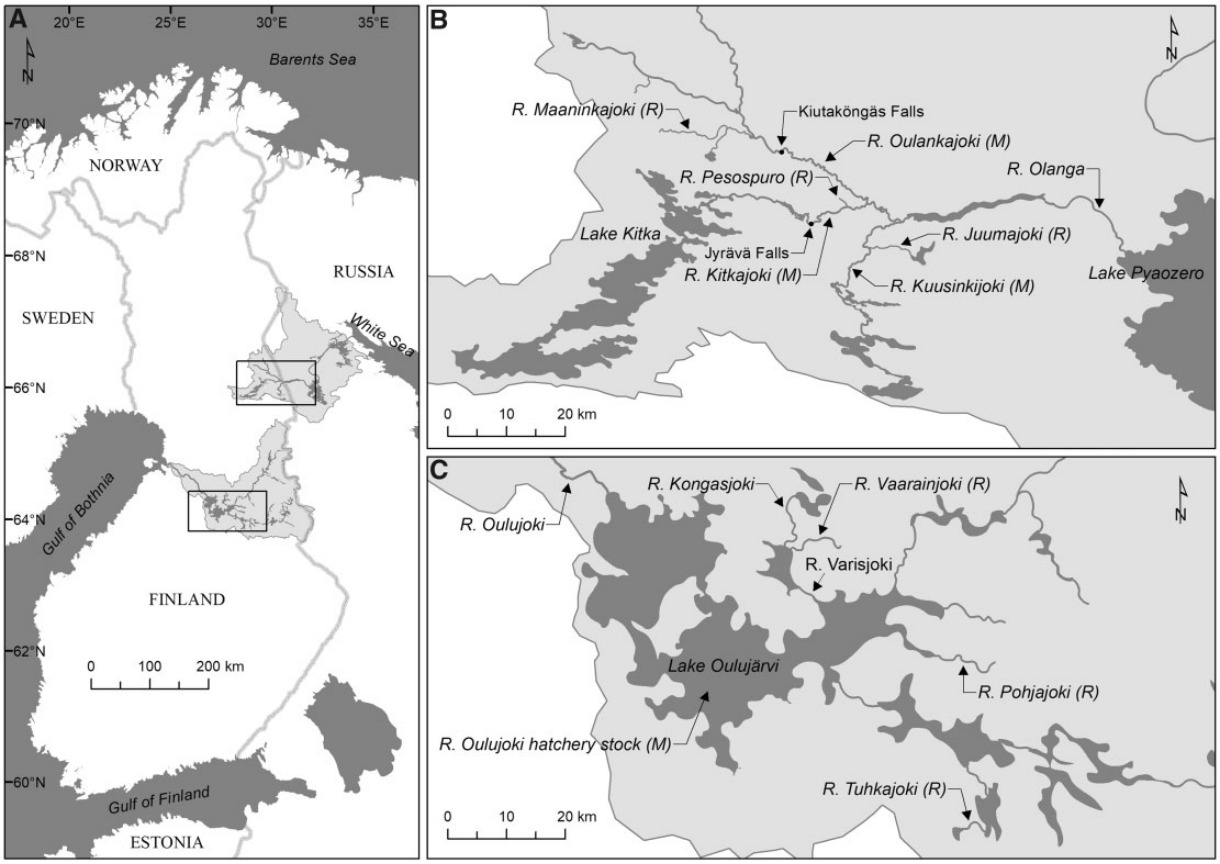

Fig. 1.—(*a*) General map of Finland and of the two watersheds locations. (*b*) Koutajoki watershed. Three resident populations (R) and three migratory populations (M) were sampled in corresponding tributaries and main stems. (*c*) Oulujoki watershed. Three resident populations (R) and one migratory populations (M) were sampled in corresponding tributaries and lake.

ethanol solution, Oy Anders Meder Ab, Helsinki, Finland), measured for total length, sampled for 2–3 scales for age determination and a small piece of pectoral fin that was preserved in pure ethanol, and released after recovery.

Oulujoki Watershed

Four populations were sampled in the Oulujoki (O) watershed above the main feeding area Lake Oulujärvi (887 km^2) (fig. 1c). Wild trout were caught by electrofishing from three putatively resident brown trout populations in rivers Pohjajoki (16.9.–17.9.2015, OR1), Tuhkajoki (17.9.2013, OR2), and Vaarainjoki (28.9.–30.9.2010, 15.9.–11.10.2011, and 2.10.2012, OR3). Lake Oulujärvi stock (OM1) was originally established by breeding adfluvial brown trout from two populations, River Varisjoki and River Kongasjoki (fig. 1c). All of these populations except for River Tuhkajoki are naturally connected.

DNA Samples

DNA samples were extracted from individuals of each population (table 1, see also Lemopoulos et al. [2017] and Prokkola et al. unpublished manuscript). The quality of total DNA was controlled with fluorometric measurements using Qubit 2.0 (Qubit dsDNA HS Assay Kit, ThermoFisher Scientific).

Genotyping

Libraries for double digest restriction-site associated DNA (ddRADseq) were taken from two previous studies (Lemopoulos et al. 2017; Prokkola et al., unpublished manuscript). The protocol used was adapted from (Peterson et al. 2012; Pukk et al. 2014). Briefly, DNA was digested using two enzymes (PstI-HF [5′CTGCAG 3′] and BamHI-HF [5′GGATCC 3′]) and then ligated using unique individual barcodes to the forwards ends. We pooled the individuals into five libraries. Each library was purified with a PCR purification kit and fragments were size selected at 280–320 bp on e-gel. They were then amplified with PCR and purified with SPRI-Beads. Finally, we examined the concentrations (Qubit 2.0) and quantities using Agilent 2100 Bioanalyzer. Sequencing was conducted at commercial provider Turku Centee for Biotechnology (BTK), Turku, Finland (www.btk.fi) with an Illumina HiSeq 2500 system.

Table 1
Summary Information of the Studied Populations and Samples

Sample Location	River Type	Putative Life-History	Number of Samples	Average Length (TL and SD) [mm]	Accronym
Juumajoki	Tributary	Resident	30	70.83 ± 12.25	KR1
Maaninkajoki	Tributary	Resident	30	86.42 ± 33.21	KR2
Pesospuro	Tributary	Resident	30	146.20 ± 24.64	KR3
Kuusinkijoki	Main stem	Migratory	30	699.13 ± 73.42	KM1
Oulankajoki	Main stem	Migratory	30	604.33 ± 56.88	KM2
Kitkajoki	Main stem	Migratory	30	767.73 ± 77.32	KM3
Pohjajoki	Tributary	Resident	11	248.5 ± 60.7	OR1
Tuhkajoki	Tributary	Resident	12	422.1 ± 55.7	OR2
Vaarainjoki	Tributary	Resident	29	459.3 ± 79.1	OR3
Migratory stock	Main stem	Migratory	28	526.9 ± 67.1	OM1

We used Stacks v1.40 (Catchen et al. 2013) *process_radtags* function to demultiplex, quality filter, clean, and trim all reads to 85 bp. Since Atlantic salmon is the evolutionarily closest relative to brown trout with an accessible genome (GenBank: GCA_000233375.4) (Crête-Lafrenière et al. 2012; Lien et al. 2016), Bowtie v. 2.3.0 (Langmead and Salzberg 2012) was used to create an index of the Atlantic salmon genome and to align all the reads against it (bowtie2 –p 2 –*s*/–summary –sensitive –end, rest of parameters to default). Reads that were aligned to several locations were assessed separately, and only the ones presenting unique best scores were retained. In total, 70% of the original reads were retained, and the average coverage after filtering was 53X. Genotype error rate (single nucleotide polymorphism [SNP] level) was 6%. We used the *refmap* and *population* functions for SNP calling. SNPs with a minimum coverage of three (i.e., each SNP position was covered by at least three reads), with loci that were present in all our populations (–p 6 and –p 4, respectively) and in 60% of the individuals retained. Further, SNPs with a minor allele frequency of at least 0.05 (Roesti et al. 2012) and maximum heterozygosity of 0.5 (Hohenlohe et al. 2011) were retained (supplementary table S4, Supplementary Material online). All other parameters were set to default values. Afterwards, we filtered the SNPs for Hardy–Weinberg equilibrium using the "adegenet" (v2.01) (Jombart 2008) package (*hw.test* function, with 1,000 repetitions) in R version 3.2.0 (R Core Team 2015). Only loci that were in equilibrium in at least 50% of the populations were kept. In total, out of 3.46×10^9 and 1.19×10^8 retained reads, respectively (supplementary tables S1–S3, Supplementary Material online), 5,519 SNPs for the Koutajoki watershed and 5,670 for the Oulujoki watershed were retained for the final analyses.

Outlier Analyses

Potential candidate SNPs influenced by divergent selection related to migratory-residency were identified using two genome scans (Bayescan [Foll and Gaggiotti 2008] and Pcadapt: [Luu et al. 2017]) and two environmental association analysis methods (LFMM: [Frichot and François 2015] and BayScEnv: [Villemereuil and Gaggiotti 2015]. *P* values were corrected (False Discovers Rate, FDR) and SNPs were identified under an alpha threshold of 0.05 for genome scans, whereas more relaxed thresholds (0.1) were applied environmental association methods in order to reduce type II error. SNPs identified as outliers in at least three of the methods were examined in detail for gene proximity and putative functions.

Bayescan

Bayescan (v2.1) (Foll and Gaggiotti 2008) was run with a burn-in of 50,000 and 50,000 iterations. Individuals were pooled according to populations, and the rest of the parameters were set to defaults.

Pcadapt

Pcadapt (v3.0.4) (Luu et al. 2017) was run with a *K* value of six (v3.3.2). SNPs *q*-values were obtained with the qvalue package (Dabney and Storey 2015). All the other parameters were set to default values.

Latent Factor Mixed Modeling (LFMM)

Using the LEA package (v1.6.0) (Frichot and François 2015) in R version 3.3.2 (R Core Team 2016), we ran the *lfmm* function. The environment file was implemented using "0" for resident populations and "1" for migratory populations. 30 repetitions were conducted with $K = 6/K = 4$,60,000 iterations and a burn-in of 30,000 and by allowing missing data. The rest of the parameters were set to defaults. Adjusted *P* values were obtained using the genomic inflation factor (λ) and Benjamini–Hochberg correction.

BayeScEnv

In the environment file of BayeScEnv (v1.1) (Villemereuil and Gaggiotti 2015), we assigned the same distance from the origin for both ecotypes, using "−0.5" for resident individuals and "0.5" for migratory. 20 pilot runs of 2,000 iterations

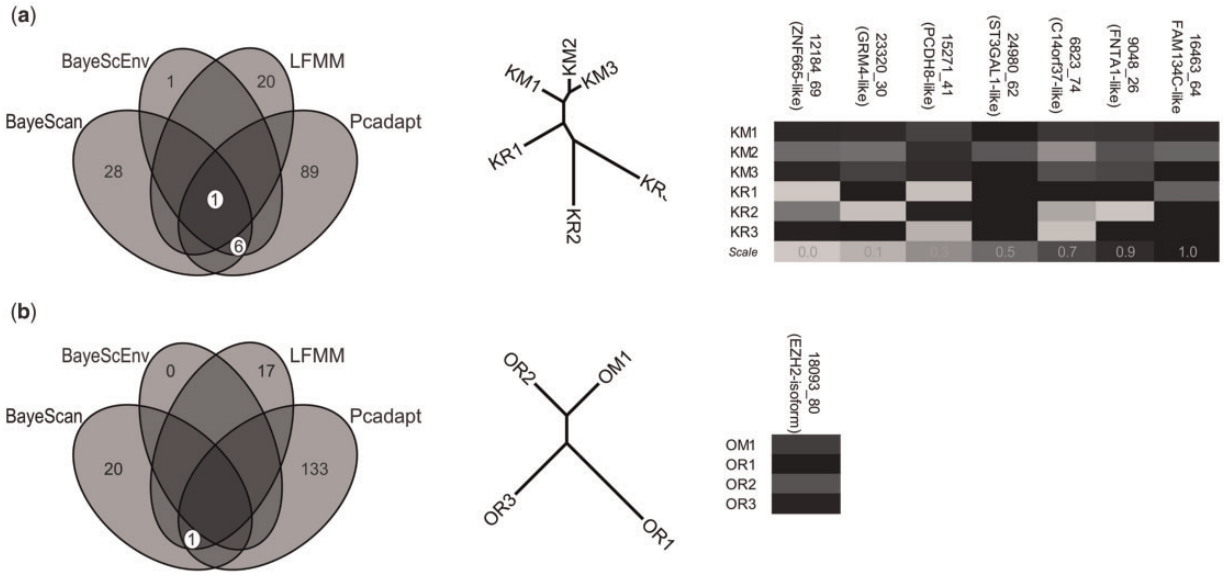

Fig. 2.—Overlap among outlier approaches (*a*) Koutajoki watershed: consistent outliers identified using multiple genome approaches are presented within white circles. UPGMA tree compiled based on 5,519 SNPs. Heatmap of the major allele frequencies of the seven most consistent outlier SNPs. (*b*) Oulujoki watershed: consistent outlier identified using multiple genome approaches is presented within white circle. UPGMA tree compiled based on 5,670 SNPs. Heatmap of the major allele frequencies of the most consistent outlier SNPs.

were ran prior to a burn-in phase of 50,000 and 1,00,000 iterations. The rest of the parameters were set to defaults.

Allele Frequency and Multivariate Analysis

Allele frequencies for the identified outliers were computed using *makefreq* function in adegenet package. Principle component analysis (PCA) using the *dudi.pca* function of ade4 package (v1.7.4; Chessel et al. 2004) was conducted based on 1) the whole data and 2) solely on the seven outliers identified in the Koutajoki watershed. Missing values were treated using the *tab* function of adegenet (v2.0.1; Jombart 2008) using the "mean" parameter; that is, replacing missing values by the mean allele frequencies. Three individuals were trimmed out from the second PCA, as they did not contain any genotype data on these seven loci.

Results

Koutajoki

Out of 5,519 SNPs, Bayescan detected 44, Pcadapt 105, LFMM 22, and BayesScEnv eight putative outliers (fig. 2*a*). Five outliers were identified by two methods (supplementary table S5, Supplementary Material online). In addition, six outlier loci were identified by three and one by all methods (fig. 2*a* and table 2). Two of the outliers were found within the coding region of the corresponding genes while five were located from 11,597 to 62,804 bp from the adjacent gene based on the Atlantic salmon reference genome. None of the seven outliers showed fixed allele frequency differences between migratory or resident populations. The seven outlier SNPs mapped against or close to different genes coding for proteins. As such, a zinc finger protein, a metabotropic glutamate receptor, a protocadherin, a sialyltransferase, an uncharacterized protein, a Farnesyltransferase and a FAM were associated to these outliers (table 2).

In general, PCA revealed similar patterns as UPGMA clustering, where migratory populations grouped together, and resident populations were more diverged (see Lemopoulos et al. 2017). PCA based on 5,519 SNP data set (fig. 3*a*) and on seven outlier SNPs (fig. 3*b*) showed comparable but more diffused patterns in the second casee. In contrast, PCA of random subsets showed weaker distinction between populations (supplementary fig. S1, Supplementary Material online).

Oulujoki

Based on the analysis of 5,670 SNPs, Bayescan identified 20, Pcadapt 133, LFMM 17, and BayeScEnv zero putative outliers (fig. 2*b*). Overall, six outliers were identified by two methods (supplementary table S6, Supplementary Material online) and only one was identified by at least three methods and was located within the coding region of *Histone-lysine N-methyltransferase isoform* gene (table 2). This SNP did not show fixation of alternative alleles between migratory and resident populations (fig. 2*b*).

Overlap between Watersheds

None of the outliers detected by any number of methods (table 2, supplementary tables S5 and S6, Supplementary

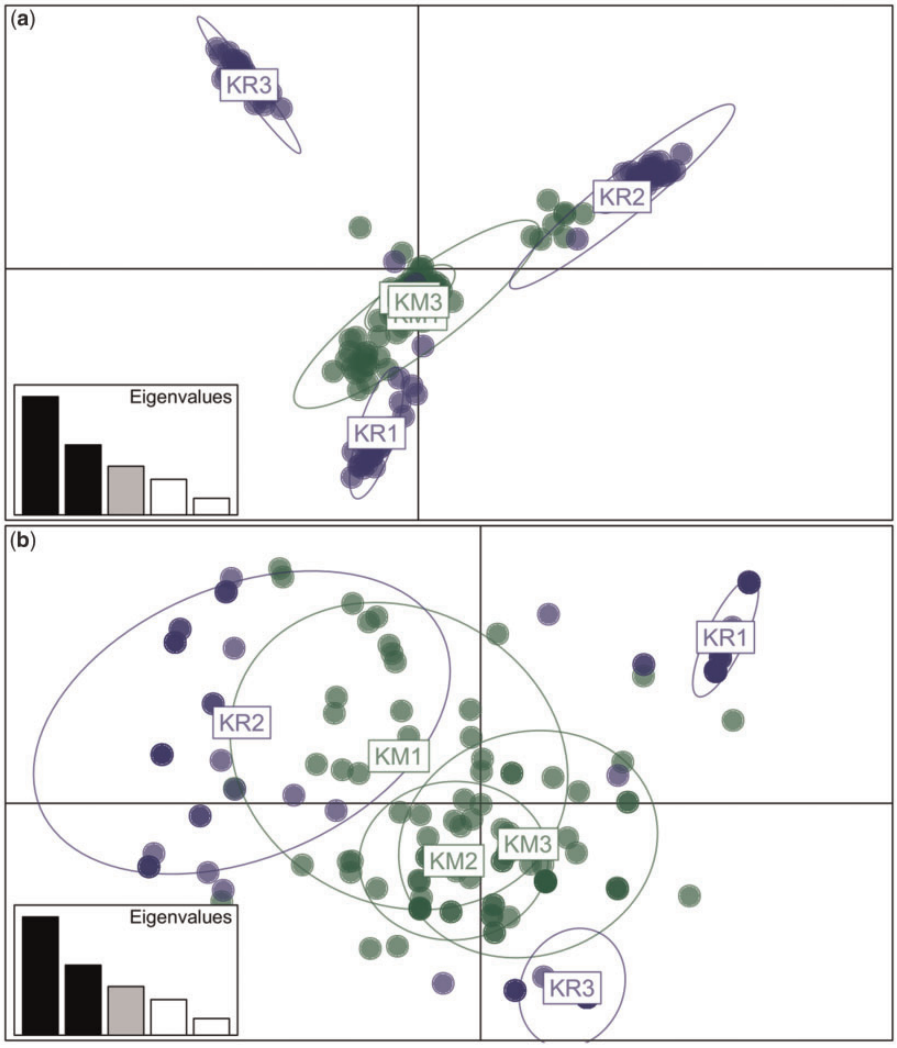

Fig. 3.—Principal component analysis of River Koutajoki samples based on (*a*) 5519 SNPs and (*b*) 7 outlier SNPs. Resident populations (KR) are plotted in blue while migratory populations (KM) are plotted in green.

Material online) were shared between the two watersheds. However, some gene families were identified within two watersheds by a limited number of methods (supplementary table S7, Supplementary Material online).

Discussion

To date, no single locus of major effect has been reported to explain the resident migratory life-history dichotomy in salmonids. Our study in brown trout agrees with the available literature in having identified multiple outlier SNPs that mapped close to genes that may play a role in migration propensity by affecting osmoregulation, and growth. Most of the involved functions of outlier genes are likely associated with smoltification in salmonids. In addition, the genes associated with thermal processes and growth may be related to adaptations required to live in small boreal brooks.

Because anadromous salmonids face similar physiological and environmental challenges during their life cycle, it is reasonable to expect that shared biological pathways related to ancestral diadromy have contributed to the evolution of migrations also in currently landlocked salmonid (McDowall 1997; Bloom et al. 2014). In the Koutajoki watershed, with historical connectivity to the White Sea, the most consistent outlier SNP between migratory and resident populations was found adjacent to the *ZNF665-like* gene. Proteins that contain zinc finger motifs have shown to be differentially expressed during smoltification in Atlantic salmon (Sear et al. 2010) and coho salmon *Onchorhynchus kisutch* (Gallagher et al. 2008), but also in the brain between progeny of experimentally bred offspring in the *O.mykiss* complex (McKinney et al. 2015). The next two outlier SNPs mapped adjacent to glutamate receptor and cadherin genes (table 2). The metabotropic glutamate receptor gene was identified to be under

Table 2
Annotation of the Eight Most Consistent Outlier SNPs

SNP ID	Watershed	Number of Methods Supporting Outlier Status of a SNP	CHROMOSOME (*S. salar*)	Distance (bp) from Closest Predicted Gene	Closest Predicted Protein (Corresponding Gene Symbol)	Biological process/ coexpression support
12184_69	Koutajoki	4	11	11,597	Zinc finger protein 665-like (*ZNF665-like)*	Gene family is differentially expressed during smoltification and in different ecotypes progeny in several salmonids (Gallagher et al., 2008; Seear et al., 2010; McKinney et al., 2015)
23320_30.3	Koutajoki	3	22	15,068	metabotropic glutamate receptor 4-like (*GRM4-like*)	Gene family involved in in rainbow trout migratory behavior (Hale et al., 2013; Baerwald et al., 2016)
15271_41	Koutajoki	3	14	59,757	protocadherin-8-like (*PCDH8-like*)	Gene family involved in rainbow trout migratory behavior (Hale et al., 2013; Baerwald et al., 2016)
24980_62.1	Koutajoki	4	25	0	acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 1-like (*ST3GAL1-like*)	Involved in mucus secretion in brown trout (Malachowicz et al., 2017)
6823_74.2	Koutajoki	3	6	15,936	Uncharacterized protein C14orf37-like	Differentially expressed between freshwater and saltwater Japanese eel. (Gu, 2014)
9048_26	Koutajoki	3	9	62,804	Farnesyltransferase/geranylgeranyltransferase type-1 subunit alpha-like (*FNTA1-like)*	Differentially expressed in low temperature in two species of gobies (Wellband and Heath 2017)
16463_64	Koutajoki	3	3	0	*FAM134C-like*	Differentially expressed according to temperature in a goby species (Logan and Somero 2010)
18093_80	Oulujoki	3	17	0	Histone-lysine N-methyltransferase isoform (*EZH2-isoform*)	Differentially expressed in trout muscle according to feeding treatment. (Rescan et al. 2017)

diversifying selection in resident and anadromous *O. mykiss* (Hale et al. 2013), whereas another recent work showed that metabotropic glutamate receptors are differentially methylated between *O. mykiss* ecotypes (Baerwald et al. 2016). Because G-protein-coupled glutamate receptors are involved in central nervous system transmission (Yin and Niswender 2014), synaptic plasticity, learning, and memory (Ohtani et al. 2014), these receptors may play an important role in the migration and homing of salmonids. Similarly, cadherins representing calcium-dependent cell adhesion proteins have been identified as putative targets of migration-driven divergent selection in *O. mykiss* (Hale et al. 2013), while also displaying differential methylation patterns between its two ecotypes (Baerwald et al. 2016).

Two other outlier SNPs mapped close to genes involved in osmoregulation (table 2). *ST3GAL1-like* gene belongs to protein glycosylation pathway and is implicated in mucus production in anadromous brown trout (Malachowicz et al. 2017). Among many functions including acting as a mechanical barrier (Desseyn et al. 2000) and protection from pathogens (Padra et al. 2014), mucus is known to be important for osmoregulation (Shephard 1994; Tacchi et al. 2015). Interestingly, a related sialyltransferase protein is overexpressed in migratory individuals of the partially migrating European blackbird *Turdus merula* (Franchini et al. 2017). Another outlier (table 2) mapped close to the *C14orf37-like* gene that is differentially expressed between freshwater and marine-phase Japanese eel *Anguilla japonica* (Gu 2014). Thus, evidence from gene expression studies supports the putative functional link between osmoregulation and both the *ST3GAL1-like* and *C14orf37-like* genes. Even though the Koutajoki trout is currently landlocked, there has been a historical connection to the White Sea (Koutaniemi 1999), and smoltification most likely still involves genes that originally facilitated migration to a marine environment, as suggested by recent studies on landlocked Atlantic and Pacific salmon

species (Piironen et al. 2013; Leitwein et al. 2017). Evolutionary consequences of the transition from saline to freshwater has been extensively investigated in nonsalmonid species such as three-spined stickleback (e.g., Hohenlohe et al. 2010; Jones et al. 2012). These studies have led to the identification of several candidate genes potentially involved in saltwater–freshwater transition adaptation. For example, among candidate genes identified by Ferchaud et al. (2014), *FAM70A* (family with sequence similarity 70), *GRID1* (glutamate receptor, ionotropic, delta 1) and *CDH20* (cadherin 20) all belong to gene families also represented among outlier loci in this study (table 2). The detection of these genes in landlocked populations may hint that physiological changes—induced by genetic components—could be driving the migratory behavior (Boel et al. 2014), rather than the opposite.

In addition, while a majority of the consistent outliers appeared to be associated with migratory behavior the possibility that some of the identified outliers reflect selection on other traits cannot be ruled out. Because resident brown trout inhabited cold, small headwater streams, they may experience strong temperature-driven selection; two other outlier genes (*FNTA1-like; FAM134-like*) have shown differential expression in different temperature conditions in gobies *Gillichthys mirabilis, Neogobius melanostomus* and *Proterorhinus semilunaris* (Logan and Somero 2010; Wellband and Heath 2017).

In contrast to Koutajoki watershed, only a single outlier was identified by at least three different methods in the Oulujoki watershed. The outlier occurred near a gene (*EZH2*) that has been shown to be differentially expressed in relation to compensatory muscle growth in rainbow trout (Rescan et al. 2017; table 2). Food availability is known to have a crucial role in brown trout smoltification (Jones et al. 2015), such that fasted trout are more likely to migrate than trout provided with abundant food (Wysujack et al. 2009; Bergman et al. 2013). Thus, it is possible that compensatory muscle growth in brown trout is linked to migration and, therefore, *EZH2* may have functional consequences for migratory-resident life-histories. In addition, epigenetic modifications of DNA (i.e., methylation) have been associated with different life-history strategies in rainbow trout (Baerwald et al. 2016) and also with saltwater adaptation in brown trout (Morán et al. 2013). Therefore, histone-lysine N-methyltransferase isoform (*EZH2*) could be involved in such processes in brown trout. However, the observed outliers may also result from unintended domestication and mixed origin that may have influenced the genetic make-up of the studied hatchery-reared migratory stock (OM1).

Interestingly, and resembling previous *O. mykiss* studies (e.g., Hale et al. 2013; Hecht et al. 2014), none of the outliers overlapped between the two watersheds (supplementary tables S5 and S6, Supplementary Material online). Technical issues such as suboptimal genome coverage intrinsic to RADseq, low number of studied populations and low sample sizes are some potential factors preventing us from getting an exhaustive list of candidate genes associated with migration (Ahrens et al. 2018). Also, while reducing Type I errors (false positive), focusing on outliers supported by multiple methodologies may increase the frequency of Type II errors (i.e., failing to identify real outliers under selection). In addition, we cannot exclude the possibility that at least some headwater streams contain low proportions of migratory individuals, as measuring individual migration patterns in natural population is extremely challenging. Therefore, it is possible that some gene families identified in the two watersheds (supplementary table S7, Supplementary Material online) contain additional variants related to migratory behavior not identified by our analysis. However, the limited overlap of migration-associated outliers between studies may also have a biological rationale. Migratory and resident life history strategies may be influenced by both population-specific effects, such as migration timing (Cauwelier et al. 2017; Prince et al. 2017), or available standing genetic variation (e.g., Barrett and Schluter, 2008). Migratory tactics in salmonids are considered threshold traits (Dodson et al. 2013) with switches occurring at certain values for heritable characteristics such as body length (Paez et al. 2010) or body mass (Martyniuk et al. 2003). It is not surprising that a large extent of the genetic variation between migration types could be unique to each population, as parallelism in ecological differentiation is not always reflected through uniform genotype patterns (Frazer and Russello 2013; Nichols et al. 2016).

The evolution of migration is not phylogenetically constrained, as alternative migration strategies exist across evolutionary distant taxa point to a parallel evolution of key biological pathways (Dingle 2006). Our results provide indirect evidence that migration in brown trout, despite being considered highly plastic (Olsson et al. 2006), is influenced by a set of putative candidate genes that appear to be shared with *O. mykiss* and potentially other Pacific salmonids. Further studies combining individual movement information within whole genome-wide association frameworks are needed to validate the role of the identified migration-related candidate loci. Nevertheless, this work, to the best of our knowledge, identifies for the first time several promising candidate genes associating with the migratory behavior of brown trout. Thus, the genes we identified represent interesting targets to further understand the evolution of migratory behavior.

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

Ethics

No animal experiments were performed. All sampled fish (License: 1013/5713-2012 by Center for Economic

Development, Transport, and the Environment) were released after sampling.

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