Parallel epigenetic modifications induced by hatchery rearing in a Pacific salmon

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Wild stocks of Pacific salmonids have experienced sharp declines in abundance over the past century. Consequently, billions of fish are released each year for enhancing abundance and sustaining fisheries. However, the beneficial role of this widely used management practice is highly debated since fitness decrease of hatchery-origin fish in the wild has been documented. Artificial selection in hatcheries has often been invoked as the most likely explanation for reduced fitness, and most studies to date have focused on finding signatures of hatchery-induced selection at the DNA level. We tested an alternative hypothesis, that captive rearing induces epigenetic reprogramming, by comparing genome-wide patterns of methylation and variation at the DNA level in hatchery-reared coho salmon (*Oncorhynchus kisutch*) with those of their wild counterparts in two geographically distant rivers. We found a highly significant proportion of epigenetic variation explained by the rearing environment that was as high as the one explained by the river of origin. The differentially methylated regions show enrichment for biological functions that may affect the capacity of hatchery-born smolts to migrate successfully in the ocean. Shared epigenetic variation between hatchery-reared salmon provides evidence for parallel epigenetic modifications induced by hatchery rearing in the absence of genetic differentiation between hatchery and natural-origin fish for each river. This study highlights epigenetic modifications induced by captive rearing as a potential explanatory mechanism for reduced fitness in hatchery-reared salmon.

epigenetics | methylation | coho salmon | hatchery | RAD sequencing

A major question in captive breeding of plants and animals for conservation efforts is how to maintain the fitness of captive-bred individuals upon release into the wild (1–3). This question is central with respect to the objective of rehabilitating declining or threatened species (4–6). For salmonid species, change in fitness-related traits and gene expression has been reported to occur in a single generation of captivity in a hatchery environment (7–9). Such rapid changes may in turn lead to maladaptation in the natural environment (8). Most studies investigating the molecular basis for rapid change in fitness-related traits occurring in hatcheries have focused on finding signatures of selection at the genome level by identifying loci with a large effect (7, 10–13). Consequently, it still remains to be elucidated if such rapid selection on complex phenotypic traits would rather induce subtle changes in allele frequency over multiple loci (5, 14, 15). Similarly, the relative roles of the genetic vs. nongenetic underlying processes responsible for such phenotypic changes are also still debated.

Numerous wild stocks of anadromous salmon and trout (genus *Oncorhynchus* and *Salmo*) have experienced fluctuating abundance over the past century, with a series of sharp declines in abundance (16–18). As a consequence, conservation hatcheries have been flourishing, with the goal of preserving ecosystem integrity, enhancing declining populations, and sustaining fisheries. This situation is common along the North American Pacific coast where billions of salmonids, all species included, are released from hatcheries each year. Despite substantial improvement in production practices (see *Supporting Information* for details), the beneficial role of hatcheries in enhancing and restoring wild stocks is still debated because many studies have provided evidence for reduced fitness and maladaptation of hatchery fish when released in the wild (7, 9, 19–24). While some discrepancies may be observed between salmonid species (25), studies of coho salmon are concordant in showing that survival of hatchery-born fish compared with their wild counterparts is significantly reduced (20–22, 24). It has also been shown that the hatchery environment may affect a wide range of fitness-related traits, including reproductive success (represented by the number of eggs and the number of eggs surviving to hatch), swimming endurance (swimming time to fatigue), and predator avoidance (20). Although some studies have shown that selection induced by the hatchery environment was involved in such fitness impairment, they also have reported that different environmental conditions (e.g., fish density) may significantly modulate the extent of physiological acclimation to the hatchery environment (8, 20, 23, 26).

In the current study, we used a genome-wide sequencing approach to compare global patterns of genetic variation and methylation in white muscle tissue of hatchery-reared juvenile (smolt) coho salmon with those of their wild counterparts in two geographically distant rivers in British Columbia, Canada. Our results show that, despite a nonsignificant genetic difference between hatchery and wild salmon originating from the same river drainage, the hatchery environment induces hypermethylation for

Significance

Captive rearing is known to impact the fitness of individuals released in the wild, but the relative role of genetic vs. nongenetic underlying processes is still debated. We measured genome-wide methylation profiles to document epigenetic differences between Pacific salmon originating from a hatchery and their natural-born congeners in two geographically distant rivers. Our results provide evidence that the epigenetic modifications induced by hatchery rearing provide a potential explanatory mechanism for reduced fitness of hatchery-reared salmon once released in the wild.

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Data deposition: The sequences reported in this paper have been deposited in the National Center for Biotechnology Information Sequence Read Archive, https://www.ncbi.nlm.nih.gov/sra/ (BioProject accession no. PRJNA389610).

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regions associated with ion homeostasis, synaptic and neuromuscular regulation, and immune and stress response, as well as control of swimming functions. This study highlights epigenetic modifications induced by captive rearing as a potential explanatory mechanism for reduced fitness previously reported in hatchery-produced coho salmon.

Results

The white dorsal muscle was sampled at the same exact place on each fish to measure variation both at the genetic and epigenetic level between hatchery (HOR) and natural (NOR) origin fish on 40 juvenile (smolt) coho salmon originating from two geographically distant rivers in British Columbia (Canada): the Quinsam and the Capilano rivers (Fig. 1). HOR fish were produced from Salmon Enhancement Program (SEP) hatcheries following a primary production strategy (see *Supporting Information* for details). Fish were sampled either before release (May 15, 2014) or in a reservoir (May 23, 2014) for the Capilano HOR and NOR fish, respectively, and in the estuary (June 19, 2014) for both NOR and HOR individuals from the Quinsam River, ~2 to 6 wk following the last production release (see *Methods* for more details).

Evidence for Parallel Epigenetic Modifications in Hatchery Environment. We used a tiling window approach to quantify the percentage of methylation over 1,000-bp regions throughout the genome masked for cytosine to thymine (C-T) polymorphism and retained only cytosines in a cytosine-phosphate-guanine (CpG) context for downstream analyses, as these regions represent the responsive methylation context in vertebrates. We used a distance-based redundancy analysis (db-RDA) to document methylation variation among HOR and NOR fish from both rivers, with river of origin, rearing environment, and sex as explanatory variables. The model was highly significant ($P < 0.001$) with an adjusted R^2 of 0.16 (Fig. 2). Both river of origin and rearing environment were significant whereas no significant effect was detected for sex (Fig. 2). Partial db-RDAs revealed that the net variation explained by rearing environment [adjusted (adj.) $R^2 = 0.08$; F = 4.34; $P < 0.05$] was identical to that explained by river of origin (adj. $R^2 = 0.08$; F = 4.66; $P < 0.01$). This shared variation between HOR salmon from both rivers relative to their NOR congeners provides evidence for similar (parallel) epigenetic modifications induced by hatchery rearing.

We identified differentially methylated regions (DMRs) (defined as having >15% overall difference; q-value < 0.001) (*Supporting Information*) between rearing environments, using a logistic regression, with sex and river of origin as covariates. We identified a total of 100 DMRs that were distributed among 27 chromosomes and 20 unmapped scaffolds (Fig. 3). The proportion of hypermethylated DMRs observed in both rivers was eight times greater in HOR relative to NOR salmon (89 vs. 11; $\chi^2 = 60.84$, df = 1, $P < 0.001$), suggesting a global pattern of down-regulation of genes associated with these DMRs in HOR salmon.

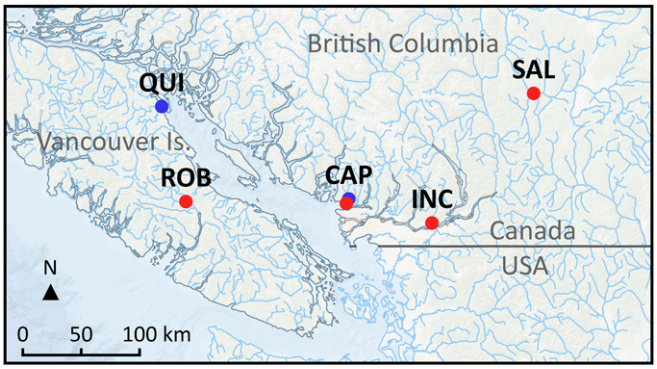

Fig. 1. Samples locations. Samples for epigenetic (methylation profiling) and population genomics analyses are shown in blue: CAP, Capilano River (n = 20), QUI, Quinsam River ($n = 19$). Samples for whole-genome resequencing ($n = 20$) are shown in red. CAP, Capilano ($n = 5$); INC, Inch Creek ($n = 5$); ROB, Robertson River ($n = 5$); SAL, Salmon River ($n = 5$). Resequencing samples were obtained from a hatchery located downstream of each river of origin, except Salmon River, for which the river of origin is represented while the fish were reared at Spius Creek hatchery, Meritt (British Columbia, BC, Canada).

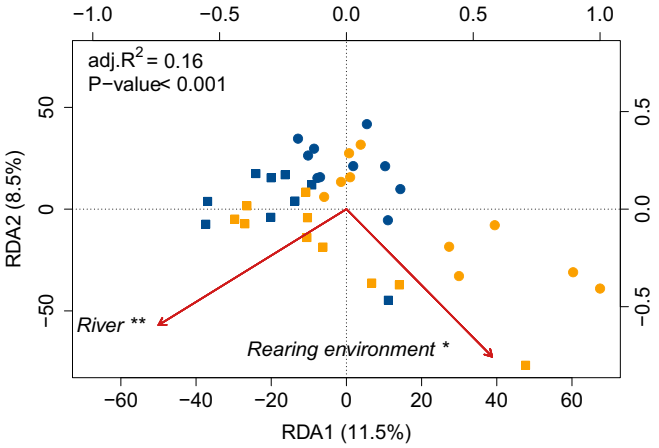

Fig. 2. Distance-base redundancy analysis (db-RDA) performed on the methylation data. A db-RDA for DNA methylation levels based on 131,807 1,000-bp sliding window regions for each individual. Symbols represent rivers: circle, Capilano; square, Quinsam. Colors represent rearing environment: blue, hatchery; yellow, wild. The db-RDA was globally significant and explained 16% of all DNA methylation regions variation (adj. $R^2 = 0.16$). River of origin and rearing environment both significantly explained 8% of the variation after controlling for each other with subsequent partial db-RDAs. $^{**}P$ value < 0.01 and $^{*}P$ value < 0.05, related to the explanatory factors.

Functional Annotation and Gene Ontology of DMRs. We identified 37 DMRs out of 100 that overlapped 52 unique transcripts and regions comprising 5 kb upstream and downstream of these transcripts. A blastx approach successfully identified 29 unique Uniprot IDs, which again revealed an excess of hypermethylation in HOR relative to NOR fish (25 hypermethylated vs. 4 hypomethylated; $\chi^2 = 15.21$, df = 1, $P < 0.001$) (Fig. 3 and Table S1). These regions were mostly located within a gene body or in UTR regions, supporting their functional roles in gene expression regulation and/or splicing events (Table S1). Gene ontology (GO) analysis revealed an overrepresentation (P value < 0.05 and at least three genes by GO term) of modules associated with ion homeostasis (GO:0055080, cation homeostasis; GO:0042592, homeostatic process; GO:0043167, ion binding; GO:0055065, metal ion homeostasis). A previous study in the closely related rainbow trout (*Oncorhynchus mykiss*) showed that hatchery rearing negatively affects acclimation to seawater by reducing the specific activity of NA^+ K^+ ATPase, which resulted in lower survival following seawater transfer (27). We also observed a significant enrichment for functions associated with the immune response (GO:0031347, regulation of defense response; GO:0050727, regulation of inflammatory response; GO:0045321, leukocyte activation), as well as synaptic signal modulation and locomotion functions (GO:0099572, postsynaptic specialization; GO:0050885, neuromuscular process controlling balance). The neuromuscular process controlling balance includes the calcium/calmodulin-dependent protein kinase type II subunit beta (CAMK2B), a main actor of the neuromuscular communication and regulating Ca^{2+} signaling in skeletal muscle tissue (28), which was hypermethylated in HOR salmon. Its activation has also been associated, together with the Ca^{2+} signaling, with sustained and endurance muscle exercise in humans and the control of muscle development and excitation

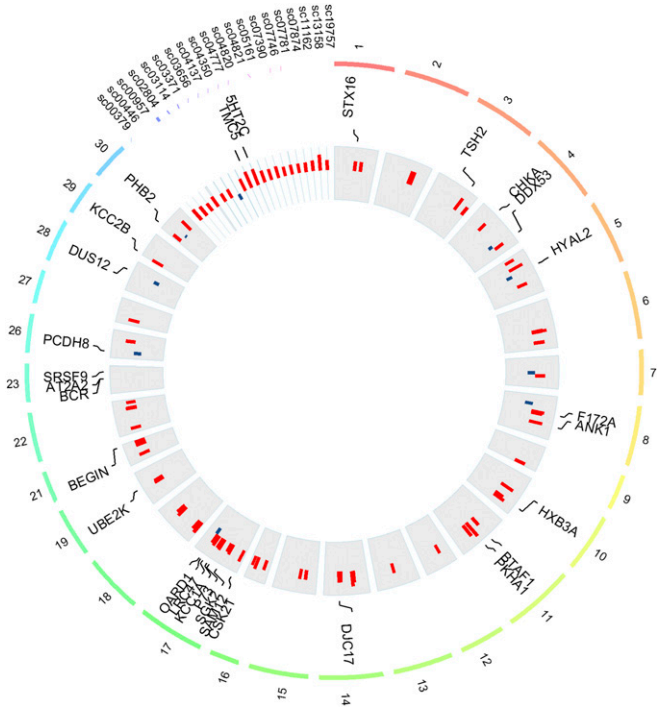

Fig. 3. Circos plot of differentially methylated regions between hatchery and wild fish. Only the chromosomes (n = 27) and scaffolds (sc) (n =20) containing differentially methylated regions are plotted. Bar plots show the difference of methylation levels between hatchery and wild fish. Red bar plots represent hypermethylated regions in hatchery fish, and blue bar plots represent hypomethylated regions in hatchery fish. Only annotated regions (blastx e-value < 10−6) are represented.

(29, 30). Lower critical swimming performance (*Uct*) has been documented in hatchery-reared coho salmon compared with their wild counterparts following transfer to seawater, and reduced average swimming speed has been documented in F1-hatchery smolts relative to wild smolts of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) (31, 32). The serotonin receptor 2C (HTR2C), which regulates appetite and feeding behavior (33), was also hypermethylated in HOR salmon. Finally, we observed a GO enrichment for transcription factors (GO:0006357, regulation of transcription from RNA polymerase II promoter), which comprised the TATA-binding protein-associated factor 172, also hypermethylated in HOR fish, which is involved in global transcription regulation. Genes under TATA box regulation are more able to respond rapidly to environmental stress, they show more variability in their expression range (phenotypic plasticity) compared with non-TATA regulated genes, and they account for the appearance of stress-induced phenotypes (34).

No Evidence for Genome-Wide Genetic Differentiation Between HOR and NOR Salmon. The principal coordinates analysis (PCoA) was performed on a Euclidean distance matrix of the 15,044 markers, and a db-RDA was produced on the genetic variation explained by these PCoA factors (response matrix), with river of origin, rearing environment, and sex as explaining variables. The model was highly significant ($P < 0.001$) with an adjusted R^2 of 0.18 (Fig. 4). Both river of origin and sex were significant whereas no significant effect was detected for rearing environment (Fig. 4). No significant outlier with a genome-scan approach (Bayescan v2.0) (35) was detected between sexes (Fig. S1). Moreover, an analysis of molecular variance (AMOVA) revealed no significant genome-wide difference between HOR and NOR salmon [genetic differentiation (Fst) = 0.005 and 0.002, for Capilano River and Quinsam River populations, respectively; $P > 0.05$] while the net difference between rivers was highly significant (36) (mean Fst = 0.038 ± 0.003; $P < 0.001$) (Table S2). Additionally, heterozygosity and inbreeding values (Gis) were not significantly different between rivers or between HOR and NOR salmon (Table S3). No outlier [false discovery rate (FDR) > 0.05] was detected between HOR and NOR fish using Bayescan v2.0 (Fig. S2) whereas random forest identified 114 covarying markers, distributed over the 30 chromosomes. Nevertheless, permutations revealed that a similar pattern of apparent polygenic selection according to the distributions of the out-of-bag (OOB) errors could indeed be obtained by chance alone (Fig. S3). Population genomics analyses confirmed the prediction that HOR and NOR salmon belong to a single panmictic population within a given river. Our results cannot rule out that selection within one generation has caused changes in allele frequencies between HOR and NOR fish in genome regions that were not screened. Nevertheless, they indicate that such an effect would be modest relative to parallel differences observed at the epigenetic level.

Discussion

The decline of many wild stocks of Pacific salmon encouraged the development of conservation hatcheries for enhancement. However, the hatchery environment during early life stages induces significant physiological and behavioral changes that may ultimately reduce the fitness of hatchery-born fish (25, 37). Hatchery fish have been shown to have higher reproductive success than their wild counterparts in hatchery conditions, but lower success when released in the wild with an accumulative impact over a generation, indicating inadvertent selection occurring after a single generation of hatchery rearing (8, 9, 37). Recent work provided evidence for a pronounced difference in gene expression between wild and hatchery fish after 1 y of captivity, despite no significant differences at the genome level (7). Similarly, a differential pattern of gene expression between domesticated and wild Atlantic salmon evolved in parallel in North America and Europe within five generations (10). Here, our results support the hypothesis that epigenetic modifications induced by hatchery rearing during early developmental stages may represent a potential explanatory mechanism for rapid change in

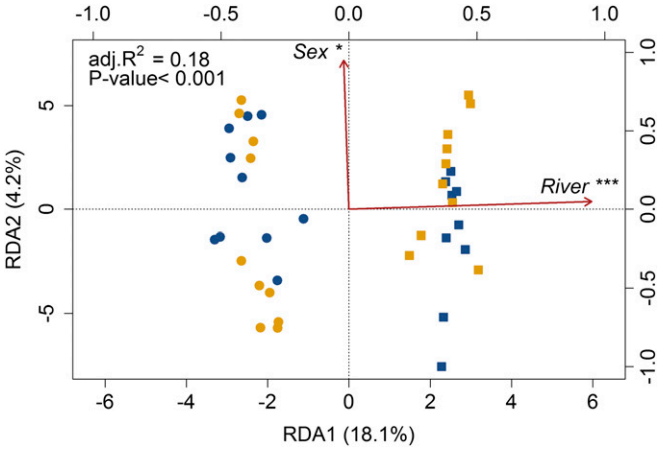

Fig. 4. Distance-base redundancy analysis (db-RDA) performed on the genetic data. The db-RDA performed on the total filtered 15,044 SNPs identified. Symbols represent rivers: circle, Capilano; square, Quinsam. Colors represent captivity treatment: blue, hatchery; yellow, wild. The db-RDA was globally significant and explained 18% of all SNPs variation (adj. R^2 = 0.18). River of origin and sex explained significantly 16% and 2%, respectively, of the variation after controlling for each other with subsequent partial db-RDAs. ***P value < 0.001 and *P value < 0.5, related to the explanatory factors.

fitness-related traits in juvenile salmon during their seaward migration. As such, our results are in line with accumulating evidence for epigenetic reprogramming caused by environmental conditions at a specific time that may induce phenotypic changes which may persist in subsequent life stages (38–41).

Strikingly, these parallel epigenetic modifications, mainly in the form of hypermethylation, were induced independently in two genetically distinct populations and in the apparent absence of overall neutral and adaptive variation between HOR and NOR salmon in these systems. As such, our results confirm that HOR and NOR coho salmon within a given river belong to a single panmictic population, as predicted based on the hatchery programs applied in these rivers. These so-called "integrated programs" are based on local populations and involve spawning in hatchery and natural environments. HOR and NOR fish in each river are not kept separate; thus HOR fish spawn in both the hatchery and the natural habitat as do NOR fish, which can maintain high gene flow in the whole system. Furthermore, no difference in genetic diversity (heterozygosity or inbreeding coefficient) was observed between HOR and NOR salmon, hence not supporting that there is no evidence for increased inbreeding depression in hatchery fish for the populations we studied. Finally, we found no evidence of either large effect or polygenic selection acting between hatchery and wild samples when using either a standard genome scan approach or a statistical framework appropriate for investigating the effect of weak selection in multiple regions of the genome. Therefore, our work corroborates a recent study on juvenile steelhead trout (*Oncorhynchus mykiss*), which showed that a single generation in captivity induced differences in the expression of hundreds of genes in offspring reared in identical environments, but produced from parents that experienced different rearing environments, in the absence of significant genetic differentiation (7). However, comparisons between both studies should be interpreted cautiously given the differences in experimental setting.

In contrast to the absence of significant genetic differences, our results revealed highly significant epigenetic differences between HOR and NOR salmon that were as pronounced as those observed between populations from different rivers. Our results differ from a study that compared hatchery-born and wild steelhead trout where no significant difference in methylation profiles was observed (38). However, rather than biological differences (differences among species and/or life stages), it is most likely that the absence of difference in levels of methylation originate from the lower resolution of the molecular technique available at that time. Indeed, the authors noted that they were only able to detect "all-or-nothing" changes (near 100% methylation) and thus could not rule out that moderate levels of differences could exist, which the reduced-representation bisulfite sequencing (RRBS) protocol allowed us to detect in our study. With an approach offering a substantial increase in genomic resolution, we found evidence for a highly significant effect of hatchery rearing on DNA methylation profiles across several regions of the coho salmon epigenome. Moreover, our results revealed that the same epigenetic modifications developed in parallel between the two independent study systems, mainly in the form of hypermethylation.

In Atlantic salmon, it has been shown that hatchery fish are not as efficient as wild fish in rapid seawater acclimation (27). In addition, acclimation to seawater induces profound, yet transient, changes in methylation levels in brown trout (*Salmo trutta* L.) (39). Here, we showed that genomic regions with differential methylation profiles between HOR and NOR salmon in both rivers were enriched for ion homeostasis and control of body fluid level functions, adding to growing evidence that hatchery rearing may affect the osmoregulatory process during smoltification. For instance, serine/threonine-protein kinase (SGK2), which was hypermethylated in HOR salmon, is a potent stimulator of epithelial Na^+ channels (40). Similarly, seawater acclimation in the killifish (*Fundulus heteroclitus*) is associated with the level of SGK1 expression (no SGK2 or SGK3 ortholog present in the killifish genome) (41). Considering the fundamental role of these biological functions during smoltification (physiological acclimation to seawater) and seaward migration of juvenile salmonids (42), we propose that hatchery-induced epigenetic modifications during early developmental stages could be partly responsible for the saltwater acclimation deficiency reported in coho salmon (43). Moreover, neuromuscular communication, through regulation of Ca^{2+} levels, was among the biological functions showing the most pronounced pattern of hypermethylation in HOR salmon, including a major regulator of motoneuron signal transmission through Ca^{2+}levels (CAMK2). This strongly suggests an alteration of the neuromuscular communication that could reduce swimming performance as documented in hatchery-reared coho salmon (31). It is noteworthy that a previous study in rainbow trout revealed differential levels of methylation in key genes responsible for muscle function, which could possibly impact swimming performance in this species (44).

In addition to functions that could impact swimming performance, it is noteworthy that, while we investigated white muscle tissue only, we observed an enrichment for overall synaptic signal control functions, which raises the hypothesis that the hatchery environment causes epigenetic modifications that could cause important physiological and endocrinal change in HOR salmon. In particular, the patterns of hypermethylation we observed at some major neurological regulators, such as HTR2C in HOR salmon, may play a role in the commonly reported behavioral differences between captive-reared and wild fish, such as increased aggressiveness, foraging, and boldness (30, 45–50). This hypothesis could be tested by comparing methylation profiles in the brain of fish with different aggressiveness, foraging, and boldness characteristics (50).

One methodological factor that may warrant a cautious inter-pretation of our results is that white muscle represents a mixed-cell tissue. Therefore, variable proportions of different cell types among individuals and/or different cell status could introduce biases in measures of methylation levels (51). However, the fact that we were still able to detect pronounced parallel changes in the form of hypermethylation between two independent systems makes our results conservative and suggests that possible biases would be inherent to biological differences between hatchery and wild individuals. Finally, because of the tissue specificity of the methylation patterns (52–54), further studies extending the experimentation on a broader range of tissues will be necessary for a more comprehensive characterization of the impact of hatchery rearing on salmon smolts.

The reduced genome representation method used here and the fact that we investigated only one tissue (yet representing 80% of young salmon body weight) resulted in only a partial coverage of all possible epigenetic differences that may exist between HOR and NOR coho salmon. Our results most likely underestimate the magnitude of epigenetic modifications incurred in the hatchery environment. Nevertheless, our results showed that hatchery-induced methylation changes happened in a similar manner in independent hatcheries, that these differences are much more pronounced than differences at the genome level, and that shared methylation changes persisted in the smolts of both rivers, despite the fact that they were exposed to different environments for several weeks after their release. It is therefore plausible that the hypermethylation of important physiological functions could have an immediate impact on the out-migrating smolt's capability to acclimate to the freshwater–saltwater transition and the short-term survival at sea, which could ultimately impact the fitness of HOR fish. Moreover, based on previous studies, it is reasonable to hypothesize that hatchery-induced epigenetic modifications during early developmental stages (postfertilization and germ cell differentiation) could impact on lifelong phenotypic changes (55, 56). However, whether or not the epigenetic modifications that persisted for several weeks in smolts are maintained in returning adults and/or are inherited and acted upon by natural selection cannot be answered from our results.

Different practices in hatchery rearing are currently evaluated to circumvent the general observation that captive rearing reduces fitness in the wild. Alternative rearing practices may differ in environmental conditions (e.g., hatchery facilities or open lake), age at release (fry or smolt), or nutrition (supplemented or not by commercial food), which may significantly affect fish survival (25, 26, 39, 57, 58). The effect of such factors could also be detected at the epigenetic level (39). Clearly, improving our understanding of the dual role of genetic and nongenetic variation induced by captive rearing will contribute to the development of the best practices for the management and conservation of salmonids and numerous other species that are managed through supplementation worldwide (1).

Methods

Hatchery Procedures and Sampling. The Salmon Enhancement Program (SEP) hatcheries have standard operating procedures employed across hatcheries, with the primary production strategy (PPS) being used for coho salmon at both Capilano and Quinsam hatcheries, British Columbia, Canada (see details in *Supporting Information*). Coho yearling smolts, defined as 1+ year after hatching, are released over a month. In this study, the progeny of fall-run 2012 Capilano and Quinsam River adult coho salmon were released in each respective river as yearling smolts in 2014. Capilano River coho salmon juveniles were collected in fresh water before production releases; the hatchery fish were collected at the hatchery on May 15, 2014 while the wild samples were caught via trap nets in the reservoir on May 23, 2014. These freshwater fish were classified as smolts as all physiological changes in preparation for saltwater had occurred, with minimal size differences in fork length or weight between the two groups: 116 mm and 16.9 g for hatchery and 111 mm and 13.7 g for wild individuals on average. Quinsam River smolts were collected via beach seine nets inside the Campbell River estuary, where the Quinsam River outflows to the sea, on June 19, 2014, ∼2 to 6 wk following the last production coho release from the hatchery. Hatchery fish were identified by their "marked" or clipped adipose fin while the wild samples were initially collected as "unmarked" coho and later confirmed as wild due to their lack of coded wire tag (CWT) detection and lack of an adipose fin clip. We collected a total of 40 coho salmon, including 10 juveniles from each river (smolt stage) reared in captivity in a local hatchery and 10 smolts born in the wild. Whole smolts were anesthetized, frozen on dry ice, transported to the Molecular Genetics Laboratory [Fisheries and Oceans Canada (DFO)] in Nanaimo, BC, and held at −80 °C until subsampled for analysis. Frozen white muscle sections were taken from whole smolts, ∼4 mm above to 4 mm below the lateral line, shipped on dry ice to Laval University, and subsampled for analysis. White muscle tissue was preferred because of its importance in both migration and homeostasis in fish (making up to 80% of the body weight) and because previous studies identified key markers linked to muscle development and activity as differentially methylated between migratory and nonmigratory ecotypes of rainbow trout (*Oncorhynchus mykiss*) (44, 59–62).

DNA Extraction and Reduced-Representation Bisulfite Sequencing Library Preparation. The RRBS library preparation was adapted from a previously published protocol (63). Libraries were sequenced on a HiSEq. 2000 platform (five individuals by lane) at the McGill University and Génome Québec Innovation Centre (Montréal, QC) using a 100-bp single-end reads approach. In parallel, sex information was inferred by PCR using a method previously described for salmonids (sdY_E2S2 5′-GTGGAGTACTG-CGAAGAGGAGGT-3′ and sdY_E2AS4 5′-CTTAAAACCACTCCACCCTCCAT-3′ primers) (64). Sex information for each individual is available in Table S4. Detailed methods are provided in *Supporting Information*.

Methylation Calling. To avoid the possibility of falsely interpreting existing C-T DNA polymorphism as epigenetic variation, we masked these SNPs from the genome of the coho salmon (GenBank assembly accession no. GCA_002021735.1). We used Bismark v0.14.5 (65) and extracted only CpGs with sufficient coverage (≥10×). CpGs were assembled in 1,000-pb regions, and a logistic regression, with the river of origin and sex as covariates, was conducted to identify differentially methylated regions (DMRs) with the MethylKit R package (66). The DMRs were retained when showing at least 15% of difference between treatment, q-value < 0.001, and when a given 1,000-bp region comprised at least three CpGs. For functional annotation, we mapped the coho salmon transcriptome (67) to the genome (65) and annotated the DMRs overlapping genes location (5 kb up- and downstream) according to ref. 68. We added more information to DMRs relative position (3′ and 5′ UTRs, gene body, and CpG islands, shores, and shelves) based on a previous paper on rainbow trout (44). Detailed methods are provided in *Supporting Information*.

Population and Rearing Environment Effect on DMR Analysis. We first computed a Euclidian distance matrix on the 131,807 regions and performed a principal coordinates analysis (PCoA). A distance-based redundancy analysis (db-RDA) was then produced with the retained PCo factors ($n = 6$) as the response matrix and the variables population, rearing environment, and sex as the explanatory matrix using a stepwise model selection. Partial db-RDAs were produced to test for the effect of the selected variables after controlling for the remaining variables. The effect of a given factor was considered significant when the P value was < 0.05. Detailed methods are provided in *Supporting Information*.

Genotyping for Genetic Data. For population genomics analysis, mapping and genotyping were conducted with the BISulfite-seq CUI Toolkit (69). Only biallelic markers with minimum and maximum depth of coverage between 5× and 100×, minor allele frequency (maf) of >0.05, minimum quality of 5, maximum missing of 20%, and in Hardy–Weinberg equilibrium (P value > 0.05) were conserved. Markers with statistical linkage disequilibrium (LD) above R^2 0.8 were also orphaned (one SNP dropped) (70). From the initial 12,375,758 SNPs, only 15,044 were retained for subsequent population genomics analysis after applying these filtering criteria. Detailed methods are provided in *Supporting Information*.

Genomic Differentiation Between Hatchery and Wild Origin Fish from Each River. Similarly to DMR analysis, we computed a Euclidian distance matrix using the 15,044 filtered SNPs to perform a principal coordinates analysis (PCoA). A db-RDA was then produced with the retained PCo factors ($n = 10$) as the response matrix and the same explanatory variables, using a stepwise model selection. Partial db-RDAs were produced to test for the effect of the selected variables, after controlling for the other variables. The effect of a given factor was considered significant when the P value was <0.05. Pairwise genetic differentiation (Fst), individual coefficients of inbreeding (Gis), and observed and expected heterozygosity within samples were estimated using GENODIVE v2.0b27 (36) (Tables S2 and S3). Detailed methods are provided in *Supporting Information*. To detect outlier loci between sexes (Fig. S1) and test for possible selective effect within a single generation between HOR and NOR fish within each river (Fig. S2), we first conducted a standard genome scan approach using Bayescan v1.2 (35) on the 15,044 filtered markers. We also tested for polygenic selection using a multilocus analysis with Random Forest while accounting for population structure (rivers). We used permutations ($n = 1{,}000$) to assess whether a signal of polygenic selection similar to the one that was detected (*Results*) could be obtained by chance (e.g., due to genetic drift or sampling error). We compiled the final out-of-bag (OOB) error statistics for each run of simulation and compared it to the final OOB statistics in our empirical dataset (Fig. S3). Detailed methods are provided in *Supporting Information*.

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1. Laikre L, Schwartz MK, Waples RS, Ryman N; GeM Working Group (2010) Compromising genetic diversity in the wild: Unmonitored large-scale release of plants and animals. *Trends Ecol Evol* 25:520–529.
2. Stockwell CA, Hendry AP, Kinnison MT (2003) Contemporary evolution meets conservation biology. *Trends Ecol Evol* 18:94–101.
3. Ford MJ (2002) Selection in captivity during supportive breeding may reduce fitness in the wild. *Conserv Biol* 16:815–825.
4. Fraser DJ (2008) How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evol Appl* 1:535–586.
5. Yeaman S (2015) Local adaptation by alleles of small effect. *Am Nat* 186(Suppl 1): S74–S89.
6. Snyder NFR, et al. (1996) Limitations of captive breeding in endangered species recovery. *Conserv Biol* 10:338–348.
7. Christie MR, Marine ML, Fox SE, French RA, Blouin MS (2016) A single generation of domestication heritably alters the expression of hundreds of genes. *Nat Commun* 7:10676.
8. Christie MR, Marine ML, French RA, Blouin MS (2012) Genetic adaptation to captivity can occur in a single generation. *Proc Natl Acad Sci USA* 109:238–242.
9. Araki H, Cooper B, Blouin MS (2007) Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science* 318:100–103.
10. Mäkinen H, Vasemägi A, McGinnity P, Cross TF, Primmer CR (2015) Population genomic analyses of early-phase Atlantic Salmon (*Salmo salar*) domestication/captive breeding. *Evol Appl* 8:93–107.

11. Xia JH, et al. (2015) Signatures of selection in tilapia revealed by whole genome resequencing. *Sci Rep* 5:14168.
12. Liu L, et al. (2016) A genome scan for selection signatures comparing farmed Atlantic salmon with two wild populations: Testing colocalization among outlier markers, candidate genes, and quantitative trait loci for production traits. *Evol Appl* 10: 276–296.
13. Tsai H-Y, et al. (2015) Genome wide association and genomic prediction for growth traits in juvenile farmed Atlantic salmon using a high density SNP array. *BMC Genomics* 16:969.
14. Laporte M, et al. (2016) RAD sequencing reveals within-generation polygenic selection in response to anthropogenic organic and metal contamination in North Atlantic Eels. *Mol Ecol* 25:219–237.
15. Bourret V, Dionne M, Bernatchez L (2014) Detecting genotypic changes associated with selective mortality at sea in Atlantic salmon: Polygenic multilocus analysis surpasses genome scan. *Mol Ecol* 23:4444–4457.
16. Noakes DJ, Beamish RJ, Kent ML (2000) On the decline of Pacific salmon and speculative links to salmon farming in British Columbia. *Aquaculture* 183:363–386.
17. Irvine JR, Fukuwaka M-A (2011) Pacific salmon abundance trends and climate change. *ICES J Mar Sci* 68:1122–1130.
18. Krkošek M, et al. (2007) Declining wild salmon populations in relation to parasites from farm salmon. *Science* 318:1772–1775.
19. Araki H, Schmid C (2010) Is hatchery stocking a help or harm? Evidence, limitations and future directions in ecological and genetic surveys. *Aquaculture* 308(Suppl 1): S2–S11.
20. Chittenden CM, et al. (2010) Genetic versus rearing-environment effects on phenotype: Hatchery and natural rearing effects on hatchery- and wild-born coho salmon. *PLoS One* 5:e12261.
21. Chittenden CM, et al. (2008) Riverine, estuarine and marine migratory behaviour and physiology of wild and hatchery-reared coho salmon *Oncorhynchus kisutch* (Walbaum) smolts descending the Campbell River, BC, Canada. *J Fish Biol* 72:614–628.
22. Irvine JR, O'Neill M, Godbout L, Schnute J (2013) Effects of smolt release timing and size on the survival of hatchery-origin coho salmon in the Strait of Georgia. *Prog Oceanogr* 115:111–118.
23. Evans ML, Wilke NF, O'Reilly PT, Fleming IA (2014) Transgenerational effects of parental rearing environment influence the survivorship of captive-born offspring in the wild. *Conserv Lett* 7:371–379.
24. Zimmerman MS, Irvine JR, O'Neill M, Anderson JH, Greene CM (2015) Spatial and temporal patterns in smolt survival of wild and hatchery coho salmon in the Salish sea. *Mar Coast Fish* 7:116–134.
25. Christie MR, Ford MJ, Blouin MS (2014) On the reproductive success of early-generation hatchery fish in the wild. *Evol Appl* 7:883–896.
26. Berejikian BA, et al. (2016) Rearing strategies alter patterns of size-selective mortality and heritable size variation in steelhead trout (*Oncorhynchus mykiss*). *Can J Fish Aquat Sci* 74:273–283.
27. McDonald DG, et al. (1998) Condition and performance of juvenile Atlantic salmon (*Salmo salar*): Effects of rearing practices on hatchery fish and comparison with wild fish. *Can J Fish Aquat Sci* 55:1208–1219.
28. Martinez-Pena y Valenzuela I, Mouslim C, Akaaboune M (2010) Calcium/calmodulin kinase II-dependent acetylcholine receptor cycling at the mammalian neuromuscular junction in vivo. *J Neurosci* 30:12455–12465.
29. Rose AJ, Kiens B, Richter EA (2006) Ca2+-calmodulin-dependent protein kinase expression and signalling in skeletal muscle during exercise. *J Physiol* 574:889–903.
30. Rose AJ, Frøsig C, Kiens B, Wojtaszewski JFP, Richter EA (2007) Effect of endurance exercise training on Ca2+ calmodulin-dependent protein kinase II expression and signalling in skeletal muscle of humans. *J Physiol* 583:785–795.
31. Brauner CJ, Iwama GK, Randall DJ (1994) The effect of short-duration seawater exposure on the swimming performance of wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) during smoltification. *Can J Fish Aquat Sci* 51: 2188–2194.
32. Pedersen L-F, Koed A, Malte H (2008) Swimming performance of wild and F1-hatchery-reared Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) smolts. *Ecol Freshwat Fish* 17:425–431.
33. Pérez Maceira JJ, Mancebo MJ, Aldegunde M (2014) The involvement of 5-HT-like receptors in the regulation of food intake in rainbow trout (*Oncorhynchus mykiss*). *Comp Biochem Physiol C Toxicol Pharmacol* 161:1–6.
34. Roelofs D, Morgan J, Stürzenbaum S (2010) The significance of genome-wide transcriptional regulation in the evolution of stress tolerance. *Evol Ecol* 24:527–539.
35. Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics* 180:977–993.
36. Meirmans PG, Van Tienderen PH (2004) Genotype and genodive: Two programs for the analysis of genetic diversity of asexual organisms. *Mol Ecol Notes* 4:792–794.
37. Araki H, Berejikian BA, Ford MJ, Blouin MS (2008) Fitness of hatchery-reared salmonids in the wild. *Evol Appl* 1:342–355.
38. Blouin MS, et al. (2010) No evidence for large differences in genomic methylation between wild and hatchery steelhead (*Oncorhynchus mykiss*). *Can J Fish Aquat Sci* 67: 217–224.
39. Morán P, Marco-Rius F, Megías M, Covelo-Soto L, Pérez-Figueroa A (2013) Environmental induced methylation changes associated with seawater adaptation in brown trout. *Aquaculture* 392–395:77–83.
40. Friedrich B, et al. (2003) The serine/threonine kinases SGK2 and SGK3 are potent stimulators of the epithelial Na+ channel αhe serine. *Pflugers Arch* 445:693–696.
41. Shaw J, et al. (2008) The role of SGK and CFTR in acute adaptation to seawater in *Fundulus heteroclitus*. *Cell Physiol Biochem* 22:69–78.
42. Boeuf G (1993) Salmonid smolting: A pre-adaptation to the oceanic environment. *Fish Ecophysiology*, Fish and Fisheries Series, eds Rankin JC, Jensen FB (Chapman & Hall, London), pp 105–135.
43. Shrimpton JM, Bernier NJ, Iwama GK, Randall DJ (1994) Differences in measurements of smolt development between wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) before and after saltwater exposure. *Can J Fish Aquat Sci* 51: 2170–2178.
44. Baerwald MR, et al. (2016) Migration-related phenotypic divergence is associated with epigenetic modifications in rainbow trout. *Mol Ecol* 25:1785–1800.
45. Olla BL, Davis MW, Ryer CH (1998) Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. *Bull Mar Sci* 62: 531–550.
46. Brown C, Laland K (2001) Social learning and life skills training for hatchery reared fish. *J Fish Biol* 59:471–493.
47. Álvarez D, Nicieza AG (2003) Predator avoidance behaviour in wild and hatchery-reared brown trout: The role of experience and domestication. *J Fish Biol* 63: 1565–1577.
48. Metcalfe NB, Valdimarsson SK, Morgan IJ (2003) The relative roles of domestication, rearing environment, prior residence and body size in deciding territorial contests between hatchery and wild juvenile salmon. *J Appl Ecol* 40:535–544.
49. Sundström LF, Johnsson JI (2001) Experience and social environment influence the ability of young brown trout to forage on live novel prey. *Anim Behav* 61:249–255.
50. Aubin-Horth N, Landry CR, Letcher BH, Hofmann HA (2005) Alternative life histories shape brain gene expression profiles in males of the same population. *Proc Biol Sci* 272:1655–1662.
51. Johnston IA, Bower NI, Macqueen DJ (2011) Growth and the regulation of myotomal muscle mass in teleost fish. *J Exp Biol* 214:1617–1628.
52. Morán P, Pérez-Figueroa A (2011) Methylation changes associated with early maturation stages in the Atlantic salmon. *BMC Genet* 12:86.
53. Venney CJ, Johansson ML, Heath DD (2016) Inbreeding effects on gene-specific DNA methylation among tissues of Chinook salmon. *Mol Ecol* 25:4521–4533.
54. Feil R, Fraga MF (2012) Epigenetics and the environment: Emerging patterns and implications. *Nat Rev Genet* 13:97–109.
55. Johnson LJ, Tricker PJ (2010) Epigenomic plasticity within populations: Its evolutionary significance and potential. *Heredity (Edinb)* 105:113–121.
56. Faulk C, Dolinoy DC (2011) Timing is everything: The when and how of environmentally induced changes in the epigenome of animals. *Epigenetics* 6:791–797.
57. Fast DE, et al. (2015) A synthesis of findings from an integrated hatchery program after three generations of spawning in the natural environment. *N Am J Aquac* 77: 377–395.
58. Tatara CP, et al. (2017) Age and method of release affect migratory performance of hatchery Steelhead. *N Am J Fish Manag* 37:700–713.
59. Parry G (1961) Osmotic and ionic changes in blood and muscle of migrating salmonids. *J Exp Biol* 38:411–427.
60. Björnsson BT, Stefansson SO, McCormick SD (2011) Environmental endocrinology of salmon smoltification. *Gen Comp Endocrinol* 170:290–298.
61. Stefansson SO, et al. (2012) Growth, osmoregulation and endocrine changes in wild Atlantic salmon smolts and post-smolts during marine migration. *Aquaculture* 362: 127–136.
62. Wilke NF, O'Reilly PT, MacDonald D, Fleming IA (2015) Can conservation-oriented, captive breeding limit behavioural and growth divergence between offspring of wild and captive origin Atlantic salmon (*Salmo salar*)? *Ecol Freshwat Fish* 24:293–304.
63. Gu H, et al. (2011) Preparation of reduced representation bisulfite sequencing libraries for genome-scale DNA methylation profiling. *Nat Protoc* 6:468–481.
64. Yano A, et al. (2013) The sexually dimorphic on the Y-chromosome gene (sdY) is a conserved male-specific Y-chromosome sequence in many salmonids. *Evol Appl* 6: 486–496.
65. Krueger F, Andrews SR (2011) Bismark: A flexible aligner and methylation caller for bisulfite-seq applications. *Bioinformatics* 27:1571–1572.
66. Akalin A, et al. (2012) methylKit: A comprehensive R package for the analysis of genome-wide DNA methylation profiles. *Genome Biol* 13:R87.
67. Kim J-H, Leong JS, Koop BF, Devlin RH (2016) Multi-tissue transcriptome profiles for coho salmon (*Oncorhynchus kisutch*), a species undergoing rediploidization following whole-genome duplication. *Mar Genomics* 25:33–37.
68. Guo JU, et al. (2011) Neuronal activity modifies the DNA methylation landscape in the adult brain. *Nat Neurosci* 14:1345–1351.
69. Zhou W (2016) biscuit-0.1.3. Zenodo. Available at doi.org/10.5281/zenodo.48262. Accessed October 3, 2016.
70. Larson WA, et al. (2014) Genotyping by sequencing resolves shallow population structure to inform conservation of Chinook salmon (*Oncorhynchus tshawytscha*). *Evol Appl* 7:355–369.

Supporting Information

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Sampling Sites

We collected samples from the Quinsam and Capilano Rivers in British Columbia (Canada), namely (Fig. S1). These systems were chosen because they are well-suited to test specifically for the effect of rearing environment on patterns of methylation, independent of the genetic background between fish born in the wild (thereafter natural origin) vs. those born in hatchery. Indeed, hatchery programs for these rivers were developed as so called "integrated programs," meaning that they are based on local populations and involve spawning in the two environments (hatchery and wild). Thus, hatchery and natural origin fish in each river are not kept separate; hatchery origin fish spawn in both the hatchery and the natural habitat as do natural origin fish. This is made possible by the fact that both hatcheries are run as "swim-in" facilities, meaning that the hatchery water flows through a stream/channel to the river. Following hatching and a rearing period of 2 y, juvenile salmon (thereafter smolts) emigrate and imprint on this hatchery water. While mostly hatchery origin fish tend to "home" back to the hatchery collecting ponds through the channel, nothing prevents gene flow between hatchery and natural origin adults since the latter can also enter the hatchery channel to reproduce. Similarly, nothing prevents hatchery origin salmon to spawn elsewhere in the watershed.

Given this setting, the prediction is that there should be one single panmictic population in each river (that spawns in two different environments), which should result in no overall genetic differentiation between hatchery and wild salmon. Therefore, observed differences in patterns of methylation between wild and hatchery fish would be very unlikely to be associated with genetically distinct populations and should instead reflect the effect of rearing environment. The only chance for differential hatchery/natural selection to occur and to cause changes in allele frequencies within a single generation would be in the eggs/juveniles between adult spawning and emigration of their juvenile (smolt) progeny.

Hatchery Procedures and Sampling

The Salmon Enhancement Program (SEP) hatcheries have standard operating procedures employed across hatcheries, with the primary production strategy (PPS) being used for coho salmon at both Capilano and Quinsam hatcheries. The PPS integrates major improvements with the aim to lessen the genetic and ecological impacts of hatchery releases in the wild, mainly by using local broodstock, maintaining seasonal timing, using an enriched environment, low density, and by promoting volitional release and imprinting (1). Briefly, adult spawning peaks occurred in late October to early November with random single-pair breeding. Pre-eye eggs are kept in troughs with low flow for ∼3 mo and are moved at the eyed stage to heath trays through the alevin stage. The fry are moved to groundwater raceway ponds in April and held until the following late fall, where they are marked and moved to larger gravel substrate ponds through the parr stage until smoltification, which is the physiological change required to allow a salmon to survive in salt water. Coho yearling smolts, as defined 1+ year after hatching, are released over a month. In this study, the progeny of fall-run 2012 Capilano and Quinsam River adult coho salmon were released in each respective river as yearling smolts in 2014. These two river systems were chosen because they are well-suited to test specifically for the effect of rearing environment on patterns of methylation, independent of the genetic background between fish born in the wild and those born in hatchery.

Authorization to collect samples in the study was provided by a scientific license issued under the provisions of the Fisheries Act passed by the Canadian Parliament in 1985 and last amended in 2016. Under the Act, the scientific license was issued by Fisheries and Oceans Canada to allow departmental staff to collect samples in the course of their work. As there is no requirement for an Institutional Animal Care and Use Committee (IACUC) or equivalent under the Act, sampling protocols were neither vetted nor approved by an IACUC.

DNA Extraction and Reduced-Representation Bisulfite Sequencing Library Preparation

The RRBS library preparation was adapted from a protocol described elsewhere (2). We used DNeasy blood and tissue kit columns for the extraction of genomic DNA (gDNA) following protocol guidelines (QIAGEN) and Quant-iT PicoGreen dsDNA assays for DNA quantification (Fluoroskan Ascent FL; ThermoFisher Scientific). Genomic DNA (600 ng) was digested using MspI restriction enzymes, cleaned using magnetic beads (volume ratio 1.8×), and rinsed twice with ethanol 80%. End-repair and A-tailing steps were conducted in solution containing Klenow fragments (5 U; New England Bio Labs, Inc.) and dNTPs (0.5 mM dATP, 0.05 m dGTP, 0.05 mM dCTP) with an incubation step (30 °C for 30 min and 37 °C for 20 min). The solution was cleaned with magnetic beads (ratio 1.8×) and rinsed twice with ethanol 80%. NEXTflex illumina DNA barcodes (Bio Scientific) were ligated using overnight incubation at 16 °C in a master mix solution containing the T4 ligase buffer (1×; New England Bio Labs, Inc.) and a T4 ligase (2,000 U). The solution was cleaned with magnetic beads (ratio 1×) and rinsed twice with ethanol 80%. A size-selection step was necessary for capturing specific fragment length (200 to 400 bp), which implied two rinses with magnetic beads (first wash 0.7× and beads were discarded, second wash ratio 0.15×). A bisulfite conversion treatment was conducted following EZ DNA Methylation-Gold Kit protocol recommendations (Zymo Research). Library quality and quantity were verified on a HiSense DNA-Bioanalyzer 2100 chip (Agilent) and with Quant-iT PicoGreen assays (Fluoroskan Ascent FL; Thermo Labsystems), respectively. Libraries were sequenced on a HiSEq. 2000 platform (five individuals by lane) at the McGill University and Génome Québec Innovation Centre (Montréal, QC) using a 100-bp single-end reads approach. In parallel, sex information was inferred by PCR using a method previously described for salmonids (sdY_E2S2 5′-GTGGAGT-ACTGCG-AAGAGGAGGT-3′ and sdY_E2AS4 5′-CTTAAAACCACTCCA-CCCTCCAT-3′ primers) (3). Sex information for each individual is available in Table S4.

Methylation Calling

The RRBS reads were first trimmed using Cutadapt software (4) implemented in Trim_Galore! v0.4.1 (www.bioinformatics.babraham.ac.uk/projects/trim_galore/). After quality filtering and sample quality check, one sample from the Quinsam River hatchery was identified as an outlier in a principal component analysis (PCA) (axes 1 to 4, drives axis 3 alone) and statistically (Grubbs test, G = 3.37, U = 0.7, P value = 0.005179). This sample was characterized by a low coverage (Methylkit *"GetCoverageStats"* function) (5) and a lower total number of unique mapped sequences compared with the other samples, which most likely occurred during library preparation. To avoid the possibility of falsely interpreting existing C-T DNA polymorphism as epigenetic variation, we then masked the draft genome assembly of the coho salmon (GenBank assembly accession no. GCA_002021735.1) for all C-T polymorphism (1,896,050 SNPs markers; maf = 0.05) identified with whole-genome resequencing of 20 salmon from five British Columbia rivers (Fig. 1)

using BEDtools v2.26.0 (6). Both mapping and genotyping were conducted with the BISulfite-seq CUI Toolkit (BISCUIT) suite (7) with default parameters, including an additional prefiltering step for low mapping quality (MAPQ < 10) conducted with SAMtools v1.19 (8).

For methylation calling, trimmed reads were mapped against the masked coho salmon genome using Bismark v0.14.5 aligner (9). Only methylation information for cytosine in a CpG context was extracted using the Bismark "*methyl extraction*" function (9). Only bases with at least 10× coverage over all individuals were conserved for subsequent analysis, and methylation levels on normalized count across individuals were compiled using a tiling window approach (step = 1,000 bp; size = 1,000 bp) with the MethylKit R package (5). Finally, a logistic regression, with the river of origin and sex as covariates, was conducted to identify differentially methylated regions (DMRs) with the "*calculateDiffMeth*" function implemented in the MethylKit R package (5). The DMRs were retained when showing at least 15% of difference between treatment, q-value < 0.001, and when a given 1,000-bp region comprised at least three CpGs.

Population and Rearing Environment Effect on DMR Analysis

We first computed a Euclidian distance matrix on the 131,807 regions and performed a principal coordinates analysis (PCoA) on this Euclidian distance matrix. PCo factors showing a relative eigenvalue higher than 2.75%, which correspond to the meaningful axes based on a broken-stick distribution, were selected as surrogate for multilocus epigenotypes (10). The Euclidean distance and the PCoA were obtained, respectively, with the functions "*daisy*" and "*pcoa*" available in the ape R package (11). To test for the effect of population (river of origin) and rearing environment (NOR or HOR), a distance-based redundancy analysis (db-RDA) was also produced with the retained PCo factors (n = 6) as a response matrix and the variables river of origin, captivity, and sex as the explanatory factors. We first produced a stepwise model selection on variables river of origin, rearing environment (natural or hatchery), and sex using the function "*ordistep*" in the vegan R package (12). Partial db-RDAs were produced to test for the effect of rearing environment or river of origin alone, after controlling for the remaining variables. The effect of a given factor was considered significant when the P value was <0.05.

Functional Annotation

For the functional analysis, we first mapped the multitissue reference transcriptome of the coho salmon (13) to the draft genome assembly (GenBank assembly accession no. GCA_002021735.1) using the splicing-tolerant Gmap (release 2016-11-07) aligner (14), and we kept the primary path for subsequent analysis. We used the GenomicRanges R package to find overlap between DMRs and genes (allowing 5 kb up- and downstream of genes) (15). The choice of 5 kb corresponds to the window used in a previous study to locate genes in the vicinity of CpG islands regions (16). Furthermore, detailed information on functional annotation based on gene location information was extracted following methods similar to a previous study on DNA methylation in rainbow trout to enhance comparison (17). The authors defined regions as follows: 3′ UTR < 1.5 kb from transcription start site (TSS); 5′ UTR < 5 kb from TSS, gene body equivalent to the longest ORF identified with the "*LongOrfs*" TransDecoder's function (18). In complement, we used the Gardiner–Garden masked approach described by Bock et al. (19) for CpG islands (CpGi) annotation based on the repeat-masked reference genome, considering CpGs shores (<2 kb up- and downstream of CpGi) and CpG shelves (2 to 4 kb up and downstream of CpGi). For this purpose, the genome was masked for repeats using WindowMasker with default parameters (20). Functional annotations are reported in Table S1 (gene body > UTRs > CpGs > CpGi shores >CpGi shelves). We then used a blastx approach against a Swissprot database (21, 22) to annotate transcript sequences. Only matches with an e-value < 1e−6 were considered. Finally, we used GOAtools v0.6.10 (23) implemented in GO enrichment workflow (https://github.com/enormandeau/go_enrichment) to identify gene ontology and assess gene enrichment. Our background list included genes that could have been localized in the vicinity of methylated regions being considered (at least 3 CpG in a 1,000-bp region and 10× coverage) and represented a total of 20,817 genes. Only GO terms with P value < 0.05 and including at least three genes were considered (Dataset S1).

Genotyping for Genetic Data

Variation at the genome level was also quantified between HOR and NOR fish in each river to test the prediction that coho salmon in each river should comprise a single panmictic population, which should translate in the overall absence of genetic differences between HOR and NOR populations within a river, as well as similar measures of genetic diversity. However, significant population structure and associated genetic differences are expected between rivers. For population genomics analysis, only biallelic markers with minimum and maximum depth of coverage between 5× and 100×, minor allele frequency (maf) > 0.05, minimum quality of 5, maximum missing of 20%, and in Hardy–Weinberg equilibrium (P value > 0.05) were conserved. Markers with statistical linkage disequilibrium (LD) above R^2 0.8 were also orphaned (one SNP dropped) (24). All filtering was conducted with VCFtools v0.1.14 software (25). From the initial 12,375,758 SNPs, only 15,044 were retained for subsequent population genomics analysis after applying these filtering criteria.

Genomic Differentiation Between Hatchery and Wild Origin Fish from Each River

Similarly to DMR analysis, we first computed a Euclidian distance matrix using the 15,044 filtered SNPs that were used to perform a principal coordinates analysis (PCoA). This procedure allowed us to avoid any effect of imputing data on the genotype database, even with our observed low global missing genotypes (2.09%) (10). Because no axis could be selected according to the broken-stick distribution, we selected all axes explaining at least 2.75% of the variation (10 axes explaining 33.9% of the variance), as previously performed with epigenetic markers (10). The Euclidean distance and the PCoA were obtained, respectively, with the functions "*daisy*" and "*pcoa*" available in the ape R package (11). We first produced a stepwise model selection on variables river of origin, rearing environment (natural or hatchery), and sex using the function "*ordistep*" in the vegan R package (12). A distance-based redundancy analysis (db-RDA) was then produced with the retained PCo factors (n = 10) as the response matrix and the variables population, rearing environment, and sex as the explanatory matrix. A db-RDA is a variant of canonical correlation analysis (CCA), which gives less weight to rare alleles that can be caused by genotypic error (26). Partial db-RDAs were produced to test for the effect of sex or river of origin alone, after controlling for the other variables. The effect of a given factor was considered significant when the P value was < 0.05. Pairwise genetic differentiation (Fst) was quantified with the multilocus AMOVAs function implemented in GENODIVE v2.0b27 software with 999 iterations (27, 28) (Table S2). Individual coefficients of inbreeding (Gis) and observed and expected heterozygosity within samples were estimated using GENODIVE v2.0b27 (27) (Table S3).

Local Selection on Loci of Major Effect and Polygenic Selection

To detect outlier loci between sexes (Fig. S1) and test for possible selective effect within a single generation between HOR and NOR fish within each river (Fig. S2), we first conducted a standard genome scan approach. We ran Bayescan v1.2 (29) on the 15,044 markers with 5,000 iterations and a burn-in length of 100,000. A permissive value of prior-odds of 10 was chosen to ensure detecting

all putative outliers. No outlier loci associated with differences between sexes were detected; thus, it is unlikely that spurious genetic differentiation could have been created from sex differentiation in subsequent population genomics analysis (30). We also tested for polygenic selection using a multilocus analysis while accounting for population structure (rivers). Thus, we first quantified the admixture coefficient for each sample with ADMIXTURE v1.23 software using k = 2 and 20,000 iterations (31, 32). To test for polygenic selection, we used a random forest framework, implemented in the randomForest R package (33), that is well-suited for a large number of genetic markers and a low number of individuals (34–37). Given the small number of missing data (2.09%), we used the "*na.roughfix*" function implemented in the randomForest R package to impute the missing data (33). To account for population structure, we used a general linear model to fit the genotype against the admixture proportion. Residuals of the models were used for the random forest analysis. A series of three random forest analyses were run with 1,000,000 trees for each run. We used the "permuted importance" statistic automatically calculated by the random forest algorithm as an indicator of the significance of the marker (in interaction with others) in discriminating HOR and NOR fish. At the end of the first two runs, the markers having an importance of <0 were removed as they are not useful in discriminating hatchery from wild fish (38). We chose the total number of random forest analyses so the final out-of-bag (OOB) error would be stable and close to 5% (38). Finally, as there is no consensus method to set the important markers from the nonimportant ones, we fixed the threshold according to the upper end elbow of the importance distribution (markers with importance of >25) (35, 38).

We used permutations (n = 1,000) to assess whether a signal of polygenic selection similar to the one that was detected (*Results*) could be obtained by chance (e.g., due to genetic drift or sampling error). Within each river, the factor hatchery or wild was randomly permutated using the "schuf" function in Linux (https://github.com/jleluyer/permutation2randomforest). As conducted above, we assessed the coefficient of membership to each population using ADMIXTURE v1.23 software (k = 2) (32). The coefficients of admixture were used to remove the river effect in the random forest analysis (1,000,000 trees, three successive runs with removal of markers with importance of <0 between each run) (33). To evaluate the probability of identifying markers under selection, we compiled the final out-of-bag (OOB) error statistics for each run of simulation and compared it with the final OOB statistics in our empirical dataset using permutations tests (Fig. S3).

1. Flagg TA, Nash CE, eds (1999) *A Conceptual Framework for Conservation Hatchery Strategies for Pacific Salmonids* (US Department of Commerce, Washington, DC), NOAA Technical Memorandum NMFS-NWFSC-38.
2. Gu H, et al. (2011) Preparation of reduced representation bisulfite sequencing libraries for genome-scale DNA methylation profiling. *Nat Protoc* 6:468–481.
3. Yano A, et al. (2013) The sexually dimorphic on the Y-chromosome gene (sdY) is a conserved male-specific Y-chromosome sequence in many salmonids. *Evol Appl* 6: 486–496.
4. Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17:10–12.
5. Akalin A, et al. (2012) methylKit: A comprehensive R package for the analysis of genome-wide DNA methylation profiles. *Genome Biol* 13:R87.
6. Quinlan AR, Hall IM (2010) BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics* 26:841–842.
7. Zhou W (2016) biscuit-0.1.3. Zenodo. Available at doi.org/10.5281/zenodo.48262. Accessed October 3, 2016.
8. Li H, et al.; 1000 Genome Project Data Processing Subgroup (2009) The sequence alignment/map format and SAMtools. *Bioinformatics* 25:2078–2079.
9. Krueger F, Andrews SR (2011) Bismark: A flexible aligner and methylation caller for bisulfite-seq applications. *Bioinformatics* 27:1571–1572.
10. Legendre P, Legendre L (2012) *Numerical Ecology* (Elsevier, Amsterdam), 3rd Ed, Vol 24.
11. Paradis E, Claude J, Strimmer K (2004) Ape: Analyses of phylogenetics and evolution in R language. *Bioinformatics* 20:289–290.
12. Oksanen J, et al. (2012) Vegan: Community Ecology Package. R package. Version 2.1-16/r2187.
13. Kim J-H, Leong JS, Koop BF, Devlin RH (2016) Multi-tissue transcriptome profiles for coho salmon (*Oncorhynchus kisutch*), a species undergoing rediploidization following whole-genome duplication. *Mar Genomics* 25:33–37.
14. Wu TD, Watanabe CK (2005) GMAP: A genomic mapping and alignment program for mRNA and EST sequences. *Bioinformatics* 21:1859–1875.
15. Lawrence M, et al. (2013) Software for computing and annotating genomic ranges. *PLoS Comput Biol* 9:e1003118.
16. Guo JU, et al. (2011) Neuronal activity modifies the DNA methylation landscape in the adult brain. *Nat Neurosci* 14:1345–1351.
17. Baerwald MR, et al. (2016) Migration-related phenotypic divergence is associated with epigenetic modifications in rainbow trout. *Mol Ecol* 25:1785–1800.
18. Haas BJ, et al. (2013) De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nat Protoc* 8:1494–1512.
19. Bock C, Walter J, Paulsen M, Lengauer T (2007) CpG island mapping by epigenome prediction. *PLoS Comput Biol* 3:e110.
20. Morgulis A, Gertz EM, Schäffer AA, Agarwala R (2006) WindowMasker: Window-based masker for sequenced genomes. *Bioinformatics* 22:134–141.
21. Altschul SF, et al. (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402.
22. Bairoch A, Apweiler R (2000) The SWISS-PROT protein sequence database and its supplement TrEMBL in 2000. *Nucleic Acids Res* 28:45–48.
23. Klopfenstein DV, et al. (2016) tanghaibao/goatools: GOATOOLS v0.6.10. Zenodo. Available at doi.org/10.5281/zenodo.159493. Accessed December 5, 2016.
24. Larson WA, et al. (2014) Genotyping by sequencing resolves shallow population structure to inform conservation of Chinook salmon (*Oncorhynchus tshawytscha*). *Evol Appl* 7:355–369.
25. Danecek P, et al.; 1000 Genomes Project Analysis Group (2011) The variant call format and VCFtools. *Bioinformatics* 27:2156–2158.
26. Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia* 129:271–280.
27. Meirmans PG, Van Tienderen PH (2004) Genotype and genodive: Two programs for the analysis of genetic diversity of asexual organisms. *Mol Ecol Notes* 4:792–794.
28. Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131:479–491.
29. Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics* 180:977–993.
30. Benestan L, et al. (June 28, 2017) Sex matters in massive parallel sequencing: Evidence for biases in genetic parameter estimation and investigation of sex determination systems. *Mol Ecol*, 10.1111/mec.14217.
31. Alexander DH, Lange K (2011) Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. *BMC Bioinformatics* 12:246.
32. Alexander DH, Novembre J, Lange K (2009) Fast model-based estimation of ancestry in unrelated individuals. *Genome Res* 19:1655–1664.
33. Liaw A, Wiener M (2002) Classification and regression by randomForest. *R News* 2: 18–22.
34. Holliday JA, Wang T, Aitken S (2012) Predicting adaptive phenotypes from multilocus genotypes in Sitka spruce (*Picea sitchensis*) using random forest. *G3 (Bethesda)* 2: 1085–1093.
35. Laporte M, et al. (2016) RAD sequencing reveals within-generation polygenic selection in response to anthropogenic organic and metal contamination in North Atlantic Eels. *Mol Ecol* 25:219–237.
36. Pavey SA, et al. (2015) RAD sequencing highlights polygenic discrimination of habitat ecotypes in the panmictic American eel. *Curr Biol* 25:1666–1671.
37. Brieuc MSO, Ono K, Drinan DP, Naish KA (2015) Integration of random forest with population-based outlier analyses provides insight on the genomic basis and evolution of run timing in Chinook salmon (*Oncorhynchus tshawytscha*). *Mol Ecol* 24: 2729–2746.
38. Goldstein BA, Polley EC, Briggs FBS (2011) Random forests for genetic association studies. *Stat Appl Genet Mol Biol* 10:32.
39. Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370.

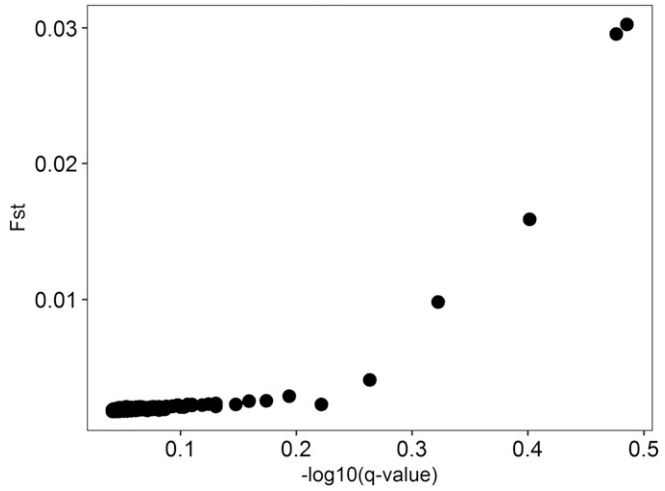

Fig. S1. BayeScan 2.0 plot of the 15,044 biallelic markers for the comparison between sexes. Fst are plotted against the −log10 q-value. The prior odd was fixed at 10. Outlier was considered significant when −log10(q-value) > 1.3 (q-value < 0.05).

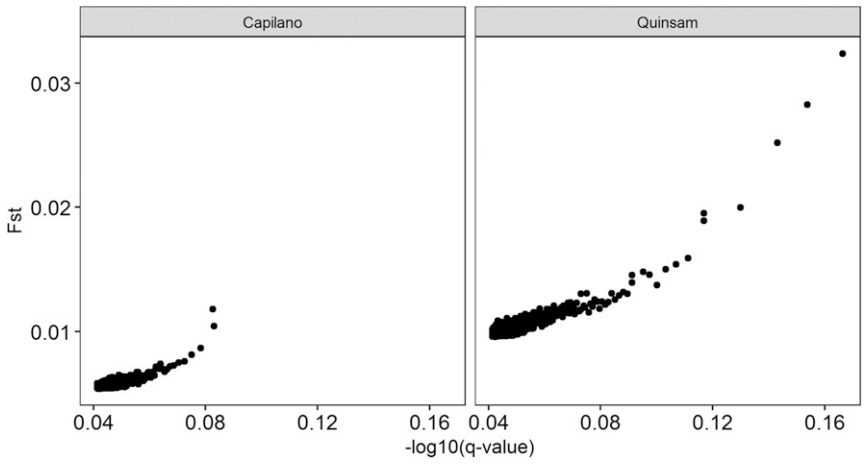

Fig. S2. BayeScan 2.0 plot of 15,044 biallelic markers for the comparison between hatchery and natural origin fish for each river. Fst are plotted against the −log10 q-value. The prior odd was fixed at 10. Outliers were considered significant when −log10 (q-value) > 1.3 (q-value < 0.05), but no marker passed this threshold.

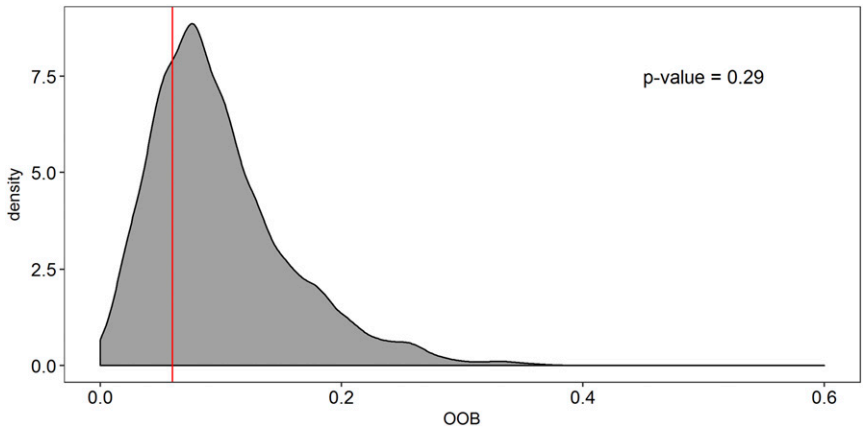

Fig. S3. Distribution of the final out-of-bag (OOB) errors from random forest analysis. The final OOB were calculated after the three runs of random forest with 1,000,000 trees for each of the 1,000 permutations. The red line indicates the final OOB value for the empirical dataset (OOB = 0.06) after three successive runs of random forest with 1,000,000 trees for each run.

Table S1. Differentially methylated regions (DMRs) and their association with Uniprot entries between hatchery and wild smolt coho salmon

Symbol	Uniprot ID	Transcript ID	Gene name	Chr./Scaff.	Met. diff.	Localization
5HT2C	Q5IS66	GDQG01000256.1	5-Hydroxytryptamine receptor 2C	Scaffold04777	23.3	Shelves
ANK1	Q02357	GDQG01029546.1	Ankyrin-1	Okis08	21.1	Gene body
AT2A2	Q03669	GDQG01041157.1	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	Okis23	15.7	Gene body
BCR	P11274	GDQG01010189.1	Breakpoint cluster region protein	Okis23	15.7	3′ UTR
BCR	Q6PAJ1	GDQG01010373.1	Breakpoint cluster region protein	Okis23	15.7	Gene body
BEGIN	Q9BUH8	GDQG01032671.1	Brain-enriched guanylate kinase-associated protein	Okis21	17.3	3′ UTR
BTAF1	O14981	GDQG01038303.1	TATA-binding protein-associated factor 172	Okis11	15.6	3′ UTR (1.5 to 5 kb)
BTAF1	O14981	GDQG01038304.1	TATA-binding protein-associated factor 172	Okis11	15.6	Gene body
CHKA	P35790	GDQG01024040.1	Choline kinase alpha	Okis04	15.6	Gene body
CSK21	Q60737	GDQG01021514.1	Casein kinase II subunit alpha	Okis17	19.6	5′ UTR
DDX53	Q86TM3	GDQG01005021.1	Probable ATP-dependent RNA helicase DDX53	Okis04	16.1	5′ UTR
DJC17	Q91WT4	GDQG01018295.1	DnaJ homolog subfamily C member 17	Okis14	17.3	Gene body
DUS12	Q9UNI6	GDQG01021214.1	Dual specificity protein phosphatase 12	Okis28	−18.9	3′ UTR (1.5 to 5 kb)
F172A	Q7T297	GDQG01019163.1	Protein FAM172A	Okis08	20.3	Gene body
HXB3A	O42368	GDQG01036924.1	Homeobox protein Hox-B3a	Okis10	15.2	Gene body
HYAL2	Q12891	GDQG01026988.1	Hyaluronidase-2	Okis05	−17.6	3′ UTR
HYAL2	Q12891	GDQG01026990.1	Hyaluronidase-2	Okis05	−17.6	Gene body
HYAL2	Q12891	GDQG01026993.1	Hyaluronidase-2	Okis05	−17.6	3′ UTR
HYAL2	Q12891	GDQG01027002.1	Hyaluronidase-2	Okis05	−17.6	Gene body
KCC1A	Q63450	GDQG01026516.1	Calcium/calmodulin-dependent protein kinase type 1	Okis17	17.9	3′ UTR (1.5 to 5 kb)
KCC2B	P28652	GDQG01028157.1	Calcium/calmodulin-dependent protein kinase type II subunit beta	Okis29	18.9	Gene body
LRC47	Q505F5	GDQG01001482.1	Leucine-rich repeat-containing protein 47	Okis17	15.6	3′ UTR (1.5 to 5 kb)
OARD1	Q9Y530	GDQG01002423.1	O-acetyl-ADP ribose deacetylase 1	Okis17	−17.1	Gene body
P73	Q9XSK8	GDQG01009063.1	Tumor protein p73	Okis17	17.5	Gene body
PCDH8	O95206	GDQG01028291.1	Protocadherin-8	Okis26	−15.9	3′ UTR (1.5 to 5 kb)
PHB2	Q5XIH7	GDQG01016094.1	Prohibitin-2	Okis30	15.5	3′ UTR (1.5 to 5 kb)
PKHA1	Q9HB21	GDQG01013238.1	Pleckstrin homology domain-containing family A member 1	Okis11	27.7	5′ UTR
SAM12	Q0VE29	GDQG01021692.1	Sterile alpha motif domain-containing protein 12	Okis17	15.1	Gene body
SGK2	Q9HBY8	GDQG01021555.1	Serine/threonine-protein kinase Sgk2	Okis17	18.8	Gene body
SRSF9	Q5PPI1	GDQG01030164.1	Serine/arginine-rich splicing factor 9	Okis23	19.2	5′ UTR
SRSF9	Q5PPI1	GDQG01030165.1	Serine/arginine-rich splicing factor 9	Okis23	19.2	5′ UTR
STX16	Q8BVI5	GDQG01021767.1	Syntaxin-16	Okis01	15.3	3′ UTR
TMC5	Q6UXY8	GDQG01032265.1	Transmembrane channel-like protein 5	Scaffold04350	27.4	Shores
TSH2	Q9NRE2	GDQG01039096.1	Teashirt homolog 2	Okis03	22.2	3′ UTR (1.5 to 5 kb)
UBE2K	P61087	GDQG01019710.1	Ubiquitin-conjugating enzyme E2 K	Okis19	15.3	5′ UTR
Unknown	Unknown	GDQG01000757.1	Unknown	Okis07	15.6	Gene body
Unknown	Unknown	GDQG01002050.1	Unknown	Scaffold07390	15.2	3′ UTR (1.5 to 5 kb)
Unknown	Unknown	GDQG01003870.1	Unknown	Okis21	17.3	Shelves
Unknown	Unknown	GDQG01005352.1	Unknown	Okis19	16.5	3′ UTR
Unknown	Unknown	GDQG01007903.1	Unknown	Okis04	16.1	3′ UTR (1.5 to 5 kb)
Unknown	Unknown	GDQG01008658.1	Unknown	Scaffold04821	18.0	3′ UTR
Unknown	Unknown	GDQG01009276.1	Unknown	Okis13	15.6	5′ UTR
Unknown	Unknown	GDQG01009277.1	Unknown	Okis13	15.6	5′ UTR
Unknown	Unknown	GDQG01020612.1	Unknown	Scaffold03114	15.0	Gene body
Unknown	Unknown	GDQG01023154.1	Unknown	Okis04	16.1	3′ UTR (1.5 to 5 kb)
Unknown	Unknown	GDQG01023155.1	Unknown	Okis04	16.1	3′ UTR (1.5 to 5 kb)
Unknown	Unknown	GDQG01023157.1	Unknown	Okis04	16.1	3′ UTR (1.5 to 5 kb)
Unknown	Unknown	GDQG01025110.1	Unknown	Okis17	19.6	5′ UTR
Unknown	Unknown	GDQG01026416.1	Unknown	Scaffold04821	18.0	3′ UTR
Unknown	Unknown	GDQG01027613.1	Unknown	Scaffold00446	17.9	3′ UTR (1.5 to 5 kb)
Unknown	Unknown	GDQG01033116.1	Unknown	Scaffold04821	18.0	5′ UTR
Unknown	Unknown	GDQG01042549.1	Unknown	Scaffold02804	19.2	Gene body

Annotation was based on a blastx approach against the Uniprot–Swissprot database (e-value < 10−6). Only significant DMRs were included [methylation difference (Met. diff.) between hatchery and wild (Meth. diff.) >15%; q-value < 0.01]. Positive values are associated with hypermethylation relative to natural origin salmon. Transcript IDs correspond to the multitissue reference transcriptome for the coho salmon (13). Each region represents a 1,000-bp portion of one of the 30 chromosomes (Chr.) (Okis) or additional scaffolds (Scaff.) from the draft coho salmon genome assembly (GenBank assembly accession no. GCA_002021735.1)

Table S2. Fst values for all pairwise comparisons

Pairwise comparisons	Fst	*P* value
Comparisons between rearing environments		
Qui-Hat/Qui-Wld	0.005	0.087
Cap-Wld/Cap-Hat	0.002	0.301
Comparisons between samples from different rivers		
Qui-Hat/Cap-Hat	0.041	0.001
Qui-Hat/Cap-Wld	0.035	0.001
Qui-Wld/Cap-Hat	0.041	0.001
Qui-Wld/Cap-Wld	0.037	0.001

Cap-Hat, Capilano-hatchery; Cap-Wld, Capilano-wild; Qui-Hat, Quinsam-hatchery; Qui-Wld, Quinsam-wild. Fst was calculated with 999 iterations using Weir and Cockerham's (39) parameter estimation (related to *No Evidence for Genome-Wide Genetic Differentiation Between HOR and NOR Salmon*).

Table S3. Measures of genetic diversity by sampling locations for 15,044 filtered SNPs

Sampling location	Ho	SD	He	SD	Gis	SD
Qui-wild	0.237	0.002	0.247	0.001	0.040	0.003
Qui-hatchery	0.241	0.002	0.249	0.001	0.031	0.003
Cap-wild	0.242	0.002	0.249	0.001	0.029	0.003
Cap-hatchery	0.327	0.002	0.248	0.001	0.045	0.003

SDs were obtained by jackknifing over loci. Gis, inbreeding coefficient; He, expected frequency of heterozygotes within samples; Ho, observed frequency of heterozygotes within samples (related to *No Evidence for Genome-Wide Genetic Differentiation Between HOR and NOR Salmon*).

Table S4. Table of individual information and bisulfite conversion results

Sample	Uniquely mapped sequence	Methylated cytosines in CpG context	Unmethylated cytosines in CpG context	Sex	River	Captivity
c1794	18,745,108	44,822,828	14,163,057	Male	Capilano	Hatchery
c1795	22,245,802	54,695,255	18,880,282	Female	Capilano	Hatchery
c1803	15,075,530	39,914,114	12,822,778	Female	Capilano	Hatchery
c1804	17,668,794	44,981,567	15,211,068	Male	Capilano	Hatchery
c1810	20,533,309	53,009,956	20,616,271	Male	Capilano	Hatchery
c1813	18,332,412	45,177,880	14,758,058	Male	Capilano	Hatchery
c1817	19,635,361	49,485,493	19,539,160	Male	Capilano	Hatchery
c1820	17,161,389	44,125,902	16,360,997	Male	Capilano	Hatchery
c1821	19,136,087	47,345,316	16,200,291	Female	Capilano	Hatchery
c1822	17,258,064	45,173,398	16,193,714	Male	Capilano	Hatchery
c4231	14,590,959	35,206,270	17,901,987	Female	Quinsam	Hatchery
c4232	16,599,541	42,783,196	15,366,059	Female	Quinsam	Hatchery
c4233	14,542,311	28,905,502	23,029,703	Female	Quinsam	Hatchery
c4234	17,493,722	46,814,905	15,350,392	Male	Quinsam	Hatchery
c4237	15,252,011	39,965,992	14,837,504	Female	Quinsam	Hatchery
c4242	20,069,147	51,358,688	18,692,043	Male	Quinsam	Hatchery
c4244	17,050,661	45,269,417	14,391,076	Male	Quinsam	Hatchery
c4245	15,763,567	41,480,785	13,747,503	Male	Quinsam	Hatchery
c4247	17,153,814	45,542,096	14,823,065	Male	Quinsam	Hatchery
c4251	15,095,394	38,971,558	15,978,062	Female	Quinsam	Wild
c4252	15,898,404	42,566,157	14,410,810	Female	Quinsam	Wild
c4254	14,509,279	39,839,585	12,777,768	Female	Quinsam	Wild
c4255	15,721,649	41,974,619	13,868,434	Male	Quinsam	Wild
c4257	20,199,795	50,191,624	18,629,320	Male	Quinsam	Wild
c4258	15,868,459	41,957,069	14,278,354	Female	Quinsam	Wild
c4260	15,750,731	41,372,870	14,961,624	Male	Quinsam	Wild
c4262	14,742,275	37,499,561	13,756,859	Male	Quinsam	Wild
c4263	16,789,054	35,586,662	25,947,096	Male	Quinsam	Wild
c4268	16,344,630	40,671,905	19,507,202	Male	Quinsam	Wild
cap06	15,702,751	40,656,552	14,070,745	Male	Capilano	Wild
cap10	16,173,511	38,457,794	20,107,091	Female	Capilano	Wild
cap11	18,215,774	46,861,209	16,394,616	Female	Capilano	Wild
cap13	15,398,650	36,558,507	12,114,735	Female	Capilano	Wild
cap17	18,409,033	45,626,631	15,919,577	Male	Capilano	Wild
cap19	18,756,014	45,577,492	21,374,067	Female	Capilano	Wild
cap21	16,506,006	40,453,837	17,214,330	Female	Capilano	Wild
cap22	16,536,356	40,163,921	17,949,988	Female	Capilano	Wild
cap23	19,567,836	50,367,430	17,878,701	Male	Capilano	Wild
cap26	18,265,660	40,606,297	26,403,499	Male	Capilano	Wild

Dataset S1. Gene ontology enrichment analysis

Dataset S1