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ARTICLE

Rearing environment affects the genetic architecture and plasticity of DNA methylation in Chinook salmon

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Abstract

Genetic architecture and phenotypic plasticity are important considerations when studying trait variation within and among populations. Since environmental change can induce shifts in the genetic architecture and plasticity of traits, it is important to consider both genetic and environmental sources of phenotypic variation. While there is overwhelming evidence for environmental effects on phenotype, the underlying mechanisms are less clear. Variation in DNA methylation is a potential mechanism mediating environmental effects on phenotype due to its sensitivity to environmental stimuli, transgenerational inheritance, and influences on transcription. To characterize the effect of environment on methylation, we created two 6×6 (North Carolina II) Chinook salmon breeding crosses and reared the offspring in two environments: uniform hatchery tanks and seminatural stream channels. We sampled the fish twice during development, at the alevin (larval) and fry (juvenile) stages. We measured DNA methylation at 13 genes using a PCR-based bisulfite sequencing protocol. The genetic architecture of DNA methylation differed between rearing environments, with greater additive and nonadditive genetic variance in hatchery fish and greater maternal effects in seminatural channel fish, though gene-specific variation was evident. We observed plasticity in methylation across all assayed genes, as well as gene-specific effects at two genes in alevin and six genes in fry, indicating developmental stage-specific effects of rearing environment on methylation. Characterizing genetic and environmental influences on methylation is critical for future studies on DNA methylation as a potential mechanism for acclimation and adaptation.

Introduction

Phenotypic plasticity refers to the ability of a genotype to produce different phenotypes depending on environmental context (Scheiner and Lyman 1989; Uller 2008; Richards et al. 2010; Guillaume et al. 2016). Since phenotypic

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plasticity can occur over short time scales compared with genetic adaptation, which requires generations of selection and adequate standing genetic variation, plasticity serves as a rapid mechanism for coping with changing environmental conditions (Guillaume et al. 2016). Studies have characterized plasticity in response to a broad range of environmental cues, including plastic changes in gene expression and jaw morphology when cichlids were fed different diets (Schneider et al. 2014), plastic colouration in reef fish, which resulted in increased prey capture success and fitness (Cortesi et al. 2015), changes in gill gene expression after rapid transfer to saltwater in killifish (Scott et al. 2004), changes in steelhead salmon brain growth depending on rearing environment complexity (Kihslinger and Nevitt 2006), and gene expression plasticity in response to confinement stress in Chinook salmon (Wellband et al. 2018). While plasticity is known to occur over short time periods within an organism's life, transgenerational plasticity also occurs when offspring phenotype is based on both parental and offspring environmental contexts (Galloway and Etterson 2007; Uller 2008). Transgenerational plasticity can

be maladaptive if the parental environment is a poor predictor of offspring environmental conditions (Galloway 2005; Uller 2008), or if there is the potential for parent–offspring conflict (Uller 2008). The main mechanism for the transmission of transgenerational plasticity is through maternal effects on offspring phenotype and development (Galloway and Etterson 2007; Marshall 2008; Uller 2008), which is often an important component of the underlying genetic architecture of early-life phenotypic traits.

When individual variation is at least partially genetically derived and not entirely determined by environment, components of an organismal phenotype can be explained by the genetic architecture of traits. Genetic architecture is the underlying quantitative genetic basis of variation in phenotypic traits, and includes gene effects, interaction effects among genes, and environmental factors affecting phenotype (Martínez et al. 2014). Often, genetic architecture is reported as maternal, additive, and nonadditive genetic variance components (Houde et al. 2013). Maternal effects are nongenetic influences of maternal genotype and environment on offspring phenotype (Marshall and Uller 2007), often through control of gamete size and deposition of proteins, hormones, and mRNA into eggs (Nodine and Bartel 2012; Perez et al. 2017), in addition to other mechanisms (e.g., Heath et al. 1996; Aykanat et al. 2012b; Nodine and Bartel 2012; Videvall et al. 2016; Falica et al. 2017). Since maternal effects can strongly influence offspring phenotype, particularly early in life (Houde et al. 2013), they can have considerable effects on offspring development and fitness (Galloway and Etterson 2007; Marshall and Uller 2007; Perez et al. 2017; Fan et al. 2019). Additive genetic effects are heritable, predictable based on genotype, and respond to selection (Houde et al. 2013) making additive genetic variation an ideal target for selective breeding programs and predicting evolutionary trajectories of populations. Nonadditive genetic effects encompass dominance effects (interactions among alleles within a locus), epistatic effects (interactions among loci), and higher-order interactions (Sheldon and Merilä 1999). While the effects of nonadditive genetic variance are difficult to predict, there is abundant evidence for nonadditive genetic effects on transcription (Aykanat et al. 2012b; Wellband et al. 2018) and fitness-related traits (Aykanat et al. 2012a; Houde et al. 2013) with the potential for nonadditive effects to contribute to fitness (Sheldon and Merilä 1999; Neff et al. 2011). The study of the underlying genetic architecture of traits is important to characterize the basis and breadth of phenotypic variation and the evolution of organisms, yet genetic architecture is often influenced by environment (Holloway et al. 1990; Etterson 2004; Yeaman and Whitlock 2011; Parsons et al. 2016; Wellband et al. 2018), resulting in genotype-by-environment (G × E) effects

on phenotype. When G × E effects on phenotype occur, environmental variation elicits different phenotypes from the same genotype, resulting in variable fitness of a single genotype dependent on environmental context (García de Leániz et al. 2007; Sae-Lim et al. 2016). Thus, an understanding of the genetic (additive, nonadditive, and maternal variance) basis of phenotypic traits, the environmental context in which organisms reside, and the interaction between genetics and the environment is critical for understanding the basis of phenotype and the evolution of organisms (Banta and Richards 2018).

Despite the importance of the role of plasticity and genetic architecture in phenotypic variation, the mechanisms behind those effects are not well characterized. Epigenetic mechanisms such as DNA methylation alter organism function without underlying changes in the DNA sequence (Bird 2007; Bossdorf et al. 2008). DNA methylation represents an exciting possible mechanism for differences in genetic architecture and phenotypic plasticity to contribute to underlying early-life trait variation. Previous studies have identified plasticity in methylation levels in response to stressors, including changes in methylation in response to pollutant exposure (Fang et al. 2013; reviewed in Head 2014; Olsvik et al. 2019), temperature changes (Anastasiadi et al. 2017; Metzger and Schulte 2017; Liew et al. 2020), elevated salinity (Morán et al. 2013; Metzger and Schulte 2018; Li et al. 2020), inbreeding (Vergeer et al. 2012; Venney et al. 2016; Berbel-Filho et al. 2019), and captive rearing and/or domestication (Nätt et al. 2012; Le Luyer et al. 2017; Rodriguez Barreto et al. 2019). In addition to its sensitivity to environmental changes, methylation can be inherited across generations (Kamstra et al. 2018; Fan et al. 2019; Santangeli et al. 2019). Methylation can exhibit additive (heritable) genetic variance (Hannon et al. 2018) and has been identified as a potential mechanism for the propagation of locus-specific maternal effects (Venney et al. 2020); both additive and maternal sources of variance are important components of the genetic architecture of traits. Due to its sensitivity to the environment and its transmission across generations, DNA methylation represents a possible novel mechanism behind environmentally labile genetic architecture and phenotypic plasticity.

Chinook salmon (Oncorhynchus tshawytscha) are an ideal species for the study of phenotypic plasticity and genetic architecture early in life. Chinook salmon undergo a single, terminal reproductive event, and lack parental care (Heath et al. 1999), eliminating the confounding effects of parental care on offspring phenotype. External fertilization and the production of large numbers of gametes enable large-scale sophisticated breeding experiments. Salmon are sensitive to environmental changes, often exhibiting G × E effects on phenotype and fitness, consistent with other evidence for local adaptation (García de Leániz et al. 2007;

Fraser et al. 2011). Many salmon species are economically and ecologically important with various supplementation and conservation efforts aimed at maintaining and supplementing Chinook salmon stocks (Fraser 2008). However, hatchery rearing often results in reduced fitness and survival in salmon (Araki et al. 2007; Blouin et al. 2010; Fraser et al. 2011; Becker et al. 2014; Le Luyer et al. 2017), even after a single generation of hatchery rearing (Araki et al. 2007). Hatchery-reared salmon exhibit altered DNA methylation patterns (Le Luyer et al. 2017; Rodriguez Barreto et al. 2019), transcription (Christie et al. 2016; Wellband et al. 2018), disease resistance (Becker et al. 2014), brain development (Kihslinger and Nevitt 2006), egg size (Heath et al. 1996), and reduced survival (Blouin et al. 2010; Becker et al. 2014). Differences in genetic architecture among salmon populations (Aykanat et al. 2012a; Houde et al. 2013, 2015) and among environments (Aykanat et al. 2012b; Wellband et al. 2018) have been reported, thus it is possible that rearing juveniles in uniform environments (hatcheries) as opposed to their natural environment influences the genetic architecture of DNA methylation in Chinook salmon. This hypothesis is supported by previous research which identified differentially methylated regions of the genome in hatchery-reared compared with wild Coho salmon (Le Luyer et al. 2017), as well as differences in the genetic architecture of transcription in hatchery-reared and semi-naturally reared Chinook salmon (Wellband et al. 2018).

Here we characterized the effect of rearing environment on the genetic architecture and plasticity of DNA methylation to determine the genetic basis of the effects of environment on DNA methylation. We created two 6×6 factorial (North Carolina II) breeding crosses using Chinook salmon and raised them in hatchery and seminatural rearing environments to determine the effect of early rearing environment on (1) the role of DNA methylation in plastic response to early-life environmental conditions, (2) the extent of $G \times E$ interactions on methylation, and (3) the genetic architecture of DNA methylation. We assayed methylation in Chinook salmon alevins (larval stage) and fry (post-exogenous feeding) at 13 genes involved in development, immune response, stress response, and metabolism using a PCR-based bisulfite sequencing protocol for next-generation sequencing (Venney et al. 2016). Since environmental differences induce changes in the genetic architecture of various traits (Holloway et al. 1990; Etterson 2004; Yeaman and Whitlock 2011; Parsons et al. 2016; Wellband et al. 2018), we predicted that different rearing environments would induce changes in the genetic architecture of DNA methylation, ultimately contributing to underlying changes in phenotype among environments. Based on previous research showing strong environmental effects on methylation (Fang et al. 2013; Morán et al. 2013;

Anastasiadi et al. 2017; Le Luyer et al. 2017), we hypothesized that rearing environment would induce changes in DNA methylation at specific genes. Based on known transgenerational transmission of methylation (Kamstra et al. 2018; Fan et al. 2019; Santangeli et al. 2019) and interactions between transmitted methylation signals and the environment, we expected to observe $G \times E$ effects on methylation. Environmental conditions influence the phenotype of organisms (Scheiner and Lyman 1989; Uller 2008; Richards et al. 2010; Guillaume et al. 2016) as well as changes in the genetic architecture underlying phenotypic traits (Fang et al. 2013; Morán et al. 2013; Anastasiadi et al. 2017; Le Luyer et al. 2017). Understanding the mechanistic and molecular genetic basis of phenotypic variation among environments is critical to quantifying variation within natural populations and understanding how environmental fluctuations influence organismal phenotype, and often fitness, in a rapidly changing world. Quantifying the sources of phenotypic variation and environmental effects on phenotype is critical to making informed conservation and management decisions, and to understanding the molecular basis of phenotype.

Materials and methods

Breeding design and sampling

Two 6×6 North Carolina II breeding crosses were set up on October 31st, 2014, using three-, four-, and five-year-old sexually mature male and female Chinook salmon at Yellow Island Aquaculture, Ltd (YIAL). The North Carolina II design allows for the estimation of additive (sire), maternal (dam–sire), and nonadditive (dam × sire interaction) variance components. Replicated 6×6 factorial crosses were made using six males and six females, resulting in 36 families per cross (72 families total). Fertilized eggs from each family were split into two replicate cells and incubated in freshwater vertical incubators following standard procedures at YIAL. On December 19th, 2014, ~40 eyed eggs per replicate cell were transferred to a Whitlock–Vibert box and buried in the gravel substrate of an artificial seminatural channel at YIAL. The seminatural channel experienced greater temperature and environmental fluctuations and served as a proxy for a more variable, natural environment.

On March 2nd, 2015, alevins were collected from the hatchery incubators and seminatural channels, humanely euthanized, and stored in a high salt buffer (25-mM sodium citrate, 10-mM EDTA, 5.3-M ammonium sulfate, pH 5.2) for later analysis. To minimize cumulative environmental effects across developmental stages, the seminatural channel was restocked with alevin from the

hatchery. This allowed us to test the effects of rearing environment on DNA methylation at both the alevin and fry stage while eliminating the possibility that shifts in methylation are simply maintained through development. The two replicate incubation tray cells for each family in the hatchery were pooled to reduce replicate effects. Approximately ten alevins per replicate were taken from the incubator trays in the hatchery and transferred to the artificial stream environment in 1 of 24 randomly assigned aluminum enclosures measuring $120 \times 60 \times 60$ cm. The enclosures consisted of a bottom tray filled with coarse gravel, and a frame extending above the surface of the artificial stream with netting from the top of the frame to below the gravel. Each enclosure contained offspring from nine families of fish. The remaining alevins from each family were split between two 200-L flow-through barrels (144 barrels total) with adequate flow and oxygenation in the hatchery. All fry were humanely euthanized and sampled after 10 weeks of hatchery or seminatural channel rearing on May 11th, 2015. The fry was cut open to expose their body cavities and preserved in a high salt buffer as described above for alevin.

DNA extraction

Digestions for DNA extractions were performed as in Venney et al. (2020). Alevins were cut in half to aid in digestion and both halves were digested in 6000 μL of digestion buffer (100-mM NaCl, 50-mM Tris-HCl pH 8.0, 10-mM EDTA, 0.5% SDS) with 10 μL of proteinase K. The fry had their livers removed for another experiment, were cut into three pieces (to help with digestion) and digested in 7000 μL of digestion buffer with 10-μL proteinase K. The liver represents a small portion of total somatic genomic DNA; thus, the removal of this organ is unlikely to significantly affect our results regardless of the metabolic importance of the liver. While studying average whole-body methylation masks potential tissue-specific methylation signals, it allowed us to study both larval (alevin) and fully developed (fry) fish. All samples were digested overnight at 37 °C before a 150-μL aliquot was used for DNA extraction via a high-throughput plate-based protocol (Venney et al. 2020) based on a protocol by Elphinstone et al. (2003).

Parentage analysis

Since multiple families of fry were combined and reared in the seminatural channel enclosures, parentage assignment was performed using microsatellite genotyping (for detailed methods, see Wellband et al. (2018)). Fin clips were taken from all fry in the seminatural channel and DNA was extracted using the high-throughput plate-based protocol (Elphinstone et al. 2003). Individuals were genotyped at five microsatellite loci by analyzing PCR fragments on a Licor 4300 DNA Analyzer. Genotypes were scored based on the sizes of parental alleles, and analyzed in Cervus v3.0.7 (Kalinowski et al. 2007) where parentage was determined using known parental pairs with an 80% confidence interval. Fish achieving a 95% confidence interval for parentage was preferentially used for further analyses.

Bisulfite conversion, PCR, and next-generation sequencing

DNA was quantified using a Quant-IT PicoGreen dsDNA Assay kit, an accurate plate-based DNA quantification method. Bisulfite conversion was performed using 500 ng of DNA and an EZ-96 DNA Methylation-Lightning kit following the manufacturer protocol.

PCR was performed using bisulfite sequencing primers for coding regions of 13 highly conserved genes involved in metabolism, stress response, and early development (Venney et al. 2016). The selected genes span a broad range of functions, are important in early development, and/or are logical targets for maternal or environmental effects. Between 136 and 225 bp were amplified per gene (2371 bp total; Table S1) after primer sequences were removed. Bisulfite sequencing libraries were generated using a two-stage PCR approach and sequencing method (Venney et al. 2016) wherein the first stage amplified the targeted gene loci, and the second stage ligated barcode sequences, sequencing adapters, and primers. Next-generation sequencing was performed on the Ion Torrent Personal Genome Machine® (PGM™) using an Ion PGM™ Sequencing 400 kit (maximum length of 400 bp) with an Ion 318™ Chip. Samples were spread across four sequencing runs.

Data processing

Sequence data were demultiplexed using mothur (Schloss et al. 2009) to remove primer sequences and generate one sequence file per individual based on barcode sequences. Bisulfite sequence data were aligned to existing sequence data for the target loci using bwa-meth (Pedersen et al. 2014) with only two non-cytosine mismatches allowed to ensure high sequence fidelity due to short read length. A table with data on average percent methylation for each CpG site in each gene in each individual was generated using bwa-meth. Data tables were imported into R (R Development Core Team 2016), which was used for all downstream analyses unless otherwise stated. Additional quality assurance was performed to ensure that CpG sites with <5 reads per gene per individual, and those that were

present in <70% of individuals, were excluded from the analysis (Venney et al. 2016). Rosner's test for extreme outliers was used to identify outlier methylation estimates, which were likely due to low read depth rather than a true biological signal.

Genetic architecture of DNA methylation

To characterize the genetic basis behind variation in DNA methylation, we measured the genetic architecture of DNA methylation by estimating additive, nonadditive, and maternal variance components. Additive genetic variance is calculated as $4 \times$ (sire component of variance), nonadditive genetic variance is calculated as $4 \times$ (sire × dam interaction variance), and maternal variance is calculated as (dam–sire) components of variance (Lynch and Walsh 1998). We studied genetic architecture at two levels: (1) across all genes combined with environment as a factor to determine how environment influences the genetic architecture of DNA methylation across all genes, and (2) for each gene in each environment for the two developmental stages, to quantify changes in genetic architecture underlying variation in DNA methylation among loci, environments, and developmental stages.

First, we tested if rearing environment affected the genetic architecture of DNA methylation across all genes. For each developmental stage, we ran a linear mixed model (LMM) in lme4 (Bates et al. 2015) to estimate the fixed effects of environment and gene, random effects of dam and sire, and all two-, three- and four-way interactions, on DNA methylation across all genes. The significance of each term was tested using likelihood ratio tests starting with higher-order interaction terms, which were excluded when they did not significantly contribute to model fit.

To assess the locus-specific genetic architecture of DNA methylation in each developmental stage and rearing environment, restricted variance analyses (genetic variance components limited to values between 0 and 1) were performed in the R package fullfact (Houde and Pitcher 2016). Briefly, LMMs were used to estimate the random effects of dam, sire, and dam × sire interaction on DNA methylation at each locus. A restricted variance analysis was performed for each gene in each developmental stage in each rearing environment to estimate the gene-specific additive, nonadditive, and maternal variance components contributing to the genetic architecture of DNA methylation. A Benjamini–Hochberg false discovery rate (FDR) correction was used to correct for multiple comparisons. Two-sided paired t-tests were used to determine whether there was a significant difference in the percent variance (additive, maternal, and nonadditive) across all genes due to environmental effects on the genetic architecture of methylation in each developmental stage.

Plasticity and G × E interactions on DNA methylation

We tested for genotype, environment, and G × E effects on methylation using full-sibling unrelated families (diagonal cells in 6×6 crosses) as a proxy for genotype to prevent inflating similarity due to half-siblings from other crosses. The R package lme4 (Bates et al. 2015) was used for all LMMs. For each developmental stage, an LMM was run across all genes to test for overall effects of gene, genotype (family), environment, G × E interaction, and all other two- and three-way interaction terms on DNA methylation. For all models, gene and environment were included as a fixed effect, while genotype and G × E interaction were specified as random effects. Terms were excluded from the model starting with higher-order interaction terms using likelihood ratio tests to assess the significance of individual terms.

To determine which genes were driving significant effects, an LMM was run for each gene in each developmental stage to determine whether genotype, environment, and G × E interaction significantly affect locus-specific methylation, and a Benjamini–Hochberg FDR correction was used to correct for multiple comparisons.

Results

Genetic architecture of DNA methylation between environments

LMMs testing for environmental, gene, dam, sire, and interaction effects across all genes in each developmental stage were simplified to exclude all three- and four-way interaction terms based on lack of statistical significance from likelihood ratio tests, except the environment × gene × dam effect was retained in the LMM for fry methylation. Environment, as well as environment × gene, gene × dam, and gene × sire interactions, all significantly affected methylation across genes at the alevin stage (all $p < 0.001$). At the fry stage, gene × sire interaction ($p < 0.001$), environment × gene and environment × gene × dam interactions (both $p < 0.001$) significantly affected methylation.

Using LMMs for each gene in each rearing environment and developmental stage, we detected significant dam effects on methylation of GTIIBS ($p < 0.05$), metA ($p < 0.01$), hsc71, and itpa (both $p < 0.001$) in hatchery alevins after FDR correction, as well as dam effects on GTIIBS and itpa in seminatural channel alevins ($p < 0.01$) after FDR correction. We detected no significant effects in the fry stage except a dam effect on GTIIBS methylation in seminatural channel fry ($p < 0.01$) after FDR correction. We observed significant sire effects on GTIIBS in hatchery alevins, and no significant dam × sire effects. Rearing environment influenced the genetic architecture underlying

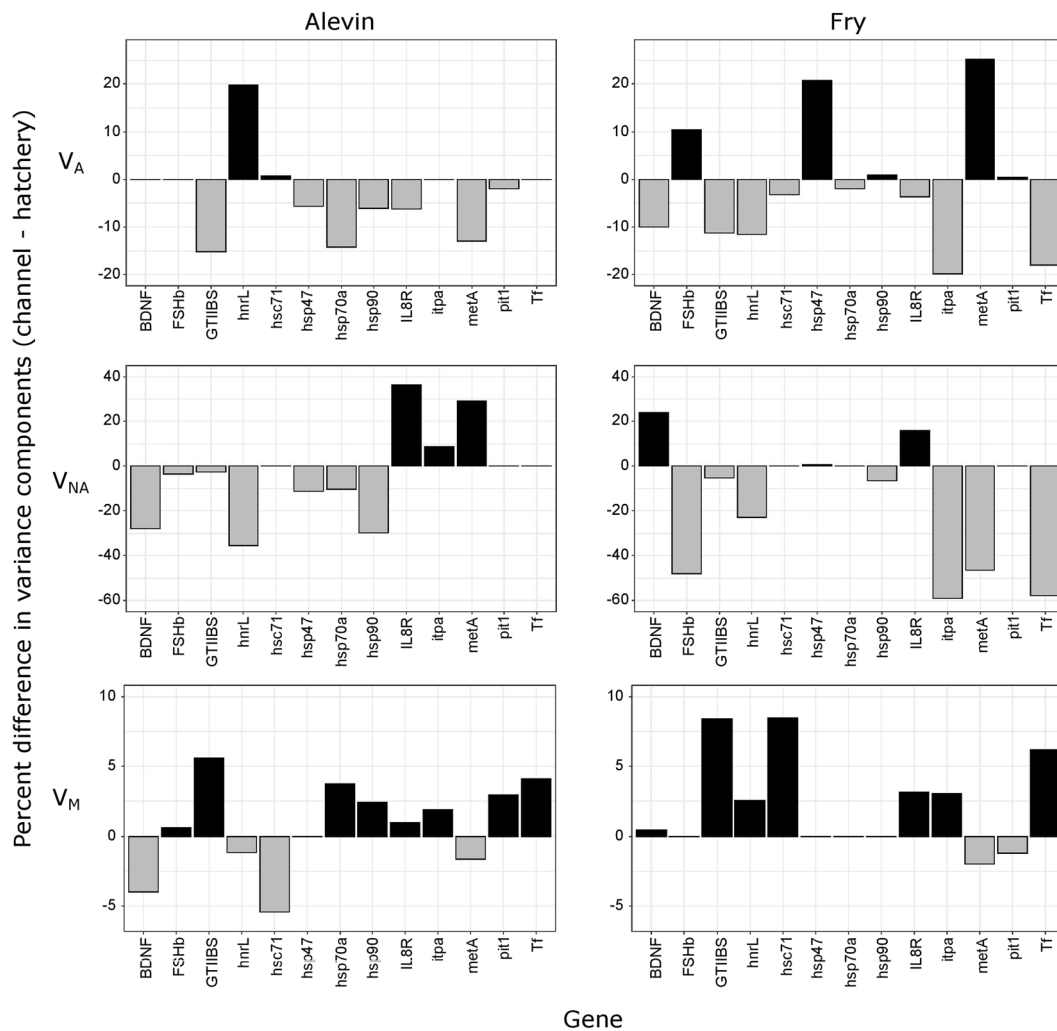**Fig. 1 Bar graph showing the effects of rearing environment on additive (V_A), nonadditive (V_{NA}), and maternal (V_M) variance components on gene-specific DNA methylation in Chinook salmon.** Bars represent the percent difference in variance components (seminatural channel—hatchery) due to early rearing environment. Black bars indicate greater contributions of the variance component to methylation status of genes in the seminatural channel while gray bars indicate greater contributions of the variance component in the hatchery.

DNA methylation in a gene-specific manner (Fig. 1 and Table S2). In general, we observed increased additive and nonadditive variation in hatchery-reared fish and increased maternal effects in seminatural channel-reared fish at both alevin and fry life stages (Fig. 2). Two-sided paired t-tests testing for differences in the percent variance (additive, maternal, and nonadditive) of methylation were non-significant except for maternal effects in the fry stage (Fig. 2).

Genotype, environment, and G × E effects on methylation

LMMs for the effects of genotype (full-sibling family), environment, gene, and all two-way interactions were run in each developmental stage and environment. Likelihood ratio tests for LMMs allowed the exclusion of the three-way interaction effect due to non-significance. LMMs identified strong environment × gene interaction effects on DNA methylation across all genes in both the alevin and fry stages ($p < 0.001$) indicating gene-specific methylation in response to rearing environment, as well as strong genotype × gene effects in the alevin stage ($p < 0.001$) indicating variation in methylation among families. Genotype, environment, gene, and genotype × environment effects were not significant in either developmental stage.

When LMMs were run for each gene in each developmental stage, we detected strong environmental effects on DNA methylation at specific loci after FDR correction (Fig. 3 and Table S3). We detected significant environmental effects on methylation at hsc71 and metA in alevin ($p < 0.001$), as well as effects on fry DNA methylation at
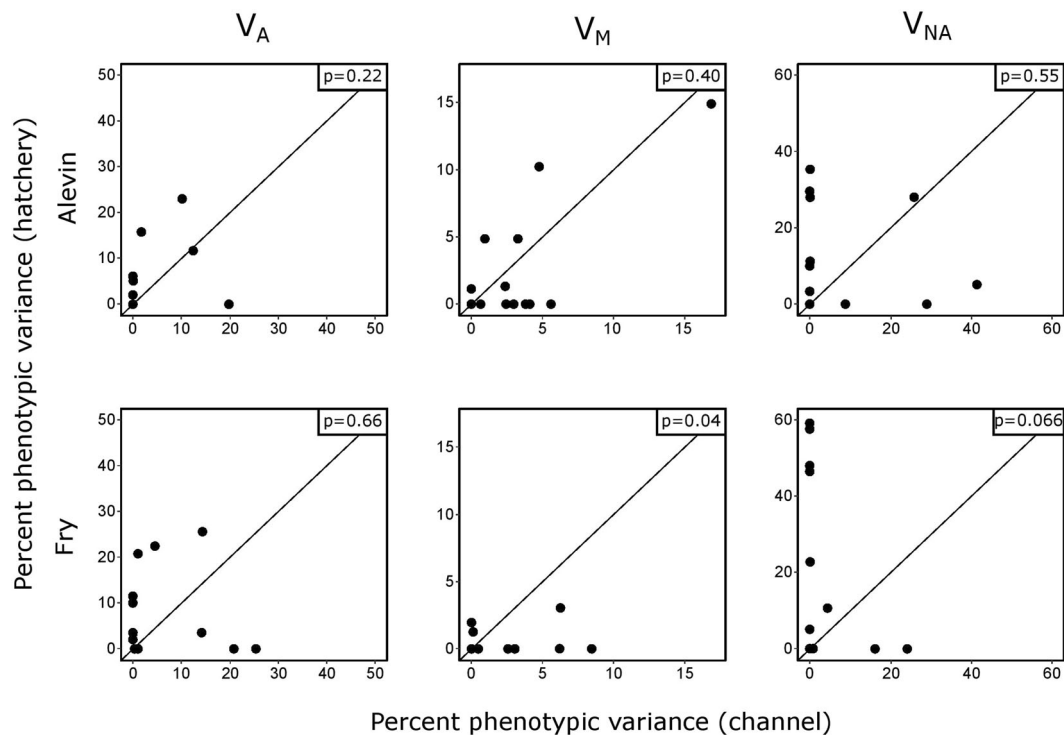
Fig. 2 Scatterplot comparing genetic variance components between rearing environments, with the 1:1 line plotted for reference. Each point represents a gene locus; points above the 1:1 line indicate that the methylation variance component is higher in hatchery-reared fish relative to seminatural channel-reared fish, while points below the line indicate the opposite. *P*-values from two-sided paired *t*-tests for each variance component in each developmental stage are reported, indicating whether rearing environment significantly affected the proportion of variance across all genes.

hsp47, hsp70a and metA (all $p < 0.001$), hsp90, and pit1 ($p < 0.01$) after FDR correction. Genotype and G × E effects were not significant.

Discussion

To fully understand the genetic basis of phenotypic variation within and among natural populations, it is crucial to characterize the genetic architecture of traits of interest, as well as the effects of the environment on that genetic architecture (Holloway et al. 1990; Etterson 2004; Yeaman and Whitlock 2011; Parsons et al. 2016; Wellband et al. 2018). Numerous previous studies have reported evidence for environmental effects on phenotype which can influence individual survival and the persistence of populations in changing environments (reviewed in Angers et al. 2010; Savolainen et al. 2013; Bernatchez 2016; Sae-Lim et al. 2016; Sheriff et al. 2017). While many studies have shown that the environment influences DNA methylation (Ball et al. 2009; Angers et al. 2010; Nätt et al. 2012; Morán et al. 2013; Fang et al. 2013; Head 2014; Anastasiadi et al. 2017; Le Luyer et al. 2017; Metzger and Schulte 2017, 2018; Olsvik et al. 2019; Li et al. 2020; Liew et al. 2020), the effects of environmental differences on the genetic

architecture of DNA methylation have not been studied. However, previous studies have provided evidence for additive inheritance of methylation targeted to specific regions of the genome (Hannon et al. 2018) a link between genotype and methylation status (Herrera and Bazaga 2010; Liu et al. 2012; Foust et al. 2016; Berbel-Filho et al. 2019), and family effects on methylation (Metzger and Schulte 2018). We observed differences in the genetic architecture of DNA methylation in Chinook salmon based on rearing environment and developmental stage, consistent with previous studies on environmental and developmental effects on genetic architecture (Etterson 2004; Aykanat et al. 2012b; Parsons et al. 2016; Wellband et al. 2018). We found significant dam × gene effects in alevin across all genes and both rearing environments, indicating locus-specific maternal effects at the alevin stage, consistent with previous research (Venney et al. 2020). Sire × gene interactions were significant at both the alevin and fry stage, indicating that additive genetic variation is targeted to specific genes. The environment × gene × dam interaction term significantly affected methylation across all genes in the fry stage, suggesting that rearing environments can facilitate or inhibit latent maternal effects. While most studies show a decline in maternal effects through development in salmon (Heath et al. 1999; Houde et al. 2015;
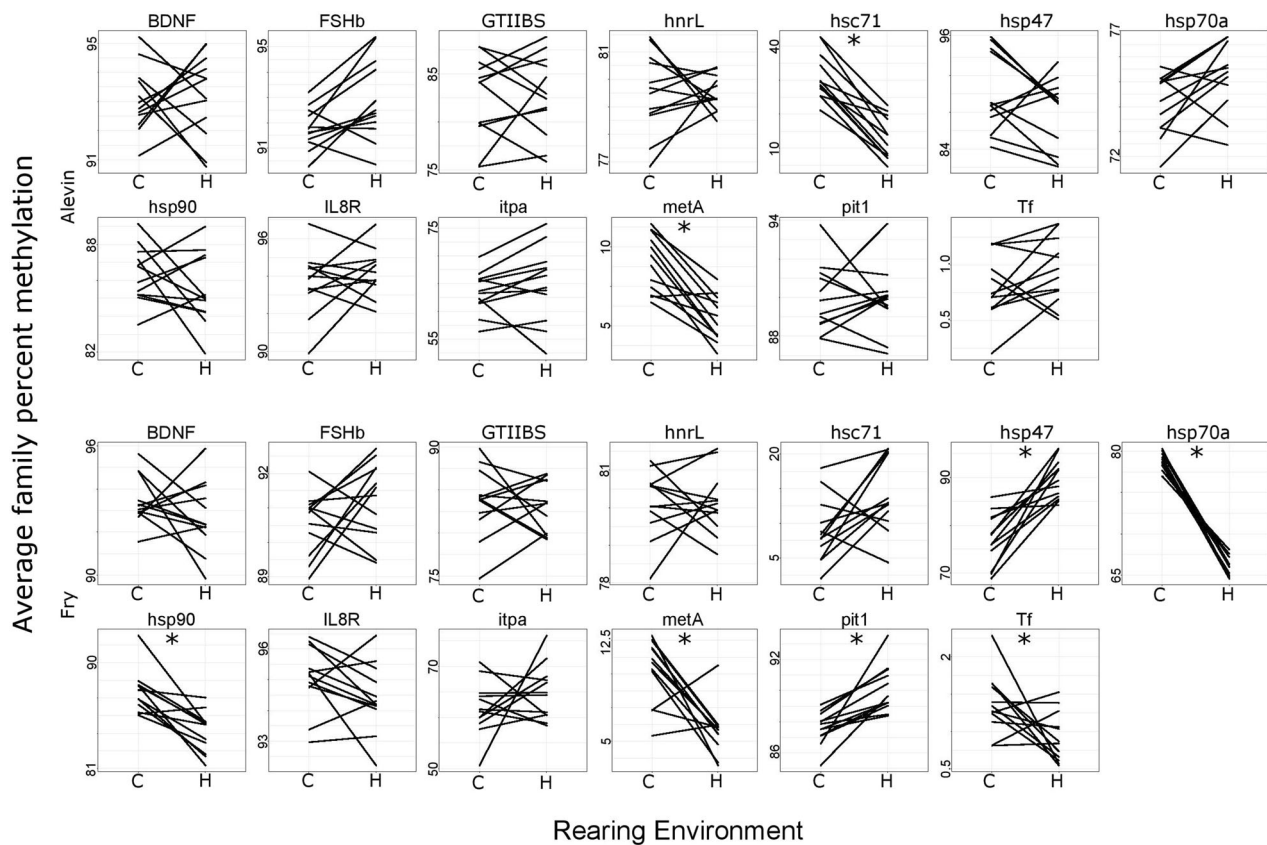
Fig. 3 Reaction norm plots showing the effects of rearing environment on gene-specific DNA methylation in Chinook salmon alevin (top half) and fry (bottom half) full-sibling families. Each line represents the average percent methylation of a full-sibling family, while asterisks denote significant environmental effects on gene-specific methylation. Genotype × gene locus effects on methylation were significant across all genes in the alevin, but not the fry stage. Genotype × environment effects were not significant. C seminatural channel, H hatchery.

Venney et al. 2020), parental effects have been shown to influence offspring at the fry stage in Chinook salmon (Falica and Higgs 2013). In general, we observed higher additive and nonadditive variation and lower maternal effects in hatchery-reared fish relative to fish reared in the seminatural channel in both the alevin and fry stage (Figs. 1, 2). Control of methylation is a complicated process involving many proteins and pathways, some of which are still being identified (Grandjean et al. 2007), and methylation is inherently sensitive to environmental stimuli (e.g., Angers et al. 2010; Morán et al. 2013; Anastasiadi et al. 2017; Le Luyer et al. 2017). Thus, it makes sense that rearing environment influences the genetic architecture of methylation through development, likely through multi-locus (i.e., epistatic) effects on methylation and demethylation processes (Grandjean et al. 2007). It remains unclear whether the emergence of additive and nonadditive effects in hatchery fish, or of maternal effects in seminatural channel-reared fish, would be beneficial to offspring. Maternal effects prepare offspring for a predicted environment based on maternal genotype and environmental experience and thus have the potential to adaptively influence offspring

fitness (Wolf and Wade 2009). However, additive and nonadditive effects on offspring traits can also prove adaptive (Neff et al. 2011). Some traits exhibit additive genetic variation, allowing for selection for or against a given trait, whereas other traits exhibit nonadditive variation due to improved fitness from the pairing of specific alleles or genetic factors with one another, resulting in a beneficial trait (Neff et al. 2011). It is unclear whether maternal effects, or additive and nonadditive effects on DNA methylation will prove beneficial to offspring, though it is important to consider environmental context when studying the genetic architecture of DNA methylation, and in epigenetic studies in general. While hatchery-reared salmon often exhibit reduced survival in the wild (Blouin et al. 2010; Becker et al. 2014), our study used a captive-bred population. Future studies on DNA methylation using wild-caught salmon as parents to quantify changes in the genetic architecture of DNA methylation in response to rearing environment may provide insights into the mechanisms behind reduced fitness of hatchery-reared salmon for applications in conservation efforts, and the relevance of G × E effects on methylation. Environmentally induced

shifts in the genetic architecture of DNA methylation could have important impacts on phenotype due to the effects of DNA methylation on gene expression (Bossdorf et al. 2008) and phenotype (Cubas et al. 1999; Bossdorf et al. 2008; Ma et al. 2018). Thus, it is important to consider the environment in which an organism resides, as well as the effects of the environment on the genetic architecture of traits when studying interindividual variation.

Numerous studies have shown plasticity in methylation in response to environmental effects (Ball et al. 2009; Angers et al. 2010; Nätt et al. 2012; Morán et al. 2013; Fang et al. 2013; Head 2014; Anastasiadi et al. 2017; Le Luyer et al. 2017; Metzger and Schulte 2017, 2018; Olsvik et al. 2019; Li et al. 2020; Liew et al. 2020). Hatchery rearing has become increasingly important in fish supplementation and conservation efforts (Fraser 2008), though the epigenetic effects of hatchery vs. (semi)natural rearing remain unclear due to conflicting results (Blouin et al. 2010; Le Luyer et al. 2017). However, rearing environment-induced plasticity in methylation has far-reaching implications in our understanding of how the environment shapes organismal function and development, particularly in stochastic environments and those influenced by climate change. In our study, rearing environment affected gene-specific methylation across genes in Chinook salmon as indicated by significant environment × gene locus interactions, as well as significant environmental effects. We observed substantial plasticity in methylation levels of heat shock proteins (hsc71 in alevin; hsp47, hsp70a, and hsp90 in fry) between rearing environments. Temperatures in the hatchery environment remain relatively stable with minor daily temperature fluctuations, whereas temperatures in the seminatural channel environment fluctuate with ambient temperature. Thus, short-term differences in seminatural channel temperature likely drive a gene-specific heat shock response (Basu et al. 2002; Lejeusne et al. 2006) and can have long-lasting effects on DNA methylation states, gene expression (Anastasiadi et al. 2017), and heat shock protein expression (Basu et al. 2002). We also observed differences between hatchery and seminatural channel-reared fish in metA methylation in both life stages, as well as Tf and pit1 methylation in fry; these loci are involved in immune response and normal growth or metabolic functions (Berczi 1997; Stafford and Belosevic 2003; Vignesh and Deepe 2017). A previous study on hatchery-reared and wild Coho salmon identified differentially methylated regions associated with immune response and metal ion processing (Le Luyer et al. 2017), consistent with our results. It is not surprising that fry exhibited more environmental effects on methylation than the alevins, as offspring experience more environmental variation over time as they develop and depart from maternal influences. Our results support DNA methylation as a mechanism for phenotypic plasticity due to

its effects on gene expression (Bossdorf et al. 2008) and phenotype (Cubas et al. 1999; Bossdorf et al. 2008; Ma et al. 2018), consistent with previous research on environmental effects on methylation (e.g., Angers et al. 2010; Morán et al. 2013; Anastasiadi et al. 2017; Le Luyer et al. 2017). The capacity for plasticity of methylation in response to environmental change highlights the potential for downstream adaptive effects on phenotype and fitness without the long lag times associated with genotypic evolutionary change (Angers et al. 2010); thus, plasticity in methylation could aid organisms in responding to rapid environmental change, prolonging organismal survival in changing environments.

Genotype and environment both influence physiological and phenotypic traits, sometimes through G × E effects wherein the environment causes differences in phenotype due to genetic differences among individuals (Sae-Lim et al. 2016). Previous studies have identified strong G × E effects on traits such as transcription in Chinook salmon (Wellband et al. 2018), survival in numerous fish species (Sae-Lim et al. 2016), and growth in transgenic Coho salmon (Sundström et al. 2007), European seabass, and other species (Dupont-Nivet et al. 2008). While methylation has been repeatedly shown to be influenced by underlying genetic factors (Herrera and Bazaga 2010; Fraser et al. 2012; Liu et al. 2012), it is unclear whether G × E interactions result in another layer of complexity underlying variation in DNA methylation. Genotype × gene interactions significantly affected methylation across all genes at the alevin stage, indicating that there is variation in gene-specific methylation among families irrespective of rearing environment. This could be due to underlying genetic control of or constraint in DNA methylation (Herrera and Bazaga 2010; Fraser et al. 2012; Liu et al. 2012), or due to significant dam (maternal) and sire (additive) genetic variation at the alevin stage. However, we found no evidence for significant G × E effects on DNA methylation in Chinook salmon, either across all genes or targeted to specific genes. While Fig. 3 shows patterns of changing methylation rank among genotypes consistent with G × E interactions at several loci, we detected no significant G × E effects on DNA methylation, though G × E effects contributed a considerable of phenotypic variance to the methylation of certain genes (Table S3). It is possible that our relatively small sample size of four siblings per 12 unrelated families (versus 72 families in previous analyses) lacks sufficient power for the detection of G × E effects (Sae-Lim et al. 2016). DNA methylation is highly variable, even within lineages of clonal fish in the absence of genetic variation, thus substantial variation in DNA methylation can exist among closely related individuals (Massicotte et al. 2011). This inherent variability contributed to the lack of significant G × E effects in our study due to low family number and high interindividual

variation. Consistent with the findings of Massicotte et al. (2011), genotype did not significantly affect methylation status in our study, though increased sample size in future studies may clarify whether there is genetic variation in the capacity for phenotypic plasticity of DNA methylation.

Environmental effects on DNA methylation have been extensively studied, yet few studies have focused on the genetic architecture or familial basis of epigenetic response to environmental differences. We show that early rearing environment influences the genetic architecture of DNA methylation at specific loci, with hatchery-reared offspring exhibiting higher additive and nonadditive genetic variation and offspring reared in the seminatural channel exhibiting higher maternal effects. Changes in the genetic architecture of traits can have significant effects on phenotype and fitness (Etterson 2004; Aykanat et al. 2012a, b; Parsons et al. 2016; Wellband et al. 2018), and thus are important considerations in evolutionary and conservation biology (Banta and Richards 2018). We show that DNA methylation exhibits phenotypic plasticity at specific loci in response to environmental change, consistent with previous studies on the effects of environment on DNA methylation (e.g., Angers et al. 2010; Morán et al. 2013; Anastasiadi et al. 2017; Le Luyer et al. 2017). We did not detect significant effects of genotype or $G \times E$ interactions on methylation when using full-sibling families as a proxy for genotype, likely due to high variance in methylation levels within full-sibling families. We present evidence for plasticity in methylation between environments, and changes in the genetic architecture of methylation which indicate that both parentage and rearing environment influence the methylation status of specific genes, consistent with previous research (Metzger and Schulte 2018). Since environmental acclimation via DNA methylation has been proposed as a novel mechanism for coping with environmental stress (Angers et al. 2010; Massicotte et al. 2011; Varriale 2014), understanding the genetic and environmental basis of DNA methylation is critical for future study of DNA methylation as a potential mechanism for environmental acclimation and local adaptation.

Data availability

Bisulfite sequencing data have been made available on Dryad for both hatchery-reared fish (https://doi.org/10.5061/dryad.5x69p8d07) and semi-naturally reared fish (https://doi.org/10.5061/dryad.0k6djh9xf).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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