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Original Article

Genome-wide DNA methylation predicts environmentally driven life history variation in a marine fish

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Abstract

Epigenetic modifications are thought to be one of the molecular mechanisms involved in plastic adaptive responses to environmental variation. However, studies reporting associations between genome-wide epigenetic changes and habitat-specific variations in life history traits (e.g., lifespan, reproduction) are still scarce, likely due to the recent application of methylome resequencing methods to non-model species. In this study, we examined associations between whole genome DNA methylation and environmentally driven life history variation in 2 lineages of a marine fish, the capelin (*Mallotus villosus*), from North America and Europe. In both lineages, capelin harbor 2 contrasting life history tactics (demersal vs. beach-spawning). Performing whole genome and methylome sequencing, we showed that life history tactics are associated with epigenetic changes in both lineages, though the effect was stronger in European capelin. Genetic differentiation between the capelin harboring different life history tactics was negligible, but we found genome-wide methylation changes in both lineages. We identified 9,125 European and 199 North American differentially methylated regions (DMRs) due to life history. Gene ontology (GO) enrichment analysis for both lineages revealed an excess of terms related to neural function. Our results suggest that environmental variation causes important epigenetic changes that are associated with contrasting life history tactics in lineages with divergent genetic backgrounds, with variable importance of genetic variation in driving epigenetic variation. Our study emphasizes the potential role of genome-wide epigenetic variation in adaptation to environmental variation.

Keywords: epigenetics, DNA methylation, life history, fish, *Mallotus villosus*

Epigenetic modifications are one of the molecular mechanisms involved in plastic phenotypic changes in response to environmental conditions (Herrel et al., 2020; Zhang et al., 2013). Environmental variation may cause epigenetic alterations that modify the phenotype without any changes in the nucleotide sequence (Bossdorf et al., 2008; Goldberg et al., 2007). Environmentally induced epigenetic variation can facilitate acclimation through altering gene expression, which can influence phenotype and fitness (Beaman et al., 2016; Ryu et al., 2018). In particular, epigenetic changes are likely to promote rapid adaptive responses to novel or changing environments (Watson et al., 2021). In addition, they could allow the expression of adaptive responses in systems where high gene flow (Lenormand, 2002) or low genetic polymorphism (Mounger et al., 2021) constrain genetic adaptation. Nonetheless, our knowledge about the role of the epigenome in habitat-specific variation in life history traits (e.g., longevity, reproduction) directly affecting fitness is still limited (Anastasiadi et al., 2021; Herrel et al., 2020; Vogt, 2021), likely due to the relatively recent application of methylome resequencing methods in non-model species. Recent research has shown that epigenetic modifications can influence longevity (e.g., *Caenorhabditis elegans*, Lee et al., 2019) and aging (e.g., *Homo sapiens*, Pagiatakis et al., 2021), and can be affected by reproductive modes (*Apis mellifera*, Remnant et al., 2016). However, the evolutionary relevance and importance of genetic variation in driving these effects are unclear (Anastasiadi et al., 2021), particularly in natural ecosystems and vertebrate systems.

DNA methylation is the most studied epigenetic mechanism (Angers et al., 2020; Hu & Barrett, 2017; Klutstein et al.,

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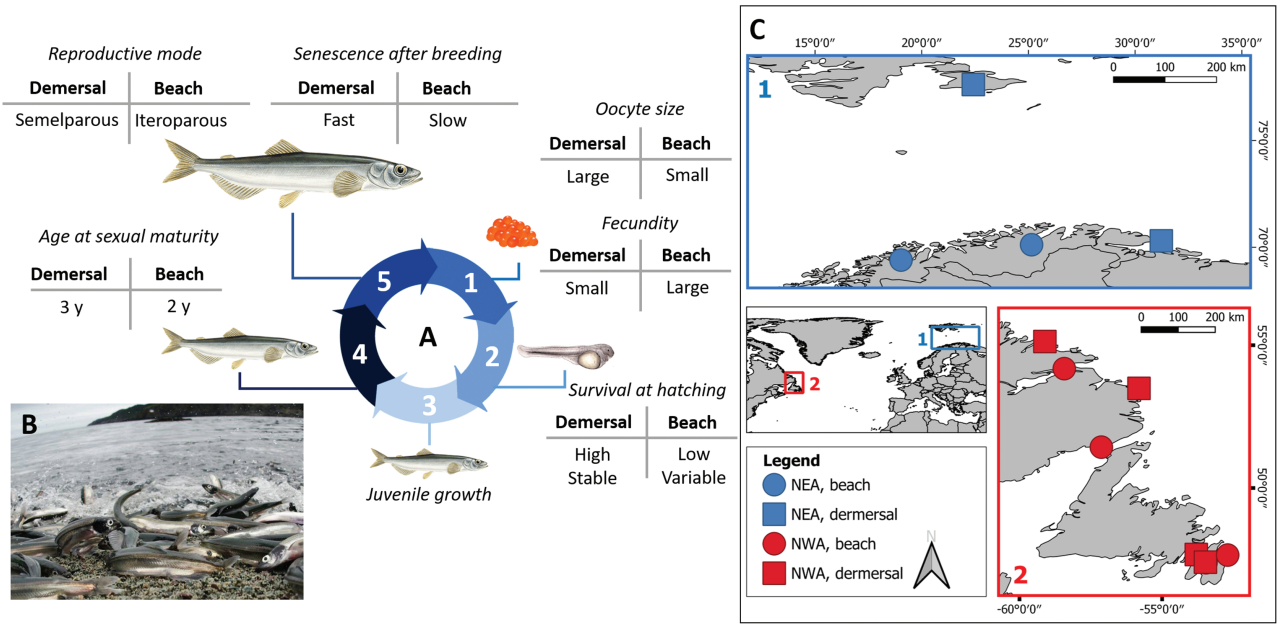

Figure 1. Alternative life history tactics in the capelin (*Mallotus villosus*) based on the work of Christiansen et al. (2008) and study area. (A) Life history differences between demersal-spawning and beach-spawning individuals. (1) Oocyte stage: demersal-spawning individuals reproduce at spawning sites located at water depth ranging from 10 to 250 m where environmental conditions are relatively cold, stable and predictable. By contrast, beach-spawning individuals breed in the intertidal zone (B) where highly variable environmental conditions (i.e., variable but generally warmer temperature and wind) are experienced. Demersal-spawning females produce less eggs than beach-spawning females, but their eggs are larger. Egg development rate is drastically shorter due to warmer temperature in beach-spawning sites than in demersal spawning individuals (Penton et al., 2012) (2–3) Larval and juvenile stages: survival at hatching is high and relatively stable over space and time in demersal spawning individuals, whereas it has the opposite characteristics in beach spawning individuals (Penton et al., 2012). Larval and juvenile growth occur at sea and no information about life history is available. (4) Subadult stage: sexual maturity is usually reached at 2 or 3 years in the two life history phenotypes (Sirois, unpublished data). (5) Adult stage: when they are sexually mature, demersal-spawning males and females reproduce once (i.e., strict semelparity) and experience a strong actuarial senescence within the weeks following reproduction. By contrast, beach-spawning individuals, especially females, may reproduce over two successive years (i.e., facultative iteroparity) and suffer a weaker actuarial senescence (i.e., increase of mortality with age). Beach-spawning females do not suffer from fecundity loss during the second reproductive event, suggesting negligible reproductive senescence. (C) Map showing the sampling sites (beach- vs. demersal- spawning sites) in North East Atlantic (NEA) and North West Atlantic (NWA).

2016; Smith & Meissner, 2013). Studies have proposed that environmental variation in early life can cause persistent epigenetic changes in the adult stage (Gavery et al., 2018; Leitwein et al., 2021; Metzger & Schulte, 2017). Thus, the natal environment is likely to permanently change the methylome, to have important consequences on the phenotype of individuals, and possibly to facilitate the non-genetic transgenerational inheritance of phenotypic traits (Herrel et al., 2020; Vogt, 2021). In three-spined sticklebacks (*Gasterosteus aculeatus*), for example, methylation variation seems to be involved in adaptive responses to variation in salinity (Artemov et al., 2017; Heckwolf et al., 2020). While some epigenetic variation is purely due to environmental acclimation, underlying genetic variation can also contribute to the methylation state of organisms, though the relative importance of genetic versus environmental factors on methylation is understudied (Anastasiadi et al., 2021). When epigenetic inheritance occurs, parents pass on epigenetic states that they acquired because of the environment they experienced to their offspring. In teleost fishes, for instance, thermal (Suárez-Bregua et al., 2020; Valdivieso et al., 2020) and captive (Wellband et al., 2021) conditions during early development cause potentially heritable modifications of methylation that are associated with changes in life history traits (e.g., juvenile growth and survival). However, studies reporting associations between methylation changes and environment-driven shifts of life history in wild populations are still scarce, limiting our ability to assess the generality of this epigenetic mechanism in plastic adaptative responses.

In this study, we examined associations between whole genome methylation and intraspecific variation in life history tactics associated with contrasting environmental spawning conditions (Figure 1). Capelin (*Mallotus villosus*), a small marine pelagic fish with a circumpolar distribution, displays two life history tactics (Cayuela et al., 2020; Christiansen et al., 2008; Figure 1), that are present in multiple ancient lineages that diverged approximatively 2.5 Mya (Dodson et al., 2007). Capelin adopting demersal spawning are strictly semelparous (i.e., single reproductive event over a lifetime) and experience a strong actuarial senescence (i.e., increase of mortality after breeding) irrespective of sex (Christiansen et al., 2008). They reproduce at spawning sites located at water depths ranging from 10 to 300 m, where environmental conditions are relatively predictable, and water temperature is low and stable (Penton et al., 2012). Fish adopting the beach spawning tactic are facultatively iteroparous (i.e., one or two breeding seasons over a lifetime) in both sexes, suffer a slower actuarial senescence (i.e., increase in mortality late in life), and reproduce in the intertidal zone, where water temperature is relatively warm but variable, and desiccation risk caused by wind makes offspring survival highly unpredictable (Frank & Leggett, 1981; Leggett et al., 1984; Penton et al., 2012). Previous studies reported negligible genetic variation among pools of breeders of the two life history tactics in

the North Atlantic (Cayuela et al., 2020; Kenchington et al., 2015; Præbel et al., 2008).

We generated whole genome sequencing (WGS) and whole genome bisulfite sequencing (WGBS) data for both demersal and beach-spawning individuals from two ancient lineages occurring in the northeast Atlantic Ocean (NEA lineage) and the northwest Atlantic Ocean (NWA lineage). First, using WGS data, we quantified the extent of genetic divergence between demersal and beach-spawning individuals both within and between lineages and re-evaluated the hypothesis of limited gene flow between these two pools of individuals (Cayuela et al., 2020; Kenchington et al., 2015). Then, using WGBS data separately for each lineage, we (a) identified differentially methylated loci (DMLs) and regions (DMRs) associated with life history tactic, (b) quantified the overall influence of life history on whole genome DNA methylation, and (c) quantified the effect of life history on whole genome methylation while controlling for the effects of genetic polymorphism.

Methods

Sample collection and whole genome resequencing data

We analyzed samples of 453 breeding adults (241 and 212 beach spawning and demersal individuals, respectively) from 12 spawning sites (6 demersal sites and 6 beach spawning sites; Figure 1) located in the northwest (8 sites, NWA lineage) and northeast Atlantic (4 sites, NEA lineage; for details see Supplementary Table S1). The DNA samples from two sites (DRL and BB65) were reused from a previous study (Kenchington et al., 2015). The fish were collected, and a piece of the dorsal fin was preserved in RNAlater or 96% EtOH. While the dorsal fin may be less metabolically active than other tissues, it is composed of a variety of different cell types (e.g., epithelial cells, blood, and lymphatic tissue) (Hou et al., 2020). The use of this tissue allowed the inclusion of the sites from Kenchington et al. (2015) since DNA methylation patterns differ among tissues (Gavery et al., 2018; Venney et al., 2016) and thus cannot be compared across tissues. DNA was extracted with a salt-based method (Aljanabi & Martinez, 1997), and an RNAse A (Qiagen) treatment was applied following the manufacturer's recommendation. DNA quality was assessed using gel electrophoresis. A total of 453 individuals from the 12 spawning sites were sequenced for the whole genome (WGS) at low coverage (~1.5× per individual) with the median sample size per site being 38 (range: 20–50) individuals. A subset of 55 individuals from 11 spawning sites (excluding the DRL site for which DNA quality was too low for WGS) was sequenced at medium-high coverage (~17× per individual) using WGBS which uses bisulfite conversion to differentiate between methylated and unmethylated cytosines (see Supplementary Table S1 for sample sizes).

Molecular analyses: whole genome sequencing

DNA quality of each extract was evaluated on a NanoDrop and on a 1% agarose gel. Only samples with acceptable ratios that showed clear high molecular weight bands were retained for library preparation. Following the approach used in Therkildsen and Palumbi (2017), we removed DNA fragments shorter than 1 kb by treating each extract with Axygen beads in a 0.4:1 ratio, and eluted the DNA in 10 mM Tris-Cl, pH 8.5. We measured DNA concentrations with Biotium Accuclear and normalized all samples at a concentration of 5 ng/μL. Then, sample DNA extracts were randomized, distributed in 17 plates (96-well), and re-normalized at 2 ng/μL.

Whole genome high-quality libraries were prepared for each fish sample according to the protocol described in previous studies (Baym et al., 2015; Therkildsen & Palumbi, 2017). Briefly, a tagmentation reaction using an enzyme from the Nextera kit, which simultaneously fragments the DNA and incorporates partial adapters, was carried out in a 2.5 μL volume with approximately 2 ng of input DNA. Then, we used a two-step polymerase chain reaction (PCR) procedure with a total of 12 cycles (8 + 4) to add the remaining Illumina adapter sequence with dual index barcodes and amplify the libraries. The PCR was conducted with the KAPA Library Amplification Kit and custom primers derived from the Nextera XT set of barcodes (384 total combinations). Amplification products were purified from primers and size-selected with a two-step Axygen beads cleaning protocol, first with a 0.5:1 ratio, keeping the supernatant (medium and short DNA fragments), then with a 0.75:1 ratio, keeping the beads (medium fragments). Final concentrations of the libraries were quantified with Biotium Accuclear and fragment size distribution was estimated with an Agilent BioAnalyzer for a subset of 10–20 samples per plate. Equimolar amounts of 84 libraries were combined into 6 separate pools for sequencing on 6 lanes of paired-end 150 bp reads on the Illumina HiSeq2000 system.

Raw reads were trimmed and filtered for quality using the default parameters with FastP (Chen et al., 2018). Reads were aligned to the reference genome of *Mallotus villosus* (Cayuela et al., 2020) with BWA-MEM (Li & Durbin, 2009) and filtered with samtools v1.8 (Li et al., 2009) to keep only unpaired, orphaned, and concordantly paired reads with a mapping quality over 10. Duplicate reads were removed with the MarkDuplicates module of Picard Tools v1.119 (http://broadinstitute.github.io/picard/). Then, we realigned reads around indels with the GATK IndelRealigner (McKenna et al., 2010). Finally, to avoid double-counting the sequencing support during single nucleotide polymorphism (SNP) calling, we used the clipOverlap program in the bamUtil package v1.0.14 (Jun et al., 2015) to soft clip overlapping read ends and we kept only the read with the highest quality score in overlapping regions. This pipeline was inspired by Therkildsen and Palumbi (2017) and is available at https://github.com/enormandeau/wgs_sample_preparation.

Molecular analyses: whole genome DNA methylation

Methylation data were generated by WGBS. Library preparation and sequencing were performed at the Centre d'Expertise et de Services Genome Québec (Montréal, QC, Canada). Regarding library preparation, genomic DNA was spiked with unmethylated λ DNA and fragmented. Fragments underwent end repair, adenylation of 3′ ends, and adaptor ligation. Adaptor-ligated DNA was bisulfite-converted followed by amplification by PCR. Library qualities were assessed using the Agilent 2100 BioAnalyzer (Agilent Technologies). Libraries were sequenced on the Illumina HiSeq2000 system to generate WGBS data. DNA methylation quantification relied on the detection of cytosine/thymine polymorphisms after bisulfite conversion. Treatment of genomic DNA with sodium bisulfite induces the deamination of unmethylated cytosine bases to uracil, while methylated cytosine bases remain unchanged. Hence, after PCR,

unmethylated cytosines are detected as thymines, whereas the remaining cytosines indicate cytosine methylation.

Raw WGBS reads were trimmed and cleaned for quality using fastprequiring a minimum length of 100 bp and a minimum phred score of 25, and the first and last bases were trimmed. Trimmed data were aligned to the reference genome using bwa-meth (https://github.com/brentp/bwa-meth), and duplicate reads were marked and removed using picard tools *MarkDuplicates* (https://github.com/broadinstitute/picard). Bias in methylation levels across the length of reads was assessed using MethylDackel's *mbias* function (https://github.com/dpryan79/Methyl-Dackel), and bases that showed skewed methylation levels due to sequencing error were trimmed off the beginnings and ends of reads. Methylation levels were tabulated using MethylDackel *extract* and outputted as a bedGraph and methylKit file for each sample. The pipeline is available at https://github.com/enormandeau/bwa-meth_pipeline.

Methylation data were further filtered to remove C/T and A/G SNPs which can interfere with methylation calling since we cannot discern a true thymine read from an unmethylated read (or an A read at the G position of a CpG site). Thus, we produced a list of C/T and A/G SNPs with a minor allele frequency of 0.01 or greater with coverage of at least one read in at least 20% of individuals and masked these sites from the methylation data using bedtools (Quinlan & Hall, 2010). Methylation data were filtered to include only CpG sites with five to 100 reads, then CpG sites with sufficient coverage in less than 80% of samples were excluded from further analysis. The pipeline is available at https://github.com/cvenney/dss_pipeline.

SNP identification and genome-wide divergence among lineages and life history tactics

Analyses of WGS data were carried out using ANGSD v0.931 (Korneliussen et al., 2014), a software specifically designed to take genotype uncertainty into account instead of basing the analysis on called genotypes. The analytical pipeline is available at https://github.com/clairemerot/angsd_pipeline. We kept input reads with a samtools flag below 255 (not primary, failure and duplicate reads, tag -remove_bads = 1), with a minimum base quality score (minQ) of 20, a minimum mapping quality score (minMapQ) of 30. We used the GATK genotype likelihood model (GL 2) to generate allelic frequency spectra, infer major and minor alleles (doMajorMinor 1), and estimate allele frequencies (doMaf 2). We filtered to keep only SNPs covered by at least one read in at least 50% of the individuals, with a total coverage below 1,812 (four times the number of individuals) to avoid including repeated regions in the analysis and with minor allele frequency above 5%. We then calculated F_{ST} between lineages and life history tactics, randomly subsampled to a similar size of 59 individuals per group. We used the realSFS function in ANGSD, providing the previously obtained list of polymorphic SNPs and their polarization as major or minor allele (options –sites and –doMajorMinor 3).

Identification and functional annotation of DMLs and DMRs associated with life history tactic

We implemented Wald tests in the *DSS* R package (Wu et al., 2013) for each continent to identify differentially methylated loci (DMLs, i.e., CpG sites) and regions (DMRs, i.e., regions with a high concentration of DMLs, as identified by DSS) between life histories. As with the SNP analysis, we analyzed methylation separately for each continent due to strong lineage effects. CpG sites with a probability of $p < .01$ after false discovery rate correction were considered DMLs. DMRs were called based on DML results, requiring a DMR to contain at least 10 CpG sites with at least 50% significant DMLs and spanning a minimum of 200 bp. DMRs within 50bp of one another were merged and treated as a single DMR. Initial DMR results indicated that BB65 samples were driving DMR detection (Supplementary Figure S1), and they were thus excluded from the analysis. Scripts are available at https://github.com/cvenney/dss_pipeline.

Gene ontology (GO) enrichment analysis was performed to determine the functional significance of methylation differences between life histories. All NWA DMRs were used in the analysis, but only a subset of NEA DMRs was used due to the large number of DMRs resulting in an overabundance of enriched GO terms. We made a subset of the top 400 DMRs for NEA based on the highest absolute value of the AreaStat parameter obtained from DSS, which reflects both the length and difference in methylation of DMRs (i.e., higher AreaStat = higher "significance level" of the DMR). The genome sequences and transcripts of the GenBank fHypTra1 assembly for *Hypomesus transpacificus* were used to locate the sets of annotated genes found within 5 kb of the different sets of DMRs of interest. These sets of genes were retained for GO enrichment analysis using the https://github.com/enormandeau/go_enrichment pipeline. Briefly, the *Hypomesus transpacificus* transcripts of the associated genes were first annotated using the Swissprot database to retrieve their Swissprot IDs. The Swissprot IDs were then used to automatically retrieve the GO annotations of each transcript. Finally, goatools Python library (https://github.com/tanghaibao/goatools) was used to find over-represented GO terms in each of the sets of genes of interest. GO enrichment results were filtered to retain significantly enriched terms with a Benjamini–Hochberg false discovery rate (FDR)-adjusted *p*-value less than .1 and excluding broad terms (i.e., levels 0–2).

Decomposition of the genetic and methylation variation associated with life history tactics

We extracted the genotype likelihood matrices of 20 European and 28 North American individuals. We excluded the BB65 samples based on DMR analysis, RIG-1 as it was not sequenced with WGS, and MAK2-DSEA-15 as it was determined to be an outlier based on PCA. For each continent, we inferred the individual covariance matrices with *PCAngsd* (Meisner & Albrechtsen, 2018) and decomposed them onto orthogonal axes using principal component analysis (PCA) using a scaling 2 transformation, which added an eigenvalue correction, to obtain the individuals PC scores (Legendre & Legendre, 2012). For epigenetic variation, PC scores were similarly obtained from the CpG percent methylation matrices for each continent produced using methylKit (Akalin et al., 2012) on the same individuals, using only CpG sites with coverage across all samples. For both the genetic and epigenetic PCAs, we retained PC axes with eigenvalues greater than one and cumulatively explaining a maximum of 80% of variance for use in redundancy analysis (RDA). We performed three separate RDAs for each continent using the vegan package in R (Oksanen et al., 2020) to test (a) the

effect of life history on DNA methylation, (b) the effect of life history on SNP variation, and (c) the effect of genetic (i.e., SNP) variation on variation in DNA methylation. Finally, we performed a partial RDA for the effect of life history on DNA methylation while controlling for genetic effects on DNA methylation. The significance of the global models, individual PC axes, and model terms were then tested with analyses of variance using 1,000 permutations of the data. The percentage of epigenetic variation explained by each model was determined based on adjusted R^2.

Results

Assessing genomic variation between lineages and life history tactics

WGS analyses showed pronounced genetic variation between NWA and NEA lineages, but negligible genetic differentiation between individuals of the two life history tactics within both lineages. We identified 6,711,583 SNPs across lineages, 6,202,586 lineage-specific SNPs in NEA, and 6,388,908 in NWA. Pairwise F_{ST} estimated between lineages and life history tactics confirmed this pattern (Table 1): F_{ST} was 0.23 between lineages and about two orders of magnitude less between life history within lineages (NWA: 0.003; NEA: 0.004). Based on the considerable genetic divergence between lineages, we performed all subsequent analyses separately for each continent.

Table 1. Pairwise F_{ST}</mathgraphic_> between lineages (NWA and NEA) and life history tactics (beach-spawning individuals BS, and demersal individuals D) within both lineages.

	NWA_BS	NWA_D	NEA_BS	NEA_D
NWA_BS	–	–	–	–
NWA_D	0.0032	–	–	–
NEA_BS	0.2250	0.2254	–	–
NEA_D	0.2369	0.2373	0.0042	–

Note. 95% CIs cannot be calculated in ANGSD and are therefore not provided.
NEA = northeast Atlantic Ocean; NWA = northwest Atlantic Ocean.

Identifying differentially methylated regions associated with lineages and life history tactics

A total of 7,450,160 CpG sites with 18X mean coverage underwent analysis after all quality filtering (see Supplementary Table S2 for detailed coverage information). We found 299,311 DMLs and 9,125 DMRs between life history tactics for the NEA capelin (Figure 2, Supplementary Tables S3 and S4). Initially, we found 83,001 DMLs and 2,626 DMRs for the NWA analysis, though the BB65 (Grebe's Nest, NL, Canada) samples were driving the methylation differences (Supplementary Figure S1). The BB65 samples were thus excluded from the NWA dataset, and the DSS analysis was rerun for the remaining 30 samples. After their exclusion, we found 6,319 DMLs and 199 DMRs due to the life history tactic in NWA fish (Figure 2, Supplementary Tables S5 and S6). We observed considerable hypomethylation in NEA beach spawners compared to demersal spawners (99.6% of 9,125 DMRs), whereas fewer DMRs were hypomethylated in NWA beach capelin (28.1% of 199 DMRs).

GO annotation of DMRs revealed enrichment of 38 terms in NEA and 13 terms in NWA after all filters. There were

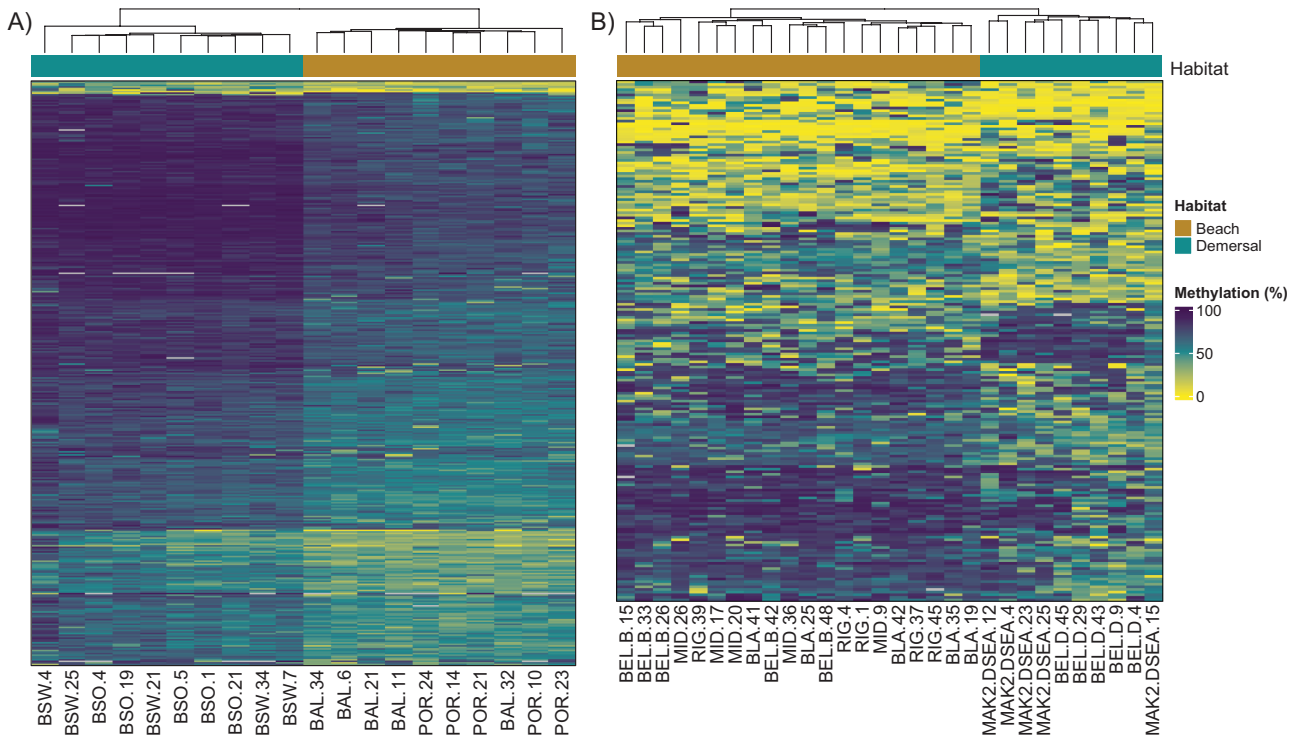

Figure 2. Differentially methylated region (DMR) analysis identified (A) 9,125 DMRs in North East Atlantic (NEA) and (B) 199 DMRs in North West Atlantic (NWA) excluding BB65 between beach- and demersal-spawning capelin. Clustering was performed based on Euclidean distance and the dendrogram clearly separates spawning types in both NEA and NWA. Each row represents a DMR, and percent methylation is communicated by block color (yellow = 0%, indigo = 100% methylation).

Table 2. GO annotation results for DMRs between beach- and demersal-spawning NWA and NEA capelin.

NWA				
GO term	**GO domain**	**Name**	**Depth**	**Adjusted *p*-value (BH-FDR)**
GO :0050877	BP	Nervous system process	3	.015
GO :0099163	BP	Synaptic signaling by nitric oxide	5	.054
GO :0099177	BP	Regulation of trans-synaptic signaling	5	.097
GO :0099543	BP	Trans-synaptic signaling by soluble gas	6	.054
GO :0050804	BP	Modulation of chemical synaptic transmission	6	.097
GO :0098923	BP	Retrograde trans-synaptic signaling by soluble gas	7	.030
GO :0048167	BP	Regulation of synaptic plasticity	7	.054
GO :0099548	BP	Trans-synaptic signaling by nitric oxide	7	.054
GO :0050806	BP	Positive regulation of synaptic transmission	7	.097
GO :0098924	BP	Retrograde trans-synaptic signaling by nitric oxide	8	.030
GO :0005886	CC	Plasma membrane	3	.018
GO :0030689	CC	Noc complex	3	.040
GO :0030692	CC	Noc4p-Nop14p complex	4	.018
NEA				
GO term	GO domain	Name	Depth	Adjusted *p*-value (BH-FDR)
GO :0016043	BP	Cellular component organization	3	1^{E}-07
GO :0050794	BP	Regulation of cellular process	3	2^{E}-04
GO :0043170	BP	Macromolecule metabolic process	3	1^{E}-02
GO :0050877	BP	Nervous system process	3	4^{E}-02
GO :0006810	BP	Transport	3	8^{E}-02
GO :0098609	BP	Cell-cell adhesion	3	9^{E}-02
GO :0048731	BP	System development	3	9^{E}-02
GO :0022607	BP	Cellular component assembly	4	6^{E}-03
GO :0043933	BP	Protein-containing complex organization	4	3^{E}-02
GO :0098742	BP	Cell-cell adhesion via plasma-membrane adhesion molecules	4	4^{E}-02
GO :0007399	BP	Nervous system development	4	4^{E}-02
GO :0001764	BP	Neuron migration	4	4^{E}-02
GO :0007281	BP	Germ cell development	4	7^{E}-02
GO :0030030	BP	Cell projection organization	4	9^{E}-02
GO :0065003	BP	Protein-containing complex assembly	5	1^{E}-02
GO :0120036	BP	Plasma membrane bounded cell projection organization	5	4^{E}-02
GO :0031175	BP	Neuron projection development	6	4^{E}-02
GO :0097485	BP	Neuron projection guidance	7	8^{E}-02
GO :0007411	BP	Axon guidance	8	8^{E}-02
GO :0120025	CC	Plasma membrane bounded cell projection	3	5^{E}-04
GO :0043229	CC	Intracellular organelle	3	2^{E}-03
GO :0043227	CC	Membrane-bounded organelle	3	5^{E}-03
GO :0016021	CC	Integral component of membrane	3	2^{E}-02
GO :0031012	CC	Extracellular matrix	3	7^{E}-02
GO :0031226	CC	Intrinsic component of plasma membrane	3	7^{E}-02
GO :0045202	CC	Synapse	3	8^{E}-02
GO :0099240	CC	Intrinsic component of synaptic membrane	4	5^{E}-03
GO :0043005	CC	Neuron projection	4	2^{E}-02
GO :0043231	CC	Intracellular membrane-bounded organelle	4	3^{E}-02
GO :0005887	CC	Integral component of plasma membrane	4	6^{E}-02
GO :0016342	CC	Catenin complex	4	7^{E}-02
GO :0099699	CC	Integral component of synaptic membrane	5	3^{E}-03
GO :0098936	CC	Intrinsic component of postsynaptic membrane	5	5^{E}-03
GO :0030424	CC	Axon	5	3^{E}-02
GO :0030425	CC	Dendrite	5	6^{E}-02

Table 2. Continued

NWA				
GO term	**GO domain**	**Name**	**Depth**	**Adjusted *p*-value (BH-FDR)**
GO :0099055	CC	Integral component of postsynaptic membrane	6	5^{E}-03
GO :0099580	MF	Ion antiporter activity involved in regulation of postsynaptic membrane potential	6	2^{E}-03
GO :1905060	MF	Calcium:cation antiporter activity involved in regulation of postsynaptic cytosolic calcium ion concentration	9	2^{E}-02

Note. DMR = differentially methylated region; GO = Gene ontology; NEA = northeast Atlantic Ocean; NWA = northwest Atlantic Ocean. Results were deemed significant with an adjusted *p*-value < .1 and were filtered to remove broad terms with depths 0–2.

significantly enriched terms related to nervous system function in both lineages (e.g., NEA: GO:0050877 nervous system development, GO:0001764 neuron migration, GO:0031175 neuron projection development, GO:0007411 axon projection guidance; NWA: GO:0050877 nervous system process, GO:0099163 synaptic signaling by nitric oxide, GO:0048167 regulation of synaptic plasticity, etc.; Table 2).

Quantifying methylation variation between life history tactics

We performed RDA with 161,764 CpG sites in NEA and 66,915 in NWA (i.e., all sites with no missing data for each lineage). Life history tactic explained a significant proportion of total methylome variation in both NEA (p = .00004, adjusted R^2 = 0.136) and NWA (p = .028, adj. R^2 = 0.010), though the effect was stronger in NEA (Figure 3A,B). In contrast, life history had no significant effect on genetic variation for either lineage (NEA: p = .360, adj. R^2 = 0.002; NWA: p = .121, adj. R^2 = 0.015). Genetic variation had no significant effect on whole genome methylation variation (Figure 3C,D, NEA: p = .234, adj. R^2 = 0.120; NWA: p = .447, adj. R^2 = 0.012), though genetic control over methylation was an order of magnitude stronger in NEA than NWA. After controlling for genetic effects on DNA methylation using partial RDA, we found no significant effect of life history tactic on DNA methylation (NEA: p = .183, adj. R^2 = 0.090; NWA: p = .343, adj. R^2 = 0.028). The amount of methylation variation explained by the life history tactic was reduced in the partial RDA controlling for genetic effects compared to the original RDA for NEA (9% vs. 13.6% of variance explained, respectively) but increased for NWA (2.8% vs. 1% of variance explained) indicating varying degrees of genetic contributions to epigenetic variation between lineages.

Discussion

Our results show that environmentally driven life history shifts were associated with epigenetic changes in both NEA and NWA despite high gene flow between pools of demersal- and beach-spawning individuals within the two lineages. We found strong genetic differentiation between lineages but little differentiation between life histories within the two lineages that diverged approximately 2.5 Mya (Cayuela et al., 2020; Dodson et al., 2007) and evolved separately on both sides of the North Atlantic. There was a greater influence of genetic variation on epigenetic variation in NEA than NWA, suggesting varying levels of epigenetic-driven plasticity between lineages.

Negligible genetic differentiation between life history tactics

Our study revealed weak genetic differentiation between capelin from the two life history tactics in both lineages. The low F_{ST} values (NWA: 0.003; NEA: 0.004) likely result from pronounced gene flow—if not panmixia—among individuals with the two life history tactics and large effective population size (N_e; Cayuela et al., 2020). Moreover, the amount of genetic variation associated with life history tactic was not significant in either lineage for the F_{ST} and RDA analyses (F_{ST} : Table 1; RDA: NEA p = .360, adj. R^2 = 0.002, NWA p = .121, adj. R^2 = 0.015), indicating that genetic variation and reproductive isolation do not contribute to the contrasting life history phenotypes observed in *Mallotus villosus*. This result is congruent with previous studies that have revealed marginal genetic variation between beach and demersal spawning sites within the NWA lineage using mitochondrial DNA, microsatellites, and SNPs (Cayuela et al., 2020; Dodson et al., 1991; Kenchington et al., 2015; Præbel et al., 2008). Nevertheless, the absence of marked genomic differentiation between demersal-spawners and beach-spawners does not rule out the possibility of a partial limited genetic control of these life history tactics through a small number of loci, particularly since there were no significant whole genome methylation differences between tactics when controlling for genetic background. Indeed, Cayuela et al. (2020) identified 105 SNP outliers associated with capelin life history tactics in the NWA lineage, suggesting that genetic variation between demersal-spawners and beach-spawners is likely maintained in some genomic regions via spatially varying selection and/or habitat matching choice despite high gene flow.

Epigenetic differentiation between life history tactics

Life history tactic had significant effects on whole genome methylation in both NEA and NWA. Thus, epigenetic differences contributed more to life history tactic than genomic differences in both lineages, supporting the role of epigenetic mechanisms in intraspecific shifts of life history tactics. However, these differences likely reflect plastic responses to the environment that are partially modulated by genetic background due to the non-significance of whole genome methylation differences after controlling for genetic variation (see next Discussion section). Methylation differences between life histories are likely due to environmental differences, as both biotic (e.g., trophic resources; Morán et al., 2013) and abiotic factors (e.g., temperature: Campos et al., 2013, Metzger &

Schulte, 2017; salinity: Artemov et al., 2017; Heckwolf et al., 2020) impact methylation patterns in both marine and freshwater fishes. The differences in methylation associated with life history tactic could be induced by water temperature experienced during embryonic development, the stage at which temperature usually influences DNA methylation patterns in fishes (Anastasiadi et al., 2017; Burgerhout et al., 2017; Lallias et al., 2021; Sävilammi et al., 2020). This interpretation is supported by an experimental study on *Dicentrarchus labrax* (Anastasiadi et al., 2017), which found that genome-wide reprogramming events of methylation marks induced by temperature usually take place shortly after fertilization and are completed during embryogenesis rather than later in life (i.e., larvae and juvenile stages). It is also noteworthy that epigenomic modifications mediated by temperature are very unlikely to occur in adults when they return to reproduce, since they usually spend less than 24 hr at spawning sites (Davoren, 2013). In capelin, Penton et al. (2012) reported higher hourly and mean daily incubation temperatures at a beach spawning site relative to a demersal spawning site on the northeast Newfoundland coast, which was supported by a literature review of temperatures at spawning locations in the wider North Atlantic. Beach-spawned eggs also experience highly variable salinity due to fluctuations with tides and freshwater surface runoff whereas salinity at demersal sites is relatively stable (Præbel et al., 2009). Therefore, it is plausible that differences in temperature and salinity during embryonic growth in beach- and demersal-spawning sites may be responsible for the observed methylation differences in adult capelin. Early-life environment could also result in stable epigenetic modifications that are passed on to future generations (Schmitt et al., 2020), leading to an accumulation of epigenetic variation that contributes to phenotypic diversification (Hu et al., 2021; Vogt, 2017). This association between juvenile environment and DNA methylation assumes that adults preferentially reproduce in their habitat of birth (i.e., habitat matching choice), but not necessarily at the same locations unlike the homing behavior observed in salmonids and other fishes (Keefer & Caudill, 2014). Habitat matching choice (sensu Edelaar et al., 2008) has previously been proposed as a mechanism to explain local adaptation to environmental conditions prevailing in capelin beaching-spawning sites despite

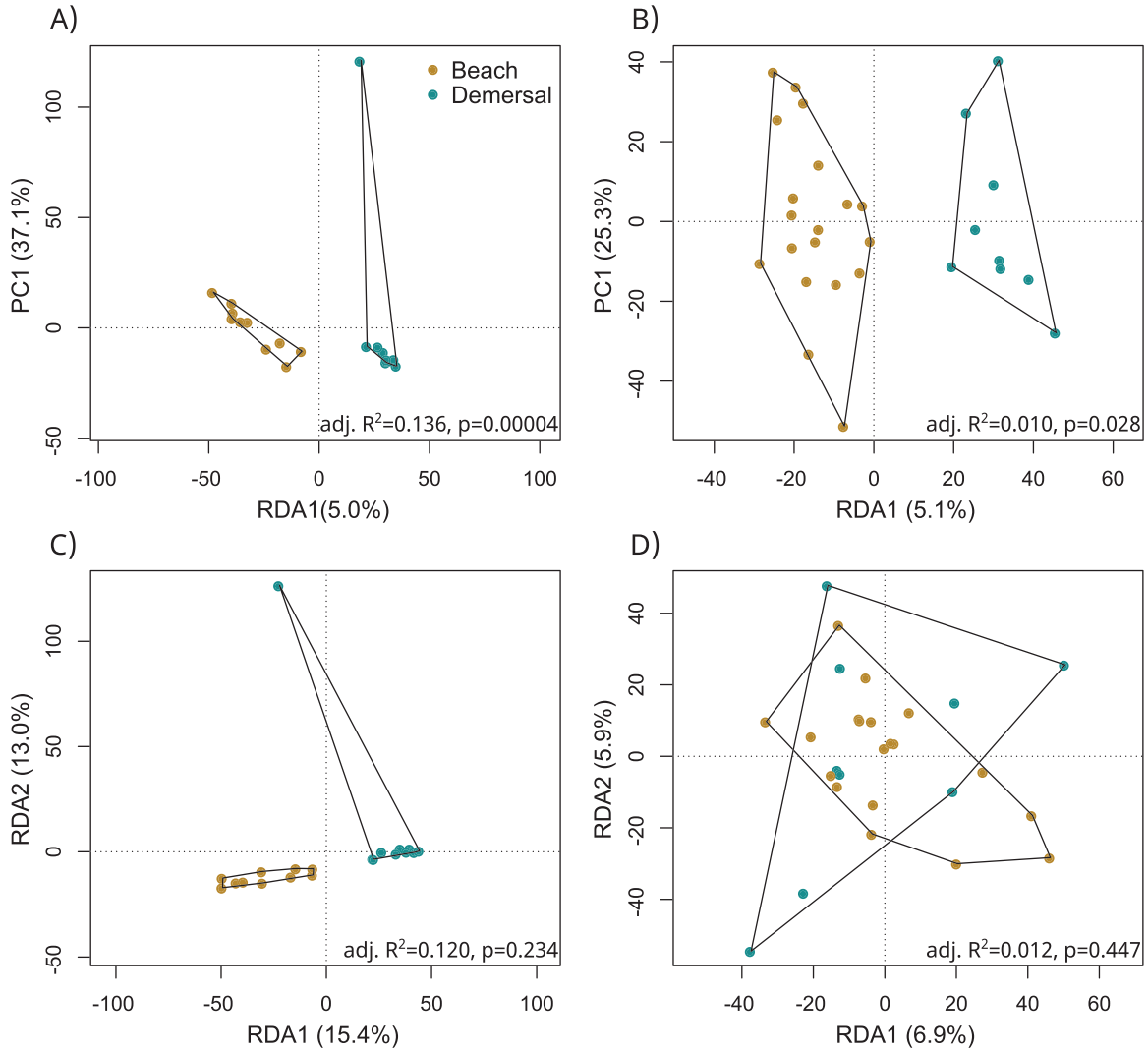

Figure 3. RDAs for the influence of habitat on whole genome DNA methylation in (A) North East Atlantic (NEA) and (B) North West Atlantic (NWA), and the influence of whole genome genetic (SNP) variation on DNA methylation in (C) NEA and (D) NWA. Adjusted R^2 and *p*-values are presented in the respective panes. Plots show RDA1 and PC1 for (A) and (B), and RDA1 and 2 for (C) and (D). Golden points denote beach samples and blue points denote demersal.

high gene flow (Cayuela et al., 2020). We show that epigenetic changes likely contribute to capelin life history tactic, though the extent of epigenetic differences between life histories varies between lineages.

There were fewer epigenetic differences between NWA beach- and demersal-spawning capelin than NEA (Figure 2; 199 vs. 9,125 DMRs, 1.0% vs. 13.6% of variance, respectively), potentially due to other mechanisms contributing to differentiation (e.g., gene expression, other epigenetic mechanisms, signaling pathways), though this could also be due to greater spawning site consistency in NEA capelin. Changes in the distribution of NEA capelin within their normal range are relatively short (~100 km) in the Barents Sea (though demersal-spawning capelin may undertake >1,000 km oceanic migrations to spawn in the Barents Sea (Gjøsæter, 1998)) whereas NWA capelin sometimes make long distance shifts in habitat use, including establishment of new spawning sites and migrations from the Pacific to Northwest Atlantic Ocean (Rose, 2005). Displacement distances are correlated to changes in sea temperature (Rose, 2005); therefore, NEA capelin may disperse less and experience more stable thermal environments. NEA capelin may also have been less affected by the last glaciation period and thought to be able to spawn between 40 and 50°N along the eastern Atlantic and Mediterranean coasts. NWA capelin were further displaced south of 40°N and underwent partial recolonization from the Pacific Ocean (Stergiou, 1989), though other studies suggest NWA capelin were displaced to a southern Grand Bank spawning site in Newfoundland during the last glacial maxima (Carscadden et al., 1989; Rose, 2005). Therefore, the two lineages likely experienced differences in ocean currents, thermohaline conditions, and habitat availability during the last glacial maxima. Together, this could lead to clearer epigenetic differentiation between spawning life histories in NEA than in NWA where historical and contemporary displacement distances are greater and spawning sites are more variable.

Within NWA, the BB65 (Grebe's Nest) samples exhibited a different methylation profile from other NWA samples and NEA capelin. They drove DMR identification when included in the analysis (Supplementary Figure S1) and were thus excluded. The causes of the divergent methylation pattern in the BB65 site remain difficult to identify. Within the different glacial lineages of capelin, the genetic structure based on SNPs is weak; Cayuela et al. (2020) demonstrated a slight isolation-by-distance pattern across the whole NWA lineage. It therefore seems unlikely that a break in gene flow with the other reproductive populations of capelin would lead to this methylation differentiation. Nevertheless, a study showed that sea temperature strongly affected certain structural variations of the genome (CNVs) of capelin, even at a fine spatial scale (Cayuela et al., 2021), which suggests that environmental factors may affect certain features of the capelin's genome in the study area. Overall, it is possible that local environmental variation (e.g., temperature, salinity) caused the methylation changes observed at the BB65 site, as previously reported in other fish (Artemov et al., 2017; Heckwolf et al., 2020; Suárez-Bregua et al., 2020; Valdivieso et al., 2020). Further research is needed to understand the factors causing the divergent methylation pattern of this sampling site.

GO enrichment analyses for NEA and NWA DMRs both identified enriched terms associated with neural function. Environmental changes result in altered brain DNA methylation and behavior in mangrove killifish (*Kryptolebias marmoratus*) with the potential for transmission to offspring regardless of offspring environment (Berbel-Filho et al., 2020). Differences in DNA methylation have also been associated with behavioral changes in rats (Anier et al., 2014; Mychasiuk et al., 2013) and zebrafish (Cuomo et al., 2021). In honeybees (*Apis mellifera carnica*), transcriptional changes in genes associated with metabolism and behavior were associated with shifts in reproductive phenotypes (Cardoen et al., 2011). Thus, it is possible that differences in capelin DNA methylation contribute to behavioral differences associated with life history tactics, though further validation of gene expression differences between life histories is needed.

Varying genetic influences on epigenetic variation between life histories

We provide evidence that genetic variation exerts some control over variation in DNA methylation. Controlling for genetic effects on DNA methylation in the partial RDA rendered the effect of life history on the methylome non-significant, indicating that genetic variation modulates the epigenetic response to the environment. A previous study in salt marsh perennials (*Spartina alterniflora* and *Borrichia frutescens*) found that controlling for genetic effects on methylation often reduced the relationship between epigenetic state and habitat (Foust et al., 2016). Similar effects of genetic background on DNA methylation have previously been reported in plants (Seymour et al., 2014) and humans (Bell et al., 2011). In fishes, genetic effects on methylation have been reported due to hybridization in snakehead (*Channa* spp.; Ou et al., 2019), and in isogenic lines of rainbow trout (*Oncorhynchus mykiss*; Lallias et al., 2021), though direct quantification of whole genome genetic and epigenetic diversity is rare. Our results support the previous assertion that genetic and epigenetic variation are often intertwined (Adrian-Kalchhauser et al., 2020).

The extent of genetic control over variation in DNA methylation differed between lineages, with greater genetic control in NEA than NWA (Figure 3C,D). This implies that the methylome is under more stable genetic control in NEA while there may be greater plasticity in NWA. Interestingly, after controlling for the influence of genetic variation in the partial RDA, life history explained more epigenetic variation in NWA than in the RDA ignoring genetic variation (2.8% vs. 1%, respectively), suggesting that the removal of genetically encoded methylation variation focuses the analysis on plastic loci exhibiting differential methylation between spawning types. The opposite effect was observed in NEA, where the partial RDA explained less variation (9% vs. 13.6%), implying that some genetically encoded epigenetic variation driving life history variation was removed in this model. It has been hypothesized that plasticity will be maintained in variable environments but will be reduced or lost in more stable environments through genetic assimilation (Crispo, 2007). While epigenetic variation can rapidly accumulate in response to environmental fluctuations to the point where epigenetic variation becomes uncoupled from genetic variation, some epimutations have been shown to be stable over long time periods in response to selection in *Arabidopsis thaliana* (Van Der Graaf et al., 2015). Due to the relatively stable spawning site locations of NEA capelin (Rose, 2005) compared to the NWA lineage (Carscadden et al., 1989; Rose, 2005; Stergiou, 1989), it is possible that some capacity for plasticity was lost in the NEA lineage due to habitat stability. Epigenetic

plasticity may be maintained in NWA due to the establishment of new spawning sites and migrations from the Pacific Ocean requiring capelin to acclimate to novel environments. Therefore, the differences in the association between genetic and epigenetic variation between lineages may be explainable based on the evolutionary history of the lineages since the last glaciation period, leading to differences in epigenetic plasticity between lineages.

Conclusion

Our study shows that epigenetic variation is associated with important habitat-specific changes in life history traits in a marine fish. We have demonstrated the association of methylation changes with life history tactics in two lineages with highly divergent genetic backgrounds, with genetic variation driving methylation status more strongly in the NEA lineage. Our study, as well as previous work on a diversity of fish species, suggests that DNA methylation is involved in phenotypic diversification and adaptive responses to habitat changes. More generally, these studies seem to confirm that epigenetic variation promotes important shifts of life history traits allowing organisms to occupy novel and unpredictable environments.

Supplementary material

Supplementary material is available online at *Evolution* (https://academic.oup.com/evolut/qpac028)

Data availability

Raw whole genome sequencing data and whole genome bisulfite sequencing data are available on NCBI (Sequence Read Archive) under accession no. PRJNA811815 and PRJNA811812, respectively.

Author contributions

H.C. and C.V. wrote the paper. H.C., C.V., C.R., C.M., M.L., and E.N. performed bioinformatics and the statistical analyses. L.B., M.C., and P.S. initiated the project, and L.B. conceptualized and coordinated the work. All authors read and edited the final manuscript version.

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References

Adrian-Kalchhauser, I., Sultan, S. E., Shama, L. N. S., Spence-Jones, H., Tiso, S., Keller Valsecchi, C. I., & Weissing, F. J. (2020). Understanding "non-genetic" inheritance: Insights from molecular-evolutionary crosstalk. *Trends in Ecology and Evolution*, 35(12), 1078–1089. https://doi.org/10.1016/j.tree.2020.08.011

Akalin, A., Kormaksson, M., Li, S., Garrett-Bakelman, F. E., Figueroa, M. E., Melnick, A., & Mason, C. E. (2012). MethylKit: A comprehensive R package for the analysis of genome-wide DNA methylation profiles. *Genome Biology*, 13, R87. https://doi.org/10.1186/gb-2012-13-10-r87

Aljanabi, S. M., & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25, 4692–4693. https://doi.org/10.1093/nar/25.22.4692

Anastasiadi, D., Díaz, N., & Piferrer, F. (2017). Small ocean temperature increases elicit stage-dependent changes in DNA methylation and gene expression in a fish, the European sea bass. *Scientific Reports*, 7, 1–12. https://doi.org/10.1038/s41598-017-10861-6

Anastasiadi, D., Venney, C. J., Bernatchez, L., & Wellenreuther, M. (2021). Epigenetic inheritance and reproductive mode in plants and animals. *Trends in Ecology and Evolution*, 36(12), 1124–1140. https://doi.org/10.1016/j.tree.2021.08.006

Angers, B., Perez, M., Menicucci, T., & Leung, C. (2020). Sources of epigenetic variation and their applications in natural populations. *Evolutionary Applications*, 13, 1262–1278. https://doi.org/10.1111/eva.12946

Anier, K., Malinovskaja, K., Pruus, K., Aonurm-Helm, A., Zharkovsky, A., & Kalda, A. (2014). Maternal separation is associated with DNA methylation and behavioural changes in adult rats. *European Neuropsychopharmacology*, 24, 459–468. https://doi.org/10.1016/j.euroneuro.2013.07.012

Artemov, A. V., Mugue, N. S., Rastorguev, S. M., Zhenilo, S., Mazur, A. M., Tsygankova, S. V., Boulygina, E. S., Kaplun, D., Nedoluzhko, A. V., Medvedeva, Y. A., & Prokhortchouk, E. B. (2017). Genome-wide DNA methylation profiling reveals epigenetic adaptation of stickleback to marine and freshwater conditions. *Molecular Biology and Evolution*, 34(9), 2203–2213. https://doi.org/10.1093/molbev/msx156

Baym, M., Kryazhimskiy, S., Lieberman, T. D., Chung, H., Desai, M. M., & Kishony, R. (2015). Inexpensive multiplexed library preparation for megabase-sized genomes. *PLoS One*, 10, e0128036. https://doi.org/10.1371/journal.pone.0128036

Beaman, J. E., White, C. R., & Seebacher, F. (2016). Evolution of plasticity: Mechanistic link between development and reversible acclimation. *Trends in Ecology and Evolution*, 31(3), 237–249. https://doi.org/10.1016/j.tree.2016.01.004

Bell, J. T., Pai, A. A., Pickrell, J. K., Gaffney, D. J., Pique-Regi, R., Degner, J. F., Gilad, Y., & Pritchard, J. K. (2011). DNA methylation patterns associate with genetic and gene expression variation in HapMap cell lines. *Genome Biology*, 12, R10. https://doi.org/10.1186/gb-2011-12-1-r10

Berbel-Filho, W. M., Berry, N., Rodríguez-Barreto, D., Rodrigues Teixeira, S., Garcia de Leaniz, C., & Consuegra, S. (2020). Environmental enrichment induces intergenerational behavioural and epigenetic effects on fish. *Molecular Ecology*, 29, 2288–2299. https://doi.org/10.1111/mec.15481

Bossdorf, O., Richards, C. L., & Pigliucci, M. (2008). Epigenetics for ecologists. *Ecology Letters*, 11(2), 106–115. https://doi.org/10.1111/j.1461-0248.2007.01130.x

Burgerhout, E., Mommens, M., Johnsen, H., Aunsmo, A., Santi, N., & Andersen, Ø. (2017). Genetic background and embryonic temperature affect DNA methylation and expression of myogenin and muscle development in Atlantic salmon (*Salmo salar*). *PLoS One*, 12, e0179918. https://doi.org/10.1371/journal.pone.0179918

Campos, C., Valente, L., Conceição, L., Engrola, S., & Fernandes, J. (2013). Temperature affects methylation of the myogenin putative promoter, its expression and muscle cellularity in Senegalese sole larvae. *Epigenetics*, 8, 389–397. https://doi.org/10.4161/epi.24178

Carscadden, J. E., Frank, K. T., & Miller, D. S. (1989). Capelin (*Mallotus villosus*) spawning on the Southeast Shoal: influence of physical factors past and present. *Canadian Journal of Fisheries and Aquatic Sciences*, 46, 1743–1754.

Cardoen, D., Wenseleers, T., Ernst, U. R., Danneels, E. L., Laget, D., De Graaf, D. C., Schoofs, L., & Verleyen, P. (2011). Genome-wide analysis of alternative reproductive phenotypes in honeybee workers. *Molecular Ecology*, 20, 4070–4084. https://doi.org/10.1111/j.1365-294X.2011.05254.x

Cayuela, H., Dorant, Y., Mérot, C., Laporte, M., Normandeau, E., Gagnon-Harvey, S., Clément, C., & Bernatchez, L. (2021). Thermal adaptation rather than demographic history drives genetic structure inferred by copy number variants in a marine fish. *Molecular Ecology*, 30, 1624–1641. https://doi.org/10.1111/mec.15835

Cayuela, H., Rougemont, Q., Laporte, M., Mérot, C., Normandeau, E., Dorant, Y., Tørresen, O. K., Hoff, S. N. K., Jentoft, S., Sirois, P., Castonguay, M., Jansen, T., Praebel, K., Clément, M., & Bernatchez, L. (2020). Shared ancestral polymorphisms and chromosomal rearrangements as potential drivers of local adaptation in a marine fish. *Molecular Ecology*, 29, 2379–2398. https://doi.org/10.1111/mec.15499

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, 34, i884–i890. https://doi.org/10.1093/bioinformatics/bty560

Christiansen, J. S., Præbel, K., Siikavuopio, S. I., & Carscadden, J. E. (2008). Facultative semelparity in capelin *Mallotus villosus* (Osmeridae)-an experimental test of a life history phenomenon in a sub-arctic fish. *Journal of Experimental Marine Biology and Ecology*, 360, 47–55. https://doi.org/10.1016/j.jembe.2008.04.003

Crispo, E. (2007). The Baldwin effect and genetic assimilation: Revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. *Evolution (N. Y)*, 61, 2469–2479. https://doi.org/10.1111/j.1558-5646.2007.00203.x

Cuomo, M., Borrelli, L., Della Monica, R., Coretti, L., De Riso, G., D'Angelo Lancellotti di Durazzo, L., Fioretti, A., Lembo, F., Dinan, T. G., Cryan, J. F., Cocozza, S., & Chiarotti, L. (2021). DNA methylation profiles of Tph1A and BDNF in gut and brain of *L. rhamnosus*-treated zebrafish. *Biomolecules*, 11, 142. https://doi.org/10.3390/biom11020142

Davoren, G. K. (2013). Divergent use of spawning habitat by male capelin (*Mallotus villosus*) in a warm and cold year. *Behavioral Ecology*, 24, 152–161.

Dodson, J. J., Carscadden, J. E., Bernatchez, L., & Colombani, F. (1991). Relationship between spawning mode and phylogeographic structure in mitochondrial DNA of North Atlantic capelin *Mallotus villosus*. *Marine Ecology Progress Series*, 76, 103–113.

Dodson, J. J., Tremblay, S., Colombani, F., Carscadden, J. E., & Lecomte, F. (2007). Trans-Arctic dispersals and the evolution of a circumpolar marine fish species complex, the capelin (*Mallotus villosus*). *Molecular Ecology*, 16, 5030–5043. https://doi.org/10.1111/j.1365-294X.2007.03559.x

Edelaar, P., Siepielski, A. M., & Clobert, J. (2008). Matching habitat choice causes directed gene flow: A neglected dimension in evolution and ecology. *Evolution*, 62, 2462–2472. https://doi.org/10.1111/j.1558-5646.2008.00459.x

Foust, C. M., Preite, V., Schrey, A. W., Alvarez, M., Robertson, M. H., Verhoeven, K. J. F., & Richards, C. L. (2016). Genetic and epigenetic differences associated with environmental gradients in replicate populations of two salt marsh perennials. *Molecular Ecology*, 25(8), 1639–1652. https://doi.org/10.1111/mec.13522

Frank, K. T., & Leggett, W. C. (1981). Wind regulation of emergence times and early larval survival in capelin (*Mallotus villosus*). *Canadian Journal of Fisheries and Aquatic Sciences*, 38(2), 215–223. https://doi.org/10.1139/f81-028

Gavery, M. R., Nichols, K. M., Goetz, G. W., Middleton, M. A., & Swanson, P. (2018). Characterization of genetic and epigenetic variation in sperm and red blood cells from adult hatchery and natural-origin steelhead, *Oncorhynchus mykiss*. *G3*, 8(11), 3723–3736. https://doi.org/10.1534/g3.118.200458

Gjøsæter, H. (1998). The population biology and exploitation of capelin (*Mallotus villosus*) in the Barents Sea. *Sarsia*, 83, 453–496. https://doi.org/10.1080/00364827.1998.10420445

Goldberg, A. D., Allis, C. D., & Bernstein, E. (2007). Epigenetics: A landscape takes shape. *Cell*, 128, 635–638. https://doi.org/10.1016/j.cell.2007.02.006

Heckwolf, M. J., Meyer, B. S., Häsler, R., Höppner, M. P., Eizaguirre, C., & Reusch, T. B. (2020). Two different epigenetic information channels in wild three-spined sticklebacks are involved in salinity adaptation. *Science Advances*, 6(12), eaaz1138. https://doi.org/10.1126/sciadv.aaz1138

Herrel, A., Joly, D., & Danchin, E. (2020). Epigenetics in ecology and evolution. *Functional Ecology*, 34(2), 381–384. https://doi.org/10.1111/1365-2435.13494

Hou, Y., Lee, H. J., Chen, Y., Ge, J., Osman, F. O. I., McAdow, A. R., Mokalled, M. H., Johnson, S. L., Zhao, G., & Wang, T. (2020). Cellular diversity of the regenerating caudal fin. *Science Advances*, 6, 1–12. https://doi.org/10.1126/sciadv.aba2084

Hu, J., & Barrett, R. D. H. (2017). Epigenetics in natural animal populations. *Journal of Evolutionary Biology*, 30, 1612–1632. https://doi.org/10.1111/jeb.13130

Hu, J., Wuitchik, S. J., Barry, T. N., Jamniczky, H. A., Rogers, S. M., & Barrett, R. D. (2021). Heritability of DNA methylation in threespine stickleback (*Gasterosteus aculeatus*). *Genetics*, 217(1), iyab001. https://doi.org/10.1093/genetics/iyab001

Jun, G., Wing, M. K., Abecasis, G. R., & Kang, H. M. (2015). An efficient and scalable analysis framework for variant extraction and refinement from population-scale DNA sequence data. *Genome Research*, 25, 918–925. https://doi.org/10.1101/gr.176552.114

Keefer, M. L., & Caudill, C. C. (2014). Homing and straying by anadromous salmonids: A review of mechanisms and rates. *Reviews in Fish Biology and Fisheries*, 24, 333–368. https://doi.org/10.1007/s11160-013-9334-6

Kenchington, E. L., Nakashima, B. S., Taggart, C. T., & Hamilton, L. C. (2015). Genetic structure of capelin (*Mallotus villosus*) in the Northwest Atlantic Ocean. *PLoS One*, 10, e0122315. https://doi.org/10.1371/journal.pone.0122315

Klutstein, M., Nejman, D., Greenfield, R., & Cedar, H. (2016). DNA methylation in cancer and aging. *Cancer Research*, 76, 3446–3450. https://doi.org/10.1158/0008-5472.CAN-15-3278

Korneliussen, T. S., Albrechtsen, A., & Nielsen, R. (2014). ANGSD: Analysis of next generation sequencing data. *BMC Bioinformatics*, 15, 1–13. https://doi.org/10.1186/s12859-014-0356-4

Lallias, D., Bernard, M., Ciobotaru, C., Dechamp, N., Labbé, L., Goardon, L., Le Calvez, J. M., Bideau, M., Fricot, A., Prézelin, A., Charles, M., Moroldo, M., Cousin, X., Bouchez, O., Roulet, A., Quillet, E., & Dupont-Nivet, M. (2021). Sources of variation of DNA methylation in rainbow trout: Combined effects of temperature and genetic background. *Epigenetics*, 16(9), 1031–1052. https://doi.org/10.1080/15592294.2020.1834924

Lee, T. W., David, H. S., Engstrom, A. K., Carpenter, B. S., & Katz, D. J. (2019). H3K9me2 protects lifespan against the transgenerational burden of germline transcription in C. elegans. *Elife*, 8, e48498.

Legendre, P., & Legendre, L. F. (2012). *Numerical ecology*. Elsevier.

Leggett, W. C., Frank, K. T., & Carscadden, J. E. (1984). Meteorological and hydrographic regulation of year-class strength in capelin (*Mallotus villosus*). *Canadian Journal of Fisheries and Aquatic Sciences*, 41, 1193–1201.

Leitwein, M., Laporte, M., Le Luyer, J., Mohns, K., Normandeau, E., Withler, R., & Bernatchez, L. (2021). Epigenomic modifications induced by hatchery rearing persist in germ line cells of adult salmon after their oceanic migration. *Evolutionary Applications*, 14(10), 2402–2413. https://doi.org/10.1111/eva.13235

Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends in Ecology and Evolution*, 17(4), 183–189. https://doi.org/10.1016/s0169-5347(02)02497-7

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, 25, 1754–1760.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R.; 1000 Genome Project Data Processing Subgroup. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*, 25(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., & DePristo, M. A. (2010). The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*. 20, 1297–1303.

Meisner, J., & Albrechtsen, A. (2018). Inferring population structure and admixture proportions in low-depth NGS data. *Genetics*, 210, 719–731. https://doi.org/10.1534/genetics.118.301336

Metzger, D. C., & Schulte, P. M. (2017). Persistent and plastic effects of temperature on DNA methylation across the genome of threespine stickleback (*Gasterosteus aculeatus*). *Proceedings of the Royal Society B*, 284, 20171667. https://doi.org/10.1098/rspb.2017.1667

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, 77–83. https://doi.org/10.1016/j.aquaculture.2013.02.006

Mounger, J., Ainouche, M. L., Bossdorf, O., Cavé-Radet, A., Li, B., Parepa, M., Salmon, A., Yang, J., & Richards, C. L. (2021). Epigenetics and the success of invasive plants. *Philosophical Transactions of the Royal Society of London, B Biological Science*, 376(1826), 20200117. https://doi.org/10.1098/rstb.2020.0117

Mychasiuk, R., Harker, A., Ilnytskyy, S., & Gibb, R. (2013). Paternal stress prior to conception alters DNA methylation and behaviour of developing rat offspring. *Neuroscience*, 241, 100–105. https://doi.org/10.1016/j.neuroscience.2013.03.025

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2020). Vegan: Community ecology package. R package version 2.5-7.

Ou, M., Mao, H., Luo, Q., Zhao, J., Liu, H., Zhu, X., Chen, K., & Xu, H. (2019). The DNA methylation level is associated with the superior growth of the hybrid fry in snakehead fish (*Channa argus* × *Channa maculata*). *Gene*, 703, 125–133. https://doi.org/10.1016/j.gene.2019.03.072

Pagiatakis, C., Musolino, E., Gornati, R., Bernardini, G., & Papait, R. (2021). Epigenetics of aging and disease: A brief overview. *Aging Clinical and Experimental Research*, 33(4), 737–745. https://doi.org/10.1007/s40520-019-01430-0

Penton, P. M., Davoren, G. K., Montevecchi, W. A., & Andrews, D. W. (2012). Beach and demersal spawning in capelin (*Mallotus villosus*) on the northeast Newfoundland coast: Egg developmental rates and mortality. *Canadian Journal of Zoology*, 90, 248–256. https://doi.org/10.1139/z11-132

Præbel, K., Christiansen, J. S., & Fevolden, S. E. (2009). Temperature and salinity conditions in a sub-Arctic intertidal spawning habitat for capelin. *Marine Biology Research*, 5, 511–514. https://doi.org/10.1080/17451000902729670

Præbel, K., Westgaard, J. I., Fevolden, S. E., & Christiansen, J. S. (2008). Circumpolar genetic population structure of capelin *Mallotus villosus*. *Marine Ecology Progress Series*, 360, 189–199. https://ui.adsabs.harvard.edu/link_gateway/2008MEPS..360..189P/doi:10.3354/meps07363

Quinlan, A. R., & Hall, I. M. (2010). BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics*, 26, 841–842. https://doi.org/10.1093/bioinformatics/btq033

Remnant, E. J., Ashe, A., Young, P. E., Buchmann, G., Beekman, M., Allsopp, M. H., Suter, C. M., Drewell, R. A., & Oldroyd, B. P. (2016). Parent-of-origin effects on genome-wide DNA methylation in the Cape honey bee (*Apis mellifera capensis*) may be confounded by allele-specific methylation. *BMC Genomics*, 17(1), 226. https://doi.org/10.1186/s12864-016-2506-8

Rose, G. A. (2005). Capelin (*Mallotus villosus*) distribution and climate: A sea "canary" for marine ecosystem change. *ICES Journal of Marine Science*, 62, 1524–1530. https://doi.org/10.1016/j.icesjms.2005.05.008

Ryu, T., Veilleux, H. D., Donelson, J. M., Munday, P. L., & Ravasi, T. (2018). The epigenetic landscape of transgenerational acclimation to ocean warming. *Nature Climate Change*, 8(6), 504–509. https://doi.org/10.1038/s41558-018-0159-0

Sävilammi, T., Papakostas, S., Leder, E. H., Vøllestad, L. A., Debes, P. V., & Primmer, C. R. (2020). Cytosine methylation patterns suggest a role of methylation in plastic and adaptive responses to temperature in European grayling (*Thymallus thymallus*) populations. *Epigenetics*, 16(3), 271–288. https://doi.org/10.1080/15592294.2020.1795597

Schmitt, C., Peterson, E., Willis, A., Kumar, N., McManus, M., Subbiah, S., & Crago, J. (2020). Transgenerational effects of developmental exposure to chlorpyrifos-oxon in zebrafish (*Danio rerio*). *Toxicology and Applied Pharmacology*, 408, 115275. https://doi.org/10.1016/j.taap.2020.115275

Seymour, D. K., Koenig, D., Hagmann, J., Becker, C., & Weigel, D. (2014). Evolution of DNA methylation patterns in the Brassicaceae is driven by differences in genome organization. *PLoS Genetics*, 10, e1004785. https://doi.org/10.1371/journal.pgen.1004785

Smith, Z. D., & Meissner, A. (2013). DNA methylation: Roles in mammalian development. *Nature Reviews Genetics*, 14, 204–220. https://doi.org/10.1038/nrg3354

Stergiou, K. (1989). Capelin *Mallotus villosus* (Pisces: Osmeridae), glaciations, and speciation: A nomothetic approach to fisheries ecology and reproductive biology. *Marine Ecology Progress Series*, 56, 211–224. https://doi.org/10.3354/meps056211

Suárez-Bregua, P., Pérez-Figueroa, A., Hernández-Urcera, J., Morán, P., & Rotllant, J. (2020). Temperature-independent genome-wide DNA methylation profile in turbot post-embryonic development. *Journal of Thermal Biology*, 88, 102483. https://doi.org/10.1016/j.jtherbio.2019.102483

Therkildsen, N. O., & Palumbi, S. R. (2017). Practical low-coverage genomewide sequencing of hundreds of individually barcoded samples for population and evolutionary genomics in nonmodel species. *Molecular Ecology Resources*, 17, 194–208. https://doi.org/10.1111/1755-0998.12593

Valdivieso, A., Ribas, L., Monleón-Getino, A., Orbán, L., & Piferrer, F. (2020). Exposure of zebrafish to elevated temperature induces sex ratio shifts and alterations in the testicular epigenome of unexposed offspring. *Environmental Research*, 186, 109601. https://doi.org/10.1016/j.envres.2020.109601

Van Der Graaf, A., Wardenaara, R., Neumann, D. A., Taudt, A., Shaw, R. G., Jansen, R. C., Schmitz, R. J., Colomé-Tatché, M., & Johannes, F. (2015). Rate, spectrum, and evolutionary dynamics of spontaneous epimutations. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 6676–6681. https://doi.org/10.1073/pnas.1424254112

Venney, C. J., Johansson, M. L., & Heath, D. D. (2016). Inbreeding effects on gene-specific DNA methylation among tissues of Chinook salmon. *Molecular Ecology*, 25, 4521–4533. https://doi.org/10.1111/mec.13777

Vogt, G. (2017). Facilitation of environmental adaptation and evolution by epigenetic phenotype variation: Insights from clonal, invasive, polyploid, and domesticated animals. *Environmental Epigenetics*, 3(1), dvx002. https://doi.org/10.1093/eep/dvx002

Vogt, G. (2021). Epigenetic variation in animal populations: Sources, extent, phenotypic implications, and ecological and evolutionary relevance. *Journal of Bioscience*, 46(1), 1–47. https://doi.org/10.1007/s12038-021-00138-6

Watson, H., Powell, D., Salmón, P., Jacobs, A., & Isaksson, C. (2021). Urbanization is associated with modifications in DNA methylation in a small passerine bird. *Evolutionary Applications*, 14(1), 85–98. https://doi.org/10.1111/eva.13160

Wellband, K., Roth, D., Linnansaari, T., Curry, R. A., & Bernatchez, L. (2021). Environment-driven reprogramming of gamete DNA methylation occurs during maturation and is transmitted intergenerationally in Atlantic Salmon. *G3*, 11(12), jkab353. https://doi.org/10.1093/g3journal/jkab353

Wu, H., Wang, C., & Wu, Z. (2013). A new shrinkage estimator for dispersion improves differential expression detection in RNA-seq data. *Biostatistics*, 14, 232–243. https://doi.org/10.1093/biostatistics/kxs033

Zhang, Y. Y., Fischer, M., Colot, V., & Bossdorf, O. (2013). Epigenetic variation creates potential for evolution of plant phenotypic plasticity. *New Phytologist*, 197(1), 314–322. https://doi.org/10.1111/nph.12010