

The Evolutionary Complexities of DNA Methylation in Animals: From Plasticity to Genetic Evolution

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Abstract

The importance of DNA methylation in plastic responses to environmental change and evolutionary dynamics is increasingly recognized. Here, we provide a Perspective piece on the diverse roles of DNA methylation on broad evolutionary timescales, including (i) short-term transient acclimation, (ii) stable phenotypic evolution, and (iii) genomic evolution. We show that epigenetic responses vary along a continuum, ranging from short-term acclimatory responses in variable environments within a generation to long-term modifications in populations and species. DNA methylation thus unlocks additional potential for organisms to rapidly acclimate to their environment over short timeframes. If these changes affect fitness, they can circumvent the need for adaptive changes at the genome level. However, methylation has a complex reciprocal relationship with genetic variation as it can be genetically controlled, yet it can also induce point mutations and contribute to genomic evolution. When habitats remain constant over many generations, or populations are separated across habitats, initially plastic phenotypes can become hardwired through epigenetically facilitated mutagenesis. It remains unclear under what circumstances plasticity contributes to evolutionary outcomes, and when plastic changes will become permanently encoded into genotype. We highlight how studies investigating the evolution of epigenetic plasticity need to carefully consider how plasticity in methylation state could evolve among different evolutionary scenarios, the possible phenotypic outcomes, its effects on genomic evolution, and the proximate energetic and ultimate fitness costs of methylation. We argue that accumulating evidence suggests that DNA methylation can contribute toward evolution on various timescales, spanning a continuum from acclimatory plasticity to genomic evolution.

Key words: DNA methylation, epigenetics, plasticity, evolution, mutation, adaptation.

Significance

DNA methylation is increasingly studied as a mechanism for phenotypic plasticity, though its potential contributions to adaptation and evolution are only beginning to be studied. We highlight the flexibility of methylation as an evolutionary mechanism affecting species on broad evolutionary timescales, including (i) short-term transient acclimation, (ii) stable phenotypic evolution, and (iii) genomic evolution. This synthesis provides our perspective on the complex role of DNA methylation in acclimation and adaptation, its relationship with genetic evolution, and the proximate and ultimate costs of methylation for organisms.

Introduction

The study of epigenetics took root over 80 years ago with the pioneering works of Conrad Waddington showing that environmentally induced phenotypes could be genetically assimilated (Waddington 1952, 1956). With the advent of modern genomic technologies, research has delved into the direct study of underlying epigenetic mechanisms in ecology and evolution. DNA methylation (hereafter often shortened to “methylation”), the addition of a methyl group to the DNA, is an epigenetic mechanism that regulates transcription and has important implications for phenotypic plasticity and evolution (Hu and Barrett 2017). DNA methylation can rapidly change in response to environmental cues (Morán et al. 2013; Huang et al. 2017; Hu et al. 2019; Beemelmanns et al. 2021), yet there is increasing evidence that some methylation marks are inherited across multiple generations in eukaryotes, including plants and animals (Jablonka and Raz 2009; Anastasiadi et al. 2021). DNA methylation also has a reciprocal and functionally interdependent relationship with genetic variation as it can be genetically controlled to varying degrees (Richards 2006; Adrian-Kalchhauser et al. 2020) and can promote DNA mutations and large-scale DNA sequence changes, generating novel genetic variation (Jablonka and Lamb 1995; Danchin et al. 2019). This complexity leads to fundamental questions on how methylation can contribute to the evolution of phenotypic plasticity in different evolutionary contexts.

In this Perspective, we discuss the intricate ways DNA methylation can influence evolution on different timescales depending on ecological and environmental contexts, spanning a continuum from transient acclimatory effects to genetic evolution. While methodological and experimental issues with epigenetic studies have been recently reviewed (Husby 2022; Laine et al. 2023), we provide an evolutionary perspective that fuses contemporary considerations on phenotypic plasticity with the current state of knowledge on DNA methylation. Due to the slightly different functions and nucleotide contexts of DNA methylation in various taxa, we focus our attention on animals. We provide a short, selective synthesis of the current literature on DNA methylation in acclimation, adaptation, and long-term evolutionary change, including the proximate energetic and ultimate fitness implications of DNA methylation.

Rapid Acclimation Through Plastic DNA Methylation

Methylation studies often focus on short-term acclimatory responses to environmental change. Methylation can aid in rapid acclimation to environmental change through altered gene expression, though the link between altered methylation and gene expression is not definite. Some

studies report a link between methylation and transcription (Anastasiadi et al. 2018; Blondeau-Bidet et al. 2023) whereas the relationship is less consistent or absent in others (Dixon et al. 2018; Beemelmanns et al. 2021; Christensen et al. 2021). Methylation may also cause qualitative transcriptional differences such as alternative splicing (Flores et al. 2012; Lev Maor et al. 2015) rather than differential expression. There is also evidence that gene expression changes may precede methylation changes (Pacis et al. 2019), contrary to what was originally thought.

It is also unclear how quickly methylation can be modified (fig. 1A). For example, invasive solitary sea squirts (*Ciona savignyi*) were exposed to different temperatures and salinities, and methylation changes were monitored from 1 to 120 h after exposure (Huang et al. 2017). Methylation changes were rapid, occurring after only 1 h in response to high-temperature exposure but a few days slower in response to other stressors, and often disappeared after several days (Huang et al. 2017). Experimental colonization of high- and low-quality islands with brown anole (*Anolis sagrei*) led to methylation changes in lizards placed in low-quality environments after 4 days (Hu et al. 2019). Methylation did not differ considerably between lizards from source and high-quality islands, indicating that not all environmental differences are sufficient to elicit epigenetic acclimation responses (Hu et al. 2019). A reciprocal transplant study on marine and freshwater three-spine stickleback (*Gasterosteus aculeatus*) found that the methylation state changed after 4-day exposure to altered salinity (Artemov et al. 2017). Rapid methylation changes have also been observed in Atlantic salmon (*Salmo salar*) after 3 days of thermal stress (Beemelmanns et al. 2021) and after brown trout (*Salmo trutta*) were fed a salty diet for 2 day (Morán et al. 2013). Many methylation differences disappeared after several weeks in both studies, showing the transience of DNA methylation.

Epigenetic changes could be adaptive or maladaptive depending on how suitable the resultant phenotypes are for the environment (O’Dea et al. 2016) and whether they occur during development or later in life (see Box 1). Capacity for plasticity can also evolve (fig. 1B), partially due to the magnitude of environmental fluctuations during development (Beaman et al. 2016), though it is unclear when high plasticity and transience of methylation state would be favored. Stable environments could lead to reduced plasticity due to reduced need for acclimation. Conversely, highly variable environments may favor reduced plasticity when the environment changes too quickly or unpredictably for methylation to induce adaptive and timely phenotypic change. A study manipulating the ability of budding yeast (*Saccharomyces cerevisiae*) to condense chromatin through histone deacetylation found that plasticity is favored when the rate of epigenetic change resembles the rate of environmental change (Stajic et al. 2022). While Stajic et al. (2022)

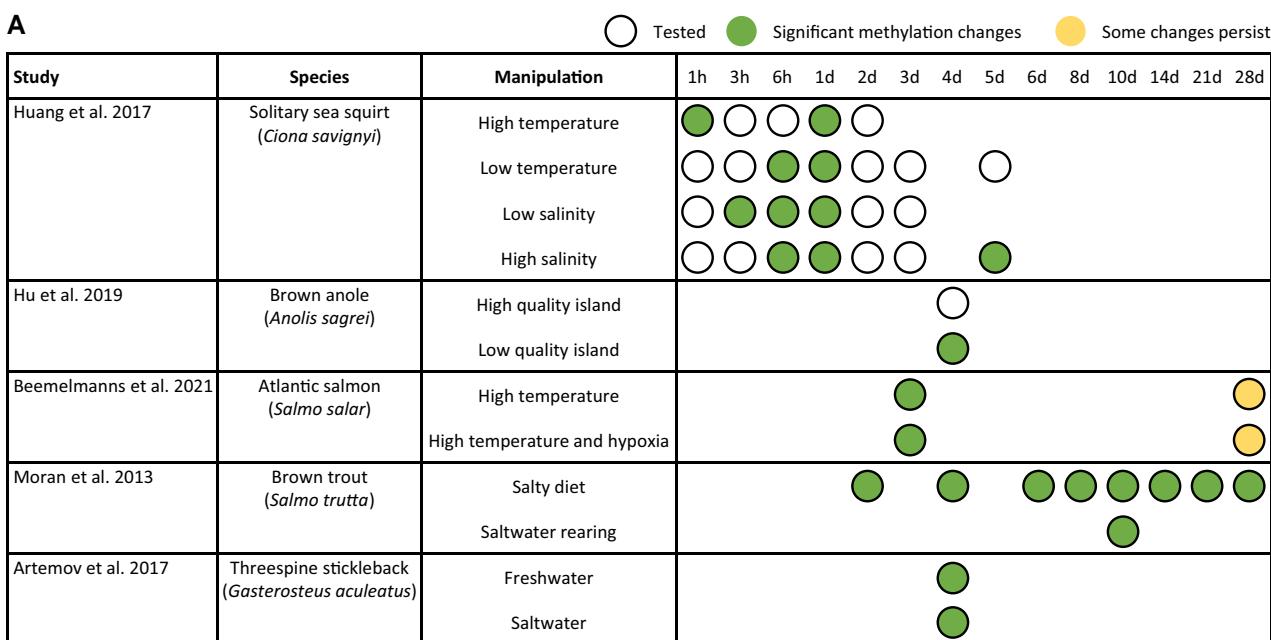
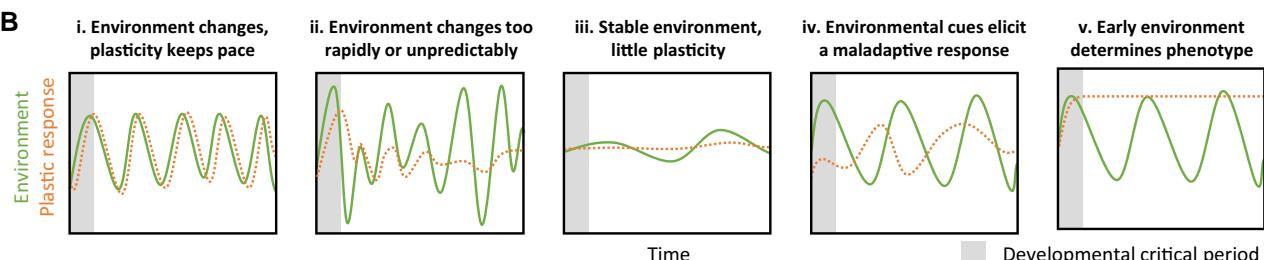
A**B**

Fig. 1.—DNA methylation can contribute toward rapid, transient acclimation. (A) Summary of five selected studies showing rapid (<4 days) epigenetic responses to environmental manipulation. (B) Hypothetical relationships between the rate of environmental change and corresponding plastic responses within a generation. Developmental critical periods are represented by the grey-shaded background. (i) The environment changes at a rate that permits plastic epigenetic responses to keep pace, with organisms able to respond to environmental cues rapidly and appropriately. (ii) The environment changes too rapidly or unpredictably for plastic methylation changes to keep up (e.g., Stajic et al. 2022) which makes plasticity costly and/or maladaptive. (iii) The environment remains relatively stable and does not elicit plastic responses. (iv) The environment changes at a speed where DNA methylation can respond, but environmental cues are not accurately interpreted, or plastic responses are maladaptive. (v) Developmental plasticity leads to developmental determination of methylation state.

studied histone modifications rather than DNA methylation, they showed that the rate of phenotypic plasticity via epigenetic mechanisms can evolve over time in response to environmental change. Further studies of the evolution of plasticity versus stability in the methylation state will improve our understanding of how methylation contributes to acclimation and fitness in changing environments. Range edges and invasive species present particularly interesting systems to study the effects of demographic features (e.g., population

size, magnitude and adaptiveness of plasticity, and the extent of genotype by environment effects) on the evolution of plasticity (Usui et al. 2023). These populations must cope with unpredictable and often novel environments as they expand their ranges through rapid adaptation via altered genetic or epigenetic state, presenting an opportunity to study the evolution of plasticity and its implications for fitness and demographics (Wellenreuther et al. 2022; Usui et al. 2023).

Box 1 Intragenerational developmental plasticity

Plasticity induced by environmental cues encountered during the gamete or embryo developmental phase is called developmental plasticity (Beaman et al. 2016; Angers et al. 2020). Such environmental impacts during early development can lead to permanent epigenetic and irreversible phenotypic outcomes. Developmental plasticity is pervasive in nature, with many environmental factors affecting the expression of different traits in a variety of species. It allows organisms to adapt to changing conditions, such as nutrition, stress, or environmental factors, thereby providing the means to cope with environmental heterogeneity.

Emblematic examples of epigenetically mediated developmental plasticity include sex determination in species with an environmental sex determination system where sex is a developmentally plastic trait and the environment (e.g., temperature) encountered during sensitive developmental windows irreversibly determines its binary fate (i.e., male or female) throughout life via differential DNA methylation and gene expression (Navarro-Martín et al. 2011; Piferrer et al. 2019). Further examples include nutrition-dependent caste determination in social insects (Smith et al. 2008) and density-dependent production of dispersing morphs in swarming locusts (Ernst et al. 2015). Plasticity can also be induced at the juvenile or adult stages; this is typically reversible and is called acclimation or reversible plasticity.

Epigenetic changes induced later in life are often more labile and have greater potential to contribute to rapid acclimation. These changes occur in loci with greater epigenetic instability or environmental sensitivity, contributing to the capacity for plasticity and trait changes throughout the organism's lifetime (Angers et al. 2020). Developmental plasticity can facilitate adaptive responses to environmental change, and its evolution can be shaped by natural selection (Lafuente and Beldade 2019).

Functional, Stable Epigenetic Impacts on Phenotype

DNA methylation is increasingly recognized as contributing to stable, ecologically important phenotypic variation in diverse animal taxa. Despite the transience of some methylation marks, others can stably encode functional phenotypes within and across generations (Anastasiadi et al. 2021).

Within a generation, methylation changes associated with developmental plasticity can irreversibly determine sex in fishes and reptiles (Piferrer 2021) and castes (female workers vs. queens) in buff-tailed bumblebee (*Bombus terrestris*) (Marshall et al. 2023). In the context of populations and species, there is growing support for the role of developmental plasticity leading to altered methylation, life history variation, and adaptation. Methylation has been linked to capelin fish (*Mallotus villosus*) reproductive tactics and life history (Venney et al. 2023), New Zealand mud snail (*Potamopyrgus antipodarum*) shell shape due to water flow rate (Thorson et al. 2017), walking stick insect (*Timema cristinae*) coloration and host plant choice (de Carvalho et al. 2023), eastern happy cichlid (*Astatotilapia calliptera*) ecotypes in early divergence (Vernaz et al. 2022), and in cavefish (*Astyanax mexicanus*) eye degeneration (Gore et al. 2018). Methylation can also lead to transitions among sexual systems, including shifts between dioecy and hermaphroditism (Piferrer 2021).

The contributions of DNA methylation to parallel freshwater adaptation of three-spine stickleback have been particularly well characterized (fig. 2A; Artemov et al. 2017; Heckwolf et al. 2020; Hu and Barrett 2023). Studies have reported considerable methylation differences between

ancestral saltwater and derived freshwater stickleback populations (Artemov et al. 2017; Heckwolf et al. 2020; Hu and Barrett 2023). There is little evidence for epigenetic parallelism among populations despite considerable genomic, transcriptomic, and phenotypic parallelism related to freshwater adaptation (Hu and Barrett 2023). Freshwater populations show higher epigenetic plasticity than marine populations which may compensate for low genetic variation in freshwater systems (Artemov et al. 2017), facilitating acclimation to novel environments. However, many sites showed low methylation plasticity in response to salinity change in another experiment, suggesting that stabilizing selection may have acted to maintain the methylation state or that there is some extent of genetic control over methylation (Heckwolf et al. 2020). Together, these studies suggest that DNA methylation contributes toward stable phenotypic variation leading to diversification and local adaptation in stickleback.

Given the environmental sensitivity of DNA methylation, it is often unclear under what circumstances putatively adaptive methylation differences would be maintained, how stable they are, and what implications they have for natural populations (fig. 2B). Dispersal to a novel environment could lead to an altered methylation state, which would then remain relatively stable for generations (Artemov et al. 2017; Thorson et al. 2017; Heckwolf et al. 2020; Vernaz et al. 2022; de Carvalho et al. 2023; Hu and Barrett 2023), possibly due to environmental perpetuation of methylation state. Stabilizing selection could act to maintain a methylation state in a given environment, though genetic and epigenetic drift can also lead to the

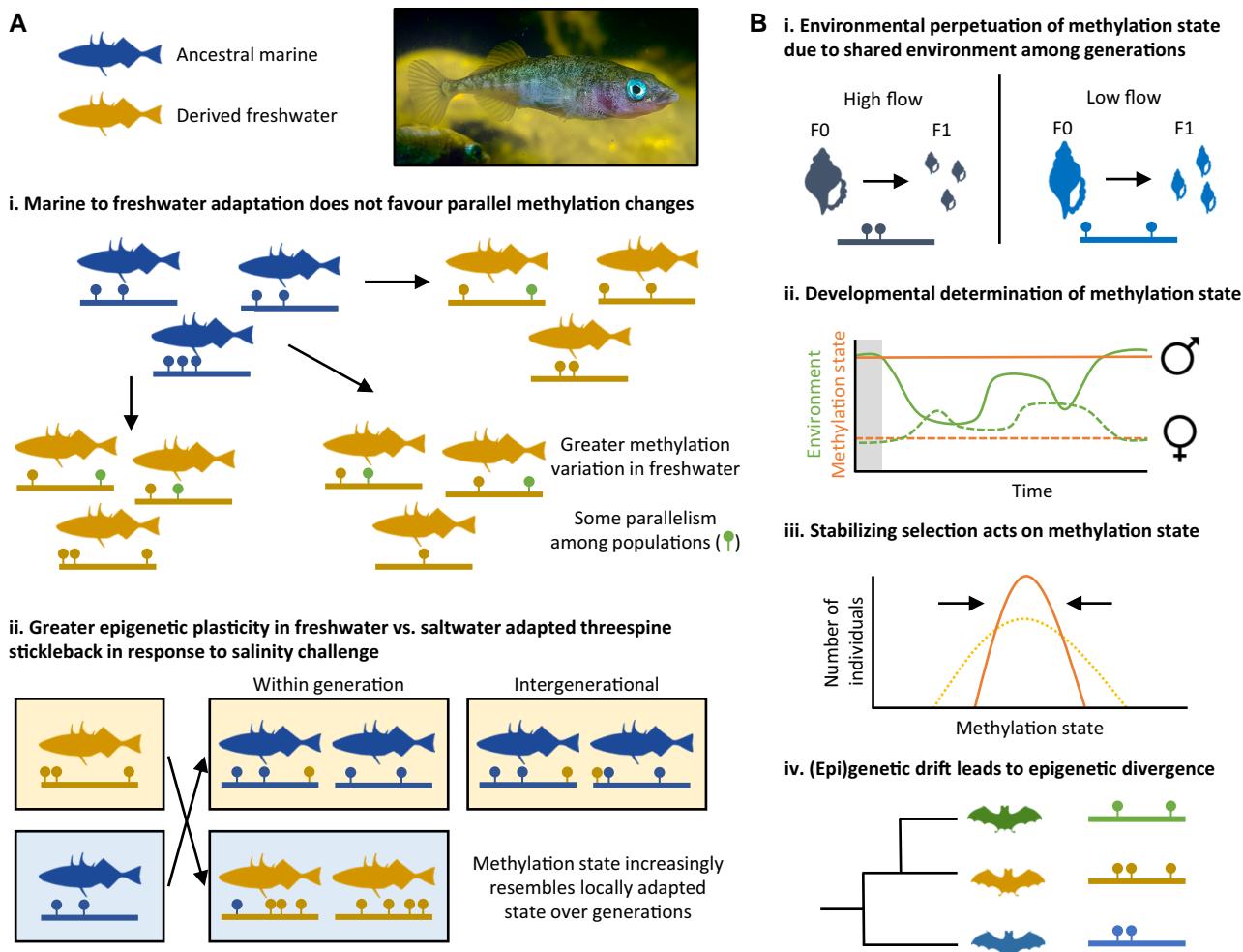


Fig. 2.—DNA methylation can contribute toward stable phenotypic variation. (A) A synthesis on salinity acclimation and adaptation in three-spine stickleback. (i) Hu and Barrett (2023) found that repeated evolution of stickleback from ancestral marine to derived freshwater environments is associated with altered DNA methylation. There was no significant trend toward parallelism versus non-parallelism, indicating that different methylation changes in different populations can achieve the same adaptive response to salinity. Lollipops signify methylated sites. (ii) The studies by Artemov et al. (2017) and Hu and Barrett (2023) found that freshwater populations had a greater capacity for plasticity during saltwater challenges, possibly compensating for reduced genetic variation due to bottlenecks. Heckwolf et al. (2020) reared stickleback from brackish environments in lower salinities, causing their methylation state to increasingly resemble that of locally adapted stickleback over generations. (B) Scenarios that would maintain methylation state leading to stable phenotypic variation. (i) Organisms experience the same environment as their parents leading to perpetuation of their methylation state. (ii) Developmental plasticity determines methylation state and consequent phenotypes. Two different individuals' phenotypes and environments are denoted by the different line types (solid and dotted). (iii) Stabilizing selection acts to maintain methylation state. (iv) Populations or species neutrally diverge through (epi)genetic drift.

accumulation of epigenetic divergence in natural populations (Liu et al. 2015; Vilgalys et al. 2019; Venney et al. 2021). Evidence for evolutionary dynamics acting on methylation and leading to adaptive variation is constantly growing, reinforcing the role of methylation in evolution beyond developmental plasticity.

A Reciprocal Relationship Between DNA Methylation and Genetic Variation

An increasing body of evidence sheds light on the degree of autonomy of methylation from genetic control in animals

(Richards 2006; Adrian-Kalchhauser et al. 2020). Recent cross-species comparisons using several hundred species have shown that methylation is fairly conserved across the evolutionary tree (Aliaga et al. 2019; Klughammer et al. 2023). Simultaneous assessment of genomic and methyome data shows that 3-mers, three nucleotide patterns in the DNA, were predictive of low versus high methylation levels among species (Klughammer et al. 2023). DNA sequence determined methylation state in a mostly consistent manner across 580 animal species, suggesting the existence of a highly conserved “genomic code,” whereas evolutionary history among species was reflected

in phyloepigenetic relationships (Klughammer et al. 2023). Gene body methylation levels tend to follow one of four “types” regardless of species, ranging from consistently low methylation to variable or bimodal methylation levels in different genes within the same species (Aliaga et al. 2019). However, these methylation types do not correspond to phylogenetic distance in the 147 species studied, though they are often conserved within clades (Aliaga et al. 2019), supporting the evolutionary conservation of methylation.

This link between genetic and methylation variation has also been observed in ecological studies (Heckwolf et al. 2020; Sepers et al. 2023; Venney et al. 2023). Methylation differences between capelin life histories were partially genetically driven, even in the absence of overall genetic differences between spawning types (Venney et al. 2023). MethylQTL analysis in partially cross-fostered wild great tit (*Parus major*) showed that methylation differences are largely due to biological brood and that methylation variation was driven by SNP variation at 24% of differentially methylated CpG sites (Sepers et al. 2023). MethylQTLs were also found in genomic regions associated with freshwater adaptation in stickleback (Hu et al. 2021). Genomic variation also described 27% of interindividual methylation differences across two populations of Olympia oyster (*Ostrea lurida*) (Silliman et al. 2023). Despite increasing evidence that methylation is partially genetically controlled and conserved across species, it remains a malleable, plastic mechanism that can change in response to short-term environmental change. Therefore, methylation changes could reflect complex genotypes by environmental responses to environmental changes. Further research into genetic control and constraints over methylation state across different environments is thus urgently needed.

Methylation can also cause hardwired DNA changes due to its inherent mutagenic effects (Tomkova and Schuster-Böckler 2018), leading to a reciprocal relationship between methylation and genetic variation. Epigenetically induced mutagenesis typically occurs due to spontaneous deamination of methylated cytosine to thymine, though C > A and C > G mutations also occur at lower frequency (Tomkova and Schuster-Böckler 2018). Studies are beginning to quantify the effects of methylation on mutagenesis in an evolutionary context (Danchin et al. 2019). A study comparing red jungle fowl and domestic chickens (*Gallus* spp.) found that SNPs are enriched in CpG sites; most of these SNPs arose neutrally and putatively contributed toward chicken speciation (Pértille et al. 2019). Sites showing differential methylation between freshwater and marine stickleback have high allelic diversity, particularly those with intermediate and variable methylation levels, suggesting that CpGs are more prone to mutate when selection on methylation state becomes relaxed (Ord et al. 2023). In domesticated European sea bass (*Dicentrarchus labrax*), genetic variants in populations selected for 25 years appeared in the same

CpGs that responded to captivity in early domesticates (Anastasiadi and Piferrer 2019). DNA methylation is also enriched at nucleotides where all possible mutations would lead to codon degeneracy and amino acid changes in buff-tailed bumble bee (fig. 3A; Marshall et al. 2023). Together, this growing body of evidence suggests that methylation could trigger DNA mutation, resulting in genetic divergence between populations and species. Ultimately, this could lead to the genetic assimilation of initially plastic phenotypes (Danchin et al. 2019), an idea originally proposed in a genetic context by Waddington (1952) to facilitate phenotypic evolution.

DNA methylation can also affect genome evolution through the activity of transposable elements (TEs), which are mobile DNA sequences that can move around the genome. High methylation of TEs promotes heterochromatin formation, which prevents their movement (Dion-Côté and Barbash 2017). When two diverging lineages harbor TEs at different genomic regions, hybridization can lead to altered offspring TE methylation, increased TE mobility, genome instability, reduced offspring fitness, and potential reproductive isolation and speciation (Dion-Côté and Barbash 2017). TE methylation has been associated with phylogenetic and transcriptional differences leading to speciation in Lake Malawi cichlids (Vernaz et al. 2021). TE hypomethylation in hybrids of benthic-limnetic lake whitefish species (*Coregonus clupeaformis*) has also been associated with reproductive isolation associated with postzygotic isolation mechanisms causing increased mortality in hybrids (Laporte et al. 2019). Therefore, DNA methylation can induce both point mutations and contribute to altered genome structure during evolution.

This reciprocal relationship between methylation and genetic variation leads to important questions regarding the coevolution of epigenetic and genetic variation, their effects on one another, and how methylation could influence and be influenced by mutational dynamics in natural populations (examples in fig. 3B). Species naturally differ in mutation rate due to differences in generation time, but also partially due to differences in DNA repair efficiency (Bromham 2009). Species also differ in genomic methylation levels, with a wide range of levels in invertebrates, high levels in marsupials and birds, and even higher levels in fishes and amphibians (Klughammer et al. 2023). Because intermediately methylated sites tend to have the highest mutation rates (Pértille et al. 2019; Ord et al. 2023), potentially due to less regulation of methylation state (Ord et al. 2023), species with intermediate methylation levels may experience greater rates of methylation-induced mutagenesis. Further multi-omic analyses on phenotypic and adaptive divergence are needed to disentangle the complex relationships between genomic and epigenomic variation and their implications for evolution.

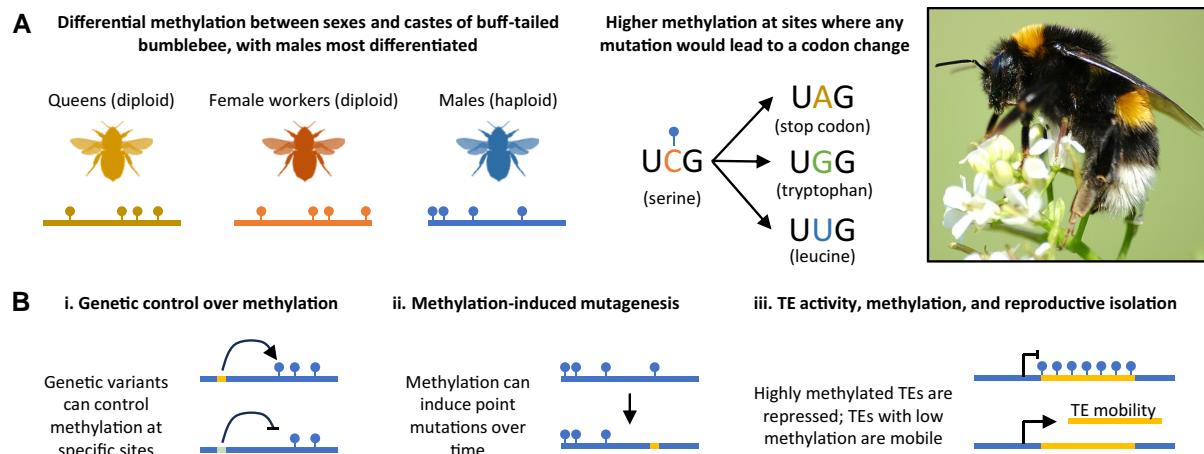


Fig. 3.—The reciprocal relationship between DNA methylation and genetic variation. (A) Marshall et al. (2023) characterized methylation differences (lollipop) between sexes and castes of buff-tailed bumblebee. Diploid queens and female worker bees had more similar methylation profiles than haploid males. Sites with higher methylation tended to be zero-fold degenerate sites (i.e., sites that will always lead to a codon change when mutation occurs). This suggests that these sites are more likely to mutate, leading to altered protein amino acid sequence. (B) Interactions between genetic and epigenetic variation that could contribute to evolution. (i) Genetic variants such as SNPs can regulate methylation at proximal or distant CpG sites, leading to genetic control over the methylation state. (ii) DNA methylation can induce point mutations at methylated sites over time, particularly if these mutations are not corrected by DNA repair. (iii) DNA methylation suppresses TE activity whereas hypomethylation increases their movement, leading to structural variation.

The Proximate and Ultimate Costs of DNA Methylation

High plasticity in methylation state requires direct energy expenditure as DNA methylation carries an energetic cost. DNA methyltransferases methylate cytosine residues using S-adenosylmethionine, a product of adenosine triphosphate (cellular energy), and methionine as a methyl donor (Kohli and Zhang 2013). Enzymatic demethylation involves 10–11 translocase enzymes which oxidize 5-methylcytosine to 5-hydroxymethylcytosine using α -ketoglutarate, the reactant in the rate-limiting step of the citric acid cycle (Kohli and Zhang 2013). Loss or dilution of DNA methylation via cell division can also lead to passive demethylation with no direct energetic cost (Kohli and Zhang 2013), though the associated energy investment is presumably lost. While the exact metabolic costs of DNA methylation are unclear, there is a cost to maintaining and altering methylation, especially considering the number of methylated nucleotides present in an organism. Higher epigenetic plasticity therefore should incur a greater metabolic cost to the organism.

Plasticity in methylation state can ultimately influence fitness even within a single generation (Dixon et al. 2018; Anastasiadi et al. 2021). While methylation can expand the phenotypic space encoded by a genome, the fitness consequences of those phenotypes depend on their stability, suitability to the environment, and potential to accurately respond to future conditions. For instance, a reciprocal transplant study in branching stony coral (*Acropora millepora*) found that transplanted corals that

adjusted methylation state to resemble local corals had improved fitness-related traits compared with corals whose methylation state did not resemble locals (Dixon et al. 2018). Therefore, low plasticity in methylation state, genetic constraint, and epigenetic inheritance could be maladaptive if they perpetuate maladaptive phenotypes in a changing environment, leading to epigenetic traps (O'Dea et al. 2016). Uncoupling of methylation and genetic variation can occur (Angers et al. 2020) as can uncoupling between methylation and transcription, especially early in development (Spruijt and Vermeulen 2014). If a genetically controlled methylation state has a negative effect on fitness, it could lead to reduced genetic control over methylation, or epigenetic control over transcription and phenotype, leading to increased plasticity or reliance on other sources of molecular variation. Conversely, if high variation in methylation state causes negative fitness effects, selection might favor increased genetic control. Carefully designed experimental evolution studies in broad taxa would help to solidify the fitness implications of DNA methylation in different environments and the potential modification of its relationship with genetic, transcriptional, and phenotypic variation.

Conclusions

Phenotypic plasticity is often considered distinct from adaptive variation, though this divide is dissolving as research on the molecular mechanisms of plasticity advances. In this Perspective, we showed that DNA methylation is

increasingly recognized as an important mediator of phenotypic plasticity and provides an additional layer of molecular variation, though we are only beginning to unravel the complex relationships among the methylome, genome, environment, and overarching evolutionary dynamics in natural systems. Further research across broad taxa is needed to understand what drives transience versus stability of methylation state, capacity for plasticity, the reciprocal relationship between DNA methylation and genetic variation, and the phenotypic and fitness implications of methylation and epigenetically induced genomic change. Incorporating methylation analyses into existing studies on the evolution of plasticity (e.g., Pigliucci 2005; Crispo and Chapman 2010; Gibbin et al. 2017) is urgently needed to bridge the gap between ecological and epigenomic research, furthering our understanding of how organisms cope with environmental change.

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online (<http://www.gbe.oxfordjournals.org/>).

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Data Availability

No new data were generated in support of this article.

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