The promise and challenge of environmental epigenomics in a rapidly changing world

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Abstract

There has been a fast-paced research effort on the role of epigenetic mechanisms in facilitating organisms' capacity to cope with rapid environmental change, highlighted by several recent reviews and special issues on this topic. What is important, along with this momentum, is to pause and reflect on both the promises and challenges of linking detailed molecular mechanisms to broad patterns of population responses. Such reflection involves considering how different epigenetic mechanisms operate, depend on genomic context and taxonomic variation in function, the stability and persistence of epigenetic marks, and approaches to measuring these phenomena in natural populations. Here, we discuss in further detail these complexities, and in so doing bring a critical perspective on both the promise and limitations of studies considering epigenetic mechanisms as a driver of climate change adaptation. We provide practical suggestions for future studies that consider how both fundamental biology and methodological issues guide the design of ecological epigenomic studies. Collectively, we advocate for an interdisciplinary approach, where insights from molecular biology and evolutionary ecology, and close interaction between theoretical models and empirical tests, are important for advancing this field.

Keywords: epigenetics, DNA methylation, histone modification, climate change adaptation, plasticity

1. Introduction

We live in an era of rapidly changing environmental conditions, with increasingly frequent and intense extreme weather events, widespread habitat loss and fragmentation, and exposure to novel pathogens among other challenges [1]. Facing this multitude of environmental stressors, populations must adapt or shift to increasingly limited ranges of their habitat to avoid extinction [2]. There has been extensive literature on the scale of these problems and the solutions, and how they vary across and within species: for example, whether certain life stages are particularly vulnerable [3], the life history characteristics of species with less capacity to adapt [4], and the effect of multiple, interacting stressors [5].

One area which has received considerable attention is the role of phenotypic plasticity in helping organisms cope with rapid, unprecedented environmental change [6]. In brief, phenotypic plasticity is the capacity of a genotype to produce multiple phenotypes in response to varying environmental inputs [7]. It is a compelling mechanism for climate change adaptation because it allows for responses at a rapid timescale, compared to the pace at which random genetic mutations would arise by chance. There is tremendous complexity in how plasticity results in responses to novel or rapidly changing environments – including adaptive and non-adaptive processes – by shifting trait means towards an optimum, or by exposing genetic variation [8,9]. Theoretical models on plasticity and environmental change, often agnostic of underlying mechanisms, have highlighted how these different processes can lead to population responses such as adaption versus extinction [6,10] and the conditions favouring the evolution of plastic responses [11,12]. Meanwhile, empirical studies face challenges of testing these predictions [13,14], due to factors such as the complexity of the environmental variation (and its dual role in inducing a phenotypic response and imposing selection [15]), the physiological response of the organism, whether sufficient power exists to measure changes in mean and variance of traits, whether responses are reversible or not, and effects across generations.

To gauge the potential of these processes for responding to environmental change, it is important to understand the underlying mechanisms that link environmental input through to phenotypic response [16,17]. Over the past few decades, there has been growing interest in the role of epigenetics as the driving force underlying population responses to environmental change (including plasticity) [18–23].

(i) Epigenetic mechanisms: a brief primer

Animal cells have evolved diverse and sophisticated epigenetic mechanisms to manage several critical cellular functions, including: 1) precise gene regulation during development and cell-type differentiation, 2) suppression of potentially harmful transposable elements (TEs), and 3) establishment of three-dimensional genome organization [24]. Here, we focus on transcriptional epigenetic processes (DNA methylation and histone modification), rather than post-transcriptional mechanisms (small RNAs) which have also gathered interest but are covered elsewhere [25,26].

One of the most intensively studied epigenetic processes is DNA methylation, whereby methyl groups are attached to cytosines and thus render areas of the chromosome (genes themselves, or promoters) inaccessible to transcriptional machinery [27,28]. DNA methylation can be complex, with methylation occurring at multiple base residues: however, for clarity, throughout this review we will refer to DNA methylation as 5mC (5-methylcytosine), the most commonly studied form, which refers to methylation occurring at cytosine residues in CpG dinucleotides. This process is catalysed by a group of enzymes called the DNA methyl transferases (DNMTs). 5mC is one of the most stable epigenetic marks, often associated with long-term gene silencing particularly at TEs, imprinted genes and CpG-rich promoter regions [24,29].

A second, major epigenetic process comes in the form of histone modifications, including methylation, acetylation, phosphorylation, and ubiquitination of specific amino acid residues, which create a complex "histone code" that dictates chromatin accessibility and transcriptional activity [30]. Histone modifications can alter the expression of nearby genes and, through changes in nuclear organisation, influence gene regulation over long-range genomic distances [31]. For example, heterochromatin formation, characterized by dense packaging of DNA around histones and enrichment of repressive marks like H3K9me3, not only silences repetitive sequences and transposons [32] but also organizes the genome by tethering specific chromosomal regions to distinct nuclear compartments, that influence gene expression of spatially clustered regions (e.g., transcriptional factories) [31].

(ii) Epigenetics under climate change: sensitivity, buffering and variability

While the primary functions of epigenetic marks are implicated in cellular differentiation and maintaining normal development, accumulating evidence suggests that epigenetic marks are sensitive to external environmental inputs. This sensitivity raises the intriguing possibility that epigenetic marks represent a mechanism through which plasticity can be achieved [33,34]. For example, work in isogenic model organisms (mouse, *C. elegans*) has highlighted the multiple epigenetic pathways through which biotic and abiotic conditions experienced across development can trigger epigenetic changes that alter gene expression and cause subsequent phenotypic change, with some of these changes persisting for several generations [35,36]. Moreover, there have been many observations of population-level differences in epigenetic marks, namely DNA methylation (5mC), between locally adapted wild populations across diverse taxa [37–43], often correlated with local abiotic environmental conditions (*e.g.*, salinity, temperature, latitude), suggestive of a potential role in adaptation to changing environments.

These studies provide compelling evidence that epigenetic marks play a key role in key environmental challenges currently facing the planet. The idea is appealing because unlike genetic mutations that emerge spontaneously and then spread through the population over multiple generations, epigenetic modifications can be rapidly established across the entire population, maintained across cell divisions, and potentially reversed, providing cells with a flexible regulatory system that can adapt to changing conditions while preserving genomic integrity. How the epigenetic mark responds to the environmental input and subsequently

affects phenotype is key for our understanding of its role in climate change adaptation. First, epigenetic marks can reflect environmental sensitivity [15,44] as explained above. Second, as for epigenetic marks primarily engaged in core cellular functions, such as genome stability and cell-type identify, their capacity for environmentally induced modification is limited. In this case, observed environmental effects may reflect developmental noise rather than functional adaptive responses, and their role in environmental responses would be through facilitating robustness early in life and buffering development in the face of environmental perturbations [45,46]. Third, epigenetic marks can arise independently of environmental input, through stochastic processes [47], and thus provide increased variability in phenotypes across the population that could increase or reduce adaptive potential, depending on factors such as their stability [48].

(iii) Emerging field of environmental epigenetics and a roadmap for future work

The potential for epigenetic responses to mediate environmentally induced phenotypic change in natural populations has been the subject of growing research attention [18–23], yet broad patterns are challenged by increasing appreciation of the complexity inherent in studying these mechanisms. Much of this complexity has been brought to light by the recent proliferation of genomic datasets from non-model organisms. Understanding how epigenetic marks facilitate adaptation to environmental change requires careful consideration of, first, functional aspects of epigenetic marks (which likely vary between systems), second, the way in which the epigenetic responds to the stressor – if at all – and what role it may play in population response (increased sensitivity, buffering, producing more variability), and, third, the persistence of the epigenetic response through to later life stages or across generations. Moreover, what constitutes environmental change can be fraught with complexity, as several interacting factors – some more challenging to measure than others – are often changing concurrently, such as, temperature, pollution, anthropogenic habitat loss and so on.

Our aim in this review is to present a broad overview of the potential for epigenetic studies for understanding how animal populations will respond to climate change, with a focus on the challenge of making generalisations across taxonomic groups, and of linking approaches from molecular biology with evolutionary ecology. We highlight the key insights to be gained from considering specific epigenetic mechanisms, how these vary in functionality across taxa, how they depend on genetic background, life stage exposure and persistence of response. We also present the importance of different approaches to understanding the issue – from in-depth molecular analyses to longer term experimental evolution and studies on natural populations. We attempt, as much as possible, to draw examples from a wide range of animal species, including vertebrates and invertebrates, and laboratory and field studies. Finally, we propose a roadmap for researchers setting out to conduct studies on this topic.

2. Taxonomic diversity of epigenetic marks and their functional roles

Recent advances in genome sequencing have enabled researchers to explore epigenetic responses to environmental change across a taxonomically diverse range of organisms,

moving the field beyond the reliance on laboratory model organisms. As this research has expanded, we have also started to appreciate the substantial taxonomic variation in epigenetic systems that exist across the tree of life. In this section, we highlight this taxonomic variation and its implications for interpreting interactions between the environment and epigenetic responses. Specifically, we focus on: (i) *which* epigenetic marks are present and prevalent (e.g., DNA methylation versus histone modifications), (ii) *where* these marks occur in the genome (e.g., gene bodies, transposable elements, or promoters), and (iii) *when* they are established or modified during development (i.e., reprogrammed).

(i) Which epigenetic marks are present and prevalent?

(a) DNA methylation (5mC)

Across the tree of life, 5mC has been gained and lost several times with significant functional consequences [49,50]. Whilst mammals possess 70-80% genome-wide 5mC coverage, holometabolous insects with retained 5mC systems exhibit much sparser coverage (1-12%) [51–53]. However, there is significant variation amongst almost every group [51,52,54]. For example, 5mC has been completely lost in *Drosophila* and *C. elegans* due to loss of DNA methyltransferase (DNMT) genes, a pattern shared by several other insect lineages [51,52]. The epigenetic machinery, including DNMTs, has likewise experienced substantial evolutionary diversification [52,53,55]. Beyond these quantitative differences, the genomic distribution and functional targets of 5mC vary dramatically across taxa. In mammals, most of 5mC silences transposable elements, with the remainder distributed at gene bodies, promoter regions and CpG-rich islands [24,29,32]. Conversely, the sparse methylation in insects and other athropods is primarily restricted to gene bodies of highly expressed housekeeping genes, representing approximately 35% of protein-coding genes [51–53]. This variation, reviewed extensively elsewhere [51,52,54], has important implications for how epigenetic mechanisms influence gene expression, phenotype and behaviour.

There have been several recent comprehensive reviews cataloguing the diverse effects of environmental variables on DNA methylation patterns across diverse taxonomic groups [18,21,56–58]. A recurring theme in these studies has been the difficulty of associating changes in 5mC with changes in gene expression and other phenotypic consequences across a broad range of species, including vertebrates and invertebrates [39,40,42,53,59,59–67]. This may be due, in part, to technical limitations in detection, species-specific functional differences (Section 2ii) or the competing effects of genetic variation (Section 3), though it may also reflect the complex biology of these marks. The lack of concordance between a change in 5mC and neighbouring gene expression may suggest that the regulatory effects 5mC are highly gene-, tissue- or cell-type specific (or a combination thereof). Moreover, the functional consequences of 5mC likely depend on the genomic context (promoter versus gene body regions, CpG-rich islands), and these functions are likely to vary across taxa (discussed further in Section 2ii).

Another important consideration is that 5mC may not work alone, and rather works in concert with other regulatory features of the genome (chromatin modifications or other post-

transcriptional processes) [68–70]. For example, a recent study examined the epigenetic basis of smoltification in salmon (*Salmo salar*), wherein juveniles undergo behavioral and physiological changes to prepare for seawater migration. The authors found that change in chromatin accessibility and transcription factor networks, rather 5mC, were associated with extensive gene expression changes underlying this environmentally-sensitive developmental transition [69]. Thus, in some cases, 5mC might follow rather than drive expression changes initiated by other epigenetic mechanisms. This does not necessarily mean that 5mC serves no regulatory role and could still serve as an important biosensor of environmental change, but demonstrating causal links between methylation changes and functional phenotypic outcomes remains a critical challenge for future studies (discussed further in Section 4).

(b) Histone modifications

While patterns of 5mC vary significantly across taxa, histone modifications show remarkable conservation suggesting they represent fundamental mechanisms for gene regulation [67,68]. This distinction is exemplified by transposable element (TE) silencing mechanisms: mammals employ both DNA methylation and H3K9me3 histone modifications to silence TEs, whereas insects rely predominantly on H3K9me3 for this function [32,53]. Moreover, other core histone marks like H3K4me3 (at active promoters), H3K27me3 (for Polycomb-mediated silencing), and H3K36me3 (at transcribed gene bodies) maintain remarkably similar regulatory roles across taxonomic groups, underscoring the fundamental conservation of chromatin-based gene regulation [24,71–73].

This functional conservation has prompted investigation into whether histone modifications are better predictors of organismal stress responses across diverse taxa. While the effects of climate stress-related variables (e.g., heat, drought, salinity) on histone modifications have been extensively studied in plants [74], emerging work reveals similar patterns across animals [70,75,76]. For example, early-life heat stress in *C. elegans* triggers histone acetylation via the chromatin SW1/SNF complex leading to long-lasting defence responses and extended lifespan [76]. Accumulating evidence demonstrates that histone states could prime organismal responses to stress. In laboratory mice, early-life stress establishes region-specific chromatin modification patterns in the brain. Individual differences in these modifications distinguish stress-resilient from stress-susceptible individuals, with susceptible mice exhibiting exaggerated responses to subsequent stressors [77,78]. Similarly, in the honeybee species complex, baseline histone methylation at heat shock protein genes distinguishes thermo-tolerant (*A. m. jemenetica*) from thermo-susceptible (*A. m. carnica*) subspecies, with susceptible bees showing exaggerated chromatin responses to heat stress [79]. These findings suggest that histone modifications may serve as more functionally conserved indicators of stress resilience across taxa than DNA methylation, despite the greater technical challenges of profiling them (discussed further in Section 4).

(ii) Where does the epigenetic mark feature in the genome?

Work in vertebrates has highlighted some of the complexities of linking changes in 5mC to functional changes in gene expression and subsequent phenotype. This is due, in part, to whether methylation occurs in promoters, gene bodies, or repetitive regions and these

patterns vary substantially across taxa (Section 2(i)). This taxonomic variation can complicate efforts to predict when environmental changes will produce epigenetic responses with phenotypic outcomes. Here we examine two contrasting scenarios to highlight the importance of genomic context in the interpretation of epigenetic studies: (a) gene body methylation in insects, where the functional role of 5mC remains debated, and (b) transposable element silencing, where epigenetic marks appear to serve a conserved function across taxa

(a) Case of gene body methylation (GBM)

Until recently, most studies linking environmental exposures to epigenetic changes assume conserved functional roles for 5mC, focusing on the role of 5mC in silencing gene expression and repetitive elements (see Section 2i). As mentioned earlier, most holometabolous insects appear to contain low levels of methylation restricted to gene bodies, often termed, gene body methylation (GBM), though there appear to be potential exceptions [51–53]. Despite these low levels of methylation, several studies have shown that levels of 5mC in insects change in response to various exposures, including ocean and atmospheric warming and life-history factors [39,40,42,59,60]. However, many of these changes were not correlated with changes in gene expression, suggesting that the functional consequences of changes in 5mC appear to depend on genomic context. Whereas changes in 5mC at CpG-rich promoters can prevent transcription factor binding and reduce levels of transcription [80,81], GBM on the other hand appears more important for stabilising the transcription of highly expressed genes and preventing transcriptional noise [33,82]. Consistent with this, using burying beetles (*N. vespilloides*), we showed that sustained parental care regimes (loss versus presence) was associated with changes in the methylation status of several genes. While changes in the methylation status of genes were not associated with changes in the level of gene expression, they were, however, associated with reduced gene expression variability [59]. This suggests that epigenetic responses to environmental change may function not only through altering gene expression levels, but also by modulating transcriptional stability, raising the possibility that these marks could promote phenotypic buffering in changing environments [83,84].

Given the sparse levels of 5mC in insects, environmental stressors may have effects on phenotype through epigenetic pathways (such as DNMTs and associated methylation machinery) independent of 5mC. For example, despite showing little to no 5mC, maternal DNMT knockdown in the red flour beetle (*Tribolium casteneum*) produces embryos that fail to develop [85]. Studies on several insect species have shown that knockdown of DNMTs perturbs oocyte development and fertility despite little impact on levels of 5mC, leading some to argue that 5mC is only retained in insects because the DNMTs are indispensable [86–89]. Expression of DNMTs can also be temperature sensitive [90,91]. For example, in the seed beetle (*Callosobruchus maculatus*), DNMT expression was positively correlated with increasing temperatures. Pharmacological manipulation of DNMT activity using 3-aminobenzamide (3-AB) and zebularine partially reversed temperature-associated reductions in fecundity and offspring viability in the F1 and F2 generations, but only under specific temperatures [91]. Although these two drugs are generally considered to have

opposing effects on DNA methylation, their shared ability to improve fitness-related traits at elevated temperatures suggests that the observed responses may reflect broader perturbations of epigenetic and DNA maintenance pathways. While these findings remain preliminary, they raise the possibility that DNMTs could be potential targets for interventions (transgenic or pharmacological) aimed at maintaining fecundity under climate stress in insects, though the mechanistic basis of this relationship requires substantial further study.

(b) Case of repetitive regions of the genome

Transposable elements (TEs) comprise mobile DNA sequences capable of copying or moving to new genomic locations, though most copies in contemporary genomes are inactive remnants of past insertion events [92]. They constitute diverse proportions of genomes, ranging from less than 10% in some compact fish and insect genomes to over 85% in some plant genomes [92]. Epigenetic mechanisms, such as 5mC and chromatin modifications (namely H3K9me3) help suppress TE activation and potentially deleterious transposition events [32,92]. However, TEs are also rich in promoters and enhancers, and epigenetic de-regulation at these sequences can influence neighbouring gene expression and alter local chromatin organisation [92–94]. The epigenetic marks on or around these repetitive regions also appears to be particularly sensitive to environmental stressors [32,93,94].

One of the classic examples demonstrating that 5mC is environmentally sensitive comes from studies of loci that are derived from transposable elements: intracisternal-A particle (IAP) elements. When an IAP is inserted into an exon of the *agouti* gene (A^{vy}), interindividual differences in the methylation status of this IAP result in a range of phenotypic outcomes including coat color pigmentation and predisposition for obesity [95,96]. Importantly, 5mC levels at this IAP are sensitive to environmental cues, such as nutritional supplementation with methyl donors or bisphenol-A (a pollutant derived from plastics), resulting in phenotypic variation that is inherited through the gametes [95–97]. Whilst such loci might be more specific to vertebrates, there is accumulating evidence across taxa demonstrating that environmental challenges, including temperature stress, pollution, and pesticide exposure, can alter epigenetic states at TEs, with downstream effects on neighbouring gene expression and even movement of TEs themselves [93,94,98–100]. For example, in insects, pesticide resistance and stress resistance, have arisen though novel TE insertions that have become fixed in the population [101,102]. Such effects can be achieved by activated TEs becoming co-opted into novel regulatory elements (e.g., transcription factor binding sites) or by spreading epigenetically modifiable sequences to neighbouring genes and shaping the subsequent chromatin environment, which can further rewire gene regulatory networks [32,92]. The extent to which this occurs depends on the origin and evolutionary history of species-specific TE loads. However, given their capacity to bridge short-term transcriptional responses and long-term evolutionary change, environmentally sensitive TEs warrant closer attention, particularly in taxa with highly active TE loads such as invertebrates [103,104].

(iii) When do marks change, and do they persist?

A final consideration for the taxonomic variability of epigenetic responses is the speed at which the response occurs, and how long it persists both during development and across

generations. In some cases, changes could persist for multiple generations independent of continued environmental exposure, while others may represent transient responses. Indeed, it is arguably the responsiveness of these epigenetic processes that have generated interest in their role as key facilitators to rapid environmental change. However, key questions remain about the persistence and stability of these marks, both within and across generations.

(a) Epigenetic reprogramming as a barrier to transgenerational persistence

DNA methylation is attractive as a possible mechanism underlying environmentally-induced epigenetic change due to the well-established mitotic heritability of DNA methylation patterns that are maintained through DNA replication and cell division [24,29], suggesting these marks could be stable across the lifespan. There is plenty of evidence suggesting that stressors applied early in life have stable effects across the lifespan (Section 2i). Epigenetic resetting, however, which is common in vertebrate development, imposes a major constraint on the heritability of epigenetic marks. This is because any acquired marks would be erased in the early embryo (during meiosis and at fertilization) [105]. When epigenetic resetting occurs, it is to ensure proper sex-specific and cell-lineage specific placement of epigenetic marks in the totipotent zygote [105]. Thus, some have argued that this constrains the window for establishing heritable epigenetic effects in vertebrates to exposures occurring during primordial germ cell development, when methylation patterns are being established in the embryo [106].

There are differences in the degree and timing of epigenetic reprogramming across taxa [51,105,107]. In the zebrafish (*Danio rerio*), the paternal methylome largely escapes reprogramming, whereas the maternal methylome persists until later stages of zygotic development, until it is eventually reprogrammed [108]. In contrast, in the honeybee (*Apis mellifera*), gene body methylation appears to be stably inherited across generations and shows no evidence of reprogramming [109], similar to findings in corals [40]. In other invertebrates, such as echinoderms and tunicates, DNA methylation shows locus-specific reprogramming, occurring at gene promoters but not uniformly across the genome, suggesting that reprogramming depends on both genomic location and functional context in gene regulation and development [110].

Even in species with extensive reprogramming, there are some loci that manage to escape reprogramming events (e.g., imprinted genes and IAP elements derived from repetitive regions). For example, the A^{vy} and $Axin^{Fu}$ locus, escape reprogramming events in the mouse, allowing interindividual differences in methylation to persist across generations [95,111]. To test whether this pattern extended beyond these well-characterised examples, genome-wide screens identified additional similar loci in the mouse genome capable of transmitting interindividual differences in 5mC across generations [112,113]. However, unlike A^{vy} and $Axin^{Fu}$ loci, the majority of these newly identified loci were insensitive to environmental perturbations (e.g., bisphenol-A or maternal diet supplementation), suggesting that loci capable of both escaping reprogramming *and* responding to environmental cues may be rare exceptions [113]. Indeed, there is generally minimal

evidence that acquired 5mC is transmitted across generations [114,115]. This is perhaps why post-transcriptional silencing mechanisms, such as small RNAs, transmitted through the germline represent a potentially more viable mechanism for transgenerational inheritance in vertebrate species [114,116], although it remains unclear if this is a viable mechanism in all species given the diversity in small RNA systems across taxa [26,117].

(b) Life history modulates transgenerational epigenetic potential

Reproductive and other life history characteristics are likely important determinants of whether epigenetic marks are likely to persist across generations [115,118]. Animals that reproduce clonally may be more likely to transmit epigenetic information across generations, as has been reported in a freshwater snail (*Potamopyrgus antipodarum*), *Daphnia* and *C. elegans* [35,43,119,120]. For example, in *Daphnia magna*, studies have shown that environmentally induced 5mC can persist across at least four generations [119]. Interestingly, such persistence occurs for more naturally occurring environmental stressors (such as zinc, temperature) and not the artificial approach of a de-methylating drug. Similarly, work in *C. elegans* has demonstrated that somatic histone methylation affecting longevity can be transmitted to the germline and inherited across generations, indicating that histone modifications may sometimes also escape reprogramming [121]. While these are compelling examples, the generalisability and selection for such epigenetic memory across species is likely to depend on species-specific life-history properties. For example, while reproductive mode clearly influences the likelihood of transgenerational inheritance, other life-history traits (e.g., social structure or parental investment) may also modulate how environmental signals are translated into epigenetic signals. In laboratory mice, the quality of maternal care environment can buffer the direct environmental impacts of epigenetic marks and phenotype — effects that are only detectable with experimental designs that account for this buffering [115,122,123]. Thus, for most species, short-term changes in epigenetic marks may alter phenotype in ways that affect the immediate response to environmental change, but for species with extensive epigenetic reprogramming these effects would be transient and require recapitulation each generation from continued exposure, more closely resembling a plastic response.

3. Sources of epigenetic variation in natural populations

Beyond taxonomic variation in epigenetic mechanisms and the technical challenges of measuring them, a fundamental question remains: what role do epigenetic changes play in adaptation to environmental stress? Answering this requires disentangling whether epigenetic differences between natural populations reflect direct environmental induction, underlying genetic divergence, or their interaction.

(i) Bidirectional relationships between genetic and epigenetic variation

Epigenetic and genetic processes may operate as complementary systems functioning across different timescales. The rapid responsiveness of epigenetic marks relative to the mutability of DNA sequence suggests they may facilitate immediate responses to environmental change or enable phenotypic change within a generation), while genetic

variation provides the substrate for longer-term evolutionary adaptation [124]. Moreover, these systems may be bidirectional: epigenetic states can influence the generation of genetic variation itself, as well as being under genetic control. DNA sequence variants can affect the establishment or maintenance of epigenetic marks at linked loci (*cis*-effects) or genome-wide (*trans*-effects). For example, single nucleotide polymorphisms within CpG sites prevent methylation at those positions, while variants in genes encoding DNA methyltransferases or chromatin modifiers can alter epigenetic landscapes across the genome [125–127]). There is also evidence that methylation marks at cytosines can increase C to T mutation rates through spontaneous deamination, meaning highly methylated regions could accumulate mutations faster than unmethylated regions [128,129]. Chromatin states can also affect mutation rates because open chromatin is more accessible to mutagens while condensed heterochromatin may experience reduced repair efficiency [130]. Additionally, loss of epigenetic silencing can activate transposable elements, generating insertional mutations and chromosomal rearrangements (Section 2ii). Thus, environmentally-induced epigenetic changes could alter the mutation landscape, potentially accelerating genetic adaptation in genomic regions where epigenetic marks have shifted in response to environmental stressors. A key question is when and how these relationships work in natural contexts.

(ii) Epigenetic differentiation in natural populations: challenges of attribution

Comparisons of natural populations that have diverged in response to environmental conditions reveal compelling epigenetic differences [131], though disentangling their causes remains challenging. In the Atlantic molly (*Poecilia mexicana*), populations have adapted to toxic hydrogen sulphide-rich springs and show stable difference in blood 5mC in genes related to sulphur metabolism and toxicity [132]. When these fish were bred in the lab for two generations, their grand offspring retained 80% of their differentially methylated regions characteristic of their wild-derived sulphide-adapted grandparents, demonstrating stable inheritance of epigenetic differences despite removal of the environmental stressor. However, the extent to which these reflect spontaneous epigenetic differences (i.e., epimutations) is currently unknown. These populations of *P. mexicana* studies are also genetically differentiated, having diverged approximately 10,000 years ago with an average F_{ST} of 0.11, and thus, differences in 5mC may have emerged through DNA sequence change [133]. Indeed, in sticklebacks (*Gasterosteus aculeatus*), some but not all population-level variation in DNA methylation is correlated with plasticity-induced by differences in water salinity [134] and some of these methylation changes have been linked to *cis* or *trans*- acting loci which show evidence of local adaptation [135]. Other studies have failed to find strong associations between observed epigenetic marks and genetic sequence [41,59,136,137]. However, many of these studies are likely to be underpowered, given that genome-wide association studies typically require thousands of samples [138]. Regardless, these mixed results likely reflect the multiple pathways through which epigenetic differences arise in natural populations, which often act simultaneously.

Several studies using clonal populations suggest that epigenetic effects might be more likely to occur when genetic variation is limited [35,43,119], thus, potentially allowing for

phenotypic adaptation in the absence of standing genetic variation. For example, American populations of the mud snail (*Potamopyrgus antipodarum*) originated from a single clone have adapted to different aquatic environments (rivers versus lakes) despite limited genetic diversity, and these adaptations are associated with distinct DNA methylation patterns [43]. More recent evidence suggests that epigenetic marks may contribute to maintaining two recently diverged ecomorphs of the cichlid fish, *Astatotilapia calliptera*, in the crater lake Masoko which show almost no genetic differences but are characterised by different diets, morphologies and lake depth preferences [37]. Whether epigenetic changes truly play a larger role in scenarios with low genetic diversity or are simply easier to detect statistically when genetic noise is reduced remains unclear. This may also explain why some of the most pronounced examples of environmentally-induced epigenetic effects come from inbred model organisms (section 2). These examples collectively illustrate the fundamental challenges of working with natural populations that make it difficult to separate the causal role of any change in epigenetic marks, while at the same time acknowledging that studying these processes in the wild is key for understanding of the role of epigenetics in climate change adaptation. Resolving this requires additional experimental approaches that can establish the time course, stability and source of epigenetic effects (discussed further in Section 4) [139].

4. A roadmap for future studies

Throughout this review, we have discussed how taxonomic diversity, genetic architecture and other factors shape the functional consequences of epigenetic marks in the potential response to environmental stressors. While this complexity requires careful consideration, it also opens doors for meaningful discovery. Thus, in this final section, we offer practical suggestions for researchers planning studies in this area (see Figure 1). These suggestions are intended as flexible guideposts that can be adapted to different systems and contexts. We conclude by highlighting how interdisciplinary collaboration can facilitate turning these complexities into opportunities for discovery in this fast-moving field.

(i) Deciding what epigenetic mechanism to measure, and in what species

Choosing which epigenetic mark(s) to measure depends on technical feasibility, known mechanistic links between the specific marks, the environmental challenge, and practicalities of the study organism. For example, 5mC is commonly measured because of its stability, but care should be taken when generalising from mammals to insects, where 5mC may serve different gene regulatory functions (Section 2i). Even in closely related taxonomic groups, there can be variation in overall levels and distribution of 5mC, which also affects the type of 5mC assay to use [140]. There have been relatively fewer studies on how histone modifications respond to climate stress, although some interesting examples are emerging [70,75,76]. This delay is likely due to the technical hurdles often posed by working with fresh nuclei and optimising species-specific antibodies for immunoprecipitation in non-model organisms. However, universal antibodies for chromatin modifications are becoming more readily available (e.g., Active Motif) making these assays much more possible than before. Additionally, broad chromatin accessibility assays (e.g., ATAC-seq) can distinguish dynamic

open (euchromatic) from closed (heterochromatic) regions genome-wide without requiring measurement of specific histone modifications, offering a more accessible entry point for non-model systems [141].

Key considerations here are: (1) is there a genome available for the study organism and how well annotated is it? (2) Are there previous functional studies on the epigenetic mark in question: what is its role under control conditions, how might it change depending on environmental stress (i.e.: act as a buffer, be environmentally sensitive, or increase variability)? Encouragingly, as genome sequencing becomes more affordable and accessible, coupled with rapidly expanding genomic resources, systems that are currently inaccessible for such studies will become increasingly tractable.

(ii) Controlling or measuring the underlying genetic background

Epigenetic responses depend not only on the mark itself and the taxonomic group considered, but also on the genetic background of the population (section 3). Many experimental epigenetic studies are conducted on clonal systems or highly inbred lines, which may amplify the detectability of epigenetic changes and overestimate their contribution to phenotypic change in response to environmental variables. Thus, the presence of background genetic variation can confound the detection of epigenetic effects, whilst extreme inbreeding may amplify the relative importance of epigenetic changes in the absence of other mechanisms.

For researchers working with natural populations or outbred laboratory strains, several approaches can help disentangle genetic and epigenetic contributions (see also [22,142]): (1) estimate genome-wide variation or inbreeding statistics and accounting for it in analyses (using whole-genome sequencing or through reads from epigenetic sequencing protocols [41,135,143] (2) use family-based designs (common of quantitative genetic studies) or common garden experiments where related individuals share genetic backgrounds but experience different environmental treatments [59,59,144,145]; (3) for field-based studies, reciprocal transplant and cross-generation designs would allow for teasing apart epigenetic changes with stable genetic variation over multiple generations [39,146].

(iii) Selecting environmental challenges to measure or manipulate, in light of ecological relevance and known functional relationships

The nature of the research question and study system will determine whether an observational approach in natural populations or an experimental approach under controlled laboratory conditions is most appropriate. Particularly for an experimental system in the laboratory, researchers will face the challenge of selecting ecologically relevant stressors within a range of current or predicted conditions, to test responses under realistic climate change scenarios. This might depend, for example, on an understanding of the environmental gradient experienced in the organism's natural habitat – which is often hard to ascertain, particularly for those well-studied lab systems where their ecology is not well characterised (e.g. *C. elegans*, Drosophila).

Climate change encompasses a suite of interacting stressors, including temperature, humidity, pollutants, pesticides, habitat loss and other factors, that rarely change in isolation [5]. Deciding which factors to measure or manipulate, requires understanding the mechanistic links between the environmental exposure, the internal physiological response and the potential epigenetic change, and how these interact. While it may not be practically feasible to measure or manipulate all components of the environment, decisions should be guided both by biological meaningfulness as well as feasibility.

(iv) Deciding which life stage to impose the environmental stress and to measure the molecular and phenotypic response

It is widely accepted that certain periods of development, often early in life, exhibit heightened sensitivity to environmental stressors [147,148]. As such, deciding at what point the environmental exposure is measured or imposed (for observational or experimental studies, respectively) and when the outcome is measured can have significant implications for the interpretation of results. Temporal dynamics of exposure and response will also depend on ecology and life-history. Related to this, some epigenetic marks are maintained across meiosis while others are reset and reprogrammed between generations in some taxa (Section 2iii). Changes in DNA methylation at birth may indeed be linked, for example, to maternal stress in utero [149], but if these do not remain stable into adulthood, the consequences of this epigenetic response may be limited or transient. Hence, timing of measurements can be critical – not only for detecting epigenetic responses to environmental challenges but also for distinguishing stable heritable changes from transient developmental plasticity.

(v) Considering whether responses are measured in specific tissues or across the whole organism

While studies often tend to measure bulk DNA methylation or epigenetic marks from accessible tissue types (e.g. blood), many epigenetic effects are likely to be tissue- or even cell type-specific. This creates a trade-off between what is practically feasible and what is biologically meaningful to measure. For example, blood samples may be convenient and non-lethal, but if the environmental stressor primarily affects reproductive tissues, neural function, or metabolic organs, then blood-derived epigenetic data may miss the most relevant changes. Indeed, tissue specific studies exist and are useful [150–153]. However, in many field studies or with protected species, such targeted tissue sampling may not be feasible. In these cases, researchers should acknowledge this limitation and interpret results accordingly. Accessible tissues can still provide valuable population-level signals or serve as proxies [153–155], but may not capture the full extent of tissue-specific responses and require additional cross-validation [153,156]. In laboratory studies, however, single-cell epigenetic profiling [157] offers exciting potential to resolve cell type-specific patterns within heterogeneous tissues, but remains technically challenging and cost-prohibitive for most ecological studies. As these technologies become more accessible, they will enable finer-scale resolution of how environmental stressors affect specific cell-types or tissues to produce phenotypic change.

(vi) Functional validation of mechanism to be measured: linking to other 'omic data and phenotypic responses

Understanding the mechanistic roles of epigenetic marks requires us to move beyond simple sequencing correlations into functional genomics where these marks and their associated enzymes can be manipulated. While the methods for editing the epigenetic marks themselves remain relatively new [158], both RNAi expression knockdown and CRISPR-Cas9 based genome editing techniques are becoming increasingly routine in non-model organisms [159–161], offering promising avenues for testing the functional role of epigenetic systems across taxa. Where the genetic tools for knocking out or editing particular genes and/or their marks may not be feasible, functionality can also be assessed using pharmacological inhibitors or provisioning individuals with dietary methionine, to experimentally manipulate levels of DNA methylation or other epigenetic marks [162]. Traditional evolutionary paradigms (e.g., experimental evolution, artificial selection) in conjunction with whole-genome sequencing could also be useful in testing functional outcomes of changes in epigenetic marks within or between closely related species [59,163].

Given evidence that epigenetic mechanisms can be interactive [164], studies that measure multiple epigenetic marks simultaneously (e.g., DNA methylation alongside histone modifications or small RNAs) can provide richer mechanistic insights than single-mark approaches. Similarly, pairing epigenetic measurements with gene transcription data is essential for establishing functional outcomes, particularly in species where these relationships are not well characterized—which is often the case in environmental epigenetic studies. Multi-omic approaches that integrate epigenetic, transcriptomic, and other molecular datasets offer the most comprehensive view of how organisms respond to environmental change (e.g., [63]). Such approaches can span multiple scales, from whole-organism methylation profiles to single-cell resolution measurements, enabling researchers to connect population-level patterns to mechanism. While technically demanding and costly, these integrative studies are becoming increasingly necessary for revealing the functional properties of these marks and identifying reliable biomarkers.

(vii) Interaction between empirical studies and theoretical model development

Finally, epigenetic studies of population responses to climate change should be interpreted against theoretical models that predict when and how such changes might be adaptive. Here, the current gap between detailed molecular research and models agnostic of mechanism can create challenges, and future approaches bringing theoreticians and empiricists together can help bridge the gap between the generation of such models and how to test them experimentally (e.g., [91]).

Three key aspects of models could benefit from input from molecular biologists. First, the epigenetic mark itself is often considered in an abstract way, without explicit consideration of the actual mechanism involved (e.g. 'level of maternal input', [165]). How might predictions change if a specific epigenetic mechanism (e.g., gene-body methylation) is modelled? Second, the phenotype and how it is selected is often considered in simple terms, for example a binary trait where selection involves a lower proportion of 'mismatched'

individuals surviving their environment [44]. The phenotype under selection is a composite of several complex traits, and measuring the strength of selection, or indeed fitness, can be challenging when only proxies are available. Third, how the environment is modelled and how it changes within and across generations is also simplified: flipping between two states, for example. We acknowledge the benefits of simplifying assumptions to make models tractable and provide general insights. However, this can present challenges for empiricists testing model predictions. Bringing insights from ecological and experimental data on the exact environmental stressors (e.g. temperature versus humidity) with longer term field data on environmental variation within and across populations (e.g. [166]), can help parameterise environmental variation in models more realistically, and allows for closer integration between empiricists and theoreticians.

5. Conclusions

While the field of ecological epigenetics has generated considerable interest and enthusiasm around the potential for epigenetic variation to facilitate rapid environmental responses, key questions remain: (i) What is the relationship between epigenetic changes and phenotypic outcomes? (ii) How do environmentally induced epigenetic changes persist across generations under natural conditions? (iii) What is the relative contribution of epigenetic versus genetic variation to phenotypic responses in wild populations? (iv) Under what circumstances do epigenetic mechanisms respond faster than selection on standing genetic variation? By taking stock of evidence across taxa and epigenetic mechanisms, this review highlights where progress has been made and where targeted advances are still needed.

Perhaps one of the biggest challenges facing this field is that we are rapidly accumulating data but lacking comprehensive conceptual frameworks in which to interpret these data. Answering the fundamental questions requires moving beyond correlative observations and towards studies that establish causal mechanistic links and quantify fitness consequences. Rather than seeking a unified framework applicable across all taxa and contexts, progress will come from targeted approaches that address the mechanistic, taxonomic, and life-history complexity we have detailed throughout this review. The path forward requires careful evaluation of existing evidence alongside continued empirical work, ensuring that interpretations remain grounded in demonstrated mechanisms. Declining sequencing costs and multi-omic integration now make these rigorous approaches increasingly feasible. By simultaneously examining genetic, epigenetic, and transcriptomic variation within well-designed experimental and theoretical frameworks, researchers can address pressing questions about how populations respond to rapid environmental change. These are not tasks for lone researchers but ones that call for interdisciplinary collaboration.

6. References

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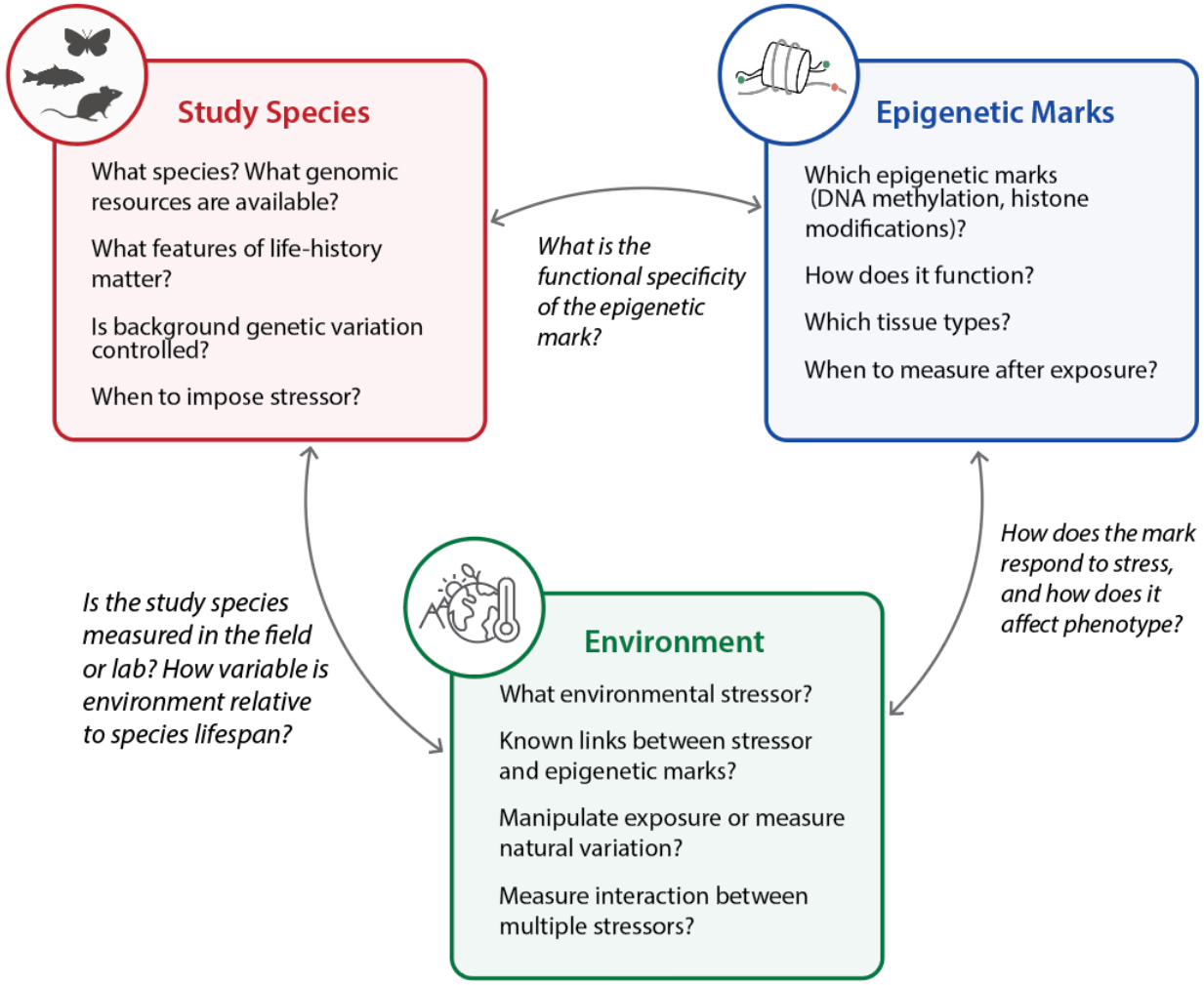

Figure 1. Key considerations for designing studies in ecological epigenetics. Robust experimental design requires addressing three interconnected axes—study species, epigenetic marks, and environment—along with the critical questions that arise at their interfaces (arrows).