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The interplay between epigenetic mechanisms and deleterious mutations: implications for fitness, evolution and conservation

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Understanding the causal effects of genetic mutations is essential for explaining fitness variation, forecasting evolutionary trajectories and assessing extinction risk, yet remains a fundamental challenge, particularly in natural populations. While amino acid substitutions can alter protein structure and function, mutations affecting gene regulation can also have significant fitness consequences. In this Opinion Piece, we argue that epigenetic mechanisms, given their central role in gene regulation, probably modulate the deleteriousness of mutations. Drawing on evidence from humans and model organisms, we identify three ways in which epigenetic mechanisms might interact with deleterious mutations. Specifically, we hypothesize that epigenetic regulation may: (i) be disrupted by deleterious mutations in non-coding regions and epigenetic regulator genes; (ii) buffer the expression of deleterious mutations; and (iii) contribute to the repair and purging of deleterious mutations. Advances in next- and third-generation sequencing and bioinformatics now allow these hypotheses to be empirically tested in wild populations. As many species face ongoing population declines, unravelling how epigenetic mechanisms influence the functional effects of mutations is vital for understanding fitness variation, guiding evolutionary predictions and informing conservation strategies.

This article is part of the theme issue 'Ecological epigenetics at the intersection of behaviour and life history variation in non-model animals'.

1. Introduction

Quantifying the functional effects of genetic mutations (box 1) poses a major challenge across medical science, agriculture, evolutionary biology and conservation [1–4]. A mutation can affect a phenotype by changing the amino acid sequence (i.e. a **coding mutation**, see the Glossary for descriptions of the terms highlighted in bold), resulting in altered or non-functional proteins, or by affecting the transcription or translation of a gene, resulting in the disruption of gene regulation (i.e. a **non-coding mutation**) [3,5]. The functional effects of a given mutation further depend on the extent to which it interacts with other genomic features [6].

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Box 1: genetic mutations

The effect of a genetic mutation depends on its genomic context and, when located within a coding region, on its impact on the resulting amino acid sequence. In this Opinion Piece, we use the term *genetic mutation* specifically to refer to single-nucleotide polymorphisms. Although other classes of mutations, such as indels, copy number variations, translocations and inversions, are also known to influence genome function, comparatively little is known about how they interact with the epigenome.

Mutations in coding regions (exons) can either be synonymous if they do not alter the amino acid sequence, or non-synonymous if they do. Mutations in non-coding regions, such as introns, untranslated regions and intergenic DNA, can influence gene regulation by affecting promoters, enhancers or other regulatory elements. In this Opinion Piece, we indicate whether a hypothesis applies to coding or non-coding mutations; if not specified, the hypothesis is assumed to apply to both.

Mutations can also be classified according to their timing and mode of transmission. Somatic mutations arise in body (somatic) cells after fertilization and are not transmitted to offspring [7]. These mutations can influence an individual's health and survival by contributing to cancers, degenerative diseases and ageing. By contrast, meiotic mutations, also known as germline mutations, occur in cells that undergo meiosis to form gametes and are therefore heritable [7]. Occurring before or during meiosis, germline mutations are a major source of inherited genetic variation, shaping both disease susceptibility and evolutionary change. Unless otherwise specified, references to mutations in this Opinion Piece include both somatic and meiotic mutations.

A mutation is considered deleterious if it reduces fitness, either by causing embryonic or premature death (i.e. lethal mutations) or by decreasing survival or reproductive success later in life. Such effects probably arise because of impairments of fitness-relevant traits, such as cognition, metabolic rate, parasite resistance, sexual trait expression, sperm quality and other biological functions [8,9–13]. The severity of these effects may also depend on environmental stressors, such as food limitation and competition. Throughout this Opinion Piece, we use the term *deleterious mutation* broadly to include both mutations that lead to premature death before an individual reaches sexual maturity and sublethal mutations that reduce fitness in adulthood, recognizing that their effect sizes, and consequently the strength of selection against them, can vary.

De novo mutations can be neutral, deleterious or advantageous, and their spread within a population depends on population genetic forces such as selection and drift [14]. The distribution of fitness effects of mutations is influenced by multiple factors, including dominance, epistatic interactions, the environmental context and adaptation [15]. Deleterious mutations (box 1) reduce fitness when expressed and contribute to an individual's **mutation load**. Experimental studies of model organisms, where mutations are induced (chemically or through ionizing radiation) or allowed to accumulate (by propagating inbred or bottlenecked lines under minimal selection for many generations), have demonstrated that deleterious mutations can reduce fitness by affecting physiological performance [16], morphology [17] and the expression of sexual traits [18,19]. Their fitness effects vary from being lethal to having weaker, context-dependent effects later in life, with corresponding variation in the strength of selection acting upon them. A detailed mechanistic understanding of the phenotypic effects of deleterious mutations therefore requires the integration of genomic and fitness data.

More than 60 years ago, Jacob & Monod [20] argued that a perfectly good enzyme could be deleterious if it were synthesized under the wrong conditions. Their work was among the first to emphasize how coding and non-coding mutations could be quantitatively different, with the latter affecting how and when enzymes are transcribed [21]. Expressing the correct genes at the right time and at the appropriate dosage is essential for quantitative traits involved in development [22], morphology [23] and behaviour [24]. Hence, non-coding mutations and other factors that disrupt the fine tuning of gene regulation could disproportionately affect quantitative traits linked to growth, survival and reproduction [21].

Epigenetic mechanisms (box 2; figure 1) influence gene expression without altering the underlying nucleotide sequence [25], making them key players in the fine tuning of **gene regulation** [26]. Examples of epigenetic mechanisms include **DNA methylation**, **histone modifications** and other marks that alter **chromatin accessibility** [25]. Epigenetic regulation primarily operates at the transcriptional level, determining whether a gene is transcribed into messenger RNA (mRNA) and the amount of mRNA produced. Epigenetic mechanisms can also influence other levels of gene regulation including translation, where mRNA is translated into protein, and post-translation, where protein activity, stability and localization are fine-tuned [27,28], for example, through the expression of RNA-binding proteins [29].

Box 2: epigenetic mechanisms

Epigenetic mechanisms are biochemical modifications that alter gene expression without changing the underlying nucleotide sequence. These modifications can influence interactions between histones and DNA, thereby modulating gene accessibility. In eukaryotic cells, DNA is wrapped around histone proteins to form nucleosomes, the basic units of chromatin (figure 1; [30]). Each nucleosome consists of eight histones: two copies each of H2A, H2B, H3 and H4 [31]. The extent to which DNA is tightly or loosely packed around these histones determines the accessibility of genes to RNA polymerase and other transcription factors [32] and thus controls transcriptional activity. When DNA is tightly packed around histones, forming heterochromatin, transcription is generally repressed. Conversely, loosely packed DNA, known as euchromatin, typically permits gene expression (figure 1). However, heterochromatin is not always associated with transcriptional repression, depending on factors such as the developmental state of an organism and the chromosomal location [33].

Histones possess amino acid tails that extend from their core and play a central role in epigenetic regulation [34]. Although these tails do not contain DNA, they serve as targets for various chemical modifications, including methylation, acetylation, phosphorylation and ubiquitination. These modifications collectively regulate DNA accessibility, gene expression, DNA repair and chromatin structure [35,36,37–41]. They can occur at different amino acid residues within the tails; for example, the

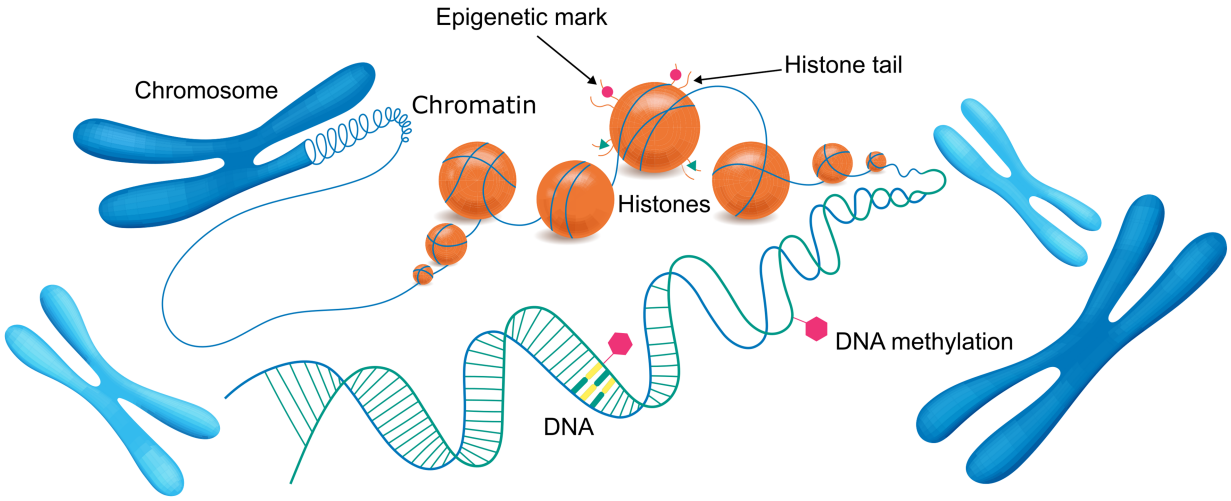

Figure 1. Schematic representation of several epigenetic mechanisms. DNA is wrapped around histone proteins to form nucleosomes, the basic building blocks of chromatin. The tails of histones, extending from the nucleosome core, contain amino acids that are targets of various epigenetic modifications, such as methylation and acetylation. These modifications influence DNA accessibility (how tightly or loosely the DNA is packed) and consequently processes such as DNA repair. The DNA itself can also be modified, most commonly through the methylation of cytosine nucleotides.

demethylation of lysine 9 (K9) in the tail of histone H3, which forms H3K9me2, alters chromatin accessibility and transcriptional activity [42]. In addition to histone modifications, epigenetic changes can also occur directly on DNA, such as methylation in promoter regions, which generally blocks transcription factor binding and represses gene expression in vertebrates [43]. Notably, DNA methylation patterns vary among taxa: while DNA methylation most frequently occurs at cytosine-phosphate-guanine (CpG) dinucleotides in vertebrates, non-CpG methylation has been observed in several fish and insect species, and methylation does not always repress transcription [44].

However, the link between epigenetic mechanisms and transcription is complex. Open chromatin generally facilitates gene transcription [45], yet some transcription factors can bind to the DNA and initiate transcription in regions with compact chromatin [46]. Additionally, the relationship between DNA methylation and gene transcription depends on the location of the methylation mark. Although evidence from vertebrates indicates that cytosine-phosphate-guanine (CpG) methylation close to transcription start sites generally suppresses transcription [47], the transcriptional effects of DNA methylation in other genomic regions, such as gene bodies, are less clear [48]. By contrast, DNA methylation in invertebrates regulates gene expression predominantly by acting on gene bodies instead of promoters [44–50]. The function of epigenetic marks therefore varies according to their genomic targets and the species in question [48].

Epigenetic mechanisms are influenced by both genetic and environmental factors. Genetic variation often underpins epigenetic variation [51,52], with specific epigenetic regulator genes playing crucial roles in establishing and maintaining **epigenetic marks** [53,54]. Environmental stressors such as malnutrition, especially when experienced during early life stages, can also alter epigenetic patterns [55], although individual differences in the sensitivity of epigenetic mechanisms to environmental stimuli may themselves be genetically determined [56]. Given this inherent link between genetic and epigenetic variation, epigenetic mechanisms could potentially mediate the phenotypic effects of deleterious mutations, a possibility that should be tested to better understand their evolutionary significance.

We hypothesize that epigenetic mechanisms, owing to their central function in regulating gene expression [25,35], may mediate the phenotypic effects of deleterious mutations. Indirect evidence for such a link comes from studies of **inbreeding**, which increases genome-wide homozygosity and thereby unmasks recessive deleterious alleles. For instance, in the plant *Scabiosa columbaria*, the disappearance of **inbreeding depression** following chemical removal of DNA methylation**,** a key epigenetic mark [57], suggests that DNA methylation can, to some extent, contribute to the manifestation of maladaptive phenotypes. This finding prompted speculation about the involvement of epigenetic mechanisms in inbreeding depression [58,59]. However, to our knowledge, no studies have directly investigated the mechanistic pathways by which DNA methylation and other epigenetic mechanisms influence the expression of deleterious mutations at the level of the nucleotide using next-generation sequencing data.

In this Opinion Piece, we outline three hypotheses linking epigenetic mechanisms to deleterious mutations, drawing on empirical evidence from model organisms ranging from yeast to humans, as well as limited but emerging data from wild animal populations (figure 2). In §2, we hypothesize that genetic mutations may induce maladaptive epigenetic patterns, leading to adverse alterations in gene expression and reduced fitness. In §3, we postulate that epigenetic modifications may buffer the expression of deleterious mutations by modulating gene activity in response to internal and external cues. In §4, we explore how epigenetic mechanisms may influence the prevalence of deleterious mutations in natural populations through their roles in DNA repair and recombination. In §5, we argue that understanding the interaction between genetic and epigenetic variation is essential for advancing evolutionary theory and informing biological conservation. Finally, in §6, we briefly outline methodological approaches and highlight empirical strategies for investigating how genetic and epigenetic variation jointly influence fitness and evolution, which is critical for conservation science.

Figure 2. Examples of wild vertebrate species in which one or two aspects of the interplay between genetic variation, epigenetic variation and/or fitness have been empirically investigated, but never all three simultaneously. Studies include: (a) in black grouse, sexual trait expression is mediated by inbreeding-dependent CpG site methylation changes at key candidate genes [60]; (b) inbreeding and epigenetic diversity are positively correlated in Kenyan [61] but not in Australian house sparrows [62]; (c) in white-footed mice sampled along a range expansion gradient, genetic and epigenetic diversity are uncorrelated [63]; (d) in killifish, interactions between parasites and inbreeding have been found to influence DNA methylation [64]; (e) in a comparative study of 60 amniote species including the green sea turtle, the CpG content of several gene promoters was found to be positively associated with lifespan [65]; (f) a similar positive association between promotor CpG content and lifespan was found across 131 mammals, including the killer whale [66]; (g) in a study of eight vertebrates including the orangutan, increased CpG density in gene promoters was found to correlate with gene expression levels [67]); (h) in the great tit, genetic effects explain a substantial proportion of the variation in DNA methylation, with *trans*-acting quantitative trait loci (QTLs) having been identified [52]; and (i) comparable findings have been reported in the three-spined stickleback, where genetic effects contribute significantly towards variation in DNA methylation, and *trans*-acting QTLs have been mapped [68]. The image used in (c) was reproduced from Charles Homler available at https://animalia.bio/white-footed-mouse/1000, licensed under CC BY-SA 4.0. The image used in (d) was reproduced from S. Hellner available at https://www.fishbase.se/summary/Kryptolebias-hermaphroditus, licenced under CC BY-NC 4.0. The image used in (i) was reproduced from WikiMedia available at https://upload.wikimedia.org/wikipedia/commons/3/37/Gasterosteus_aculeatus_-_Epinoche_-_Three-spined_stickleback.jpg, licenced under CC BY-SA-2.0. All other photos are courtesy of Oliver Krüger and used with permission.

2. Hypothesis 1: deleterious mutations may disrupt epigenetic regulation

Epigenetic patterns are established and maintained by epigenetic modifier genes [69], while genetic variation across the genome can also influence epigenetic marks [52,70,71]. Consequently, genetic mutations may, in certain cases, disrupt epigenetic regulation. Specifically, we hypothesize that deleterious mutations may give rise to maladaptive epigenetic patterns and reduce fitness, particularly when they: (i) affect key epigenetic regulator genes; (ii) involve C > T transitions; (iii) influence epigenetic marks in *trans* across the genome; (iv) are located in micro ribonucleic acid (miRNA) genes; and/or (v) interfere with alternative splicing, as detailed below.

(a) Mutations in epigenetic modifier genes

Epigenetic modifier genes are essential for establishing epigenetic marks, maintaining genome stability and regulating global epigenetic changes. These genes are involved in processes such as the maintenance of genome-wide methylation (***DNMT1*** gene), the control of de novo methylation (***DNMT3*** genes), active demethylation (***TET*** genes) and transcriptional regulation (***SETDB1*** gene). Mutations in these genes therefore have the potential to induce global epigenetic changes with severe fitness consequences (figure 3a), a prediction supported by multiple empirical studies.

Research on humans and mice has established that specific mutations or classes of mutations in epigenetic modifier genes lead to altered epigenetic states implicated in cancer and other diseases [72]. For example, knock-down of *DNMT1* can result in genome-wide hypomethylation [73], while mutations in *DNMT3A* can cause hypomethylation [74] and genomic instability [75]. Similarly, mutations in *TET* genes can disrupt normal DNA demethylation processes [76–80], while a deletion in *SETDB1* has been shown to alter DNA methylation and upregulate the expression of zinc-finger genes, disrupting cellular homeostasis [81]. These disrupted epigenetic patterns arising from genetic mutations are characteristic of many cancers [73,76–81], immune diseases [82] and metabolic disorders [83], and they sometimes lead to embryonic lethality [84].

Knock-out studies in teleost fishes and insects further emphasize the importance of *DNMT* for survival and reproduction. In insects, CRISPR/Cas9-induced knock-down of *DNMT* genes or their paralogues can cause embryonic lethality [85], reduced longevity and sterility [86]. However, the fitness effects of mutations depend on which genes are affected and their functional importance, which can vary across species. For example, a mutation in *DNMT3aa* (a *DNMT3a* orthologue) disrupts gametogenesis in tilapia, whereas a mutation in *DNMT3ab* (a *DNMT3a* and *DNMT3b* orthologue) does not impair gonadal development [87]. Similarly, knock-out of *DNMT3a* in zebrafish does not substantially reduce survival but does alter thermal plasticity [88].

Based on this evidence, we argue that naturally occurring deleterious mutations in epigenetic modifier genes could influence a wide range of quantitative traits in natural populations. Although this hypothesis remains untested, the evolutionarily conserved roles of epigenetic regulators across diverse species [69,89] suggest that mutations in these genes have the potential to reduce fitness without necessarily causing lethality or sterility, instead disrupting gene expression networks involved in development, reproduction, ageing or other key life-history traits. Notably, naturally occurring variation in the expression of *DNMT* and *TET* genes has been documented both among and within populations of wild house sparrows across a range expansion [90], which may reflect phenotypic plasticity or genetic differences associated with the colonization of new habitats. Such variation could provide a foundation for future studies aiming to link epigenetic modifier gene expression to fitness outcomes in the wild.

(b) C > T transitions

In vertebrates, DNA methylation predominantly occurs at cytosine residues, notably at **CpG sites** within gene **promoter** regions, where it typically represses gene transcription in somatic cells [43]. However, methylated cytosines are chemically unstable owing to increased electron density, making them prone to spontaneous deamination [91]. This process results in **C > T transitions** that convert methylated cytosines into unmethylated thymines [92,93]. When an unmethylated cytosine undergoes deamination, it becomes uracil, which is readily recognized as abnormal and efficiently repaired. By contrast, the deamination of methylated cytosine yields thymine, a natural DNA base that is not recognized as abnormal and therefore often escapes repair [94]. This process gives rise to mutation hotspots at methylated cytosines. Such methylation associated C > T mutations effectively erase methylation marks and, when located in promoter regions, can trigger aberrant gene activation [95]. Conversely, increased CpG content in promoter regions may be adaptive, as CpG sites enable DNA methylation to regulate gene expression, facilitating phenotypic plasticity in response to environmental cues [96,97], a phenomenon that could contribute towards an individual's 'epigenetic potential' [97]. We therefore hypothesize that C > T mutations in vertebrate promoter regions may reduce fitness by impairing the capacity of CpG methylation to regulate gene expression (figure 3b).

In line with this hypothesis, the majority of single-nucleotide polymorphisms (SNPs) at CpG sites in humans are associated with methylation differences [98], with the largest effects observed for C > T transitions, which are known to contribute disproportionately to cancer formation [95]. The loss of DNA methylation has also been shown to promote tumorigenesis via the transcriptional activation of mutated genes that have the potential to cause cancer (i.e. oncogenes), stressing the importance of DNA methylation in the silencing of deleterious alleles [99]. Moreover, promotor regions with high densities of CpG sites are frequently found in housekeeping genes [100], which are essential for cellular processes across multiple tissues and developmental stages [101]. This emphasizes the functional importance of promotor CpG sites for maintaining organismal integrity and suggests that mutations at these loci are likely to have negative fitness consequences.

Further evidence from wild systems supports this view. For example, **CpG site density**, which is affected by various factors including C > T mutations, has been linked to longevity [97,65,66]. It has been suggested that, when CpG density is high, a change in methylation at a single site has a smaller effect, so overall gene regulation remains more stable [102]. This epigenetic stability may confer greater resistance to age-related DNA methylation changes [102]. Studies of wild animals indeed show that species with lower CpG site densities in the promotor regions of several genes have shorter lifespans compared to those with higher densities [65,66,103]. Although these studies did not directly examine C > T mutations or investigate intraspecific variation in CpG site density, they imply that the loss of CpG sites may entail fitness costs. Thus, C > T transitions at CpG sites are expected to contribute towards maladaptive epigenetic patterns, phenotypic dysregulation and reduced fitness.

(c) *Trans*-acting hotspots

Regions of the genome that influence quantitative variation in DNA methylation at CpG sites are referred to as methylation quantitative trait loci (meQTLs). A single meQTL can sometimes affect the epigenetic state of multiple CpG sites, either in close proximity to the locus (a *cis*-meQTL) or at distant genomic locations (a *trans*-meQTL) [104]. When a *trans*-meQTL affects numerous CpG sites across the genome, forming a '*trans*-acting hotspot', genetic variation at this locus can modulate the expression of multiple genes. Consequently, mutations at *trans*-acting hotspots, which can be located in coding or non-coding regions of the genome, are expected to have substantial fitness consequences (figure 3c).

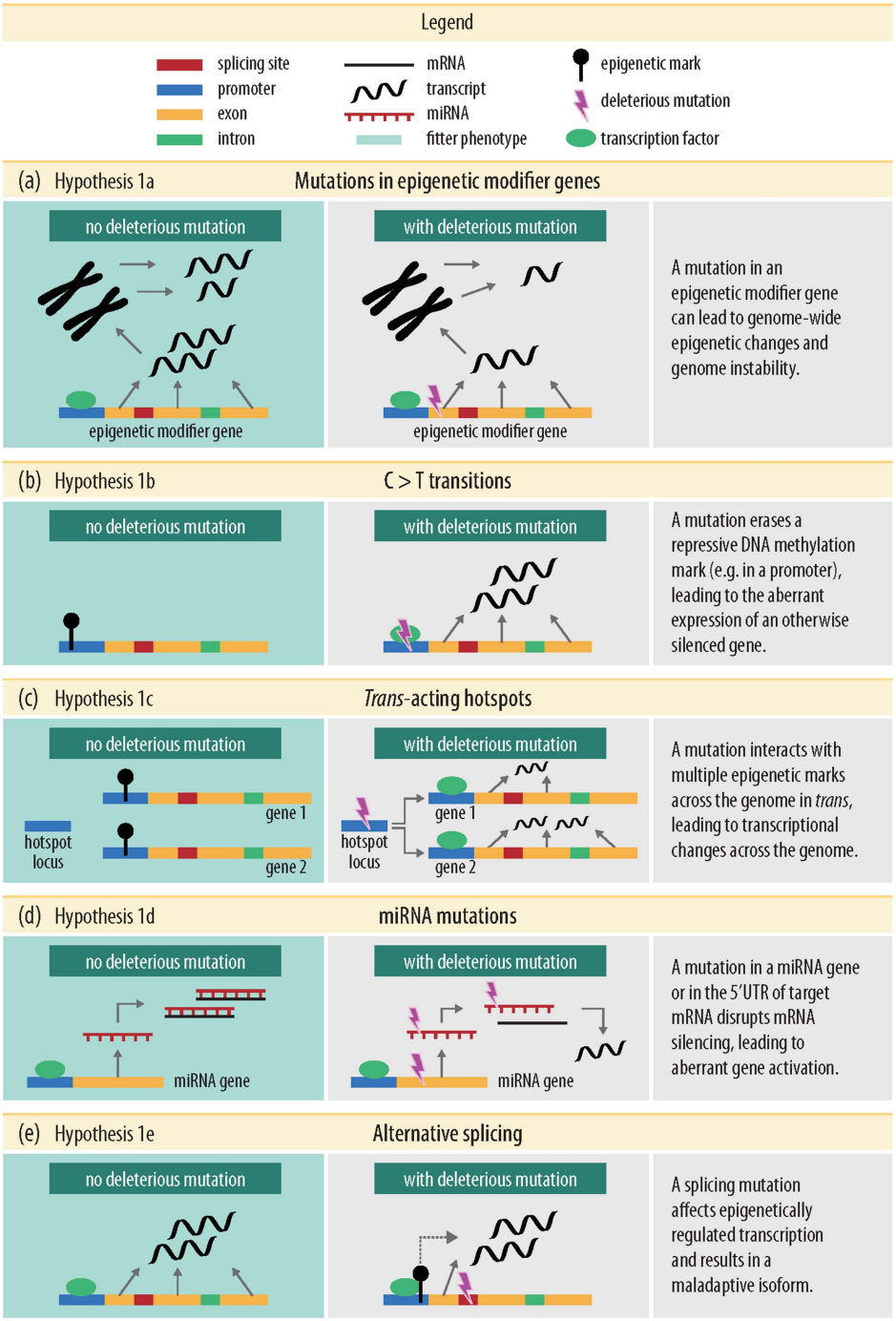

Figure 3. Schematic representation of hypothesis 1, divided into five sub-hypotheses (a–e); (§2a–e). Each sub-hypothesis represents a distinct way in which a deleterious mutation may reduce fitness via interactions with epigenetic mechanisms (middle column) compared with genotypes lacking the deleterious mutation (left column). Brief verbal explanations can be found in the right column.

Numerous meQTLs have been identified in humans and other model organisms (e.g. [105–107]). By linking these meQTLs to genome-wide association study (GWAS) hits, researchers have uncovered *trans*-meQTL hotspots associated with diseases including cardiovascular conditions [105,108] and COVID-19 severity [105], as well as with complex traits such as lifespan [107]. While the molecular mechanism(s) underlying *trans*-meQTLs remain largely unknown, current evidence suggests that loci harboring *trans*-meQTL probably affect transcription-regulating genes in *cis* [106,108]. These genes, in turn, influence DNA methylation at distal CpG sites in *trans*, thereby modulating gene regulation across the genome [106,108].

Trans-acting hotspots have also been identified in wild animals, including great tits [52] and stickleback [68]. In these studies, individual genetic variants have been associated with the methylation state of tens to hundreds of distal CpG sites. Furthermore, particularly striking pleiotropic *trans*-acting hotspots were identified in domesticated chickens, with five genetic loci collectively explaining methylation variation at over 1300 distal CpG sites [71]. However, future research is needed to determine whether these hotspots are enriched for genes related to transcriptional regulation, whether they are associated with broad-scale changes in gene expression, and to what extent genetic variation at *trans*-acting hotspots contributes to fitness variation in wild populations.

(d) Mutations in miRNA genes

miRNAs are small **non-coding RNA** molecules that post-transcriptionally regulate gene expression by inducing the translational silencing or transcript decay of target mRNAs [109]. miRNA genes are transcribed by RNA polymerase II, and the resulting miRNA complexes bind to complementary sequences, typically located in the 3′ **untranslated region** (3′ UTR) of the target mRNAs [109]. A critical component of this interaction is the 'seed region', a short sequence of two to seven nucleotides in the 5′ UTR that recognizes and binds to the complementary target mRNA sequence [109]. miRNAs are important in epigenetic control [110], while their own expression is also epigenetically regulated [111]. Consequently, we hypothesize that deleterious mutations in miRNA genes, particularly within the seed region or the 3′ UTR, may impair miRNA–mRNA binding, disrupt post-transcriptional gene regulation [112] and reduce fitness (figure 3d), as previously discussed by Arumugam *et al*. [113].

Research from model systems has shown that mutations within miRNA genes, mRNA target sites and miRNA-binding sites can disrupt transcriptional or post-transcriptional gene regulation as well as miRNA maturation (reviewed in [114] and [115]). For example, mutations in the regulatory regions of miRNAs can alter their transcription, leading to aberrant mRNA expression patterns [115]. Similarly, mutations in miRNA sequences or their target sites in mRNAs can interfere with the production of mature miRNAs and/or miRNA expression [116]. Such miRNA-mediated transcriptional dysregulation has been linked to disease susceptibility, developmental abnormalities [117] and impaired behaviour [118], and is therefore likely to carry fitness costs.

Despite these insights, miRNA research in wild species faces two major challenges: reliably identifying *bona fide* miRNAs [119] and detecting polymorphisms in miRNA loci [120]. Although methods to detect miRNAs are generally well established [121], the lack of curated miRNA databases complicates the identification and validation of miRNAs in natural populations of non-model species. Nevertheless, the field is advancing for non-model organisms too, with expanding miRNA databases facilitating improved annotations across various taxa [119,122,123]. While some miRNA genes are highly conserved across species [119,124], indicating that they play essential roles in core biological processes, others appear to be lineage-specific and might signify recent adaptation [125]. SNPs have already been detected in the miRNA genes of several domesticated [120,126,127] and zoo animals [119], indicating appreciable interspecific variability. Such SNPs, particularly in conserved miRNA genes, are expected to alter miRNA expression and/or the expression of their target genes, although no studies to our knowledge have tested for their effects on fitness.

(e) Splicing mutations

The process of **alternative splicing** allows a single precursor mRNA to produce multiple distinct mature mRNA transcripts by varying exon composition from a single precursor mRNA, thereby producing different protein isoforms from the same gene [128,129]. This process is regulated by various epigenetic mechanisms, including DNA methylation, histone modifications, chromatin conformation and **long non-coding RNAs** (lncRNAs) [see for overviews 130,131]. For example, lncRNAs [132] and histone modifications [133–136] can influence transcript length and modulate the activity of RNA-binding proteins involved in splicing by recruiting, interacting with or blocking them. There is a complex interplay between epigenetic factors and alternative splicing, as epigenetic regulators can themselves be modulated by alternative splicing [137]. Given that aberrant splicing is a hallmark of many diseases [138,139], we hypothesize that mutations disrupting the epigenetic regulation of alternative splicing are likely to have negative effects on fitness (figure 3e). Specifically, research in mice has shown that methylation-dependent alternative splicing is regulated by the *HP1* gene [140]. Owing to the importance of this gene in alternative splicing regulation, genetic mutations in *HP1* are expected to disrupt methylation-dependent splicing patterns. Similarly, *Hu* genes regulate alternative splicing by modulating local histone modification patterns surrounding alternative exons [141]. Numerous other known and as yet undiscovered genes are likely to contribute to the interaction between epigenetic regulation and alternative splicing [140].

We are not aware of any empirical studies linking genetic mutations to alternative splicing patterns that influence fitness in natural populations. However, differential isoforms of a gene involved in pheomelanin synthesis have been linked to differences in plumage coloration between two species of pheasant [142]. Given that plumage coloration is important for crypsis, social signalling and sexual selection [143], this finding supports the argument that alternative splicing could potentially give rise to variation in fitness-relevant traits. Furthermore, splicing patterns have been shown to vary among wild house mice sampled along a latitudinal gradient [144] and between seasonal morphs of African butterflies [145]. These findings suggest that alternative splicing may contribute to local adaptation and highlight its potentially important yet underexplored role in generating phenotypic diversity in natural populations.

3. Hypothesis 2: epigenetic mechanisms may buffer the expression of deleterious mutations

Epigenetic mechanisms can respond to internal and environmental cues [146], acting as dynamic regulators of gene expression. If epigenetic modifications can adjust gene activity to optimize fitness in changing contexts [147], it is plausible that they may also modulate gene expression in response to the presence of maladaptive genetic variants. We hypothesize that epigenetic mechanisms may buffer the expression of deleterious mutations through one or more of the following processes: (i) compensatory modulation of gene expression patterns; (ii) silencing of deleterious gene expression; and/or (iii) alternative splicing.

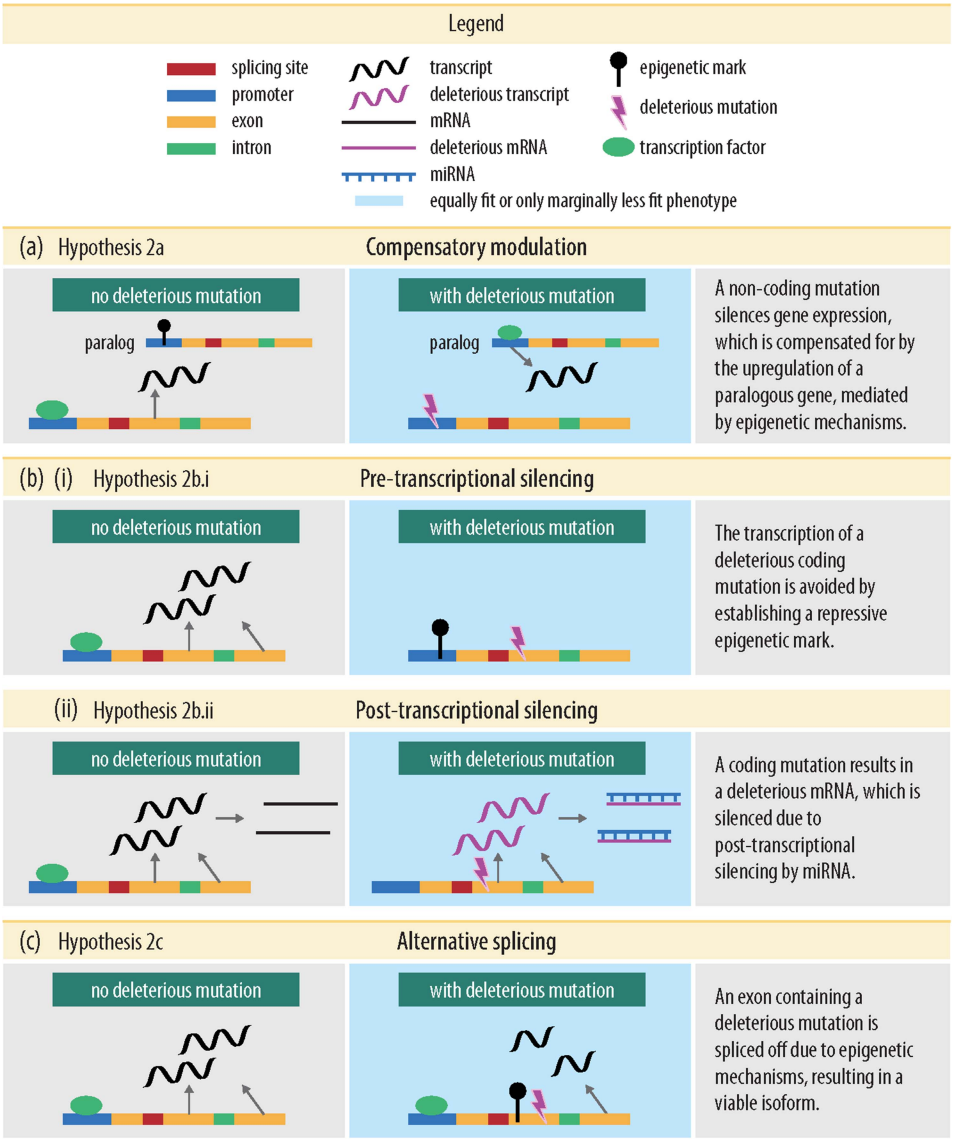

Figure 4. Schematic representation of hypothesis 2, subdivided into four sub-hypotheses (a–c); (§3a–c). Epigenetic mechanisms may buffer against the deleterious effects of a mutation (middle column), leading to equally fit or marginally less fit phenotypes compared to genotypes without the deleterious mutation (left column). Brief verbal explanations can be found in the right column.

(a) Compensatory modulation of gene expression patterns

When a mutation causes a loss of functional gene expression (i.e. a **loss of function (LOF) mutation**), its phenotypic effects may be counteracted by the activity of other genes, a phenomenon termed **genetic compensation** [148]. Such compensatory transcriptional responses may involve the upregulation of **paralogous genes**, which share sequence similarities and can overlap in expression pattern and function (e.g. [149–152]), changes in the expression of genes within the same regulatory or cellular network as the mutated gene [148,153] or changes in allele-specific expression through the downregulation of deleterious alleles and/or the upregulation of ancestral alleles, although empirical evidence for the latter is currently lacking. We hypothesize that genetic mutations resulting in the loss of gene expression or the production of abnormal mRNAs may trigger epigenetic modifications at compensatory genes (figure 4a). These modifications could increase the accessibility of transcription factors that upregulate compensatory genes, thereby mitigating the deleterious effects of the mutation [148]. In vertebrates, such epigenetic changes are likely to occur at promoter sites, where CpG demethylation generally facilitates transcription [50]. In invertebrates, they may instead occur within gene bodies, where DNA methylation plays a key role in transcriptional regulation [49].

In support of this hypothesis, laboratory studies of fruit flies have shown that gene expression changes induced by inbreeding are associated with alleviated inbreeding depression [154,155], suggesting that deleterious mutations may trigger compensatory transcriptional responses. Similar compensatory gene expression has also been observed in nematodes [156]. Evidence from other systems indicates that such responses may be triggered by mRNA degradation, a post-transcriptional process that prevents the translation of faulty transcripts and limits the accumulation of dysfunctional proteins [157]. In mice and zebrafish, the degradation of mutant mRNAs has been shown to initiate the upregulation of paralogous genes, representing a form of genetic compensation [158,159]. This response is often accompanied by increased chromatin accessibility and/or histone modifications at the paralogous

loci [158,159]. Together, these findings suggest that epigenetically mediated compensatory gene expression can occur in animals in response to genetic mutations, although its prevalence and fitness consequences in wild populations remain unclear.

(b) Silencing of deleterious gene expression

Coding mutations can result in the production of maladaptive protein isoforms, depending on how they affect amino acid sequences, protein structure and stability [160]. By contrast, non-coding mutations can cause ectopic gene expression, that is, expression in inappropriate tissues, developmental stages or seasonal contexts. Developmental and seasonal events, such as migration and reproduction, require coordinated physiological, morphological and behavioural changes that are orchestrated by tightly regulated **gene expression programmes** [161,162]. Consequently, misexpression in terms of timing, location or magnitude could potentially impact development, reproduction and survival [163,164]. Epigenetic mechanisms are known to play a central role in the spatiotemporal expression of gene regulation, particularly during developmental and seasonal transitions [165–171], and are increasingly being recognized as important in ecological contexts such as hibernation, migration and reproduction [172–176]. Based on this, we speculate that epigenetic mechanisms might help to silence deleterious gene expression and reduce maladaptive gene activity. Such silencing could plausibly occur via (i) pre- and/or (ii) post-transcriptional regulation, as described below:

(i) mutations can only affect fitness if they are expressed or affect expression levels. Gene expression at the pre-transcriptional stage is regulated by targeted epigenetic modifications, such as increased DNA methylation or histone marks at promoter regions in vertebrates. These modifications reduce chromatin accessibility, thereby inhibiting transcription and limiting maladaptive gene expression [35,36–41]. We hypothesize that epigenetic mechanisms could potentially suppress the transcription of deleterious coding mutations, which might alleviate the associated fitness costs (figure 4b(i)). However, there is currently no empirical evidence for the epigenetic silencing of deleterious mutations; and/or

(ii) alternatively, deleterious mutations might be silenced post-transcriptionally. Regulation at this stage can occur via non-coding RNAs (ncRNAs) (e.g. small interfering RNAs, antisense RNAs, miRNAs and lncRNAs), which can destabilize, cleave or hybridize with mRNA transcripts, preventing translation and blocking the production of maladaptive gene products [177]. We hypothesize that ncRNAs could silence the expression of deleterious mutations through these post-transcriptional mechanisms (figure 4b(ii)). In humans, miRNAs have been used to silence mutated genes responsible for neurodegenerative diseases, resulting in improved neuropathological and behavioural phenotypes [178]. This suggests that post-transcriptional regulation can buffer the phenotypic effects of deleterious mutations in clinical contexts and raises the possibility that similar mechanisms might have evolved to modulate the expression of deleterious mutations in wild animals. However, empirical support for such buffering effects in natural populations is currently lacking.

(c) Alternative splicing

As discussed in §2e, alternative splicing enables a single precursor mRNA to be processed into multiple mRNA isoforms post-transcriptionally, a process known to be influenced by various epigenetic mechanisms. Consequently, rather than silencing an entire mutated gene, we hypothesize that epigenetic modifications might instead promote alternative splicing patterns that exclude or compensate for the affected regions (figure 4c). If such epigenetic changes facilitate the splicing of mutated exons in a way that yields viable, functional and non-degrading isoforms, they could partially or fully restore gene functions that would otherwise be compromised by deleterious mutations.

Evidence from model organisms supports the idea that alternative splicing can functionally compensate for the effects of deleterious mutations through isoform diversification. For example, frameshift mutations in human tumour suppressor genes, such as *TP53*, can be partially bypassed by exon skipping or cryptic splice site usage, resulting in truncated yet partially functional protein isoforms that retain tumour suppressive activity [179]. Similarly, mutations in genes such as *DYSF* (dystrophy-associated fer-1-like 1 gene) and *TTN* (titin gene), which are linked to muscle disorders, can be mitigated by alternative splicing events that generate isoforms which compensate for lost protein function [180]. Furthermore, the fruit fly study described above (§3a) found that alleviated inbreeding depression was associated with the upregulation of genes involved in alternative splicing [154,155]. Although these studies did not directly investigate the epigenetic regulation of alternative splicing, they highlight the possible yet largely unexplored role of epigenetic mechanisms in facilitating beneficial splicing outcomes.

4. Hypothesis 3: epigenetic mechanisms may mediate the repair and purging of deleterious mutations

Whereas hypotheses 1 and 2 explore how epigenetic regulation may mediate the fitness effects of deleterious mutations, it is also conceivable that epigenetic mechanisms could help organisms to avoid these costs altogether, effectively acting as ‘protectors’. This could theoretically occur via (i) the targeted identification and repair of deleterious mutations, and/or (ii) the selective elimination (purging) of cells or lineages carrying such mutations.

(a) DNA repair

Mutations arise from errors during DNA replication or exposure to environmental mutagens such as UV radiation or chemicals, which can lead to chemical groups becoming attached to DNA bases [181]. Most mutations are corrected by DNA repair mechanisms that prevent their propagation within an organism (via mitosis) or their transmission to the next generation (via meiosis). These repair systems function by excising and replacing damaged bases or by directly reversing chemical changes to DNA bases (e.g. removing the chemical groups). One key mechanism, DNA mismatch repair, corrects mispaired nucleotides and small insertions/deletions (indels), mainly during the S and G2 phases of the cell cycle when DNA is replicated and subsequently scanned for errors [182]. In humans, this repair mechanism is initiated by a specific form of DNA methylation: the addition of trimethyl (three methyl groups; me3) to lysine 36 (K36) on histone H3 (box 2) to form the histone modification H3K36me3. The hMSH6 protein has a histone reader domain that recognizes and binds to H3K36me3, which helps to localize the mismatch repair complex to chromatin, allowing the DNA to be scanned for mismatches (e.g. [183,184]). If an error is found, downstream repair proteins, such as DNA polymerase, are recruited to restore the correct sequence. The functional importance of H3K36me3 is reflected by its enrichment in expressed exons [185] and its widespread presence in actively transcribed genes [185–190].

Although the epigenetic regulation of DNA mismatch repair has been well characterized in humans, very little is known about whether similar mechanisms operate in non-human animals. However, the histone reader domains fused to hMSH6, which are critical for recognizing H3K36me3 and initiating mismatch repair, appear to be conserved across most deuterostomes (e.g. vertebrates) as well as lophotrochozoans, arthropods and cnideria [191]. This conservation suggests that epigenetic mechanisms may play an important role in limiting the accumulation of deleterious mutations in many organisms by reducing the number of mutations that escape repair (figure 3a). However, this remains a largely untested hypothesis that calls for further empirical investigation.

Given that epigenetically mediated DNA repair is advantageous because it prevents the accumulation of deleterious mutations, genetic mutations that disrupt this mechanism are likely to be highly deleterious. In humans, mutations that impair the methylation-dependent interaction between hMSH6 and H3K36me3 have been shown to compromise DNA mismatch repair and lead to the development of paediatric gliomas [192]. Similarly, mutations in mismatch repair genes (e.g. *hMLH1*, *hMSH2* and *hPMS2*), as well as hypermethylation of their promoter regions, can disrupt the function and expression of these genes, resulting in defective repair and increased mutation rates, which have been linked to various forms of cancer in humans [193,194]. These findings imply that mutations affecting components of this epigenetically regulated DNA repair pathway could have severe fitness consequences by allowing deleterious mutations to accumulate unchecked.

(b) Purging

If DNA repair mechanisms fail and deleterious mutations become incorporated into the genome, they may still be removed from the population through **purging**. This process is thought to be facilitated in part by **meiotic recombination**, the exchange of DNA segments between homologous chromosomes during gametogenesis. By reshuffling genetic material, recombination creates novel combinations in gametes and ultimately in offspring. This genetic mixing can help prevent the accumulation of deleterious mutations through two distinct mechanisms [195–200]. First, recombination breaks down linkage disequilibrium between deleterious and beneficial mutations [201], generating new haplotypes on which selection can act independently. Over time, this allows beneficial mutations to increase in frequency while facilitating the selective removal of deleterious ones [196]. Second, recombination can concentrate multiple deleterious mutations on the same chromosomal segment, creating a 'high-load' haplotype that can be more efficiently eliminated by natural selection [196,202]. Together, these mechanisms illustrate how recombination enhances the efficacy of selection, promoting the removal of harmful mutations while preserving beneficial genetic variation.

Importantly, growing evidence suggests that epigenetic modifications and meiotic recombination may be tightly interconnected [203–205]. In mammals, recombination frequently occurs at recombination hotspots, where the zinc finger protein PR domain-containing 9 (PRDM9) promotes recombination by binding to specific motifs [206]. However, many taxa, including birds, canids and some fishes, lack the PRDM9 binding site [204,207–210]. Despite this, they still exhibit considerable variation in both the rate and genomic distribution of recombination events across individuals and populations [204,207–210]. In these species, recombination hotspots instead tend to coincide with gene regulatory elements, such as CpG islands, transcription start sites and gene promoter regions, which are typically characterized by low levels of DNA methylation and open chromatin enriched for H3K4me3 [203–206,208,209,211]. We therefore hypothesize that epigenetic mechanisms may alter the accessibility of DNA to the recombination machinery by manipulating chromatin accessibility [204]. If this holds true, altered epigenetic states might affect the rate at which novel haplotypes are generated and, by extension, the efficiency with which deleterious mutations can be purged.

Moreover, if a mutation in an epigenetic modifier gene leads to global epigenetic changes (§2a), this might also affect meiotic recombination. Such a mutation might, for example, increase chromatin accessibility in recombination coldspots or decrease it in recombination hotspots. This effect could be especially pronounced in species lacking the PRDM9 binding site, where recombination targeting relies more heavily on chromatin features. A broad restructuring of recombination activity might not only disrupt purging but could also interfere with meiotic fidelity, with important implications for fertility (for an overview, see [196]). Consequently, it seems plausible that mutations which globally alter recombination patterns via epigenetic changes may exert deleterious effects.

5. What are the implications of the hypothesized mechanisms?

A deeper knowledge of how epigenetic mechanisms influence the phenotypic effects of mutations is essential for understanding fitness variation. Although bioinformatic predictions and GWAS can identify putatively functional mutations, their actual fitness effects may depend heavily on the epigenetic context. For instance, if deleterious mutations induce epigenetic changes with pleiotropic and/or genome-wide consequences (§2), their effects on fitness could be substantial. In such cases, failing to account for epigenetic variation may lead to their true effects on fitness being underestimated. Conversely, if epigenetic mechanisms act to buffer or silence deleterious mutations (§3), phenotypic traits may be more robust than expected to the presence of harmful mutations. Here, overlooking epigenetic mechanisms could lead to the fitness effects of mutations being overestimated.

Beyond individual fitness, the interplay between genetic mutations (specifically those that occur in the germline) and epigenetic mechanisms may shape evolutionary outcomes by influencing the extent to which mutations are subject to purifying selection or **genetic drift**. For example, if deleterious mutations are epigenetically silenced, they may evade purging by natural selection and persist at higher than expected frequencies in natural populations. Over evolutionary timescales, this could shift the focus of natural selection: rather than acting directly on a genetic mutation, selection may instead favour epigenetic mechanisms that control its expression. This logic mirrors the Baldwin effect [212,213], in which selection is argued to favour plasticity rather than acting on traits directly. More broadly, the evolutionary fate of mutations depends largely on their selection coefficients. If these coefficients are substantially altered by epigenetic factors, then understanding this dependency becomes crucial for predicting evolutionary dynamics. Without incorporating epigenetic influences, models of mutation load, adaptation and long-term genome evolution may remain incomplete.

If epigenetic mechanisms truly influence fitness, understanding their mediating role could also be important for biological conservation. Conservation genetics has traditionally prioritized preserving genetic diversity and minimizing inbreeding to maximize population fitness [214,215]. However, if epigenetic variation also contributes to individual fitness, population persistence and evolutionary potential, then maintaining epigenetic diversity should likewise become a conservation priority. This is particularly relevant for small, vulnerable populations, where strong genetic drift can drive deleterious mutations to high frequency [216], increasing the risk of mutational meltdown [217]. In such cases, epigenetic buffering may help alleviate the effects of harmful mutations, whereas high mutation loads could disrupt epigenetic regulation and further compromise population viability.

Because epimutations arise more frequently than genetic mutations [218,219], epigenetic diversity may change more rapidly across generations, potentially enhancing phenotypic plasticity. Understanding these dynamics is essential before epigenetic variation can be integrated into conservation practise. This will require a clearer picture of the fitness effects of epigenetic variants and, ideally, the identification of specific epigenetic marks with substantial phenotypic effects. Conservation strategies could then extend to monitoring epigenetic diversity across space and time, with efforts to promote beneficial marks and minimize harmful ones. Existing approaches, such as promoting gene flow or implementing genetic rescue, might be adapted for this purpose. Nonetheless, the potential for adaptive epigenetic responses remains largely hypothetical and demands rigorous empirical investigation. Moreover, the mediating role of epigenetic mechanisms might depend on the environmental conditions and whether these conditions are changing, and if so, at what speed and at which level of predictability. We expect that the environmental context helps to determine the relative contributions of the genome and the environment in explaining fitness variation, and hence the possibility for epigenetic mediation to significantly contribute to fitness. However, while environmental changes can induce epigenetic changes (e.g. [220–222]), the majority of epigenetic variation is explained by genetic variation (e.g. [52,68,223]). Thus, we see relatively little opportunity for such plasticity of epigenetic mediation.

6. Disentangling the interplay between epigenetic mechanisms and deleterious mutations

Building on the conceptual framework outlined in §§2–4, we now turn to empirical strategies for investigating whether epigenetic mechanisms could function as exacerbators (§2), buffers (§3) or protectors (§4) in natural populations. While the preceding sections established the theoretical basis for these roles, several key questions remain unresolved. For example, are certain epigenetic mechanisms more prevalent than others and might this depend on the gene or mutation in question? Do multiple mechanisms co-occur, and if so, are their effects additive, synergistic or antagonistic? What is the threshold of mutational effect size required to trigger an epigenetic buffering response? Furthermore, to what extent do these dynamics depend on genomic architecture, population history or ecological context? Addressing these questions will require empirical studies of wild animal systems (figure 2) embedded within their natural ecological and evolutionary settings. Integrating multi-omics approaches with data on phenotypic and life-history traits in these systems holds great promise for deepening our understanding of how genetic and epigenetic variation jointly shape fitness.

Fortunately, recent advances in sequencing technologies, bioinformatics and the expansion of genomic resources [224–227] are making studies of this kind increasingly feasible. High-quality reference genomes and whole genome resequencing data are becoming widely available for non-model species, driven by declining sequencing costs [228] and improved protocols for analysing low-quality samples [229,230]. These resources facilitate the construction of **linkage maps**, offering detailed insights into **recombination landscapes**, including the locations of recombination hotspots and cold spots. Concurrently, expanding databases of regulatory elements (e.g. miRNAs [122,123,231]) are improving the annotation of genes involved in regulatory processes, while comparative analyses of model organisms can facilitate the identification orthologous genes in related taxa [232]. In parallel,

simulation models are increasingly powerful tools for predicting the long-term evolutionary trajectories of deleterious mutations [233] and exploring the epigenetic modulation of their expression [234,235].

Alongside these developments, a suite of bioinformatic tools now allows the prediction of deleterious mutations from whole genome resequencing data. Tools such as GERP [236] evaluate evolutionary constraint to identify potentially harmful mutations, operating on the assumption that variants in highly conserved genomic regions are more likely to disrupt essential biological functions and reduce fitness. Other tools such as SnpEff [237], VEP [238] and SIFT [239] predict the functional consequences of coding variants by determining the likely effects of amino acid changes on protein structure and function. These tools can identify specific mutation types including LOF mutations and other predicted 'high impact' mutations, which can be aggregated to estimate **genomic mutation loads** at the individual, population or species levels. While recent studies have begun to test for associations between genomic mutation loads and fitness [8,233,240–241], more research is needed to determine the phenotypic consequences of predicted deleterious mutations, evaluate their use as indicators of population viability [242] and explore their interactions with epigenetic mechanisms.

Moving beyond correlative evidence requires the integration of data across multiple layers of biological organization. Transcriptomic and proteomic approaches are invaluable in this regard. RNA sequencing (RNA-seq) [243] enables the precise quantification of gene expression changes driven by epigenetic mechanisms, while long-read sequencing technologies (e.g. PacBio, Oxford Nanopore) improve the detection of alternative splicing variants and allele-specific transcripts [243,244]. Proteomic approaches, such as mass spectrometry and cryo-electron microscopy, further allow for the quantification of protein abundance, post-translational modifications and interaction networks [245]. Integrating these multi-omics approaches with machine learning could help to unravel the causal biological pathways linking mutations to phenotypic and fitness outcomes via molecular intermediates.

Crucially, connecting molecular mechanisms to fitness outcomes requires robust, high-quality fitness proxies. However, fitness itself is complex and can be realized in diverse ways. For example, individuals vary in how they allocate resources to reproduction and survival across their lifetimes, reflecting different life-history strategies [246]. Capturing this complexity requires comprehensive datasets spanning morphological, physiological and behavioural traits. Because these traits vary in their heritability [247], exposure to selection [248] and sensitivity to environmental conditions [249], their potential for epigenetic modulation may vary accordingly. Longitudinal studies that gather molecular and phenotypic data across environmental gradients will be especially valuable for disentangling how the hypothesized mechanisms influence phenotypic and fitness variation, both within individual lifespans and across generations.

Researchers studying wild animal systems can already begin testing our hypotheses using correlative approaches, provided that genomic, epigenomic, transcriptomic and/or fitness data are available. For example, by combining predicted deleterious mutations with epigenetic data, one could investigate whether mutations in specific genes (such as epigenetic modifiers, §2a or miRNA genes, §2d) are associated with distinct epigenetic patterns and consequently, fitness differences. When both SNP and RNA-seq data are available, it should also be possible to test whether predicted deleterious exonic mutations in genes with paralogues are associated with the upregulation of the corresponding paralogue by comparing gene expression patterns among individuals with and without the mutation (§3a). Additionally, the theoretical mechanism of deleterious gene silencing (§3b) could be investigated empirically by determining whether genes carrying predicted deleterious mutations show higher CpG methylation in their promoters than genes carrying neutral or no mutations. Furthermore, naturally occurring variation in population density [250] offers a test bed for investigating whether epigenetic mechanisms can buffer the effects of deleterious mutations under stressful, more competitive conditions [251].

In situ manipulations and laboratory-based studies provide opportunities to investigate causal relationships between genetic and epigenetic variation under controlled or semi-controlled conditions. *In situ* experimental manipulations, such as **cross-fostering**, can disentangle genetic and environmental contributions to epigenetic variation. This approach has already been applied in wild birds such as the great tit [52] (figure 2) as well as in laboratory mice [252]. Additionally, **mutation accumulation or induction experiments**, long used in model organisms like fruit flies [253] and more recently extended to house mice [17], could be adapted to non-model species to test whether artificially elevated mutation loads elicit compensatory epigenetic and transcriptomic responses. More targeted **genome editing** tools, such as CRISPR/Cas9 [254], have also been applied in wild species, for instance, to pinpoint causal evolutionarily relevant loci in sticklebacks [255] and could be used to understand whether the introduction of genetic mutations can induce epigenetic responses.

Laboratory experiments manipulating molecular states (e.g. methylation, chromatin accessibility) and directly measuring organismal performance could further interrogate causal relationships between epigenetic variation and fitness. For example, the global distribution of epigenetic marks could be manipulated by administering methylation inhibitors or methyl donors, respectively, as demonstrated in zebrafish [256], ducks [257] and Japanese quail [258], allowing fitness comparisons both within- and among-individuals. Likewise, artificial selection on genetic features (i.e. genomic selection [259]) has already been performed in great tits [260] and could be adapted to create selection lines that differ in the presence of e.g. putatively buffering epigenetic marks. Releasing individuals from these lines into semi-natural enclosures [175] and measuring fitness proxies would then allow tests of whether associations between genetic mutations and epigenetic patterns arise because certain epigenetic marks confer a fitness advantage (§3). While ethical and logistical considerations may limit the applicability of some experimental or interventionist approaches, these examples highlight their potential applicability to uncover mechanistic insights into the interplay between genetic and epigenetic variation and its fitness consequences.

7. Summary

While the precise functional effects of many genetic mutations remain elusive, they are probably intricately linked to the epigenome in ways that are only beginning to be understood. In this Opinion Piece, we hypothesize several mechanisms through which epigenetic mechanisms may interact with genetic mutations to influence phenotypic variation and fitness outcomes. We emphasize that empirical testing of these mechanisms has become increasingly feasible in wild systems owing to methodological advances, accelerating data availability and powerful bioinformatic tools. Ultimately, a comprehensive understanding of how genetic and epigenetic factors interact is essential for uncovering the determinants of individual fitness, predicting long-term evolutionary dynamics and informing conservation strategies.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.
Data accessibility. This article has no additional data.
Declaration of AI use. We have not used AI-assisted technologies in creating this article.
Authors' contributions. R.S.C.: conceptualization, investigation, visualization, writing—original draft, writing—review and editing; B.S.: conceptualization, funding acquisition, investigation, visualization, writing—original draft, writing—review and editing; K.v.O.: funding acquisition, writing—review and editing; J.H.: funding acquisition, supervision, writing—original draft, writing—review and editing.

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Glossary

Alternative splicing: the process by which different combinations of exons are selectively included or excluded from a single precursor messenger RNA to form multiple mature messenger RNA isoforms that encode distinct protein variants from the same gene [128,129].

C > T transition or **C > T mutation:** a point mutation where a cytosine mutates into a thymine nucleotide.

Chromatin accessibility: chromatin refers to the packaging of DNA around histones. The tightness of this packaging determines how accessible the chromatin is to DNA-binding proteins, such as transcription factors. Chromatin accessibility is a dynamic property of DNA that is influenced by epigenetic modifications that alter the structure of chromatin [261].

Coding mutation: a mutation located in a coding region of a gene, such as an exon. Depending on the specific nucleotide change, it can alter the amino acid sequence of the resulting protein, potentially affecting its structure and function.

CpG site: a DNA sequence consisting of a cytosine (C) followed by a guanine (G), separated by a phosphate group (p). CpG sites are often enriched in promoter regions and are typically unmethylated, facilitating transcription factor binding.

CpG site density: the number of CpG dinucleotides within a given stretch of DNA [262]. Regions of high CpG density, known as CpG islands, can be 300–3000 bp long depending on the genomic location [263] and species [264,265], and are often found in gene promoters, where they play key roles in regulating gene expression [262]. By contrast, CpG shores are lower density regions that flank CpG islands. CpG site density can be influenced by multiple factors, including DNA methylation, selective pressures, chromatin structure, recombination rate and GC content [262,266,267].

Cross-fostering: an experimental method in which offspring are raised by foster rather than biological parents to disentangle genetic and environmental influences on phenotypes. Cross-fostering can be partial, where only some offspring in a brood or litter are exchanged, or full, where entire broods or litters are swapped.

DNA methylation: an epigenetic modification involving the addition of a methyl group to a DNA nucleotide. In vertebrates, DNA methylation in promoter regions generally inhibits transcription factor binding and represses gene expression [36,268], whereas DNA methylation within gene bodies can activate gene expression in insects [48]. Methylation at other genomic regions, such as enhancers and insulators, may also be functionally important, although these effects are less well understood [48].

***DNMT1*:** the *DNA-methyltransferase 1* gene is responsible for maintaining DNA methylation patterns by methylating DNA daughter strands during replication, thereby preserving genome-wide methylation [269].

DNMT3: *DNA-methyltransferase 3* genes (DNMTA3a and DNMTA3b) are responsible for de novo DNA methylation and the establishment of new methylation patterns during early development. This process provides the mechanistic foundation for cellular differentiation and enables epigenetic modifications [69].

Epigenetic mark: a specific type of epigenetic mechanism that includes the physical modification of DNA or histones, such as DNA methylation or histone acetylation.

Epigenetic mechanisms: biochemical modifications that alter gene expression without changing the underlying nucleotide sequence [25]. They include DNA methylation, non-coding RNAs and chromatin modifications.

Gene expression programme: the dynamic, tissue-specific and context-dependent regulation of gene activity across an individual's life history. It involves the coordinated up- and downregulation of individual genes and gene networks to support development, physiological function and responses to environmental cues.

Gene regulation: the control of gene expression, which governs when, where (i.e. in which tissue) and to what extent gene is expressed [270].

Genetic compensation: changes in RNA or protein levels of one or more genes, often paralogues, that functionally compensate for the loss of function of another gene, thereby buffering against the phenotypic effects of that loss [148].

Genetic drift: random changes in allele frequencies that occur in finite populations owing to chance.

Genome editing: the alteration of genetic material by inserting, replacing, modifying or deleting a DNA sequence.

Genomic mutation load: the cumulative burden of predicted deleterious mutations in an individual, typically including both homozygous and heterozygous mutations.

Histone modifications: epigenetic marks involving chemical modifications to the tails of histone proteins [271,272]. These modifications influence how tightly DNA is wound around the histones. When histone-DNA interactions result in a tightly packed chromatin structure (heterochromatin), the transcriptional machinery cannot access the DNA, leading to gene silencing. Conversely, looser chromatin (euchromatin) facilitates gene expression.

Inbreeding: the mating of individuals that are closely related through common ancestry.

Inbreeding depression: the reduced fitness of offspring born to closely related parents.

Linkage map: a genetic map showing the relative positions of genetic markers along a chromosome based on how frequently they are inherited together. Distances are measured in centimorgans (cM), a unit that reflects how often recombination occurs between them during meiosis.

Long non-coding RNA (lncRNA): RNA molecules longer than 100 nucleotides that do not encode proteins but play key roles in regulating gene expression. They are involved in chromatin remodelling, the modulation of histone and DNA methylation and acetylation and regulation at both the pre- and post-transcriptional and translational levels.

Loss of function (LOF) mutation: a genetic mutation that reduces or abolishes the activity of a protein. This can result from the introduction of a premature stop-codon (nonsense mutation), or insertions/deletions that disrupt the transcript's reading frame or cause exon loss.

Meiotic recombination: the exchange of genetic material between homologous chromosomes during meiosis that generates new combinations of alleles.

Micro ribonucleic acid (miRNA): small non-coding RNAs that post-transcriptionally regulate gene expression by binding to target mRNA molecules, leading to translation repression or mRNA degradation [112].

Mutation accumulation experiments: experiments in which multiple replicate lines of an organism are propagated for multiple generations under relaxed selection, often through repeated population bottlenecks. This allows mutations to accumulate at random and their fitness effects to be assessed.

Mutation induction experiments: experiments in which organisms are exposed to mutagens, such as ionizing radiation or chemicals, to artificially increase mutation rates. This enables testing of the effects of increasing mutation loads on fitness.

Mutation load: the reduction in fitness owing to the accumulation of deleterious mutations.

Non-coding mutation: a mutation occurring in a non-coding region of the genome, including intergenic and intronic regions, untranslated regions (UTRs), promoters and distal regulatory elements.

Non-coding RNA: RNA molecules that do not encode proteins but play roles in regulating gene expression at the post-transcriptional level. They include microRNAs (miRNAs), small interfering RNAs (siRNAs), long non-coding RNAs (lncRNAs), transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs)

Paralogous genes (paralogues): homologous genes that arise from the duplication of an ancestral gene within the same genome.

Promoter: a DNA sequence upstream of a gene's transcription start site (TSS) that serves as a binding site for transcription factors and other proteins to initiate transcription.

Purging: the process by which natural selection removes deleterious mutations from a population, reducing their frequency.

Recombination landscapes: variation in recombination rates along chromosomes [273], which is influenced by factors such as chromosome size, proximity to centromeres or telomeres and sex.

***SETDB1*:** the *SET domain bifurcated histone lysine methyltransferase 1* gene encodes a histone methyltransferase that regulates histone methylation, gene silencing and transcriptional repression [274].

Untranslated region (UTR): a genetic sequence located at the 5′ or 3′ end of a gene that flanks the coding region but is not translated into a protein. While they do not code for amino acids, UTRs influence mRNA stability, localization and translation efficiency.

***TET* genes:** *ten-eleven-translocation* genes encode enzymes that mediate DNA demethylation by oxidating 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) [53].