Running title: Epigenetics and Temperature

Title: Epigenetic responses to temperature and climate

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## ABSTRACT

Epigenetics represents a widely accepted set of mechanisms for how organisms respond to the environment by regulating phenotypic plasticity and life history transitions. Understanding the effects of environmental control on phenotypes and fitness, via epigenetic mechanisms, is essential for understanding the ability of organisms to rapidly adapt to environmental change. This review highlights the significance of environmental temperature on epigenetic control of phenotypic variation, with the aim of furthering our understanding of how epigenetics might help or hinder species' adaptation to climate change. It outlines how epigenetic modifications, including DNA methylation and histone/chromatin modification, i) respond to temperature and regulate thermal stress responses in different kingdoms of life, ii) regulate temperature-dependent expression of key developmental processes and seasonal phenotypes, iii) facilitate transgenerational epigenetic inheritance of thermal adaptation, iv) adapt populations to local and global climate gradients and finally v) facilitate in biological invasions. Although the evidence points towards a conserved role of epigenetics in responding to temperature change, there appears to be an element of temperature- and species-specificity in the specific effects of temperature change on epigenetic modifications and resulting phenotypic responses. The review identifies areas of future research in epigenetic responses to environmental temperature change.

## Introduction

Physiological responses to the environment have long been attributed to variation in underpinning genetic and environmental drivers which interact to shape developmental and phenotypic variation (DeWitt and Scheiner 2004; Skinner 2015). Epigenetic mechanisms have recently become identified as a factor mediating this interaction, promoting rapid phenotypic variation under environmental change (Burggren and Crews 2014; Burggren 2016). Epigenetic mechanisms represent a set of, often inherited, molecules that modify DNA accessibility to enzymes, and thus affect gene expression and patterns of mRNA splicing. Because these mechanisms are themselves differentially environmentally sensitive, they add complexity to the standard genotype-environment paradigm for understanding phenotype expression and development (Burggren 2020). Understanding how epigenetic mechanisms respond to thermal environments, and how they in turn affect thermally responsive phenotypes, is therefore a critical piece of the puzzle in understanding and predicting organismal responses to climate change.

## Functional Significance of DNA Methylation and Chromatin Modification Across Taxa

The epigenome comprises molecular modifications that are superimposed within the genome/chromatin and control gene expression. Modifications of the epigenome through chemical marking will affect gene expression without altering the DNA sequence (Lyko and Maleszka 2011). Due to its reversibility, epigenetic modifications can create adaptive, short- or long-term, phenotypic plasticity of gene expression under environmental stress, and may increase the potential to adapt to environmental change (Skinner 2015; Burggren 2016; Herrel et al. 2020), or buffer organisms against deleterious environmental effects (O'Dea et al. 2016).

One well-studied mechanism of epigenetic modification is DNA methylation (Jaenisch and Bird 2003), a process by which methyl groups are removed from S-adenyl methionine and are usually covalently added to a cytosine, 5' to a guanosine, within a dinucleotide CpG site (Moore et al. 2013; Varriale 2014). This process is catalysed by the DNA (cytosine-C5) methyltransferase (Dnmt) family of enzymes. For a full review of all Dnmt forms and functionality, see Lyko (2018) and Table 2 for *dnmts* commented in this review. In vertebrates, generally, the most accepted role of DNA methylation is to regulate gene expression by controlling the binding of transcription factors to gene promoter regions (Jones 2012). However, in plants and

invertebrates, gene-body methylation is proving to be more common than in vertebrates (Sarda et al. 2012; Takuno and Gaut 2013), and has been associated with regulating RNA splicing (Sarda et al. 2012; Bewick and Schmitz 2017) and preventing transposon shuffling (Mandrioli and Volpi 2003; Cokus et al. 2008). DNA demethylation also plays a crucial role in regulating phenotype development across taxa and is catalysed by demethylation enzymes (Kohli and Zhang 2013). Demethylation is essential for embryonic cell differentiation (Kohli and Zhang 2013), immune system development (Deleris et al. 2016; Tsagaratou et al. 2017), and phenological processes (e.g., flowering time (Gallego-Bartolomé et al. 2018)).

Histone modification is another epigenetic mechanism that influences the probability of gene transcription. Histone proteins are key components of chromatin structure and are subjected to epigenetic marks such as methyl or acetyl tags, which are regulated by their associated enzymes (Tessarz and Kouzarides 2014). Generally, histone acetylation correlates to an active chromatin state and thus transcriptional activation, while histone methylation is linked with chromatin and transcriptional repression (Bannister and Kouzarides 2011). Unlike DNA methylation, the genomic localizations and functions of many histone modifications are highly conserved among taxa (Bernstein et al. 2005; Woo and Li 2012; Jambhekar et al. 2019; Zhao et al. 2019). Interestingly, DNA methylation and histone modifications can 'communicate' in a crosstalk manner and is associated with chromatin regulation (Cedar and Bergman 2009; Du et al. 2015; Castillo-Aguilera et al. 2017; Hughes et al. 2020).

## Environmental Regulation of Epigenetic Modifications

The emerging field of environmental epigenetics is contributing to understanding how epigenomic modifications can flexibly regulate phenotypic responses to the changes in the environment (Bossdorf et al. 2008). In this review, we focus specifically on temperature-induced alterations of the epigenome, and the role of epigenetics in shaping temperature-induced phenotypic variation. Below we highlight (i) epigenetic control of thermal stress responses, (ii) temperature-induced epigenetic control of development and seasonality and (iii) heritability of temperature-induced epigenetic regulation. We then conclude with (iv) the role of epigenetics in adapting to local climates, (v) its role in biological invasions, and how epigenetic variation

could contribute to increasing the evolvability of organisms to environmental temperature change. For details on temperature regimes and genes studied, see Tables 1 and 2.

## i) Epigenetic Control of Thermal Stress Response

### Cold stress response

Epigenetic control of cold stress responses is well documented in plants and affect diverse responses from defence to metabolism and life history transitions. For instance, in upland cotton (*Gossypium hirsutum*), cold treatment resulted in a global decrease in DNA methylation and, consequently, an increase in expression of trehalose-6-phosphate synthase-like gene (*ghtps*) – a gene involved in plant global defence (Fan et al. 2013). Similar changes in DNA methylation on cold-response defence genes, including *HbICE1* (Inducer of C-repeat binding factor Expression 1 from *H. brasiliensis*), were observed in rubber trees (*Hevea brasiliensis*) following cold treatment (Tang et al. 2018), suggesting the cold response mechanism of cotton and rubber trees involves regulating defence-related genes through DNA methylation changes. Conversely, the alpine plant *Chorispora bungeana* exhibited whole-genome methylation and demethylation changes in response to chilling vs. freezing temperatures on metabolic and growth-regulating genes (Song et al. 2015). Patterns of whole-genome demethylation changes under short-term cold stress, that were unlinked to thermal acclimation processes, were also reported in maize (*Zea mays*), allowing regulation of transposons and abiotic stress-responsive genes (Shan et al. 2013). Moreover, cold-acclimated *Arabidopsis thaliana* displayed freeze tolerance following freezing treatment, with greater survival rates compared to non-acclimated plants. This was due to histone acetylation on promoters of cold-responsive *COR* genes, facilitated by the HOS15-mediated degradation of histone deacetylase 2C, allowing the switch from repressive to active chromatin and thus *COR* gene transcription essential for the cold-stress response (Park et al. 2018). Together, these studies demonstrate diversity in cold stress responses in plants and the underlying epigenetic mechanism responding to cold stress is species-specific.

The role of epigenetic responses to cold stress in animal study systems has been less described, although epigenetic modifications have a strong role in initiating and maintaining animal cold acclimation or coping style strategies. For example, differential expression of *dnmt*, histone acetyltransferase and

demethylation enzymes following freeze treatment in the freeze-tolerant goldenrod gall fly (*Eurosta solidaginis*) and the goldenrod gall moth (*Epiblema scudderiana*) suggests epigenetic regulation is an essential mechanism involved in freeze survival strategies (Williamson 2017). Investigating the epigenetic marks associated with key genes involved in these strategies would further prove the role of epigenetics in freeze tolerance in these species. In addition, tissue-specific epigenetic changes, specifically histone methylation and DNA methylation in liver and skeletal muscle, were observed in freeze-tolerant wood frogs (*Rana sylvatica*) during freeze-thaw cycles, potentially facilitating in the transcriptional regulation of metabolic processes to enter a state of hypometabolism essential for freezing survival during winter months (Hawkins and Storey 2018; Zhang et al. 2019). Moreover, in the zebrafish (*Danio rerio*), short- (5 days) vs. long-term (30 days) exposure to colder temperatures had opposing effects on whole-genome methylation levels, with the promoters of many cold-responsive genes affected (Han et al. 2016).

### *Heat stress response*

The impact of heat stress on epigenetic regulation of biological processes is particularly important to investigate with regards to increasing global temperatures and has been studied across different epigenetic mechanisms and diverse taxa. For instance, histone acetyltransferase Gcn5 positively regulates thermotolerance in *Arabidopsis* through increased acetylation of the promoter regions of heat stress response genes including heat shock transcription factor A3 (*hsfa3*) and ultraviolet hypersensitive6 (*uvh6*) genes, resulting in successful germination and plant survival under heat stress (Hu et al. 2015). Additionally, short-term heat stress in *Arabidopsis* induced heat shock protein 101 (*hsp101*) expression, but expression declined upon repeated short stress treatment (Scheid et al. 2010), suggesting that the plant epigenome is adapted to recover from short-term temperature stress. Moreover, heat shock exposure of *Drosophila melanogaster* Kc 'embryo' cells resulted in overall chromatin remodelling and activation of heat shock protein 70 (*hsp70*) and other temperature-responding factors, such as Poly(ADP) Ribose Polymerase – which potentially aids in thermotolerance in *Drosophila* (Petesch and Lis 2008). Similarly, chromatin remodelling and differential expression of *dnmt*1 and *dnmt*3 was found to be essential for heat shock survival and chill coma recovery in

an invasive white fly (*Bemisia tabaci*) Mediterranean (MED) cryptic species (Dai et al. 2017; Dai et al. 2018; Ji et al. 2020).

Epigenetic mechanisms are also often involved in longer-term acclimation to stressful temperatures. In broiler chickens (*Gallus gallus domesticus*), elevated developmental temperatures resulted in temperature-induced epigenetic regulation of *hsps* and a stress regulator gene, corticotropin-releasing hormone (*crh*) gene, via DNA methylation and demethylation (Cramer et al. 2015; Cramer et al. 2018; Vinoth et al. 2018; Cramer et al. 2019), providing protection against thermal stress that carries over across life stages. Metzger and Schulte (2017) also studied the effects of early exposure temperature change in three-spined stickleback (*Gasterosteus aculeatus*) larvae on temperature acclimation in adults. It was found that 25% of the differentially methylated regions (DMRs) associated with variation in developmental temperature were also differentially methylated in response to temperature acclimation in adults. One of the DMRs was in an E3 ubiquitin ligase gene known to regulate protein production during development and environmental stress in sea urchins (*Strongylocentrotus purpuratus*) (Pespeni et al. 2012). This suggests that a common epigenetic thermal acclimation response within multiple species responsible for anticipating future thermal conditions and thus coordinating responses across life stages. Epigenetic mechanisms may also regulate transgenerational thermal acclimation processes, and examples of this are reviewed below (section iii).

In general, under cold stress, target response genes under epigenetic control are often involved in defence mechanisms and stress coping strategies. In contrast, epigenetic response to heat stress more often affects genes which function in protein assembly and stability (Vabulas et al. 2010) (Table 2). Moreover, the reviewed studies using *Rana sylvatica* and *Bemisia tabaci* as model systems investigated temperature effects on multiple epigenetic mechanisms, highlighting the importance of studying more than one epigenetic mechanism to create a full picture of the epigenetic response to temperature change.

**ii) Temperature-Induced Epigenetic Control of Development and Seasonality**

Changes in temperature often influence many aspects of ontogeny or seasonal development across taxa, including morphological development (Andrews et al. 2000; Hammond et al. 2007) and seasonal or

developmental timing of life history events (Thakur et al. 2010). However, the molecular mechanisms underlying these effects are only recently becoming understood.

### *Temperature dependent epigenetic effects on development*

Early developmental mechanisms are largely regulated by epigenetic modifications that control stage-specific gene expression and cell differentiation (Skinner 2011). Developmental temperature stress during egg incubation or juvenile development can directly impact the epigenome structure of the developing embryo, affecting developmental outcomes. Incubating wall lizard (*Podarcis muralis*) eggs at sub-critical temperatures had a strong influence on gene expression associated with epigenetic processes during embryonic development, including histone methyltransferase, histone deacetylase and a chromobox protein homolog associated with chromatin remodelling/organisation. Widespread chromatin remodelling under this process can lead to the transcriptional regulation of embryonic developmental processes, such as zygote cellular differentiation (Feiner et al. 2018). In addition, Metzger and Schulte (2017) identified 2130 differentially methylated regions (DMRs) that responded to changes in developmental temperature in three-spine stickleback larvae. Hypermethylation of DMRs was more common than hypomethylation in response to both warm and cold developmental temperatures, indicating a potential for hypermethylation to regulate transcriptional activation or inactivation of specific genes involved in early development (Metzger and Schulte 2017).

Temperature stress can also influence epigenetic enzymes essential for stage-specific development. Yan et al. (2015) discovered that a 1°C increase in Pekin duck (*Anas platyrhynchos domesticus*) egg incubation temperature during critical stages of embryonic development resulted in the upregulation of *dnmts* and embryonic stage-specific enzyme activities of Dnmt1 and Dnmt3a within specific tissues, suggesting that *dnmts* are involved in temperature-induced epigenetic regulation of embryonic development. Similarly, Anastasiadi et al. (2017) found a developmental stage-dependence effect of elevating temperatures on global DNA methylation and overexpression of *dnmt1*, *dnmt3* and *myogenin* (used for muscle growth) in European sea bass (*Dicentrarchus labrax*) larvae but not in juveniles.

It is also important to study the phenotypic outcomes within the developing organism to determine how the developmental temperature change influences specific phenotypes. This has been studied in a variety of aquaculture fish. One study found that exposing European eel males (*Anguilla anguilla*) to a constant cold temperature during development resulted in increased differentially expressed genes within the brain-pituitary-gonad (BPG) axis (Rozenfeld et al. 2019). Genes involved in reproduction were differentially expressed in the gonads, while histone H3-K9 methylation and histone H3 deacetylation genes were upregulated in brain and pituitary tissues (Rozenfeld et al. 2019). This differential activity in the BPG-axis was correlated with the early induction of sexual development, as indicated with increased spermatogonial cell counts within the testes. This study therefore demonstrates a clear link between epigenetic control of expression of genes involved in reproduction and developmental phenotypic outcomes. In addition, *dnmt*s were sensitive to acute and continuous thermal stress in Atlantic cod (*Gadus morhua*) embryos (Skjærven et al. 2014), which may correlate with increased developmental rates and muscle phenotypic variation (Iversen and Danielssen 1984; Hall and Johnston 2003). Similarly, Campos and colleagues (2013) found a significant difference in muscle growth in metamorphic Senegalese sole (*Solea senegalensis*) larvae, depending on rearing temperatures, due to temperature-regulated expression *dnmt1* and *dnmt3b* and thus epigenetic control of *myogenin* in skeletal muscle via promotor methylation. These studies provide evidence of temperature-induced epigenetic control of growth plasticity in aquaculture fish.

Phenotypic outcomes following epigenetic change in response to developmental temperature stress have also been studied in individuals from wild populations and species that are likely to be facing climate change. The colonial ascidian, *Didemnum vexillum*, is an invasive marine invertebrate that has colonised marine habitats worldwide due to their ability to tolerate a variety of environmental conditions such as temperature and salinity fluctuations (Lambert 2001; Zhan et al. 2015). It was found that, after just three days of exposure to increasing water temperature, global DNA methylation significantly increased as well as a slower growth rate in *D. vexillum* colonies (Hawes et al. 2018). This epigenetic adjustment of growth rate in response to temperature may provide an adaptive, temporary survival solution until conditions improve. In addition, heat stressed marine Antarctic polychaete, *Spiophanes tcherniai* adults displayed a net increase in methylation at CpG sites after four weeks, which coincided with time of return of normal, baseline metabolic rates (Marsh

and Pasqualone 2014), suggesting DNA methylation may have a putative role in regulating metabolism to match thermal conditions. Conversely, maladaptive temperature dependent epigenetic effects have also been reported. Cooler egg incubation in wild caught wall lizards resulted in DNA hypomethylation in the brain tissues of newly hatched lizards – which correlated with the expression of deleterious phenotypic traits including smaller body sizes and scale malformations (Paredes et al. 2016). This latter example demonstrates that temperature change during development can have detrimental as well as adaptive effects, depending on how selection has shaped the underpinning epigenetic mechanisms.

### *Temperature-dependent sex determination and sex reversal*

In many poikilotherm vertebrates, such as fish and reptiles, changes in environmental temperatures during critical stages of development can determine gonadal and phenotypic sex of the individual through temperature-induced alterations of physiological pathways associated with sex determination, such as hormone production (Valenzuela and Lance 2004). Epigenetic regulation of temperature-dependent sex determination (TSD) and sex reversal has been investigated in many species, specifically on temperature-induced epigenetic control of expression of sex-determining genes: doublesex and mad-3 related transcription factor 1 (*dmrt1*) and sex determining region Y box 9 (*sox9*), male-bias genes, and oestrogen synthetase/aromatase (*cyp19a1*), a female-bias gene (Ge et al. 2018; Hammond et al. 2016).

TSD has been described extensively in reptiles, however only few have studied the underlying epigenetic mechanisms of TSD. For instance, incubating American alligator (*Alligator mississippiensis*) eggs at higher, male-producing temperatures resulted in increased methylation and thus downregulation of female-bias *cyp19a1* gene in embryo gonads, with identical epigenetic control of male-bias *sox9* in embryos at female-producing temperatures (Parrott et al. 2014). In addition, in red-eared slider turtles (*Trachemys scripta*), decreasing egg incubation temperature from a female-producing temperature of 31°C to a male-producing temperature of 26°C during the thermosensitive period resulted in increased methylation of the *cyp19a1* promoter and thus its decreased expression in turtle embryos (Matsumoto et al. 2016). Moreover, *Kdm6b*, a histone demethylase specific to *dmrt1* and known to play a role in male sex determination, is upregulated in

response to lower, male-producing temperatures, resulting in the demethylation and thus transcriptional activation of target gene *dmrt1* (Ge et al. 2018).

Even in species with genetically determined sexes, temperature-dependent epigenetic processes can lead to masculinization or feminization of phenotypes. In European sea bass, DNA methylation levels in the *cyp19a1* promoter are lower in females than in males. Rearing genetically-female juveniles at high temperatures during the thermosensitive period of development increased DNA methylation in the *cyp19a1* promoter similar to levels in males, which correlated with decreased aromatase transcription in the gonads and led to phenotypic masculinization (Navarro-Martín et al. 2011). In addition, rearing Nile tilapia (*Oreochromis niloticus*) larvae at a high temperature resulted in 5 of the 89 genetic females undergoing sex reversal into masculinized tilapia, known as pseudo-males, due to changes in DNA methylation on *cyp19a1a* and *dmrt1* within the gonads, thus changing their expression and resulting in the development of testis similar to genetic males (Wang et al. 2019). Conversely, sex reversal in genotypic female tiger pufferfish (*Takifugu rubripes*) was observed following low temperature-induced masculinization, and was associated with *pfcyp19a* DNA hypermethylation and thus its decreased expression (Zhou et al. 2019).

These studies not only demonstrate temperature-dependent epigenetic regulation of expression of genes involved in sex differentiation, but also indicate that, like patterns of TSD and sec reversal themselves, the underpinning epigenetic regulators often evolve in temperature- and species-specific ways, although common targets (e.g., *cyp19a1*) are often involved.

### *Epigenetics and temperature control of seasonal phenotypes*

Temperature-induced changes in epigenetics are often found to be important for seasonal developmental processes. Flowering plants rely on winter temperature cues to initiate vernalisation, which enable plants to flower in the following spring or early summer in conditions favourable for seed production (Amasino 2010; Xu and Chong 2018). The underlying epigenetic mechanisms for vernalisation regulation have been studied in plants such as *Arabidopsis* and temperate grasses, as temperate plants experience great seasonal variation in temperature. In *Arabidopsis*, prolonged cold treatment in seedlings induced epigenetic silencing of Flowering Locus C (*flc*) – a flowering repressor locus – via trimethylation of histone H3 at lysine 27 and was

facilitated by a cold memory element (Yuan et al. 2016). This epigenetic regulatory pathway appears to be specific to *Arabidopsis*, as many temperate grasses have a *flc*-independent epigenetic network despite being temperature-dependent (Ruelens et al. 2013; Bouché et al. 2017; Sharma et al. 2017; Luo and He 2020), indicating the existence of multiple distinct temperature-dependent epigenetic pathways underlying vernalisation regulation across plant taxa. Studies on the epigenetic effects of temperature on seasonality in wild animals are scarce. However, one study observed that temperature increase from January to July (seasonal changes in temperature) caused slight global DNA hypomethylation in female great tits (*Parus major*) – which could allow the expression of genes involved in the onset of reproduction such as *dio2* and *rora* (Viitaniemi et al. 2019).

The studies reviewed in this section show that epigenetic mechanisms are typically critical for controlling temperature dependent developmental and seasonal processes. Better understanding of these mechanisms in more study systems is especially important in industries such as agriculture and aquaculture, where manipulating the incubation or rearing temperature may alter desired characteristics of the farmed animal, or for conservation of wild organisms that are particularly sensitive to temperature-induced epigenetic control during development, for example in animals subject to temperature-dependent sex determination or exhibiting critical timing of seasonal phenotypes (Thackeray et al. 2016).

### iii) Temperature-Induced Heritable Changes in Epigenetic Variation

Epigenetic effects of thermal stress responses experienced by the parent may be passed on to the next generation through transgenerational epigenetic inheritance (Burggren 2016). Inheriting temperature-responsive epigenetic patterns, i.e., direct inheritance of specific DNA or histone methylation or acetylation marks, is a form of transgenerational thermal plasticity. This response can evolve under natural selection, and often allows the next generation to differentially express advantageous traits in response to temperature changes predicted by the parental environment (Weyrich et al. 2016).

Most studies investigating transgenerational epigenetic inheritance have been limited to studying only two generations within their study system due to time constraints of the species' developmental period.

Nonetheless, results from these studies have been promising. A study investigating the effect of paternal heat exposure on wild guinea pig (*Cavia aperea*) sons found that global DNA methylation levels in sons sired by heat-exposed fathers differed from sons from the same father before the father was exposed to heat (Weyrich et al. 2016). In addition, sons from heat-exposed fathers displayed DNA hypermethylation on CpG sites of the thermoregulation gene, Signal Transducer and Activator of Transcription 3 (*stat3*) which correlated with the downregulation of this gene, indicating paternal epigenetic inheritance of *stat3* regulation (Weyrich et al. 2016). Other pathways, including cell motility, muscle structure, energy production and immune functions, were also influenced by paternal heat exposure, indicating that adaptive, temperature-induced epigenetic regulation of multiple pathways can persist for at least two generations (Weyrich et al. 2019). Further studies on the phenotypic outcomes associated with these affected pathways will provide a greater understanding of the transgenerational epigenetic effects of temperature on offspring fitness.

The phenotypic consequences of transgenerational inheritance were investigated in coral reef fish, *Acanthochromis polyacanthus*. Subjecting fish to a gradual increase in water temperature across two generations improved reproductive and aerobic performance, due to differential methylation of genes involved in energy homeostasis and thermogenesis, suggesting epigenetic regulation of these genes is key for transgenerational thermal acclimation in *A. polyacanthus* (Ryu et al. 2018).

There are a few model systems that enable researchers to study transgenerational epigenetic inheritance across more than two generations. For instance, Klosin et al. (2017) observed transgenerational inheritance of epigenetic control of heterochromatin derepression and thus *daf-21* (*hsp90*) overexpression for at least 14 generations following thermal stress in F0 *Caenorhabditis elegans*. Derepression at *daf-21* was associated with histone methyltransferase downregulation and thus histone trimethylation depletion. Transgenerational inheritance of thermal stress response over multiple generations was also studied in transgenic *Arabidopsis* within three generations of extreme temperature exposure. Extreme temperature stress alleviated transcriptional silencing of *TS-GUS*, a previously inserted reporter transgene, and LINE retrotransposons in F0 plants via increased histone acetylation (Lang-Mladek et al. 2010). Greater *TS-GUS* activity remained detectable in somatic cells from both F1 and F2 unstressed generations, indicating immediate and heritable effects of temperature on transgenic reactivation. However, *TS-GUS* activity in the F3 generation returned to

levels similar to non-stressed plants due to resetting during seed ageing, suggesting that epigenetic heritability is often time limited (Lang-Mladek et al. 2010). Transgenerational inheritance of thermotolerance was also observed in brine shrimp (*Artemia franciscana*). Epigenetic control of *hsp70* via histone acetylation was observed across three subsequent unstressed generations following daily nonlethal heat shocks in F0 progeny - which correlated with increased survival in the subsequent progenies (Norouzitallab et al. 2014). This indicates inheritance of *hsp70* epigenetic regulation ensures cytoprotective memory in subsequent generations in preparation for potential stress (Horowitz 2016).

Mechanisms of transgenerational thermal plasticity are continuing to be described. How and why different epigenetic modifications show carryover effects across generations requires further study, both to understand the regulation of epigenetic inheritance, as well as to understand whether and how this is shaped by natural selection.

## iv) The Role of Epigenetics in Climate Adaptation

Many organisms exhibit variation in epigenetic mechanisms in response to climate, resulting in among-population divergence that may be entirely plastic or heritable in nature. The role of epigenetics in response to climate variation is important in long-lived organisms such as trees, as their long generation times constrain their ability to adapt to rapid climatic changes via genetic modifications (Franks and Hoffmann 2012; Bräutigam et al. 2013). In valley oak trees (*Quercus lobata*) sampled from areas of different climate gradients in California (Gugger et al. 2016), there was a significant correlation between CG-single methylation variants and the mean maximum temperature of the warmest month. Genes located near these methylation variants may play a role in environmental stress response, for example, a gene encoding a dehydration-responsive element-binding-like protein (Gugger et al. 2016). This provides evidence of how epigenetically controlled thermal plasticity can facilitate local adaptation to changing climates.

The role of epigenetics in response to climate variation has also been studied in wild animal populations. For instance, flat tree oysters (*Isognomon alatus*), sampled from a mangrove ecosystem subject to multiple natural stressors, displayed lower global DNA methylation levels during the wet and warmer

season (Suarez-Ulloa et al. 2019). Moreover, oysters sampled from locations with different stress regimes displayed different seasonal global DNA methylation patterns, suggesting that populations are differentially adapted to thermal stressors depending on the conditions they experience, and that seasonal changes to epigenetic stress response is an important component of climate adaptation in mangrove ecosystems. Among-population variation in epigenetic machinery in response to natural temperature variation has also been observed in geographically distant populations of the invasive ascidian, *Ciona robusta* (Pu and Zhan 2017) and of female great roundleaf bats (*Hipposideros armiger*) (Liu et al. 2012), indicating a conserved role of epigenetic variation in local climate adaptation. Furthermore, fish adapted to polar region temperatures, and particularly Antarctic fishes, have higher 5mC methylation levels compared to fish adapted to temperate or tropical temperatures, the latter having the lowest 5mC methylation levels (Varriale and Bernardi 2006). This negative correlation between DNA methylation and temperature has been maintained over evolutionary time which may facilitate adaptive potential of these species.

Overall, these studies highlight the importance of studying the role of epigenetic variation in climate adaptation in natural wild populations that are exposed to true environmental factors, as opposed to exposing lab-reared or non-lab-reared organisms to artificial environments. These studies also highlight that phenotypic plasticity, including transgenerational plasticity, is an important mechanism for reducing detrimental impacts of climate change. However, evolved changes in epigenetic machinery can also result in locally adapted levels of whole-genome methylation or histone modifications. Permanent changes to the epigenome, potentially at first induced by thermal environments but later canalised by underpinning sequence divergence, may facilitate local adaptation and promote the adaptive potential within the species. To understand the interplay between epigenetics and adaptation, it is important to study epigenetic variation among wild populations that are exposed to different climates, and the developmental or genetic basis of such variation.

## v) Epigenetics and Temperature During Biological Invasions

The impact of temperature change on epigenetic regulation of biological processes could facilitate biological invasions of species by facilitating their rapid response to new environments and habitats (Jeremias

et al. 2018). The cowpea seed beetle (*Callosobruchus maculatus*) is an agricultural pest that can rapidly adapt to novel host species (Price et al. 2017). In addition, subjecting beetles to novel temperatures for two generations with chemically induced artificial epimutations facilitated plastic shifts in female reproductive life history trade-off allocation, namely fecundity and offspring viability, in response to temperature change, revealing an underpinning temperature-dependence of the optimal trade-off strategy (McCaw et al. in preparation). This study suggests epigenetic control of life history fitness traits may facilitate in the rapid and successful colonisation of novel environments by invasive insect pest species. In addition, the significant changes in global DNA methylation observed in the invasive colonial ascidian, *Didemnum vexillum*, after exposure to increasing temperatures, suggests that the spread of *D. vexillum* from temperate regions into subtropical regions of the Mediterranean Sea could be facilitated with changes in global DNA methylation (Ordóñez et al. 2015; Hawes et al. 2018). Similarly, epigenetic control of *hsp90* transcriptional expression through gene-body methylation may play a role in local adaptation of the invasive ascidian, *Ciona robusta*, in response to novel thermal stressors in the invaded range (Pu and Zhan 2017). Moreover, seasonal acclimatization and thermal acclimation can induce global histone modifications in liver of the invasive American bullfrog (*Lithobates catesbeianus*) tadpole – which may contribute to maintaining energy balance by modifying key metabolites, thus providing an adaptive mechanism for coping with a wide range of temperatures and thus increasing its invasive capabilities (Ishihara et al. 2019).

Recent studies are highly suggestive that epigenetic mechanisms are an important component of successful invasions. More work is needed to understand whether and if invasive species possess more labile epigenetic machinery than species or introductions which fail to establish and spread. See Hanson et al. (this issue) for further discussion of how high 'epigenetic potential' may facilitate species range shifts more generally.

**General Conclusions and Future Directions**

Due to its sensitivity to the environment, the epigenome is well suited to facilitating differential expression of ecologically important traits under different thermal regimes, transmission of these phenotypes

to future generations, and affecting population fitness and local adaptation. Due to the rapidity of their effects, epigenetic responses to temperature may be essential to facilitate population persistence or expansion in the context of global warming, particularly for longer-lived organisms where genetic change may be prohibitively slow. Further studies of epigenetically controlled thermal responses may aid in predicting the consequences of climate change on organisms including phenology, life history trade-offs, and, more broadly, on natural ecosystem function and biodiversity.

There are many taxonomic gaps in the literature, as many studies have focused on model organisms, such as *Arabidopsis thaliana*, due to the large amount of genomic and functional information already available. Tools for studying genetics and epigenetics in non-model organisms are becoming more readily available (e.g., Crotti et al. 2020), making it increasingly feasible to study epigenetic effects of temperature in diverse taxa. However, more detailed functional studies may still be challenging to carry out in non-model organisms. The epigenetic effects of temperature on protozoans, algae and fungi are particularly lacking, and this knowledge gap is important to rectify, given their important ecological roles within both marine and terrestrial ecosystems. However, some studies are starting to indicate a role of epigenetic mechanisms in bacterial and archaeal thermal stress tolerance and thermophily (Huss et al. 2016; Blum and Payne 2019), with critical implications for microbial processes in a warming world.

Although evidence points towards a conserved role of epigenetics in responding to temperature change and regulating biological processes, there appears to be an element of temperature- and species-specificity in specific epigenetic mechanisms and thermal responses. This review has identified many genes that under epigenetic control in response to temperature stress in diverse taxa (Table 2). These genes could be used as possible targets for further interrogation in multiple organisms to better understand whether temperature-dependent epigenetic mechanisms exhibit commonalities across the tree of life, or if responses are idiosyncratic within species or populations. Lastly, future studies should aim to investigate how multiple epigenetic mechanisms interact across generations to get an overall picture of the relationship between environmental cues, an organism's epigenome, and climate adaptation.

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**Table 1. Comparative analyses on the effects of temperature on epigenetic regulation of physiological and/or phenotypic expression.**

| Common name | Latin name | Temperature | Control temperature | Epigenetic mechanism | Effect | Citation |
|---|---|---|---|---|---|---|
| Upland cotton | *Gossypium hirsutum* | 4°C | 25°C-30°C | DNA methylation | Decreased DNA methylation – *ghtps* expression | Fan et al. 2013 |
| Pará rubber tree | *Hevea brasiliensis* | 4°C, 19°C | 28°C | DNA demethylation | DNA hypomethylation of cold-response defence genes | Tang et al. 2018 |
| Alpine subnival plant | *Chorispora bungeana* | -4°C, 4°C | 23°C | DNA methylation | Differential patterns of whole-genome methylation changes | Song et al. 2015 |
| Maize | *Zea mays* | 6°C | 25°C | DNA demethylation | Whole-genome demethylation | Shan et al. 2013 |
| Thale cress | *Arabidopsis thaliana* | 4°C, 0°C--10°C | 23°C | Chromatin modification | Histone acetylation on *COR* genes – active chromatin | Park et al. 2018 |
| Thale cress | *Arabidopsis thaliana* | 38°C, 45°C | 22°C | Histone modification | Histone acetylation on *hsfa3* and *uvh6* | Hu et al. 2015 |
| Thale cress | *Arabidopsis thaliana* | 37°C | 21°C | Chromatin modification | Heterochromatin decondensation – *gus* & *rpp4* regulation | Scheid et al. 2010 |
| Thale cress | *Arabidopsis thaliana* | 4°C | 22°C | Chromatin modification | Epigenetic silencing of *flc* - vernalisation | Yuan et al. 2016 |
| Thale cress | *Arabidopsis thaliana* | 4°C-42°C | "Room temperature" | Histone modification | Release of *TS-GUS* and retrotransposon transcriptional silencing (limited heritability) | Lang-Mladek et al. 2010 |
| Valley oak | *Quercus lobata* | Varying climate gradients | NA | DNA methylation | Spatial variation in DNA methylation | Gugger et al. 2016 |
| Goldenrod gall fly/gall moth | *Eurosta solidaginis* (fly), *Epiblema scudderiana* (moth) | 5°C, -15°C | 15°C | Epigenetic enzyme expression | Differential expression of *dnmt*, histone acetyltransferase and *tet* enzymes | Williamson 2017 |
| Common fruit fly | *Drosophila melanogaster* | 36.5°C | 25°C | Histone/chromatin modifications | Loss of histones/chromatin decondensation at *hsp70* | Petesch & Lis 2008 |
| Silverleaf whitefly | *Bemisia tabaci* | -5°C, 45°C | 26°C | Chromatin modification & DNA methyltransferase expression | Chromatin remodelling, *dnmt1* & *dnmt3* expression - high survival rates | Dai et al. 2017; Dai et al. 2018; Ji et al. 2020 |
| Carpet sea squirt | *Didemnum vexillum* | 27°C | 19°C | DNA methylation | Increased DNA methylation - reduced growth | Hawes et al. 2018 |
| Antarctic polychaete | *Spiophanes tcherniai* | 4°C | -1.5°C | DNA methylation | Decreased DNA methylation - normal metabolism | Marsh & Pasqualone 2014 |
| Roundworm | *Caenorhabditis elegans* | 25°C | 20°C | Chromatin/DNA methylation | DNA hypomethylation/chromatin derepression at *daf-21* (*hsp90*) | Klosin et al. 2017 |
| Brine shrimp | *Artemia franciscana* | 35°C, 38°C | 28°C | Histone modification | Histone acetylation at hsp70 | Norouzitallab et al. 2014 |

| Flat tree oyster | *Isognomon alatus* | Local water temperatures | NA | DNA methylation | Seasonal and spatial variation in DNA methylation | Suarez-Ulloa et al. 2019 |
|---|---|---|---|---|---|---|
| Invasive sea squirt | *Ciona robusta* | Local water temperatures | NA | DNA methylation | DNA hypermethylation at *hsp90* & population epigenetic variation | Pu & Zhan 2017 |
| Zebrafish | *Danio rerio* | 18°C | 28°C | DNA methylation | Opposing effects of short- & long-term treatment on DNA methylation | Han et al. 2016 |
| Three-spined stickleback | *Gasterosteus aculeatus* | 12°C, 24°C | 18°C | DNA methylation | DNA hypermethylation (larvae and thermally acclimated adults) | Metzger & Schulte 2017 |
| European sea bass | *Dicentrarchus labrax* | 15°C, 17°C, 19°C, 21°C | 15°C | DNA methylation | Larval stage-dependence | Anastasiadi et al. 2017 |
| European sea bass | *Dicentrarchus labrax* | 21°C | 15°C | DNA methylation | Temperature-induced masculinization | Navarro-Martin et al. 2011 |
| European eel | *Anguilla anguilla* | 10°C | 20°C | Histone modification | Cold-induced early sexual maturation | Rozenfeld et al. 2019 |
| Atlantic cod | *Gadus morhua* | 10°C | 6°C | DNA methyltransferase expression | DNMTs sensitive - increased development rate & muscle phenotypic variation | Skjærven et al. 2014 |
| Senegalese sole | *Solea senegalensis* | 15°C, 18°C, 21°C | NA | DNA methylation | Temperature-induced epigenetic control of muscle growth | Campos et al. 2013 |
| Nile tilapia | *Oreochromis niloticus* | 36°C | 28°C | DNA methylation | Temperature-induced sex reversal | Wang et al. 2019 |
| Tiger pufferfish | *Takifugu rubripes* | 13°C, 15°C, 17°C | NA | DNA methylation | Low-temperature induced masculinization | Zhou et al. 2019 |
| Spiny chromis | *Acanthochromis polyacanthus* | 0°C, 1.5°C, 3°C | 0°C | DNA methylation | Transgenerational thermal acclimation | Ryu et al. 2018 |
| Wood frog | *Rana sylvatica* | -4°C, -2.5°C, 5°C | 5°C | Histone & DNA methylation | Tissue-specific epigenetic changes | Hawkins & Storey 2018; Zhang et al. 2019 |
| American bullfrog | *Lithobates catesbeianus* | 3-8°C, 20-26°C (season); 4°C, 21°C (acclim.) | 4°C (warm acclimation control) | Histone modification | Histone acetylation rapidly increased, H2A.Z gradually decreased | Ishihara et al. 2019 |
| Common wall lizard | *Podarcis muralis* | 15°C | 24°C | Histone/chromatin modification | Temperature-dependent expression of histone modifying genes | Feiner et al. 2018 |
| Common wall lizard | *Podarcis muralis* | 20°C | 24°C | DNA methylation | DNA hypomethylation of brain tissues - small body size & scale malformations | Paredes et al. 2016 |
| American alligator | *Alligator mississippiensis* | 33.5°C | 30°C | DNA methylation | Differential DNA methylation pattern & expression of *cyp19a1* & *sox9* | Parrott et al. 2014 |
| Red-eared slider turtle | *Trachemys scripta (elegans)* | 26°C, 31°C | Constant temperature (either 26°C or 31°C) | DNA/histone methylation | Temperature-dependent differential epigenetic regulation of *cyp19a1* & *dmrt1* | Matsumoto et al. 2016; Ge et al. 2018 |

| Broiler chicken | *Gallus gallus (domesticus)* | 34°C, 36°C, 40.5°C | 37.5°C (incubation), 25°C (rearing) | DNA methylation | DNA hypomethylation at *hsps* at 40.5°C - postnatal thermotolerance | Vinoth et al. 2018 |
|---|---|---|---|---|---|---|
| Broiler chicken | *Gallus gallus (domesticus)* | 36°C | 30°C | DNA methylation/demethylation | DNA methylation/demethylation on *crh* – heat resilience | Cramer et al. 2015; Cramer et al. 2018, 2019 |
| Domestic Pekin duck | *Anas platyrhynchos (domesticus)* | 38.8°C | 37.8°C | DNA methyltransferase expression | Upregulation & increased activity of DNMTs | Yan et al. 2015 |
| Great tit | *Parus major* | Seasonal increase in temperature | NA | DNA methylation | DNA hypomethylation – *dio2 & rora* expression | Viitaniemi et al. 2019 |
| Brazilian guinea pig | *Cavia aperea* | 30°C | 20°C | DNA methylation | DNA hypermethylation at *stat3*, DMRs of genes within multiple pathways (paternal inheritance) | Weyrich et al. 2016; Weyrich et al. 2019 |
| Great groundleaf bat | *Hipposideros armiger* | 14.7°C, 14.9°C, 18.4°C, 18.7°C, 20.4°C | NA | DNA methylation | Methylation variation among populations | Liu et al. 2012 |

**Table 2. Key genes and their function.**

| Gene abbreviation | Full name | Function | Citation |
|---|---|---|---|
| *dnmt1* | DNA methyltransferase 1 | Post-replication DNA methylation maintenance | Gruenbaum et al. 1982; Bestor et al. 1988; Campos et al. 2013; Yan et al. 2015; Anastasiadi et al. 2017; Dai et al. 2017 |
| *dnmt2* | DNA methyltransferase 2 | Aspartic acid transfer RNA (tRNA$^{Asp}$) methylation | Goll et al. 2006 |
| *dnmt3(a/b/L)* | DNA methyltransferase 3 | DNA methylation establishment | Sasaki & Matsui 2008; Campos et al. 2013; Yan et al. 2015; Anastasiadi et al. 2017; Dai et al. 2018 |
| *ghtps* | Trehalose-6-phosphate synthase-like | Plant global defence | Fan et al. 2013 |
| *HbICE1* | Inducer of C-repeat binding factor Expression 1 from *H. brasiliensis* | Cold-response defence | Tang et al. 2018 |
| *COR* | Cold-responsive genes | Cold response | Park et al. 2018 |
| *hsfa3, uvh6* | Heat shock transcription factor A3; Ultraviolet hypersensitive6 | Heat stress response | Hu et al. 2015 |
| *hsps* (*hsp70, hsp90* (*daf-21*), *hsp101)* | Heat shock proteins | Prevent protein unfolding | Petesch & Lis 2008; Scheid et al. 2010; Norouzitallab et al. 2014; Klosin et al. 2017; Pu & Zhan 2017 |
| *crh* | Corticotropin-releasing hormone | Stress regulator | Cramer et al. 2015; Cramer et al. 2018, 2019 |
| *E3 ubiquitin ligase* | E3 ubiquitin ligase | Protein production regulation | Pespeni et al. 2012; Metzger & Schulte 2017 |
| *myog* | Myogenin | Muscle growth | Campos et al. 2013; Anastasiadi et al. 2017 |
| *dmrt1, sox9, cyp19a1* | Doublesex and mab-3 related transcription factor 1; Sex determining region Y box 9; Oestrogen synthetase/aromatase | Sex-determining genes | Navarro-Martín et al. 2011; Parrott et al. 2014; Hammond et al. 2016; Matsumoto et al. 2016; Ge et al. 2018; Wang et al. 2019; Zhou et al. 2019 |
| *kdm6b* | Lysine demethylase 6B | Histone demethylase specific to *dmrt1* | Ge et al. 2018 |
| *flc* | Flowering Locus C | Flowering repressor | Yuan et al. 2016 |
| *dio2, rora* | Deiodinase 2, Retinoic acid-related orphan receptor alpha | Onset of reproduction | Viitaniemi et al. 2019 |
| *stat3* | Signal transducer and activator of transcription 3 | Thermoregulation, embryogenesis & immune response | Weyrich et al. 2016 |
| *TS-GUS, LINEs* | Transcriptionally silenced β-glucuronidase; Long interspersed nuclear element | Reporter transgene, retrotransposons | Lang-Mladek et al. 2010 |