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REVIEW

Epigenetics in natural animal populations

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Abstract

Phenotypic plasticity is an important mechanism for populations to buffer themselves from environmental change. While it has long been appreciated that natural populations possess genetic variation in the extent of plasticity, a surge of recent evidence suggests that epigenetic variation could also play an important role in shaping phenotypic responses. Compared with genetic variation, epigenetic variation is more likely to have higher spontaneous rates of mutation and a more sensitive reaction to environmental inputs. In our review, we first provide an overview of recent studies on epigenetically encoded thermal plasticity in animals to illustrate environmentally-mediated epigenetic effects within and across generations. Second, we discuss the role of epigenetic effects during adaptation by exploring population epigenetics in natural animal populations. Finally, we evaluate the evolutionary potential of epigenetic variation depending on its autonomy from genetic variation and its transgenerational stability. Although many of the causal links between epigenetic variation and phenotypic plasticity remain elusive, new data has explored the role of epigenetic variation in facilitating evolution in natural populations. This recent progress in ecological epigenetics will be helpful for generating predictive models of the capacity of organisms to adapt to changing climates.

Introduction

Rapid climate change produces a range of new selection pressures on natural populations. As a consequence, depending on the rate and magnitude of environmental change, as well as factors such as habitat fragmentation and natural barriers, many species are experiencing conditions outside their physiological tolerances and are therefore vulnerable to decline and extinction (Hoffmann & Sgrò, 2011). One important mechanism that may reduce the detrimental effects of environmental change on organisms is phenotypic plasticity, for example, temperature acclimation (Angilletta, 2009) via the adjustment of breeding time in birds (Charmantier *et al.*, 2008) or fibre-type composition in the swimming muscles of fish (Scott & Johnston, 2016). Although studies on the evolution of phenotypic plasticity have typically used classic quantitative genetics to partition phenotypic variance (V_P) into genetic (V_G), environmental (V_E) and genotype-by-environment variance ($V_{G\times E}$), and focused on how selection acts on genetically based phenotypic plasticity (Pigliucci, 2005; Chevin & Lande, 2010, 2011; Chevin *et al.*, 2010), it has been suggested that there may be insufficient genetic variation to permit this kind of phenotypic response to climate change in many natural populations (e.g. Przybylo *et al.*, 2000; Réale *et al.*, 2003; Møller & Merilä, 2004; Charmantier *et al.*, 2008). Recently, both empirical and theoretical studies have demonstrated that epigenetic variation can either independently contribute to phenotypic plasticity, or mediate a genetically encoded plastic response (Richards *et al.*, 2010; Duncan *et al.*, 2014). Moreover, several recent findings have shed light on the range of different roles that epigenetic variation may play during evolution. First, unlike genetic variation that is caused by random mutation and is typically independent from environmental change, epigenetic variation may respond to

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environmental change in some situations (e.g. Waterland & Jirtle, 2003; Kucharski *et al.*, 2008). Second, epigenetic variation may be heritable, although the degree and mechanisms of heritability are not fully understood (e.g. Weaver *et al.*, 2004; Seong *et al.*, 2011). Third, with a higher spontaneous mutation rate than nucleotide mutations (e.g. the epimutation rate was found to be three orders of magnitude higher than the genetic mutation rate in *Arabidopsis thaliana*; Schmitz *et al.*, 2011), depending on its long-term transgenerational stability, epigenetic variation may provide the raw material for phenotypic selection when genetic variation is limited (Becker *et al.*, 2011; Schmitz *et al.*, 2011; Zhang *et al.*, 2013) (Fig. 1). These findings suggest that epigenetic variation could play an important role in regulating phenotypic plasticity and facilitating evolutionary adaptation.

The field of epigenetics has a complex history, beginning in the early 1940s when Waddington first coined the term (Waddington, 1942). As a developmental biologist, Waddington was broadly interested in how genotypes give rise to phenotypes during differentiation and development, with no particular interest in transgenerational events. In recent years, epigenetics has been more narrowly defined to refer to mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in gene sequence (Youngson & Whitelaw, 2008). Epigenetic modifications are mainly based on DNA methylation, histone modification, and small RNA regulation (Duncan *et al.*, 2014) (Box 1). DNA methylation, which typically involves the addition of a methyl group to cytosine within CpG dinucleotides in animals, is perhaps the most extensively characterized epigenetic mechanism in eukaryotes (Jones, 2012). Although DNA methylation has been found in many clades, its pattern and genomic distribution vary widely, suggesting that it may have diverse functions and different modes of targeting specific DNA elements in different taxa (Roberts & Gavery, 2012; Schübeler, 2015). Both histone post-translational modification (PTM) and small RNA regulation can impact gene expression but occur through different mechanisms (Lowdon *et al.*, 2016). Specific histone configurations are known to regulate gene expression by altering the accessibility of the underlying DNA sequences to transcription factors (Zhou *et al.*, 2011), whereas small RNAs can be partially or fully complementary to mRNAs, resulting in repression or degradation of target

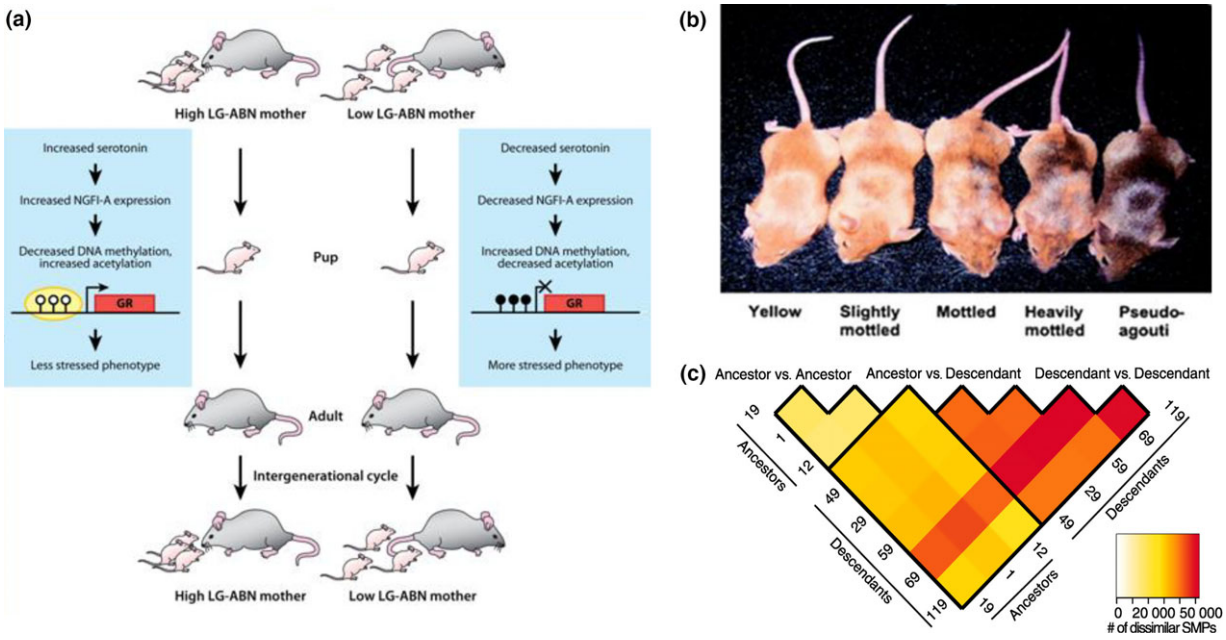

Fig. 1 Examples of roles of epigenetic variation in evolution. (a) Transgenerational inheritance of mothering style and stress in rats. Mothering style (licking/grooming (LG) and arched-back nursing (ABN)) that results in different DNA methylation and histone acetylation status at the promoter of the glucocorticoid receptor (GR) gene provokes the occurrence of the same epigenetic markers in the offspring. (b) Maternal dietary methyl supplementation and coat colour phenotype of A^{vy}/a offspring. The methylation status of a transposable element at viable yellow agouti gene (A^{vy}) controls coat colours of isogenic A^{vy}/a mice. The A^{vy} alleles of yellow mice (left) are hypomethylated, allowing maximal ectopic agouti expression. A^{vy} hypermethylation silences ectopic agouti expression in pseudo-agouti animals, recapitulating the agouti phenotype (right). (c) A heatmap indicating the number of CG single methylation polymorphisms (CG-SMPs) that differ between ancestral and descendant *Arabidopsis* populations. Although the total number of CG-SMPs was similar between all lines, the conservation of these polymorphisms among and between ancestral and descendant populations was different. Reproduced with permission from (a) Youngson & Whitelaw (b) Waterland & Jirtle and (c) Schmitz *et al.*

Box 1 Glossary

Epigenetic modifications: chromatin and DNA modifications that influence genome function but do not change the underlying DNA sequence.

Epimutation: Heritable stochastic change in chromatin state at a given position or region. In the context of cytosine methylation, epimutations are defined as heritable stochastic changes in the methylation status of a single cytosine or of a region or cluster of cytosines. Such changes do not necessarily imply changes in gene expression.

DNA methylation: the addition of methyl groups, usually to a cytosine base, as a means of chemical DNA modification.

Histone modification: a covalent post-translation change to a histone residue, including lysine acetylation, methylation and ubiquitylation, serine phosphorylation, arginine methylation, etc., each catalysed by one or more protein-modifying enzymes, many of which also have nonhistone substrates.

Histone biotinylation: a covalent binding of biotin to distinct lysine residues in histones, catalysed by holocarboxylase synthetase (HCS) and biotinidase (BTD). Histone biotinylation has been implicated in heterochromatin structures, DNA repair and mitotic chromosome condensation.

Small RNAs: a group of RNAs including microRNA (miRNA) and small interfering RNA (siRNA) that are typically <25 nucleotides and can influence gene expression through targeted degradation of mRNA or induction of methylation at complementary DNA sequences.

CpG dinucleotides: a cytosine followed (5′–3′) by a guanine. Cytosines at CpG dinucleotides constitute the principal target of DNA methylation in vertebrates. In invertebrates, cytosine methylation also occurs in other sequence contexts such as CHG (where H is any nucleotide except for C).

CpG islands: GC-rich DNA sequences that have a high density of CpG dinucleotides.

DNA methyltransferases (Dnmts): a family of enzymes that catalyse the transfer of a methyl group to DNA. There are typically three Dnmts in animals: Dnmt 1 – responsible for maintaining DNA methylation patterns; Dnmt 3a and 3b – required for de novo methylation.

Methylation-sensitive amplified fragment-length polymorphism (MS-AFLP): a commonly used technique for screening variation in DNA methylation. It can identify genomewide methylation patterns by replacing standard AFLP restriction enzymes with methylation-sensitive enzymes.

Reduced Representation Bisulphite Sequencing (RRBS): A procedure for single-base-resolution methylation analysis using bisulphite DNA sequencing of a subsection of a genome.

Epigenetic stability: the persistence of modifications in gene expression and/or epigenetic markers that influence gene expression across generations.

Phenotypic plasticity: the ability of a genotype to yield different phenotypes in response to environmental changes.

sequences (Biggar & Storey, 2015). Although our knowledge of the epigenetic machinery underlying cell signalling (i.e. how cells perceive external and internal signals, and transmit the signals to cellular machinery to activate responses) is rapidly improving, the ecological and evolutionary consequences of different epigenetic mechanisms remain poorly understood.

To achieve a more comprehensive understanding of the roles that epigenetic mechanisms may play in facilitating phenotypic plasticity and evolution, it is important to consider epigenetic processes at the population level (Johnson & Tricker, 2010). A number of recent studies have investigated the epigenetic mechanisms underlying environmentally induced phenotypes under laboratory settings (e.g. in animals: Dolinoy *et al.*, 2007; Kucharski *et al.*, 2008; in plants: Vaugh *et al.*, 2007; Salmon *et al.*, 2008). An important next step will be to study epigenetic processes under field conditions with natural levels of environmental and genetic heterogeneity (Ledon-Rettig, 2013). Here, we first review existing literature on epigenetically encoded plasticity in animals, with a specific focus on thermal plasticity as these studies provide some of the clearest examples for understanding mechanisms of generating population epigenetic variation. Second, we assess the levels of epigenetic variation in natural animal populations, emphasizing the relationship between epigenetic variation and genetic variation during adaptation. Third, we evaluate the evolutionary potential of epigenetic variation depending on its autonomy from genetic variation and its transgenerational stability. Finally, we review theoretical models that discuss epigenetic inheritance within ecological contexts. We do not cover epigenetic effects on phenotypic plasticity in plants or plant population epigenetics as these topics have already been discussed elsewhere (Richards, 2008; Hirsch *et al.*, 2012; Liu *et al.*, 2015). Furthermore, different epigenetic mechanisms and dynamics of plasticity may exist between plants and animals: sessile plants, unlike typically more mobile animals, cannot move to favourable environments, and plastic responses to biotic and abiotic stresses are more ubiquitous in plants than animals (Agrawal, 2001). Thus, the mechanisms by which epigenetics contributes to plant phenotypic plasticity and adaptation may differ for animals (Suzuki & Bird, 2008; Youngson & Whitelaw, 2008) (Box 2). Although there is a rich body of literature on the relationship between epigenetic variation and genetic variation in human populations

Box 2 Differences in DNA methylation between animals and plants

DNA methylation is the most well-characterized epigenetic mechanism in plants and animals, but there are some important differences in how and where it occurs. Five of the most significant differences between animals and plants are: 1) the presence of non-CpG methylation in plants that is targeted to transposable elements (TEs) and is typically regulated by small interfering RNAs (siRNAs) (Mette *et al.*, 2000; Chan *et al.*, 2004, 2005). 2) The timing of germline separation from somatic tissues is typically different between animals and plants. For example, in mammals, primordial germ cells (PGCs) are derived from the epiblast and arise in the posterior primitive streak during gastrulation. Thus, there is limited time for epigenetic alterations to be transmitted into germline cells. In contrast, there is no early separation of germline and soma in plants, and the gametes are derived from vegetative tissue nearing completion of development. This may provide plants with a greater opportunity for 'soft' inheritance than mammals (Youngson & Whitelaw, 2008). 3) The targets of DNA methylation between animals and plants are different. In vertebrates, gene bodies are typically methylated, whereas CpG gene promoter regions called CpG islands are often unmethylated (Suzuki & Bird, 2008). In invertebrates, methylation predominantly occurs in exons (Feng *et al.*, 2010; Zemach *et al.*, 2010). In contrast, methylation in plants typically occurs on repetitive DNA elements and TEs. 4) In general, DNA methylation occurs globally in vertebrates, with ~ 70–80% of cytosines in CpG dinucleotides being methylated (Bird & Taggart, 1980). In contrast, plants are more similar to most invertebrates in that they typically have mosaic DNA methylation patterns characterized by domains of heavily methylated DNA interspersed with domains that are methylation free (Tweedie *et al.*, 1997; Suzuki & Bird, 2008). 5) The transgenerational stability of DNA methylation between animals and plants are different. In mammals, a global reset of DNA methylation occurs both in the germline and in the zygote immediately after fertilization (Heard & Martienssen, 2014). In contrast, in plants, most of DNA methylation in CG and CHG (where H is A, C, T) sequence contexts is stable during meiosis and embryogenesis, but CHH methylation is specifically reduced (Calarco *et al.*, 2012).

(e.g. Bell *et al.*, 2012; Gutierrez-Arcelus *et al.*, 2013; Banovich *et al.*, 2014; McRae *et al.*, 2014), we do not discuss epigenetic heritability in humans here as these studies do not typically consider ecological context.

Our discussion is structured around key questions concerning the mechanisms and consequences of epigenetics in evolutionary processes: How does epigenetic variation shape phenotypic plasticity? Is epigenetic variation transgenerationally stable? What is the relationship between epigenetic patterns and adaptation in natural animal populations? Do empirical studies support theoretical models linking epigenetics, phenotypic plasticity and evolution? And finally, what are the implications of epigenetic variation for the 'evolvability' of natural populations in changing environments? Addressing these questions will be useful for identifying gaps in our understanding of epigenetic processes, and provide new scope for future ecological and evolutionary research into how animals may respond to global climate change.

Epigenetically encoded plasticity in animals

Understanding the mechanisms by which animals perceive and respond to environmental signals is of fundamental importance to ecology and evolution. In many recent studies, environmentally induced changes in gene expression have been associated with altered DNA methylation patterns or with altered histone modification (Feil & Fraga, 2012). In particular, a burgeoning area for research into epigenetic responses to environmental change has been the investigation of epigenetically encoded thermal plasticity in animals. Recent studies have characterized within-generational and potentially transgenerational epigenetic effects, which are two specific mechanisms that generate population variation, and both chromatin- and nucleic acid-based mechanisms have been explored (Table 1). Below, we review the key findings from these studies, which provide some of the best examples for understanding the relationships between epigenetic variation, phenotypic plasticity and evolution in natural populations of animals experiencing changing environmental conditions.

Within-generational epigenetic effects

Studies of within-generational epigenetic effects have shown that epigenetics can regulate diverse phenotypes associated with responses to temperature change. Below we provide a number of examples to help illustrate this diversity. However, it is important to recognize two key points about these studies. First, they typically have not explicitly considered genetic variation when different epigenetic responses were observed, so in cases where multiple populations or families are investigated, it is unclear if intraspecific epigenetic variation was induced by different environments or determined by genotype. Second, recent studies have involved laboratory-reared populations of animals and thus may not accurately reflect the epigenetic processes occurring in more natural settings. The increased genetic and environmental heterogeneity expected in the wild may result in more complex ecological and evolutionary dynamics and outcomes relative to what has been found in the laboratory.

In some fish and reptile species, sex determination is triggered by temperature changes during gametogenesis

Table 1 Overview of studies demonstrating epigenetically encoded plasticity in animals.

Animal species	Phenotype(s)	Epigenetic modification	Generations assayed (effect detected)	Richard's framework	Ref.
European sea bass (*Dicentrarchus labrax*)	Sex ratio	DNA methylation	1 (1)	Putatively obligatory (*cis*)	Navarro-Martin *et al.* (2011)
Red-ear slider turtle *(Trachemys scripta)*	Sex ratio	DNA methylation	1 (1)	Putatively obligatory (*cis*)	Matsumoto *et al.* (2013)
American alligator (*Alligator mississippiensis*)	Sex ratio	DNA methylation	1 (1)	Putatively obligatory (*cis*)	Parrott *et al.* (2014)
Senegalese sole (*Solea senegalensis*)	Muscle fibre diameter	DNA methylation	1 (1)	Putatively obligatory (*trans*)	Campos *et al.* (2013)
Zebrafish (*Danio rerio*)	Muscle phenotype (hyperplastic vs. hypertrophic)	microRNA	1 (1)	Putatively obligatory (*trans*)	Johnston *et al.* (2009)
Cobb chick (*Gallus gallus domesticus*)	Thermotolerance acquisition	Histone modification	1 (1)	Putatively obligatory (*trans*)	Kisliouk & Meiri (2009)
Cobb chick (*Gallus gallus domesticus*)	Thermotolerance acquisition	microRNA	1 (1)	Putatively obligatory (*trans*)	Kisliouk *et al.* (2011)
Antarctic polychaete (*Spiophanes tcherniai*)	Metabolic rates	DNA methylation	1 (1)	Putatively obligatory (*trans*)	Marsh & Pasqualone (2014)
Carp (*Cyprinus carpio*)	Nucleolar organization	Chromatin structure	1 (1)	Unknown	Alvarez *et al.* (2006)
Fruit fly (*Drosophila melanogaster*)	Life span, heat tolerance	Histone modification	1 (1)	Putatively obligatory (*trans*)	Camporeale *et al.* (2006)
	Life span, heat tolerance, fertility, metabolism		1 (1)	Putatively obligatory (*trans*)	Smith *et al.* (2007)
	Eye colour	Chromatin structure	5 (2)	Putatively obligatory (*cis* and *trans*)	Seong *et al.* (2011)
Guinea pigs (*Cavia aperea*)	None	DNA methylation	2 (2)	Both obligatory and facilitated/pure	Weyrich *et al.* (2016)
Reef corals (*Acropora hyacinthus*, *A. millepora*, *A. palmate*, *Pocillopora damicornis*, *Porites astreoides*, *Stylophora pistillata*)	None	DNA methylation	2 (possible 2)	Obligatory (*cis*)	Dimond & Roberts (2016)

(Valenzuela & Lance, 2004). The sex ratio depends on the activity of the gonadal aromatase Cyp19a, a product of the *cyp19a* gene, which irreversibly converts androgens into oestrogens (Navarro-Martin *et al.*, 2011). However, the molecular mechanisms by which temperature during early development influences *cyp19a* expression have remained elusive until recently. Several studies over the last few years have demonstrated that temperature changes can drive epigenetically encoded sex ratio shifts. For example, exposure of European sea bass (*Dicentrarchus labrax*) to high temperature during a critical period in early development led to an increase in DNA methylation at the *cyp19a* promoter region, and resulted in a greater proportion of males (Navarro-Martin *et al.*, 2011). The increased methylation was only found in gonad tissue and not in the brain, and only at the promoter of the *cyp19a* gene and not at the housekeeping gene *β-actin*, suggesting that sex and temperature differences in methylation levels are both tissue and gene specific. Furthermore, there was no effect of oestrogen treatments on gonadal *cyp19a* promoter methylation level, supporting the relationship between methylation of the promoter and gender bias. Another intriguing result is that several CpGs were found near transcription binding sites at the *cyp19a* promoter, suggesting potential *cis*-regulation on methylation changes. Similar DNA methylation changes have been observed in the red-eared slider turtle (*Trachemys scripta*) (Matsumoto *et al.*, 2013) and the American alligator (*Alligator mississippiensis*) (Parrott *et al.*, 2014), where high temperatures repressed gonadal aromatase expression in embryos or larvae resulting in male-biased populations. However, studies in turtles and alligators have also found different methylation patterns in other sex determination genes. For example, promoter methylation at *SOX9* showed a converse methylation pattern

compared to *cyp19a1* in alligator gonads (Parrott *et al.*, 2014). These results suggest that DNA methylation could act as a key mediator integrating temperature into molecular mechanisms that determine sex in some animal species.

Embryonic temperature or temperature at early critical periods during the establishment of thermal control has also been demonstrated to have other long-term phenotypic effects. Campos *et al.* (2013) provided evidence that DNA methylation patterns were associated with a temperature-induced muscle growth change in the Senegalese sole (*Solea senegalensis*). When embryos were reared at low temperatures, there was a significant increase in promoter methylation of a critical myogenesis regulation gene, *myog*, in larvae skeletal muscle due to the action of two DNA methyltransferases, *Dnmt1* and *Dnmt3*. As a result, fish reared at low temperatures produced smaller muscle fibres than fish reared at high temperatures. However, it remains unclear whether the changes in DNA methylation variation were specific to *myog* alone because methylation changes at other loci were not investigated. Johnston *et al.* (2009) provided evidence that in addition to DNA methylation, microRNA expression at different embryonic temperatures can also be associated with the transition from hyperplastic to hypertrophic muscle growth phenotype in adult zebrafish (*Danio rerio*). Effects of microRNA on thermal plasticity are not only confined to simple developmental transitions, but have also been shown to be involved in complex neuronal network remodelling. For example, demethylation of histone H3 at lysine 27 (H3K27) in the promoter of the brain-derived neurotrophic factor (*Bdnf*) can help build thermotolerance acquisition in chicks (Kisliouk & Meiri, 2009). Chicks injected with a microRNA, miR-138, during a critical period in establishment of thermal control exhibit difficulties in controlling body temperature after being exposed to heat stress. It is thought that the disruption of thermoregulation arises because miR-138 prevents *Bdnf* promoters from gaining methylation (Kisliouk *et al.*, 2011). Furthermore, antisense knockdown of H3K27-specific lysine histone methyltransferase (HMT), which was correlated with the demethylation of H3K27, has been shown to disrupt thermoregulation establishment and inhibit *Bdnf* mRNA expression (Kisliouk & Meiri, 2009). The above examples suggest that instead of isolated epigenetic mechanisms, it is often suites of epigenetic mechanisms that act in concert to influence animal responses to temperature change.

In addition to developmental transitions, physiological activity in animals is also closely related with temperature change. Marsh & Pasqualone (2014) showed that temperature altered metabolic rates of an Antarctic polychaete, *Spiophanes tcherniai*, and that these changes were associated with methylation gains at specific CpG sites. Interestingly, metabolic rates at high temperatures returned to control levels after a 4-week acclimation period, which suggests that DNA methylation might be responsible for regulatory shifts that differentiate metabolic activities. Other examples of the association between water temperature and epigenetic patterns include polar fish that exhibit higher global methylation levels than tropical and temperate fish (Varriale & Bernardi, 2006), and differences in nucleolar organization between winter and summer acclimated carp (*Cyprinus carpio*) (Alvarez *et al.*, 2006). However, the precise mechanisms by which phenotypic and epigenetic patterns are linked, and whether the changes in methylation are adaptive in these cases remain unclear.

The majority of studies investigating the relationship between DNA methylation and thermal plasticity have used nonmodel organisms. This may be due to a lack of recognizable *Dnmt*-like genes and limited DNA methylation patterns in several well-studied model systems (Suzuki & Bird, 2008; Roberts & Gavery, 2012). For example, the worm *Caenorhabditis elegans* essentially lacks DNA methylation, and there is no transposable element methylation in the honeybee, *Apis mellifera* (Simpson *et al.*, 1986; Wang *et al.*, 2006). Thus far, only a few studies have been conducted in the classic model organism for thermal biology, *Drosophila melanogaster*, and have mainly focused on the associations between histone modification, heat tolerance, and lifespan. These studies have yielded contrasting results regarding the effects of histone biotinylation on phenotypic variation. After comparing the biotinylation levels of the same lysine residues (K9BioH3 and K18BioH3) with controls, Camporeale *et al.* (2006) showed that reduced biotinylation in histones caused by knocking down a major catalytic enzyme (holocarboxylase synthetase, HCS) led to decreased lifespan and heat tolerance in treated flies compared to controls within one generation. In contrast, although Smith *et al.* (2007) found that flies fed on a biotin-deficient diet for 12 generations also exhibited decreased biotinylated histones, their lifespan and resistance to heat stress actually increased relative to control lines. The divergent results in these two studies may imply that lifespan and heat stress resistance are impacted differently by short-term decreased histone biotinylation vs. adaptation to histone biotinylation deficiency over multiple generations. A possible explanation is the hypothetical 'transgenerational washout' epigenetic effect (Burggren, 2015), where the level of epigenetically caused phenotypic modification, in this case reduced lifespan and heat stress resistance, progressively declines across generations to subdetectable levels. This decline may result from rapid adaptation caused by switching between epigenetic variants in periodic environments, as indicated by recent models (Furrow & Feldman, 2014; Uller *et al.*, 2015; Kuijper & Johnstone, 2016).

In summary, the current literature investigating within-generational epigenetic effects suggests that

temperature changes can strongly influence epigenetic patterns and the phenotypes associated with these epigenetic modifications. However, most studies have not explicitly considered the source of epigenetic variation, for example, environmental or genetic variation, and they have typically been conducted under laboratory conditions. In addition, almost all studies were conducted within one generation, which has precluded testing of transgenerational epigenetic effects. This is of course important because the evolutionary relevance of epigenetic effects rests on whether the responses are heritable (Richards, 2006; Heard & Martienssen, 2014).

Transgenerational epigenetic effects

Although the resetting of some epigenetic marks at one or more points during an organism's life cycle inhibits the inheritance of epigenetic modifications across generations, a growing number of examples of transgenerational epigenetic inheritance in model systems have been reported in recent years (Jablonka & Raz, 2009; Daxinger & Whitelaw, 2012; Lim & Brunet, 2013). Notably, a number of recent examples have considered transgenerational responses to heat exposure. For example, Seong *et al.* (2011) showed that heat shock can induce the repression of the *white* gene as a result of heterochromatic disruption via drosophila activation transcription factor 2 (dATF-2), a transcription factor functioning in heterochromatin nucleation (Jia *et al.*, 2004), and that the effect is maintained over several generations before returning to the normal state. This result implies that although the epigenome can be altered under environmental stress, the chromatin state retains its capacity to be reset once the environmental cues that initially induced the variation have disappeared. However, evidence that transgenerational inheritance has an epigenetic basis is generally lacking in mammals, especially in nonmodel mammals. One exception is Weyrich *et al.* (2016), who exposed adult male guinea pigs (*Cavia aperea*) to increased ambient temperature during spermatogenesis, and allowed them to mate with the same female before and after the heat exposure. There were immediate epigenetic responses to increased temperature in these males, and importantly, modified methylation was also detected in the testes of their sons, which suggests that the epimutations can possibly persist to the F2 generation. This is important because heritable epigenetic effects that contribute to a fitness increase in offspring will clearly have evolutionary consequences. Another example comes from the analysis of CpG depletion in several coral species responding to thermal stress (Dimond & Roberts, 2016). The analysis of CpG depletion is based on the hypermutability of methylated cytosines, which readily deaminate to thymine residues over evolutionary time (Roberts & Gavery, 2012). This results in a reduction in CpG dinucleotides, which implies that hypermethylated genomic regions are associated with a reduced number of CpGs, whereas genomic regions enriched with CpGs are hypomethylated. Dimond & Roberts showed that historically hypomethylated regions are enriched in differentially expressed genes that are responsive to thermal stress. These results add to a small but growing body of evidence supporting an association between transgenerational hypomethylation and stress-induced responses (Anway *et al.*, 2005; Luna & Ton, 2012; Luna *et al.*, 2012; Olson & Roberts, 2014). These studies are intriguing because they suggest a possible link between DNA methylation and plastic responses to long-term environmental change. However, it is important to note that this work has not been able to establish causal links between epigenetic variation and fitness differences, and thus, it is difficult to know if the observed epigenetic variation has been selected for via evolutionary processes or only represents the stochastic transmission of epimutations. In addition, recent studies have not been designed to distinguish between temperature-induced epigenetic variation that is transmitted only across a single generation and variation that can be inherited for several generations regardless of whether the temperature stress in maintained. Discriminating between these scenarios would help to better understand the evolutionary significance of environmentally induced transgenerational epigenetic variation in animals.

In summary, studies of epigenetically encoded thermal plasticity have started to tackle several important questions in ecological epigenetics, for example, how does epigenetic variation shape ecologically relevant phenotypes, and is environment-induced epigenetic variation transgenerationally stable? Although epigenetic analysis of thermal plasticity is still in its infancy (Box 4), results from these studies suggest that methylation patterns can be inherited and also play an important role in transgenerational responses to thermal stress. This work will serve as an important reference for future studies to investigate long-term epigenetic responses to the diverse stressors caused by environmental change.

Population epigenetics in the wild

We now know that epigenetic variation can be triggered by exposure to different environmental conditions and can sometimes be transmitted across generations. Further understanding of epigenetic processes will be aided by empirical assessment of the amount of population variation that results from either within- or transgenerational epigenetic variation. This is important because epigenetic variation can explain some phenotypic variation that cannot be attributed to genetic variation, and could thereby facilitate responses to environmental change (Bossdorf *et al.*, 2008). However, despite abundant speculation about the potential ecological and evolutionary implications of epigenetic variation, most studies have been carried out on laboratory-raised animals, and thus, the importance of

epigenetic processes in natural populations remains unclear. Furthermore, epigenetic variation has been typically studied at the individual level, which has made it difficult to discern its implications for population-level evolutionary responses. Evidence that epigenetic variation contributes to adaptation should ultimately come from studies in natural populations (Burggren, 2015). Although most of the well-documented cases of epigenetic variation in nature are from plant populations (Kalisz & Purugganan, 2004; Richards, 2006; Hirsch *et al.*, 2012), recent studies in wild animal populations have also suggested links between epigenetic variation, especially DNA methylation, and local adaptation. Below, we review key findings related to epigenetic variation in wild animal populations.

In most wild animal populations examined to date, there has been an excess of DNA methylation variation relative to genetic variation (Massicotte *et al.*, 2011; Morán & Pérez-Figueroa, 2011; Liu *et al.*, 2012; Massicotte & Angers, 2012; Schrey *et al.*, 2012; Liebl *et al.*, 2013; Skinner *et al.*, 2014; Wenzel & Piertney, 2014). First investigations into epigenetic variation in wild animal populations involved the salmonid, *Oncorhynchus mykiss* (Blouin *et al.*, 2010). The authors tested if distinct levels of DNA methylation variation could explain differential survival rates between fish in two different habitats, but found no significant differences, possibly due to small sample size (six fish in total) and low-resolution methods (MS-AFLP). However, recent studies in clonal fish (*Chrosomus eos-neogaeus*) (Massicotte *et al.*, 2011; Massicotte & Angers, 2012), round-leaf bats (*Hipposideros armiger*) (Liu *et al.*, 2012), house sparrows (*Passer domesticus*) (Schrey *et al.*, 2012; Liebl *et al.*, 2013), Atlantic salmon (*Salmo salar*) (Morán & Pérez-Figueroa, 2011), Darwin's finches (Skinner *et al.*, 2014), red grouse (*Lagopus lagopus scotica*) (Wenzel & Piertney, 2014), *Daphnia* (Schield *et al.*, 2015), yellow baboon (*Papio cynocephalus*) (Lea *et al.*, 2016), Tessellated darter (*Etheostoma olmstedi*) (Smith *et al.*, 2016) and threespine stickleback (*Gasterosteus aculeatus*) (Smith *et al.*, 2015; Trucchi *et al.*, 2016) all showed population, habitat or species-specific DNA methylation patterns. These patterns may indicate that epigenetic variation is both environmentally sensitive and common among wild animal populations, and could play an important role in regulating phenotypic traits during local adaptation. However, none of the empirical work to date has been designed to assess the degree of autonomy between epigenetic variation and genetic variation. This is important because, as we will discuss in the next section, the effects of epigenetic variation on phenotypic plasticity and evolution can be subsumed into the effects of genetic variation if epimutation is guided by underlying genetic variation. Moreover, most population epigenetic work to date has focused on DNA methylation variation, and it is important to note that other epigenetic mechanisms, for example, histone modification, chromatin remodelling and non-coding RNAs, may also play important roles in shaping phenotypic variation between populations.

In summary, empirical studies with wild animal populations have demonstrated that epigenetic variation can be documented outside of the laboratory. However, the number of examples is still small, and the traits that natural epigenetic variation has been associated with are largely limited to developmental and morphological phenotypes. Moreover, the extent to which genetic variation controls epigenetic variation, and the stability of population epigenetic variation remain unclear. Further studies that broaden the search for epigenetic variation in natural populations and assess the importance of such variation for adaptation would be valuable.

The evolutionary potential of epigenetic variation

Experimental studies investigating the role of epigenetic variation in adaptive evolution are in their initial stages (Verhoeven *et al.*, 2016). The evolutionary relevance of epigenetic variation rests on whether epigenetically induced responses are under genetic control (Richards, 2006), and whether epigenetic variation can improve species persistence. Although it is clear that epigenetically induced responses can be inherited over several generations in the laboratory (Jablonka & Raz, 2009; Daxinger & Whitelaw, 2012; Lim & Brunet, 2013), the stability of these responses over longer evolutionary timescales is unclear. In this section, we will discuss the potential evolutionary impact of epigenetic variation by focusing on two key questions: (1) How autonomous is epigenetic variation from genetic variation? (2) How stable is transgenerational epigenetic variation?

How autonomous is epigenetic variation from genetic variation?

The extent to which epigenetic variation is under genetic control is an important first step in assessing the evolutionary potential of epigenetic processes (Richards, 2006; Bossdorf *et al.*, 2008). To simplify the relationship between genetic and epigenetic variation, Richards (2006) defined three classes of epigenetic variation: obligatory, which is completely dependent on genetic variation (e.g. differentially methylated sites were frequently found within repetitive DNA in dogs; Janowitz Koch *et al.*, 2016); facilitated, which is directed or loosely potentiated by genotype (e.g. *Agouti* viable yellow epialleles in mice; Morgan *et al.*, 1999); and pure, which is typically generated by stochastic events, and is largely independent of genetic variation (e.g. growing divergence in epigenotype during ageing in monozygotic twins; Fraga *et al.*, 2005). Because undetected genetic changes might influence epigenetic

variation, it can be difficult to distinguish between pure and facilitated epigenetic variation (Richards, 2006), and thus, we mainly discuss the two extremes of epigenetic variation: obligatory and pure (Fig. 2).

Both obligatory and pure epigenetic variation could play crucial roles in phenotypic plasticity and evolution, but to date empirical examples of epigenetic variation can largely be categorized as obligatory. Examples include the marginal effects of methylation variation on expression variation when taking SNP effects into account in *Arabidopsis* (Meng *et al.*, 2016), and the targeted methylation of a transposon within the gene *Axin* that induces a unique transcript in a strain of inbred mice (Rakyan *et al.*, 2003). These examples suggest a key role of obligatory epigenetic variation in regulating the active status of transposable elements (TEs), which can be highly sensitive to environmental change, and thus facilitate responses to changing conditions. For instance, elevated temperature alters the expression of piRNAs in *Drosophila melanogaster*. As a result, the mobilization activity of transposons also changes (Brennecke *et al.*, 2008). This process can generate genetic variation and phenotypic plasticity because high mobility of TEs can increase transposon insertion polymorphisms, and the insertion of TEs into a coding or promoter region can affect gene expression (Rey *et al.*, 2016). Pure epigenetic variation may also help populations respond to environmental change. A recent theoretical study has suggested that when selection acts on pure epigenetic variation as opposed to obligatory epigenetic variation, adaptive phenotypes can occur before genotypic change due to the higher rate of epimutation permitting faster exploration of the fitness landscape (Klironomos *et al.*, 2013). However, none of the empirical work to date has addressed pure epigenetic variation in animals, possibly due to the difficulty of establishing genetically identical populations. Even in plants, there is currently very little data beyond model systems to shed light on the dynamics of pure epigenetic variation during environmental change. A recent study in *Arabidopsis thaliana* suggested that the independent contributions of pure epigenetic variation may be limited, as a large proportion of DNA methylation variants are associated with specific genetic variants (Dubin *et al.*, 2015). Thus, characterizing pure epigenetic variation should be a goal for future work, as it is crucial for understanding whether epigenetic variation can autonomously impact phenotypic variation.

One way to identify epigenetic responses under natural conditions is to look for correlations between epigenetic variation and phenotypic variation or environmental factors that are statistically independent from genetic relatedness of the individuals or populations. Many studies have quantified epigenetic variation by applying standard statistical measures used in population genetics (Box 3). To provide conclusive evidence that epigenetic variation can result in ecologically relevant phenotypic changes that are autonomous from genotypic variation, we suggest that future studies of pure epigenetic variation could transplant different populations into common environments, and test for the contributions of genetic effects to epigenetic variation by testing for genome- or epigenome-wide associations in sample individuals and their offspring (GWAS and EWAS, respectively), after correcting for confounding by genetic background using a kinship matrix (Dubin *et al.*, 2015; Orozco *et al.*, 2015; Lea *et al.*, 2016; Gugger *et al.*, 2016). Several recent studies have also developed statistical approaches to partition environmental and genetic effects on epigenetic variation. For example, a linear mixed model or binomial mixed effect model, which treats environment as a fixed effect, and the contributions from *cis*- and *trans*- genetic variants as random effects, has been used to successfully delineate phenotypic variation into components that are sensitive to temperature treatments (Amanda *et al.*, 2016). Alternatively, a leading principal coordinate analysis can be used when both GWAS and EWAS are available (Rakyan *et al.*, 2011). Although these approaches to identify environmental factors are promising, there are several caveats when interpreting results from such experimental designs. For example, detecting *cis* association between SNP and epigenetic variation does not necessarily imply genetic regulation but may simply be due to linkage disequilibrium (LD) between epigenetic variation at a locus and its proximal SNPs (Taudt *et al.*, 2016). Moreover, the causality between genetic variation and epigenetic variation may be reversed. An emerging view suggests a reciprocal relationship between TEs and epigenetic variation where epigenetic changes can also induce TE-associated genetic variation (Rey *et al.*, 2016), although many detected *cis* associations have been found in the context of SNP-mediated epigenetic silencing of nearby transposable elements (TEs). This reciprocal relationship has implications for inferring mechanisms underlying temperature-induced response. For instance, elevated temperatures were shown to alter the expression of piRNAs in *Drosophila melanogaster*, and as a result, the mobilization activity of transposons also changed (Brennecke *et al.*, 2008). If the high mobility of TEs accelerates mutation rates, and the insertion of TEs into a coding or promoter region affects gene expression, the causal explanation for gene expression changes will be epigenetic variation but not genetic regulation.

Because genetic variation can obscure the role of epigenetic variation, simplified experimental systems in which confounding effects of genetic variation have been reduced to a minimum may be useful for isolating the contributions of epigenetic processes. Bossdorf *et al.* (2008) have proposed three approaches to control for the effects of genetic variation when studying the effects of epigenotype on phenotype by either reducing epigenetic or genetic heterogeneity: first, to use species with known deficiencies in epigenetic mechanisms;

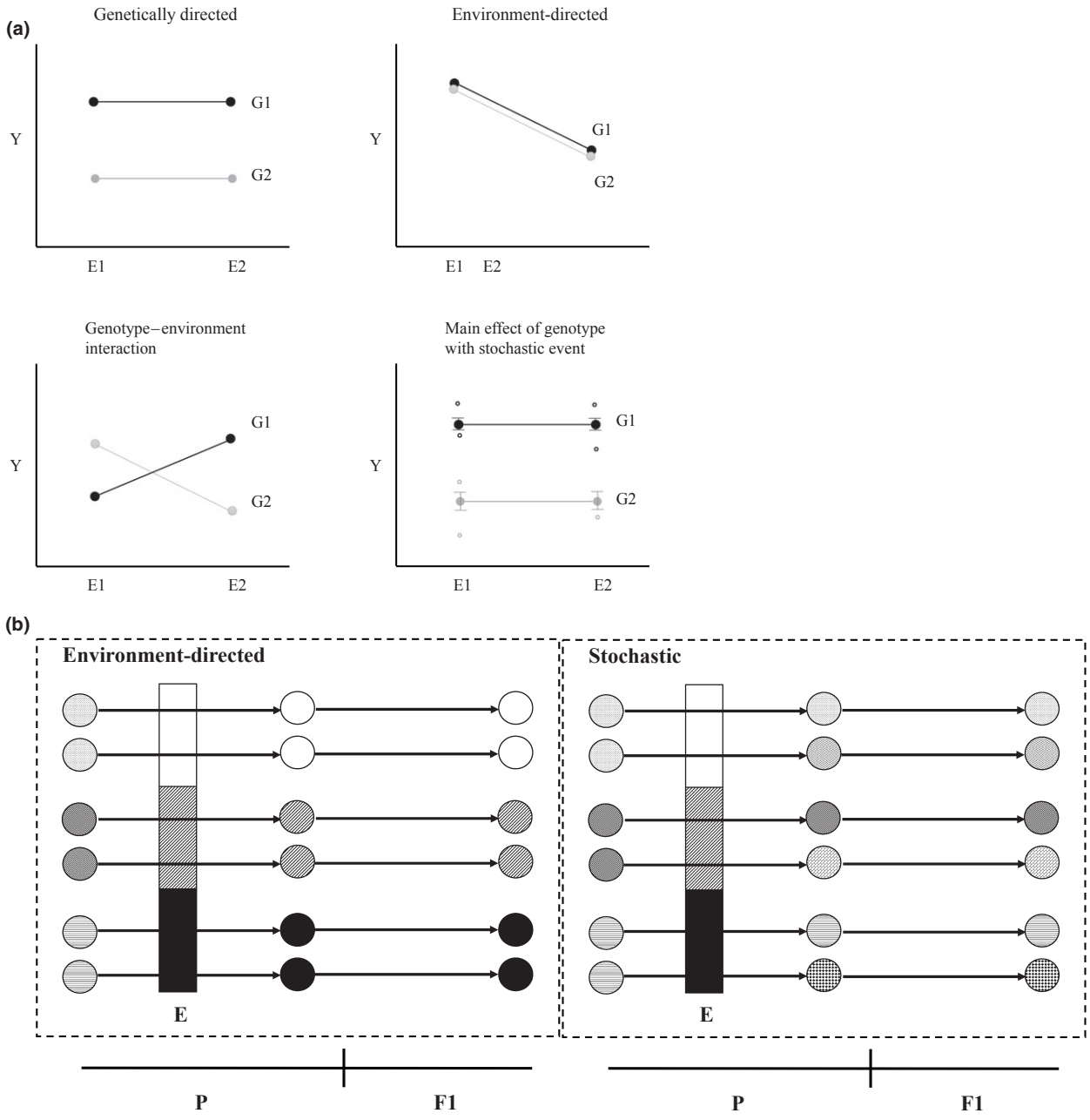

Fig. 2 A schematic of the source and stability of epigenetic variation during adaptation at the population level. (a) Interaction plots of different main sources of epigenetic variation. Here, we assume a scenario with two genotypes (G1 and G2) and two environments (E1 and E2). The *Y*-axis plots the chromatin state. Lines connect means of each genotype in each environment. We show 95% confidence interval around means, and hollow dots represent outliers. (b) Relationships between environment-directed epigenetic variation, stochastic epigenetic variation, and environmental change. Here, we assume a starting population (P) with epigenetic variation among individuals (dot, hatched and lined circles). P individuals respond to environmental change (E) and produce epigenotypes that are stably transmitted to the F1 generation. In the scenario of environment-directed epigenetic variation, epigenotypes are produced based on the cues that P individuals experience from the environment (indicated by matching of epigenotype pattern and temperature pattern), and the amount of epigenetic variation remains constant. In the scenario of stochastic epigenetic variation, new epigenotypes are produced at random and without regard to environmental conditions (wave, brick and checker board), and thus, the epigenetic variation is increased.

Box 3 How to quantify epigenetic variation and associate it with genetic and environmental variation

Genetic and epigenetic estimates of variation are fundamentally different. Genetic variation refers to diversity in allele frequencies between individuals or populations, whereas epigenetic variation refers to the presence or absence of epigenetic markers (e.g. DNA methylation) without implying changes in the underlying DNA sequence. The magnitude of genetic vs. epigenetic variation is thus difficult to compare, but patterns of change in estimates of variation can be used to contrast genetic and epigenetic diversity. Initially, researchers have utilized methylation-sensitive AFLP (MS-AFLP) to identify genome-wide methylation patterns (Schrey *et al.*, 2013; Verhoeven *et al.*, 2016). MS-AFLP identifies a multilocus epigenotype for each individual by substituting methylation-sensitive isochizomeric enzymes *Msp*I and *Hpa*II for *Mse*I in a standard AFLP protocol (Vos *et al.*, 1995). The enzymes *Msp*I and *Hpa*II have different sensitivities in recognizing cytosine methylation in the CCGG context (Salmon *et al.*, 2008). *Msp*I does not cut when the inner cytosine is methylated, and *Hap*II does not cut when either or both cytosines are fully methylated or hemi-methylated (Roberts *et al.*, 2007). Using this technique and multivariate statistical approaches, Foust *et al.* (2016) identified DNA methylation patterns (but not genetic variation) that were correlated with environmental gradients. Herrera *et al.* (2016) proposed a new analysis, epigenetic isolation by distance (IBD), to infer environmental effects on natural epigenetic variation using genetic IBD as a null model. Their results suggest that local environments are major drivers of epigenetic spatial structure in populations.

Several recent studies have also applied next-generation sequencing techniques to simultaneously analyse the relationships between genetic variation, epigenetic variation, and the environment (e.g. Schmitz *et al.*, 2011; Platt *et al.*, 2015; Gugger *et al.*, 2016). In these studies, genomic DNA treated with sodium bisulphite was sequenced. Sodium bisulphite causes deamination of unmethylated cytosines and results in the conversion of these unmethylated cytosines to uracil, leaving methylated cytosines unconverted (Laird, 2010). Untreated genomic DNA was also sequenced to provide information regarding genetic variation. Using markers generated by bisulphite sequencing, several studies have demonstrated a strong correlation between methylation variation and climate variables (Gugger *et al.*, 2016; Keller *et al.*, 2016).

Despite differences in the type of variation being estimated, some of the standard statistical measures used in population genetics for describing patterns of genetic variation should be transferable to epigenetic variation. For example, statistics that describe the frequency and diversity of alleles may be applied to epiallelic diversity, and measures such as F_{ST}, which describes genetic population structure (e.g. Liebl *et al.*, 2013), *h*, which describes the haplotype diversity (e.g. Richards *et al.*, 2012), analysis of molecular variance (AMOVA), which detects population differentiation utilizing molecular markers (e.g. Herrera & Bazaga, 2010), and principal coordinate analyses (PCoA) can be equally useful in describing population differentiation at the epigenetic level (e.g. Gao *et al.*, 2010; Wenzel & Piertney, 2014; Preite *et al.*, 2015). Correlations between population statistics for genetic vs. epigenetic variation can potentially be analysed using a Mantel test (e.g. Cervera *et al.*, 2002; Wenzel & Piertney, 2014; Foust *et al.*, 2016). In summary, genome-wide genetic variation and epigenetic variation can be quantified simultaneously and statistical methods can help elucidate the degree of autonomy between epigenetic and genetic variation, and the relative importance of genetic and epigenetic variation in facilitating population divergence and adaptation.

second, to use demethylating agents to inhibit activities of DNA methyltransferases (Dnmts), and thereby induce experimental demethylation; and third, to choose a study system with a known lack of genetic variation. As mutants with known deficiencies in epigenetic mechanisms do not yet exist for most animal systems, and the use of *in vivo* demethylating agents can lead to undesired side effects caused by untargeted, genomewide demethylation (Verhoeven *et al.*, 2016), many population epigenetic studies to date have used the last strategy. For example, researchers have used populations with limited genetic variation resulting from clonal reproduction (e.g. clonal fish, Massicotte *et al.*, 2011; Massicotte & Angers, 2012) or bottlenecks following invasion (e.g. house sparrows, Schrey *et al.*, 2012; Liebl *et al.*, 2013). Following from classic investigations in plants (Cubas *et al.*, 1999; Kalisz & Purugganan, 2004), these studies provide the first indications from animals that DNA methylation may sometimes act independently from underlying genetic variation, and facilitate a clearer evaluation of the consequences of epigenetic variation. However, mutation accumulation is still possible in clonal lines, and even the reduced genetic variation in bottlenecked populations may still be sufficient to contribute to epigenetic responses. In summary, although it remains challenging to explicitly partition the genetic basis of epigenetic variation, the ability of autonomous epigenetic variation to cause phenotypic change is increasingly appreciated, and should be considered as a potential mechanism when adaptive traits cannot be explained by common genetic variants.

How stable is transgenerational epigenetic variation?

A major barrier to transgenerational epigenetic inheritance is germline reprogramming. In contrast to plants (Verhoeven *et al.*, 2016), in animals, especially mammals, extensive erasing of epigenetic modifications occurs both in the germline and in the zygote immediately after fertilization. Thus, it is more difficult to inherit epigenetic marks that are not associated with sequence variants across generations in animals. Indeed, heritable epigenetic variation that is independent from genetic control seems to be more common in plants (Taudt *et al.*,

2016), but emerging evidence has shown that such epigenetic inheritance may also exist in animals (Youngson & Whitelaw, 2008; Jablonka & Raz, 2009; Daxinger & Whitelaw, 2012; Lim & Brunet, 2013; Heard & Martienssen, 2014). We distinguish between three main sources of transgenerational epigenetic variation: genetically directed, environment-directed, and stochastic (Shea *et al.*, 2011; Taudt *et al.*, 2016). Genetically directed epigenetic variation is regulated by *cis*- or *trans*-acting genetic variation. Environment-directed epigenetic variation is the result of exposure to current or past environmental factors. In contrast, stochastic epigenetic variation, such as epigenetic drift or epimutation, is more analogous to random genetic mutation, and may arise when organisms are exposed to stressful environments. These types of epigenetic variation can all be heritable and may share molecular mechanisms (e.g. DNA methylation), but differ in their implications for evolution (Fig. 2). When epigenetic variation is genetically directed, the effects of epigenetic variation on phenotype could be considered as a component of the genetic effects. Thus, here we focus on environment-directed and stochastic epigenetic variation that may still be stably transmitted despite not being controlled by genotype.

Typical cases of environment-directed epigenetic variation are epigenetics-mediated phenotypic plasticity in changing environments, resulting in environment-specific phenotypes (Verhoeven & Preite, 2014). Depending on the stability of such epigenetic variation, induced phenotypes can be transmitted to offspring if the epigenetic marks can resist resetting between generations, but may not persist in organisms after the environmental cue that initially induced the variation has disappeared. Empirical studies have supported the role of environment-directed epigenetic variation in mediating phenotypic plasticity within a single generation, and across generations as we reviewed in the above section. The evolutionary implications and adaptive benefits of within- and between-generation phenotypic plasticity have been discussed in Herman & Sultan (2011). In general, environment-directed epigenetic changes beyond one generation alter adaptive dynamics due to the partial uncoupling of the phenotype from the underlying genotype (Bonduriansky & Day, 2009). Such epigenetic variation could be adaptive if parents can predict the offspring environment to some extent, and the effects of epigenetic variation increase both parental and offspring fitness with low cost (Herman *et al.*, 2014). In contrast to environment-directed epigenetic variation, which is expected to show the same pattern in different individuals with the same genotype when exposed to the same environment, stochastic epigenetic variation can contribute to heritable variation that is shaped by natural selection (Shea *et al.*, 2011), and thus will be indistinguishable from genetic variation in a standard heritability analysis (Johannes *et al.*, 2008; Helanterä & Uller, 2010; Tal *et al.*, 2010). When organisms are maladapted or in stressful environments, stochastic epigenetic variation has the potential to facilitate short-term adaptation by producing phenotypically diverse offspring. This may be favourable by allowing greater exploration of phenotypic space, thereby increasing the probability of producing a phenotype that is closer to the optimum (Pál, 1998; Pál & Miklos, 1999). For longer-term adaptation, in a constant environment, unless the strength of selection is high (Klironomos *et al.*, 2013), stable transmission of stochastic epigenetic variation for many generations will be required for natural selection to produce adaptations based on epiallelic variation (Slatkin, 2009). Importantly, because stressful environments can trigger enhanced epimutation rates (Rapp & Wendel, 2005; Verhoeven *et al.*, 2010), rates of stochastic epimutation may slow after adaptation to the current environment has been achieved.

In a summary, whether epigenetic changes are environment-directed or stochastic is likely to influence their adaptive value, but both sources of epigenetic variation may maintain an adaptive phenotype long enough for new genetic mutations to arise and stabilize the phenotype (Klironomos *et al.*, 2013). Under fluctuating environmental conditions, appropriate rates of epigenetic stability from both types of variation may contribute to transient adaptation by allowing organisms to respond to environmental variation without changing their genomes (Lachmann & Jablonka, 1996; Rando & Verstrepen, 2007; Salathé *et al.*, 2009; Verhoeven & Preite, 2014). However, the costs associated with each source of variation are different. Costs of environment-directed epigenetic variation mainly accrue through the resources required to maintain sensing machinery, and there is also a potentially detrimental time delay between sensing environmental change and making a phenotypic response (Rando & Verstrepen, 2007), and stochastic epigenetic variation can be costly because it will produce some maladaptive phenotypes in every generation. Both environment-directed and stochastic epigenetic variation may compensate for evolutionary potential that is otherwise constrained by the inability to generate phenotypic variation through recombination or genetic variation (Verhoeven & Preite, 2014). Furthermore, germline resetting may also affect the evolutionary potential of environment-directed and stochastic epigenetic variation in different ways. There is no obvious conflict between environment-directed epigenetic variation and resetting because when resetting of epigenetic marks happens, paternally mediated alterations to these markers can still occur after the resetting process. For example, the typically prolonged relationship between mother and offspring in mammals may result in transgenerational persistence of an environment-directed effect (Weaver *et al.*, 2004). As for stochastic epigenetic variation, whether germline resetting is piecemeal or

global will affect its evolutionary potential. When global resetting occurs, the evolutionary potential of stochastic epigenetic variation will be reduced because stochastic epigenetic variation requires stable transmission across generations to form the basis of long-term adaptation. In contrast, if the resetting is piecemeal or incomplete, then epigenetic loci can more consistently transmit the impact of natural selection on allelic variation between generations.

Heritable epigenetic mutations and evolution: theoretical approaches

Empirical studies of epigenetic inheritance induced by genetic and environmental perturbations have been reviewed elsewhere (Youngson & Whitelaw, 2008; Jablonka & Raz, 2009; Daxinger & Whitelaw, 2012; Lim & Brunet, 2013; Heard & Martienssen, 2014). Here, we mainly survey theoretical work of epigenetic inheritance within ecological contexts. In general, current theoretical studies have applied two approaches to investigate the effects of epigenetic variation on evolution. In the first approach, the main aim is to investigate the effects of stable levels of epigenetic mutation on the maintenance of genetic or phenotypic variation (Pál, 1998; Pál & Miklos, 1999; Day & Bonduriansky, 2011; Carja & Feldman, 2012; Geoghegan & Spencer, 2012, 2013; Klironomos *et al.*, 2013; Kronholm & Collins, 2015; Day, 2016). In the second approach, the switching rate of epigenetic variation between generations has been identified as an evolutionary variable, which can evolve in response to different environments without interacting with genotypes (Jablonka *et al.*, 1995; Lachmann & Jablonka, 1996; Salathé *et al.*, 2009; Feinberg & Irizarry, 2010; Furrow & Feldman, 2014; Uller *et al.*, 2015; Kuijper & Johnstone, 2016; Table 2). In general, models of epigenetic switching rates have concluded that the rate of temporal environmental change is a key factor controlling epigenetic variation. In predictable environments, epigenetic switching rate evolves to approximately the inverse of the length of time between environmental changes (Lachmann & Jablonka, 1996; Salathé *et al.*, 2009). In contrast, under unpredictable environmental conditions, epigenetic variation allows the production of phenotypically diverse offspring, which increases the probability of producing a phenotype that is closer to the optimum, and can make epigenetic switching analogous to a genetically encoded bet-hedging strategy in fluctuating environments (Veening *et al.*, 2008; Day, 2016). This is intriguing because some recent findings studied under the context of bet-hedging may be directly translatable to epigenetic switching (e.g. Kussell & Leibler, 2005; Carja *et al.*, 2014).

Results from models that analyse the interactions between heritable epigenetic variation, genetic variation and phenotypic variation have suggested that adaptation to changing environments can be accelerated by epigenetic variation in a manner analogous to that proposed for within-generational phenotypic plasticity, which facilitates persistence, followed by genetic assimilation, and a reduction in phenotypic plasticity (Via & Lande, 1985; West-Eberhard, 2003; DeWitt & Scheiner, 2004; Richards 2006; Lande, 2009). Such adaptations can be enhanced by heritable epigenetic variation, thus helping organisms inhabit novel environments (Jablonka & Raz, 2009). However, the conditions under which stochastic vs. environment-directed epigenetic variation may be favoured are unclear because current models have only considered each mechanism in isolation of the other. For example, Furrow & Feldman (2014) suggested that epigenetic variation that is environmentally responsive is advantageous under fluctuating environments because the cost of such epigenetic variation is minimal with stably induced transgenerational phenotypic plasticity. In this case, the authors assumed that the potential cost of maladaptive epigenetic variation is high, and suggested that only epigenetic variation that is environmentally directed towards the optimal fitness state can be favoured. In contrast, Feinberg & Irizarry (2010) considered a model in which stochastic epigenetic variation was the only source of epigenetic variation, and concluded that it could be an important driver of evolutionary adaptation by increasing the range of phenotypes that could be produced by a given genotype in changing environments.

In summary, although recent studies have made progress in exploring the effects of epigenetic variation during adaptive evolution, initial results have yielded inconclusive messages about the predicted effects of epigenetic variation under environmental change, and the relative importance of environment-directed and stochastic epigenetic variation during adaptation. Further theoretical work is warranted to better understand these issues.

Conclusions

Here, we use studies of epigenetically encoded thermal plasticity in animals to provide specific examples for understanding the relationship between epigenetic variation and phenotypic plasticity. We then reviewed the patterns and potential evolutionary consequences of epigenetic variation in wild populations. Specific epigenetic patterns are well documented in some animal populations, but their prevalence and relationships with fitness remain under debate. Moreover, although studies in plants and humans have shown a strong correlation between patterns of epigenetic variation and underlying genetic variants, comparable investigations in wild animals have not yet systematically explored the relative contributions of genetic vs. epigenetic variation in explaining the heritability of phenotypic traits. Building upon the characterization of molecular mechanisms underlying epigenetic modifications, a number of recent theoretical studies investigating the stability of heritable epigenetic variation have suggested that

Table 2 Models of heritable epigenetic variation and evolution.

Approach	Relationship with genetic variation	Changing environment?	Effects of epigenetic variation	Example Theor. refs	Example Empir. refs
Effects of switching between epigenetic variants on phenotypes	Not mentioned	Yes	Long-term inheritance of epigenetic variants results in reduced fitness variance and greater population growth in random and temporally patchy environment.	Jablonka *et al.* (1995)	None
	Not mentioned	Yes	In an environment that both induces and selects variants, epigenetic variants that have a switching rate corresponding to environmental periodicity will be advantageous.	Lachmann & Jablonka (1996)	Soll *et al.* (1993)
	Controlled by genetic variation	Yes	Switching rate of epigenetic variation will reflect the rate of environmental change when selection is strong.	Salathé *et al.* (2009)	None
	Controlled by genetic variation	Yes	Increased phenotypic variability and fitness in changing environments, without changes in the mean phenotype.	Feinberg & Irizarry (2010)	Feinberg & Irizarry (2010)
	Independent from genetic variation	Yes	In the short term, epigenetic changes are equivalent to mutations and are likely to be in LD with SNPs. In the long term, epigenetic changes have limited contribution to heritability and recurrence risk in disease.	Slatkin (2009)	None
	Controlled by genetic variation	Yes	An equilibrium exists for the frequencies of the alleles at the locus controlling the epigenetic states. The equilibrium depends on the mutation rate, the fitness landscape and the period of the environmental fluctuation.	Carja & Feldman (2012)	None
	Not mentioned	Yes	Maintained phenotypic variation in changing environments, even without genetic variation.	Geoghegan & Spencer (2012, 2013)	Herrera *et al.* (2013)
	Controlled by genetic variation	Yes	Serve as a mechanism for plasticity, phenotypic switching or stable inheritance of phenotypic states, depending on the frequency of environmental changes.	Furrow & Feldman (2014)	None
	Not mentioned	Yes	Transmission of epigenetic states prevents mismatched phenotypes when the environment changes infrequently relative to generation time and when maternal and environmental cues are unreliable.	Uller *et al.* (2015)	None
	Controlled by genetic variation	Yes	Selection can favour epigenetic variation that generates a positive correlation between parental and offspring phenotype under relatively stable environments, and generates a negative correlation between parental and offspring phenotype under unstable environments.	Kuijper & Johnstone (2016)	Lewis (2010); Schmitz *et al.* (2011)
Effects of epigenetic mutation on maintaining genetic or phenotypic variation	Controlled by genetic variation	No, but with changes in microenvironments	Promote population persistence, particularly when the mean phenotype is far from the optimum. Genetic assimilation occurs much closer to the fitness peak.	Pál (1998); Pál & Miklos (1999)	Ho *et al.* (1983)
	Not mentioned	Yes	Determine phenotypic variation, and decouple phenotypic change from evolutionary dynamics of genotype.	Day & Bonduriansky (2011)	Skinner *et al.* (2014)
	Independent of genetic variation	Yes	Decouple fitness from genetic variation, allow populations to have higher level of genetic variation and allow populations to respond to environmental change even without genetic variation.	Klironomos *et al.* (2013)	None
	Independent of genetic variation	No	Depending on stability and fitness effects relative to genetic variation, epigenetic variation can accelerate the initial stage of adaptation, but cause lower final fitness values, or *vice versa*.	Kronholm & Collins (2015)	None
	Not mentioned	Yes	The combination of epigenetic inheritance and developmental noise can also explain some bet-hedging strategies that were previously explained solely by genetic mechanisms	Day (2016)	None

Box 4 Outstanding questions about epigenetically encoded thermal plasticity

Numerous questions remain regarding epigenetically encoded thermal plasticity in natural animal populations, their ecological and evolutionary importance, and potential implications for population responses to climate change.

- How taxonomically widespread is epigenetic variation in thermal plasticity, and is it linked to particular geographical or environmental gradients?

Substantial differences in epigenetic mechanisms and patterns can exist between and within taxa experiencing changes in temperature (Feng *et al.*, 2010; Zemach *et al.*, 2010), for example, differences in methylation maintenance machinery (Alonso *et al.*, 2015; Willing *et al.*, 2015), and different strategies to maintain body temperature between ectotherms and endotherms. Epigenetic variation may also be linked to particular life history or habitat features (Herman *et al.*, 2014; Verhoeven & Preite, 2014). Thus, studies of epigenetic variation should be conducted in a wide range of taxa, and between populations inhabiting different thermal environments to determine whether patterns of epigenetic variation are conserved across deep phylogenies and various habitats.

- Are there particular features that make a system a good model for studying epigenetically encoded thermal plasticity?

To study the epigenetic basic of phenotypic variation, it is useful for a system to exhibit a significant degree of phenotypic plasticity when responding to environmental changes (Dimond & Roberts, 2016). Good long-term data sets connecting environmental parameters with changes in phenotype and gene expression can also help to place functional work within a broader environmental context. For example, Barshis *et al.* (2013) provided useful long-term data on acclimation of coral species to climate change. In particular, species that can resist reprogramming during meiosis and embryogenesis, and transmit changes in DNA methylation to offspring, will help facilitate understanding of transgenerational epigenetic processes (Duncan *et al.*, 2014). For example, Schield *et al.* (2015) characterized methylation variation of clonal *Daphnia ambigua* in response to fish predator cues, showing consistent changes in the epigenome in successive generations. Finally, a well-annotated genome will clearly aid in improving inferences about the epigenetic basis of phenotypic plasticity and local adaptation. Alternative approaches for analysing DNA methylation variation are also available, for example, using a reference-free approach that combines an optimized RRBS protocol with a tailored bioinformatics pipeline (Klughammer *et al.*, 2015), or mapping reads to *de novo* constructed genomes. However, these approaches usually come with limitations, for example, detecting fewer covered CpGs than using reference-based analysis due to repetitive elements (Klughammer *et al.*, 2015) or inaccurate mapping due to assembly errors in *de novo*-assembled genomes (Earl *et al.*, 2011).

- From descriptive data to causal and quantitative effects in epigenetically encoded thermal plasticity

Genome-wide epigenetic markers, especially DNA methylation, have provided a useful tool for studying thermal plasticity. However, it remains difficult to predict from these descriptive data which of the markers, features, and profiles are indicative of causal and quantitative effects of epigenetics on thermal plasticity. Furthermore, very few studies currently account for the confounding effects of genetic variation in producing thermal plasticity. Thus, we think it will be important to adopt statistical methods (e.g. linear mixed models, binomial mixed models) that account for genetic contributions to thermal plasticity. In addition, in model systems that already have epigenetic candidate loci for thermal plasticity, new experimental approaches (e.g. site-specific DNA methylation editing with a catalytically inactive variant of the Cas9 nuclease; Liu *et al.*, 2016) may provide a means to pinpoint functional epigenetic effects on thermal plasticity.

- How are epigenetic changes induced by temperature maintained via the germline, and how is the duration of maintenance determined?

The extent to which environmentally induced epigenetic variants persist across generations remains controversial among evolutionary biologists, especially in mammals where germline resetting is more extensive than plants. It is now clear that some molecular mechanisms, for example, small interfering RNAs (RNAs), Piwi-interacting RNAs (piRNAs), and miRNAs can facilitate epigenetic inheritance via the germline in model animals (Lim & Brunet, 2013); however, the generality of these mechanisms in animal thermal plasticity warrants further empirical study. It is also unclear how epigenetic machinery affects the duration of epigenetic changes when organisms face temperature changes. DeWitt *et al.* (1998) suggested potential costs and limits of phenotypic plasticity, and if thermal plasticity is regulated by epigenetic changes, similar costs and limits will also be associated with epigenetic variation, making the trade-offs required for maintenance of epigenetic variation another interesting area to be explored.

- What is the proportion of the epigenome that is found to be under genetic control, the relative contributions of *cis*- and *trans*-acting genetic factors, their average effect sizes and their mechanisms of action in animal thermal plasticity?

A deep understanding of the heritable basis of population epigenetic variation in animals has come mainly from studies of the relationship between DNA methylation and phenotypic traits, for example, disease in humans. Results from these studies have suggested a predominant correlation between *cis*-acting genetic variants and epigenomic variation (Taudt *et al.*, 2016). If this is also true for epigenetically encoded thermal plasticity, levels of genetic variation may determine the vulnerability of organisms to changing temperatures. However, these studies also suggested stochasticity in allele-specific epigenetic variation. Stochastic epigenetic variation may compensate for the loss of phenotypic plasticity (Verhoeven & Preite, 2014). Thus, it is necessary to first

Box 4 *(Continued)*

distinguish between different sources of epigenetic variation using fine resolution sequencing techniques and statistical models, and then study the effects of each epigenetic modification on animal thermal plasticity. Techniques used in human studies (e.g. chromatin immunoprecipitation followed by sequencing (ChIP-seq)) are still financially prohibitive for many laboratory groups. More cost-effective approaches, for example, reduced representation bisulphite sequencing (RRBS), may be a good alternative for collecting data with single-nucleotide resolution. These techniques will allow for more detailed understanding of methylation variation at specific and functionally characterized loci, and will aid epigenome-wide association studies that link epigenetic variation to ecologically relevant traits such as thermal tolerance.

- Under which temperature regimes will environment-directed vs. stochastic epigenetic variation be favoured?

The relative importance of these two types of heritable epigenetic variation has not yet been resolved. Which type of epigenetic variation will be favoured may be determined by the degree of environmental variability, or the strength of natural selection (Verhoeven & Preite, 2014). Thurman & Barrett (2016) found that selection on genetic variants was strongest over relatively short timescales (<200 generations), with the greatest magnitude of selection occurring within a single generation. Thus, we predict that stochastic epigenetic variation may be favoured over short time periods when thermal environments are stressful or unpredictable. This is because stochastic epigenetic variation allows the production of phenotypes that are closer to the optimum, or rapid phenotypic switching in fluctuating environments (Veening *et al.*, 2008; Day, 2016). In contrast, environment-directed epigenetic variation will be favoured over longer time periods of consistent environmental conditions, when the process of acquiring information about the changing climate is less risky. Efforts should be made to develop integrated theoretical models that include both types of epigenetic variation, and consider the strength and timescale of selection.

transgenerational epigenetic markers can play an important role in increasing the 'evolvability' of natural populations in changing environments.

Consideration of epigenetic variation allows an expansion of current concepts of variation and evolution in natural populations to consider additional, non-genetic sources of heritable variation that natural selection may act on. However, even with the progress that we describe here, several challenges remain (Box 4). One of the most important applications of increasing knowledge of epigenetic variation may be to address the challenge that only a small proportion of variance in complex traits is explained by common genetic variants (Danchin *et al.*, 2011). By helping to fill the missing heritability gaps between genotypes and phenotypes, epigenetics may aid in predicting evolutionary responses to environmental change. Although epigenetic research in natural animal populations is at an early stage, current studies have built a solid foundation for future work to investigate the role of epigenetic variation in regulating phenotypic plasticity in natural environments, and to link this variation with fitness and long-term evolutionary consequences.

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