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EPIGENETIC STUDIES IN ECOLOGY AND EVOLUTION

INTRODUCTION

Epigenetics in ecology and evolution: what we know and what we need to know

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'Epigenetics' is often broadly defined and vaguely used (Bird 2007). In fact, Adrien Bird has been quoted as saying: 'Epigenetics is a useful word if you don't know what's going on – if you do, you use something else' (Ledford 2008). However, many do use the word, epigenetics, and some more narrowly than others. These days, the word is often applied to the study of chemical tags on DNA (such as histone modifications, DNA methylation, and the small RNAs that guide them) that have the potential to affect gene expression in response to developmental and environmental triggers (e.g. Richards 2011). Others restrict the term to denote the transgenerational heritability of these mechanisms and their phenotypic effects (epigenetic *inheritance*), which largely limits the mechanism to relatively stable chemical DNA tags with potential for transgenerational persistence, such as DNA methylation (Feng *et al.* 2010; Schmitz *et al.* 2013; Eichten *et al.* 2014). It is important to distinguish between these uses of the term as some epigenetic DNA tags (e.g. histone and chromatin modifications) are not heritable, even though they may be influenced by genes that are heritable (Ptashne 2007; Eichten *et al.* 2014). Chromatin modifications, small RNAs and transgenerational epigenetics may influence phenotype (Law & Jacobsen 2010), but ecologists and evolutionary biologists are likely to be most interested in transgenerational epigenetic changes that can shape fitness-related phenotypes. For this reason, it is not surprising that most epigenetic studies in ecology and evolutionary biology in this Special Issue focus on one specific epigenetic mechanism, DNA methylation. In recent years, many review articles and conceptual publications have argued for a role of epigenetic processes in ecology and evolution (e.g. Bossdorf *et al.* 2008; Jablonka & Raz 2009; Richards *et al.* 2010; Mirouze & Paszkowski 2011; Donohue 2014; Duncan *et al.* 2014; Schmitz 2014), but we have insufficient evidence to determine the depth and breadth of that role. In this introduction to the Special Issue in Molecular Ecology on 'Epigenetic Studies in Ecology and Evolution', we provide a brief overview of the field. What is known? What is not? And what, in fact, is the type of empirical evidence that would convince us of the relevance of epigenetics for ecology and evolution?

Why do we care about epigenetics? From an ecological perspective, epigenetic mechanisms could promote phenotypic plasticity and persistence in heterogeneous environments (Bossdorf *et al.* 2008; Castonguay & Angers 2012; Zhang *et al.* 2013). From an evolutionary perspective, epigenetic effects could have stable transgenerational transmission that influences phenotypic traits that affect fitness, but that are not entirely predicted by the underlying genotype. Without epigenetic inheritance, traits are manifestations of gene expression states that reflect genetic control and induction by the environment. With an epigenetic perspective, the induced component can have long-term effects, and the inherited component is not necessarily restricted to what is encoded in the DNA sequence – it could be due to inherited epigenetic variants, possibly under the influence of the environments in previous

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generations. This perspective offers several opportunities that are additional to what is possible under genetic inheritance only. With such a mechanism linked to phenotypic variation, there is the capacity for sustained memory (including transgenerational inheritance) of environmental responses, a selection response based on stable epigenetic variants, and effects on dynamics of genetic adaptation (Jablonka & Raz 2009). The question, of course, is what the empirical evidence is for such epigenetic effects and their impact on ecological and evolutionary processes. This Special Issue brings together recent epigenetic work in a variety of study systems, allowing for an evaluation of the current status of evidence. Here, we evaluate the studies in the Special Issue in the context of several key questions that relate to (i) environmental effects on epigenetic variation; (ii) effects of epigenetic variation on phenotypes; and (iii) the evolutionary potential of epigenetics, for which it is important to understand the relation between epigenetics and underlying genetic variation.

Environmental epigenetic effects

How much epigenetic variation is environmentally induced?

Environmental effects on DNA methylation variation have been documented in plant and animal species (Dowen *et al.* 2012; Radford *et al.* 2014). These effects might play a role in mediating phenotypic plasticity, shifting phenotypic traits depending on environmental conditions, and could potentially also increase nontargeted (stochastic) epimutation rate (Herman *et al.* 2014). They are most easily identified when experimental designs or field sampling of natural populations can separate the effects of environmental induction from selection or drift when inferring environment–epigenetic associations.

One way to identify environmental effects under natural conditions is to look for associations between natural epigenetic variation and habitat or other environmental characteristics that are statistically independent from genetic relatedness of the samples. Initially, many ecologists have utilized methylation-sensitive AFLP (MS-AFLP) markers, which are similar to well-known AFLP markers with the inclusion of methylation-sensitive restriction enzymes, to detect DNA methylation differences between samples at the restriction sites (and to distinguish this from genetic restriction site polymorphisms). Using these markers and multivariate statistical approaches, Foust *et al.* (2016) show that DNA methylation patterns in salt marsh plants are correlated with steep and replicated ecological gradients differently than the associations of genetic gradients with those gradients – providing evidence for environmental effects on DNA methylation variation. Herrera *et al.* (2016) present a method to infer environmental (or other nongenetic) effects on natural epigenetic variation by evaluating the pattern of 'epigenetic isolation by distance' to a null expectation defined by the observed genetic isolation by distance, which often shapes intrapopulation genetic diversity. They find much smaller scale epigenetic spatial autocorrelation compared to genetic spatial patterns within a single *Helleborus* population, which is what we would expect if the environment is shaping DNA methylation patterns. Despite limitations of MS-AFLPs in identifying the genetic context for methylation, the findings included in this Special Issue and earlier studies (as reviewed by Alonso *et al.* 2016) have drawn attention to the potential role of the environment in shaping phenotypes through methylation of DNA sequences.

The next level of epigenetic evidence in natural populations is coming from the analysis of natural methylation variation using a bisulfite sequencing methodology, which is based on treatment of DNA samples with bisulfite that converts unmethylated, but not methylated, cytosines to uracil, providing a basis for the recognition of methylated cytosines upon subsequent sequencing and comparison to a proper control. Using reduced representation bisulfite sequencing to track DNA methylation along a geographic and climatological gradient in long-lived Valley Oak trees, Gugger *et al.* (2016) identify maximum temperature as an environmental predictor of methylation variation (whereas *genetic* variation is predicted best by other variables). Using sequence information, Gugger *et al.* also identify 43 climate-associated single methylated variants (SMVs) out of 57,488 (SMVs), and most of them were CG-SMVs that tended to occur in or near genes, several of which have known involvement in plant response to environment. In wild baboons, Lea *et al.* (2016) show that DNA methylation differs between wild-feeding animals and animals that have grown accustomed to 'easy feeding' near a lodge. Differences were enriched in genes and pathways associated with metabolism, providing support that methylation differences are triggered by the different access to resources.

Even when some loci show modified DNA methylation due to environmental effects, typically a large proportion of the methylome is relatively stable. As Trucchi *et al.* (2016) point out, using a newly developed method for reduced representation bisulfite sequencing based on RAD sequencing, the overall DNA methylation patterns can be surprisingly well conserved between ecotypes from different ecological habitats, both in plant and in animal species.

Is environment-induced epigenetic variation transgenerationally stable?

It has become clear in recent years that not all epigenetic modifications are reset between generations. In both plants and animals, epigenetic reprogramming takes place between generations during gametogenesis and early embryonic development (Feng *et al.* 2010; Kawashima & Berger 2014) that involves DNA methylation erasure and re-establishment. In mammals, measurable reductions in methylation are thought to be due to rapid generation of new DNA molecules through replication, with new strands lacking all epigenetic modifications until methyltransferases restore methylation through memory functions (Lee *et al.* 2014; Bohacek & Mansuy 2015). But especially in plants, and especially for DNA methylation in CpG contexts (Saze *et al.* 2003), this resetting seems far from complete. Incomplete erasure can contribute to epigenetic divergence across generations. As illustrated by *Arabidopsis* mutation accumulation lines, erasure escape was evidenced as increasing divergence in gene body methylation over multiple generations due to *de novo* epimutations in CpG methylation (van der Graaf *et al.* 2015). It has also been shown that that heritable methylated regions can underlie complex phenotypic traits in *Arabidopsis* (Cortijo *et al.* 2014).

Whereas transgenerational stability of some epigenetic marks is well established, it remains a controversial question if, or to what extent, *environment-induced* epigenetic variants persist across generations (Heard & Martienssen 2014). Epigenetic mechanisms can underpin plastic responses to environmental change, which may be particularly relevant for the persistence of clonal species, those thriving in rapidly changing environments (e.g. climatic unpredictability; Rubenstein *et al.* 2016) or long-lived individuals like trees (Gugger *et al.* 2016). Importantly, through epigenetic mechanisms, maternal environmental experiences may be transferred to developing embryos to affect offspring phenotypes, thus extending phenotypic plasticity across generation boundaries. Specifically, bias either in transmission or in phenotype presentation has been documented in offspring subsequent to maternal environments. For instance, in superb starling chicks the DNA methylation status of a glucocorticoid receptor gene is associated with pre-breeding rainfall, and may have functional consequences for later breeding behaviour (Rubenstein *et al.* 2016). In guinea pigs, Weyrich *et al.* (2016) identified heritable patterns of DNA methylation with paternal transmission patterns. Males were heat-treated during spermatogenesis with induced methylation patterns identified that were transmitted to offspring. Modified methylation was detectable also in testes of F1 sons which suggests that methylation changes possibly persist throught to the F2 generation. The capacity for transgenerational environmental effects, which in mammals could in principle persist into second-generation offspring also when induced epigenetic marks are reset when gravid females are exposed, because a germline is set aside already within the developing embryo, can have consequences for evolutionary trajectories.

These studies add supporting evidence for transgenerational epigenetic effects – but it remains an open question how common such effects are. The transient nature of environment-induced modifications is, for instance, highlighted by a histone modification screen in *Schistosoma* parasitic flatworms (a species in which DNA methylation is present only at very low levels; Mourao *et al.* 2012) developing in native versus foreign host snails. Roquis *et al.* (2016) found evidence both for environment-targeted modifications (shared between replicated individuals) and also for seemingly stochastic modifications (not shared between replicates), but the environment-induced variants are shorter-lived than stochastic variants.

The above studies add intriguing observations to a growing body of evidence that patterns of natural epigenetic variation are shaped to some extent by environments and do not just reflect underlying patterns of genetic variation. It is useful to remember that such deviations from underlying genetic variation do not imply 'uncoupling' from genetics – just like a phenotypically plastic phenotype, the plastic epigenome can show a genetics-based response to environments. Future research will need to examine whole genomes and whole epigenomes to thoroughly explore the genetic basis for DNA methylation and its environmental plasticity. These new observations from natural populations will motivate deeper analyses, in terms of experimental designs and (epi)genetic resolution, which is necessary to pinpoint environmental effects, stability and functional relevance of the observed DNA methylation polymorphisms, and any underlying genetic variation.

Linking epigenetic variation to phenotypes

How does epigenetic variation shape phenotype?

The epigenetic contribution to phenotypic variation is a largely unexplored question in ecological epigenetics, with recent focus in particular on an epigenetic mechanism as a means of rapidly producing plastic – and possibly adaptive – phenotypic variation (Bossdorf *et al.* 2008). There are still many unknowns about causal effects of DNA methylation on gene expression (Jones 2012) although it has been explored across various experimental designs. One method of determining the impact of

epigenetics on phenotypic variation is through direct experimental manipulation. For example, a previous study demonstrated a yet unknown epigenetic mechanism that may explain trait depression as a result of inbreeding in the perennial plant *Scabiosa columbaria* (Vergeer *et al.* 2012). Through treatment of inbred seeds with a DNA methylation-inhibiting agent, Vergeer *et al.* (2012) were able to restore the wild-type phenotype, significantly different from controls and indistinguishable from outbred individuals. Similarly, Wilschut *et al.* (2016) surveyed variation in DNA methylation between clone members within a single widespread apomictic (asexual) lineage of the common dandelion (*Taraxacum officinale* s. lat.) and identified a correlation between heritable differences in flowering time and genomewide methylation patterns. Further, upon treatment of seedlings with a DNA methylation inhibitor, phenotypic variation was reduced and flowering was synchronized among clone members, suggesting that flowering divergence within the lineage depended on DNA methylation differences. The use of such *in vivo* demethylating agents is not without its challenges, for instance due to possible undesired side effects. But in the absence of knockout mutants with compromised DNA methylation machinery, this is the only currently available tool for experimental manipulation that is readily available to nonmodel systems. Another system that inherently contains genotype independence is the eusocial European paper wasp (*Polistes dominula*). As is the case in many eusocial insects, the queen and workers arise from the same genome – a phenomenon known as a polyphenism or environmentally induced phenotypic plasticity. DNA methylation can mediate such caste polyphenism in eusocial insects (Lyko *et al.* 2010). However, Standage *et al.* (2016) identify intercaste transcriptional signatures, enriched for neurotransmitters and metabolism genes, as well as a plethora of alternatively spliced genes, but these differences were observed in the absence of any appreciable DNA methylation.

Oftentimes, research in ecological systems cannot establish the control (internal or external) found among model systems. Thus, a survey of natural epigenetic variation across wild populations is highly desirable. If amenable, one such control can be similar in kind to a quantitative epigenetic study: specific hybrid crosses to track phenotypic variation. In this issue, Bearwald *et al.* (2016) take advantage of the heritable complex behaviour, migration in trout (*Oncorhynchus mykiss*) raised in a common garden experiment. Trout undergo major morphological shifts if they develop into the migratory (steelhead trout) or resident form (rainbow). In a survey of methylation of an F_2 cross, Baerwald and colleagues linked the morphological and behavioural variation to divergent patterns of methylation at genes that function in body weight, energy availability and the circadian rhythm pathway. In selection lines of great tit (*Parus major*) artificially selected for divergent exploratory behaviour for several generations, Verhulst *et al.* (2016) identified DNA methylation divergence between the selection lines at a main candidate gene, DRD4, that is thought to be involved in the behavioural phenotype under selection. This is suggestive of selectable phenotypic variation in exploratory behaviour that is mediated by DRD4 methylation.

These different studies take the first steps towards demonstrating the influence of epigenetic variation on phenotypic variation, through identifying causality and unravelling epigenetic from genetic effects. One striking idea about the phenotypic effects of epigenetic variation that is developed by Rice *et al.* (2016) and that requires further empirical testing is that occasional failure to erase epigenetic marks that canalize sex-specific development can lead to gonad–trait mismatches in a subsequent generation. Such mismatches might, for instance, explain overlap in the masculinity/femininity phenotype spectrum.

Evolutionary potential of epigenetic variation

To what extent does the genotype control the epigenetic state?

The degree to which epigenetic variation can be predicted from the underlying genotype is a key question in assessing the evolutionary potential of epigenetics (Richards 2006). Methylation variants can be under genetic control (e.g. when a gene is silenced by methylation due to small RNA-guided methylation of a nearby transposable element; Slotkin & Martienssen 2007), but environmental effects (possibly from previous generations) and stochastic epimutation can decouple epigenetic variants from the genotype. Searching for such an independent epigenetic component is an important aim of population epigenetics studies – this is the component where epigenetics might explain something that is not explained already from genetics. Recent work in *A. thaliana* indicates that such a genetics-independent component of methylation divergence may be limited (Dubin *et al.* 2015). They described a large proportion of DNA methylation variants associated with specific genetic polymorphisms, often in *trans* and partly associated with sequence variation within a methyltransferase. This study suggests that natural variation in methylomes could reflect local adaptation, but at least in part as a downstream effect of selection and adaptation at genetic loci whose phenotypic effects are mediated via modified DNA methylation.

There are currently very little data beyond model systems that shed light on this important issue. Several studies attempt to minimize the possibilities for genetic control by studying clonal or completely inbred study species, or alternatively, statistical approaches are used to uncover patterns of epigenetic variation that are not predictable from patterns of genetic variation (genetic relatedness) (Foust *et al.* 2016; Gugger *et al.* 2016; Herrera *et al.* 2016). These approaches provide first indications of epigenetic variation that does not follow directly from the underlying genotype, and may point at direct environmental effects when screening is based on field-collected samples. When screening is based on offspring generations from field-collected samples raised in controlled environments, heritable epigenetic differences are revealed. For instance, Keller *et al.* (2016) reveal heritable epigenetic variation across large panels of *Arabidopsis thaliana* accessions that associates with climatic variables.

There is still quite a gap between 'first indications' and actual proof of epigenetic variants that are autonomous from underlying genetic variation. First, environmentally induced variation can reflect a genetically controlled environmental response and, unless this response is transgenerationally stable, few would look at such a plastic response as being autonomous. Second, fine-grained genomic and statistical approaches are necessary (e.g. WGBS and GWAS to detect associations between genetic variants and epigenetic variation; Dubin *et al.* 2015) to identify relevant autonomous epigenetic variants. For instance, epigenetic variation within clonal lineages can be caused by genetic variation within the lineage that goes undetected in low-resolution genetic screening.

Some findings confirm genotype dependence of DNA methylation variation. Janowitz Koch *et al.* (2016) identified methylation patterns that are highly correlated with the presence of repetitive elements in the dog genome, consistent with previous hypotheses that increased methylation levels follow rapid transposon amplification events to silent transposition (Capy *et al.* 2000; Slotkin & Martienssen 2007; Chenais *et al.* 2012). For example, targeted methylation of a transposon within the gene *Axin* produces a mutant transcript in a strain of inbred mice (Rakyan *et al.* 2003). Future research will need to assess whether other mechanisms of inducing methylation play a role in shaping gene expression.

Does epigenetic inheritance affect adaptation?

Epigenetic 'adaptation' could be viewed as selection of transgenerationally stable epimutations, or alternatively, that such epimutations hasten genetic adaptation through accommodation (Klironomos *et al.* 2013). In the latter case, epigenetic variation is useful to explore the phenotypic space and initially 'hold' a phenotype while genetic mutations take over later and stabilize the phenotype. If the trait is successful, it is assumed that it will ultimately be integrated into the genome through genetic accommodation. Modelling studies suggest that the dynamics of genetic adaptation are significantly affected by epimutations, also when fitness effects of the epimutations are relatively small and reversal rates are relatively high (i.e. well within the range of empirically observed reversal rates; Kronholm & Collins 2016). Modelling studies also show that some seemingly adapted 'bet-hedging' strategies could be explained also by epigenetic inheritance combined with some developmental noise (Day 2016). Empirical evidence for epigenetic effects on adaptation has remained elusive (see Zhang *et al.* 2013; for an example of adaptive potential based on artificially induced and heritable DNA methylation variants). Heritable DNA methylation variants may be involved in traits or functional genes that are potentially under selection (Gugger *et al.* 2016; Keller *et al.* 2016; Verhulst *et al.* 2016; Wilschut *et al.* 2016) but at this stage it remains undemonstrated if such variation is autonomous or a product of underlying genetic variation.

Intriguing observations over longer evolutionary timescales come from comparisons between closely related species. In darter fish, reproductive isolation between closely related species is predicted better by DNA methylation divergence than between genetic divergence between the species (Smith *et al.* 2016), possibly indicating that epigenetic variation can affect the evolution of reproductive isolation. Other long-term methylation effects are inferred from CpG depletion in genomes. Due to higher mutation rates of methylated cytosines, hypermethylated genomic regions show a reduction in CpG dinucleotides over evolutionary time. In several coral species, such inferred methylation patterns reveal historical hypomethylation of genes that are responsive to thermal stress and ocean acidification. This suggests a possible link between DNA methylation and plastic responses to environmental change (Dimond & Roberts 2016). Also these intriguing observations warrant follow-up studies to shed light on the issue of genetic control over the relevant DNA methylation variation.

The difficulty is drawing the line between genetic-dependent and independent epigenetic variation, and exploring the mechanisms by which natural selection can act on these dynamics marks. When considering an epigenetically linked phenotype, four mechanisms are commonly linked to macro-evolutionary change: heterotopy (location), heterochrony (timing), heterometry (amount) or heterotypy (kind) of gene expression (Arthur 2004). For example, a study of Darwin's finches has successfully detailed the link between heterometric variation in

transcription and variation in a fitness-related trait, beak size and shape. This polyphenism (thin/shallow versus deep/broad beaks) is influenced by the amount of transcriptional product from two genes expressed during embryonic development (Abzhanov *et al.* 2004). The resulting phenotypic variation is acted upon by natural selection, which subsequently produced niche specialization and should be detectable with modified neutrality tests. As theory infers specific modes of selection from shifts in synonymous or replacement mutations, similar treatments of epiallele frequency spectrum data can begin to provide insights into epigenetic adaptation. Janowitz Koch *et al.* (2016) applied a modified Tajima's D (Wang & Fan 2015) to identify non-neutral coding methylation variation in domesticated dogs, possibly a consequence of strong recent artificial selection. Yet, epigenetic adaptation is likely an extreme case, with the majority of this variation often interpreted in the light of genetic accommodation.

The way forward

The studies in this Special Issue illustrate several points about the current status of ecological and evolutionary epigenetics:

Epigenetic analysis has taken off in ecology and evolution

Natural epigenetic variation (mostly DNA methylation) is currently being studied across a very diverse range of species and study designs. This diversity of species and contexts may add to our insight in the scope of epigenetic effects and their ecological and evolutionary relevance. Epigenetic mechanisms and epigenetic landscapes differ between taxa and even within taxa substantial differences can exist, for instance related to transposable element content of the genome or due to differences in methylation maintenance machinery (Alonso *et al.* 2015; Willing *et al.* 2015). In addition, epigenetic variation may be of different relevance to species depending on life history and habitat characteristics (Herman *et al.* 2014; Verhoeven & Preite 2014). While basic questions remain open concerning the mechanisms underlying epigenetic variation and epigenetic inheritance, the evaluation of the ecological and evolutionary relevance of these mechanisms will be facilitated by broad insight across a diversity of systems.

In many species and study systems, epigenetic analysis is in its infancy

Because the research effort of ecologists and evolutionary biologists is distributed over a diversity of species and systems, rather than focusing cumulative effort on a single model system, many of the presented results are 'first observations' that require more detailed follow-up work to flesh out epigenetic details of the systems. The studies presented in this Special Issue contribute intriguing observations on associations between epigenetics and habitat, traits, selection and ecological parameters. This is encouraging, and one important contribution is that these studies provide abundant evidence from natural systems that there are interesting epigenetic patterns that warrant further in-depth analysis. But we think that it will be important to also take the next step in these systems. For some systems, this includes adopting higher-resolution techniques to pinpoint epigenetic variants; for other systems, this means analysis in more extensive or controlled study designs. Ultimately, it is only high-resolution analysis in proper study designs that can pinpoint functional epigenetic effects and that can expose their relation to environments and underlying genetic variation.

Tools for epigenetic analysis in ecological study systems are maturing

Until recently, much epigenetic research in ecological study systems used methylation-sensitive AFLPs to screen methylation variation. MS-AFLPs provide very limited information on DNA methylation but the method is cost-effective, easily applied to species without a reference genome, and not computationally demanding. These initial studies have provided motivating first observations in several systems, and also in this Special Issue, several studies with large numbers of samples successfully use MS-AFLPs to capture environmental effects in natural habitats (e.g. Foust *et al.* 2016). However, the future of these studies is in bisulfite sequencing-based techniques, which provide high-resolution information of cytosine methylation and its genomic and sequence contexts. As illustrated by the studies in this Special Issue, draft reference genomes are becoming available for several of the ecological study systems, to facilitate bisulfite-based methylation analysis of candidate genes (e.g. Verhulst *et al.* 2016), whole genomes (Keller *et al.* 2016; Standage *et al.* 2016), or reduced representations of genomes (RRBS; Janowitz Koch *et al.* 2016; Gugger *et al.* 2016; Weyrich *et al.* 2016; Lea *et al.* 2016; Bearwald *et al.* 2016). Cost-effective RRBS approaches are very suitable for ecological designs with many samples, replacing MS-AFLPs as the method of choice, especially with the advent of cheap and flexible RRBS methods that harness the flexibility of RADseq (Trucchi *et al.* 2016) or genotyping-by-sequencing (Van Gurp *et al.* 2016). With such bisulfite sequencing-based methods becoming well established

in the field of ecological epigenetics, we expect the field to make important progress in describing and understanding epigenetic diversity in natural systems.

Conclusions

Assessing the importance of epigenetic effects to ecology and evolution requires the surveillance of epigenetic variation in natural populations, integrated with a detailed mechanistic understanding of their relation to the underlying genotype, the linked phenotype(s) and their environmental sensitivity. Recent empirical studies have demonstrated the potential of epigenetic effects and the exciting steps taken in a variety of natural systems. Perhaps most encouragingly, bisulfite sequencing methods are becoming widely adopted – an approach that is absolutely necessary for detailed characterization of natural variation in DNA methylation. Yet, ultimately, high-resolution genomewide screening of epigenetic as well as genetic variation, combined with expression analysis, will be necessary to pinpoint the contributions of epigenetic variation to ecology and evolution.

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