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Richards, Christina L.; Alonso, Conchita; Becker, Claude; Bossdorf, Oliver; Bucher, Etienne; Colomé-Tatché, Maria; Durka, Walter; Engelhardt, Jan; Gaspar, Bence; Gogol-Döring, Andreas; Grosse, Ivo; van Gurp, Thomas P.; Heer, Katrin; Kronholm, Ilkka; Lampei, Christian; Latzel, Vít; Mirouze, Marie; Opgenoorth, Lars; Paun, Ovidiu; Prohaska, Sonja J.; Rensing, Stefan A.; Stadler, Peter F.; Trucchi, Emiliano; Ullrich, Kristian; Verhoeven, Koen J. F.

published in
Ecology Letters
2017

DOI (link to publisher)
10.1111/ele.12858

document version
Publisher's PDF, also known as Version of record

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ECOLOGY LETTERS

Ecology Letters, (2017) **20**: 1576–1590 doi: 10.1111/ele.12858

REVIEW AND SYNTHESIS

Ecological plant epigenetics: Evidence from model and non-model species, and the way forward

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Abstract

Growing evidence shows that epigenetic mechanisms contribute to complex traits, with implications across many fields of biology. In plant ecology, recent studies have attempted to merge ecological experiments with epigenetic analyses to elucidate the contribution of epigenetics to plant phenotypes, stress responses, adaptation to habitat, and range distributions. While there has been some progress in revealing the role of epigenetics in ecological processes, studies with non-model species have so far been limited to describing broad patterns based on anonymous markers of DNA methylation. In contrast, studies with model species have benefited from powerful genomic resources, which contribute to a more mechanistic understanding but have limited ecological realism. Understanding the significance of epigenetics for plant ecology requires increased transfer of knowledge and methods from model species research to genomes of evolutionarily divergent species, and examination of responses to complex natural environments at a more mechanistic level. This requires transforming genomics tools specifically for studying non-model species, which is challenging given the large and often polyploid genomes of plants. Collaboration among molecular geneticists, ecologists and bioinformaticians promises to enhance our understanding of the mutual links between genome function and ecological processes.

Keywords

Bioinformatics, ecological epigenetics, genomics, phenotypic plasticity, response to environment.

Ecology Letters (2017) **20**: 1576–1590

INTRODUCTION

The DNA of all higher organisms is subject to different chemical modifications that influence gene activity and expression, and that are summed up under the term 'epigenetics'. One of these processes is DNA methylation, the addition of a methyl group to one of the four bases in the DNA molecule (usually cytosine). The idea that within-species variation in such epigenetic modifications may be important for the ecology and evolution of species has captivated biologists during recent years.

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Earlier evidence of natural variation in DNA methylation, as well as of the inheritance and phenotypic effects of this epigenetic variation (e.g. Cubas *et al.* 1999), led to several conceptual papers that suggested its potential relevance to ecology and evolution (e.g. Richards 2006; Bossdorf *et al.* 2008; Jablonka & Raz 2009; Richards *et al.* 2010), and empirical work has been catching up slowly. Ecologists and evolutionary biologists are particularly interested in the unique contributions that epigenetic mechanisms might make. First, environment-sensitive epigenetic mechanisms could transmit responses to environmental changes across generation boundaries. Second, heritable epigenetic variants that arise stochastically, i.e. epimutations, that affect phenotypes may be under natural selection and might contribute to adaptation, independently from DNA sequence variation.

More specifically, research in ecological and evolutionary epigenetics is concerned with (A) patterns of natural epigenetic variation, (B) the origins and drivers of this variation and (C) its ecological and evolutionary consequences (Bossdorf *et al.* 2008). Understanding these patterns, causes and consequences requires insight into a number of key questions that span research fields from molecular biology to ecology (Fig. 1): (A) What is the extent and structure of epigenetic variation in natural populations? (B1) What is the interplay between genetic variation and epigenetic variation? (B2) How frequently do spontaneous epimutations occur and how stable are they? (B3) To what extent can environmental changes induce heritable epigenetic changes? (C1) What is the relative importance of genetic vs. epigenetic variation in determining phenotypes? (C2) How important is epigenetic

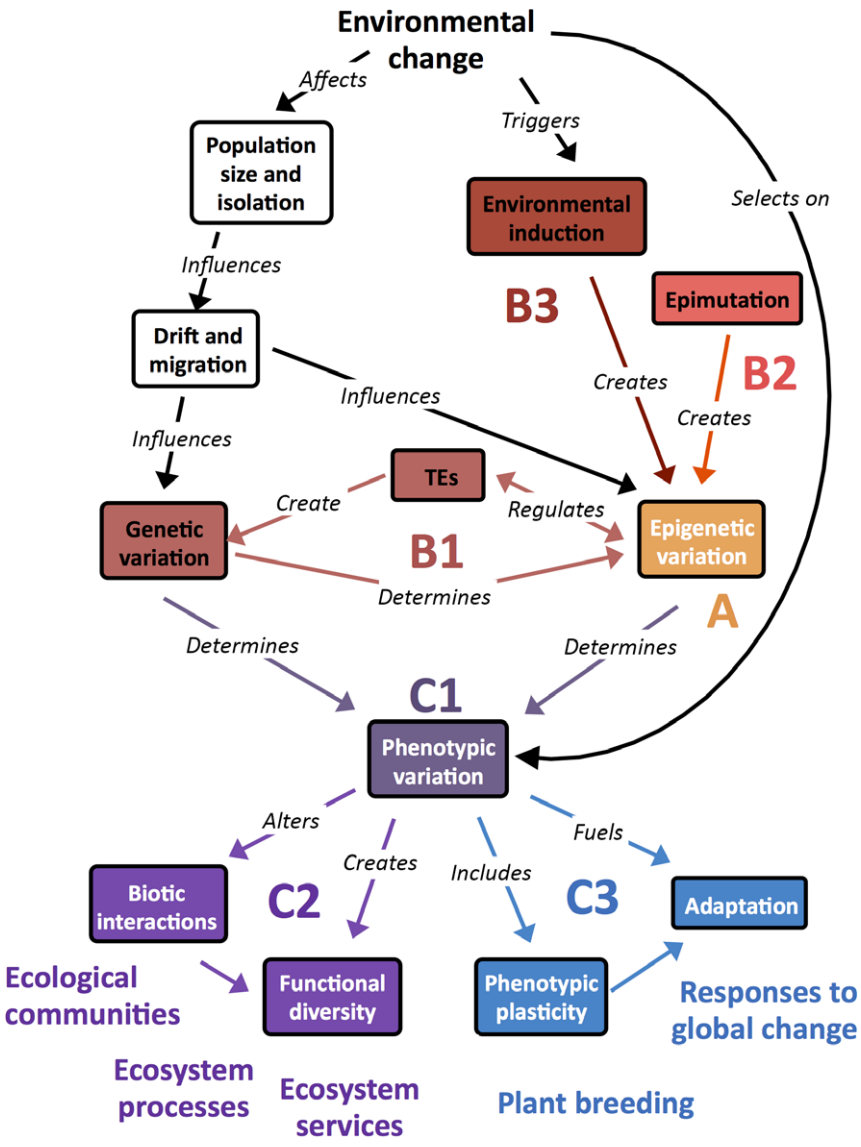

Figure 1 Research in ecological and evolutionary epigenetics is concerned with (A) patterns of natural epigenetic variation, (B) the origins and drivers of this variation and (C) its phenotypic, ecological and evolutionary consequences. Text outside of coloured boxes indicates additional contributions and down stream effects of these sources of variation that contribute to the seven key questions outlined in the text. Here, 'Environmental change' includes habitat reduction and fragmentation, which are primary causes of declines in population size, and can result in selection on phenotypic variation within populations.

variation for biotic interactions, biodiversity and the structure and functioning of communities and ecosystems? (C3) Does epigenetic variation play a role in adaptation and the evolution of populations?

For the most part these questions have been studied separately in two different research fields: molecular genetics and evolutionary ecology. In the plant sciences in particular, molecular genetics has made progress in understanding the mechanisms and dynamics of epigenetic variation by applying high-resolution genomic analysis tools to model species like *Arabidopsis thaliana*, *Oryza sativa* and *Zea mays*. Evolutionary ecology on the other hand has started to explore epigenetic variation in a broad range of non-model species (that lack extensive genomic resources), and in natural settings. This adds the complexity of real environmental conditions to experimental investigations, and has uncovered correlations between epigenetic molecular markers and environmental variation. However, the lack of high-resolution genomic tools in most non-model plant species has made it difficult to firmly establish mechanistic links among genotype, epigenotype, phenotype and environment.

To advance the study of ecological and evolutionary plant epigenetics, we need to better integrate the fields of molecular genetics and evolutionary ecology, by adding more ecological context and ecological questions to model species research (e.g. Latzel *et al*. 2013; Hagmann *et al*. 2015), and by adopting higher resolution tools in non-model species research (e.g. Platt *et al*. 2015; Xie *et al*. 2015; Gugger *et al*. 2016; van Gurp *et al*. 2016; Trucchi *et al*. 2016). In fact, a similar transition has recently been taking place in ecological and evolutionary genomics (Pavey *et al*. 2012; Narum *et al*. 2013; Alvarez *et al*. 2015), which has demonstrated the importance of testing hypotheses in natural environments. For example several studies have found that organismal responses under laboratory conditions may not reflect performance under natural conditions, and that non-adaptive processes may result in apparent genomic signatures of natural selection (Pavey *et al*. 2012; Alvarez *et al*. 2015).

This review focuses on the progress in ecological plant epigenetics accomplished by molecular epigenetics as well as evolutionary ecology, following the series of seven key questions outlined above. Our review is largely restricted to studies of DNA methylation since DNA methylation is the most frequently studied and best-understood epigenetic process to date. However, it is important to note that histone modifications and small RNAs are involved in regulating epigenetic modifications (see e.g. Matzke & Mosher 2014; Kim *et al*. 2015), and the potential for interconnection among different epigenetic mechanisms is not yet fully understood (Becker *et al*. 2011). On the basis of our review of current progress, we identify next steps and discuss strategies and methodological challenges for future ecological epigenetics research.

CURRENT PROGRESS IN ECOLOGICAL EPIGENETICS

Patterns of natural epigenetic variation

What is the extent and structure of epigenetic variation in natural populations?

Across the genome, DNA methylation varies widely within and among plant species (4–40% of cytosines; Fig. 2; Alonso *et al*. 2014, 2015; Niederhuth *et al*. 2016). Whole-genome bisulfite sequencing (Box 1) provides information on the methylation of individual cytosines (Cokus *et al*. 2008), but this method has so far only been used in model plant species. Studies in *A. thaliana* (Schmitz *et al*. 2013; Kawakatsu *et al*. 2016) and rice (He *et al*. 2010; Chodavarapu *et al*. 2012; Li *et al*. 2012) found variable DNA methylation among different lines/genotypes, and that within genomes, DNA methylation depends on the sequence context (i.e. CG, CHG or CHH) and the type of genomic region (gene promoters, gene bodies, transposable elements [TEs]). While such detailed information on genomic context is lacking for non-model species, studies in wild plant populations have documented extensive natural intraspecific variation in DNA methylation, based on anonymous markers (Schrey *et al*. 2013), global DNA methylation estimates (Alonso *et al*. 2015) or analyses of specific genes (Xie *et al*. 2015; see Box 1).

DNA methylation variation can result from genetic control, environmental induction and stochastic epimutations, and can in principle be shaped further by drift and natural selection. Consequently, the interpretation of patterns of natural epigenetic variation is not straightforward. Several ecological studies have searched for epigenetic variation that correlates with habitat or with population differentiation, and have found (1) that variation in DNA methylation usually exceeds variation in DNA sequence markers when populations from contrasting habitats are compared (e.g. Lira-Medeiros *et al*. 2010; Herrera & Bazaga 2010; Richards *et al*. 2012; Medrano *et al*. 2014; Schulz *et al*. 2014; but see Foust *et al*. 2016; Robertson *et al*. 2017), (2) that epigenetic differences are often correlated with ecological factors and (3) that some of these relationships are unrelated to patterns of genetic relatedness (Richards *et al*.

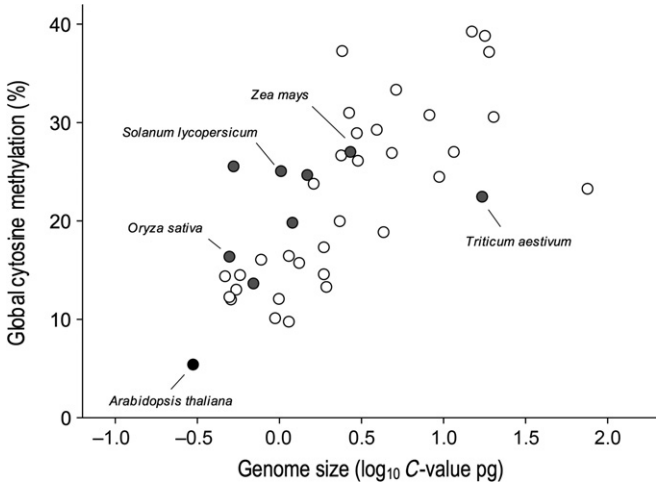

Figure 2 Features and limitations of *Arabidopsis thaliana* as a model system for epigenetic studies. Rapid development, selfing ability and reduced chromosome number and genome size (C-value = 0.30 pg) with relatively few repetitive and transposable elements, facilitate experimentation and simplify molecular analyses in *A. thaliana*. However, *A. thaliana* (black dot) is unusual within the range of variation in genome size and global cytosine methylation in study species, including some with a fully sequenced reference genome (filled dots) like rice (*Oryza sativa*), tomato (*Solanum lycopersicum*), maize (*Zea mays*) and wheat (*Triticum aestivum*). Redrawn from Alonso *et al*. (2015).

Box 1 Genome-wide screening for epigenetic variation in ecological epigenetics

GLOBAL METHYLATION

Global levels of DNA methylation can be assessed through HPLC- and ELISA-based assays that estimate the proportion of methylated cytosines across the entire genome. These methods do not require any sequence knowledge, and they do not distinguish between different genomic locations or contexts (CG, CHG, CHH) of cytosine methylation. Nevertheless, they can help to clarify magnitudes of overall DNA methylation, its variation across species (Alonso *et al*. 2015), the structuring of natural intraspecific variation (Alonso *et al*. 2014) or the global response to environmental changes.

METHYLATION-SENSITIVE MARKERS

Genome-wide patterns of DNA methylation can be captured by molecular markers obtained with methylation-sensitive restriction enzymes. **Methylation-sensitive AFLP** (Reyna-Lopez *et al*. 1997; MS-AFLP/MSAP) follows a standard AFLP protocol but uses pairs of restriction enzymes, often *HpaII* and *MspI*, that have the same recognition sequence but differential sensitivity to DNA methylation. MS-AFLP typically evaluates a few hundred restriction sites. AFLP and MS-AFLP can be applied in parallel to compare genetic and epigenetic structures of populations, and their environmental correlates (e.g. Herrera & Bazaga 2010; Richards *et al*. 2012; Schulz *et al*. 2014; Foust *et al*. 2016; Robertson *et al*. 2017). Because of its easy application, the fact that no reference genome or advanced bioinformatics skills are required, and because of a lack of alternative methods for non-model organisms, the MS-AFLP method has been popular in ecological epigenetics. However, it is now gradually being replaced by bisulfite sequencing-based methods. For example **EpiRADseq** (Schield *et al*. 2015) combines methylation-sensitive restriction enzymes with next-generation sequencing. Similar to MS-AFLP, EpiRADseq detects methylation differences only in recognition sequences, but produces a much greater number of loci and thus characterises methylation patterns at much higher resolution. This approach also provides sequence information at the interrogated loci.

BISULFITE SEQUENCING METHODS

Bisulfite treatment converts unmethylated cytosines to uracil, allowing for the identification of methylated cytosines by comparing a treated sample to a reference sample (Cokus *et al*. 2008). Bisulfite sequencing is the gold standard of DNA methylation analysis, as it evaluates individual cytosines in a target sequence or for essentially all cytosines in a genome (i.e. **whole-genome bisulfite sequencing** or **WGBS**). While WGBS enables detailed analyses of DNA methylation variation, it is restricted to species with a high-quality reference genome, and its costs may be prohibitive for large sample sizes and in species with large genomes, limiting its use for ecological studies. However, by restricting sequencing to specific subsets of the genome, bisulfite sequencing can be applied more broadly. If a high-quality reference transcriptome is available, bisulfite sequencing combined with **exome capture** permits methylation analyses of the expressed regions of the genome (and their flanking regions; Lee *et al*. 2011). Bisulfite sequencing can also be targeted to a selection of genomic fragments that are isolated with restriction enzymes (**Reduced representation bisulfite sequencing**, **RRBS**; Gu *et al*. 2011), thus providing single-nucleotide resolution of DNA methylation within each of the fragments. Availability of both sequence and methylation variation from the same large set of loci allows direct comparison of genetic and epigenetic information, and the evaluation of the contributions of SNPs vs. DMPs to population divergence. RRBS has been adopted for plant population studies (Platt *et al*. 2015), and methods have recently been developed to incorporate bisulfite sequencing into popular reduced-representation sequencing approaches that can be applied to species for which no reference genomes are available (bsRADseq and epiGBS; Trucchi *et al*. 2016; van Gurp *et al*. 2016). Having precise sequence information for methylation polymorphisms contributes to functional analyses that can link DNA methylation variation to gene expression, which can then be linked to phenotypes that contribute to population divergence and local adaptation.

CHIP-SEQUENCING

Chromatin immunoprecipitation followed by NGS (ChIP-seq) determines the modification state of histone proteins (Park 2009). Specific antibodies bind to the histone modification of interest and immunoprecipitate fragments of DNA that are wrapped around the modified histones. These DNA fragments can be sequenced and mapped to the reference genome to determine specific regions where the modifications were present. ChIP-seq methods have not been used yet in ecological epigenetics.

sRNA-SEQUENCING

Diversity and abundance of small RNAs can be obtained by deep sequencing of small RNA molecules (Studholme 2012). Functional interpretations are aided by a reference genome or transcriptome, but it is possible to quantify differential sRNA abundance across populations, environments or treatments, even in the absence of such references (e.g. Morgado et al. 2017).

2012; Schulz *et al*. 2014; Foust *et al*. 2016; Gugger *et al*. 2016). In summary, the extent and pattern of variation in DNA methylation in natural populations suggest that epigenetic variation might be relevant for ecological studies. Still, without whole-genome and -methylome data it is impossible to determine whether or not observed epigenetic patterns are under genetic control or make contributions independently from DNA sequence. Furthermore, in studies conducted in natural populations under field conditions, we cannot know whether observed differences in DNA methylation, and their correlations with environmental factors, reflect heritable (and thus potentially adaptive) variation or are merely repeatedly induced.

Origins and drivers of epigenetic variation

What is the interplay between genetic variation and epigenetic variation?

Epigenetic variation may contribute to heritable trait variation, but if the epigenetic differences are entirely under genetic control then epigenetic variation simply reflects the underlying genetic variation (Richards 2006). Such genetic control of epigenetic variation has been shown in *A. thaliana* where sequence changes in genes related to the epigenetic machinery can have dramatic effects on the epigenome (Becker *et al*. 2011; Dubin *et al*. 2015; see also Box 2: Epigenetic mutants, and 'What can be transferred from model to non-model species?' below). Quantitative genetic studies in model plant species also suggest that many DNA methylation differences among individuals are associated with underlying genetic differences. For example the majority of differentially methylated regions (DMRs; Box 3) stably segregated with the local DNA sequence allele in maize (*cis* inheritance; Li *et al*. 2014). In contrast, only 35% of DMRs were associated with DNA sequence polymorphisms among 142 natural *A. thaliana* accessions (Schmitz *et al*. 2013). Although associations between DMRs and local genetic polymorphisms can indicate genetic control over DNA methylation, such associations can also arise in the absence of genetic control, when a spontaneous epimutation is stably inherited through epigenetic inheritance (Taudt *et al*. 2016).

On the other hand, epigenetic changes can also influence genetic variation. Since DNA methylation is associated with the silencing of TEs, epigenetic change can induce novel genetic variation through regulating TE activity (Fig. 1). Reduction in DNA methylation can result in increased movement of TEs, thereby creating variation in DNA sequence. Such transposition may affect phenotypes where TEs insert near genes, attract DNA methylation and influence gene expression. In fact, many well-characterised epialleles are associated with TEs or repeats that can cause DNA methylation via RNA-directed DNA methylation (Paszkowski & Grossniklaus 2011; O'Malley & Ecker 2012; Matzke & Mosher 2014).

Many previous epigenetic studies in non-model plants have attempted to control for genetic variation using species that have naturally low levels of genetic diversity such as asexually reproducing plants (e.g. Verhoeven *et al*. 2010; Richards *et al*. 2012; Verhoeven & Preite 2014). These studies cannot address the relationship between genetic and epigenetic variation. However, a few studies on outcrossing species have used statistical approaches to make inferences about the independence of genetic and epigenetic variation by evaluating how well overall similarities in DNA methylation profiles among individuals can be predicted from their DNA sequence similarities (Herrera & Bazaga 2010; Schulz *et al*. 2014; Foust *et al*. 2016). While these studies have frequently found that epigenetic variation appeared to be independent from genetic control, they usually used low-resolution molecular markers and thus could not rule out the possibility that critical genetic polymorphisms had gone unnoticed (Becker *et al*. 2011; Dubin *et al*. 2015).

How often do spontaneous epimutations occur?

Spontaneous epimutations have the potential to contribute to heritable trait variation in a way that is not predictable from DNA sequence variation, but their evolutionary potential is determined by the rate at which they appear and revert. Epimutation rates have been estimated in a study of the accumulation of mutations in *A. thaliana*, where DNA methylation polymorphisms accumulated at a much higher frequency (van der Graaf *et al*. 2015) than genetic mutations (Ossowski *et al*. 2010). Epimutations occurred more frequently in genic regions than in TEs. This pattern is consistent with the known RNA-directed repair mechanism, which limits the development of DNA methylation polymorphisms in TEs (Teixeira *et al*. 2009). Spontaneous epimutations also occurred at the level of DMRs, i.e. within particular regions rather than at specific single sites. These epimutations showed more functional relevance to gene expression, but they also occurred at much lower frequency, comparable to the rate at which genetic mutations arise (Becker *et al*. 2011).

So far, there are virtually no data on epimutation rates in non-model plant species. A study on apomictic dandelions by Verhoeven *et al*. (2010) showed that even in a constant environment, appreciable DNA methylation differences developed among individual plants, and that most of these changes were passed on to offspring. However, further studies are needed that determine the rate and stability of epimutations across different species before we can draw any conclusions about their potential ecological and evolutionary significance.

To what extent can environmental changes induce heritable epigenetic changes?

Studies in *A. thaliana* have shown that the epigenome reacts to environmental changes such as abiotic and biotic stress (e.g. Dowen *et al*. 2012; Slaughter *et al*. 2012; Sani *et al*. 2013), and that these epigenetic changes are sometimes associated with changes in gene expression throughout the genome (Secco *et al*. 2015; Wibowo *et al*. 2016). Other studies have reported inheritance of stress-induced changes in DNA methylation (e.g. in rice: Kou *et al*. 2011; in *A. thaliana*: Bilichak *et al*. 2012). In particular, herbivore or pathogen effects can be passed on to offspring, and some of the best current evidence for epigenetically based inheritance of induced effects is in the context of such biotic interactions (Luna *et al*. 2012; Rasmann *et al*. 2012). However, several studies also found only limited inheritance of stress-induced DNA methylation

Box 2 Linking epigenetic variation to phenotype

Establishing the causal links between epigenetic variation and phenotypes is one of the key challenges in ecological epigenetics, and a number of different approaches have been used to address this question.

CANDIDATE GENES

Detailed observational studies can reveal epigenetic polymorphisms at candidate loci that are associated with expression and phenotypic differences in a genetically uniform background (such as completely inbred lines, asexually propagated individuals or within the same individual, e.g. Cubas *et al*. 1999; Xie *et al*. 2015). Such observations also suggest autonomous epigenetic determinants of phenotypic variation. However, so far such studies have been restricted to a very limited number of phenotypes in model species.

EPIGENETIC MUTANTS

A specific tool in plant epigenetic research is epigenetic mutants. These are plant lines that carry mutations in genes required for proper functioning of the epigenetic machinery, e.g. those coding enzymes for initiating (*de novo*) or maintaining DNA methylation. Such mutations can cause genome-wide epigenetic alterations (in otherwise genetically uniform backgrounds), which can be exploited for proof-of-principle studies of whether certain phenotypes are influenced by epigenetic mechanisms. Epigenetic mutants have also been useful in experiments on environmental induction and epigenetic inheritance. Evidence for a role of epigenetics is provided if a certain environment cannot induce an expected phenotypic change in an epigenetic mutant (Luna *et al*. 2012; Rasmann *et al*. 2012), or the transmission of a phenotype is altered in the mutant (Crevillen *et al*. 2014).

CHEMICAL MANIPULATION

Mutant lines are often not available in non-model organisms, but there are chemicals that can be used to manipulate epigenetic variation. Examples are 5-azacytidine (Jones 1985) or zebularine (Cheng *et al*. 2003) that both inhibit DNA methylation. There are also chemicals that target different epigenetic mechanisms, such as inhibition of histone demethylation (Kruidenier *et al*. 2012). However, many of these chemicals have cytotoxic or other off-target effects, and they can be biased to specific loci (Hagemann *et al*. 2011). In ecological studies, DNA methylation inhibitors have been used to demonstrate the importance of DNA methylation for plant responses to environmental conditions, and genotypic variation therein (Bossdorf *et al*. 2010; Herrera *et al*. 2012), for maintaining the effects of inbreeding (Vergeer & Ouborg 2012), flowering time differences (Wilschut *et al*. 2016) and inheritance of induced phenotypes (Herman *et al*. 2016).

EPIGENETIC ASSOCIATION AND QTL-MAPPING

Ecologists are most interested in natural variation, and in principle the same methods of quantitative genetics that are applicable for the study of natural genetic variation can be used to investigate natural epigenetic variation. Epigenetic variation can then be treated in two distinct ways: First, it can be treated as a phenotype, and then one can screen for genetic markers that contribute to differences in DNA methylation patterns (e.g. Dubin *et al*. 2015), which helps to reveal genetically controlled vs. autonomous methylation variation. Second, DMPs or DMRs that are stably inherited across generations can be treated the same way as conventional genetic markers and used in mapping approaches to explain phenotypic variation. Long *et al*. (2011) and Li *et al*. (2014) found that many methylation polymorphisms segregated in a normal Mendelian fashion, and could be used for epi-QTL-mapping in *Brassica napus* and maize respectively. Epigenetic markers have also been used for QTL analysis in plants in the absence of DNA sequence polymorphisms (*i.e*. *A. thaliana* epiRILs; Cortijo *et al*. 2014). We are not aware of any studies that use such markers for association mapping in non-model species other than in some low-resolution methylation sensitive AFLP studies with plants. Such marker-based mapping studies can shed light on the epigenetic contribution to phenotypic variation. However, a statistical epigenetic marker-trait association does not necessarily imply a true epigenetic contribution to the observed trait variation, as phenotypic effects may generally also be caused by a tightly linked genetic polymorphism. Follow-up studies are thus required to functionally characterise suspected epi-QTLs and evaluate them in manipulative studies.

changes in *A. thaliana* and maize (Pecinka & Mittelsten Scheid 2012; Eichten & Springer 2015; Wibowo *et al*. 2016).

Studies in non-model species have found that heritable changes in DNA methylation can occur in response to different environmental stresses, but that the strength of these effects depends on environmental conditions (Verhoeven *et al*. 2010; Alonso *et al*. 2016). For instance, Verhoeven *et al*. (2010) showed that plants with identical genotypes had a range of DNA methylation changes in response to different stresses, and that the degree to which these were inherited also varied. Richards *et al*. (2012) measured epigenetic differences in a dominant haplotype of the highly invasive clonal plant

Japanese knotweed (*Fallopia japonica*) collected from different habitats, but grown in a common environment. They found that part of the observed DNA methylation variation correlated with habitat of origin, suggesting environment-specific DNA methylation changes that persisted through clonal propagation in a common environment. Similarly, after growing the invasive plant *Ageratina adenophora* under controlled conditions, Xie *et al*. (2015) found that stable inheritance of demethylation at the promoter region of a specific gene was correlated with variation in cold tolerance. All of these studies thus indicate a correlation between environment and stable epigenetic variation. However, heritable epigenetic differences between populations may also be the result of neutral processes. Further, the changes that are potentially advantageous can result not only from environmental induction and subsequent inheritance, but may also reflect natural selection acting on variation created by spontaneous epimutations. The studies above usually cannot discriminate between these two possible explanations.

Environmental responses that are mediated by epigenetic mechanisms could be particularly relevant for asexually reproducing plants (Verhoeven & Preite 2014; Douhovnikoff & Dodd 2015; Rendina González *et al*. 2016; Spens & Douhovnikoff 2016). While some DNA methylation is stable through sexual reproduction, epigenetic changes that are environmentally induced often show limited meiotic stability, or epigenetic resetting during meiosis (Wibowo *et al*. 2016). Asexual plants use vegetative (clonal) reproduction, so meiotic epigenetic reset does not occur. The mitotic stability of epigenetic responses might allow different plant parts to respond to different microenvironments, and transmit environmentally induced DNA methylation changes. Therefore, different ramets of the same clonal individual may show different epigenetic profiles in an otherwise uniform genetic background (Rendina González *et al*. 2016; Spens & Douhovnikoff 2016). For instance environmental effects on DNA methylation in CHH contexts tend to occur at TE loci (Dubin *et al*. 2015), but the epigenetic silencing of such loci can be reinforced via small RNAs during sexual reproduction (Martínez *et al*. 2016). By circumventing this process, stable TE-associated epialleles may arise in clonal plants, as observed in clonally propagated oil palm (Ong-Abdullah *et al*. 2015).

The Consequences of Epigenetic Variation

What is the contribution of DNA methylation to phenotypes?
So far, only a few natural epialleles have been functionally characterised using various approaches (Box 2; Cubas *et al*. 1999; Manning *et al*. 2006; Paszkowski & Grossniklaus 2011; Xie *et al*. 2015). Model plants offer powerful tools to isolate epigenetic effects from genetic effects on phenotype. Of particular note are two collections of *A. thaliana* lines that were derived from crosses between the Columbia wild type, and mutants in the Columbia background that have decreased DNA methylation genome-wide (*ddm1* and *met1* mutants; Johannes *et al*. 2009; Reinders *et al*. 2009; see below in 'What can be transferred from model to non-model species?'). These epigenetic recombinant inbred lines (epiRILs) are therefore nearly identical in DNA sequence genome-wide. However, individual lines from the epiRIL collections differ from each other in DNA methylation because their genomes are mosaics of the wild type methylation patterns, and the methylation patterns of the parent with decreased DNA methylation (Johannes *et al*. 2009). Such populations allow for an assessment of the phenotypic consequences of epigenetic variation independent of variation in DNA sequence. By specifically isolating epigenetic from genetic information, the epiRILs allow for the study of the dynamics and phenotypic consequences of DNA methylation in the almost complete absence of DNA sequence variation. Both *met1* and *ddm1* epiRIL populations displayed significant phenotypic variation (Zhang *et al*. 2013; Cortijo *et al*. 2014), and linkage mapping in the *ddm1* epiRILs identified DMRs that explained heritable phenotypic effects for root length and flowering time (QTL^{epi}; Cortijo *et al*. 2014). Interestingly, a significant fraction of the DMRs created in the epiRILs are also variable in natural *A. thaliana* populations, and it is possible that they are also functionally important in the wild.

In non-model species, several studies have found correlations between anonymous methylation-sensitive AFLP markers and leaf traits (Herrera & Bazaga 2013), flower morphology (Herrera & Bazaga 2010) and fitness-related traits (Medrano *et al*. 2014) in natural populations. While it is tempting to interpret these correlations in terms of contributions to adaptation, they may be simply the result of genetic differences. Experiments will be required to identify which loci are involved, and confirm causal relationships between epigenetic variation and phenotypes.

In quantitative and ecological genetics, phenotypic variation is described with the classic equation $V_P = V_G + V_E + V_{G\times E}$ where V_P is the total variance in phenotype, and V_G, V_E and $V_{G\times E}$ are the fractions of V_P that can be ascribed to genetic variation, environmental influences or the interaction between the two. If epigenetically based heritable phenotypes exist not only in epiRILs but also in nature, then the equation might be improved to $V_P = V_G + V_{EPI} + V_E + V_{G\times EPI} + V_{G\times E} + V_{EPI\times E} + V_{G\times EPI\times E}$ (see also Gorelick 2005). So far, nearly all evidence for this is correlative and therefore inconclusive, but with increasing possibilities for whole-genome and -methylome sequencing, we should now be able to begin to explore this equation with empirical data.

What are the ecological consequences of epigenetic variation?
By mediating phenotypic plasticity, DNA methylation can facilitate response to various biotic and abiotic conditions, and persistence in different environments. To test this hypothesis, Herrera *et al*. (2012) investigated the relationship between epigenetic variation and environmental conditions in an easily manipulated yeast. Using *in vivo* demethylation by the methylation inhibitor azacytidine, they showed that the exploitation of nectar of varying sugar concentrations in flowers depended on DNA methylation. This type of experiment is ideal for isolating phenotypic plasticity, but more difficult to achieve for many long-lived plants. Nevertheless, a handful of studies have provided evidence that DNA methylation may contribute to the response to environmental factors under natural conditions. For example Herrera & Bazaga (2011) found both DNA sequence and methylation polymorphisms in *Viola*

cazorlensis were correlated with herbivory damage and habitat. In addition, variation in genomic DNA methylation has been correlated with shifts in species range (Richards *et al.* 2012; Xie *et al.* 2015), and differentiation of populations (Platt *et al.* 2015; Foust *et al.* 2016; but see Robertson *et al.* 2017). Interactions between plants and biotic or abiotic stressors can prime plants for a more rapid or vigorous response upon a second exposure in the future (Conrath *et al.* 2002), and additional epigenetic mechanisms such as histone modifications may be responsible for such sustained stress memory (e.g. Jaskiewicz *et al.* 2011).

In the model plant *A. thaliana*, there has been some evidence suggesting that the epigenetic contribution to response to ecologically important factors like nutrient availability varied among natural accessions (Bossdorf *et al.* 2010). Experiments with epiRILs demonstrated that epigenetic variation among lines led to functional diversity that had very similar effects on population and ecosystem functioning as found for genetic and species diversity effects: higher epigenetic variation created variation in phenotypes that translated into increased productivity and resistance of experimental populations (Latzel *et al.* 2013). The combination of studies in natural accessions and epiRILs suggests that epigenetic diversity may be an important component of functional biodiversity, and that epigenetic variation can be indirectly involved in evolution by modifying natural selection at the community level.

What are the evolutionary consequences of epigenetic variation? Many studies have investigated the role of environmentally induced and spontaneous epigenetic modifications in evolutionary theory (Jablonka & Raz 2009; Slatkin 2009; Day & Bonduriansky 2011; Geoghegan & Spencer 2012; Klironomos *et al.* 2013; Charlesworth & Jain 2014; Furrow 2014; Wang & Fan 2014; Kronholm & Collins 2016). Ultimately, the impact of spontaneous epigenetic variation in evolution will depend on the rates and stability of epigenetic changes, and the distribution of their phenotypic effects (Kronholm & Collins 2016). Modelling studies show that if spontaneous epigenetic changes occur at faster rates than genetic changes, this could lead to evolutionary dynamics where phenotypic changes are first driven by epigenetic changes, and become genetically encoded later (Klironomos *et al.* 2013; Kronholm & Collins 2016). These modelling studies have also shown that environmentally induced epigenetic changes that are inherited across generations could be adaptive in rapidly changing environments (Robertson & Richards 2015). However, the significance of such environmentally induced variation for adaptation will strongly depend on its persistence.

While theoretical models show that epigenetic variation has the potential to change evolutionary dynamics, more data are needed to clarify the role of epigenetics. For instance detailed analyses in *A. thaliana* have revealed little evidence for environment-induced epigenetic variation that persists for several generations (Hagmann *et al.* 2015). We also have little insight into what determines transmissibility of epigenetic variants, and how this varies between species or contexts. In *Helleborus foetidus*, there was natural variation in transmission of DNA methylation to pollen, and this was correlated with seed size and seedling recruitment success (Herrera *et al.* 2014). This suggests that transmission fidelity may be a selectable trait. Long-term data on epigenetic response to environmental conditions in different species and ecological contexts would provide critical insight about when epigenetic mechanisms are potentially important for evolutionary processes.

WHAT CAN BE TRANSFERRED FROM MODEL TO NON-MODEL SPECIES?

The transfer of information from model systems combined with advances in sequencing and bioinformatics approaches has initiated a powerful next step for ecological epigenetics due to more precise insights into function, and an increase in genome coverage. Some of the detailed information on epigenetic mechanisms that we have learned from model species is already useful in non-model systems. Gene annotations in model species provide information on genes that code for conserved components of the epigenetic machinery or genes that are epigenetically regulated. Studies of how DNA methylation is directed to specific genomic regions via small RNAs (RNA directed DNA methylation [RdDM]; Khraiwesh *et al.* 2010), methyltransferases (Noy-Malka *et al.* 2014), and other epigenetic modifications like histone modifications demonstrate that epigenetic mechanisms are controlled by evolutionarily conserved machinery (Rensing *et al.* 2008; Widiez *et al.* 2014). This information can be used to identify homologs in non-model species, which can then be targets for knock-outs or transformation to validate function in future studies (Kobayashi *et al.* 2013; Alvarez *et al.* 2015; Xie *et al.* 2015).

Mutations occurring in genes related to the epigenetic machinery can have strong effects on epigenetic variation. In a study of the accumulation of mutations in replicate *A. thaliana* lines, one line showed a single spontaneous mutation in a methyltransferase, *MEE57*. This mutation appears to have led to an increased rate of epimutation at CG sites, resulting in 40% more methylation differences after 30 generations in this line compared to the other lines (Becker *et al.* 2011). Similarly, in natural *A. thaliana* populations, Dubin *et al.* (2015) found a link between alternative alleles of the DNA methyltransferase *CMT2* (responsible for CHG and CHH methylation of certain classes of TEs) and the epigenome's capacity to respond to temperature changes. In contrast, the CMT3 homolog double mutants in maize are not viable (Li *et al.* 2014), indicating that loss of methylation can have more drastic effects in some species.

Studies in *A. thaliana* have exposed how DNA methylation effects are often associated with TE silencing. The vast majority of small RNAs (more than 60%) are complementary to TE sequences, and are involved in guiding DNA methylation to TEs through RdDM (Matzke & Mosher 2014). Small RNAs target DNA methylation to long terminal repeats (LTRs) of retrotransposons, and inhibit TE transcription. Reduction in DNA methylation at that location can be associated with the upregulation of TE expression, potentially creating new genetic variants, and phenotypes. Studies in RdDM deficient *A. thaliana* have shown that TEs get activated and introduce new copies of themselves in the genome (Mirouze *et al.* 2009; Ito *et al.* 2011). Thus, RdDM could be an important mechanism that protects the genome from TE

Box 3 Bioinformatics challenges for epigenetic sequence analysis

The analysis of epigenetic next-generation sequencing (NGS) data is a complex problem that involves several specific tasks combined in a modular fashion into analysis pipelines or workflows. Such pipelines are typically written as specific software, but it is possible to combine individual components in a customised fashion using generic bioinformatic workflow management systems such as Taverna, KNIME or Galaxy.

The construction of a **reference genome** facilitates NGS analyses since many approaches require complete genomic reference sequences. In most non-model species, no reference genome exists and genomes are often complex, highly polymorphic or polyploid. Researchers are then likely to use reduced-representation methods. Specialised tools can cluster nearly identical reads, and then assemble them locally into reference sequences (van Gurp *et al*. 2016). However, differentiating paralogs, orthologs and homologues is still challenging in large and complex genomes (Aversano *et al*. 2012; Hirsch & Buell 2013; Boutte *et al*. 2016a,b).

Another important step in bioinformatic analyses is **read mapping**, where observed sequences (reads) are assigned to specific locations in a reference sequence. For this task, software like Segemehl (Hoffmann *et al*. 2014) is optimised for large deviations between query reads and targets and can therefore also be used for bisulfite-sequencing data. Other methods, such as Bismark (Krueger & Andrews 2011) or BWA-meth (http://arxiv.org/abs/1401.1129), are specifically designed to analyse bisulfite-sequencing data.

After mapping the reads, **variant calling** identifies the positions in the reference where aligned reads deviate from a reference sequence, including the detection of SNPs, indels and structural variants. Several tools for variant calling are available (e.g. GATK, McKenna *et al*. 2010; ANGSD, Korneliussen *et al*. 2014). Some tools like Bis-SNP (Liu *et al*. 2012) and MethylExtract (Barturen *et al*. 2014) combine calling for SNPs and methylation differences from bisulfite-treated data.

Differential methylation calling is a type of variant calling that identifies differentially methylated cytosines. Several methods have been developed and integrated into specific software (Bock 2012; e.g. Methpipe, Bismark; Song *et al*. 2013; Krueger & Andrews 2011). However, comparative analyses have shown that the detection of differential methylation is highly coverage- and sequence context-dependent. Since methylation rates at CHG and CHH sites are generally low, differences at these sites are detectable only at high sequencing coverage (> 100×; Becker *et al*. 2011). In *A. thaliana*, this results in a biased detection of DMPs in CG contexts. This bias is even more pronounced in large genomes (lower coverage).

A key problem in functional epigenetics is the **identification of differentially methylated regions (DMRs)**. Studies have found that methylation changes across larger chromosomal stretches (DMRs) are more likely to influence transcriptional activity at nearby loci and contribute to phenotypic change than at single cytosines (DMPs). While it has been common practice to call DMRs by either looking for clusters of DMPs (Becker *et al*. 2011) or by applying a sliding window approach along the entire genome (Schmitz *et al*. 2013), these approaches lead to high rates of false-negative and false-positive calls respectively. The DMP clustering approach suffers from the same bias of sequence context as single DMPs, and the sliding-window approach leads to the detection of DMRs at many weakly methylated regions of the genome. In the latter case, the number of false positives inevitably increases with genome size, making it not useful for many non-model species. The first generation of region callers for BS-seq data includes MOABs (Sun *et al*. 2014), BSmooth (Hansen *et al*. 2012), DMRcate (Peters *et al*. 2015) and metilene (Jühling *et al*. 2016). A more neutral method for DMR detection is provided by an adaptation of a Hidden-Markov-Model-based approach (Molaro *et al*. 2011; Hagmann *et al*. 2015; Lea *et al*. 2015), which first identifies regions of dense methylation that are then tested for differential methylation. The method has been successfully used with *A. thaliana* (Hagmann *et al*. 2015), *A. lyrata* and *Capsella rubella* (Seymour *et al*. 2014), *Arabis alpina* (Willing *et al*. 2015), *P. patens*, rice and maize (Becker *et al*. unpublished data). It is thus applicable to genomes of different sizes, and with diverse methylation distributions. DMR calling is challenging even using WGBS data; its application to RRBS-based data from non-model species without a reference genome is further complicated by small fragment sizes.

In ChIP-seq analysis and other approaches that enrich DNA or RNA from particular genomic regions, **peak calling** is another task. It identifies mapped reads that are over-represented in particular genomic regions. These local 'peaks' indicate the position of nucleosomes carrying specific chemical modifications (Bailey *et al*. 2013).

proliferation. TEs can also play an important role when they integrate near genes, and influence gene expression. A tight interplay exists among TEs, the anti-TE activity of RdDM, and epigenetic regulation of gene expression.

In addition to the detailed information about genes involved in the epigenetic machinery, studies with the epiRIL populations have shed light on the mechanisms involved in inheritance of DNA methylation and the phenotypic impact of epigenomic alterations. DNA methylation in some genomic regions is inherited in a stable Mendelian fashion (Colomé-Tatché *et al*. 2012). In the *ddm1* epiRIL population, linkage mapping identified segregating DMRs that explained observed heritable phenotypic effects (Cortijo *et al*. 2014). This demonstrated, for the first time in any organism, that DMRs can be stably inherited for many generations independently of DNA sequence and that they can act as epigenetic quantitative trait loci (QTL^{epi}). From a phenotypic evolution point of view these QTL^{epi} have all the necessary properties to become targets of natural or artificial selection. The *ddm1* epiRILs showed heritable variation in phenotypic plasticity (Zhang

et al. 2013), and epigenetically diverse plant populations were more productive and more stable than epigenetically uniform populations (Latzel *et al*. 2013).

Further studies with *ddm1* epiRILs demonstrated that some DMRs showed patterns of non-Mendelian inheritance, mainly due to the gradual re-methylation of TEs that were demethylated in the *ddm1* parent of the epiRIL cross (Teixeira *et al*. 2009). In the *met1* epiRIL population, some aberrant phenotypes were linked to TE mobilisation (Mirouze *et al*. 2009): certain hypomethylated TEs segregated in the population and proliferated until they were eventually silenced again through post-transcriptional, and then transcriptional mechanisms (Marí-Ordóñez *et al*. 2013).

Despite all of the insight from studies of model plants, there are limitations to the transfer of knowledge to non-model species that we must keep in mind when we try to adapt these findings to non-model species, and realistic ecological settings. In particular, the genome and epigenome of *A. thaliana* are atypical for most plants that have been surveyed (Fig. 2; Alonso *et al*. 2015). Studies across diverse taxa have demonstrated that TEs can be tightly linked to epigenetic regulation of plant gene expression, but the comparatively small *A. thaliana* genome has relatively few TEs, which are mainly clustered around the centromeres. Larger plant genomes contain proportionally more TEs, and they are typically more evenly distributed. For example the 500 Mbp *Physcomitrella patens* genome contains 50% TEs with no discernible peak regions (Rensing *et al*. 2008). Recent analysis has indicated that even among plants with small genomes, *A. thaliana* might constitute an 'epigenetic outlier' due to its conspicuously reduced number of TEs: the genome of the close relative *A. lyrata* has a considerably larger TE density and consequently contains more methylated sites and regions (Seymour *et al*. 2014). In addition, TEs are not well conserved evolutionarily even among closely related species (Seymour *et al*. 2014), so insights on causes of methylation changes identified in one species may not always transfer to another. Furthermore, plant genomes differ even in the classes of TEs that they harbour. For example nucleocytoplasmic large DNA virus (NCLDV) insertions have only been shown in non-seed plant genomes (Maumus *et al*. 2014), and the maize genome is subject to reshuffling specifically by Helitrons (rolling-circle replicating transposons) that are estimated to be involved in expression of 25% of the maize transcriptome (Barbaglia *et al*. 2012). In studies of other epigenetic mechanisms, the repressing histone H3K9 methylation varies with genome size. Whereas small genomes such as *A. thaliana* show H3K9 methylation in constitutive heterochromatin, genomes of 500 Mbp and larger display H3K9 methylation in euchromatic regions as well (Houben *et al*. 2003). Analyses of epigenetic mechanisms in species with bigger and more complex genomes, including other model and non-model species, will allow for a better understanding of the relevance of epigenetic mechanisms in short-term and long-term response to natural environments.

FUTURE DIRECTIONS

Ultimately, ecological epigenetics studies are interested in understanding the epigenetic contributions to ecological and evolutionary processes in nature. High-resolution genomic analyses in controlled experiments, particularly in *A. thaliana*, have provided concrete evidence for epigenetic variants with heritable phenotypic effects, the selective potential of empirically observed epimutation rates, and the inheritance of environment-induced epigenetic effects. However, analyses of patterns in natural populations have suggested that natural DNA methylation variation in *A. thaliana* may be largely under genetic control, and showed little evidence for epigenetic legacies from environments experienced in previous generations. In contrast, in non-model species, a variety of studies showed that DNA methylation variation correlates with ecological factors in a way that is not simply predicted from patterns of genetic relatedness, suggesting a role for epigenetics that is at least partly independent of genetics. Nonetheless, the low genomic resolution of many studies on non-model organisms precludes pinpointing causality of epigenetic effects.

A priority for ecological epigenetics in the coming years is therefore to incorporate tools that enable higher resolution analyses in more realistic scenarios, and in a wider diversity of systems. This is important because the role of epigenetics is expected to vary among species, with different genomic features and ploidy levels (Ainouche *et al*. 2009; Niederhuth *et al*. 2016; Springer *et al*. 2016; Takuno *et al*. 2016). Furthermore, natural selection may have shaped the genetic capacity for transgenerational epigenetic effects differently in different environments and species. This is partly because the selective advantages of phenotypic plasticity and transgenerational effects differ among species depending on habitat predictability and life history characteristics (Herman *et al*. 2014; Verhoeven & Preite 2014; Douhovnikoff & Dodd 2015).

Addressing the questions related to epigenetic contributions to ecological and evolutionary processes will continue to be challenging not only because of the tools required to tie functionality to epigenetic changes, but also the complicated relationship between epigenetic variation and DNA sequence variation, and the labile nature of epigenetic variation (Richards *et al*. 2010). Pinpointing causality of autonomous epigenetic variation is challenging even in model systems, and studies that extend analysis into ecological settings and non-model species face a trade-off among precision, realism and generality of results (Levins 1966). With better tools available, what kind of evidence should we expect from future ecological epigenetics studies?

Functional relevance

Field studies of epigenetics provide insight about which habitat conditions may lead to interesting patterns of epigenetic divergence, but attempts to link methylation markers to phenotype or habitat in the field cannot isolate the functional importance of epigenetics. However, the phenotypic effect of field-based observations of genome-wide methylation or methylation of candidate genes can be validated using mutants, knockouts or genetically engineered organisms that are altered in genes involved in the epigenetic machinery, or that are differentially methylated (e.g. epiRILs). Subsequently, the ecological relevance of the variant can be determined in

ecological experiments in the greenhouse or field. When these types of genetic resources are not available, the broad functional importance of methylation in various ecological and evolutionary processes can be explored with chemical reduction in DNA methylation variation, which has been useful in exploring the role of DNA methylation in a variety of ecological and evolutionary processes (Box 2).

Disentangling epigenetics and genetics

Although several authors have suggested that functional DNA methylation is largely under genetic control (e.g. Li *et al*. 2012; Dubin *et al*. 2015), we have very little data in any system that can address to what extent there is a component of epigenetic variation independent of genetic variation that contributes to organismal function. For model species, it is possible to test associations between genetic alleles and epialleles from quantitative trait locus (QTL) or genome-wide association study (GWAS) mapping (e.g. Dubin *et al*. 2015), which are indicative of genetic control over epigenetic variation especially in the case of *trans* associations. In non-model species, we have described the effective use of clonal or asexual plant species for isolating the role of epigenetics, but low-level genetic variation that arises from somatic mutations in natural clonal lineages cannot be excluded. Considering that ecological studies are often focused on collections from natural populations *in situ*, future analyses will need to accommodate a simultaneous comparison of genetic and epigenetic data sets to examine how much of the overall epigenetic variation can be predicted from pairwise genetic relatedness, and identify differences in genetic and epigenetic patterns (Herrera & Bazaga 2010; Gugger *et al*. 2016; Lea *et al*. 2015; Foust *et al*. 2016; Herrera *et al*. 2016). These approaches have become standard using anonymous molecular markers, but approaches based on next-generation bisulfite sequencing methods (Box 1) will be more powerful to identify epigenetic associations with gene expression, phenotype or habitat that are not predicted by the observed patterns of genetic variation. Once these associations are identified, more detailed experiments can be used to investigate the independence and importance of the observed epigenetic effects. Follow-up experiments can include targeted bisulfite sequencing or expression of the candidate loci across different genetic backgrounds, knockouts or transgenic organisms.

Environmental effects

Several studies of natural epigenetic variation have found correlations between environmental variation and epigenetic differences, leaving open the question of whether the epigenetic variation is induced or selected by the environment, or simply a side effect of genetic structure. Environmentally induced epigenetic effects may be either transient or persistent across generations, and heritable changes may be either selected on or linked to something that is selected. Future analyses should consider that part of the epigenetic variation is similar to phenotypic variation, and carefully designed experiments are necessary to characterise both genetic and environmental contributions to epigenetic variation (Richards *et al*. 2010). Observational field surveys of natural populations can provide information on how epigenetic variation is structured on the landscape. When they simultaneously measure genetic and epigenetic data on the same individuals, statistical approaches can identify epigenetic variation that is not explained by genetic variation (Dubin *et al*. 2015; Foust *et al*. 2016). This so-called 'genetic-independent' epigenetic variation may be induced transiently by the environment or it may be stably inherited. To discriminate between these two possibilities, one must grow individuals in a common garden and analyse which epigenetic changes persist. Moreover, to isolate how much epigenetic variation is induced by environment requires experiments where both genetics and environment are controlled. Here, clonal organisms or inbred lines are particularly useful because they allow for growing genetically identical offspring in different environments. In outcrossing species, different breeding designs, such as half-sibs or full sibs, and quantitative genetics mapping approaches such as GWAS and epigenome-wide association studies (EWAS) can approximate genetic and epigenetic associations with phenotype.

To date, many studies of the environmental effects on epigenetic variation have tested for the existence and inheritance of environmentally induced effects, but there is limited insight into the adaptive significance of these effects. Building on theory of the evolution of phenotypic plasticity (Herman *et al*. 2014), there are good *a priori* hypotheses of how the capacity to adjust epigenetic modifications, and the potential for inheritance of these modifications will have different adaptive benefits depending on species life history traits and habitat characteristics, such as spatial or temporal heterogeneity and dispersal mode. Genotype-specificity in epigenetic or transgenerational effects may be common (e.g. Herman & Sultan 2016), and future ecological epigenetics studies should explore the evidence for adaptive variation in the capacity for such effects.

Technical challenges and opportunities

While computational analyses of genome-wide data have become routine in model species, not all approaches are easily transferred to ecological epigenetics (Box 3). The main challenge for developing sequencing approaches in ecological epigenetics is that existing workflows usually require at least high-quality transcriptomes, if not complete reference genomes. Two important methodological developments have the potential to advance studies in ecological epigenetics: First, recently developed approaches based on reduced-representation methods (RRBS, Box 1) allow a base-pair resolution of DNA methylation detection, and can be applied to species for which no genomic resources are available (van Gurp *et al*. 2016; Trucchi *et al*. 2016). Only short fragments of a small fraction of the genome are reconstructed in RRBS, representing only a subset of DNA methylation polymorphisms. Nevertheless, RRBS methods have successfully detected DNA methylation variation in several species (Gugger *et al*. 2016; van Gurp *et al*. 2016; Trucchi *et al*. 2016). The use of these methods in ecological experiments will help to identify DNA methylation variants that impact performance, and motivate detailed follow-up experiments to characterise candidate loci,

although there may always be undetected genetic variants that control observed DNA methylation patterns. Second, even crude draft transcriptomes and genomes support many of the standard workflows in epigenomics data analysis, therefore the additional efforts of constructing and annotating these resources will be instrumental for a more sophisticated understanding of epigenomics in non-model systems. Currently, the number of draft genomes of ecological study species is limited, but continued reduction in sequencing cost, and advances in long-read sequencing methods are rapidly bringing draft assemblies within reach. For plant genomes in particular, these genomics approaches still face the significant challenges of correctly identifying gene and genome duplication, heterozygosity, ploidy and repetitive sequences (Aversano *et al*. 2012; Hirsch & Buell 2013; Boutte *et al*. 2016a,b).

Along with the development of genomics resources, there is also a need for further development of data analysis methods. For instance identifying proper statistical testing approaches for differential DNA methylation in complex ecological experimental designs (including random effects like population and genotype) is an ongoing challenge (e.g. Lea *et al*. 2015). The definition and identification of DMRs holds additional challenges, particularly for RRBS fragments (which are often only the length of a so-called 'region'), and for DNA methylation in different genomic contexts. Existing software tools do not yet cope with these complexities (Box 3). Furthermore, while DNA methylation has been the most studied epigenetic modification in the context of natural variation, variation in histone methylation also can be inherited (Gaydos *et al*. 2014; Ragunathan *et al*. 2014; Audergon *et al*. 2015), and there is evidence for small RNA-dependent epialleles (Calo *et al*. 2014). Thus, epigenetic mechanisms other than DNA methylation need to be investigated in an ecological context, too.

Since organisms in natural settings are continuously exposed to multiple environmental signals and must respond appropriately to dynamic conditions, an ecological context provides a unique opportunity to discover information about epigenetic variation that cannot be gleaned from controlled laboratory settings. Recent studies in natural settings have found gene expression patterns that are only exposed under complex natural stimuli (Pavey *et al*. 2012; Alvarez *et al*. 2015), but the role of epigenetics is almost unexplored. Studies in non-model organisms may also yield functional information about genomic elements that are not annotated in model species, have no homolog in their most closely related model organism, or have taken on a novel function. Applying new tools and understanding of epigenetics and genome function in general to a robust ecological design will be powerful for assessing the importance of both genetic and epigenetic mechanisms in the real world.

ACKNOWLEDGEMENTS

This paper is a joint effort of the working group 'sEpiDiv – Towards understanding the causes and consequences of epigenetic diversity' organised by K.H. and L.O., kindly supported by sDiv, the Synthesis Centre of the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, funded by the German Research Foundation (FZT 118). All authors contributed to the discussions and participated in drafting the manuscript, and the writing was partially supported by funding from the National Science Foundation DEB-1419960 and the Franco-American Fulbright Commission (to C.L.R.).

AUTHORSHIP

All authors contributed to writing of the first draft of manuscript, figures and boxes, and many rounds of revisions. CLR finalised revisions and all authors approved of the final submitted version.

REFERENCES

Ainouche, M.L., Fortune, P.M., Salmon, A., Parisod, C., Grandbastien, M.A., Fukunaga, K. *et al*. (2009). Hybridization, polyploidy and invasion: lessons from Spartina (Poaceae). *Biol. Invasions*, 11(5), 1159–1173.

Alonso, C., Pérez, R., Bazaga, P., Medrano, M. & Herrera, C.M. (2014). Individual variation in size and fecundity is correlated with differences in global DNA cytosine methylation in the perennial herb *Helleborus foetidus* (Ranunculaceae). *Am. J. Bot*., 101, 1309–1313.

Alonso, C., Pérez, R., Bazaga, P. & Herrera, C.M. (2015). Global DNA cytosine methylation as an evolving trait: phylogenetic signal and correlated evolution with genome size in angiosperms. *Front. Genet*., 6, 4.

Alonso, C., Pérez, R., Bazaga, P., Medrano, M. & Herrera, C.M. (2016). MSAP markers and global cytosine methylation in plants: a literature survey and comparative analysis for a wild growing species. *Mol. Ecol. Res*., 16, 80–90. https://doi.org/10.1111/1755-0998.12426.

Alvarez, M., Schrey, A.W. & Richards, C.L. (2015). Ten years of transcriptomics in wild populations: what have we learned about their ecology and evolution? *Mol. Ecol*., 24, 710–725.

Audergon, P.N.C.B., Catania, S., Kagansky, A., Tong, P., Shukla, M., Pidoux, A.L. *et al*. (2015). Epigenetics. Restricted epigenetic inheritance of H3K9 methylation. *Science*, 348, 132–135.

Aversano, R., Raffaella Ercolano, M., Caruso, I., Fasano, C., Rosellini, D. & Carputo, D. (2012). Molecular tools for exploring polyploid genomes in plants. *Int. J. Mol. Sci*., 13, 10316–10335.

Bailey, P.K., Ladunga, I., Lefebvre, C., Li, Q., Liu, T., Madrigal, P. *et al*. (2013). Practical guidelines for the comprehensive analysis of ChIP-seq data. *PLoS Comp. Biol*., 9(11), e1003326. https://doi.org/10.1371/journal.pcbi.100332.

Barbaglia, A.M., Klusman, K.M., Higgins, J., Shaw, J.R., Hannah, L.C. & Lal, S.K. (2012). Gene capture by Helitron transposons reshuffles the transcriptome of maize. *Genetics*, 190, 965–975.

Barturen, G., Rueda, A., Oliver, J.L. & Hackenberg, M. (2014). MethylExtract: High-Quality methylation maps and SNV calling from whole genome bisulfite sequencing data. Version 2. *F1000Res*., 2, 217. https://doi.org/10.12688/f1000research.2-217.v2. eCollection. [revised 21 February 2014].

Becker, C., Hagmann, J., Müller, J., Koenig, D., Stegle, O., Borgwardt, K. *et al*. (2011). Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome. *Nature*, 480, 245–249.

Bilichak, A., Ilnystkyy, Y., Hollunder, J. & Kovalchuk, I. (2012). The progeny of *Arabidopsis thaliana* plants exposed to salt exhibit changes in DNA Methylation, histone modifications and gene expression. *PLoS ONE*, 7(1), e30515.

Bock, C. (2012). Analysing and interpreting DNA methylation data. *Nat. Rev. Genet*., 13, 705–719.

Bossdorf, O., Richards, C.L. & Pigliucci, M. (2008). Epigenetics for ecologists. *Ecol. Lett*., 11, 106–115.

Bossdorf, O., Arcurri, D., Richards, C.L. & Pigliucci, M. (2010). Experimental alteration of DNA methylation affects the phenotypic

plasticity of ecologically relevant traits in *Arabidopsis thaliana*. *Evol. Ecol.*, 24, 541–553.

Boutte, J.J., Ferreira de Carvalho, J., Rousseau-Gueutin, M., Poulain, J., Da Silva, C., Wincke, P. *et al.* (2016a). Reference transcriptomes and detection of duplicated copies in hexaploid and allododecaploid *Spartina* species (Poaceae). *Genome Biol Evol*, 8, 3030–3044.

Boutte, J.J., Aliaga, B., Lima, O., Ferreira de Carvalho, J., Ainouche, A., Macas, J. *et al.* (2016b). Haplotype detection from Next-Generation Sequencing in high-ploidy-level species: 45S rDNA gene copies in the hexaploid *Spartina maritima*. *G3*, 6, 29–40.

Calo, S., Shertz-Wall, C., Lee, S.C., Bastidas, R.J., Nicolas, F.E., Granek, J.A. *et al.* (2014). Antifungal drug resistance evoked via RNAi-dependent epimutations. *Nature*, 513, 555–558.

Charlesworth, B. & Jain, K. (2014). Purifying selection, drift, and reversible mutation with arbitrarily high mutation rates. *Genetics*, 198 (4), 1587–1602.

Cheng, J.C., Matsen, C.B., Gonzales, F.A., Ye, W., Greer, S., Marquez, V.E. *et al.* (2003). Inhibition of DNA methylation and reactivation of silenced genes by Zebularine. *J. Natl Cancer Inst.*, 95, 399–409.

Chodavarapu, R.K., Feng, S., Ding, B., Simon, S.A., Lopez, D., Jia, Y. *et al.* (2012). Transcriptome and methylome interactions in rice hybrids. *Proc. Natl Acad. Sci. USA*, 109, 12040–12045.

Cokus, S.J., Feng, S., Zhang, X., Chen, Z., Merriman, B., Haudenschild, C. D. *et al.* (2008). Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. *Nature*, 452, 215–219.

Colomé-Tatché, M., Cortijo, S., Wardenaar, R., Lahouze, B., Etcheverry, M., Martin, A. *et al.* (2012). Features of the *Arabidopsis* recombination landscape resulting from the combined loss of sequence variation and DNA methylation. *Proc. Natl Acad. Sci. USA*, 149, 16240–16245.

Conrath, U., Pieterse, C.M.J. & Mauch-Mani, B. (2002). Priming in plant–pathogen interactions. *Trends in Plant Sci.*, 7(5), 210–216.

Cortijo, S., Wardenaar, R., Colomé-Tatché, M., Gilly, A., Etcheverry, M., Labadie, K. *et al.* (2014). Mapping the epigenetic basis of complex traits. *Science*, 343(6175), 1145–1148.

Crevillen, P., Yang, H., Cui, X., Greeff, C., Trick, M., Qiu, Q. *et al.* (2014). Epigenetic reprogramming that prevents transgenerational inheritance of the vernalized state. *Nature*, 515, 587–590.

Cubas, P., Vincent, C. & Coen, E. (1999). An epigenetic mutation responsible for natural variation in floral symmetry. *Nature*, 401, 157–161.

Day, T. & Bonduriansky, R. (2011). A unified approach to evolutionary consequences of genetic and nongenetic inheritance. *Am. Nat.*, 178, E18–E36.

Douhovnikoff, V. & Dodd, R.S. (2015). Epigenetics: a potential mechanism for clonal plant success. *Plant Ecol.*, 216, 227–233.

Dowen, R.H., Pelizzola, M., Schmitz, R.J., Lister, R., Dowen, J.M., Nery, J.R. *et al.* (2012). Widespread dynamic DNA methylation in response to biotic stress. *Proc. Natl Acad. Sci. USA*, 109, E2183–E2191.

Dubin, M.J., Zhang, P., Meng, D., Remigereau, M.-S., Osborne, E.J., Casale, F.P. *et al.* (2015). DNA methylation variation in Arabidopsis has a genetic basis and shows evidence of local adaptation. *eLife*, 4, e05255, doi: 10.7554/eLife.05255.

Eichten, S.R. & Springer, N.M. (2015). Minimal evidence for consistent changes in maize DNA methylation patterns following environmental stress. *Front. Plant Sci.*, 6, 308.

Foust, C.M., Preite, V., Schrey, A.W., Verhoeven, K.J.F. & Richards, C.L. (2016). Genetic and epigenetic differences associated with environmental gradients in replicate populations of two salt marsh perennials. *Mol. Ecol.*, 25, 1639–1652.

Furrow, R.E. (2014). Epigenetic inheritance, epimutation, and the response to selection. *PLoS ONE*, 9(7), e101559.

Gaydos, L.J., Wang, W. & Strome, S. (2014). H3K27me and PRC2 transmit a memory of repression across generations and during development. *Science*, 345, 1515–1518.

Geoghegan, J.L. & Spencer, H.G. (2012). Population-epigenetic models of selection. *Theor. Popul. Biol.*, 81(3), 232–242.

Gorelick, R. (2005). Environmentally alterable additive genetic effects. *Evol. Ecol. Res.*, 7, 371–379.

van der Graaf, A., Wardenaar, R., Neumann, D.A., Taudt, A., Shaw, R.G., Jansen, R.C. *et al.* (2015). Rate, spectrum and evolutionary dynamics of spontaneous epimutations. *Proc. Natl Acad. Sci. USA*, 112, 6676–6681.

Gu, H., Smith, Z.D., Bock, C., Boyle, P., Gnirke, A. & Meissner, A. (2011). Preparation of reduced representation bisulfite sequencing libraries for genome-scale DNA methylation profiling. *Nat. Protoc.*, 6(4), 468–481.

Gugger, P., Fitz-Gibbon, S., Pellegrini, M. & Sork, V.L. (2016). Species wide patterns of DNA methylation variation in Quercus lobata and its association with climate gradients. *Mol. Ecol.*, 25, 1665–1680.

van Gurp, T.P., Wagemaker, N.C.A.M., Wouters, B., Vergeer, P., Ouborg, J.N.J. & Verhoeven, K.J.F. (2016). epiGBS: reference-free reduced representation bisulfite sequencing. *Nat. Meth.*, 13, 322–324.

Hagemann, S., Heil, O., Lyko, F. & Brueckner, B. (2011). Azacytidine and decitabine induce gene-specific and non-random DNA demethylation in human cancer cell lines. *PLoS ONE*, 6, e17388.

Hagmann, J., Becker, C., Müller, J., Stegle, O., Meyer, R.C., Wang, G. *et al.* (2015). Century-scale methylome stability in a recently diverged *Arabidopsis thaliana* lineage. *PLoS Genet.*, 11(1), e1004920.

Hansen, K.D., Langmea, B. & Irizarry, R.A. (2012). BSmooth: from whole genome bisulfite sequencing reads to differentially methylated regions. *Genome Biol.*, 13, R83.

He, G., Zhu, X., Elling, A.A., Chen, L., Wang, X., Guo, L. *et al.* (2010). Global epigenetic and transcriptional trends among two rice subspecies and their reciprocal hybrids. *Plant Cell*, 22, 17–33.

Herman, J.J. & Sultan, S.E. (2016). DNA methylation mediates genetic variation for adaptive transgenerational plasticity. *Proc. Biol. Sci.*, 283, 20160988. https://doi.org/10.1098/rspb.2016.0988.

Herman, J.J., Spencer, H.G., Donohue, K. & Sultan, S.E. (2014). How stable 'should' epigenetic modifications be? Insights from adaptive plasticity and bet hedging. *Evolution*, 68, 632–643.

Herrera, C.M. & Bazaga, P. (2010). Epigenetic differentiation and relationship to adaptive genetic divergence in discrete populations of the violet *Viola cazorlensis*. *New Phytol.*, 187(3), 867–876.

Herrera, C.M. & Bazaga, P. (2011). Untangling individual variation in natural populations: ecological, genetic and epigenetic correlates of long-term inequality in herbivory. *Mol. Ecol.*, 20, 1675–1688.

Herrera, C.M. & Bazaga, P. (2013). Epigenetic correlates of plant phenotypic plasticity: DNA methylation differs between prickly and nonprickly leaves in heterophyllous *Ilex aquifolium* (Aquifoliaceae) trees. *Bot. J. Linean Soc.*, 171, 441–452.

Herrera, C.M., Pozo, M.I. & Bazaga, P. (2012). Jack of all nectars, master of most: DNA methylation and the epigenetic basis of niche width in a flower-living yeast. *Mol. Ecol.*, 21, 2602–2616.

Herrera, C.M., Medrano, M. & Bazaga, P. (2014). Variation in DNA methylation transmissibility, genetic heterogeneity and fecundity-related traits in natural populations of the perennial herb *Helleborus foetidus*. *Mol. Ecol.*, 23(522), 1085–1095.

Herrera, C.M., Medrano, M. & Bazaga, P. (2016). Comparative spatial genetics and epigenetics of plant populations: heuristic value and a proof of concept. *Mol. Ecol.*, 25, 1653–1664.

Hirsch, C.N. & Buell, C.R. (2013). Tapping the promise of genomics in species with complex, nonmodel genomes. *Annu. Rev. Plant Biol.*, 2013 (64), 89–110.

Hoffmann, S., Otto, C., Doose, G., Tanzer, A., Langenberger, D., Christ, S. *et al.* (2014). A multi-split mapping algorithm for circular RNA, splicing, *trans*-splicing and fusion detection. *Genome Biol.*, 15(2), R34. https://doi.org/10.1186/gb-2014-15-2-r34.

Houben, A., Demidov, D., Gernand, D., Meister, A., Leach, C.R. & Schubert, I. (2003). Methylation of histone H3 in euchromatin of plant chromosomes depends on basic nuclear DNA content. *Plant J.*, 33, 967–973.

Ito, H., Gaubert, H., Bucher, E., Mirouze, M., Vaillant, I. & Paszkowski, J. (2011). An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. *Nature*, 472, 115–119.

Jablonka, E.B. & Raz, G. (2009). Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Q. Rev. Biol.*, 84(2), 131–176.

Jaskiewicz, M. *et al*. (2011). Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. *EMBO Rep*., 12, 50–55.

Johannes, F., Porcher, E., Teixeira, F., Saliba-Colombani, V., Simon, M., Agier, N. *et al*. (2009). Assessing the impact of transgenerational epigenetic variation on complex traits. *PLoS Genet*., 5, e1000530.

Jones, P.A. (1985). Altering gene expression with 5-azacytidine. *Cell*, 40, 485–486.

Jühling, F., Kretzmer, H., Bernhart, S.H., Otto, C., Stadler, P.F. & Hoffmann, S. (2016). metilene: Fast and sensitive calling of differentially methylated regions from bisulfite sequencing data. *Genome Res*., 26, 256–262.

Kawakatsu, T., Huang, S.C., Jupe, F., Sasaki, E., Schmitz, R.J., Urich, M.A. *et al*. (2016). Epigenomic diversity in a global collection of *Arabidopsis thaliana* accessions. *Cell*, 166, 492–505.

Khraiwesh, B., Arif, M.A., Seumel, G.I., Ossowski, S., Weigel, D., Reski, R. *et al*. (2010). Transcriptional control of gene expression by microRNAs. *Cell*, 140, 111–122.

Kim, J.-M., Sasaki, T., Ueda, M., Sako, K. & Seki, M. (2015). Chromatin changes in response to drought, salinity, heat, and cold stresses in plants. *Front. Plant Sci*., 6, 114.

Klironomos, F., Berg, J. & Collins, S. (2013). How epigenetic mutations can affect genetic evolution: model and mechanism. *BioEssays*, 35, 571–578.

Kobayashi, M.J., Takeuchi, Y., Kenta, T., Kume, T., Diway, B. & Shimizu, K.K. (2013). Mass flowering of the tropical tree *Shorea beccariana* was preceded by expression changes in flowering and drought-responsive genes. *Mol. Ecol*., 22, 4767–4782.

Korneliussen, T.S., Anders, A. & Nielsen, R. (2014). ANGSD: analysis of next generation sequencing data. *BMC Bioinformatics*, 15(1), 356.

Kou, H., Li, Y., Song, X., Ou, X., Xing, S., Ma, J. *et al*. (2011). Heritable alteration in DNA methylation induced by nitrogen-deficiency stress accompanies enhanced tolerance by progenies to the stress in rice (*Oryza sativa* L.). *J. Plant Physiol*., 168, 1685–1693.

Kronholm, I. & Collins, S. (2016). Epigenetic mutations can both help and hinder adaptive evolution. *Mol. Ecol*., 25, 1856–1868.

Krueger, F. & Andrews, S.R. (2011). Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications. *Bioinformatics*, 27(11), 1571–1572.

Kruidenier, L., Chung, C.-W., Cheng, Z., Liddle, J., Che, K., Joberty, G. *et al*. (2012). A selective jumonji H3K27 demethylase inhibitor modulates the proinflammatory macrophage response. *Nature*, 488, 404–408.

Latzel, V., Allan, E., Silveira, A.B., Colot, V., Fischer, M. & Bossdorf, O. (2013). Epigenetic diversity increases the productivity and stability of plant populations. *Nat. Commun*., 4, 2875.

Lea, A.J., Tung, J. & Zhou, X. (2015). A flexible, efficient binomial mixed model for identifying differential DNA methylation in bisulfite sequencing data. *PLoS Genet*., 11(11), e1005650.

Lee, E.J., Pei, L., Srivastava, G., Joshi, T., Kushwaha, G., Choi, J.H. *et al*. (2011). Targeted bisulfite sequencing by solution hybrid selection and massively parallel sequencing. *Nucl. Acids Res*., 39(19), e127.

Levins, R. (1966). The strategy of model building in population biology. *Am. Sci*., 54, 421–431.

Li, X., Zhu, J., Hu, F., Ge, S., Ye, M., Xiang, H. *et al*. (2012). Single-base resolution maps of cultivated and wild rice methylomes and regulatory roles of DNA methylation in plant gene expression. *BMC Genom*., 13, 300.

Li, Q., Eichten, S.R., Hermanson, P.J. & Springer, N.M. (2014). Inheritance Patterns and Stability of DNA methylation variation in maize near-isogenic lines. *Genetics*, 196(3), 667–676.

Lira-Medeiros, C.F. *et al*. (2010). Epigenetic variation in mangrove plants occurring in contrasting natural environment. *PLoS ONE*, 5, e10326.

Liu, Y., Siegmund, K.D., Laird, P.W. & Berman, B.P. (2012). Bis-SNP: combined DNA methylation and SNP calling for Bisulfite-seq data. *Genome Biol*., 13(7), R61.

Long, Y., Xia, W., Li, R., Wang, J., Shao, M., Feng, J. *et al*. (2011). Epigenetic QTL mapping in *Brassica napus*. *Genetics*, 189, 1093–1102.

Luna, E., Bruce, T., Roberts, M., Flors, V. & Ton, J. (2012). Next generation systemic acquired resistance. *Plant Physiol*., 158, 844–853.

Manning, K., Tor, M., Poole, M. *et al*. (2006). A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nat. Genet*., 38, 948–952.

Marí-Ordóñez, A., Marchais, A., Etcheverry, M., Martin, A., Colot, V. & Voinnet, O. (2013). Reconstructing de novo silencing of an active plant retrotransposon. *Nat. Genet*., 45(9), 1029–1039.

Martínez, G., Panda, K., Köhler, C. & Slotkin, R.K. (2016). Silencing in sperm cells is directed by RNA movement from the surrounding nurse cell. *Nat. Plants*, 2, 16030. doi: 10.1038/nplants.2016.30

Matzke, M.A. & Mosher, R.A. (2014). RNA-directed DNA methylation: an epigenetic pathway of increasing complexity. *Nat. Rev. Genet*., 15, 394–408.

Maumus, F., Epert, A., Nogue, F. & Blanc, G. (2014). Plant genomes enclose footprints of past infections by giant virus relatives. *Nat. Commun*., 5, 4268.

McKenna, A. *et al*. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*., 20(9), 1297–1303.

Medrano, M., Herrera, C.M. & Bazaga, P. (2014). Epigenetic variation predicts regional and local intraspecific functional diversity in a perennial herb. *Mol. Ecol*., 23, 4926–4938.

Mirouze, M., Reinders, J., Bucher, E., Nishimura, T., Schneeberger, K., Ossowski, S. *et al*. (2009). Selective epigenetic control of retrotransposition in *Arabidopsis*. *Nature*, 461, 427–430.

Morgado, L., Preite, V., Oplaat, C., Anava, S., Ferreira de Carvalho, J., Rechavi, O., Johannes, F. & Verhoeven, K. (2017). Small RNAs reflect grandparental environments in apomictic dandelion. *Mol. Biol. Evol*., 34, 2035–2040.

Narum, S.R., Buerkle, C.A., Davey, J.W., Miller, M.R. & Hohenlohe, P.A. (2013). Genotyping-by-sequencing in ecological and conservation genomics. *Mol. Ecol*., 22, 2841–2847.

Niederhuth, C.E.*, Bewick, A.J.*, Ji, L., Alabday, M., Kim, K.D., Li, Q. *et al*. (2016). Widespread natural variation of DNA methylation within angiosperms. *Genome Biol*., 17, 194.

Noy-Malka, C., Yaari, R., Itzhaki, R., Mosquna, A., Auerbach Gershovitz, N., Katz, A. *et al*. (2014). A single CMT methyltransferase homolog is involved in CHG DNA methylation and development of *Physcomitrella patens*. *Plant Mol. Biol*., 84, 719–735.

O'Malley, R.C. & Ecker, J.R. (2012). Epiallelic variation in *Arabidopsis thaliana*. *Cold Spring Harb. Symp. Quant. Biol*., 77, 135–145.

Ong-Abdullah, M., Ordway, J.M., Jiang, N., Ooi, S.E., Kok, S.Y., Sarpan, N. *et al*. (2015). Loss of Karma transposon methylation underlies the mantled somaclonal variant of oil palm. *Nature*, 525 (7570), 533–537.

Ossowski, S., Schneeberger, K., Lucas-Lledo, J.I., Warthmann, N., Clark, R.M., Shaw, R.G. *et al*. (2010). The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. *Science*, 327, 92–94.

Park, P. (2009). ChIP-seq: advantages and challenges of a maturing technology. *Nat. Rev. Genet*., 10, 669–680.

Paszkowski, J. & Grossniklaus, U. (2011). Selected aspects of transgenerational epigenetic inheritance and resetting in plants. *Curr. Opin. Plant Biol*., 14, 195–203.

Pavey, S.A., Bernatchez, L., Aubin-Horth, N. & Landry, C.R. (2012). What is needed for next-generation ecological and evolutionary genomics? *Trends Ecol. Evol*., 27(12), 673–678.

Pecinka, A. & Mittelsten Scheid, O. (2012). Stress-induced chromatin changes: a critical view on their heritability. *Plant Cell Physiol*., 53, 801–808.

Peters, T.J., Buckley, M.J., Statham, A.L., Pidsley, R., Samaras, K., Lord, R.V. *et al*. (2015). De novo identification of differentially methylated regions in the human genome. *Epigenetics Chromatin*, 8, 6.

Platt, A., Gugger, P. & Sork, V.L. (2015). Genome-wide signature of local adaptation linked to variable CpG methylation in oak populations. *Mol. Ecol*., 24, 3823–3830.

Ragunathan, K., Jih, G. & Moazed, D. (2014). Epigenetic inheritance uncoupled from sequence-specific recruitment. *Science*, 348, 1258699. https://doi.org/10.1126/science.1258699.

Rasmann, S., De Vos, M., Casteel, C.L., Tian, D., Halitschke, R., Sun, J.Y. *et al.* (2012). Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant Physiol.*, 158, 854–863.

Reinders, J., Wulff, B.B., Mirouze, M., Marí-Ordóñez, A., Dapp, M., Rozhon, W. *et al.* (2009). Compromised stability of DNA methylation and transposon immobilization in mosaic *Arabidopsis* epigenomes. *Genes Dev.*, 23, 939–950.

Rendina González, A.P., Chrtek, J., Dobrev, P.I., Dumalasová, V., Fehrer, J., Mráz, P. *et al.* (2016). Stress-induced memory alters growth of clonal off spring of white clover (*Trifolium repens*). *Am. J. Bot.*, 103 (9), 1567–1574.

Rensing, S.A., Lang, D., Zimmer, A.D., Terry, A., Salamov, A., Shapiro, H. *et al.* (2008). The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. *Science*, 319, 64–69.

Reyna-Lopez, G.E., Simpson, J. & Ruiz-Herrera, J. (1997). Differences in DNA methylation patterns are detectable during the dimorphic transition of fungi by amplification of restriction polymorphims. *Mol. Genet. Genomics*, 253, 703–710.

Richards, E.J. (2006). Inherited epigenetic variation – revisiting soft inheritance. *Nat. Rev. Genet.*, 7, 395–401.

Richards, C.L., Bossdorf, O. & Verhoeven, K.J.F. (2010). Understanding natural epigenetic variation. *New Phytol.*, 187, 562–564.

Richards, C.L., Schrey, A.W. & Pigliucci, M. (2012). Invasion of diverse habitats by few Japanese knotweed genotypes is correlated with high epigenetic differentiation. *Ecol. Lett.*, 15, 1016–1025.

Robertson, M.H. & Richards, C.L. (2015). Non-genetic inheritance in evolutionary theory – the importance of plant studies. *Non-Gen. Inherit.*, 2, 3–11.

Robertson, M.H., Schrey, A.W., Shayter, A., Moss, C.J. & Richards, C.L. (2017). Genetic and epigenetic variation in *Spartina alterniflora* following the *Deepwater Horizon* oil spill. *Evol. Appl. (Special issue)*, https://doi.org/10.1111/eva.12482.

Sani, E., Herzyk, P., Perrella, G., Colot, V. & Amtmann, A. (2013). Hyperosmotic priming of *Arabidopsis* seedlings establish a long-term somatic memory accompanied by specific changes of the epigenome. *Genome Biol.*, 14, R59.

Schield, D.R., Walsh, M.R., Card, D.C., Andrew, A.L., Adams, R.H. & Castoe, T.A. (2016). EpiRADseq: scalable analysis of genomewide patterns of methylation using next-generation sequencing. *Meth. Ecol. Evol.*, 7, 60–69.

Schmitz, R.J., Schultz, M.D., Urich, M.A., Nery, J.R., Pelizzola, M., Libiger, O. *et al.* (2013). Patterns of population epigenomic diversity. *Nature*, 495, 193–198.

Schrey, A.W., Alvarez, M., Foust, C.M., Kilvitis, H.J., Lee, J.D., Liebl, A.L. *et al.* (2013). Ecological Epigenetics: beyond MS-AFLP. *Integr. Comp. Biol.*, 53, 340–350.

Schulz, B., Eckstein, R.L. & Durka, W. (2014). Epigenetic variation reflects dynamic habitat conditions in a rare floodplain herb. *Mol. Ecol.*, 23, 3523–3537.

Secco, D., Wang, C., Shou, H., Schultz, M.D., Chiarenza, S., Nussaume, L. *et al.* (2015). Stress induced gene expression drives transient DNA methylation changes at adjacent repetitive elements. *eLife*, 4, doi: 10.7554/eLife.09343.

Seymour, D.K., Koenig, D., Hagmann, J., Becker, C. & Weigel, D. (2014). Evolution of DNA methylation patterns in the Brassicaceae is driven by differences in genome organization. *PLoS Genet.*, 10, e1004785.

Slatkin, M. (2009). Epigenetic inheritance and the missing heritability problem. *Genetics*, 182(3), 845–850.

Slaughter, A., Daniel, X., Flors, V., Luna, E., Hohn, B. & Mauch-Mani, B. (2012). Descendants of primed *Arabidopsis* plants exhibit resistance to biotic stress. *Plant Physiol.*, 158, 835–843.

Song, Q., Decato, B., Hong, E.E., Zhou, M., Fang, F., Qu, J. *et al.* (2013). A reference methylome database and analysis pipeline to facilitate integrative and comparative epigenomics. *PLoS ONE*, 8(12), e81148.

Spens, A.E. & Douhovnikoff, V. (2016). Epigenetic variation within Phragmites australis among lineages, genotypes, and ramets. *Biol. Invasions*, 18, 2457.

Springer, N.M., Lisch, D. & Li, Q. (2016). Creating order from chaos: epigenome dynamics in plants with complex genomes. *Plant Cell*, 28, 314–325.

Studholme, D.J. (2012). Deep sequencing of small RNAs in plants: applied bioinformatics. *Briefings in Functional Genomics*, 11, 71–85, https://doi.org/10.1093/bfgp/elr039

Sun, D., Xi, Y., Rodriguez, B., Park, H.J., Tong, P., Meong, M. *et al.* (2014). MOABS: model based analysis of bisulfite sequencing data. *Genome Biol.*, 15, R38.

Takuno, S., Ran, J.H. & Gaut, B.S. (2016). Evolutionary patterns of genic DNA methylation vary across land plants. *Nat. Plants*, 2, 15222.

Taudt, A., Colomé-Tatché, M. & Johannes, F. (2016). Genetic sources of population epigenomic variation. *Nat. Rev. Genet.*, 17(6), 319–332.

Teixeira, F.K., Heredia, F., Sarazin, A., Roudier, F., Boccara, M., Ciaudo, C. *et al.* (2009). A role for RNAi in the selective correction of DNA methylation defects. *Science*, 323(5921), 1600–1604.

Trucchi, E., Mazzarella, A.B., Gilfillan, G.D., Romero, M.T. & Paun, O. (2016). BsRADseq screening DNA methylation in natural populations of non-model species. *Mol. Ecol.*, 25, 1697–1713.

Vergeer, P. & Ouborg, N.J. (2012). Evidence for an epigenetic role in inbreeding depression. *Biol. Lett.*, 8, 798–801.

Verhoeven, K.J.F. & Preite, V. (2014). Epigenetic variation in asexually reproducing organisms. *Evol.*, 68, 644–655.

Verhoeven, K.J.F., Jansen, J.J., van Dijk, P.J. & Biere, A. (2010). Stress-induced DNA methylation changes and their heritability in asexual dandelions. *New Phyt.*, 185(4), 1108–1118.

Wang, J. & Fan, C. (2014). A neutrality test for detecting selection on DNA methylation using single methylation polymorphism frequency spectrum. *Genome Biol Evol.*, 7(1), 154–171. https://doi.org/10.1093/gbe/evu271.

Wibowo, A.T., Becker, C., Marconi, G., Durr, J., Price, J., Hagmann, J. *et al.* (2016). Hyperosmotic stress memory in *Arabidopsis* is mediated by distinct epigenetically labile sites in the genome and is restricted in the male germline by DNA glycosylase activity. *eLife*, 5, e13546.

Widiez, T., Symeonidi, A., Luo, C., Lam, E., Lawton, M. & Rensing, S.A. (2014). The chromatin landscape of the moss *Physcomitrella patens* and its dynamics during development and drought stress. *Plant J.*, 79, 67–81.

Willing, E.-M., Rawat, V., Mandáková, T., Maumus, F., James, G.V., Nordström, K.J.V. *et al.* (2015). Genome expansion of *Arabis alpina* linked with retrotransposition and reduced symmetric DNA methylation. *Nat. Plants*, 1, 14023, doi: 10.1038/nplants.2014.23.

Wilschut, R.A., Oplaat, C., Snoek, L.B., Kirschner, J. & Verhoeven, K.J.F. (2016). Natural epigenetic variation contributes to heritable flowering divergence in a widespread asexual dandelion lineage. *Mol. Ecol.*, 25, 1759–1768.

Xie, H.J., Li, A.H., Liu, A.D., Dai, W.M., He, J.Y., Lin, S. *et al.* (2015). ICE1 demethylation drives the range expansion of a plant invader through cold tolerance divergence. *Mol. Ecol.*, 24, 835–850.

Zhang, Y.Y., Fischer, M., Colot, V. & Bossdorf, O. (2013). Epigenetic variation creates potential for evolution of plant phenotypic plasticity. *New Phytol.*, 197(1), 314–322.

Editor, Tim Coulson
Manuscript received 26 April 2017
First decision made 15 June 2017
Second decision made 11 August 2017
Manuscript accepted 4 September 2017