# Ecological Epigenetics

# 10

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**Abstract**

Biologists have assumed that heritable variation due to DNA sequence differences (i.e., genetic variation) allows populations of organisms to be both robust and adaptable to extreme environmental conditions. Natural selection acts on the variation among different genotypes and ultimately changes the genetic composition of the population. While there is compelling evidence about the importance of genetic polymorphisms, evidence is accumulating that epigenetic mechanisms (e.g., chromatin modifications, DNA methylation) can affect ecologically important traits, even in the absence of genetic variation. In this chapter, we review this evidence and discuss the consequences of epigenetic variation in natural populations. We begin by defining the term epigenetics, providing a brief overview of various epigenetic mechanisms, and noting the potential importance of epigenetics in the study of ecology. We continue with a review of the ecological epigenetics literature to demonstrate what is currently known about the amount and distribution of epigenetic variation in natural populations. Then, we consider the various ecological contexts in which epigenetics has proven particularly insightful and discuss the potential evolutionary consequences of epigenetic variation. Finally, we conclude with suggestions for future directions of ecological epigenetics research.

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## 10.1 Introduction

Understanding ecology requires the examination of complex questions that motivate studies at every biological level, from molecules through ecosystems. At the molecular level, controlled laboratory and sequencing studies have revealed

that the genome is a dynamic system of interacting elements. Although we know that environmental factors have tremendous power to shape the genome (McClintock 1984; Karrenberg et al. 2007; Boyko and Kovalchuk 2011), our understanding of how complex phenotypes arise from a given genotype, and how important environmental factors are in this process, remains limited (Richards et al. 2009, 2012a; Pigliucci 2010; Martin et al. 2011).

Biologists have assumed that heritable variation due to DNA sequence differences (i.e., genetic variation) allows populations of organisms to be both robust and adaptable to extreme environmental conditions. Natural selection acts on the variation among different genotypes and ultimately changes the genetic composition of the population. While there is compelling evidence about the importance of genetic polymorphisms, evidence is accumulating that epigenetic mechanisms (e.g., chromatin modifications, DNA methylation) can affect ecologically important traits, even in the absence of genetic variation. In this chapter, we review this evidence and discuss the consequences of epigenetic variation in natural populations. We begin by defining the term epigenetics, providing a brief overview of various epigenetic mechanisms, and noting the potential importance of epigenetics in the study of ecology. We continue with a review of the ecological epigenetics literature to demonstrate what is currently known about the amount and distribution of epigenetic variation in natural populations. Then, we consider the various ecological contexts in which epigenetics has proven particularly insightful and discuss the potential evolutionary consequences of epigenetic variation. Finally, we conclude with suggestions for future directions of ecological epigenetics research.

## 10.1.1 History of Epigenetics

The term 'epigenetics' was originally coined in the early 1940s by Conrad Waddington, who is widely recognized for investigating the concepts of canalization and genetic assimilation in the context of developmental biology (Waddington 1942, 1953; Jablonka and Lamb 2002). Waddington's use of the term 'epigenetics' was very broad, referring to all developmental events which lead to the manifestation of an organism's phenotype (Holliday 2006). Since Waddington, interpretations of the term epigenetics have evolved, especially in light of discoveries of molecular processes that regulate gene activity and the inheritance of cellular phenotypes (Jablonka and Lamb 2002). These findings revealed that there is an alternative form of inheritance not easily explained by traditional Mendelian genetics (Holliday 2006). Molecular biologist Robin Holliday, was among the first to recognize this concept of non-genetic inheritance by defining epigenetics as the study of alterations in gene expression and the mitotic inheritance of gene expression patterns (Holliday 1994; Jablonka and Lamb 2002).

In recent years, epigenetics has been commonly defined as the study of heritable changes in gene expression not explained by changes in the DNA sequence (Holliday 1994; Richards 2006; Bird 2007; Bossdorf et al. 2008). However, the definition of epigenetics continues to be a topic of debate among biologists, primarily because of the inclusion of the term "heritable" in most modern definitions (Bird 2007). Depending on the field of study (e.g., molecular/cellular biology, developmental biology, or ecological/evolutionary biology), some believe that the study of epigenetics encompasses all processes aside from DNA sequence that produce the phenotype in organisms (Hallgrímsson and Hall 2011), heritable or not. Others argue that because epigenetics is often associated with "soft inheritance," heritability is a necessary component of the definition (Ho and Burggren 2010; Kovalchuk 2012). Due to these opposing views, we differentiate the term "epigenetics" from "epigenetic inheritance" (sensu Jablonka and Raz 2009). Jablonka and Raz (2009) argue that epigenetics is the study of both cellular-level and organismal-level processes underlying developmental plasticity and canalization that lead to enduring developmental effects. Alternatively,

**Fig. 10.1** The classic example of phenotypic effects of natural epigenetic variation and epigenetic inheritance. Cubas et al. (1999) showed that the change from normal bilateral (*right*) to radial symmetry (*left*) of *Linaria vulgaris* was associated with methylation and silencing of the *Lcyc* gene (Photos Reprinted from Palevitz 1999)

epigenetic inheritance is an extension of epigenetics that occurs when variations in phenotype, not caused by DNA sequence variation, are mitotically and/or meiotically inherited by future generations (Jablonka and Raz 2009). For the purpose of this chapter, we define epigenetics as the study of molecular-level mechanisms that affect gene expression without altering the underlying DNA sequence, which may lead to potentially heritable changes in phenotype (Bossdorf et al. 2008; Richards et al. 2010a; Richards 2011; Ledón-Rettig et al. 2013).

## 10.1.2  Epigenetic Mechanisms

There are several epigenetic mechanisms that can alter gene expression (e.g., chromatin remodeling, histone modifications, small interfering RNAs), yet DNA methylation of cytosines is by far the most-common mechanism studied in ecological epigenetics (Schrey et al. 2013). In many eukaryotes, DNA methylation occurs at the fifth carbon of a cytosine – as 5-methylcytosine – located within one of the following DNA sequence motifs: CpG (in animals) or CpCpG, CpHpH, CpNpG (in plants) (Rapp and Wendel 2005; Zilberman and Henikoff 2007; Zhang et al. 2008; Laird 2010; Bock 2012). Extensive research shows that DNA methylation plays an integral role in numerous biological processes including organismal development (Monk et al. 1987), genomic imprinting (Li et al. 1993), mammalian X-chromosome inactivation (Kaslow and Migeon 1987), and polyploidy/hybridization in plants (Salmon et al. 2005) (reviewed in Rapp and Wendel 2005; Zilberman and Henikoff 2007; Bock 2012; Richards et al. 2012a). A complex relationship between DNA methylation and gene expression patterns has been demonstrated (Nätt et al. 2012) and the interaction between DNA methylation and transcription machinery can directly influence an organism's phenotype (e.g., floral symmetry in *Linaria vulgaris* – Fig. 10.1 – (Cubas et al. 1999); reviewed in Jaenisch and Bird 2003; Bossdorf et al. 2008; Bock 2012). The stability of DNA methylation varies across the genome, but some loci can be directly influenced by the environment, remain stable throughout an individual's lifetime, and be inherited by future generations (Johannes et al. 2009; Angers et al. 2010; Verhoeven et al. 2010). Molecular tools have been developed to screen both genome-wide and gene-specific patterns of methylation that can be applied to study many biological taxa (reviewed in Laird 2003; Rapp and Wendel 2005; Bossdorf et al. 2008; Schrey et al. 2013). These characteristics make DNA methylation particularly useful for studying epigenetics in an ecological context.

Although the current ecological epigenetics literature is primarily focused on DNA

methylation, other epigenetic modifications can alter gene expression. Histone modifications alter the way DNA is packaged and change the accessibility of the packaged DNA for transcription. These modifications can also interact with DNA methylation (Richards and Elgin 2002; Rapp and Wendel 2005). The activity of transposable elements, regions of DNA that have the ability to move within the genome and integrate into new sites, are regulated primarily by small interfering RNAs or by DNA methylation (Kazazian 2004; Kejnovsky et al. 2012; Richards et al. 2012a; Slotkin et al. 2012). Transposable elements have the potential to alter gene expression and function when inserted within coding regions, so regulation of these areas of the genome is highly important (Kazazian 2004; Feschotte 2008). Small interfering RNAs are active in DNA methylation pathways and histone methylation pathways. Similarities between these pathways in animals and plants suggest evolutionary conservation in these epigenetic processes (Saze et al. 2012).

### 10.1.3  Epigenetics in the Study of Ecology

The field of ecological genomics has provided valuable insight into the genetic basis of ecologically and evolutionarily relevant phenotypic variation within and among natural populations (Ungerer et al. 2008). However, there are ecologically relevant phenomena that cannot be entirely explained by genetic variation (Bossdorf et al. 2008). Ecological epigenetics aims to address how epigenetic processes may also be important mediators of phenotypic variation in populations (Bossdorf et al. 2008). Just as genomics is a sub-discipline in genetics, epigenomics is a discipline within the broader field of epigenetics, which focuses specifically on characterizing epigenetic processes on a genome-wide scale. Thus for the purpose of this review, we use the more encompassing term ecological epigenetics which include genome-wide assessments of epigenetic variation (e.g., MS-AFLP studies), as well as

more gene-specific approaches used to determine the effects of epigenetic processes on phenotypic variation. One of the critical differences between ecological genomics generally and ecological epigenetics is that epigenetic variation is typically more labile and responsive to external environmental factors (e.g., via alteration of DNA methylation), often within ecological time scales (Fig. 10.2) (Bossdorf et al. 2008; Angers et al. 2010). Research on the epigenetic basis of ecologically relevant traits has posited that epigenetically-mediated response to environment can be heritable across generations and may have major implications for our understanding of evolutionary processes (Richards 2006; Bossdorf et al. 2008; Jablonka and Raz 2009).

Until recently, the vast majority of our knowledge about epigenetic mechanisms has stemmed from laboratory studies of model organisms, such as mice (Morgan et al. 1999) and *Arabidopsis thaliana* (Lippman et al. 2004). However, with the birth of ecological epigenetics, researchers are now attempting to decipher the role and significance of epigenetic processes in the context of ecology and evolution (Richards 2006; Bossdorf et al. 2008; Jablonka and Raz 2009; Richards et al. 2012a, b).

## 10.2  The Extent and Structure of Epigenetic Variation Within and Among Natural Populations

Among the most fundamental objectives underlying ecological epigenetics is understanding the importance of epigenetic variation in natural environments (Bossdorf et al. 2008; Richards et al. 2012a). To achieve this objective, various molecular techniques have been used to assess DNA methylation patterns at the individual and population level (reviewed in Dahl and Guldberg 2003; Laird 2003; Liu and Maekawa 2003; Zilberman and Henikoff 2007; Schrey et al. 2013). Thus far, methylation sensitive-AFLP (MS-AFLP; Reyna-Lopez et al. 1997) has been the most commonly used method in ecological epigenetics (reviewed by Schrey et al. 2013). This technique has been
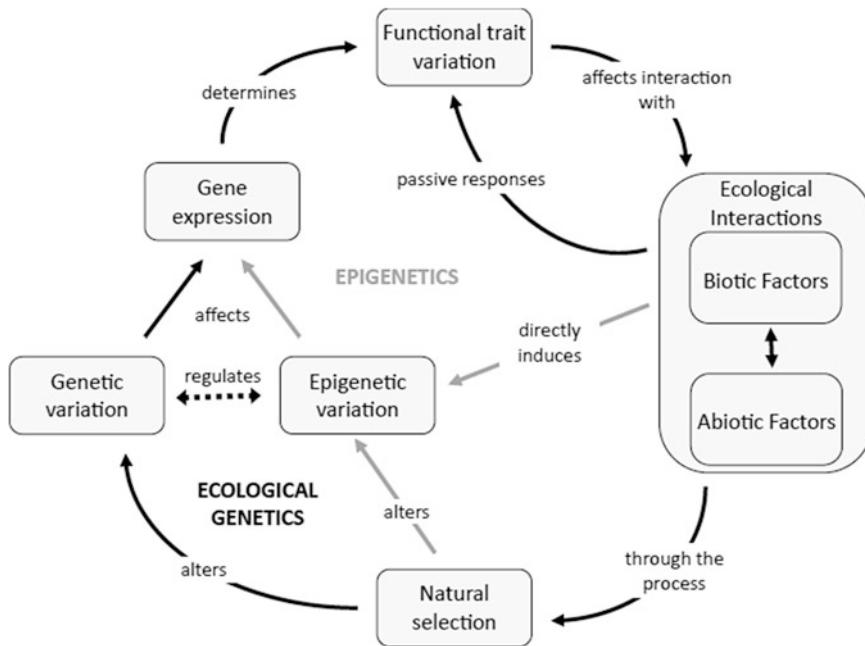
**Fig. 10.2** Genetic processes (in *black*) interact with epigenetic processes (in *grey*) and the environment. Here, focus on functional trait variation emphasizes the need for data that links epigenetic loci and specific ecologically relevant phenotypes. We also highlight that ecological interactions include biotic and abiotic factors which can both play roles in creating and maintaining epigenetic variation (Modified with permission from Bossdorf et al. 2008)

used in a variety of studies to determine the extent and structure of epigenetic variation in populations. MS-AFLP is a modification of the standard AFLP protocol (Vos et al. 1995) in that it uses methylation-sensitive isoschizomeric enzymes (e.g., substituting *MspI* and *HpaII* for *MseI*) to detect genome-wide variation in DNA methylation (Cervera et al. 2002; Salmon et al. 2005). The enzymes *MspI* and *HpaII* have different sensitivities to cytosine methylation of the CCGG recognition sequence (McClelland et al. 1994; Roberts et al. 2007). Comparing the banding pattern from independent reactions with *MspI* and *HpaII* allows for the identification of the methylation state at a particular restriction site (Salmon et al. 2008). MS-AFLP screens variation in DNA methylation at many restriction sites, generating a multi-locus epigenotype for each individual.

There are several benefits associated with using MS-AFLP to study epigenetic variation in natural populations (Schrey et al. 2013). First, this technique enables researchers to assess epigenetic variation in non-model organisms that lack a sequenced genome. The technique is also economical, which is important for ecological studies with large sample sizes. MS-AFLP also requires the same equipment and technical skills as traditional AFLP, which makes it easier for labs to couple epigenetic questions to their genetic (i.e., AFLP) work. MS-AFLP is especially useful for population epigenetic studies because it provides a genome-wide scan and allows for many individuals to be screened at multiple loci concurrently. Lastly, MS-AFLP data obtained from appropriately designed studies can demonstrate that heritable epigenetic variation – specifically DNA methylation – may provide an additional source of variation important in the process of natural selection. An extensive review of the benefits and weaknesses of MS-AFLP can be found in Schrey et al. (2013).

**Fig. 10.3** Spanish violets (*Viola cazorlensis*) studied by Herrera and Bazaga (2010, 2011) (Image courtesy of © Carlos M. Herrera. All Rights Reserved)

### 10.2.1 Insights from the Ecological Epigenetics Literature

Understanding the mechanisms of local adaptation to different habitats is an enduring quest in ecology. Since epigenetic mechanisms can respond to the environment and cause heritable variation in traits, epigenetics may contribute to the process of adaptation. This will be reflected in an association of epigenetic structure by habitat, which has been supported in several recent studies (Herrera and Bazaga 2010; Lira-Medeiros et al. 2010; Massicotte et al. 2011; Richards et al. 2012b). For example, Herrera and Bazaga (2010) examined the distribution of genetic and epigenetic variation within and among wild populations of Spanish violet (*Viola cazorlensis* – Fig. 10.3) using AFLP and MS-AFLP. They detected population differentiation at both the genetic and epigenetic levels, but also that epigenetic variation exceeded genetic variation. Identifying more variation at epigenetic markers suggests that epigenetic mechanisms could contribute a significant amount of variation in this species. They also found an association between the patterns of variation observed at epigenetic markers and the genetic loci that were implicated in adaptive differentiation in floral traits between the populations (Herrera and Bazaga 2010). This study was one of the first to find that adaptive

genetic divergence may be associated with epigenetic differentiation between populations.

Epigenetic differentiation also exists between populations of white mangroves (*Laguncularia racemosa*) located either in a river basin or near a salt marsh habitat in Brazil (Fig. 10.4). Plants in the river basin exhibited several phenotypic traits (e.g., height, diameter at breast height, leaf width and leaf area) that were significantly different from plants located near the salt marsh (Lira-Medeiros et al. 2010). Genetic analysis, using AFLP, failed to differentiate populations; however, the study found significant epigenetic differentiation between populations using MS-AFLP (Lira-Medeiros et al. 2010). These findings illustrate an association between epigenetic differences and environmental factors, and suggest that changes in methylation among salt marsh plants could be important in response to the salt marsh environment (Lira-Medeiros et al. 2010, see also Salmon et al. 2005).

In one of the few studies of wild animal populations, Massicotte et al. (2011) found a higher rate of epigenetic variation than genetic variation in the clonal fish, *Chrosomus eos-neogaeus*, from multiple lakes in Canada using MS-AFLP. Going beyond MS-AFLP, the authors also incorporated bisulfite sequencing, which clearly shows the benefits gained by using multiple techniques. They first excised and sequenced 15 randomly chosen MS-AFLP fragments. These fragments were then compared to the zebrafish genome and 11 were found to have putative similarity to zebrafish sequences. One locus that showed homology with DIRS1, a transposable element in zebrafish was then bisulfite sequenced and the researchers were able to identify five epigenetic variants at this locus (Massicotte et al. 2011). Thus, the combination of techniques was able to add context to the epigenetic differences observed by MS-AFLP and indicated that at least some of the differences detected by MS-AFLP may target important genetic elements.

One of the fundamental differences between genetic and epigenetic variation is that the latter is more environmentally labile and potentially reversible (Richards et al. 2010a, b). Therefore, patterns of epigenetic differentiation among field populations measured in different
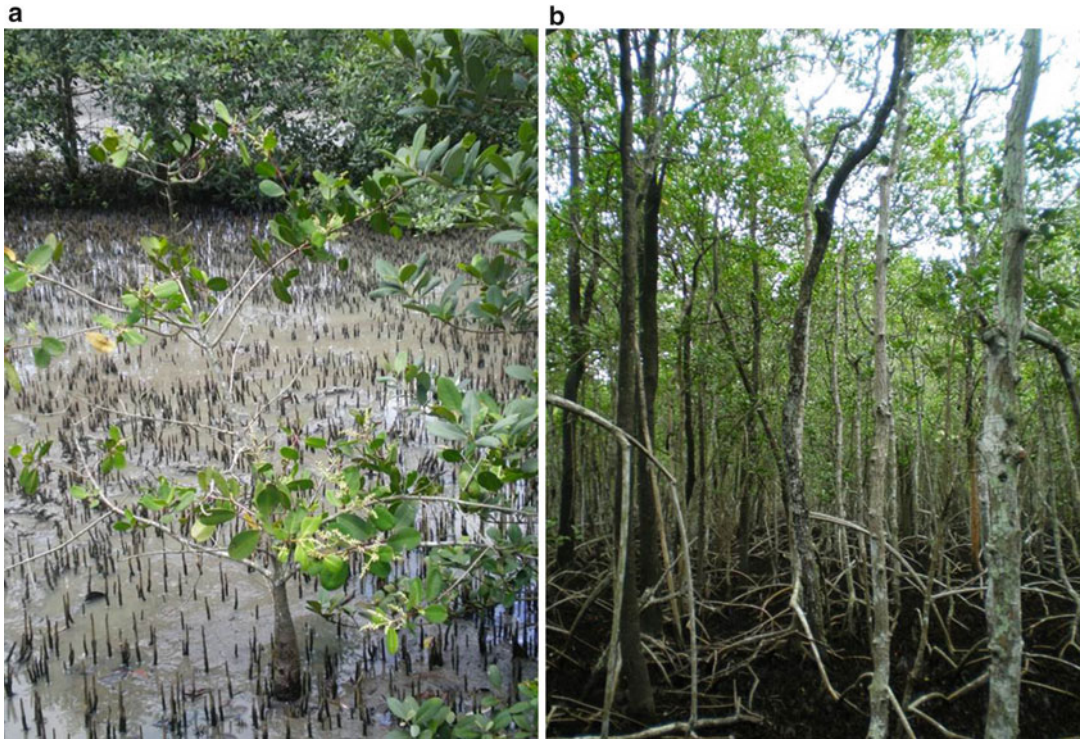
**Fig. 10.4** Adult white mangroves (*Laguncularia racemosa*) from the salt marsh (*left panel*) and from the riverine habitat (*right panel*; Lira-Medeiros et al. 2010) (Images courtesy of © Catarina F. Lira-Medeiros. All Rights Reserved)

environments – like the ones observed in the majority of these studies – include both a reversible component due to phenotypic plasticity and a non-reversible or relatively stable component due to heritable epigenetic differentiation. In this respect, analyses of epigenetic variation are similar to analyses of phenotypic variation, and common garden experiments are necessary to separate plastic and heritable components of variation (Richards et al. 2010a, b). For this reason, future studies should account for environmentally induced epigenetic effects by growing organisms in common environments and performing MS-AFLP analyses under these conditions. For example, Richards et al. (2012b), collected Japanese knotweed rhizomes from 16 different populations across three different habitat types, but grew them in a common glasshouse environment before sampling for MS-AFLP analyses. This design confirms that methylation patterns were in fact persistent and not just induced by habitat.

## 10.3 Ecological Consequences of Epigenetic Variation

Despite the recent progress that has been made in understanding the magnitude of epigenetic variation within and among populations, the extent to which epigenetic processes are associated with ecologically-relevant traits is surprisingly understudied. There have been few ecological studies that have directly linked epigenotype to phenotype or that have assessed the effects of epigenetically-mediated phenotypic differences on organismal fitness. The classic example of how epigenetics may affect ecologically important traits is the epimutation occurring in the plant *Linaria vulgaris* (Fig. 10.1) which was first observed over 250 years ago by Linneaus (Cubas et al. 1999). The epimutation results in silencing of the *Lcyc* gene, which regulates flower symmetry. Plants with a hypomethylated epimutation have flowers with radial symmetry
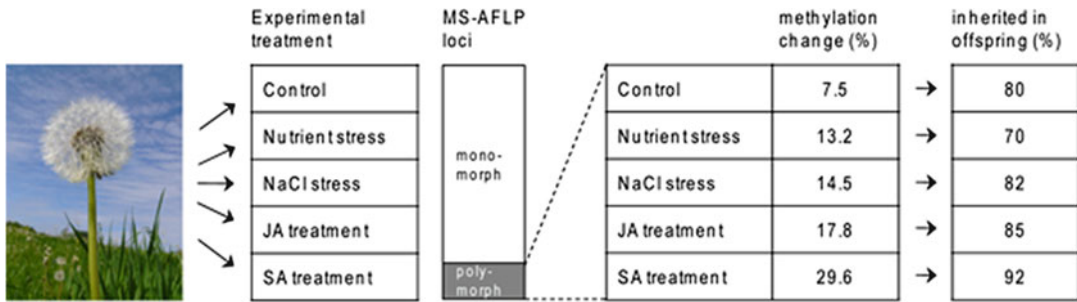
**Fig. 10.5** Induction of DNA methylation changes by ecological stresses and their heritability in asexual dandelions (*Taraxacum officinale*) from Verhoeven et al. (2010). A single apomictic dandelion genotype was exposed to different experimental environments. Although these 'susceptible' loci showed some background level of methylation change also in the control group, the rate of methylation change that was observed within the subset of susceptible loci increased significantly due to stress treatment, particularly due to treatment with jasmonic (JA) or salicylic (SA) acid. Most of the induced methylation changes were inherited in apomictic offspring that were not exposed to stress but raised in a common control environment (Reprinted with permission from Richards et al. 2012a)

whereas those without the epimutation exhibit dorsoventral symmetry (Cubas et al. 1999). This epigenetic change in flower morphology is inherited by future generations, which has important implications for flower pollination and evolutionary questions pertaining to *L. vulgaris*. However, this study did not directly attempt to characterize how these phenotypic differences influence fitness, which should be a consideration in future ecological epigenetics research.

While we know of no study that has made a direct causal link between epigenotype, phenotype and fitness in an ecological setting, several exemplary studies indicate that making these connections could enhance our understanding of ecological processes. We review below how epigenetic mechanisms may play a role in several important ecological phenomena: response to environmental stimuli, trophic interactions, niche breadth, invasive species, behavioral variation, disease susceptibility, and speciation events.

### 10.3.1 Response to Environmental Stimuli

A unique characteristic that differentiates epigenetic from genetic variation is that epigenetic processes (i.e., DNA methylation) are more responsive to the environment (Bossdorf et al. 2008; Richards et al. 2010b). Verhoeven et al. (2010) were some of the first researchers to assess how interaction with abiotic and biotic environmental factors influences DNA methylation. Verhoeven et al. (2010) used genetically identical lines of dandelion (*Taraxacum officinale*) to assess the impact of five different biotic and abiotic conditions on epigenetic variation: low nutrients, salt stress, jasmonic acid (to mimic herbivore damage), salicylic acid (to mimic pathogen damage), and control treatment. Using MS-AFLP, the authors found significantly more methylation changes genome-wide in treated plants than in controls (Fig. 10.5). Moreover, data collected on offspring from each of the treated plants raised in a common garden environment showed that the majority of the changes were also inherited (Verhoeven et al. 2010).

In a similar study, Dowen et al. (2012) exposed *Arabidopsis thaliana* wild type and mutants defective in methylation maintenance machinery to bacterial pathogens, avirulent bacteria, and salicylic acid to determine the effects on the epigenome. Each treatment resulted in different methylation patterns, suggesting that environmental stimuli not only affect global methylation, but that the epigenome responds uniquely to each stimulus and regulates gene expression dynamically (Dowen et al. 2012). In addition, salicylic acid elicited a response in transposable elements and/or their proximal genes through differential methylation, lending support to the

idea that dynamic methylation of TEs may be an important component of response to this stress.

### 10.3.2  Trophic Interactions

In addition to response to environmental stimuli, epigenetic mechanisms may play a role in response to trophic interactions, such as herbivory. Herrera and Bazaga (2011) continued their work on epigenetic and genetic variation in Spanish populations of *V. cazorlensis* by investigating the response to herbivory. DNA methylation in populations of *V. cazorlensis* exposed to long-term ungulate herbivory varied considerably, which was partially explained by browsing damage (Herrera and Bazaga 2011). The methylation state at some variable loci was also associated with specific AFLP markers that were associated with herbivory levels (Herrera and Bazaga 2011). This study was one of the first to compare both genetic and epigenetic variation contemporaneously in response to variation in a natural environmental stressor and emphasizes the importance of disentangling these two components of an organism's response. They used structural equation modeling (SEM) to show that genotype contributed directly to herbivory damage and epigenotype, but could not discriminate the relationship between epigenotype and herbivory damage. One of the SEM models predicted a consequential role between epigenetic variation and herbivory suggesting that the epigenetic patterns are induced by herbivory. Another, equally likely SEM model predicted a causal role between epigenetic variation and herbivory such that the likelihood of herbivory depended on epigenotype (Herrera and Bazaga 2011). Another possibility is that random epigenetic mutations arise and build up rapidly within isolated populations, potentially resulting in (neutral) epigenetic differences between populations that correlate with genetic differentiation of the populations (Richards et al. 2012a). Overall, the study suggests that a complex relationship exists among genotype, epigenotype, and herbivory damage requiring controlled studies to tease apart the relationships.

A persistent problem we face in understanding the importance of epigenetics in ecology is that there is a complex relationship between genetic and epigenetic effects. We must therefore separate the distinct contributions of each source of variation in order to understand if epigenetic effects provide something not attributable to genetic effects. Richards (2006) summarized the problem another way: some epigenetic effects are entirely determined by genotype, while others may be "facilitated" by specific genotypes, or may be completely independent from genotype. Some portion of variation in DNA methylation is likely attributable to underlying DNA sequence variation, for example differences in the genetic sequence of methyltransferase enzymes (Bird 2002). Therefore, disentangling these different possibilities is complicated not only because we know little about genetic-epigenetic interactions, but also because the genetic basis of most complex traits is still not well understood. To date we know of no studies that have characterized the effects of natural variation in the epigenetic machinery. However, studies in non-model systems can explore variation among genotypes for response at epigenetic markers to different environmental factors. Using a classic phenotypic plasticity design with clonal replicates of *Spartina alterniflora*, Richards and colleagues have shown that genotypes vary in the magnitude of response to community make-up and that there is a correlation between phenotypic variation and epigenetic variation among genotypes (Richards et al. Unpublished). Further study is needed to determine the relative contribution of genotype to epigenotype and the extent to which this interaction governs phenotypic variation in natural populations.

### 10.3.3  Niche Breadth

Another ongoing endeavor in ecology is to determine the mechanisms that underlie the ability of some organisms to occupy a broad niche within a community. A recent study of a nectar-living yeast (*Metschnikowia reukaufii*)

**Fig. 10.6** Six focal species from which flower nectar sugar environments were studied: from *left* to *right*, *top row*, *Digitalis obscura*, *Gladiolus illyricus*, *Aquilegia vul-* *garis*, *bottom row*, *Helleborus foetidus*, *Atropa baetica*, and *Primula vulgaris* (Herrera et al. 2012) (Images courtesy of © Carlos M. Herrera. All Rights Reserved)

showed that methylation changes are a critical component of its ability to use resources from a wide range of host environments, particularly harsh environments (Fig. 10.6) (Herrera et al. 2012). Herrera et al. (2012) grew yeast lines in multiple media of varying concentrations of sucrose, glucose, and fructose, and applied the demethylating agent 5-Azacytidine (5-AzaC). 5-AzaC had an inhibitory effect on growth, which was more pronounced in the challenging environment of high sugar concentrations. These data suggest that DNA methylation in *M. reukaufii* responds to different nectar conditions, and the epigenetic response allows the yeast to occupy a wide range of nectars and flowers (Fig. 10.7).
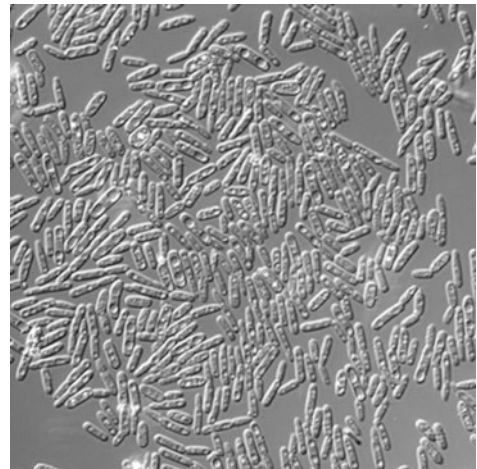


**Fig. 10.7** The nectar-living yeast *Metschnikowia reukaufii* (Herrera et al. 2012) (Image courtesy of © Carlos M. Herrera. All Rights Reserved)

### 10.3.4 Invasive Species

Introduced species should be at a major disadvantage throughout the invasion process because they may not be well-adapted to their new habitat and often experience reduced genetic variation and inbreeding due to the small size of the founding population (Pérez et al. 2006; Schrey et al. 2012). Despite these challenges, invasive species are surprisingly successful in colonizing new environments. In some cases, invasive species display

extensive phenotypic variation, which results in a genetic paradox (Pérez et al. 2006; Richards et al. 2008, 2012b; Schrey et al. 2012; Liebl et al. 2013). Several studies suggest that epigenetic variation may compensate for reduced genetic diversity and potentially mediate phenotypic plasticity in traits associated with "invasiveness" (e.g., rapid growth or reproductive output, increased competitive ability, etc.) (Pérez et al. 2006; Richards et al. 2012b; Schrey et al. 2012).

Japanese knotweed (*Fallopia japonica*) is a highly invasive plant species in Europe and has recently colonized the northeastern United States, where it occupies roadside, marsh, and beach habitats (Fig. 10.8). Richards et al. (2008, 2012b) sampled populations from these diverse habitats in Long Island, NY and grew them in common garden. Plants from the different populations displayed almost no genetic diversity, but maintained a high degree of epigenetic and phenotypic variation, and phenotypic plasticity in response to controlled salt treatments (Richards et al. 2008, 2012b). These findings suggest that epigenetic variation, rather than genetic variation, may be facilitating the rapid colonization of knotweed across a range of environments (Figs. 10.9 and 10.10).

Similarly, the house sparrow (*Passer domesticus*) is one of the most globally distributed bird species, having been successfully introduced on every continent except Antarctica (Anderson 2006; Schrey et al. 2012). Extensive phenotypic variability has been observed across native and introduced ranges, indicating the species has overcome many limitations associated with population bottlenecks (Johnston and Selander 1973; Martin 2005; Martin et al. 2005). Schrey et al. (2012) found that Nairobi (introduction 50 years ago) and Tampa (introduction 150 years ago) populations shared similar levels of epigenetic variation, while Nairobi had less genetic diversity than Tampa. Within Kenya, epigenetic diversity was negatively correlated with genetic diversity and positively correlated with inbreeding across the range expansion (Liebl et al. 2013). These results suggest that epigenetic variation could be a factor underlying the



**Fig. 10.8** Japanese knotweed (*Fallopia japonica*) populations across beach, salt marsh and roadside habitats on Long Island, NY (Image courtesy of Christina L. Richards)

phenotypic diversification often observed in these recently introduced populations. However, more research is needed to determine how methylation at specific restriction sites is functionally linked to phenotypic variation (Schrey et al. 2012; Liebl et al. 2013).

**Fig. 10.9** AFLP markers indicating no polymorphism across ramets collected from a roadside (*left*) and marsh (*right*) populations of *Fallopia japonica* (Richards et al. 2008) (Modified from Richards et al. 2012a)
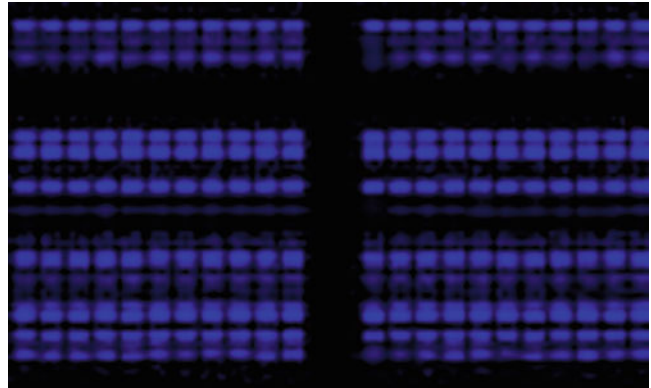


**Fig. 10.10** Response of replicates of rhizomes collected at a marsh (-*dashed*) and nearby roadside (*solid*) sites. AFLP showed that all individuals from these two populations were the same genotype (Richards et al. 2008) (Modified from Richards et al. 2012a)
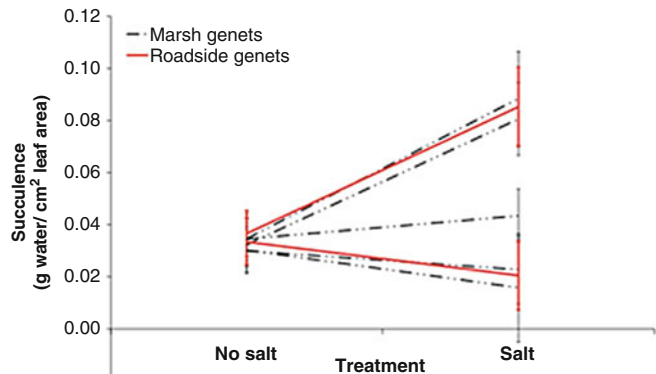


## 10.3.5 Behavioral Variation

Behavior is often considered one of the most flexible and environmentally-sensitive phenotypic traits (West-Eberhard 2003). Unlike changing other aspects of an organism's phenotype (e.g., morphology), the ability to alter behavior allows for a more rapid and less costly response to environmental cues (West-Eberhard 2003). Extensive research has demonstrated that behavioral variation is fairly common within and among populations and is often associated with different selection pressures exerted by the environment (Ledón-Rettig et al. 2013). Laboratory studies have shown that epigenetic mechanisms can affect behavioral variation in association with environmental conditions. For example, differences in larval diet can influence behavioral variation associated with the caste system in honeybees via epigenetic mechanisms (Kucharski et al. 2008; Miklos and Maleszka 2011). Despite being genetically identical, larvae

that are fed royal jelly develop into reproductive queens which are behaviorally more aggressive, whereas those that consume lower quality diets become non reproductive workers that spend much of their lives displaying behaviors associated with foraging (Kucharski et al. 2008; Miklos and Maleszka 2011). These differences in behavioral phenotype were correlated with differences in DNA methylation in the brain, such that queens had reduced DNA methylation of certain genes when compared to workers (Kucharski et al. 2008; Miklos and Maleszka 2011). Interestingly, experimental injection of larvae with a small interfering-RNA caused downregulation of the DNA methyltransferase system, which led to the production of more queens than a control group (Kucharski et al. 2008; Miklos and Maleszka 2011).

Another well-known study demonstrates how variation in maternal care behaviors within the first week of life of neonatal rats can lead to individual phenotypic differences in behavior and

stress responsiveness, which persist into adulthood (Weaver et al. 2004). Offspring of mothers who displayed high licking and grooming behavior (high-LG), grew up to be less fearful and had more attenuated stress responses when compared to offspring of mothers who displayed low licking and grooming (low-LG) behavior (Weaver et al. 2004). These major phenotypic differences were associated with a difference in the methylation status of the glucocorticoid receptor (GR) promoter in the hippocampus (Weaver et al. 2004, 2005, 2006). A cross-fostering study revealed that offspring phenotype was determined by the behavioral (high or low-LG) phenotype of the foster mother – rather than the biological mother – providing evidence that epigenetic, rather than genetic processes, are responsible for these phenotypic differences (Weaver et al. 2004).

### 10.3.6 Disease Susceptibility

Environmental perturbations experienced within critical periods of development have been implicated in the risk of disease development (e.g., cancer, heart disease, diabetes and schizophrenia) (Jirtle and Skinner 2007; Skinner et al. 2010). The genetic and environmental basis of certain diseases is well-documented in epidemiological studies (Skinner et al. 2010; Tost 2010), yet the molecular mechanisms by which environmental factors contribute to disease etiology have only recently been explored (Skinner et al. 2010). There is mounting evidence that environmentally-sensitive epigenetic processes play an important role in regulating disease susceptibility (Jirtle and Skinner 2007; Skinner et al. 2010; Tost 2010). One of the best characterized examples comes from the study of the metastable *Avy* allele of the agouti gene in mice (Morgan et al. 1999; Jirtle and Skinner 2007; Skinner et al. 2010). In animals with the *Avy* allele, expression is mediated by variable DNA methylation of a transposable element located upstream of the agouti gene. Low levels of methylation of the *Avy* allele

result in yellow coat color whereas increasing methylation of the transposable element causes a shift toward the wild-type pseudo-agouti (brown) coat color (Fig. 10.11) (Morgan et al. 1999; Jirtle and Skinner 2007; Skinner et al. 2010). Furthermore, the unmethylated state is also associated with obesity and increased susceptibility to diabetes and tumor formation (Jirtle and Skinner 2007; Skinner et al. 2010). Maternal nutritional supplementation with methyl-donors and the phytoestrogen, genistein, increases DNA methylation, which leads to a shift in offspring coat color from yellow to brown and significantly reduces the incidence of obesity, diabetes and cancer in pseudo-agouti offspring (Waterland and Jirtle 2003, 2004; Dolinoy et al. 2006, 2007). While many of the details of the epigenetic regulation of pseudo-agouti coat color have been worked out in laboratory experiments, the implications for natural populations of mammals have not been explored at all (Ledón-Rettig et al. 2013). Intuitively, the link between the mother's diet and disease susceptibility in offspring should have important ecological implications for wild populations. Thus, understanding the molecular mechanisms underlying the dramatic response to diet and its influence on disease trajectories will impact our understanding of disease dynamics.

Another series of studies have shown that exposure to certain chemicals in early life also influences disease susceptibility through direct effects on the epigenome (Jirtle and Skinner 2007; Skinner et al. 2010; Tost 2010). Environmental toxins with endocrine disruptor activity (e.g., fungicides, pesticides, plastic by-products and pharmacological substances) have been found to impact disease phenotype and fitness in adulthood (Crews et al. 2007; Skinner et al. 2010). For example, developmental exposure to environmentally relevant amounts of bisphenol A (BPA), a residue found in many plastic materials, produced changes in DNA methylation associated with increased susceptibility to cancer in rats (Ho et al. 2006; Skinner et al. 2010). Transient embryonic exposure to fungicides and pesticides also led to reduced spermatogenic capacity

**Fig. 10.11** Phenotypes of isogenic *Avy* littermates range from *pure yellow* and obese (*left*) through *mottled yellow*/agouti to lean fully agouti (called pseudoagouti, *right*) (Reprinted with permission from © (Cropley et al. 2010). All Rights Reserved)



and male infertility in rats, and the decreased male fertility was transmitted transgenerationally via alterations in DNA methylation in the male germ-line (Anway et al. 2005; Skinner et al. 2010). Although data from the biomedical literature have improved our understanding about the role of epigenetic mechanisms in the environmental basis of disease, there have been no studies to investigate the degree to which these processes influence disease susceptibility or the ecology of infectious diseases in natural populations. Studying these processes in an ecological context may reveal how increasing anthropogenic disturbances are impacting the health of populations and may be useful for ongoing conservation efforts.

### 10.3.7 Speciation Events

Epigenetic mechanisms are often involved in polyploidy and hybridization events in plants (Rapp and Wendel 2005; Richards et al. 2012a). Epigenetic mechanisms may be involved with dosage regulation of replicate genes which could allow for the separate genomes to persist or merge without gene interaction problems (Liu 2003). Ainouche and colleagues have demonstrated the importance of epigenetic mechanisms in the genus *Spartina*, which has evolved through multiple allopolyploid and hybridization speciation events (Fortune et al. 2007). In particular, *Spartina alterniflora* and *S. maritima* have formed two distinct hybrids

(*S. x townsendii* and *S. x neyrautii*) in the past century, and *S. anglica* has since formed as an allopolyploid from *S. x townsendii* (Ainouche et al. 2004). *Spartina anglica* has increased physiological tolerance over its progenitors to multiple stresses of the intertidal zone, and has become extremely invasive around the world (Ainouche et al. 2009). Using MS-AFLP, transposon display, and the Agilent rice microarray, Ainouche's group showed that changes in DNA sequence in the hybrid species were more or less additive compared to the parental species, but genome methylation and gene expression were not (Salmon et al. 2005; Parisod et al. 2010; Chelaifa et al. 2010a, b). These studies suggest that changes in DNA methylation may help explain the dramatic differences in phenotype that allow members of this genus to successfully occupy novel habitats (Salmon et al. 2005). However, these ecologically-oriented questions have not yet been addressed.

Paun et al. (2010) provide another example of how epigenetic mechanisms may be important in polyploid speciation. The orchids *Dactylorhiza majalis*, *D. traunsteineri s.l.*, and *D. ebudensis* (Fig. 10.12) all arose from independent hybridization events of the diploids *D. fuchsii* and *D. incarnata* followed by allopolyploidization. *Dactylorhiza majalis* has a wide range while *D. ebudensis* is a narrow endemic living in a single coastal dune slack habitat. *Dactylorhiza traunsteineri s.l.* has an intermediate range, but narrow tolerances of both soil moisture and pH, and

**Fig. 10.12** The allotetraploid *Dactylorhiza traunsteineri* at a natural site in Yorkshire, England (Image courtesy of © (Paun et al. 2010). All Rights Reserved)

grows in calcareous fens and marshes (Paun et al. 2010). Genome-wide methylation patterns, obtained using MS-AFLP, showed differentiation of the three species (Paun et al. 2010). Because the different species have arisen from independent hybridization events of the same parents, the authors suggest that epigenetic mechanisms could be important to the process of differentiation and contrasting environmental tolerance of these species. However, the extent that the epigenetic differences were the cause or the consequence of the lineages inhabiting different environments remain to be elucidated.

## 10.4 Evolutionary Consequences of Epigenetic Variation

Current data on epigenetic variation and its influence on phenotype have provocative implications for evolution. In at least some cases, induced epigenetic changes have been shown to be heritable through meiosis without reset for both plants and animals (Crews et al. 2007; Feng et al. 2010; Verhoeven et al. 2010; Grossniklaus et al. 2013). A number of theoretical models have been proposed to describe the evolutionary value of epigenetic variation in natural populations (Jablonka and Lamb 1989; Jablonka et al. 1992, 1995; Lachmann and Jablonka 1996; Pal and Miklos 1999; Day and Bonduriansky 2011; Geoghegen and Spencer 2012). Recent models have been limited, primarily due to a paucity of information on the behavior of epigenetic marks, but they have demonstrated that because the epigenetic code can be more dynamic and reversible than the DNA code, it can add adaptive flexibility (Jablonka and Lamb 1989; Jablonka et al. 1995; Lachmann and Jablonka 1996; Pal and Miklos 1999). Variation in epigenetic mechanisms can contribute to phenotypic variation, which is not necessarily adaptive (Pal 1998; Rapp and Wendel 2005; Richards et al. 2010b). However, epigenetic memory could be adaptive in changing environments, where epigenetic variation creates a buffering system against high rates of environmental change (Jablonka et al. 1995; Lachmann and Jablonka 1996). Epigenetic modifications could 'hold' a potentially advantageous phenotype for multiple generations, allowing time for more durable genetic processes to stabilize the phenotype (i.e., canalization or genetic assimilation; Waddington 1942, 1953; West-Eberhard 2005; Richards et al. 2012a). The ability to generate heritable epigenetic variation can speed up the process of reaching a fitness peak in the adaptive landscape, facilitate peak shifts, or facilitate the transition from one fit genotypic state to another (Pal and Miklos 1999), and create the potential for novel evolutionary outcomes in the absence of genetic variation (Tal et al. 2010; Geoghegen and Spencer

2012). Still, most models assume epigenetic motifs all have the same likelihood of reset, and that they can be easily reset even though the rate at which epigenetic maintenance and erasure occurs has been shown to vary across different sites within the genome (Rakyan et al. 2001; Feng et al. 2010; Zhou et al. 2011; Grossniklaus et al. 2013).

## 10.5 Conclusions and Future Directions

It is becoming clearer that our knowledge about important ecological processes will be informed by understanding how the epigenome functions in natural environments. Significant progress has been made in understanding the extent and distribution of epigenetic variation in natural populations as well as the potential ecological and evolutionary consequences of such variation. However, due to the relative infancy of the field of ecological epigenetics, there are still many questions that remain unanswered.

Like ecological genomics, the future of ecological epigenetics will require carefully designed studies that can account for genotype and environment effects. With experimental studies on genotypic replicates exposed to different environments, future studies can investigate the behavior of epialleles and interactions with annotated components of the genome including functional genes, regulatory elements and non-coding regions such as transposable elements. Creative new approaches to modeling the importance of both genetic and non-genetic inheritance will lend important insight for understanding the dynamic nature of genome function (*sensu* Day and Bonduriansky 2011).

Although models for epigenetically controlled traits have found that epigenetic effects may enhance the adaptive possibilities of a variety of taxa, particularly in response to novel environments (Jablonka and Lamb 1989; Geoghegen and Spencer 2012), these models are limited by a lack of data on epigenetic response to environmental factors. Ultimately, models will better inform our understanding of evolution if we can char-

acterize behavior of epigenetic marks at specific genomic elements (e.g., the promoters of ecologically important genes or activity of transposable elements). To date, this type of information has been available only for model organisms such as *Arabidopsis thaliana* (Lippman et al. 2004; Slotkin and Martienssen 2007; Vaughn et al. 2007) or mice (Morgan et al. 1999; Weaver et al. 2004). The use of next generation sequencing technology, like restriction-site-associated DNA sequencing (RAD-seq), has expanded the possibilities for non-model systems that have no reference genome. RAD-seq reduces the complexity of the genome sampled and increases the power to identify repeat or duplicate sequences (Etter et al. 2011). RAD-seq can also incorporate paired-end sequencing (RAD-PE), allowing for the assembly of larger continuous sequences from short Illumina sequences (Etter et al. 2011). RAD-PE has not been used to study methylation patterns yet, but methylation sensitive enzymes have been used to target low copy number, gene rich regions to exclude highly repetitive DNA regions that are highly methylated (Chutimanitsakun et al. 2011). This indicates that the same methodology could be used in an experimental context to compare replicates of the same genotype exposed to different conditions to allow for a genome wide probing of changes in methylation.

While most models assume that all epigenetic marks behave similarly, a recent model proposed by Day and Bonduriansky (2011) posits that some genetic elements are more likely to acquire methyl groups than others. Future studies can test this model with data from MS-AFLP or novel next generation sequencing approaches on organisms from natural populations and over clonal generations of experimental transplants and greenhouse experiments. This will allow for a genome-wide characterization of the stability and behavior of different methylation marks, how they affect phenotype and how this varies by genotype. Considering that the research community has made little progress in understanding how the genome actually functions to create complex traits and adapt to complex environments (Richards et al. 2009, 2012a; Pigliucci 2010; Martin et al. 2011), characterizing the role of epi-

genetic effects in natural systems could transform our understanding of ecological and evolutionary processes.

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