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SYMPOSIUM

Ecological Epigenetics: An Introduction to the Symposium

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Synopsis Phenotypic variation arises from interactions between environmental and genetic variation, and the emergence of such variation is, in part, mediated by epigenetic mechanisms: factors that modify gene expression but do not change the gene sequence, per se. The role of epigenetic variation and inheritance in natural populations, however, remains poorly understood. The budding field of Ecological Epigenetics seeks to extend our knowledge of epigenetic mechanisms and processes to natural populations, and recent conceptual and technical advances have made progress toward this goal more feasible. In light of these breakthroughs, now is a particularly opportune time to develop a framework that will guide and facilitate exceptional studies in Ecological Epigenetics. Toward this goal, the *Ecological Epigenetics* symposium brought together researchers with diverse strengths in theory, developmental genetics, ecology, and evolution, and the proceedings from their talks are presented in this issue. By characterizing environmentally dependent epigenetic variation in natural populations, we will enhance our understanding of developmental, ecological, and evolutionary phenomena. In particular, ecological epigenetics has the potential to explain how populations endure (or fail to endure) profound and rapid environmental change. Here, my goal is to introduce some of the common goals and challenges shared by those pursuing this critical field.

Introduction

Environmentally dependent variation has been historically recognized (Baldwin 1896; Schmalhausen 1949; Waddington 1953), but was not considered to be of particular importance to ecological and evolutionary phenomena during the Modern Synthesis. More recently, empirical studies have demonstrated that non-genetic phenotypic variation can be stably induced and inherited (Weaver et al. 2004; Anway et al. 2005; Scoville et al. 2011), and theoretical studies have demonstrated that such inherited variation can contribute to the direction and speed of evolution (Jablonka et al. 1992; Lachmann and Jablonka 1996; Day and Bonduriansky 2011). In light of such studies, it has become more accepted that this variation must have ecological and evolutionary consequences. This realization has spurred interest in characterizing environmentally dependent, and sometimes heritable, variation in natural populations (West-Eberhard 2003; Gilbert and Epel 2009; Hallgrímsson and Hall 2012). In particular, focus has been given to molecular-level factors that are thought to underlie, or influence, many environmentally sensitive morphologies, physiologies, and behaviors (Bossdorf et al. 2008; Ledón-Rettig et al. 2013).

Yet, it has been difficult understand the scope, degree, and ecological relevance of environmentally dependent epigenetic variation, in part because natural populations are genetically heterogeneous and encounter environmental variability (Johannes et al. 2008). This difficulty is reflected by a dearth of ecological epigenetic studies in the literature: while studies addressing the medical implications of epigenetic inheritance have increased dramatically over the past 10 years, studies that address the ecological and evolutionary implications of epigenetic inheritance have lagged. In organizing the symposium *Ecological Epigenetics* for the 2013 annual meeting of the Society of Integrative and Comparative Biology, we hoped to synthesize the state of this budding field by highlighting some of the most important technological, analytical, and theoretical advances that have been made. The proceedings from this symposium are presented in this issue of *Integrative and Comparative Biology*.

In my introduction, I address the following questions to reveal some of the goals we share, and challenges we face in Ecological Epigenetics:

(1) Why is it important to put epigenetic studies in the field, and study phenotypes in non-model organisms?

(2) Is it important to have molecular markers for epigenetic variation? What should molecular markers represent and what questions would they help us to answer?

(3) What technologies do we have for creating such markers?

(4) What experimental evidence would advance the field of ecological epigenetics?

Here, I will be using the term "epigenetics" in its broad sense that includes any non-genetic mechanism (e.g., nutrients, hormones, or cultural memes) (Bonduriansky et al. 2012) that enables the emergence of a phenotype. The term has been used in many ways since its inception (Waddington 1957); for instance, it is also used in a more narrow sense to describe specific molecular-level factors just above the DNA sequence or structure (Jablonka and Raz 2009). When referring to these more proximate epigenetic mechanisms (e.g., DNA and histone modifications and small noncoding RNAs), I will specify them as molecular-level epigenetic factors.

Why is it important to put epigenetic studies in the field, and study phenotypes in non-model organisms?

Because great strides have been made in determining what epigenetic mechanisms underlie environmentally induced phenotypes in laboratory settings (e.g., Weaver et al. 2004; Anway et al. 2005), it is important to delineate how ecological epigenetic studies differ, and why we should willingly complicate an established field.

Ecological and evolutionary consequences cannot be known outside natural conditions

Fitness is environmentally dependent. Therefore, the consequences of environmentally induced, epigenetically based phenotypes cannot be known without first understanding the environments in which they will be selected. At least one study suggests that the fitness consequences of environmentally induced, molecular-level epigenetic variation might vary considerably based on the environmental conditions under which it is selected. When tested under low-stress conditions, adult mice that had received high maternal care as pups demonstrated enhanced hippocampal-dependent learning relative to adult mice that had received low maternal care. Conversely, under high-stress conditions, adult mice that received low maternal care as pups learned faster (Champagne et al. 2008). Thus, it is impossible to know whether the phenotypes induced by maternal care in these mice are adaptive without knowing what environmental conditions they will encounter as adults.

Although no molecular-level epigenetic marks were assessed in this particular study, similar maternally dependent behaviors in mice are mediated in part by epigenetic programming of gene expression (Weaver et al. 2004). Few studies have used molecular-level epigenetic signatures ascertained from laboratory studies (i.e., candidate epigenes; Ledón-Rettig et al. 2013) to tie epigenetic phenotypes to fitness in nature (but see Herrera et al. 2012). This would be a fruitful avenue to pursue, although it would be critical to ensure whether the effect of an epigenetic mark that has been determined for a laboratory population behaves similarly in individuals from natural populations.

Real world environmental variation: Complex cues interact in producing phenotypes

Natural environmental cues may differ from, and are likely more dimensional than, the environmental treatments that we apply to organisms in a laboratory setting. Indeed, discrepancies between phenotypic responses to cues often occur between laboratory studies and field studies (Miner et al. 2005; Pigliucci 2005). Further, interactions between multiple environmental factors might mute or exacerbate the phenotype of interest. Thus, while laboratory studies are in most cases required to isolate environmentally dependent phenotypes from other sources of variation (e.g., genetic), the behavior and consequences of such phenotypes in nature can only be known if field studies are conducted, in parallel.

Real world genetic variation: Natural populations are genetically heterogeneous

Generally, in laboratory studies, a great deal of effort is made to standardize genetic backgrounds (e.g., recombinant inbred lines) and for good reason: this improves the ability of investigators to attribute phenotypic variation to a specific disease state or environmental manipulation. Additionally, some studies have contributed to our understanding of gene by environment interactions by assessing environmental effects over several genetic lines (Churchill 2007; Johannes et al. 2009). Still, the numbers of recombinant inbred lines used in these studies are likely too

small to address complex phenotypes, and capture only a small fraction of the variation in natural populations (Churchill 2007). This is relevant to the field of ecological epigenetics because the evolutionary implications of environmentally dependent epigenetic variation will vary, depending on whether genetic variation is available. For instance, some have questioned whether epigenetic effects induced in the soma (as opposed to the germline) are evolutionarily relevant because they do not persist in the absence of the inducing cue (Crews 2008). Although it is true that selective breeding of isogenic populations in the laboratory might never stabilize these environmentally induced effects, it is well established that natural populations harbor genetic variation in their abilities to respond to environmental cues (West-Eberhard 2003; Pigliucci 2005), and selection on this genetic variation (e.g., "modifier alleles"; Schlichting 2008) would likely modify the regulation of such induced effects if they caused variation in fitness (stabilizing or suppressing them if they were adaptive or maladaptive, respectively). Extrapolating the responses of laboratory populations that are genetically depauperate to natural populations would, in such cases, be incorrect.

Thus, while environmentally induced epigenetic variation that is independent of genetic variation might allow organisms to adapt to environmental variation on intermediate timescales, populations or species might accumulate genetic differences over long periods of time that influence how they respond, epigenetically. How, and whether, this occurs are questions that can only be addressed by comparing populations that are naturally genetically diverse. By making these comparisons, we can begin to understand how different types of populations or species respond epigenetically to environmental change.

Real-world problems

Finally, by developing the conceptual, analytical, and technological tools to deal with real-world environmental and genetic variation, we can begin to understand how environmental changes of intermediate length (the time scale at which inherited, non-genetic effects should have the greatest impact) (Lachmann and Jablonka 1996), such as biological invasions, global warming, and habitat destruction, influence the health of non-model organisms, including ourselves.

Is it important to have molecular markers for epigenetic variation?

Many of our symposium participants use molecular techniques (e.g., methylation sensitive AFLP, next-generation sequencing). Is it necessary to assess epigenetic variation at a molecular level to understand its role in ecological and evolutionary processes? It has been argued that molecular-level epigenetic factors probably are not more important than other non-genetic, heritable factors in ecological, and evolutionary phenomena (Bonduriansky 2013). If this is so, we risk obscuring our understanding of non-genetic inheritance by overestimating or underestimating its relevance in natural populations by concentrating solely on those environmentally-induced phenotypes for which molecular-level epigenetic mechanisms have been identified.

No environmentally induced phenotype will be entirely determined by molecular-level epigenetic factors: molecular epigenetic marks will have non-molecular dependencies (substances and circumstances that allow gene expression and development) (Day and Bonduriansky 2011; Moczek 2012), and non-molecular factors will have molecular-level underpinnings. Thus, while understanding the molecular mechanisms underlying phenotypes might help shed light on the potential or constraint for the evolution of an epigenetic regulatory system, molecular-level epigenetic factors per se will not better reveal the importance of environmentally dependent variation in ecological and evolutionary processes as opposed to any other measure of non-genetic variation and inheritance. However, molecular tools have the potential to reveal non-genetic variation and inheritance in a way that we have not seen before: in action in rapidly evolving, natural populations. Molecular-level epigenetic factors are becoming more feasible to characterize in non-model systems, especially in contrast to non-genetic processes that are more difficult to measure such as cultural inheritance (Danchin and Wagner 2010). If there are situations in which epialleles or epigenetic profiles can serve as proxies for environmentally induced traits that can be assessed quickly across several individuals and populations, we can begin to answer the question, "What is the prevalence and relevance of epigenetic variation in nature?" The expectations for such markers are described below.

What would these markers represent, and what questions would they answer?

Epiallelic signatures should vary among individuals with different environmental histories that have resulted in divergent phenotypes. One challenge associated with identifying such epigenetic variants is distinguishing which molecular-level epigenetic modifications are responsible for the phenotype of interest, and which have been induced as a result of the

environmental cue but are not related to the phenotype of interest. Comparisons between individuals exposed to a stimulus that do develop a phenotype versus those that are exposed and do not develop the phenotype might help isolate epigenetic marks specific for the phenotype under investigation (Fig. 1).

Additionally, beyond their environmental dependence, epigenetic states, and their resulting phenotypes possess an additional dimension that must be characterized: their reversibility or permanence. This quality distinguishes epigenetic variation and its consequences from relatively stable genetic variation on one hand, and from relatively flexible physiological plasticity on the other (Lachmann and Jablonka 1996). Thus, epigenetic markers that will be useful for assessing the role of environmentally induced traits in ecological phenomena will reflect not only an environmental exposure, they will also reflect the induced state's flexibility (i.e., transmission rates) (Lachmann and Jablonka 1996). This parameter can be determined, for instance, when epialleles or epigenetic patterns that reflect environmental effects within generations are distinct from patterns that occur across generations (Fig. 2).

As has been discussed in previous reviews, care must be taken to disentangle environmentally dependent epigenetic variation from epigenetic variation that reflects genetic variation (Richards 2008; Ledón-Rettig et al. 2013). Molecular-level epigenetic markers that are independent of genotypic variation (they are similarly induced across genotypes) will be particularly useful for describing the dynamics of epigenetic variation across rapid timescales. In contrast, molecular-level epigenetic markers that are induced only against certain genetic backgrounds might be less useful for understanding the spread of environmentally dependent epigenetic variation, as such markers would not always accurately indicate an organism's environmental history. Epigenetic variants that are completely independent of genetic variation probably do not exist, but in certain situations when population-genetic variation is expected to be, or determined to be, low (e.g., population bottlenecks, founder events, or in clonal species) (Richards et al. 2012; Liebl et al. 2013, this issue), environmentally induced epigenetic variants likely behave similarly among individuals. When such proxies are developed, they can be used to answer ecological questions such as, "How does environmentally dependent epigenetic variation contribute to fitness and to the persistence (or decline) of populations across variable and fluctuating environments?"

The goal of obtaining genetically independent epigenetic markers will change when evolutionary questions are being asked. This is because the ability to respond to environmental change with epigenetic change per se can evolve, and will almost certainly involve changes in genes, resulting in interactions of

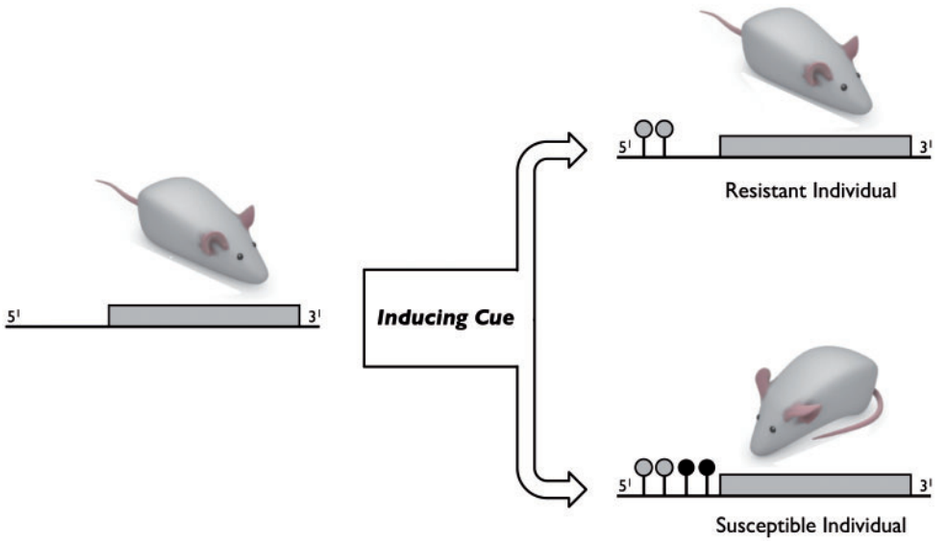

Fig. 1 Epigenetic markers for environmentally dependent phenotypes should be specific for the trait of interest. Often, researchers must expose organisms to environmental cues to induce a phenotype of interest, and this may influence epigenetic marks that are coincident with, but not underlie, the trait of interest (gray markers). Comparing individuals that develop (susceptible) and do not develop (resistant) a phenotype in response to the same environmental cue will reveal epigenetic markers that more specifically underlie the trait of interest (black markers).

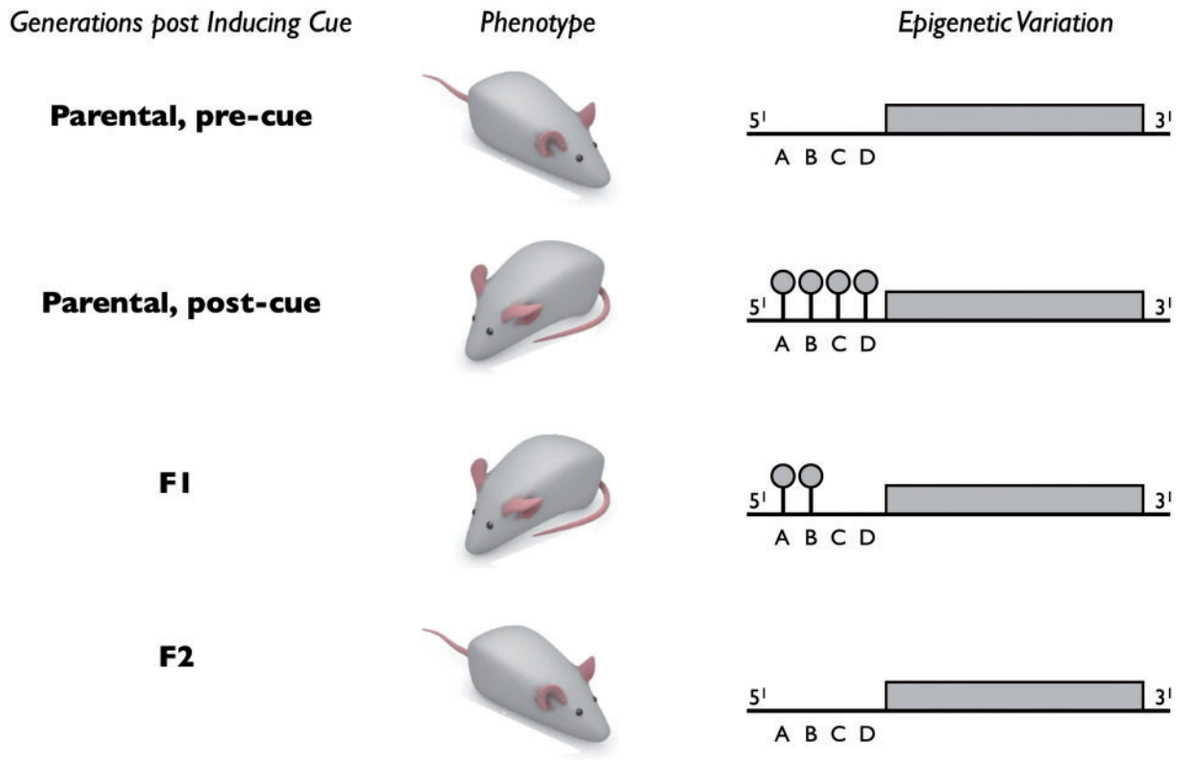

Fig. 2 Determining the persistence of epigenetic markers for environmentally dependent phenotypes will be useful for understanding their ecological relevance. For instance, induced epigenetic marks (at positions **A**, **B**, **C**, and **D**) that occur only in the generation experiencing the inducing cue might be distinguishable from marks observed in a following generation that does not experience the inducing cue, but exhibits the induced phenotype (at positions A and B). This may help an investigator to understand whether, and how, environmentally dependent and heritable epigenetic variation contributes to fitness and population persistence (or decline) across variable or fluctuating environments.

genes with the environment (Pigliucci 2005). In such situations, proxies developed because they are independent of underlying genetic variation in one population might become uninformative in other populations if they do not represent the same environmental effect. However, this phenomenon itself is a fascinating and remarkably unaddressed question: ''How do traits become more plastic or canalized, and what type of genetic modification does that entail?'' Using some of the techniques described below, we can demonstrate population genetic divergence in the ability to respond to environmental cues (Fig. 3).

What options do we have for finding these markers?

Most ecological epigenetic studies have assessed variation in a single molecular-level epigenetic factor, DNA methylation. DNA methylation is an epigenetic modification of cytosine residues (most commonly at ''CpG'' dinucleotides) found across a range of eukaryotic lineages, and is associated with either suppression or activation of gene expression depending on whether it occurs in promoter regions or in gene bodies (Suzuki and Bird 2008). Two technological developments, methylation-sensitive enzyme restriction assays and bisulfite conversion, have contributed to the ease of measuring variation in methylation across taxa (neither technology has the ability to distinguish between methylation and hydroxymethylation; however, I will use ''methylation'' here to be consistent with the primary literature). Although several alternate molecular-level epigenetic factors (e.g., histone methylation and acetylation) most certainly contribute to environmentally sensitive phenotypes, methylation-based assays are currently the most tractable option for those wishing to use epigenetic analyses with non-model systems.

Even within the restricted scope of methylation-based assays, it may be difficult for a researcher to decide what technologies and analyses to use; rapid developments are being made such that the costs of some technologies (e.g., next-generation sequencing) (Lister and Ecker 2009) are being reduced. Additionally, the organism, trait, and question at hand might

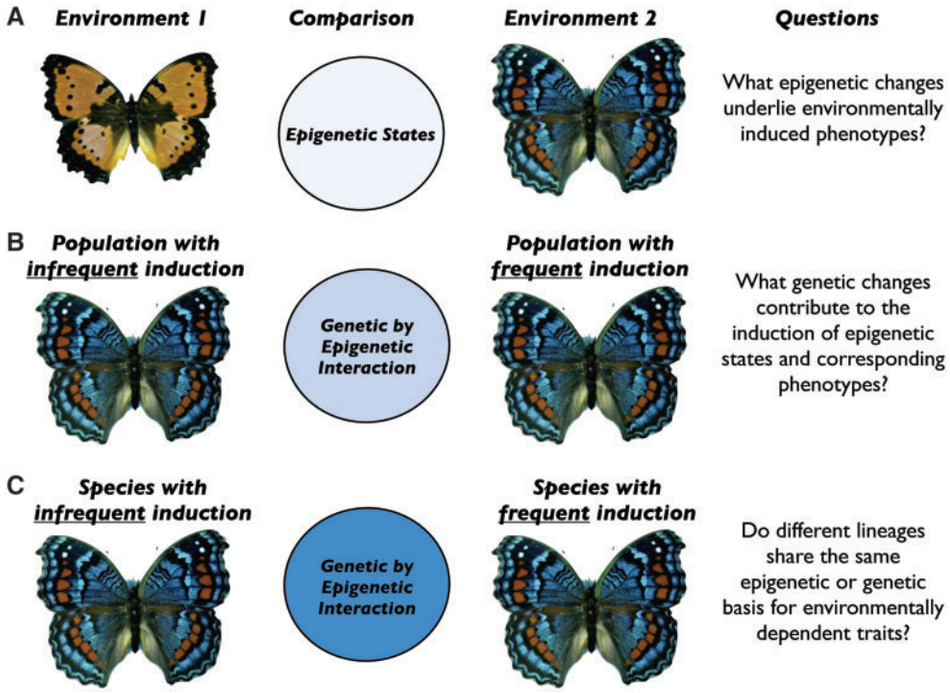

Fig. 3 Comparisons for ecological and evolutionary questions. Comparing closely related (or genetically identical) individuals of the same population will provide information on genetically independent, epigenetic patterns underlying environmentally dependent phenotypes (**A**). The two environmentally sensitive phenotypes in this example are summer (left) and winter (right) morphs of the butterfly *Precis octavia* (the Gaudy Commodore). In *P. octavia*, the winter morph is induced by low temperatures during development. By comparing induced individuals from two populations under common environmental conditions (i.e., exposed to the same environmental cues), you remove the effect of environment, and can now reveal genetic influences on the induced phenotype that have diverged between the populations (**B**). If these populations are more or less environmentally sensitive (exhibit frequent or infrequent induction of phenotype), such genetic differences may have contributed to the process of genetic accommodation (sensu West-Eberhard 2003). Finally, comparisons between more distally related lineages (e.g., species) under common environmental conditions can reveal whether different species employ similar or different epigenetic mechanisms for the induction of phenotypes (**C**).

determine the optimal approach for assessing epigenetic variation. This section outlines certain benefits and caveats involved with different techniques, and their appropriateness for different systems. It will also touch on certain challenges that are unique to describing epigenetic variation, regardless of the technique being used.

Methylation-sensitive amplified fragment length polymorphism

The methylation-sensitive variant of the amplified fragment length polymorphism protocol (MS-AFLP) provides a whole-genome approach to identifying epigenetic variation among individuals that might reflect differences in their environmental histories. Typical AFLP methods rely on enzymes that target restriction sites distributed randomly throughout the genome and produce anonymous markers (Vos et al. 1995). Correlating variation in these sites with variation in traits or fitness rests on the assumption that all randomly distributed sites are influenced similarly by global evolutionary forces (e.g., genetic drift or gene flow), but in addition, some occur in linkage disequilibrium with gene regions under selection (Luikart et al. 2003; Storz 2005). Loci that are associated with gene regions under selection will thereby exhibit deviant behavior, and are often referred to as ''outlier loci'' (Bonin et al. 2007).

Many of the benefits and caveats of MS-AFLP are outlined in detail in this issue (Schrey et al. 2013, this issue) and elsewhere (Bonin et al. 2007), so I will address them only briefly, here. MS-AFLP is a tractable technique for non-model systems because it does not rely on known genomic sequences, is relatively inexpensive, and provides several hundred markers for genome-wide coverage. The MS-AFLP technique has been used to demonstrate different facets of epigenetic variation in natural populations. For instance, MS-AFLP has been used to show that environmentally induced molecular-level epigenetic variation is sometimes transmissible across

generations (Verhoeven et al. 2010) and segregates with environmental variation (Herrera and Bazaga 2011), implying the existence of selection of phenotypes associated with induced epigenetic variation. Additionally, MS-AFLP markers have demonstrated a correlation between epigenetic states and fitness across environmental manipulations, indicating that induced changes in methylation might underlie phenotypic plasticity (Snell-Rood et al. 2013). Finally, in combination with a chemical inhibitor of methylation (5-azacytidine), one study used MS-AFLP to show that inhibiting changes in methylation reduces both plasticity and fitness (Herrera et al. 2012). However, outlier analyses can provide only limited information about the connection between the variant marker and the trait of interest. Because AFLP (and MS-AFLP) markers are anonymous, they are challenging to isolate and identify. Thus, while differential methylation of a genomic region might correspond to variation in a trait of interest, it will subsequently be difficult to understand why. This might explain why few studies that have identified correlations between AFLP markers and traits (or fitness) have actually characterized those markers. For instance, despite over 25 AFLP studies in non-model species, only a few have sequenced their candidate loci (reviewed by Nunes et al. 2012). Even if sequence data around markers can be obtained, there is no guarantee that they will provide meaningful information about the loci (Nunes 2012).

As a last caveat, the ability of MS-AFLP to detect differentially methylated loci associated with phenotypic plasticity might vary, depending on the organism and trait under investigation. For instance, in honeybees, developmental plasticity has been linked to intermediate levels of epigenetic modification of gene bodies (Lyko et al. 2010). In this type of situation, a random locus that was differentially methylated might be in linkage disequilibrium with others that are also environmentally modified on the same gene body. In contrast, multiple studies in vertebrates have demonstrated the importance of knowing the methylation status of individual CpG sites (Prendergast and Ziff 1991; Weaver et al. 2004, 2007; although see Eckhardt et al. 2006). Thus, in vertebrates, it might be a rare occasion when a differentially methylated restriction site is correlated with the presence of a differentially methylated and phenotypically relevant target, unless the restriction site, itself, is the target.

Nevertheless, the identity of a candidate, outlier loci might not be of consequence to a researcher interested in characterizing environmentally dependent epigenetic variation in their system. As long as candidate loci accurately predict environmentally induced (and possible heritable) traits, they might still be useful for understanding ecological processes that occur over rapid time scales.

Methods that rely on bisulfite conversion

A breakthrough in the detection of DNA methylation was made with the development of the bisulfite conversion technique (Frommer et al. 1992). Bisulfite conversion is a process in which DNA is treated with sodium bisulfite, resulting in the conversion of cytosine but not methylcytosines to uracil (Zemach et al. 2010). The original methylation status of the DNA can be revealed by comparing treated DNA to untreated DNA from the same sample, and determining whether the base at an original cytosine position is a thymine (originally unmethylated) or cytosine (originally methylated; Zilberman and Henikoff 2007). Bisulfite conversion has precipitated several types of epigenetic assays, only three of which will be addressed here: clonal analysis, next-generation sequencing, and array techniques.

Clonal analysis

This technique is most useful for assessing methylation patterns of a single, candidate gene of interest. After bisulfite conversion and amplification using locus-specific primers, the PCR product is subcloned, and the resulting clones are isolated and sequenced (Zhang et al. 2009). Each clone represents an individual DNA molecule, allowing researchers to see, with high resolution, the frequency with which a single base pair is methylated, both within and between samples. This is an ideal approach to use when there is a specific target in mind; preferably a target that is known to exhibit environmentally induced methylation (e.g., candidate epigenes involved with behavioral programming) (Weaver et al. 2004; Elliott et al. 2010). However, this locus-specific approach becomes impractical in terms of time and cost as the number of genomic loci being studied increases (Lister and Ecker 2009). To achieve a global picture of putative epigenetic processes, higher sequencing throughput is needed.

Next-generation sequencing

It is now becoming more feasible and economical for researchers to use whole-genome sequencing to resolve nucleotide-resolution of DNA methylation by comparing sequenced unconverted genomic DNA with bisulfite-converted genomic DNA (commonly referred to as "BS-Seq") (Cokus et al. 2008).

Multiple "reads"—short pieces of DNA created from random digestion—from unconverted DNA are assembled on the basis of their overlapping areas, and then reads from the converted data are aligned to this scaffold for comparison. One complication associated with aligning converted data to this scaffold is that converted DNA consists of only three base pairs, making alignment with a reference genome difficult. However, computer simulations appear to handle this reduction in complexity (Lister and Ecker 2009). The next-generation sequencing approach is desirable because it allows for an unbiased assessment of methylation profiles, and does not rely on organism-specific genomic resources (i.e., it can be used in non-model species). Furthermore, data can be reanalyzed as more genomic information is obtained (in contrast to array-based methods in which the array is only as informative as the genomic information at hand) (Zilberman and Henikoff 2007).

Although costs of sequencing have been reduced, the ultimate cost for a given level of coverage will depend on the size of the genome and the number of samples needed to answer a question, and this may be prohibitively high for some laboratories. A way to circumvent this limitation is through enrichment techniques, whereby regions of DNA to be sequenced are pre-selected on the basis of characteristics pertaining to methylation. For instance, non-informative reads can be reduced by first digesting the DNA with the restriction enzyme *Msp*I, which contains in its recognition motif a CpG site, thus ensuring that each sequenced read will contain at least one informative position (Meissner et al 2005; Heyn and Esteller 2012; although in some cases where the genome is heavily methylated, as in humans, it is more informative to enrich the sample for unmethylated DNA; Illingworth et al. 2008). Likewise, immunoprecipitation techniques (e.g., MeDIP) (Weber et al. 2005), or columns that contain a methyl-binding domain can be used to enrich samples for methylated sequences (Cross et al. 1994). Enriched samples can subsequently be interrogated using next-generation sequencing techniques or array-based methods (described below).

Array-based methods

Array-based methods pre-date next-generation sequencing, but have been primarily used in species that have a sequenced genome. To use array-based methods for species that lack genomic resources, an annotated genome and validated microarray must first be developed (a task accomplished through next-generation sequencing) (Marinković et al. 2012). Reads are created from genomic DNA, assembled de novo (without a reference genome) with a genome assembler, and libraries of microarray probes are designed that target the assembled sequences (Marinković et al. 2012). The probes are adhered to chips, and then samples are hybridized to the probes on the chips. How DNA is hybridized to the chips depends on the technology used to make the chip, (reviewed by Zilberman and Henikoff 2007), but all variations face the limitation that—for non-model species—there is a considerable amount of work involved in designing the chip, itself. Thus, array-based methods are most useful when there are genomic resources for the system of interest, and a large number of differentially methylated loci are already known (Zilberman and Henikoff 2007).

Once any of the above approaches works to identify a marker—or combination of markers—associated with an environmentally dependent phenotype, traditional methods of genotyping can be used to reveal how this marker is distributed over several individuals and populations.

The importance of place and time

One challenge that is unique to the description of epigenetic variation is that key epigenetic changes can be tissue-specific or even cell-specific. Even though some tissues or organs are prime candidates for epigenetic change (brain, germline, and liver for behavioral, reproductive, and metabolic programming, respectively) (Weaver et al. 2004; Anway et al. 2005; Lillycrop et al. 2007), sampling these tissues often is destructive. This limitation can be circumvented if a non-destructive tissue or material (e.g., skin, blood, saliva, or feces) can be sampled, and exhibits an epigenetic marker that is consistently associated with the trait of interest. However, this type of approach has met limited success; one recent study found that methylation profiles derived from the hippocampi of mice that had received varying levels of postnatal maternal care were correlated with their adult behavior, but profiles derived from their fecal DNA were not (Liberman et al. 2012).

Additionally, key epigenetic changes that influence stable, adult phenotypes may occur during a certain developmental window and then disappear. For instance, gonadal hormones, such as testosterone, influence DNA methylation in the developing brains of mice, but these patterns disappear later in development before reappearing at sexual maturation (Kudwa et al. 2006). Likewise, DNA methylation is involved with distinguishing queen honeybees from workers during early development, but these

differences disappear by the time of emergence (Wang et al. 2006; Herb et al. 2012). Thus, identifying key epigenetic modifications that best predict individual differences in phenotypes will be a complex task that integrates biological information about the likely timing and location of epigenetic changes.

What experimental evidence would advance ecological epigenetics?

No one study will reveal the prevalence and relevance of epigenetic variation in natural systems. Instead, these trends will emerge from the accumulation of studies that investigate varied taxa and environments. Here, I outline a few trends gleaned from such studies, as well as key, unanswered questions that warrant future research.

What we know

Correlations between environmental variation, epigenetic marks, and fitness

A few recent studies have been successful in linking environmentally induced epigenetic variants with measures of fitness. Snell-Rood et al. (2013) found that environmental manipulation caused epigenetic modification at (MS-AFLP) loci in horned beetles (genus *Onthophagus*), and that a subset of these differences were correlated with performance in each of those environments, suggesting that nutritional plasticity in horned beetles may be related to environmentally induced methylation. Likewise, Herrera et al. (2012) harnessed the clonal nature of a yeast system (genus *Metschnikowia*) to link epigenetic loci to specific traits involved in sugar metabolism, and, in turn, the yeast's performance on media that differed in sugar concentration and mimicked natural variation faced by *Metschnikowia*. In this study, Herrera et al. also inhibited the induction of methylation using the chemical 5-azacytidine, and this manipulation significantly depressed the performance of the yeast in high-sugar environments, suggesting a causal link between environmentally dependent methylation and phenotypic plasticity in this system.

In short, there is mounting evidence that environmentally induced epigenetic modifications contribute both to plasticity and performance under natural (or semi-natural) conditions. The characteristic of these studies that separates them from typical laboratory studies of epigenetic variation is that they use environmental cues that reflect conditions their organisms would experience in nature, and measure the performance of the resulting phenotypes within the selecting environment.

What we would like to know

The influence of epigenetic variation on population persistence or decline

As previously mentioned, environmentally induced epigenetic variation has the potential of being the most powerful at intermediate time scales (Lachmann and Jablonka 1996). Thus, it could potentially enable persistence of populations during rapid environmental change, in particular when genetic diversity is low (e.g., Richards et al. 2012). Rapid environmental change is, unfortunately, a common contemporary theme due to global warming, human-exacerbated biological invasions, and habitat fragmentation. It has become of great interest to understand which lineages will best endure these rapid environmental changes, and whether their success will have anything to do with environmentally dependent epigenetic variation.

In this issue, Liebl et al. (2013) assess whether epigenetic variation has been involved in the success of an invasion by the house sparrow (*Passer domesticus*) in Kenya. Intriguingly, when comparing populations that varied in their time since colonization (younger populations being established over the most recent 5 years, and older populations dating back 50 years) they found that levels of epigenetic variation (as measured by MS-AFLP) were inversely correlated with levels of genetic variation. The authors suggest that epigenetic variation may be compensating for decreased levels of genetic variation (and corresponding phenotypic variation) during this range expansion. This study represents an admirable start toward describing epigenetic variation during contemporary evolution. However, to fully understand whether invasion success is dependent on environmentally dependent epigenetic variation, it will be necessary to compare populations and species that are in the process of successfully enduring environmental change with closely related lineages that have had the same opportunity but are not enduring environmental change (Miller and Ruiz 2009). The latter type can be failed invaders or species that have remained in their ancestral range.

Likewise, the study conducted on yeast by Herrera et al. (2012) has important implications for the role of environmentally dependent epigenetic variation in a lineage's niche breadth. The epigenetic modifications induced by the variable sugar environments allowed *M. reukaufii* to exploit a wide range of sugar environments, suggesting that the naturally broad population niche width of the yeast was largely a consequence of plasticity in resource use arising from environmentally induced epigenetic changes in

DNA methylation. Future studies with this system could address whether yeast species that have a more restricted niche breadth have a less robust or nonexistent environmental induction of epigenetic marks.

Finally, although most studies so far have focused on the role of environmentally induced molecular-level epigenetics in population persistence, it will be also important to assess its possible role in population decline. Even if an induced epigenetic change is initially adaptive, it could result in mismatches between phenotype and environment if the modification persists after the environment has subsequently changed (Gluckman et al. 2009). Epigenetic inheritance can cause population decline when phenotypes induced by ancestral environments are not appropriate for current environmental conditions, and in some cases, it is hard to imagine any context in which epigenetic marks increase fitness (e.g., infertility) (Anway et al. 2005).

The evolution of epigenetic responses and epigenetic inheritance

As previously mentioned, an exciting and practically unaddressed question is, "What genetic and epigenetic modifications enable plastic traits to become canalized or enhanced?" (Moczek et al. 2011). Answers to this question may start to emerge as the accessibility of next-generation sequencing becomes more available to those with non-models systems. For instance, in honeybees, next-generation sequencing was used to identify a suite of differentially methylated regions between nurse honeybees and foragers, two environmentally dependent forms of worker bees (Herb et al. 2012). Because next-generation sequencing yields both genetic and epigenetic data, it could be used to compare populations or species that are divergent in their inducibility to better understand the types of genetic and epigenetic changes that contribute to the evolution of plasticity. Fortunately, there are several ecological systems that possess populations and species that have diverged in their sensitivity to environmental cues (e.g., Ledón-Rettig and Pfennig 2011; Valena and Moczek 2012) and would be ideal for this type of analysis.

Conclusions

Interest in Ecological Epigenetics has gained considerable momentum over the past decade. In the wake of this burgeoning interest, it will be crucial to establish reasons why we should direct our efforts and resources to this field. For instance, some have argued that other processes, unrelated to epigenetics, are more likely than epigenetic variation and inheritance to contribute to adaptation (reviewed by Gupta 2013). This sentiment reflects a misunderstanding of the field. It is not the expectation of ecological epigeneticists that epigenetic variation will replace genetic variation as the sole source of evolutionary change, or specifically, adaptation (although, as discussed, there may be scenarios in which it may be the primary source of phenotypic variation). Instead, we are addressing the expectation that epigenetic change most likely influences both the direction and speed of evolutionary change, and is therefore critical for us to understand, especially in instances of rapid environmental change. However, it will be hard for us to understand the prevalence and relevance of environmentally induced epigenetic variation in ecology and evolution without taking it to the field.

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