

IDEA AND PERSPECTIVE

Epigenetics for ecologists

Oliver Bossdorf,^{1*} Christina L. Richards² and Massimo Pigliucci³

¹Department of Community Ecology, Helmholtz Centre for Environmental Research-UFZ, Theodor-Lieser-Str. 4, D-06120 Halle, Germany

²Department of Biology and Center for Genomics and Systems Biology, New York University, New York, NY 10003, USA

³Department of Ecology & Evolution, Stony Brook University, Stony Brook, NY 11794, USA

*Correspondence: E-mail: oliver.bossdorf@ufz.de

Abstract

There is now mounting evidence that heritable variation in ecologically relevant traits can be generated through a suite of epigenetic mechanisms, even in the absence of genetic variation. Moreover, recent studies indicate that epigenetic variation in natural populations can be independent from genetic variation, and that in some cases environmentally induced epigenetic changes may be inherited by future generations. These novel findings are potentially highly relevant to ecologists because they could significantly improve our understanding of the mechanisms underlying natural phenotypic variation and the responses of organisms to environmental change. To understand the full significance of epigenetic processes, however, it is imperative to study them in an ecological context. Ecologists should therefore start using a combination of experimental approaches borrowed from ecological genetics, novel techniques to analyse and manipulate epigenetic variation, and genomic tools, to investigate the extent and structure of epigenetic variation within and among natural populations, as well as the interrelations between epigenetic variation, phenotypic variation and ecological interactions.

Keywords

Adaptation, DNA methylation, ecological genetics, epialleles, inheritance, maternal effects, natural variation, rapid evolution.

Ecology Letters (2008) 11: 106–115

INTRODUCTION

Species and their traits are not fixed but are subject to genetic variation and evolutionary change. Not only are ecologically important traits often genetically differentiated in natural populations (Linhart & Grant 1996; Mousseau *et al.* 2000; Merilä & Crnokrak 2001), there is also accumulating evidence that they can evolve rapidly (Thompson 1998; Hairston *et al.* 2005; Carroll *et al.* 2007). Genetic variation and microevolution are therefore increasingly recognized as relevant to basic ecological research (e.g. Whitham *et al.* 2006; Johnson & Stinchcombe 2007) and applied issues such as ecological restoration (Rice & Emery 2003; Bischoff *et al.* 2006), the invasion of exotic species (Mooney & Cleland 2001; Bossdorf *et al.* 2005; Strauss *et al.* 2006) and the response of ecological communities to global environmental change (Davis & Shaw 2001; Davis *et al.* 2005; Jump & Penuelas 2005; Parmesan 2006). However, while ecologists are still struggling to conceptually and methodologically incorporate genetics into their work, the situation is now likely to become even more complex, as recent research suggests that *epigenetic* processes, too,

could play a significant role in natural variation and microevolution.

The epigenetic code

Epigenetics is the study of heritable changes in gene expression and function that cannot be explained by changes in DNA sequence (Richards 2006; Bird 2007). These epigenetic changes are based on a set of molecular processes that can activate, reduce or completely disable the activity of particular genes: (i) methylation of cytosine residues in the DNA, (ii) remodelling of chromatin structure through chemical modification, in particular acetylation or methylation, of histone proteins and (iii) regulatory processes mediated by small RNA molecules. The different classes of processes are not independent from each other but often regulate gene activity in a complex, interactive fashion (Grant-Downton & Dickinson 2005; Berger 2007).

In the past, the term ‘epigenetics’ has sometimes also been used in a much broader sense to include all processes that determine how the genotype translates into the

phenotype, thereby encompassing much of the field of developmental biology. However, this definition of epigenetics, relating to Waddington's concept of 'epigenesis', is outdated and has been replaced by the new definition given above (Richards 2006; Bird 2007).

The currently best-studied epigenetic mechanism is DNA methylation (Jaenisch & Bird 2003; Bender 2004), which usually involves the addition of a methyl group to a CpG site, a cytosine followed by a guanine in the DNA sequence. CpG sites are often clustered in the regulatory region of genes, and the methylation of these so-called CpG islands is often (but not always) associated with reduced activity of the associated genes. The methylation reaction is catalysed by several methyltransferase enzymes.

While the stability of epigenetic modifications through cell divisions has been studied extensively in the last decades – after all, it is a major component of what modern molecular developmental biology is concerned with – there is now mounting evidence that epigenetic modifications can also be inherited across generations (Chong & Whitelaw 2004; Richards 2006). Meiotic inheritance of epigenetic alleles (epialleles) differing in DNA methylation but not DNA sequence has been demonstrated, for instance, in toadflax (Cubas *et al.* 1999), *Arabidopsis thaliana* (Mittelsten Scheid *et al.* 2003; Rangwala *et al.* 2006; Vaughn *et al.* 2007) and mice (Rakyan *et al.* 2003; Blewitt *et al.* 2006). In plants, transgenerational inheritance of DNA methylation appears to rely on a methyltransferase enzyme that replicates methylation patterns during both mitosis and meiosis (Takeda & Paszkowski 2006).

It is important to point out that in the molecular biological literature the term 'epigenetic inheritance' is used for both mitotic and meiotic inheritance of epigenetic modifications. This is somewhat unfortunate and a potential source of confusion, because in classical genetics and evolutionary biology the term 'inheritance' is usually restricted to the description of transgenerational phenomena, i.e. meiosis. In this paper, we focus exclusively on the evolutionarily relevant inheritance of epigenetic variation across generations.

Another important insight from recent epigenetics research is that there can be natural variation in epigenetic modifications that is at least partly independent from variation in the DNA sequence (e.g. Cubas *et al.* 1999; Cervera *et al.* 2002; Riddle & Richards 2002; Keyte *et al.* 2006; Shindo *et al.* 2006; Vaughn *et al.* 2007). For instance, Cervera *et al.* (2002) and Vaughn *et al.* (2007) found large and consistent ecotypic variation of DNA methylation in *A. thaliana* that was not correlated with genetic variation. Keyte *et al.* (2006) explored DNA methylation polymorphism in 20 accessions of cotton and found that the levels of epigenetic variation greatly exceeded genetically based estimates of variation.

Finally, what makes epigenetic processes fundamentally different from genetic processes is that in some cases environmentally induced epigenetic changes may be inherited by future generations (Richards 2006; Whitelaw & Whitelaw 2006; Jirtle & Skinner 2007). For instance, Fieldes & Amyot (1999) experimentally altered DNA methylation in flax and showed that this significantly affected the phenotypes of at least four generations of progeny. In mice, environmental toxins (Anway *et al.* 2005; Crews *et al.* 2007) and dietary supplements (Cropley *et al.* 2006) induce changes in DNA methylation that are inherited over several generations. In *Drosophila*, experimental reduction of the heat shock protein Hsp90 (which also occurs in response to environmental stress) causes stable phenotypic changes which appear to be due to the release of hidden epigenetic variation (Sollars *et al.* 2003). The latter study is particularly intriguing because it provides a hypothesis for the actual mechanism that connects environmental stimulus and epigenetic change.

Taken together, these results seem to pose a challenge to the Modern Evolutionary Synthesis (Jablonka & Lamb 1998, 2005; Grant-Downton & Dickinson 2006; Richards 2006), which is based on the assumptions that the only source of heritable variation in natural populations is genetic, and that evolution by natural selection depends on the existence of genetic variation whose ultimate origin is random mutations (Mayr & Provine 1980). Yet, how serious this challenge really is we currently cannot even guess, because there is a dearth of studies that have addressed epigenetic questions in a real-world context (Kalisz & Purugganan 2004; Richards 2006). This is where ecologists should come into play.

Why ecologists should be interested

Ultimately, we would like to know how important epigenetic variation and epigenetic inheritance are in the real world. To get at this question, however, it is imperative to place these processes in an ecological perspective and study their causes and consequences in natural populations. This, in turn, can only be accomplished if evolutionary ecologists begin to incorporate epigenetics into their thinking and join forces with geneticists and molecular biologists in their empirical research.

From an ecologist's point of view, there are several reasons why epigenetics should be an exciting area of research. First, epigenetic processes could explain some of the heritable phenotypic variation observed in natural populations that cannot be explained by differences in DNA sequence. Taking epigenetics into account will therefore improve our understanding of the mechanisms underlying natural variation in ecologically important traits. Second, studying epigenetics will provide insights into the mechanisms that allow organisms to respond to the

environment. Epigenetic processes are at the core of several types of phenotypic plasticity, such as the environmentally induced transition to flowering in plants (Bastow *et al.* 2004; He & Amasino 2005), and they apparently mediate some types of maternal environmental effects (Rossiter 1996; see e.g. Anway *et al.* 2005; Cropley *et al.* 2006).

Recently, Crews *et al.* (2007) demonstrated that heritable epigenetic variation can even affect animal behaviour. When rats were treated only once with a toxin that altered DNA methylation, this still significantly affected the mate choice behaviour of the F₃ generation. As behaviour is often regarded to be the most responsive aspect of animal phenotypes (West-Eberhard 2003), such epigenetic effects on behaviour may have particularly profound evolutionary consequences.

More generally, epigenetic processes may increase the evolutionary potential of organisms in response to abiotic stress and other environmental challenges, which could potentially be highly relevant in the context of global environmental change.

Finally, there is increasing evidence that epigenetic processes are an important component of hybridization and polyploidization events, and may therefore play a key role in speciation and the biology of many invasive species (Ellstrand & Schierenbeck 2000; Liu & Wendel 2003; Rapp & Wendel 2005; Salmon *et al.* 2005; Chen & Ni 2006).

While several recent review articles have highlighted the importance of epigenetic processes to evolutionary questions (e.g. Jablonka & Lamb 1998, 2005; Kalisz &

Purugganan 2004; Grant-Downton & Dickinson 2005, 2006; Rapp & Wendel 2005; Richards 2006), these contributions have only occasionally mentioned a need for ecological experiments in epigenetics, let alone its relevance to ecologists. Below, we sketch a new field of ecological epigenetics, and, to provide some specific food for thought, we suggest a set of fundamental questions that need to be addressed, together with a brief outline of the methods and experiments that will allow answering these questions.

A FRAMEWORK FOR ECOLOGICAL EPIGENETICS

There are two ways by which epigenetic processes may contribute to microevolution in natural populations. On the one hand, if heritable epigenetic variation translates into phenotypic variation and, ultimately, fitness differences among individuals, then epigenetic processes may provide a second system of heritable variation for natural selection to act upon, similar to the one based upon genetic variation (Fig. 1). On the other hand, epigenetic variation, unlike genetic variation, may be altered directly by ecological interactions (Fieldes and Amyot 1999; Anway *et al.* 2005; Cropley *et al.* 2006; Richards 2006; Whitelaw & Whitelaw 2006) and may therefore provide an additional, accelerated pathway for evolutionary change (Fig. 1).

Ecological genetics is the study of genetic processes in an ecological context, i.e. of the interplay between heritable genetic variation in ecologically important traits, ecological

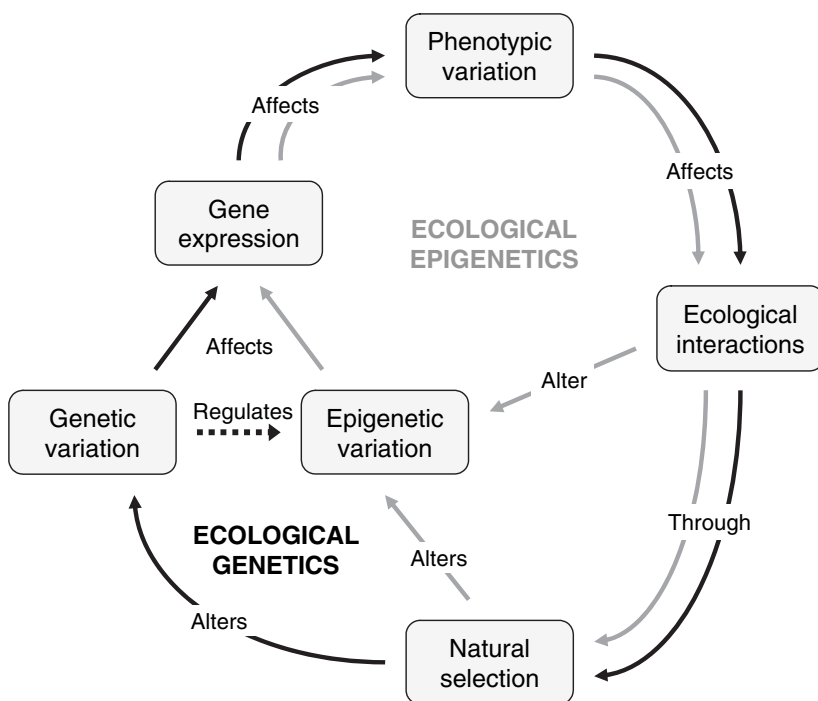


Figure 1 Differences and similarities between ecological genetics (black arrows) and ecological epigenetics (grey arrows). On one hand, epigenetic processes may provide a second inheritance system, very similar to the genetic inheritance system, that allows evolution by natural selection. On the other hand, epigenetic variation, unlike genetic variation, may be altered directly by ecological interactions and therefore provide an additional, accelerated pathway for evolutionary change.

interactions and mechanisms of evolutionary change in natural populations (Ford 1964; Conner & Hartl 2004). As a discipline, ecological genetics complements molecular genetics by placing it in an ecological perspective, i.e. by studying the causes and consequences, and relative importance, of genetic processes in natural populations. In a conceptually analogous manner, a new field of ecological *epigenetics* could complement molecular epigenetics by studying epigenetic processes in an ecological context. Several of the questions addressed will be parallel to those addressed in ecological genetics. Consequently, we should be able to use many of the standard methodological approaches of ecological genetics – such as common garden and selection studies, and in particular the combined manipulation of genetic and ecological factors – in ecological epigenetics, too.

The most fundamental questions in ecological epigenetics are: (i) What is the extent and structure of epigenetic variation within and among natural populations? (ii) Does epigenetic variation affect phenotypic variation in ecologically relevant traits? (iii) What is the relative importance of epigenetic variation in determining the outcome of ecological interactions? (iv) To what extent can biotic and abiotic environmental factors induce heritable changes in epigenetic variation? In the following, we elaborate on each of these questions, and how to address them.

In all of the questions and approaches outlined below, an important conceptual issue is the autonomy of epigenetic variation (Richards 2006). As many developmental processes have a genetic and an epigenetic component, genetic variation among populations should often be accompanied by some degree of corresponding epigenetic variation (Fig. 2). In many of these cases, epigenetic variation may be largely under genetic control, and therefore its quantification is not going to provide any insight (i.e. explanation of phenotypic variance) beyond that already obtained from the study of genetic variation. However, epigenetic variation can (and sometimes will) be partly or completely autonomous from genetic variation (Richards 2006), and it is those cases that ecological epigenetics should focus on.

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

One of the basic questions in ecological epigenetics is how much heritable epigenetic variation exists in natural populations, and how this variation is distributed within and among populations. Also, we would like to know whether there are systematic patterns of epigenetic variation in relation to particular environmental factors, and how patterns of epigenetic variation differ across species or phyla.

To separate heritable epigenetic variation from non-heritable epigenetic variation (resulting from developmental plasticity in response to different environments) it is necessary to study the progeny of different natural populations and/or maternal families in a common environment (Fig. 2), and to use the resemblance of epigenetic pattern among relatives as indication of epigenetic inheritance.

The greatest range of methods for quantifying epigenetic variation across individuals and populations is currently available for DNA methylation, though it is probably only a matter of time until it will be possible to conduct population screenings of other types of epigenetic variation. There are well-established techniques for studying the methylation status of specific genes, and for assaying genome-wide patterns of DNA methylation (Laird 2003). Until recently, however, these methods have almost exclusively been used in cell and molecular biology, and in particular cancer research.

In the context of ecological epigenetics, a particularly useful approach is the study of methylation-sensitive markers such as MS-AFLP (Cervera *et al.* 2002). MS-AFLP is a modification of the standard AFLP technique for genetic fingerprinting, which uses methylation-specific restriction enzymes and can therefore detect differences in DNA methylation. It can provide rapid epigenetic fingerprints for large number of samples and will therefore in many cases be a good starting point for investigating epigenetic variation in natural populations. Another advantage of MS-AFLP is that it can be used in non-model organisms. The technique has recently been successfully applied to compare methylation patterns across plant populations and species (e.g. Cervera *et al.* 2002; Salmon *et al.* 2005; Keyte *et al.* 2006) and even fungi (Reyna-Lopez *et al.* 1997).

Obviously, some of the standard statistical measures used in population genetics for describing patterns of genetic variation should be transferable to the description of epigenetic variation, even though this has not been taken advantage of so far. For instance, statistics that describe the frequency and diversity of alleles may be equally applied to epiallelic diversity, and measures such as F_{ST} , which describe genetic population structuring, should be equally useful to describe population differentiation at the epigenetic level.

Another group of methods that can be used for broad, genome-wide analyses of epigenetic patterns are high-throughput epigenomic profiling methods based on microarrays (Van Steensel & Henikoff 2003; Martienssen *et al.* 2005) or direct sequencing of chromatin immunoprecipitated (ChIP) DNA (e.g. Barski *et al.* 2007; Mikkelsen *et al.* 2007). Currently, these techniques are used only on model organisms (e.g. Vaughn *et al.* 2007; Zhang *et al.* 2007; Zilberman *et al.* 2007). However, genomic tools developed

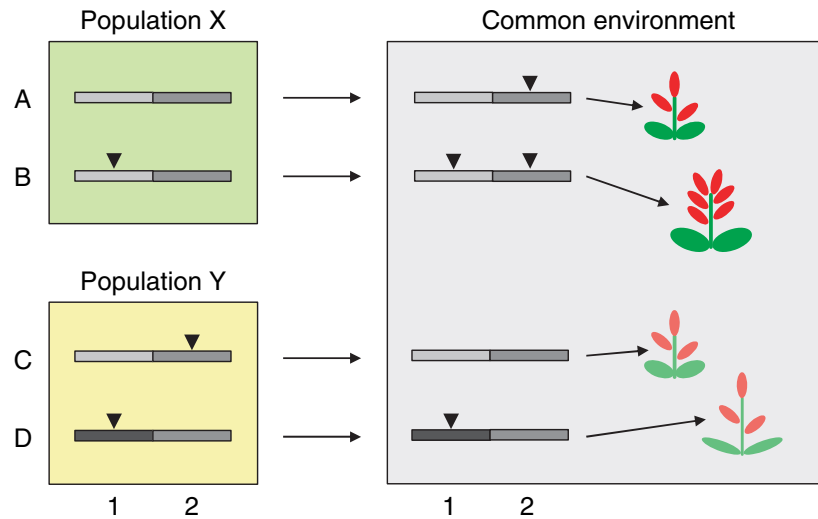


Figure 2 Hypothetical relationships between genetic, epigenetic and phenotypic variation in natural populations. Shown are two genes for each of two individuals in two populations. The horizontal bars are the DNA, with differences in DNA sequence indicated by different shades of grey. Epigenetic modifications at a particular gene are indicated by the black triangles. Natural epigenetic variation may be found within (A1 vs. B1) or between (A2/B2 vs. C2/D2) populations. Epigenetic variation can be independent of (A1 vs. B1) or confounded with (C1 vs. D1) genetic variation. Some epigenetic variation in natural populations may result from phenotypic plasticity and may therefore be non-heritable, i.e. it will not persist in a common environment (C2 vs. D2). If independent epigenetic variation persists in a common environment (as in A1/B1), this is evidence for epigenetic inheritance. If this heritable epigenetic variation translates into phenotypic and fitness differences (as illustrated above), it is ecologically and evolutionarily relevant.

on model organisms can often be used on related species, too. In fact, a recent study by Horvath *et al.* (2003) showed that *Arabidopsis* microarrays could be used to analyse gene expression in several distant species, including leafy spurge and poplar. As the technological progress in epigenomics is very rapid, and these methods are continuously becoming faster and cheaper, it is conceivable that epigenomic tools will eventually start playing a role in ecological epigenetics, just as genomic tools are now increasingly considered in ecological genetics (Thomas & Klaper 2004; Ouborg & Vriezen 2007).

How does epigenetic variation affect phenotypic variation in ecologically important traits?

Another basic but important task in ecological epigenetics is to establish a functional connection between heritable epigenetic variation and phenotypic variation in ecologically relevant traits. Only if naturally occurring epigenetic variation significantly affects phenotypic traits and, ultimately, fitness, can it be relevant to the ecology and evolution of natural populations. Again, to separate heritable from non-heritable epigenetic variation, this research must be done in a common environment (Fig. 2).

There are several possible approaches to testing for a relationship between epigenotype and phenotype. All of them

share the common challenge that to demonstrate the phenotypic consequences of epigenetic variation, one must at the same time control for the effects of genetic variation (although it is interesting to think that classic studies of genetic variation should also account for the converse possibility that some of the observed variability is due to epigenetic factors). One way to achieve this is to use natural epimutations (Das & Messing 1994; Cubas *et al.* 1999), or mutants of model species with known deficiencies in epigenetic mechanisms, such as methylation-insensitive genotypes of *Arabidopsis* (Vongs *et al.* 1993; Kankel *et al.* 2003), and study their phenotype in comparison to controls with the same genetic background in a common environment.

A related technique is the use of the demethylating agent 5-azacytidine (Jones 1985), which inhibits the enzyme methyltransferase and thereby causes demethylation of the DNA, for experimental alteration (epimutagenesis) of DNA methylation patterns to demonstrate the phenotypic consequences of such alterations (e.g. Burn *et al.* 1993). If organisms from different natural populations respond differently to the 5-azacytidine treatments, this can be taken to be indirect evidence of natural epigenetic variation. Moreover, if the degree of population similarity in this response is not correlated with population relatedness, this may indicate autonomous epigenetic variation (*sensu* Richards 2006). Of course, to establish evolutionary significance of artificial epimutations, it is desirable to conduct these

studies over several generations (e.g. Fieldes 1994; Fieldes & Amyot 1999).

Another solution to avoid a confounding between genetic and epigenetic effects would be to choose study systems with a known lack of genetic variation. For instance, several highly invasive exotic plant species, such as alligator weed (*Alternanthera philoxeroides*), Japanese knotweed (*Fallopia japonica*) or fountain grass (*Pennisetum setaceum*) do not, in spite of their broad ecological distribution, appear to possess any genetic variation in their introduced ranges (Hollingsworth & Bailey 2000; Xu *et al.* 2003; Mandák *et al.* 2005; Le Roux *et al.* 2007). If different populations of these species show significant phenotypic variation in a common environment, it would certainly be interesting to screen them for epigenetic variation with the methods described above. Demonstrating that natural populations with zero genetic variation (and therefore, according to the common framework of evolutionary biology, zero immediate potential for evolutionary change) are in fact epigenetically diverse and may therefore evolve rapidly, would be an important achievement with potentially far-reaching implications.

In the case of genetically uniform species, it should also be possible to infer epigenetic variation from patterns of gene or protein expression, using microarrays (Kammenga *et al.* 2007) or two-dimensional electrophoresis (Gorg *et al.* 2004), because in the absence of genetic variation any significant population differentiation in gene or protein expression must be due to underlying epigenetic variation (negative operational definition of epigenetics; Richards 2006). A great advantage of these methods is that they integrate over different epigenetic mechanisms and are therefore more likely to detect epigenetic divergence than methods such as MS-AFLP, which examine only one mechanism at a time.

Finally, the link between epigenetic variation and phenotypic traits can be studied at a more detailed, functional level using QTL mapping approaches that are based on methylation-sensitive marker data (Garfinkel *et al.* 2004). Parental lines that are known to differ significantly in the degree and pattern of DNA methylation, or known methylation mutants of model species, could be used in a crossing scheme to produce 'epi-recombinant inbred lines' (RILs) characterized by varying DNA methylation patterns. These lines could then be used to identify specific epigenomic regions that are associated with the observed phenotypic variation.

What is the relative importance of epigenetic variation in determining the outcome of ecological interactions?

Having established a link between epigenetic and phenotypic variation, the next logical step in ecological epigenetics will be to investigate the degree to which epigenetic

variation can affect important ecological interactions. This should include both (i) relationships between organisms and abiotic environmental factors, e.g. the phenotypic plasticity and stress tolerance of plants or animals in response to important resources such as light, water or nutrients and (ii) biotic interactions among different organisms, e.g. the degree to which epigenetic variation affects competitive ability, resistance to predators and pathogens, etc.

Methodologically, these questions can be approached in a very similar manner to the ones described above, except that the experimental designs must now include a manipulation of abiotic or biotic ecological factors, and the phenotypes of organisms are expanded by 'traits' such as phenotypic plasticity, pathogen resistance or competitive ability, which quantify the direction and strength of ecological interactions. As above, it is important to control for the effect of genetic variation by using natural epimutations, epi-RILs, populations with a natural lack of genetic variation or 5-azacytidine to create artificial variation in DNA methylation. The greatest challenge will be to develop experimental designs that incorporate both genetic and epigenetic variation, and are therefore able to assess their relative importance and test for their interplay in determining the outcome of ecological interactions.

We found only one published study that could be regarded as an example for what we have outlined above. Tatra *et al.* (2000) subjected two ecotypes of the perennial plant *Stellaria longipes* to a factorial combination of light and 5-azacytidine. They found that in one genotype the effect of light on plant growth was altered through the 5-azacytidine treatments, whereas in the other genotype it was not. This suggests that (artificial) epigenetic variation can affect ecological interactions, and that genotype and epigenotype may interact in this respect. However, the replication in this study was extremely low and no statistical test was carried out, so the results should be regarded as very preliminary. We are not aware of any published study that has addressed the effect of epigenetic variation on ecological interactions with a solid and well-replicated experimental design.

To what extent can biotic and abiotic environmental factors induce heritable changes in epigenetic variation?

What makes epigenetic processes unique is that, unlike genetic variation, epigenetic variation can be altered directly by the environment, and in some cases these epigenetic changes may be inherited by the next generations. This possibility for environmentally induced epigenetic inheritance is particularly intriguing because it would, in a sense, represent a case of 'soft inheritance' (Mayr & Provine 1980; Richards 2006), a concept that has met with considerable resistance in evolutionary biology for a long time because of its Lamarckian flavour (Jablonka & Lamb 1998; Chong &

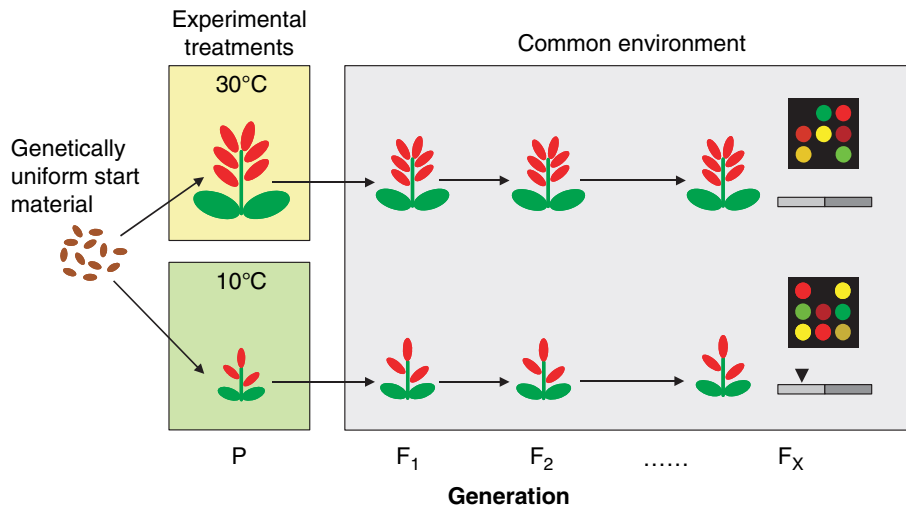


Figure 3 Outline of an experimental design that tests for environmentally induced rapid epigenetic evolution. First, the same plant or animal genotypes are subjected to contrasting environments for at least one generation. Second, their progeny is bred in a common environment for several generations, to examine whether epigenetic changes and associated phenotypic differences are passed on to the following generations. Ideally, one should demonstrate that at the end of the experiment the phenotypes and patterns of DNA methylation (black triangle) or gene expression of these environmental lines are different, but not the DNA sequence (grey horizontal bars).

Whitelaw 2004; Richards 2006). One of the most exciting issues in ecological epigenetics, therefore, will be to attempt to track down such evolutionary responses to environmental change that are mediated by epigenetic inheritance.

In practice, we have two main options for studying these phenomena: first, we can use standard ecological genetic approaches, such as reciprocal transplants or common garden experiments, to test for adaptation to local environmental conditions. However, in this case, only in study systems without genetic variation (see above) will be able to unambiguously ascribe observed phenotypic differences to underlying epigenetic variation.

As an alternative to studying the results of past selection, we may instead choose to study epigenetic evolution in action. We have outlined an appropriate experimental design in Fig. 3. Generally, it must involve three steps: (i) the same plant or animal genotypes are subjected to contrasting environments; (ii) their offspring are bred in a common environment over several generations; after which (iii) phenotypic and epigenetic differences are quantified and statistically compared. If we find that the descendants of those lines that experienced different environments remain phenotypically different, and at the same time they show significant divergence in patterns of DNA methylation, gene or protein expression – in spite of being still identical at the DNA level – this will be evidence for rapid epigenetically based evolution. Conducting experiments over several generations, not just two, will allow us to discern between transient effects and permanent epigenetic changes.

As outlined above, methods that quantify gene function in a way that integrates over different epigenetic mechanisms, such as expression microarrays or protein profiling by two-dimensional electrophoresis, will generally be most likely to detect epigenetic divergence in such experiments.

CONCLUSIONS

Increasing empirical evidence for natural epigenetic variation and epigenetic inheritance suggests that we might need to expand our concept of variation and evolution in natural populations, taking into account several (likely interacting) ecologically relevant inheritance systems. Potentially, this may result in a significant expansion (though by all means not a negation) of the Modern Evolutionary Synthesis as well as in more conceptual and empirical integration between ecology and evolution.

Ecologists should be particularly interested in the study of epigenetic processes as this could significantly improve their understanding of the mechanisms underlying natural phenotypic variation and the responses of organisms to environmental change. It is urgent for ecologists to recognize the relevance of epigenetic processes to their field, and start incorporating epigenetic questions into their research.

When planning their research, ecologists should generally bear in mind that (i) to be of broad ecological–evolutionary relevance, epigenetic variation must be heritable across generations and (ii) only such epigenetic variation that is at least partly independent from genetic variation will have the potential to provide truly novel insights.

In this paper, we have focused on epigenetic variation within species and its ecological relevance, because we felt this perspective was missing from the current literature. We did not discuss the potentially important role of epigenetic inheritance in hybridization and polyploidization, because this aspect of epigenetics, with its relevance to speciation and macroevolution, has been highlighted elsewhere (e.g. Jablonka & Lamb 1998; Liu & Wendel 2003; Rapp & Wendel 2005; Chen & Ni 2006; Grant-Downton & Dickinson 2006). And, of course we did not attempt to cover all questions about epigenetics that ecologists could possibly ask. For instance, is epigenetic diversity an important component of biodiversity, and therefore relevant to questions about ecosystem functioning? Is epigenetically driven evolution a significant part of the responses of ecological systems to global environmental change? Questions like these would certainly deserve further attention and should be explored in the future.

Another important challenge for future research will be to develop theoretical models and novel statistical approaches for analysing complex epigenetic data, and for understanding and predicting epigenetic evolution in natural populations. In particular, there is currently no established statistical framework for predicting the evolution of traits influenced jointly by genetic and epigenetic variation. Also, we know virtually nothing about rates of spontaneous epimutations in natural populations, let alone their stability over time. Clearly, there are still many pieces missing from the epigenetic puzzle.

How important is epigenetic inheritance in the real world, when compared to genetic inheritance? This question is a matter of heated debate. A prime example is the seminal 1998 review paper by Jablonka & Lamb (1998) and the great variety of responses it provoked (all published in the same journal issue). Some researchers argue that epigenetic inheritance is possible but rather unimportant, whereas others think it of overriding importance. However, there is currently little empirical data to support either view, and the issue can certainly not be settled *a priori*. To understand the full significance of epigenetic variation and inheritance, it is imperative to place these processes in an ecological perspective and study their causes and consequences in natural populations. Eventually, only a combination of ecological with molecular and genomic approaches will allow us to better understand the role of epigenetic processes in natural populations.

ACKNOWLEDGEMENTS

We thank the members of the Pigliucci lab for many fruitful discussions, Harald Auge, Daniel Prati, Richard Lindroth and three anonymous referees for their comments on the manu-

script, and Jonathan Wendel for his advice. This research was partially supported by the NSF grant IOB-0450240, New York Sea Grant and by Stony Brook University.

REFERENCES

- Anway, M.D., Cupp, A.S., Uzumcu, M. & Skinner, M.K. (2005). Epigenetic transgenerational actions of endocrine disruptors and mate fertility. *Science*, 308, 1466–1469.
- Barski, A., Cuddapah, S., Cui, K.R., Roh, T.Y., Schones, D.E., Wang, Z.B. *et al.* (2007). High-resolution profiling of histone methylations in the human genome. *Cell*, 129, 823–837.
- Bastow, R., Mylne, J.S., Lister, C., Lippman, Z., Martienssen, R.A. & Dean, C. (2004). Vernalization requires epigenetic silencing of FLC by histone methylation. *Nature*, 427, 164–167.
- Bender, J. (2004). DNA methylation and epigenetics. *Annu. Rev. Plant Biol.*, 55, 41–68.
- Berger, S.L. (2007). The complex language of chromatin regulation during transcription. *Nature*, 447, 407–412.
- Bird, A. (2007). Perceptions of epigenetics. *Nature*, 447, 396–398.
- Bischoff, A., Vonlanthen, B., Steinger, T. & Müller-Schärer, H. (2006). Seed provenance matters – effects on germination of four plant species used for ecological restoration. *Basic Appl. Ecol.*, 7, 347–359.
- Blewitt, M.E., Vickaryous, N.K., Paldi, A., Koseki, H. & Whitelaw, E. (2006). Dynamic reprogramming of DNA methylation at an epigenetically sensitive allele in mice. *PLoS Genet.*, 2, e49.
- Bossdorf, O., Auge, H., Lafuma, L., Rogers, W.E., Siemann, E. & Prati, D. (2005). Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, 144, 1–11.
- Burn, J.E., Bagnall, D.J., Metzger, J.D., Dennis, E.S. & Peacock, W.J. (1993). DNA methylation, vernalization, and the initiation of flowering. *Proc. Natl Acad. Sci. U.S.A.*, 90, 287–291.
- Carroll, S.P., Hendry, A.P., Reznick, D.N. & Fox, C.W. (2007). Evolution on ecological time-scales. *Funct. Ecol.*, 21, 387–393.
- Cervera, M.-T., Ruiz-Garcia, L. & Martinez-Zapater, J. (2002). Analysis of DNA methylation in *Arabidopsis thaliana* based on methylation-sensitive AFLP markers. *Mol. Genet. Genomics*, 268, 543–552.
- Chen, Z.J. & Ni, Z. (2006). Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. *BioEssays*, 28, 240–252.
- Chong, S. & Whitelaw, E. (2004). Epigenetic germline inheritance. *Curr. Opin. Genet. Dev.*, 14, 692–696.
- Conner, J.K. & Hartl, D.L. (2004). *A Primer of Ecological Genetics*. Sinauer Associates, Sunderland.
- Crews, D., Gore, A.C., Hsu, T.S., Dangleben, N.L., Spinetta, M., Schallert, T. *et al.* (2007). Transgenerational epigenetic imprints on mate preference. *Proc. Natl Acad. Sci. U.S.A.*, 104, 5942–5946.
- Cropley, J.E., Suter, C.M., Beckman, K.B. & Martin, D.I.K. (2006). Germ-line epigenetic modification of the murine A(vy) allele by nutritional supplementation. *Proc. Natl Acad. Sci. U.S.A.*, 103, 17308–17312.
- Cubas, P., Vincent, C. & Coen, E. (1999). An epigenetic mutation responsible for natural variation in floral symmetry. *Nature*, 401, 157–161.
- Das, O.P. & Messing, J. (1994). Variegated phenotype and developmental methylation changes of a maize allele originating from epimutation. *Genetics*, 136, 1121–1141.

- Davis, M.B. & Shaw, R.G. (2001). Range shifts and adaptive responses to quaternary climate change. *Science*, 292, 673–679.
- Davis, M.B., Shaw, R.G. & Etterson, J.R. (2005). Evolutionary responses to changing climate. *Ecology*, 86, 1704–1714.
- Ellstrand, N.C. & Schierenbeck, K.A. (2000). Hybridization as a stimulus for the evolution of invasiveness. *Proc. Natl Acad. Sci. U.S.A.*, 97, 7043–7050.
- Fieldes, M.A. (1994). Heritable effects of 5-azacytidine treatments on the growth and development of flax (*Linum usitatissimum*) genotrophs and genotypes. *Genome*, 37, 1–11.
- Fieldes, M.A. & Amyot, L.M. (1999). Epigenetic control of early flowering in flax lines induced by 5-azacytidine applied to germinating seed. *J. Hered.*, 90, 199–206.
- Ford, E.B. (1964). *Ecological Genetics*. Methuen, London.
- Garfinkel, M.D., Sollars, V.E., Lu, X. & Ruden, D.M. (2004). Multigenerational selection and detection of altered histone acetylation and methylation patterns: toward a quantitative epigenetics in *Drosophila*. *Methods Mol. Biol.*, 287, 151–168.
- Gorg, A., Weiss, W. & Dunn, M.J. (2004). Current two-dimensional electrophoresis technology for proteomics. *Proteomics*, 4, 3665–3685.
- Grant-Downton, R.T. & Dickinson, H.G. (2005). Epigenetics and its implications for plant biology: 1. The epigenetic network in plants. *Ann. Bot.*, 96, 1143–1164.
- Grant-Downton, R.T. & Dickinson, H.G. (2006). Epigenetics and its implications for plant biology: 2. The ‘epigenetic epiphany’: epigenetics, evolution and beyond. *Ann. Bot.*, 97, 11–27.
- Hairston, N.G., Ellner, S.P., Geber, M.A., Yoshida, T. & Fox, J.A. (2005). Rapid evolution and the convergence of ecological and evolutionary time. *Ecol. Lett.*, 8, 1114–1127.
- He, Y.H. & Amasino, R.M. (2005). Role of chromatin modification in flowering-time control. *Trends Plant Sci.*, 10, 30–35.
- Hollingsworth, M.L. & Bailey, J.P. (2000). Evidence for massive clonal growth in the invasive weed *Fallopia japonica* (Japanese knotweed). *Biol. J. Linn. Soc.*, 133, 463–472.
- Horvath, D.P., Schaffer, R., West, M. & Wisman, E. (2003). *Arabidopsis* microarrays identify conserved and differentially expressed genes involved in shoot growth and development from distantly related plant species. *Plant J.*, 34, 125–134.
- Jablonka, E. & Lamb, M.J. (1998). Epigenetic inheritance in evolution. *J. Evol. Biol.*, 11, 159–183.
- Jablonka, E. & Lamb, M.J. (2005). *Evolution in Four Dimensions: Genetic, Epigenetic, Behavioral, and Symbolic Variation in the History of Life*. MIT Press, Cambridge.
- Jaenisch, R. & Bird, A. (2003). Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet.*, 33, 245–254.
- Jirtle, R.L. & Skinner, M.K. (2007). Environmental epigenomics and disease susceptibility. *Nat. Rev. Genet.*, 8, 253–262.
- Johnson, M.T.J. & Stinchcombe, J.R. (2007). An emerging synthesis between community ecology and evolutionary biology. *Trends Ecol. Evol.*, 22, 250–257.
- Jones, P.A. (1985). Altering gene expression with 5-azacytidine. *Cell*, 40, 485–486.
- Jump, A.S. & Penuelas, J. (2005). Running to stand still: adaptation and the response of plants to rapid climate change. *Ecol. Lett.*, 8, 1010–1020.
- Kalisz, S. & Purugganan, M.D. (2004). Epialleles via DNA methylation: consequences for plant evolution. *Trends Ecol. Evol.*, 19, 309–314.
- Kammenga, J.E., Herman, M.A., Ouborg, N.J., Johnson, L. & Breitling, R. (2007). Microarray challenges in ecology. *Trends Ecol. Evol.*, 22, 273–279.
- Kankel, M.W., Ramsey, D.E., Stokes, T.L., Flowers, S.K., Haag, J.R., Jeddeloh, J.A. *et al.* (2003). *Arabidopsis* *MET1* cytosine methyltransferase mutants. *Genetics*, 163, 1109–1122.
- Keyte, A.L., Percifield, R., Liu, B. & Wendel, J.F. (2006). Intra-specific DNA methylation polymorphism in cotton (*Gossypium hirsutum* L.). *J. Hered.*, 97, 444–450.
- Laird, P.W. (2003). The power and the promise of DNA methylation markers. *Nat. Rev. Cancer*, 3, 253–266.
- Le Roux, J.J., Wicczorek, A.M., Wright, M.G. & Tran, C.T. (2007). Super-genotype: global monoclonality defies the odds of nature. *PLoS ONE*, 2, e590.
- Linhart, Y.B. & Grant, M.C. (1996). Evolutionary significance of local genetic differentiation in plants. *Annu. Rev. Ecol. Syst.*, 27, 237–277.
- Liu, B. & Wendel, J.F. (2003). Epigenetic phenomena and the evolution of plant allopolyploids. *Mol. Phylog. Evol.*, 29, 365–379.
- Mandák, B., Bímová, K., Pyšek, P., Štěpánek, J. & Plačková, I. (2005). Isoenzyme diversity in *Reynoutria* (Polygonaceae) taxa: escape from sterility by hybridization. *Plant Syst. Evol.*, 253, 219–230.
- Martienssen, R.A., Doerge, R.W. & Colot, V. (2005). Epigenomic mapping in *Arabidopsis* using tiling microarrays. *Chromosome Res.*, 13, 299–308.
- Mayr, E. & Provine, W.B. (1980). *The Evolutionary Synthesis: Perspectives on the Unification of Biology*. Harvard University Press, Cambridge.
- Merilä, J. & Crnokrak, P. (2001). Comparison of genetic differentiation at marker loci and quantitative traits. *J. Evol. Biol.*, 14, 892–903.
- Mikkelsen, T.S., Ku, M.C., Jaffe, D.B., Issac, B., Lieberman, E., Giannoukos, G. *et al.* (2007). Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature*, 448, 553–560.
- Mittelsten Scheid, O., Afsar, K. & Paszkowski, J. (2003). Formation of stable epialleles and their paramutation-like interaction in tetraploid *Arabidopsis thaliana*. *Nat. Genet.*, 34, 450–454.
- Mooney, H.A. & Cleland, E.E. (2001). The evolutionary impact of invasive species. *Proc. Natl Acad. Sci. U.S.A.*, 98, 5446–5451.
- Mousseau, T.A., Sinervo, B. & Endler, J.A. (2000). *Adaptive Genetic Variation in the Wild*. Oxford University Press, Oxford.
- Ouborg, N.J. & Vriezen, W.H. (2007). An ecologist’s guide to ecogenomics. *J. Ecol.*, 95, 8–16.
- Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Syst.*, 37, 637–669.
- Rakyan, V.K., Chong, S., Champ, M.E., Cuthbert, P.C., Morgan, H.D., Luu, K.V.K. *et al.* (2003). Transgenerational inheritance of epigenetic states at the murine Axin(Fu) allele occurs after maternal and paternal transmission. *Proc. Natl Acad. Sci. U.S.A.*, 100, 2538–2543.
- Rangwala, S.H., Elumalai, R., Vanier, C., Ozkan, H., Galbraith, D.W. & Richards, E.J. (2006). Meiotically stable natural epialleles of Sadhu, a novel *Arabidopsis* retroposon. *PLoS Genet.*, 2, e36.
- Rapp, R.A. & Wendel, J.F. (2005). Epigenetics and plant evolution. *New Phytol.*, 168, 81–91.
- Reyna-Lopez, G., Simpson, J. & Ruiz-Herrera, J. (1997). Differences in DNA methylation patterns are detectable during the

- dimorphic transition of fungi by amplification of restriction polymorphisms. *Mol. Genet. Genomics*, 253, 703–710.
- Rice, K.J. & Emery, N.C. (2003). Managing microevolution: restoration in the face of global change. *Front. Ecol. Environ.*, 1, 469–478.
- Richards, E.J. (2006). Inherited epigenetic variation – revisiting soft inheritance. *Nat. Rev. Genet.*, 7, 395–401.
- Riddle, N.C. & Richards, E.J. (2002). The control of natural variation in cytosine methylation in *Arabidopsis*. *Genetics*, 162, 355–363.
- Rossiter, M.C. (1996). Incidence and consequences of inherited environmental effects. *Annu. Rev. Ecol. Syst.*, 27, 451–476.
- Salmon, A., Ainouche, M.L. & Wendel, J.F. (2005). Genetic and epigenetic consequences of recent hybridization and polyploidy in *Spartina* (Poaceae). *Mol. Ecol.*, 14, 1163–1175.
- Shindo, C., Lister, C., Crevillen, P., Nordborg, M. & Dean, C. (2006). Variation in the epigenetic silencing of FLC contributes to natural variation in *Arabidopsis* vernalization response. *Genes Dev.*, 20, 3079–3083.
- Sollars, V., Lu, X., Xiao, L., Wang, X., Garfinkel, M.D. & Ruden, D.M. (2003). Evidence for an epigenetic mechanism by which Hsp90 acts as a capacitor for morphological evolution. *Nat. Genet.*, 33, 70–74.
- Strauss, S.Y., Lau, J.A. & Carroll, S.P. (2006). Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecol. Lett.*, 9, 354–371.
- Takeda, S. & Paszkowski, J. (2006). DNA methylation and epigenetic inheritance during plant gametogenesis. *Chromosoma*, 115, 27–35.
- Tatra, G.S., Miranda, J., Chinnappa, C.C. & Reid, D.M. (2000). Effect of light quality and 5-azacytidine on genomic methylation and stem elongation in two ecotypes of *Stellaria longipes*. *Physiol. Plant.*, 109, 313–321.
- Thomas, M.A. & Klaper, R. (2004). Genomics for the ecological toolbox. *Trends Ecol. Evol.*, 19, 439–445.
- Thompson, J.N. (1998). Rapid evolution as an ecological process. *Trends Ecol. Evol.*, 13, 329–332.
- Van Steensel, B. & Henikoff, S. (2003). Epigenomic profiling using microarrays. *Biotechniques*, 35, 346–357.
- Vaughn, M.W., Tanurdzic, M., Lippman, Z., Jiang, H., Carrasquillo, R., Rabinowicz, P.D. *et al.* (2007). Epigenetic natural variation in *Arabidopsis thaliana*. *PLoS Biol.*, 5, e174.
- Vongs, A., Kakutani, T., Martienssen, R.A. & Richards, E.J. (1993). *Arabidopsis thaliana* DNA methylation mutants. *Science*, 260, 1926–1928.
- West-Eberhard, M.J. (2003). *Developmental Plasticity and Evolution*. Oxford University Press, New York.
- Whitelaw, N.C. & Whitelaw, E. (2006). How lifetimes shape epigenotype within and across generations. *Hum. Mol. Genet.*, 15, R131–R137.
- Whitham, T.G., Bailey, J.K., Schweitzer, J.A., Shuster, S.M., Bangert, R.K., Leroy, C.J. *et al.* (2006). A framework for community and ecosystem genetics: from genes to ecosystems. *Nat. Rev. Genet.*, 7, 510–523.
- Xu, C.-Y., Zhang, W.J., Fu, C.-Z. & Lu, B.-R. (2003). Genetic diversity of alligator weed in China by RAPD analysis. *Biodivers. Conserv.*, 12, 637–645.
- Zhang, X., Clarenz, O., Cokus, S., Bernatavichute, Y.V., Pellegrini, M., Goodrich, J. *et al.* (2007). Whole-genome analysis of histone H3 lysine 27 trimethylation in *Arabidopsis*. *PLoS Biol.*, 5, e129.
- Zilberman, D., Gehring, M., Tran, R.K., Ballinger, T. & Henikoff, S. (2007). Genome-wide analysis of *Arabidopsis thaliana* DNA methylation uncovers an interdependence between methylation and transcription. *Nat. Genet.*, 39, 61–69.

Editor, Richard Lindroth

Manuscript received 8 August 2007

First decision made 10 September 2007

Manuscript accepted 10 October 2007