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How might epigenetics contribute to ecological speciation?

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Abstract Speciation research has seen a renewed interest in ecological speciation, which emphasises divergent ecological selection leading to the evolution of reproductive isolation. Selection from divergent ecologies means that phenotypic plasticity can play an important role in ecological speciation. Phenotypic plasticity involves the induction of phenotypes over the lifetime of an organism and emerging evidence suggests that epigenetic marks such as cytosine and protein (histone) modifications might regulate such environmental induction. Epigenetic marks play a wide role in a variety of processes including development, sex differentiation and allocation, sexual conflict, regulation of transposable elements and phenotypic plasticity. Here we describe recent studies that investigate epigenetic mechanisms in a variety of contexts. There is mounting evidence for environmentally induced epigenetic variation and for the stable inheritance of epigenetic marks between generations. Thus, epigenetically-based phenotypic plasticity may play a role in adaptation and ecological speciation. However, there is less evidence for the inheritance of induced epigenetic variation across multiple generations in animals. Currently few studies of ecological speciation incorporate the potential for the involvement of epigenetically-based induction of phenotypes, and we argue that this is an important omission [*Current Zoology* 59 (5): 686–696, 2013].

Keywords Ecological speciation, Adaptation, Reproductive isolation, Phenotypic plasticity, Epigenetic mechanisms

Recent studies of speciation have included a renewed interest in the role of ecology with an increasing focus on ecological speciation (Nosil, 2012; Schluter, 2001). Ecological speciation occurs via the adaptation of populations to different environments and the concurrent evolution of reproductive isolation, leading to an increased incompatibility of genomic regions (Nosil et al., 2009a; Nosil et al., 2009b; Rundle and Nosil, 2005). Exactly how ecological speciation differs from other modes of speciation is subtle, mainly because in most cases speciation will involve an organism's interactions with its ecology (Sobel et al., 2010). However, a key factor in ecological speciation is the presence of ecologically-based divergent selection driving adaptation and isolation (a counter-example being mutation order speciation where environmental selection is uniform and the source of variation is through the accumulation of different mutations that arise between groups; Schluter, 2009; Unckless and Orr, 2009). Ecological speciation requires divergent selection from either the abiotic or biotic environment and because of this essential role of ecology, phenotypic plasticity might be an important process during ecological speciation as it may accelerate adaptation to or persistence in a novel envi-

ronment (Fitzpatrick, 2012; Pfennig et al., 2010; Thibert-Plante and Hendry, 2011; Wund, 2012).

Ecological speciation is now being examined at the genomic level, with influential models such as divergence hitchhiking, which predict genomic 'islands' and 'continents' of divergence, coming to the fore (Feder and Nosil, 2010). These stem from the idea that genomic divergence during speciation is heterogeneous, with some regions being genetically more divergent due to selection and others being homogenised by gene flow (Smadja et al., 2008; Turner et al., 2005; Via and West, 2008). Speciation is likely to start as a slow process with restricted gene flow at only a few key loci (or genomic islands) involved in local adaptation, mate choice or other selective processes (Butlin, 2010). These regions of divergence may then spread out across the genome due to reduced gene flow influencing linked regions, creating genomic 'continents', until populations are reproductively isolated, facilitating increased divergence across the genome (Charlesworth et al., 1997; Nosil, et al., 2009a; Feder and Nosil, 2010). Processes such as phenotypic plasticity may play a role during the initial stages of population divergence, and contribute to localised genomic divergence (Fitzpatrick, 2012).

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Here we briefly describe what is known about the roles of phenotypic plasticity and epigenetic variation in evolution, specifically ecological speciation. We start by re-stating the role of phenotypic plasticity in ecological speciation. Heritable variation for plasticity exists in natural populations and can be shaped by selection, and plasticity can alter the targets of selection, influencing the outcome of adaptive evolution (Wund, 2012). A potential role for plasticity in influencing assortative mating is important, but poorly studied. Next, we examine some criticisms of the role of epigenetic marks (a suite of effects that can alter gene expression without changes in DNA sequence) in evolution and conclude that there is now much evidence for their heritability, at least in the short term. Further, the induction of epigenetic marks by the environment suggests that such marks could underlie the evolution of phenotypic plasticity. Thus epigenetic variation could promote ecological speciation (Pal and Miklós, 1999) and we discuss how epigenetic mechanisms might contribute to adaptation and reproductive isolation. We conclude with some example study systems that seem particularly promising for furthering our understanding of the role of epigenetics in ecological speciation.

1 Phenotypic Plasticity

Phenotypic plasticity has been poorly defined in the past with many uses of the term applied to a multitude of phenotypes. A useful, if broad, definition was proposed by Schlichting and Smith (2002) as ‘any change in an organism’s characteristics in response to an environmental signal’. This encompasses all hierarchical levels of response (from gene expression to the phenotype) as well as applying to all phenotypic categories (life history, physiological, morphological or behavioural, including learning). Here we refer to plasticity in the broadest sense for an inclusive examination of the role of trait plasticity in speciation, but the role of learning in speciation has been reviewed elsewhere (e.g. Verzijden et al., 2012). Our definition incorporates adaptive, suboptimal and nonadaptive responses because an initially nonadaptive response can facilitate adaptive change, for example simply by increasing phenotypic variation (Feinberg and Irizarry, 2010). Because of the broad spectrum of phenotypes and the potentially large number of different evolutionary outcomes, it has been a challenge to determine the precise role of plasticity in adaptation and speciation (Wund, 2012).

Phenotypic plasticity often allows organisms to respond adaptively to environmental factors, and might be particularly important when populations encounter a novel environment following dispersal or rapid environmental change. Adaptation to a range of new environments may follow a single invasion event, for example when fish invade glacial or other new lakes (Skúlason et al., 1999), or occur with on-going gene flow or fluctuating environments. Demonstration of the importance of plasticity is complex due to the operation of processes over different timescales; developmental plasticity over a single organismal lifetime, and the evolution of plasticity and evolutionary repercussions over generations. For example, genetic accommodation can occur via selection on the population mean of a trait, following a plastic response to a new environment, leading to shifts in the trait mean and increased or reduced trait plasticity. If genetic accommodation leads to reduced plasticity this may result in genetic assimilation, or fixation, of a trait (Pigliucci et al., 2006). Thus, divergent selection across environments might lead to speciation via genetic assimilation (Pal and Miklós, 1999). Distinguishing the relative importance of plasticity versus ‘conventional’ sources of genetic variation in longer-term divergence is particularly difficult (Pfennig et al., 2010). However, evidence suggests that plasticity can aid in the persistence of species encountering novel environments, result in the release of cryptic genetic variation, alter the mean and variance of phenotypes on which selection can then act and facilitate rapid adaptation (Gibson and Dworkin, 2004; Lande, 2009; Le Rouzic and Carlborg, 2008; Schlichting, 2008; Scoville and Pfrender, 2010). Thus, plasticity and other sources of genetic variation involved in ecological adaptation could be synergistic. Phenotypic plasticity has been reviewed extensively (see Fitzpatrick, 2012 and Pfennig et al., 2010 for recent reviews), and a number of theoretical models have examined plasticity across both spatially and temporally varying environments (see Thibert-Plante and Hendry, 2011 for a summary).

Phenotypic plasticity has been suggested to be a facilitator of speciation (West-Eberhard, 1989; West-Eberhard, 2003) and there is evidence from a number of different study systems to support this. For example plasticity in host plant utilisation has led to diversification in butterflies (Nylin and Janz, 2009) and *Drosophila* (Smith et al., 2013), plasticity in foraging behaviour has aided in white fish radiations (Lundsgaard-Hensen et al., 2013), in intralacustrine speciation of

Arctic charr (Adams and Huntingford, 2004), and plasticity in feeding morphologies in cichlid fish has contributed to trophic radiations (Stauffer and van Snik Gray, 2004).

For ecological adaptation to influence speciation, it must initiate or increase reproductive isolation between diverging forms (Price et al., 2003; Thibert-Plante and Hendry, 2011; West-Eberhard, 2003; West-Eberhard, 2005; West-Eberhard, 1989; Wund et al., 2008). For this to happen, assortative mating or another cause of reduced gene flow must occur in response to the plastic changes in phenotype. This might be almost inevitable when there are intimate associations between traits under ecological selection and assortative mating, such as those seen for flowering time in plants (Hall et al., 2006) or nest structure and body size in sticklebacks (Ólafsdóttir et al., 2006). The importance of ecological plasticity to significant variation in mating signals or preferences is poorly understood (Olvido and Mousseau, 1995), in particular we know of no study which has addressed if plasticity could influence multiple effect (or 'magic') traits by facilitating the phenotypic linkage of ecological adaptation and assortative mating.

A number of studies have examined the evolutionary consequences of phenotypic plasticity, but few have considered ecological speciation directly. However, Thibert-Plante and Hendry (2011) used individual-based simulations to explore how phenotypic plasticity can influence the evolution of reproductive barriers during ecological speciation. They showed that adaptive plasticity evolves readily in the presence of dispersal between populations in different environments, and facilitates the colonization of highly divergent novel environments. Without phenotypic plasticity new environments may never be successfully colonized. Once new environments have been colonized and populations persist they will be under divergent selection, potentially on different traits to those allowing the initial persistence, potentially leading to genetic accommodation or assimilation.

Phenotypic plasticity can also influence natural selection on migrants and such selection is potentially more important than both sexual selection and selection against hybrids in the formation of reproductive barriers (Thibert-Plante and Hendry, 2011). Typically, individuals develop before they migrate. Thus selection against migrant individuals forms an ecologically-based reproductive barrier, which may be particularly effective because natural selection on migrants acts earlier than sexual selection (and before hybrids are formed) due to

migrant individuals being less suited to their new environment (Hendry 2004; Nosil et al., 2005). It has been noted that such plasticity might only cause weak reproductive barriers yet these barriers occur *before* genetic divergence, due to selection against phenotypes induced in the alternative environment (Thibert-Plante and Hendry, 2011). Therefore, such selection might be very important at the initial stages of ecological speciation, reducing gene flow and allowing adaptive divergence (the spread of genomic 'islands' of divergence; Feder and Nosil, 2010).

2 Epigenetic Mechanisms and Evolution

The consensus definition of an epigenetic-based trait is 'a stably heritable phenotype resulting from changes in a chromosome without alternations in the DNA sequence' (Berger et al., 2009). Similar to allelic variation, epialleles are defined as 'alternative chromatin states at a given locus defined with respect to individuals in a population at a given time point and tissue type' (Johannes et al., 2008). Note that epigenetic marks are not necessarily heritable and may simply be a result of chance or environmental induction in each generation. Historically there have been several different uses of the word 'epigenetic' (Bird, 2007). Waddington (1942) originally used this word in the context of 'epigenesis', or how phenotypes arise during the development of an organism from the zygote. Inherent in his idea was the fact that epigenetic mechanisms were wiped clean and reset anew each generation (Richards, 2006). Here, we use the word 'epigenetic mechanism' and 'epigenetic mark' to refer generally to any chemical modification to DNA, RNA or protein that might influence chromatin state and gene expression, referring specifically to 'epigenetic inheritance' as the stable inheritance of epigenetic marks across multiple generations.

Epigenetic marks encompass a range of modifications that provide an additional layer of information above that of the DNA sequence. These include modification of cytosine residues (most commonly methylation but also hydroxymethylation; Tahiliani et al., 2009), protein modifications (most commonly acetylation, deacetylation, ubiquitination, pseudouridylation and/or methylation of histones) and RNA-based regulatory systems (a number of small and large noncoding RNAs such as small nuclear RNAs, small nucleolar RNAs, microRNAs and small interfering RNAs; Jablonka, 2012a). Epigenetic modifications often interact and are components of complex pathways that regulate gene

expression levels, switching genes on and off and regulating alternative splicing (Bossdorf et al., 2008; Luco et al., 2011; Luco et al., 2010). DNA methylation is the most thoroughly studied type of epigenetic mark and thus much of our knowledge of the role epigenetics in evolution focuses on studies of DNA methylation.

Epigenetic marks are dynamic and essential for normal development but, crucially, the environment can modify them, suggesting a mechanistic role for epigenetics in phenotypic plasticity. There are numerous examples of environmentally induced epigenetic marks in plants (e.g. the effects of temperature exposure on flowering time; Gendall et al., 2001), and in mammals (e.g. the effects of maternal behaviour on offspring behaviour; Weaver et al., 2004). Generally, much work on the environmental induction of marks has focused on nutrition and chemical exposure in medicine and ecotoxicology (Harris et al., 2012; Vandegehuchte and Janssen, 2011). In mice, dietary supplements increase the methyl donor metabolite S-adenosylmethionine, elevating DNA methylation and causing suppression of *Agouti* gene overexpression, leading to increased health and longevity (Cooney et al., 2002). Epigenetic modifications have been intensively studied in cancer research and maternal exposure to carcinogens, such as DEHP (Di-2-(ethylhexyl) phthalate), can modify DNA methylation patterns in the testes of offspring (Wu et al., 2010). More relevantly, exposure to toxic chemicals such as heavy metals (e.g. Cadmium; Takiguchi et al., 2003) and organic pollutants (e.g. Benzo(a)pyrene; Shugart, 1990) can cause a variety of epigenetic modifications (see Vandegehuchte and Janssen, 2011 for a thorough review) and these changes are often maintained across subsequent generations, even when the inducer is removed (Anway et al., 2005). Such studies indicate that epigenetic variation may prove to be a common mechanism underlying environmentally induced phenotypes (Jaenisch and Bird, 2003; Johnson and Tricker, 2010; Levine et al., 2011; Richards et al., 2010; Richards, 2011; Smith et al., 2013).

Epigenetic mechanisms exist in almost every living organism and while virtually unstudied with respect to ecological speciation, these mechanisms have the potential to contribute to rapid adaptation to a changing environment. The role of epigenetic marks as a mechanism of phenotypic plasticity is not controversial, but more contentious is whether or not induced epigenetic marks can be stably inherited for a number of generations without continued induction and independently from the underlying genetic variation. If such marks can

be induced in the germ line, for example if a mechanism by which external cues are parsed to the gametes were to exist, then this induced and heritable epigenetic mark would not only 'program' future generations for their environment but also be under the influence of selection (Richards, 2008; Jablonka, 2012a). This means that epigenetic variation could contribute to ecological speciation across relatively stable novel environments.

2.1 Transgenerational epigenetic inheritance

There is growing evidence that some epigenetic marks are heritable, suggesting that epigenetically controlled plasticity could carry over into subsequent generations. In mammals, DNA methylation is removed from the male genome hours after fertilization and the female genome is passively demethylated through a lack of re-established methylation on newly synthesised strands in subsequent zygotic cellular divisions (Jaenisch and Bird, 2003). This is followed by *de novo* methylation of the zygotic genome after attachment to the uterine wall (Jaenisch, 1997), with marks then often being stable throughout development. One argument against a major role for epigenetic marks in adaptive evolution is that epigenetic marks are erased during meiosis as well as early embryogenesis. This makes it difficult to see how a mark is then inherited in the next generation in a stable manner. Yet the erasure of DNA methylation during meiosis may be limited to mammals, and even then is by no means complete (Richards, 2006). Recent evidence from genome-wide bisulfite sequencing in zebrafish demonstrates that DNA methylation associated with specific genes is maintained in sperm but not oocytes, with paternal methylation patterns being carried through embryogenesis and maternal patterns being passively removed (Jiang et al., 2013). Thus, in this fish species, paternal epigenetic patterns are stably inherited and marks at specific loci are copied to the maternal genome early in embryogenesis.

Transgenerational epigenetic inheritance has been demonstrated in plants (Goll and Bestor, 2005; Johannes et al., 2009), nematodes (Greer et al., 2011) and fish (Walter et al., 2002; Jiang et al., 2013). However, two distinctions must be noted; first, the difference between heritable marks, and *induced* heritable marks: second, differentiation of marks that are induced each generation, from those truly inherited. For marks to be truly heritable they must be present in the F3 and subsequent generations once the cause of the induction has been removed (Harris et al., 2012). If the transmission of environmentally induced epigenetic variation to offspring does occur, it must be either through the produc-

tion of gametes from induced somatic cells (as in plants), through the induction of marks in the soma and germ line together during early development (e.g. exposure in the womb in mammals; Skinner, 2011) or through an active process targeting the gametes (see section 2.2.2).

2.2 Criticisms of epigenetic marks in evolution

The suggestion that epigenetic marks play a role in adaptive evolution is still controversial and we believe that three main factors contribute to this controversy centered on the view that epigenetics is a pseudo-Lamarckian process (Jablonka and Lamb, 1995). Firstly, epigenetic variation is not seen as a primary driver of evolution in the same way as novel mutations (Dickins and Rahman, 2012). Secondly, an important aspect of neo-Darwinism is that evolution is not 'directed' and thus because epigenetic variation might be induced and stably inherited at non-random genomic locations, it can be seen as directed. Finally, epigenetic marks are seen as dependent on DNA and thus do not evolve independently of genetic variation (Dickins and Rahman, 2012). However, such criticisms focus on epigenetics as an 'unorthodox' mechanism and ignore recent evidence of the ecological importance of epigenetic variation and heritability of marks. Far from being Lamarckian, epigenetic marks may evolve under Darwinian processes, and have an important role in phenotypic evolution (Haig, 2007). These three aspects of epigenetics are explored in turn below.

2.2.1 Epimutations in evolution

Evidence suggests that epimutations can arise stochastically, be heritable, underlie quantitative traits, have similar mutation rates to genetic mutations and contribute to phenotypic evolution. Generally, novel epimutations can occur spontaneously across generations, in somatic cells with aging, in the male germline during meiosis (De Boer et al., 2010) and through mistakes during post-fertilization reprogramming. Schmitz et al. (2011) demonstrated spontaneously occurring DNA methylation at more than 100,000 CpG (a cytosine and guanine dinucleotide on the same DNA strand separated by a phosphodiester bond) positions in *Arabidopsis thaliana* occurring over 30 generations, the majority of which were meiotically stable. Johannes et al. (2009) discovered stable epigenetic variation over at least 8 generations that may have contributed up to 30% of heritability in flowering time and plant height.

Epimutations at individual positions in *A. thaliana* are much more frequent than DNA mutations. The lower bound of epimutation rates was found to be 4.46

$\times 10^{-4}$ methylation polymorphisms per CpG site per generation (Schmitz et al., 2011), compared with genetic mutation rates of 7×10^{-9} base substitutions per site per generation in the same *A. thaliana* lines (Ossowski et al., 2010). However, epimutation rates in contiguous regions of methylation, such as differentially methylated regions (DMRs), which are more likely to be functional, are more similar to that of DNA mutational change (Becker, et al., 2011). Becker et al. (2011) found 249 DMRs that tended to be more stable than individual positions over the 30 generations examined. These data indicate that epimutations occur as frequently as genetic mutations and are seen more often at certain key functional genomic positions. This is also true for natural populations of *A. thaliana* where methylation variation underlies heritable phenotypic variation (Roux et al., 2011) and high levels of epimutation polymorphism exist among ecotypes (Vaughn et al., 2007). Thus there is tantalizing evidence for the importance of epigenetic novelty as a source of Darwinian evolution. Further, such evidence is not limited to plants, with increasing numbers of studies demonstrating epigenetic variation in mammals (Daxinger and Whitelaw, 2012), insects (Seong et al., 2011) and nematodes (Greer et al., 2011).

2.2.2 Induction and stable inheritance of epigenetic marks

Transgenerational epigenetic variation has been demonstrated in a number of species, but can induced epigenetic marks become stably inherited after induction and if so what roles do they play in adaptive evolution? There is now some evidence in mammals (Franklin and Mansuy, 2010; Daxinger and Whitelaw, 2012) and *Drosophila* (Seong, et al., 2011) that induced epigenetic inheritance does occur. Theoretical models suggest that such a mechanism could provide an adaptive advantage in stochastic environments, providing a link between short-term individual adaptive responses and long-term evolutionary change (Jablonka et al., 1995; Duckworth, 2012). This mechanism would not be 'directed' evolution as such, but more akin to phenotypic plasticity in that it is an evolved mechanism to deal with environmental change.

Several studies in *A. thaliana* lines that differ in DNA methylation but not genetic variation show heritable phenotypic variation and plasticity in ecologically relevant traits (Johannes et al., 2009; Zhang et al., 2013). These studies utilized epigenetic recombinant inbred lines (epiRILs) that are genetically almost identical but vary in levels of DNA methylation. The epiRIL lines

were created through crossing closely related parent plants from a single accession that differed little genetically except at the decrease in DNA methylation (*DDMI*) locus. At this locus, one parent was homozygous for the wild type allele and the other for a mutant allele, causing genome-wide variation in methylation patterns in offspring. The resulting phenotypes varied substantially (Johannes, et al., 2009) and estimates of heritability after exposure of epiRIL lines to drought and increased nutrient conditions were substantial (ranging 7%–46%; Zhang et al., 2013). Some traits such as plant height, flowering time and total biomass showed significant levels of heritability comparable to that of classic genetic recombinant inbred lines. Further, heritable variation for phenotypic plasticity was seen, suggesting an epigenetic basis to the evolution of plasticity (rather than a simple role of epigenetics in developmentally plastic responses; Zhang et al., 2013). Such epigenetic variation could be considered non-genetic “plasticity loci” (Schlichting and Pigliucci, 1993), and play a particularly important role in rapid adaptation to changing environments.

2.2.3 Associations between genetic and epigenetic variation

Ultimately, epigenetic modifications are associated with genomic sequence variation to some degree because DNA underlies the proteins that create marks, and epigenetic marks involve changes to DNA, RNA or proteins. Richards (2006) set out three categories of epigenetic mark, highlighting the importance of epigenetic variation and its association with genetic variation. These are; obligatory epigenetic variation that is entirely dependent on genetic variation, facilitated epigenetic variation in which the genotype directs epigenetic marks in a probabilistic but not deterministic manner, and independent epigenetic variation generated by stochastic events, largely independent from the DNA sequence. These categories were later expanded to distinguish between induced epigenetic variation (developmental) and inherited variation (transgenerational) each having all three levels of genetic dependence (Jablonka, 2012b). Thus genotype does not always predict the epigenotype (Richards, 2008).

The link between genetic and epigenetic variation is important and some theoretical models have examined epigenetics in terms of genetic variation that changes phenotypic variance but not the mean of traits, mediated epigenetically (Carja and Feldman, 2012; Feinberg and Irizarry, 2010). Feinberg and Irizarry (2010) found evidence for genetic variants that influence epigenetic

variation at key genomic locations. They empirically examined DNA methylation in mice and humans discovering stochastic epigenetic variation in variably methylated regions (VMRs; regions of increased inter-individual DNA methylation variability). Further, they saw that many DMRs across species differed in their underlying DNA sequence (loss or gain of CpG dinucleotides), thus identifying heritable genetic variation that influences phenotypic variability through epigenetic mechanisms. VMRs were often discovered near DMRs, suggesting that these genomic features may evolve into one another over time. Feinberg and Irizarry (2010) simulated the effect of selection on genetic loci that can modify the variance of phenotypes (through epigenetic variation), without influencing the mean of a trait and showed that such a mechanism provides an adaptive advantage in a fluctuating environment. This demonstrates the importance of interacting genetic and epigenetic variation.

Epigenetic marks may also feedback into genetic change, contributing to long-term evolution. There are several ways in which epigenetic marks might do this, such as the regulation of transposable elements or an effect on recombination. Additionally, 5-methylcytosine is subject to spontaneous deamination, turning a methylated C into a T, at ~12 times higher a rate than traditional DNA mutations (Laird and Jaenisch, 1994, Sved and Bird, 1990). CpG depletion can occur over generations in regions that are targeted for methylation (Flores and Amdam, 2011). Ossowski et al. (2010) examined mutation accumulation lines in *A. thaliana* and found more G:C > A:T transitions than expected by chance. Methylated sites had a higher probability of mutating in this manner, and they concluded that a combination of UV-induced mutagenesis and deamination of methylated cytosines could account for these mutations. The production of facultative heterochromatin involves several histone cores that wrap DNA, preventing gene expression by blocking the attachment of transcriptional machinery (Grewal and Moazed, 2003). This process could shield silenced regions from the action of selection, allowing mutations to accumulate, in much the same way as heterochromatin accumulates transposable elements (Dimitri and Junakovic, 1999). When these genes are once again expressed, accumulated mutations will be open to the action of selection. Such mechanisms might be linked to the wide range of stress-induced responses such as heat shock, which in flies can produce novel phenotypes that can be selected and fixed in only 14 generations (Waddington, 1953). Generally, a

number of stress responses demonstrate an epigenetic basis (Crews et al., 2012; Stern et al., 2012). Thus epigenetic variation can be linked to genetic variation to different degrees and epigenetic variation might feed-back into the genome.

3 Epigenetics in Ecological Speciation

We have seen above that epigenetic novelty can arise in much the same way as mutational novelty, be heritable to some extent and contribute to variation in quantitative characters that can be shaped by natural selection (Becker et al., 2011; Johannes et al., 2009; Richards et al., 2010). Epigenetic variation is likely to play a role in the initial stages of ecological speciation by facilitating adaptation to novel or differing ecological environments. Phenotypic plasticity can allow for the colonization of novel habitats and might lead to divergence of ecological and reproductive traits through genetic accommodation or assimilation. Epigenetic mechanisms might facilitate this process by providing the environmentally sensitive developmental response. Epigenetic marks themselves would be under selection if stable and heritable, however selection will also be targeting genetic variants controlling epigenetic patterns. Once selection has acted on epigenetically-based phenotypic variation, epigenetic patterns might be maintained for numbers of generations (e.g. bison DNA shows stable patterns of methylation between ancient and contemporary DNA samples; Llamas et al., 2012).

An important issue is whether epigenetic changes could directly contribute to reproductive isolation. Initially, during ecological speciation, this might occur through direct or indirect selection on reproductive traits (Rundle and Schluter, 2004). This potentially includes 'magic traits' if epigenetic marking of genes underlying ecological traits would also influence reproductive traits. Epigenetic marks can play a role in mediating environmentally dependent behaviours (reviewed in Ledón-Rettig et al., 2013) and thus could influence speciation through pre-mating reproductive isolation. Further, epigenetic incompatibilities may occur through environmental induction or accumulate over time between divergent environments, leading to population divergence (Jablonka, 2012b). Incompatibilities that arise through exposure to alternative environments might lead to reduced hybrid fitness, inviability or sterility. This could be especially true if divergent epigenetic patterns occur on the sex chromosomes, which are likely targets of genes imprinted due to sexual conflict (Crespi and Nosil, 2013; Vrana, 2007). For example, the

Igf2 gene is imprinted with DNA methylation patterns in the gametes, which are specific to the sex of the parent from which it originates. Crosses between two rodent species, *Peromyscus polionotus* and *P. maniculatus*, result in imprinting disruption in the hybrid offspring at a number of genes, including *Igf2* (Vrana et al., 1998) and this is accompanied by growth defects in the hybrids.

Epigenetic incompatibilities may also arise through genetic changes between diverging populations (reviewed in Michalak, 2009). For example, epigenetic incompatibility between strains of *A. thaliana* can occur through gene duplication events leading to the transposition of genes and a re-patterning of DNA methylation (Durand et al., 2012). Epigenetic incompatibilities have been found in plants both within (Tarutani et al., 2010) and between (Ishikawa and Kinoshita, 2009) species. However, there is little direct evidence as yet to suggest that ecologically induced epigenetic variation frequently leads to incompatibility and reproductive isolation.

4 Potential Experimental Systems

Much knowledge has been gained through the examination of epigenetics in *A. thaliana*, and it is possible that some of this data can be generalized to non-plant species. However, plants have slightly different mechanisms for the production of epigenetic marks (Goll and Bestor, 2005), thus it is necessary to consider mechanisms within animals separately. Clonal organisms such as *Daphnia* have the potential to be powerful organisms for the study of epigenetic variation in evolution and speciation because they can reproduce clonally or sexually (Harris et al., 2012). Sex determination and sexual reproduction in *Daphnia* species are epigenetically determined and there is much ecological, morphological and physiological data on their response to a variety of environmental inducers, including predators (Weider and Pijanowska, 1993) and toxic conditions (Vandegheuchte and Janssen, 2011). In addition, one species has recently had their genome sequenced (*Daphnia pulex*; Colbourne et al., 2011), transgenic techniques exist for genetic manipulations and some epigenetic information has already been gathered in terms of DNA methylation and histone modification data (Robichaud et al., 2012; Vandegheuchte et al., 2009).

Other species need to be examined to understand whether a role for epigenetic changes in ecology and evolution might be widespread. Social insect caste determination has recently been demonstrated to involve epigenetic changes (Chittka et al., 2012). *Drosophila*

species are one of the 'Dmmt2 only' species that have only one DNA methyltransferase in their genome, suggesting either a lesser role of epigenetics, or a role for other mechanisms of epigenetic variation (Krauss and Reuter, 2011). The nematode worm *Caenorhabditis elegans* has gained attention, with recent work uncovering the role of chromatin modification and induced epigenetic inheritance associated with longevity (Greer et al., 2011). Thus a number of species have the potential to give insight into evolutionary epigenetics. To examine ecological speciation, the threespine stickleback *Gasterosteus aculeatus* might prove a useful system because populations evolve rapidly from marine to freshwater ecotypes in response to the environment (Bell and Foster, 1994) leading to reproductive isolation, with evidence indicating that plasticity and genetic accommodation facilitate this divergence (Wund et al., 2008). A role of plasticity in morph differentiation in lacustrine fish has been well documented (Skúlason et al., 1999), but how such variation might lead to stable distinct morphs is poorly understood, and remains an important empirical challenge.

5 The Future of Epigenetics in Ecological Speciation

Epigenetic mechanisms perform a number of different biological roles and it is likely that the primary importance of epigenetic marks in evolution is as a mechanism underlying developmental plasticity, which is a key to survival in novel environments. However, there is accumulating evidence that epigenetic marks can be stably inherited. Although some evidence for induced epigenetic inheritance exists, more evidence is certainly required to determine how widespread this is across organisms and how it may therefore contribute to adaptation and ecological speciation.

Future work examining the role of epigenetics in speciation should try to partition the roles of different epigenetic mechanisms and assess how they contribute to evolutionary change. For instance, which marks underlie adaptive environmentally induced variation and are they stably inherited? How frequently does induced epigenetic inheritance occur in response to environmental change? What is the role of genetically dependent compared to independent epigenetic variation and how do they differ in terms of inducing phenotypic variation? How much influence does feedback from epigenetic variation have on genetic variation and evolution? Empirical studies are required to examine these questions across a range of different organisms, and

emerging fields such as population epigenetics will be essential for such investigations. The most fully developed approaches for detecting genomic effects on ecological speciation seeks outliers in genomic differentiation (Feder and Nosil, 2010). It remains to be seen if such regions may sometimes involve aspects of the machinery of epigenetic regulation, or be the targets of such effects, but it also seems timely that studies of ecological speciation should incorporate gene expression variation and the potential for epigenetic marking of key genes (Smith et al., 2013).

Alongside empirical work we also need to build a base of theoretical knowledge on how epigenetic-based traits evolve. Epigenetic changes are reversible and environmentally sensitive, thus the dynamics of evolving epigenetic-based phenotypes will be different from that of genetic traits. Additionally, there is the complexity derived from the feedback between genetic and epigenetic variation. This complexity is only now being dissected, and processes such as conditional changes in one locus caused by another (analogous to gene conversion; Jablonka, 2012b) or epigenetic regulation of transposable elements (leading to change in phenotypic variation; Rebollo et al., 2010) are still far from understood. However, from emerging results it is clear that the context of epigenetic variation is important. Some epigenetic marks are important for developmental plasticity, yet may not be passed to the next generation. Alternatively, some marks may be heritable but show variable patterns in genomes across individuals (VMRs), or demonstrate more concerted differences (DMRs), often dependent on genomic position. Some marks are sensitive to environmental change and others are not. Thus environmental context, genomic context, biological role and heritability are key elements for understanding the role of epigenetic marks in adaptation and speciation.

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References

- Adams S, Vinkenoog R, Spielman M, Dickinson HG, Scott RJ, 2000. Parent-of-origin effects on seed development in *Arabidopsis thaliana* require DNA methylation. *Development* 127: 2493–2502.
- Adams CE, Huntingford FA, 2004. Incipient speciation driven by phenotypic plasticity? Evidence from sympatric populations of Arctic charr. *Biological Journal of the Linnean Society* 81: 611–618.
- Anway MD, Cupp AS, Uzumcu M, Skinner MK, 2005. Epige-

- netic transgenerational actions of endocrine disruptors and male fertility. *Science* 308: 1466–1469.
- Becker C, Hagmann J, Muller J, Koenig D, Stegle O et al., 2011. Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome. *Nature* 480: 245–249.
- Bell MA, Foster SA, 1994. *The Evolutionary Biology of the Threespine Stickleback*. Oxford: Oxford University Press.
- Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A, 2009. An operational definition of epigenetics. *Genes & Development* 23: 781–783.
- Bird A, 2007. Perceptions of epigenetics. *Nature* 447: 396–398.
- Bossdorf O, Richards CL, Pigliucci M, 2008. Epigenetics for ecologists. *Ecology Letters* 11: 106–115.
- Butlin RK, 2010. Population genomics and speciation. *Genetica* 138: 409–418.
- Carja O, Feldman MW, 2012. An equilibrium for phenotypic variance in fluctuating environments owing to epigenetics. *Journal of the Royal Society Interface* 9: 613–623.
- Charlesworth B, Nordborg M, Charlesworth D, 1997. The effects of local selection, balanced polymorphism and background selection on equilibrium patterns of genetic diversity in subdivided populations. *Genetical Research* 70: 155–174.
- Chittka A, Wurm Y, Chittka L, 2012. Epigenetics: The making of ant castes. *Current Biology* 22: R836–R838.
- Colbourne JK, Pfrender ME, Gilbert D, Thomas WK, Tucker A et al., 2011. The ecoresponsive genome of *Daphnia pulex*. *Science* 331: 555–561.
- Cooney CA, Dave AA, Wolff GL, 2002. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. *Journal of Nutrition* 132: 2393S–2400S.
- Crespi B, Nosil P, 2013. Conflictual speciation: Species formation via genomic conflict. *Trends in Ecology & Evolution* 28: 48–57.
- Crews D, Gillette R, Scarpino SV, Manikkam M, Savenkova MI et al., 2012. Epigenetic transgenerational inheritance of altered stress responses. *Proceedings of the National Academy of Sciences, USA* 109: 9143–9148.
- Daxinger L, Whitelaw E, 2012. Understanding transgenerational epigenetic inheritance via the gametes in mammals. *Nature Reviews Genetics* 13: 153–162.
- De Boer P, Ramos L, De Vries M, Gochhait S, 2010. Memoirs of an insult: Sperm as a possible source of transgenerational epimutations and genetic instability. *Molecular Human Reproduction* 16: 48–56.
- Dickins TE, Rahman Q, 2012. The extended evolutionary synthesis and the role of soft inheritance in evolution. *Proceedings of the Royal Society of London, Series B: Biological Sciences* 279: 2913–2921.
- Dimitri P, Junakovic N, 1999. Revising the selfish DNA hypothesis: New evidence on accumulation of transposable elements in heterochromatin. *Trends in Genetics* 15: 123–124.
- Duckworth RA, 2012. Epigenetic inheritance systems act as a bridge between ecological and evolutionary timescales. *Behavioral Ecology* 24: 327–328.
- Durand S, Bouché N, Perez Strand E, Loudet O, Camilleri C, 2012. Rapid establishment of genetic incompatibility through natural epigenetic variation. *Current Biology* 22: 326–331.
- Feder JL, Nosil P, 2010. The efficacy of divergence hitchhiking in generating genomic islands during ecological speciation. *Evolution* 64: 1729–1747.
- Feinberg AP, Irizarry RA, 2010. Stochastic epigenetic variation as a driving force of development, evolutionary adaptation, and disease. *Proceedings of the National Academy of Sciences, USA* 107: 1757–1764.
- Fitzpatrick BM, 2012. Underappreciated consequences of phenotypic plasticity for ecological speciation. *International Journal of Ecology* doi:10.1155/2012/256017.
- Flores KB, Amdam GV, 2011. Deciphering a methylome: What can we read into patterns of DNA methylation? *The Journal of Experimental Biology* 214: 3155–3163.
- Franklin TB, Mansuy IM, 2010. Epigenetic inheritance in mammals: Evidence for the impact of adverse environmental effects. *Neurobiology of Disease* 39: 61–65.
- Gendall AR, Levy YY, Wilson A, Dean C, 2001. The VERNALIZATION 2 gene mediates the epigenetic regulation of vernalization in *Arabidopsis*. *Cell* 107: 525–535.
- Gibson G, Dworkin I, 2004. Uncovering cryptic genetic variation. *Nature Reviews Genetics* 5: 681–690.
- Goll MG, Bestor TH, 2005. Eukaryotic cytosine methyltransferases. *Annual Review of Biochemistry* 74: 481–514.
- Greer EL, Maures TJ, Ucar D, Hauswirth AG, Mancini E et al., 2011. Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*. *Nature* 479: 365–371.
- Grewal SIS, Moazed D, 2003. Heterochromatin and epigenetic control of gene expression. *Science* 301: 798–802.
- Haig D, 2007. Weismann rules! OK? Epigenetics and the Lamarckian temptation. *Biology and Philosophy* 22: 415–428.
- Hall MC, Basten CJ, Willis JH, 2006. Pleiotropic quantitative trait loci contribute to population divergence in traits associated with life history variation in *Mimulus guttatus*. *Genetics* 172: 1829–1844.
- Harris KD, Bartlett NJ, Lloyd VK, 2012. *Daphnia* as an emerging epigenetic model organism. *Genetics Research International* 2012. doi:10.1155/2012/147892
- Hendry AP, 2004. Selection against migrants contributes to the rapid evolution of ecologically dependent reproductive isolation. *Evolutionary Ecology Research* 6: 1219–1236.
- Ishikawa R, Kinoshita T, 2009. Epigenetic programming: The challenge to species hybridization. *Molecular Plant* 2: 589–599.
- Jablonka E, 2012a. Epigenetic inheritance and plasticity: The responsive germline. *Progress in Biophysics & Molecular Biology* 111: 99–107.
- Jablonka E, 2012b. Epigenetic variations in heredity and evolution. *Clinical Pharmacology & Therapeutics* 92: 683–688.
- Jablonka E, Lamb MJ, 1995. *Epigenetic Inheritance and Evolution: The Lamarckian Dimension*. Oxford: Oxford University Press.
- Jablonka E, Oborny B, Molnar I, Kisdi E, Hofbauer J et al., 1995. The adaptive advantage of phenotypic memory in changing environments. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 350: 133–141.
- Jaenisch R, 1997. DNA methylation and imprinting: Why bother? *Trends in Genetics* 13: 323–329.
- Jaenisch R, Bird A, 2003. Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. *Nature Genetics* 33: 245–254.
- Jiang L, Zhang J, Wang J-J, Wang L, Zhang L et al., 2013. Sperm, but not oocyte, DNA methylome is inherited by zebrafish early embryos. *Cell* 153: 773–784.

- Johannes F, Colot V, Jansen RC, 2008. Epigenome dynamics: A quantitative genetics perspective. *Nature Reviews Genetics* 9: 883–890.
- Johannes F, Porcher E, Teixeira FK, Saliba-Colombani V, Simon M et al., 2009. Assessing the impact of transgenerational epigenetic variation on complex traits. *Plos Genetics* 5: e1000530.
- Johnson LJ, Tricker PJ, 2010. Epigenomic plasticity within populations: Its evolutionary significance and potential. *Heredity* 105: 113–121.
- Krauss V, Reuter G, 2011. DNA methylation in *Drosophila*: A critical evaluation. *Progress in Molecular Biology and Translational Science* 101: 177–191.
- Laird PW, Jaenisch R, 1994. DNA methylation and cancer. *Human Molecular Genetics* 3: 1487–1495.
- Lande R, 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology* 22: 1435–1446.
- Le Rouzic A, Carlborg O, 2008. Evolutionary potential of hidden genetic variation. *Trends in Ecology & Evolution* 23: 33–37.
- Ledón-Rettig CC, Richards CL, Martin LB, 2012. Epigenetics for behavioral ecologists. *Behavioral Ecology* 24: 311–324.
- Levine MT, Eckert ML, Begun DJ, 2011. Whole-genome expression plasticity across tropical and temperate *Drosophila melanogaster* populations from Eastern Australia. *Molecular Biology and Evolution* 28: 249–256.
- Llamas B, Holland ML, Chen K, Cropley JE, Cooper A et al., 2012. High-resolution analysis of cytosine methylation in ancient DNA. *PLoS One* 7: e30226.
- Luco RF, Allo M, Schor IE, Kornbliht AR, Misteli T, 2011. Epigenetics in alternative pre-mRNA splicing. *Cell* 144: 16–26.
- Luco RF, Pan Q, Tominaga K, Blencowe BJ, Pereira-Smith OM et al., 2010. Regulation of alternative splicing by histone modifications. *Science* 327: 996–1000.
- Lundsgaard-Hansen B, Matthews B, Vonlanthen P, Taverna A, Seehausen O, 2013. Adaptive plasticity and genetic divergence in feeding efficiency during parallel adaptive radiation in whitefish (*Coregonus* spp.). *Journal of Evolutionary Biology* 26: 483–498.
- Martin CC, McGowan R, 1995. Parent-of-origin specific effects on the methylation of a transgene in the zebrafish *Danio rerio*. *Developmental Genetics* 17: 233–239.
- Michalak P, 2009. Epigenetic, transposon and small RNA determinants of hybrid dysfunctions. *Heredity* 102: 45–50.
- Nosil P, Harmon LJ, Seehausen O, 2009b. Ecological explanations for (incomplete) speciation. *Trends in Ecology & Evolution* 24: 145–156.
- Nosil P, Vines TH, Funk DJ, 2005. Perspective: Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution* 59: 705–719.
- Nosil P, Funk DJ, Ortiz-Barrientos D, 2009a. Divergent selection and heterogeneous genomic divergence. *Molecular Ecology* 18: 375–402.
- Nosil P, 2012. *Ecological Speciation*. Oxford: Oxford University Press.
- Nylin S, Janz N, 2009. Butterfly host plant range: An example of plasticity as a promoter of speciation? *Evolutionary Ecology* 23: 137–146.
- Ólafsdóttir GA, Ritchie MG, Snorrason SS, 2006. Positive assortative mating between recently described sympatric morphs of Icelandic sticklebacks. *Biology Letters* 2: 250–252.
- Olvido AE, Mousseau TA, 1995. Effect of rearing environment on calling-song plasticity in the striped ground cricket. *Evolution* 49: 1271–1277.
- Ossowski S, Schneeberger K, Lucas-Lledo JI, Warthmann N, Clark RM et al., 2010. The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. *Science* 327: 92–94.
- Pal C, Miklós I, 1999. Epigenetic inheritance, genetic assimilation and speciation. *Journal of Theoretical Biology* 200: 19–37.
- Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD et al., 2010. Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology & Evolution* 25: 459–467.
- Pigliucci M, Murren CJ, Schlichting CD, 2006. Phenotypic plasticity and evolution by genetic assimilation. *The Journal of Experimental Biology* 209: 2362–2367.
- Price TD, Qvarnstrom A, Irwin DE, 2003. The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London Series B-Biological Sciences* 270: 1433–1440.
- Rebollo R, Horard B, Hubert B, Vieira C, 2010. Jumping genes and epigenetics: Towards new species. *Gene* 454: 1–7.
- Richards CL, Bossdorf O, Pigliucci M, 2010. What role does heritable epigenetic variation play in phenotypic evolution? *Bioscience* 60: 232–237.
- Richards EJ, 2006. Inherited epigenetic variation: Revisiting soft inheritance. *Nature Reviews Genetics* 7: 395–401.
- Richards EJ, 2011. Natural epigenetic variation in plant species: A view from the field. *Current Opinion in Plant Biology* 14: 204–209.
- Richards EJ, 2008. Population epigenetics. *Current Opinion in Genetics & Development* 18: 221–226.
- Robichaud NF, Sassine J, Beaton MJ, Lloyd VK, 2012. The epigenetic repertoire of *Daphnia magna* includes modified histones. *Genetics Research International* doi:10.1155/2012/174860.
- Roux F, Colomé-Tatché M, Edelist C, Wardenaar R, Guerche P et al., 2011. Genome-wide epigenetic perturbation jump-starts patterns of heritable variation found in nature. *Genetics* 188: 1015–1017.
- Rundle HD, Nosil P, 2005. Ecological speciation. *Ecology Letters* 8: 336–352.
- Rundle HD, Schluter D, 2004. Natural selection and ecological speciation in sticklebacks. In: Dieckmann U, Doebeli M, Metz J, Tautz D ed. *Adaptive Speciation*. Cambridge: Cambridge University Press, 192–209.
- Schlichting CD, 2008. Hidden reaction norms, cryptic genetic variation, and evolvability. *Annals of the New York Academy of Sciences* 1133: 187–203.
- Schlichting CD, Pigliucci M, 1993. Control of phenotypic plasticity via regulatory genes. *American Naturalist* 142: 366–370.
- Schlichting CD, Smith H, 2002. Phenotypic plasticity: Linking molecular mechanisms with evolutionary outcomes. *Evolutionary Ecology* 16: 189–211.
- Schluter D, 2001. Ecology and the origin of species. *Trends in Ecology & Evolution* 16: 372–380.
- Schluter D, 2009. Evidence for ecological speciation and its alternative. *Science* 323: 737–741.

- Schmitz RJ, Schultz MD, Lewsey MG, O'Malley RC, Urich MA et al., 2011. Transgenerational epigenetic instability is a source of novel methylation variants. *Science* 334: 369–373.
- Scoville AG, Pfrender ME, 2010. Phenotypic plasticity facilitates recurrent rapid adaptation to introduced predators. *Proceedings of the National Academy of Sciences, USA* 107: 4260–4263.
- Seong KH, Li D, Shimizu H, Nakamura R, Ishii S, 2011. Inheritance of stress-induced, ATF-2-dependent epigenetic change. *Cell* 145: 1049–1061.
- Shugart LR, 1990. 5-Methyl deoxycytidine content of DNA from bluegill sunfish *Lepomis macrochirus* exposed to benzo[a]pyrene. *Environmental Toxicology & Chemistry* 9: 205–208.
- Skinner MK, 2011. Environmental epigenetic transgenerational inheritance and somatic epigenetic mitotic stability. *Epigenetics* 6: 838–842.
- Skúlason S, Snorrason SS, Jónsson B, 1999. Sympatric morphs, populations and speciation in freshwater fish with emphasis on Arctic charr. In: Magurran AE, May RM ed. *Evolution and Biological Diversity*. Oxford: Oxford University Press, 70–92.
- Smadja C, Galindo J, Butlin R, 2008. Hitching a lift on the road to speciation. *Molecular Ecology* 17: 4177–4180.
- Smith G, Fang Y, Liu X, Kenny J, Cossins A et al., 2013. Transcriptome-wide expression variation associated with environmental plasticity and mating success in cactophilic *Drosophila mojavensis*. *Evolution* 67: 1950–1963.
- Sobel JM, Chen GF, Watt LR, Schemske DW, 2010. The biology of speciation. *Evolution* 64: 295–315.
- Stauffer JR, van Snik Gray E, 2004. Phenotypic plasticity: Its role in trophic radiation and explosive speciation in cichlids (Teleostei: Cichlidae). *Animal Biology* 54: 137–158.
- Stern S, Fridmann-Sirkis Y, Braun E, Soen Y, 2012. Epigenetically heritable alteration of fly development in response to toxic challenge. *Cell Reports*. 1: 528–542.
- Sved J, Bird A, 1990. The expected equilibrium of the CpG dinucleotide in vertebrate genomes under a mutation model. *Proceedings of the National Academy of Sciences, USA* 87: 4692–4696.
- Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H et al., 2009. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 324: 930–935.
- Takiguchi M, Achanzar WE, Qu W, Li G, Waalkes MP, 2003. Effects of cadmium on DNA-(Cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation. *Experimental Cell Research* 286: 355–365.
- Tarutani Y, Shiba H, Iwano M, Kakizaki T, Suzuki G et al., 2010. Trans-acting small RNA determines dominance relationships in *Brassica* self-incompatibility. *Nature* 466: 983–986.
- Thibert-Plante X, Hendry AP, 2011. The consequences of phenotypic plasticity for ecological speciation. *Journal of Evolutionary Biology* 24: 326–342.
- Turner TL, Hahn MW, Nuzhdin SV, 2005. Genomic islands of speciation in *Anopheles gambiae*. *PLoS Biology* 3: 1572–1578.
- Unckless RL, Orr HA, 2009. Dobzhansky-Muller incompatibilities and adaptation to a shared environment. *Heredity* 102: 214–217.
- Vandegheuchte MB, Janssen CR, 2011. Epigenetics and its implications for ecotoxicology. *Ecotoxicology* 20: 607–624.
- Vandegheuchte MB, Lemiere F, Janssen CR, 2009. Quantitative DNA-methylation in *Daphnia magna* and effects of multi-generation Zn exposure. *Comparative Biochemistry & Physiology Part C: Toxicology & Pharmacology* 150: 343–348.
- Vaughn MW, Tanurdzic M, Lippman Z, Jiang H, Carrasquillo R, et al., 2007. Epigenetic natural variation in *Arabidopsis thaliana*. *PLoS Biology* 5: 1617–1629.
- Verzijden MN, ten Cate C, Servedio MR, Kozak GM, Boughman JW et al., 2012. The impact of learning on sexual selection and speciation. *Trends in Ecology & Evolution* 27: 511–519.
- Via S, West J, 2008. The genetic mosaic suggests a new role for hitchhiking in ecological speciation. *Molecular Ecology* 17: 4334–4345.
- Vrana PB, Guan XJ, Ingram RS, Tilghman SM, 1998. Genomic imprinting is disrupted in interspecific *Peromyscus* hybrids. *Nature Genetics* 20: 362–365.
- Vrana PB, 2007. Genomic imprinting as a mechanism of reproductive isolation in mammals. *Journal of Mammalogy* 88: 5–23.
- Waddington CH, 1942. Canalization of development and the inheritance of acquired characters. *Nature* 150: 563–565.
- Waddington CH, 1953. Genetic assimilation of an acquired character. *Evolution* 7: 118–126.
- Walter RB, Li HY, Intano GW, Kazianis S, Walter CA, 2002. Absence of global genomic cytosine methylation pattern erasure during medaka *Oryzias latipes* early embryo development. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 133: 597–607.
- Weaver ICG, Cervoni N, Champagne FA, D'Alessio AC, Sharma S et al., 2004. Epigenetic programming by maternal behavior. *Nature Neuroscience* 7: 847–854.
- Weider LJ, Pijanowska J, 1993. Plasticity of *Daphnia* life histories in response to chemical cues from predators. *Oikos* 67: 385–392.
- West-Eberhard MJ, 2003. *Developmental Plasticity and Evolution*. Oxford: Oxford University Press.
- West-Eberhard MJ, 2005. Developmental plasticity and the origin of species differences. *Proceedings of the National Academy of Sciences, USA* 102: 6543–6549.
- West-Eberhard MJ, 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* 20: 249–278.
- Wu SD, Zhu J, Li YS, Lin T, Gan LQ et al., 2010. Dynamic epigenetic changes involved in testicular toxicity induced by Di-2-(ethyl-hexyl) phthalate in mice. *Basic & Clinical Pharmacology & Toxicology* 106: 118–123.
- Wund MA, 2012. Assessing the impacts of phenotypic plasticity on evolution. *Integrative Computational Biology* 52: 5–15.
- Wund MA, Baker JA, Clancy B, Golub JL, Foster SA, 2008. A test of the "Flexible stem" model of evolution: Ancestral plasticity, genetic accommodation, and morphological divergence in the threespine stickleback radiation. *American Naturalist* 172: 449–462.
- Zhang YY, Fischer M, Colot V, Bossdorf O, 2013. Epigenetic variation creates potential for evolution of plant phenotypic plasticity. *New Phytologist* 197: 314–322.