Review

Cell PRESS

Transgenerational defense induction and epigenetic inheritance in plants

Liza M. Holeski[1], Georg Jander[2] and Anurag A. Agrawal[3]

[1] University of Wisconsin-Madison, Department of Entomology, Madison, WI 53706, USA
[2] Boyce Thompson Institute for Plant Research, Ithaca, NY 14853, USA
[3] Cornell University, Department of Ecology and Evolutionary Biology, and Department of Entomology, Ithaca, NY 14853, USA

Rapidly accumulating evidence shows that herbivore and pathogen attack of plants can generate particular defense phenotypes across generations. What was once thought to be an oddity of plant defense induction now appears to be a taxonomically widespread phenomenon with strong potential to impact the ecology and evolution of species interactions. DNA methylation, histone modifications, and small RNAs each contribute to transgenerational defense initiation; examples in several species demonstrate that this induction can last for multiple generations. Priming of the offspring generation for more rapid induction following subsequent attack has also been reported. The extent to which transgenerational induction is predictable, detectable in nature, and subject to manipulation will determine the ability of researchers to decipher its role in plant–herbivore and plant–pathogen interactions.

Genotype, environment, and. . . ?

In *Philosophie Zoologique*, Jean-Baptiste Lamarck [1], proposed two laws: that organisms change adaptively in response to the environment, and that such changes are heritable. Phenotypic plasticity in response to environmental heterogeneity occurs abundantly in nature, is often adaptive, and has become a central concept in modern ecology and evolutionary biology. Particularly in sessile organisms, such as plants, which cannot move to favorable environments, within-generation plastic responses to biotic and abiotic stresses are ubiquitous [2–6]. Lamarck's second law, which focused on heritable changes resulting from the use or disuse of macroscopic organs, was discredited during the development of the theory of evolution by natural selection and Mendelian inheritance via specific germ-line cells. Nevertheless, recent examples of transgenerational effects on progeny phenotypes [7–13], as well as advances in the study of molecular mechanisms that can mediate such inheritance of acquired traits [14–18], suggest that Lamarck had greater insight into the topic than is generally recognized.

For ecologists and evolutionary biologists interested in understanding the sources of phenotypic variation, transgenerational responses are an important addition to the typical focus on environmental effects within a single generation and the effects of natural selection mediated via classical Mendelian inheritance. Transgenerational induction, as we define it here (see Glossary), is a change in offspring phenotype that is cued by an environmental signal in the parental generation, and is expressed independently of changes in the offspring genotype. Such effects can occur through maternal and/or epigenetic effects, two phenomena that differ in both mechanism and outcome. Maternal effects, whereby the maternal genotype influences fitness irrespective of the progeny genotype, have been investigated for decades, mostly through studies emphasizing the effects of offspring size and nutrient provisioning by the mother [19,20]. For example, maternal effects on seed size, which influence germination characteristics and seedling size, have been found in multiple species [19]. In plants, the maternal tissue of the seed coat can also provide a means for inheritance of maternal resources, or even regulation by maternally encoded genes, without actual transfer of metabolites to the embryo or endosperm. The relatively recent inclusion of epigenetic modifications, which allow vertical transmission of acquired traits without alteration of the underlying DNA sequence (e.g., DNA methylation

Glossary

Constitutive defense: plant defensive traits that are expressed before attack (induction) by herbivores or pathogens.
Epigenetic changes: heritable changes in phenotype that are mediated by mechanisms other than alterations in the DNA sequence. Stable epigenetic modifications, which can be inherited both maternally and paternally, can occur through self-reinforcing mechanisms that include DNA methylation, histone modifications, and RNAi.
Epigenotype: the stable pattern of epigenetic marks that is outside the actual DNA sequence. Variation among epigenotypes might also be called 'heritable epigenetic variation' or 'transgenerational epigenetic variation'.
Induced defense: the upregulation of plant defense following attack by herbivores or pathogens. This can involve the enhancement of constitutive defenses or the production of novel defenses.
Maternal effect: the genotype of the maternal organism dictates the progeny phenotype, irrespective of the progeny genotype. This can occur through provisioning of the seeds with nutrients, protein, or small RNAs. Maternal effects can also be propagated via the maternal tissue in the seed coat.
Maternal environmental effect: irrespective of the maternal genotype, the maternal environment influences the progeny phenotype. This can occur via epigenetic changes in the maternal genome that are transmitted to the progeny.
Priming: a condition whereby plants that have been subjected to prior attack will respond more quickly or more strongly to a subsequent attack. Given that resources are not committed until the threat returns, priming is thought to be a relatively low-cost mechanism of advancing plant defense.
Tolerance: the degree to which plant fitness is affected by herbivore damage relative to fitness in the undamaged state [88].
Transgenerational induction: a change in offspring phenotype that is cued by an environmental signal in the parental generation, and is expressed independently of changes in the offspring genotype. It can occur via epigenetic inheritance or due to maternal effects.

Corresponding author: Holeski, L.M. (holeski@wisc.edu).

0169-5347/$ – see front matter http://dx.doi.org/10.1016/j.tree.2012.07.011 Trends in Ecology and Evolution xx (2012) 1–9

[21–24]), into theories of evolutionary ecology and genetics has brought new vigor to research on transgenerational inheritance in plants and other organisms [7,25–30]. For instance, a recent issue of *Plant Physiology* included three publications detailing the role of epigenetic mechanisms in transgenerational induction of defenses in *Arabidopsis* (*Arabidopsis thaliana*) and tomato (*Solanum lycopersicum*) [31–33].

In many cases, transgenerational effects occur via unspecified mechanisms, and it is unclear whether they are caused by maternal provisioning or epigenetic mechanisms, or both. Nonetheless, a key component of epigenetic inheritance is the self-propagating nature of the signal through DNA modification (Box 1). Thus, epigenetic signals potentially can be transmitted through more than one generation and might not be evidenced by changes in seed provisioning. Such epigenetic effects are fascinating and perhaps counterintuitive, but can be adaptive if the environmental cues received by the parents are predictive of the environment that will be experienced by their progeny [34–36]. In a manner similar to random genetic mutations, random epigenetic DNA changes may also be subject to selection that provides fortuitous adaptation to variation in the natural environment.

Transgenerational effects have been proposed to impact population dynamics (e.g., [37]), community interactions [38], and the evolutionary potential of species [35,39]. However, there has not yet been a strong connection between mechanistic studies and efforts to understand the ecological and evolutionary implications in natural environments. In this review, we focus on the transgenerational induction of plant resistance to insect and microbial attack. Plant phenotypic plasticity in responses to herbivores and pathogens is often adaptive, with well-understood signaling mechanisms, gene expression responses, and quantifiable phenotypes. Moreover, several recent studies not only demonstrate transgenerational induction of defense responses, but also implicate underlying epigenetic mechanisms [31–33,40,41]. These remarkable discoveries suggest that the current genotype–environment framework for understanding plant defense needs expansion.

Box 1. Mechanisms of epigenetic effects on the phenotype

The mechanisms outlined below have a wide temporal spectrum of consequences, and are not necessarily independent of one another.

DNA methylation

Cytosine methylation, most commonly detected at CG, CHG, and CHHDNA sequence sites in plants, influences gene expression by altering transcription or chromatin structure. Once DNA has been methylated, the methylation state can be maintained during DNA replication in both mitotic and meiotic cell division [89–91]. Thus, because the DNA methylation state can change in response to biotic stress during the vegetative growth, it provides a medium for epigenetic inheritance and effects on gene expression that are maintained from one generation to the next [7].

Histone modification

DNA in eukaryotic cells is tightly packed around histone octamers. The structure of chromatin (i.e., the combination of DNA and histone proteins) can make DNA more or less accessible for transcription. Different post-translational modifications of histone proteins, in particular methylation (lysine and arginine) and acetylation (lysine) in the *N*-terminal segment, influence the local chromatin structure and can have both positive and negative effects on gene expression [14]. DNA methylation and histone modification are closely intertwined, and it has not yet been determined whether one or the other is causal [92].

Small RNA

miRNA and siRNA are short RNA molecules, typically less than 25 nucleotides in length, that influence gene expression through targeted degradation of mRNA or induction of methylation at complementary DNA sequences. By altering DNA methylation, small RNA can trigger epigenetic inheritance. Although both miRNA and siRNA can regulate gene expression, they differ in their biogenesis and functional effects [7,14].

Enhanced homologous recombination

Homologous recombination can result in DNA sequence inversions, deletions, duplications, or translocations. In the case of duplicated genes, recombination between paralogs can alter patterns of gene expression. Thus, enhanced levels of homologous recombination can repair damaged DNA and/or create increased genetic flexibility (via mutations) in stressful environments [93,94]. By promoting homologous recombination and/or transposition, epigenetic changes also can lead to stably inherited genetic changes [18].

Transgenerational plant resistance: current state of the field

Although it had long been known that environmental factors impact offspring phenotypes [19], until the early 1980s there was no systematic effort to study the transgenerational impacts of herbivory and pathogen attack (Table 1). In 1983, a preliminary study by Roberts [42] showed that inoculation with *tobacco mosaic virus* (TMV) induced resistance in the progeny of infected tobacco (*Nicotiana tabacum*) compared with the progeny of uninfected plants. Other studies demonstrated that plants attacked by herbivores or pathogens produced seeds containing higher concentrations of defense compounds compared with control plants that were not infested [43,44]. These studies provided a proof of concept, but did not identify ecological consequences of transgenerational induction. A series of papers at the turn of the millennium demonstrated that insect herbivory during the vegetative phase of wild radish (*Raphanus raphanistrum*), induced resistance in young seedlings of progeny plants [38,45,46]. This transgenerational effect was triggered by lepidopteran herbivory or foliar application of jasmonic acid, but not leaf clipping. Although there were fitness impacts of this transgenerational induction when plants were grown in the field, no mechanism was identified and the observed increase in resistance was transient (i.e., the effect was relaxed at the second true leaf stage). Nevertheless, these experiments provided a foundation upon which more mechanistic studies could be built.

Recent publications contain a rapidly growing number of examples of transgenerational induction of resistance to pathogens and herbivores (Table 1). It has been confirmed that TMV infection of tobacco causes increased resistance in the progeny generation, not only against the virus, but also against bacterial (*Pseudomonas syringae*) and oomycete (*Phytophthora nicotianae*) pathogens [40]. Similar effects were seen with *Arabidopsis*, where *P. syringae* infection enhanced resistance in the next generation to both *P. syringae* and an oomycete, *Hyaloperonospora*

Table 1. Overview of studies demonstrating transgenerational induction of resistance to herbivores or pathogens[a]

Plant species	Year	Parental treatment	Offspring measure	Generations assayed (effect detected)	Refs
Arabidopsis thaliana (Brassicaceae)	2002	*Peronospora parasitica*, chemical elicitors INA and BTH; treatments applied to seedlings	Homologous recombination	1(1)	[98]
	2006	Flagellin (a bacterial elicitor of plant defenses); treatments applied to seedlings	Homologous recombination	4(1)	[94]
	2012	*Pseudomonas syringae* pv. *tomato* (DC3000); treatments applied to seedling rosettes	Priming state of SA- and JA-inducible genes, resistance to pathogens, seed mass, and offspring growth	2(2)	[32]
	2012	*Pieris rapae* and other lepidopteran herbivory during vegetative phase	Herbivore performance (multiple species), glucosinolates, and seed characteristics	3(2)	[33]
	2012	Seedlings primed with β-aminobutyric acid (BABA), avirulent *P. syringae* pv. *tomato* (DC3000 *avrRpt2*)	Gene expression within SA pathway, resistance to virulent *P. syringae*, and resistance to *Hyaloperonospora arabidopsidis*	2(1)	[31]
Hordeum vulgare (Poaceae)	2012	Vegetative plants sprayed with ASM or saccharin	Relative growth rate, and resistance to *Rhynchosporium commune*	1(1)	[58]
Lotus wrangelianus (Fabaceae)	2012	Herbivory (field) throughout life time of plant	Field assay of herbivory resistance	1(1)	[60]
Mimulus guttatus (Phrymaceae)	2007	Simulated herbivory during vegetative and reproductive phases	Trichomes	1(1)	[47]
	2011	Simulated herbivory during vegetative and reproductive phases	Trichomes	1(1)	[41]
Nicotiana attenuate (Solanaceae)	2001	MeJA application and leaf removal; treatments applied at seedling rosette phase	Nitrogen allocation to seed (defense is positively correlated with nitrogen)	1(1)	[99]
Nicotiana tabacum (Solanaceae)	1983	TMV infection of seedlings	Lesion size following challenge-inoculations of TMV	2(1)	[42]
	2010	TMV infection of seedlings	Recombination, methylation, resistance to three pathogens, gene expression (pathogenesis-related protein 1; *PR1*), and callose deposition	2(2)	[40]
Populus sp. (Salicaceae)	2012	*Chrysomela confluens* herbivory during immature fruit stage	Phytochemical defense	1(1)	Holeski *et al.*, unpublished
Raphanus raphanistrum (Brassicaceae)	1999-2002	*Pieris rapae* herbivory, JA, leaf clipping (all during the vegetation stage)	Resistance to a specialist caterpillar, seed glucosinolates, trichomes, field assay of attack and fitness	1(1)	[45,46]
Solanum lycopersicum (Solanaceae)	2012	MeJA during immature fruit stage	Resistance to *Helicoverpa zea* caterpillars	1(1)	[33]
Taraxacum officinale (Asteraceae)	2009	JA and/or SA applied to seedlings.	Methylation patterns (no phenotype identified)	1(1)	[69]
Viola cazorlensis (Violaceae)	2011	Browsing (field) throughout life time of plant	Methylation patterns and resistance (field)	Multiyear(20)	[100]

[a]Abbreviations: BTH, benzothiadiazole; ASM, acibenzolar-S-methyl; INA, 2,6-dichloroisonicotinic acid; JA, jasmonic acid; MeJA, methyl jasmonate; SA, salicylic acid; TMV, tobacco mosaic virus.

arabidopsidis [31]. In another *Arabidopsis* study, repeated *P. syringae* inoculation increased resistance in two subsequent generations [32]. Although insect feeding has been studied less extensively, caterpillar herbivory on both *Arabidopsis* and tomato increased resistance in the subsequent progeny generation [33]. Finally, simulated herbivory on monkeyflower (*Mimulus guttatus*) induced greater abundance of a physical and chemical defense, glandular leaf trichomes, in the damaged plants and also in their progeny [41,47].

These recent experiments, involving several different plant species, not only provide solid support for transgenerational induction of resistance to attack, but also suggest molecular mechanisms that might mediate the plant responses. Increased resistance to multiple pathogens [31–33,40], as well as similar transgenerational defense elicitation by more than one lepidopteran species [38], suggests a low specificity in these responses. Consistent with this observation, transgenerational resistance to attack involves regulation of the salicylate- and jasmonate-mediated defense signaling pathways, which induce broad-spectrum resistance against pathogens and herbivores, respectively. Antagonistic regulation, whereby jasmonate suppresses salicylate regulation [48] and vice versa [49], can augment plant responses. For example, plants induced with *P. syringae* had more histone H3 lysine 9 (H3K9)

acetylation at salicylate-responsive promoters, consistent with their higher transcription [32] and, conversely, a jasmonate-inducible promoter in these plants had elevated histone H3 lysine 27 (H3K27) methylation, which could repress transcription. Thus, it is conceivable, although not yet proven, that *P. syringae* infection could induce transgenerational sensitivity to herbivores.

Arabidopsis resistance to *P. syringae* was correlated with overall hypomethylation of DNA [32]. Moreover, the transgenerational resistance phenotype could be recapitulated with a *DNA methyltransferase 1* (*drm1*; GenBank accession number **NM_121542**) *drm2* (GenBank accession number **NM_121466**) *chromomethylase 3* (*cmt3*; GenBank accession number **NM_105646**) mutant [50], which has knockout mutations of three non-CG DNA methyltransferases, suggesting that the DNA methylation state by itself has a role in the inheritance [32]. Given that *Arabidopsis* is self-pollinating, these experiments did not determine whether this epigenetic inheritance of pathogen resistance occurred via the ovule or the pollen, or both.

Transgenerational resistance to lepidopteran herbivory persisted for two generations and required small RNA synthesis and processing [38]. Given that small RNA is phloem mobile and can lead to altered target-site methylation in the genome, it is conceivable that there is an induced signal that travels from vegetative tissue to developing seeds to induce stable epigenetic changes (e.g., DNA methylation) in the progeny genome [7,33]. Several additional studies, including work in non-model systems, have demonstrated impacts of herbivory or pathogen infection on genomic methylation and recombination, without measures of the offspring phenotype (Table 1).

Priming of transgenerational responses

To further unravel the mechanisms of transgenerational induction of plant defense, it is useful to distinguish between induced defenses and priming for enhanced responses to subsequent attack. Transgenerational-induced responses involve an inherently higher level of resistance after attack has occurred in the previous generation, and can come in the form of elevated accumulation of secondary metabolites or physical barriers, such as trichomes. By contrast, primed progeny plants have the same level of constitutive defenses as the progeny of plants that were not subjected to infection or herbivory in the previous generation, but the trigger for defense induction is set such that their response to subsequent attack is faster or stronger [51]. Both induced and primed responses are thought to be a cost-saving strategy and have been well studied within a single generation [52–57]. Nonetheless, transgenerational defense priming has only been described during the past few years and, yet, it appears to be general.

A few studies have now experimentally addressed how primed defense phenotypes are shaped by environmental cues in the parental generation, the offspring generation, and their interaction. Luna *et al.* [32] noted transgenerational changes in responses to both salicylate and jasmonate in progeny of pathogen-infected *Arabidopsis.* Chemical elicitation of barley (*Hordeum vulgare*) primed the subsequent generation of resistance to a fungal pathogen, *Rhynchosporium commune* [58]. In both *Arabidopsis* and tomato [33], herbivore damage in the parental generation resulted in enhanced jasmonate-responsiveness to damage in the offspring generation. Similar transgenerational priming of transcriptional responses in response to pathogen attack was also found for *Arabidopsis* [31]. Other experiments with *Arabidopsis* demonstrated that epigenetically inherited changes can strongly alter plant responses to jasmonate and salicylate [59]. Nonetheless, a reverse transgenerational priming effect was observed in wild radish; plants that were induced with jasmonate in the maternal generation showed reduced responses to caterpillars in the offspring generation [45]. Finally, in a recent field experiment with *Lotus wrangelianus*, terHorst and Lau [60] showed that both plant resistance to herbivores and plant fitness were dependent on the environment (exposure to insect herbivory) in the parental and offspring generations. Thus, although there is not universal evidence for priming across generations, it is clear that the parental environment consistently modifies the responsiveness of offspring to induction cues.

Ecological implications

The ecological impact of transgenerational induction of defenses rests upon the fact that organismal phenotypes are critical for mediating ecological interactions, and that the impact of the phenotype usually depends on the environmental context. As such, transgenerational induction may have all the impacts of phenotypic plasticity, with the added twist of delays and gaps in the expression. A few other key points are relevant. The generality or specificity in the elicitation of the transgenerationally induced defense, in combination with herbivore and host plant life history and population dynamics, will govern the impacts of the defense. For example, the life history of the plant (e.g., number of generations per growing season or degree of seed dormancy) and the life history and population dynamics of the herbivore have considerably increased importance when the transgenerational effect is specific to a particular herbivore, because a mismatch is probable.

Transgenerational defense induction, when adaptive, should affect interspecies competitive interactions and plant community dynamics. Consider, for example, the context of the pervasive, yet controversial, Janzen–Connell hypothesis, which predicts that seedling survival will increase with increasing distance from the parent plant due to reduced pressures from host-specific herbivores and pathogens [61–63]. The expected result is larger gaps between conspecific plants than would be expected given patterns of seed distribution, along with increased plant species richness, because heterospecifics have an advantage near parent plants. Although many tropical and temperate species have shown this pattern, the results are highly species specific [64,65]. The mechanisms behind this correspondence (or lack thereof) are still being debated [63]. We hypothesize that transgenerational defense induction or priming could be a major factor impacting Janzen–Connell spatial effects. In particular, when a maternal plant (especially a tree, with relatively weak dispersal) is heavily attacked, transgenerational induction may provide seedlings with a competitive edge in their parental environment. Given that transgenerational

defense induction appears common, and that the competitive environment as well as abiotic conductions can affect various aspects of plant dispersal and life history [66,67], we expect that important discoveries will be made in the ecological impacts of transgenerational defense induction.

Epigenetic effects, natural selection, and evolution

The evolutionary relevance of transgenerational induction of defense rests on whether responses are adaptive and whether there is heritable genetic or epigenetic variation for the epigenetic inheritance [24,34,39,68] (Figure 1). Perhaps the best evidence for adaptive transgenerational

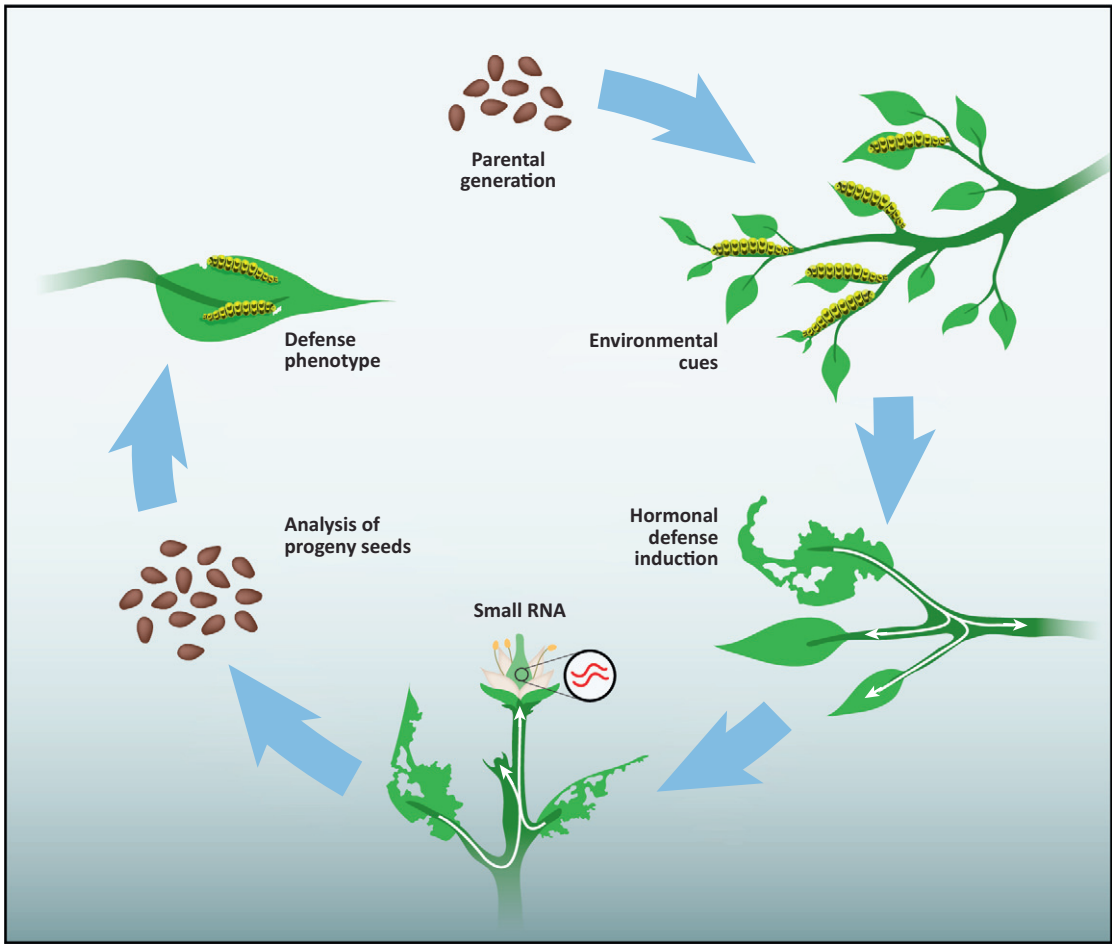

Figure 1. A schematic of the steps involved in the 'life cycle' of transgenerational induction. These steps include genetic background, environmental cues, defense induction, small RNA, analysis of progeny seeds, and the defense phenotype. The schematic includes suggestions for experimental design to maximize the ability to detect or investigate each step.

(a) Genetic background. The effects of transgenerational inheritance on the phenotype can be most clearly pinpointed when isolated from the effects of genetic and environment variation. Separation of epigenetic from genetic variation is critical for predicting the evolution of epigenetic effects and demonstrating their consequences. Genetic variation among replicate plants can be minimized through inbreeding over multiple generations (e.g., [40,72]). In the case of the commonly studied model plant *Arabidopsis*, which is largely self-pollinating, most natural isolates are already highly inbred. Epigenetic recombinant inbred lines (epiRILs) have been instrumental in examining epigenetic variation in a relatively standardized genetic background [74,96,97].

(b) Environmental cue. Insect feeding, pathogen infection, treatment with chemical elicitors, and mechanical damage are used to trigger defense responses in plants. Application of such environmental cues must be timed such that the plant perceives a stress, but is not killed before seed set. Treatments before and/or after the initiation of reproduction have been shown to trigger transgenerational induction responses (Table 1, main text).

(c) Defense induction. Successful application of herbivores and pathogens in the treated generation can be assessed through analysis of characteristic plant responses [33]. These include changes in gene expression, production of defense signaling hormones, and increased accumulation of defense-related end products (i.e., chemical and physical defenses). In some plant species, such as *Arabidopsis* and tomato, available mutants can be used to test for the involvement of specific defense pathways in transgenerational resistance.

(d) Small RNA. In addition to small molecules, phloem-mobile small RNA can provide a signal that allows transfer of information from vegetative tissue to developing seeds. High-throughput sequencing approaches can provide a global view of changes in the small RNA profile in response to the applied stress. As with defense induction, mutant plants can be used to examine the specific involvement of small RNA biogenesis in epigenetic inheritance.

(e) Analysis of progeny seeds. Ultimately, some type of 'memory' of the applied stress must be maintained in the seeds of the afflicted plants. Size of the seeds, provision of nutrients, and accumulation of defensive secondary metabolites in response to biotic stress are all measurable and can influence plant resistance in the next generation. Small RNA in the seeds might represent a form of stored information that influences gene expression in the subsequent generation. Chromatin modifications, such as DNA methylation and histone acetylation, can be assessed in the seeds by bisulfite sequencing and proteomic approaches to determine whether there are reproducible differences between stressed and unstressed plants.

(f) Defense phenotype. Increased resistance in the progeny generation can take the form of constitutively elevated defenses or priming for enhanced responses to subsequent threats. Plant defense status at the level of gene expression, signaling molecules, and defensive metabolites can be measured as in the parental generation to determine whether progeny have constitutively higher defenses. Repeated treatment in the progeny generation, in combination with a time-course analysis of defense responses, will demonstrate whether offspring of maternally damaged plants respond more strongly or more rapidly to subsequent threats than do offspring of control plants.

effects in the wild comes from a study of American bellflower (*Campanulastrum americanum*) plants in divergent light environments [67]. When parental and offspring phenotypes were matched to the parental environment, plant fitness was more than threefold higher than in plants with mismatched environments. For plant resistance to herbivores or pathogens, relatively few studies have examined transgenerational effects on fitness. In the two field studies to date, parental exposure to herbivores impacted offspring fitness, but these effects were complex and were not completely consistent with the transgenerational effects being adaptive [38,60].

Despite some evidence for adaptive transgenerational effects in plants, if there is no heritable variation for the epigenotype itself, there cannot be a response to natural selection. The published studies that have been designed to detect heritable epigenetic variation have generally found such variation, including the study of American bellflower described above [67] and studies of plant resistance in wild radish and yellow monkeyflower [38,41,45,47,69]. Moreover, DNA methylation, small RNA accumulation, and histone modifications, three probable mechanisms required for the acquisition of epigenetic inheritance, show heritable variation among *Arabidopsis* accessions [70–73]. Such natural variation could contribute to epigenetic differences in pest and pathogen resistance that can be passed from one generation to the next.

Recent studies using *Arabidopsis* epigenetic recombinant inbred lines (epiRILs) have demonstrated substantial heritable epigenetic-based variation for traits such as responses to defense hormones, flowering time, plant height, and growth rate [59,74]. Multiple DNA methylation variants in the epiRILs showed stable inheritance across generations [74]. The observed differences in phenotype can be reliably assigned to epigenetic origins, because the variation among epiRILs is heritable (i.e., is greater among than within lines). However, with the exception of recent transposon movement, there is essentially no DNA sequence variation among these same lines. In some cases, the extent of variation among epiRILs appears equivalent to that between genetically diverged ecotypes of *Arabidopsis* [75]. In addition, correlations for ten traits were consistent across accessions and epiRILs, suggesting not only that the effects of epigenetic variation on the phenotype are substantial, but also that evolutionary responses to selection of accessions and epiRILs would be similarly constrained due to correlations among traits [75]. A limitation of these studies is that the epiRILs were made through chemical treatments or introgression of methylation mutations. To increase the ecological relevance, further research in this area should focus on making crosses with plants containing epigenetic changes induced by natural levels of biotic or abiotic stress.

In the face of heritable epigenetic variation, the relevance and applicability of traditional, Mendelian-based models of evolution depend on the relation between genetic and epigenetic variation. A recent predictive model incorporating both of these modes of inheritance suggests that the two interact with one another in numerous direct and indirect ways, with widely disparate results [39]. Epigenetic variation would have no detectable impact on evolutionary trajectories, for example, if variation in the epigenotype is strongly positively correlated with genotype. In this case, the effect of epigenetic state on the phenotype can be subsumed into the genotypic effect [24]. By contrast, epigenetic variation has the potential to change the adaptive landscape of genes, resulting in long-term effects on evolution, if variation in the epigenotype is weakly or uncorrelated with the genotype. In this case, selection can act directly (and solely) upon epigenotypic variation. Thus, phenotypic change can be decoupled from the genotype. In another scenario, epigenetic changes can promote DNA recombination and transposition [18], thereby leading to stably inherited genome alterations. The resulting evolutionary dynamics can deviate substantially from Mendelian model predictions, and use of a new theoretical framework appears essential [35,39].

When epigenetic inheritance does exist for a particular plant defense trait, what does this departure from the Mendelian model of inheritance mean for natural plant populations? Rather than confounding understanding of, and ability to predict, evolution, transgenerational epigenetic variation can provide a missing link between sequence variation and phenotypic variation. For example, whereas genome-wide association studies are relatively new in the study of plant defense, they have been feasible for substantially longer in the study of human genetics. These studies demonstrated that the observed variability in human genomic data does not account for the heritabilities of several complex human diseases [76]. However, when epigenetic inheritance was considered, the 'missing heritability' problem was substantially reduced [77,78]. Similar patterns have been suggested, but not as comprehensively investigated, for the epigenetic inheritance of complex traits in plants [79].

Despite all of these advances, no multigenerational experiments have evaluated the relative contribution of epigenetic inheritance in response to natural selection. Such studies are clearly needed to test model predictions and illustrate the relative role of transgenerational epigenetic induction.

Concluding remarks

Even with the recent progress that we describe here, numerous basic questions remain (Box 2). Current technology provides the ability to address many of these questions by isolating specific causal factors and investigating mechanistic details of transgenerational induction (Figure 1). Some of the most important applications of increasing knowledge of transgenerational induction might be in addressing the challenges faced in agricultural pest management and understanding and/or predicting patterns of plant adaptation to rapid environmental change (e.g., introduced pests and climate change [80,81]). The increasing ability to fill in the 'missing heritability' links between genotype and phenotype should aid in predicting evolutionary responses to environmental change.

Transgenerational induction might be critical to the long-term survivorship of plant species that have survived for multiple generations under relaxed selection from herbivores. Adaptation to an altered environment could be

Box 2. Outstanding questions

Numerous questions remain regarding patterns of transgenerational inheritance of defense induction, their ecological and evolutionary importance, and potential implications.

- **How taxonomically widespread is transgenerational induction and is it linked to particular plant life-history strategies?**
  - Is transgenerational induction more prevalent in rapidly germinating plants than in those with prolonged dormancy?
  - Are small-seeded species more prone to epigenetic inheritance, whereas large-seeded species are more likely to show maternal effects?
  - Is transgenerational induction more prevalent in annual compared with perennial plants?
  - How do the effects and cost of transgenerational induction in an annual plant compare with those of 'long-term' induction in a perennial plant?

- **To what extent is transgenerational induction of defense correlated with constitutive defense and/or within-generational induction?**
  - Do the genes and/or genetic regions overlap between these three types of defense? For example, to what extent is the up- or down-regulation of constitutive genes involved, rather than novel regulatory factors and gene expression?
  - How variable within and/or across populations is transgenerational induction of defense to the same environmental cue?

- **What are the costs of transgenerational induction of defense?**
  - Are there resource allocation or ecological costs associated with transgenerational induction of resistance?
  - If so, how do these costs compare to the costs of constitutive and/or within-generation induction of defense?
  - How often do the herbivory triggers of transgenerational induction accurately predict herbivore pressures in the next generation?
  - Are costs of transgenerational induction incurred by the maternal plant or the progeny, or both? How do costs of transgenerational induction compare to those of maternal provisioning [95]?

- **Do maternal effects (provisioning) and epigenetic effects work in concert or independently in transgenerational induction?**
  - Is the transgenerational induction of all physical- and chemical-resistance traits under epigenetic control?
  - Can tolerance be transgenerationally induced? If so, is the induction via provisioning, epigenetic mechanisms, or provisioning governed by epigenetic mechanisms?

- **What are the relative contributions of transgenerational induction, genotype, and within-generation plasticity to variation in plant phenotypes?**
  - Is the relative ecological importance proportional to the percent of phenotypic variation explained by transgenerational induction?
  - In natural populations, does transgenerational induction of resistance impact the reproductive fitness of the offspring?

- **Are laboratory or domesticated crop plant strains likely to show particularly strong transgenerational effects compared with collections with a more varied history?**
  - Does the extent of transgenerational induction of a genotype (or epigenotype) depend on its past history of association with plant parasites, and are particular laboratory strains or domesticated crop lines artificially relaxed?

- **Are there potential agricultural applications of transgenerational induction of defense and epigenetics?**
  - Is priming of parent plants for transgenerational induction of resistance a viable option for increasing offspring resistance without decreasing offspring yield? How might the costs and/or benefits compare to the priming of seeds for within-generation induction?
  - How specific is transgenerational induction of resistance to eliciting cues, relative to within-generation induction of resistance to the same cues?

- **Does an understanding of transgenerational and epigenetic effects improve the ability to predict organismal responses to climate change [81]?**
  - Can epigenetic variation enhance the rates of plant evolutionary responses to rapid environmental change, including the introduction of exotic pests [23,80,87]?

accelerated in a manner analogous to that proposed for within-generation phenotypic plasticity (phenotypic plasticity facilitates survival, followed by genetic assimilation, with a reduction in phenotypic plasticity [82–86]). Such adaptation could be enhanced by transgenerational epigenetic induction, thus helping plant populations expand into novel environments or adapt to other forms of environmental change [23,81,87]. In agriculture, epigenetic priming of plant defenses, as has been accomplished with tomato [33], barley [58], and tobacco [40,42], has the potential to increase productivity without time-consuming breeding approaches. It is clear that, despite recent rapid advances, the importance, mechanisms, and consequences of transgenerational induction of defense for plants and their associated insects and microbes are only beginning to be unraveled.

Acknowledgments

This review was supported by NSF-DEB 0841609 (L.H.) NSF-DEB 1118783 (A.A.A.) and NSF-IOS-1139329 (G.J.). We thank Martin Heil (who suggested transgenerational impacts on Janzen–Connell dynamics), Rick Lindroth, Ken Raffa, Christina Richards, Eric Richards, and anonymous reviewers for helpful comments on the manuscript, and Sergio Rasmann for reviving this topic at Cornell University.

References

1 de Monet de Lamarck, J.B.P.A. (1809) *Philosophie Zoologique ou Exposition des Considérations Relatives à L'histoire Naturelle des Animaux*, Oxford University (in French)
2 Donohue, K. *et al.* (2001) Adaptive divergence in plasticity in natural populations of *Impatiens capensis* and its consequences for performance in novel habitats. *Evolution* 55, 692–702
3 Dudley, S. and Schmitt, J. (1996) Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *Am. Nat.* 147, 445–465
4 Baythavong, B.S. (2011) Linking the spatial scale of environmental variation and the evolution of phenotypic plasticity: selection favors adaptive plasticity in fine-grained environments. *Am. Nat.* 178, 75–87
5 Agrawal, A.A. (2001) Phenotypic plasticity in the interactions and evolution of species. *Science* 294, 321–326
6 Callaway, R.M. *et al.* (2003) Phenotypic plasticity and interactions among plants. *Ecology* 84, 1115–1128
7 Richards, E.J. (2011) Natural epigenetic variation in plant species: a view from the field. *Curr. Opin. Plant Biol.* 14, 204–209
8 Danchin, É. *et al.* (2011) Beyond DNA: integrating inclusive inheritance into an extended theory of evolution. *Nat. Rev. Genet.* 12, 475–486
9 Feil, R. and Fraga, M.F. (2012) Epigenetics and the environment: emerging patterns and implications. *Nat. Rev. Genet.* 13, 97–109
10 Hauser, M-T. *et al.* (2011) Transgenerational epigenetic inheritance in plants. *Biochim. Biophys. Acta* 1809, 459–468

11 Dyer, A.R. *et al.* (2010) Synthesis: the role of adaptive trans-generational plasticity in biological invasions of plants. *Evol. Appl.* 3, 179–192
12 Sultan, S.E. *et al.* (2009) Contrasting patterns of transgenerational plasticity in ecologically distinct congeners. *Ecology* 90, 1831–1839
13 Whittle, C.A. *et al.* (2009) Adaptive epigenetic memory of ancestral temperature regime in *Arabidopsis thaliana*. *Botany* 87, 650–657
14 Rapp, R.A. and Wendel, J.F. (2005) Epigenetics and plant evolution. *New Phytol.* 168, 81–91
15 Richards, C.L. and Wendel, J.F. (2011) The hairy problem of epigenetics in evolution. *New Phytol.* 191, 7–9
16 Richards, E.J. (2008) Population epigenetics. *Curr. Opin. Genet. Dev.* 18, 221–226
17 Pieterse, C. (2012) Prime time for transgenerational defense. *Plant Physiol.* 158, 545
18 Boyko, A. and Kovalchuk, I. (2011) Genome instability and epigenetic modification: heritable responses to environmental stress? *Curr. Opin. Plant Biol.* 14, 260–266
19 Roach, D.A. and Wulff, R.D. (1987) Maternal effects in plants. *Annu. Rev. Ecol. Syst.* 18, 209–235
20 Lynch, M. and Walsh, B. (1998) *Genetics and Analysis of Quantitative Traits*, Sinauer Associates
21 Paszkowski, J. and Grossniklaus, U. (2011) Selected aspects of transgenerational epigenetic inheritance and resetting in plants. *Curr. Opin. Plant Biol.* 14, 195–203
22 Henderson, I.R. *et al.* (2006) Dissecting *Arabidopsis thaliana* DICER function in small RNA processing, gene silencing and DNA methylation patterning. *Nat. Genet.* 38, 721–725
23 Jablonka, E. and Raz, G. (2009) Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Q. Rev. Biol.* 84, 131–176
24 Richards, E.J. (2006) Inherited epigenetic variation: revisiting soft inheritance. *Nat. Rev. Genet.* 7, 395–401
25 Bossdorf, O. *et al.* (2007) Epigenetics for ecologists. *Ecol. Lett.* 11, 106–115
26 Richards, C.L. *et al.* (2010) What role does heritable epigenetic variation play in phenotypic evolution? *Bioscience* 60, 232–237
27 Boyko, A. and Kovalchuk, I. (2011) Genetic and epigenetic effects of plant–pathogen interactions: an evolutionary perspective. *Mol. Plant* 4, 1014–1023
28 Bossdorf, O. and Zhang, Y. (2011) A truly ecological epigenetics study. *Mol. Ecol.* 20, 1572–1574
29 Bonduriansky, R. (2012) Rethinking heredity, again. *Trends Ecol. Evol.* 27, 330–336
30 Hunter, B. *et al.* (2012) Epigenetic inheritance: what news for evolution? *Curr. Biol.* 22, R54–R56
31 Slaughter, A. *et al.* (2012) Descendants of primed *Arabidopsis* plants exhibit resistance to biotic stress. *Plant Physiol.* 158, 835–843
32 Luna, E. *et al.* (2012) Next-generation systemic acquired resistance. *Plant Physiol.* 158, 844–853
33 Rasmann, S. *et al.* (2012) Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant Physiol.* 158, 854–863
34 Mousseau, T. and Fox, C.W. (1998) *Maternal Effects as Adaptations*, Oxford University Press
35 Bonduriansky, R. and Day, T. (2009) Nongenetic inheritance and its evolutionary implications. *Annu. Rev. Ecol. Evol. Syst.* 40, 103–125
36 Gluckman, P. *et al.* (2009) Epigenetic mechanisms that underpin metabolic and cardiovascular diseases. *Nat. Rev. Endocrinol.* 7, 401–408
37 Rossiter, M. (1996) Incidence and consequences of inherited environmental effects. *Annu. Rev. Ecol. Syst.* 27, 451–476
38 Agrawal, A.A. (2001) Transgenerational consequences of plant responses to herbivory: an adaptive maternal effect? *Am. Nat.* 157, 555–569
39 Day, T. and Bonduriansky, R. (2011) A unified approach to the evolutionary consequences of genetic and nongenetic inheritance. *Am. Nat.* 178, E18–E36
40 Kathiria, P. *et al.* (2010) Tobacco mosaic virus infection results in an increase in recombination frequency and resistance to viral, bacterial, and fungal pathogens in the progeny of infected tobacco plants. *Plant Physiol.* 153, 1859–1870
41 Scoville, A.G. *et al.* (2011) Differential regulation of a MYB transcription factor is correlated with transgenerational epigenetic inheritance of trichome density in *Mimulus guttatus*. *New Phytol.* 191, 251–263
42 Roberts, D. (1983) Acquired resistance to tobacco mosaic virus transmitted to the progeny of hypersensitive tobacco. *Virology* 124, 161–163
43 Lammerink, J. *et al.* (1984) Effect of the cabbage aphid (*Brevicoryne brassicae*) on total glucosinolate in the seed of oilseed rape (*Brassica napus*). *N. Z. J. Agr. Res.* 27, 89–92
44 Shattuck, V.I. (1993) Glucosinolates and glucosinolate degradation in seeds from turnip mosaic virus-infected rapid cycle *Brassica campestris* L. plants. *J. Exp. Bot.* 44, 963–970
45 Agrawal, A.A. (2002) Herbivory and maternal effects: mechanisms and consequences of transgenerational induced plant resistance. *Ecology* 83, 3408–3415
46 Agrawal, A.A. *et al.* (1999) Transgenerational induction of defences in animals and plants. *Nature* 401, 60–63
47 Holeski, L.M. (2007) Within and between generation phenotypic plasticity in trichome density of *Mimulus guttatus*. *J. Evol. Biol.* 20, 2092–2100
48 Zheng, X.Y. *et al.* (2012) Coronatine promotes *Pseudomonas syringae* virulence in plants by activating a signaling cascade that inhibits salicylic acid accumulation. *Cell Host Microbe* 11, 587–596
49 Thaler, J.S. *et al.* (2012) Evolution of jasmonate and salicylate signal crosstalk. *Trends Plant Sci.* 17, 260–270
50 Chan, SW-L. *et al.* (2006) RNAi, DRD1, and histone methylation actively target developmentally important non-CG DNA methylation in *Arabidopsis*. *PLoS Genet.* 2, e83
51 Conrath, U. *et al.* (2002) Priming in plant–pathogen interactions. *Trends Plant Sci.* 7, 210–216
52 Hilker, M. and Meiners, T. (2010) How do plants 'notice' attack by herbivorous arthropods? *Biol. Rev.* 85, 267–280
53 Karban, R. (2010) The ecology and evolution of induced resistance against herbivores. *Funct. Ecol.* 25, 339–347
54 Johnson, M.T.J. (2011) Evolutionary ecology of plant defences against herbivores. *Funct. Ecol.* 25, 305–311
55 Worrall, D. *et al.* (2011) Treating seeds with activators of plant defence generates long-lasting priming of resistance to pests and pathogens. *New Phytol.* 193, 770–778
56 van Hulten, M. *et al.* (2006) Costs and benefits of priming for defense in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 103, 5602–5607
57 Walters, D.R. *et al.* (2008) Priming for plant defense in barley provides benefits only under high disease pressure. *Phys. Mol. Plant Path.* 73, 95–100
58 Walters, D. and Patterson, L. (2012) Parents lend a helping hand to their offspring in plant defence. *Biol. Lett.* http://dx.doi.org/10.1098/rsbl.2012.0416
59 Latzel, V. *et al.* (2012) Epigenetic variation in plant response to defense hormones. *Ann. Bot.* http://dx.doi.org/10.1093/aob/mcs088
60 terHorst, C.P. and Lau, J.A. (2012) Direct and indirect transgenerational effects alter plant–herbivore interactions. *Evol. Ecol.* http://dx.doi.org/10.1007/s10682-012-9560-8
61 Janzen, D. (1970) Herbivores and the number of tree species in tropical forests. *Am. Nat.* 104, 501–528
62 Connell, J. (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in forest trees. In *Dynamics of Populations* (Den Boer, P.J. and Gradwell, G.R., eds), pp. 298–312, Centre for Agricultural Publishing and Documentation
63 Clark, J.S. (2010) Individuals and the variation needed for high species diversity in forest trees. *Science* 327, 1129–1132
64 Hyatt, L. *et al.* (2003) The distance dependence prediction of the Janzen–Connell hypothesis: a meta-analysis. *Oikos* 103, 590–602
65 Schupp, E.W. and Jordano, P. (2011) The full path of Janzen–Connell effects: genetic tracking of seeds to adult plant recruitment. *Mol. Ecol.* 20, 3953–3955
66 Donohue, K. (1999) Seed dispersal as a maternally influenced character: mechanistic basis of maternal effects and selection on maternal characters in an annual plant. *Am. Nat.* 154, 674–689
67 Galloway, L.F. and Etterson, J.R. (2007) Transgenerational plasticity is adaptive in the wild. *Science* 318, 1134–1136
68 Kalisz, S. and Purugganan, M.D. (2004) Epialleles via DNA methylation: consequences for plant evolution. *Trends Ecol. Evol.* 19, 309–314
69 Verhoeven, K.J.F. *et al.* (2009) Stress-induced DNA methylation changes and their heritability in asexual dandelions. *New Phytol.* 185, 1108–1118

70 Zhai, J. *et al.* (2008) Small RNA-directed epigenetic natural variation in *Arabidopsis thaliana*. *PLoS Genet.* 4, e1000056
71 Shindo, C. *et al.* (2006) Variation in the epigenetic silencing of FLC contributes to natural variation in *Arabidopsis* vernalization response. *Genes Dev.* 20, 3079–3083
72 Todesco, M. *et al.* (2012) Natural variation in biogenesis efficiency of individual *Arabidopsis thaliana* microRNAs. *Curr. Biol.* 22, 166–170
73 Vaughn, M.W. *et al.* (2007) Epigenetic natural variation in *Arabidopsis thaliana*. *PLoS Biol.* 5, e174–e1629
74 Johannes, F. *et al.* (2009) Assessing the impact of transgenerational epigenetic variation on complex traits. *PLoS Genet.* 5, e1000530
75 Roux, F. *et al.* (2011) Genome-wide epigenetic perturbation jump-starts patterns of heritable variation found in nature. *Genetics* 188, 1015–1017
76 Goldstein, D.B. (2009) Common genetic variation and human traits. *N. Engl. J. Med.* 360, 1696–1698
77 Slatkin, M. (2009) Epigenetic inheritance and the missing heritability problem. *Genetics* 182, 845–850
78 Furrow, R.E. *et al.* (2011) Environment–sensitive epigenetics and the heritability of complex diseases. *Genetics* 189, 1377–1387
79 Weigel, D. (2012) Natural variation in *Arabidopsis*: from molecular genetics to ecological genomics. *Plant Physiol.* 158, 2–22
80 Bonduriansky, R. *et al.* (2012) The implications of nongenetic inheritance for evolution in changing environments. *Evol. Appl.* 5, 192–201
81 Nicotra, A.B. *et al.* (2010) Plant phenotypic plasticity in a changing climate. *Trends Plant Sci.* 15, 684–692
82 Via, S. and Lande, R. (1985) Genotype–environment interaction and the evolution of phenotypic plasticity. *Evolution* 39, 505–522
83 DeWitt, T.J. and Scheiner, S. (2004) *Phenotypic Plasticity: Functional and Conceptual Approaches*, Oxford University Press
84 West-Eberhard, M. (2003) *Developmental Plasticity and Evolution*, Oxford University Press
85 Lande, R. (2009) Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *J. Evol. Biol.* 22, 1435–1446
86 Richards, C.L. *et al.* (2010) What role does heritable epigenetic variation play in phenotypic evolution? *BioScience* 60, 232–237
87 Pál, C. and Miklós, I. (1999) Epigenetic inheritance, genetic assimilation and speciation. *J. Theor. Biol.* 200, 19–37
88 Strauss, S.Y. and Agrawal, A.A. (1999) The ecology and evolution of plant tolerance to herbivory. *Trends Ecol. Evol.* 14, 179–185
89 Cubas, P. *et al.* (1999) An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* 401, 157–161
90 Richards, E.J. (2009) Quantitative epigenetics: DNA sequence variation need not apply. *Genes Dev.* 23, 1601–1605
91 Takeda, S. and Paszkowski, J. (2006) DNA methylation and epigenetic inheritance during plant gametogenesis. *Chromosoma* 115, 27–35
92 Grewal, S.I.S. and Moazed, D. (2003) Heterochromatin and epigenetic control of gene expression. *Science* 301, 798–802
93 Petes, T.D. and Hill, C.W. (1988) Recombination between repeated genes in microorganisms. *Annu. Rev. Genet.* 22, 147–168
94 Molinier, J. *et al.* (2006) Transgenerational memory of stress in plants. *Nature* 442, 1046–1049
95 Elwell, A.L. *et al.* (2011) Separating parental environment from seed size effects on next generation growth and development in *Arabidopsis*. *Plant Cell Environ.* 34, 291–301
96 Reinders, J. *et al.* (2009) Compromised stability of DNA methylation and transposon immobilization in mosaic *Arabidopsis* epigenomes. *Genes Dev.* 23, 939–950
97 Johannes, F. and Colome-Tatche, M. (2011) Quantitative epigenetics through epigenomic perturbation of isogenic lines. *Genetics* 188, 215–227
98 Lucht, J.M. *et al.* (2002) Pathogen stress increases somatic recombination frequency in *Arabidopsis*. *Nat. Genet.* 30, 311–314
99 Van Dam, N. and Baldwin, I. (2001) Competition mediates costs of jasmonate-induced defences, nitrogen acquisition and transgenerational plasticity in *Nicotiana attenuata*. *Funct. Ecol.* 15, 406–415
100 Herrera, C.M. and Bazaga, P. (2011) Untangling individual variation in natural populations: ecological, genetic and epigenetic correlates of long-term inequality in herbivory. *Mol. Ecol.* 20, 1675–1688