

COMMENTARY

Plant epigenetics: phenotypic and functional diversity beyond the DNA sequence

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Phenotypic variation determines the capacity of plants to adapt to changing environments and to colonize new habitats. Deciphering the mechanisms contributing to plant phenotypic variation and their effects on plant ecological interactions and evolutionary dynamics is thus central to all biological disciplines. In the past few decades, research on plant epigenetics is showing that (1) epigenetic variation is related to phenotypic variation and that some epigenetic marks drive major phenotypic changes in plants; (2) plant epigenomes are highly diverse, dynamic, and can respond rapidly to a variety of biotic and abiotic stimuli; (3) epigenetic variation can respond to selection and therefore play a role in adaptive evolution. Yet, current information in terms of species, geographic ranges, and ecological contexts analyzed so far is too limited to allow for generalizations about the relevance of epigenetic regulation in phenotypic innovation and plant adaptation across taxa. In this report, we contextualize the potential role of the epigenome in plant adaptation to the environment and describe the latest research in this field presented during the symposium “Plant epigenetics: phenotypic and functional diversity beyond the DNA sequence” held within the Botany 2020 conference framework in summer 2020.

KEY WORDS DNA methylation; environmental change; epigenetic inheritance; evolution; rapid adaptation.

Plants have diversified into many different habitats on earth, colonizing even the most extreme ecosystems (e.g., the poles, high altitude ecosystems, deserts, salt marshes, and geothermal vents). Understanding how plants cope with the environment has inspired countless research across all fields of biological sciences. This knowledge has become even more relevant now as plants are facing very rapid shifts in environmental conditions due to the impact of human activities on the planet. It is well established that the capacity of plants to respond to the environment ultimately depends on their levels of genetic diversity, phenotypic variation, and plasticity (e.g., Björklund et al., 2009; Anderson et al., 2012; Henn et al., 2018). Further evidence suggests that DNA sequence variation alone is not responsible for all standing phenotypic variation in plants, and epigenetic variation further contributes to extant phenotypic and functional diversity. For instance, one of the now classic examples is the switch from bilateral to radial floral symmetry in the toadflax *Linnaria vulgaris* (Cubas et al., 1999). Also, the colorless nonripening

natural strain of tomato is a result of an epimutation of the SBP-box transcription factor (Manning et al., 2006); the accumulation of vitamin E in tomato is determined by spontaneous changes in DNA methylation of a SINE transposon located in the promoter region of the gene *VTE3(1)* (Quadrana et al., 2014); the prickly leaves of the holly tree are associated with lower DNA cytosine methylation at certain markers (Herrera and Bazaga, 2013); the spontaneous epigenetic silencing of the *DWARF1 (D1)* gene in rice leads to a dwarf phenotype (Miura et al., 2009); and the transition from male to female flowers in a gynodioecious melon line is epigenetically controlled (Martin et al., 2009). With this evidence in mind, we organized the symposium “Plant epigenetics: phenotypic and functional diversity beyond the DNA sequence” within the Botany 2020 thematic framework “Plants at the extremes”, held virtually from 27 to 31 July 2020. The main scope of this symposium was to foster discussion on the latest research about the relevance of epigenetic regulation in phenotypic innovation and plant adaptation to contrasting environments.

Plant epigenetics is a multidisciplinary research field that aims to understand how plants have their phenotypes adjusted to external conditions through mechanisms other than DNA sequence modifications, and the ecological and evolutionary consequences of the phenotypic variation brought about by those mechanisms. This research field studies the set of chromatin modifications that regulate chromatin structure and its effect on gene expression, the stability of plant genomes, the ways in which epigenetic variants emerge and can be transmitted, and their phenotypic consequences and impacts on plant population dynamics and evolutionary processes. Epigenetic mechanisms, encompassing chemical modifications to the DNA (i.e., DNA methylation) and histone proteins, and presence and abundance of other regulatory molecules such as noncoding RNAs determine how genes are expressed and therefore how the genotype translates into a phenotype. This process is crucial for sessile organisms such as plants, as they rely heavily on changes in gene expression to respond to their surrounding environment.

Population epigenetic studies showed that epigenetic variation can be spatially structured among and within plant populations and, if so, such structure can be associated with environmental variation and phenotypic differentiation (Lira-Medeiros et al., 2010; Paun et al., 2010; Cara et al., 2013; Medrano et al., 2014; Schulz et al., 2014; Foust et al., 2016; Herrera et al., 2017; Gáspár et al., 2019). Thus, epigenetic variation could help plants to better adjust their phenotypes to local environmental conditions. The frequency and generality of epigenetic population structure is still uncertain because current information in terms of number of species and geographic ranges analyzed is too limited, provided the broad diversity of plant epigenomic features. For example, genome-wide cytosine DNA methylation levels (as the percentage of all methylated cytosines) range between 5 and 43% in a set of 34 angiosperm species (Alonso et al., 2015; Niederhuth et al., 2016) and from 4.8 to 42.2% in a larger set of 279 angiosperm species (Alonso et al., 2019). Further, CG methylation in the transcribed regions of genes, i.e., gene body methylation, ranges between 2 and 86% in 77 different species across Viridiplantae (Bewick et al., 2017); histone H3 methylation at lysine 9 (H3K9me2) shows different distribution patterns between euchromatic and heterochromatic regions across the genomes of 23 angiosperms and one moss (Houben et al., 2003); H3K9me2 and CHG methylation are widespread throughout the maize genome, whereas they are limited to specific regions of the *Arabidopsis thaliana* genome (Fig. 1 in Springer et al., 2016). Some of these epigenomic features exhibit a strong phylogenetic signal (Alonso et al., 2015, 2019; Bewick et al., 2017), suggesting that ecological epigenetic responses can largely differ across species in a way shaped by long-term evolution.

Furthermore, we also know that environmental conditions such as heat and salt stress and biotic stresses such as viral infections can alter epigenetic marks contributing to plant epigenomic diversity and phenotypic adaptation (Erdmann and Picard, 2020). Some of these environmentally induced epigenetic marks can be transmitted from parents to offspring, either transiently or while the conditions that induced them last (Rasmann et al., 2012; Herman and Sultan, 2016). These environmentally induced changes could provide the substrate for phenotypic variation that, when related to individual fitness, provides more opportunities for adaptive evolution by broadening the phenotypic space available to selection (Fig. 1). The contribution of environmentally induced epigenetic changes to phenotypic variation could be crucial for predominantly clonal plants. The canonical view is that genetic variation is key to long-term survival

of populations (Bijlsma and Loeschcke, 2012; Allendorf, 2017), suggesting that clonal plants could be more susceptible to extinction, especially in dynamic environments (Holsinger, 2000; Bijlsma and Loeschcke, 2012). Recent evidence suggests that epigenetic variation could be a source of phenotypic variation in clonal or asexually reproducing plants. Thus, epigenetic variation might compensate and complement their potentially low levels of genetic variation, allowing them to adapt and respond to environmental challenges (e.g., Raj et al., 2011; Wilschut et al., 2016; Jueterbock et al., 2020).

Finally, for those more interested in understanding the molecular mechanisms underlying epigenetic processes, the implementation of next-generation sequencing techniques has particularly enlightened the dynamics of DNA methylation in the model plant *A. thaliana*, providing guidelines for more detailed analyses in other species. DNA methylation is one of the most-studied epigenetic marks, which, in plants, occurs in cytosines located within three different DNA sequence contexts: CG, CHG, and CHH (where H stands for A, C, or T). Methylation in the different contexts is maintained or established de novo through different enzymatic pathways. DNA methylation can occur in different genomic contexts (e.g., genes, repetitive elements), can have different transgenerational stabilities and may thus differ in its biological function and relevance. For example, transposable elements are typically methylated in the three contexts, which silences them, while genes are usually unmethylated or methylated in the CG context only (Schmitz et al., 2019). CG and CHG methylation are symmetrical (they happen on both complementary DNA strands), while CHH methylation is not. During gametogenesis, many epigenetic marks are either removed or added de novo (a phenomenon called epigenetic reprogramming), which could limit the heritability of some epigenetic marks (Kawashima and Berger, 2014). As shown by epimutation accumulation lines, i.e., selfing lines that accumulate spontaneous changes in the methylation status of a certain cytosine over time, CG DNA methylation is largely stable and heritable. However, gains and losses of CG methylation accumulate gradually across the genome and at a much higher rate than genetic mutations (Becker et al., 2011; Schmitz et al., 2011; van der Graaf et al., 2015). CG methylation is maintained with a high fidelity through both mitotic and meiotic cell divisions by METHYLTRANSFERASE 1 (MET1), which methylates hemimethylated DNA after replication (Schmitz et al., 2019). Newly acquired CHG and CHH epimutations could be heritable over short periods of time, depending on the efficiency of self-reinforcing loops (i.e., a loop of chemical reactions in which each product enforces the next reaction) involving the histone mark H3K9me2 and two different CHROMOMETHYLASES (CMT3 and CMT2, for CHG and CHH, respectively (Du et al., 2012); these CMTs have a lower fidelity than MET1 (Erdmann and Picard, 2020). Finally, 24-nt small interfering RNA (siRNA) can lead to de novo DNA methylation in the three contexts through a pathway called RNA-directed DNA methylation (RdDM) (Erdmann and Picard, 2020). This pathway is also self-reinforcing, which could further contribute to transgenerational transmission of DNA methylation in the three contexts (Erdmann and Picard, 2020).

Our Botany 2020 conference symposium gathered a set of speakers at different stages of their research career who approached the study of plant epigenetics from different perspectives, including manipulative experiments and broad field surveys. The studied plant species also represented a wide range of taxa with varied life history characteristics including bryophytes and annual and perennial herbs and trees (Fig. 1). We believe that a more comprehensive

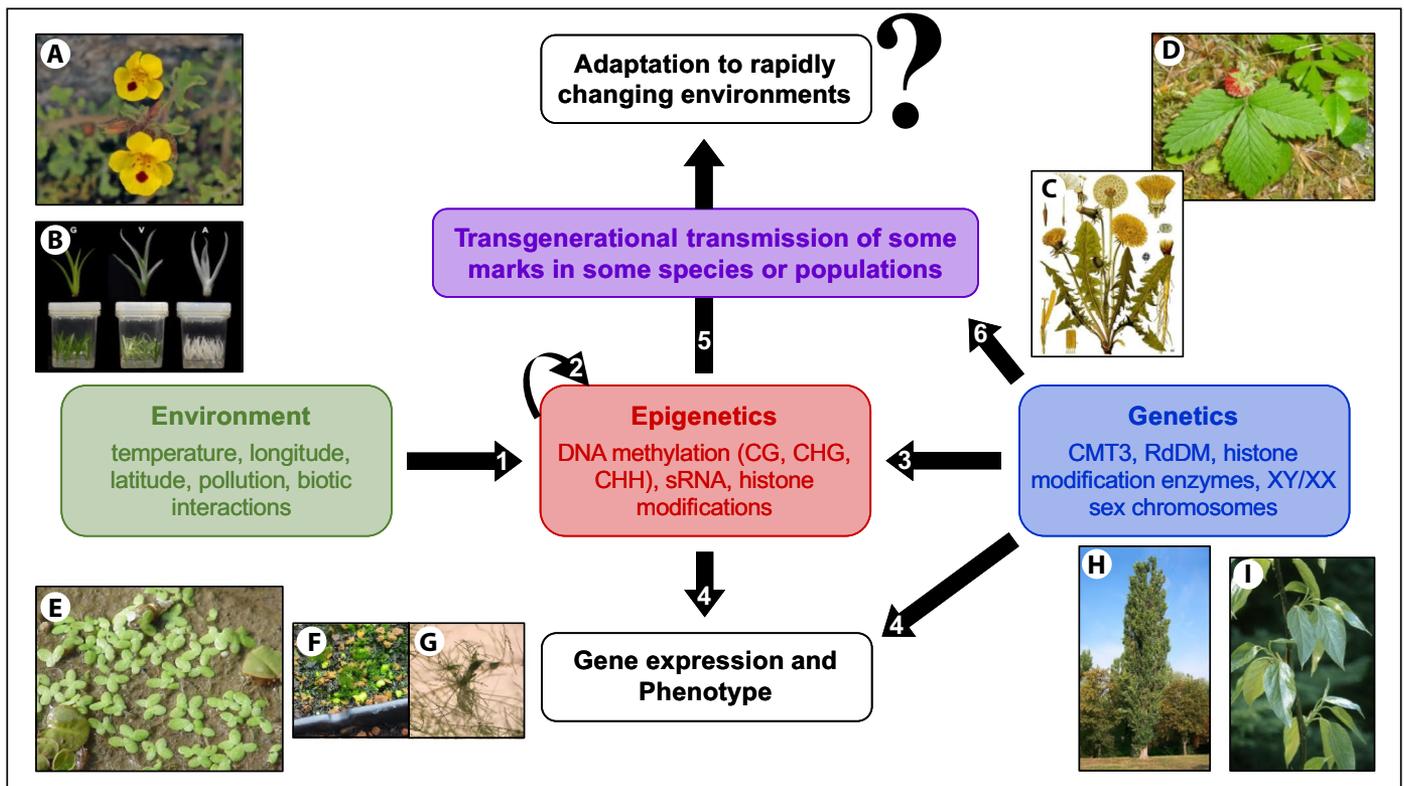


FIGURE 1. Present experimental evidence supports that plant epigenetic variation can be induced by the biotic and abiotic environment (1), spontaneously generated by means of random epimutations (2), and determined by sequence variation in genes associated with the epigenetic machinery (3). Genetic and epigenetic variation influence plant phenotypes (4) and, like DNA sequence variants, some epigenetic marks can be transmitted across generations (5). Evidence suggests that genetics influences the transmissibility of epigenetic marks across generations (6). Despite this evidence, the question “Can epigenetics allow rapid adaptive evolution in plants?” remains unanswered. The answer is not straightforward because, most likely, the significance of epigenetics in plant adaptation and evolution is species-, genotype-, and environment-specific. The speakers invited to the “Plant epigenetics: phenotypic and functional diversity beyond the DNA sequence” symposium made exciting contributions to the field by studying epigenetic regulation in a broad set of plants with different life histories: the annual herb *Mimulus laciniatus* (A: ©2011 Ron Wolf); the perennial herbs *Agave angustifolia* (B: extracted from Duarte-Aké et al., 2016), *Taraxacum officinale* (C), *Fragaria vesca* (D: ©Lindsey Koepke), and *Lemna minor* (E: ©Kurt Stüber); the terrestrial mosses *Scopelophila cataractae* (F: ©Teresa Boquete) and *Ceratodon purpureus* (G: ©Teresa Boquete); and the trees *Populus nigra* (H) and *P. balsamifera* (I).

understanding of the role of epigenetics in plant evolution and its interaction with the environment require the combination of these varied approaches and study systems. During the symposium, we discussed two main research lines: (1) the underlying epigenetic basis of striking plant phenotypic switches and (2) the analysis of natural or stress-induced epigenetic variants and their potential impact in plant adaptation. In the following sections, we provide a framework for these studies and the most exciting findings presented by our set of speakers. All the talks are available to the Botany 2020 conference attendees through the conference platform. Other interested readers should contact the speakers to request more information.

EPIGENETIC REGULATION WITH MAJOR PHENOTYPIC EFFECTS

Clelia de la Peña addressed the epigenetic changes underlying the albino phenotype of *Agave angustifolia*. Using plant tissue culture techniques, her group established a large collection of almost

genetically identical plants of this species with striking phenotypic variation: green plants (i.e., normal phenotype), albino plants (i.e., phenotype lacking photosynthetic and other pigments), and variegated plants (i.e., phenotype combining both normal and albino cells within the same plant). The three phenotypes differed markedly in terms of (1) density of some histone marks, (2) the expression of DNA methylases and in global DNA methylation levels, and (3) the expression of enzymes involved in histone modifications (Duarte-Aké et al. [2016] and Us-Camas et al. [2017] give more details.). De la Peña also reported pronounced decreases in global DNA methylation levels right before the transition from green to variegated and albino plants took place (Us-Camas et al., 2017), and before reversion from variegated to green phenotypes. The *A. angustifolia* example illustrates how multiple interconnected epigenetic mechanisms have the potential to induce major heritable phenotypic changes.

Katharina Bräutigam studies the mechanisms of sex determination in the dioecious tree *Populus balsamifera*. Combining a series of smartly designed experimental approaches, Bräutigam and her

collaborators discovered a single gene, *ARR17*, that had a differential methylation pattern between male and female trees in vegetative tissues that allowed the discrimination between male and female individuals (Bräutigam et al., 2017). When methylated, this gene was not expressed and generated males; when unmethylated, this gene was highly expressed and led to females. Her latest results showed that the methylation status of *ARR17* could be linked to the RdDM pathway that was activated in males by the presence of a partial and reversed copy of this gene on the Y chromosome. Interestingly, sex-specific gene expression patterns observed during *P. balsamifera* reproductive development were nested within a series of chromatin reorganization events, that is, the sets of differentially expressed genes between males and females at each specific time point in reproductive development was influenced by a series of development-specific changes in epigenetic pathways such as DNA methylation, polycomb group protein complexes, RdDM, histone modifications, etc. This finding provides further support to the idea that epigenetic changes provide a flexible mechanism for chromatin remodeling through which plants rapidly respond to developmental cues and adjust developmental processes depending on genetic determinants such as XX or XY chromosomes (Ahmad et al., 2010; Berr and Shen, 2010; Bräutigam and Cronk, 2018).

EPIGENETIC VARIATION IN RELATION TO ENVIRONMENTAL CHANGE AND STRESS

Tuomas Toivainen contributed work to this line of inquiry by studying the signature of historical and current climatic data in natural populations of woodland strawberry (*Fragaria vesca*) across the species distribution range. Toivainen presented evidence for both genetic and epigenetic structure in this perennial herb; populations grouped by regions nested within two clearly separated clusters, eastern and western European populations. CG DNA methylation level in *F. vesca* correlated significantly and positively with latitude, a phenomenon that has previously been observed in *A. thaliana* (Kawakatsu et al., 2016). CHG and CHH methylation levels, on the other hand, were negatively correlated to longitude. What is more, non-CG methylation was inherited in introgressed regions of hybrids from two of the populations. Interestingly, genome scans also showed evidence of natural selection acting on the enzymatic machinery involved in the establishment of epigenetic marks in natural *F. vesca* populations, including *CMT3* and enzymes that affect histone modifications. It is currently uncertain whether the DNA methylation population structure observed in *F. vesca* is independent from the underlying genetic structure and if it could play its own role in plant adaptation to the environment.

Jack Colicchio further highlighted the intricate relationship between genetics, epigenetics, environment, and plant phenotypes by studying the effect of the parental environment on the phenotype expressed by the offspring, i.e. transgenerational plasticity (Roach and Wulff, 1987; Donohue and Schmitt, 1998; Herman and Sultan, 2011). The transmission of environmental effects across generations can happen, among other mechanisms through heritable epigenetic modifications (e.g., Herman and Sultan, 2011; Jablonka, 2013; Colicchio et al., 2018). Transgenerational plasticity would be positively selected, i.e., adaptive, when there is high environmental autocorrelation, that is, when the parental environment predicts the offspring environment (Colicchio and Herman, 2020). Using a novel experimental design, Colicchio examined transgenerational

plasticity to temperature in the annual *Mimulus laciniatus* by exposing several genotypes to different temperatures in the laboratory and then exposing their offspring to the same range of temperature regimes both in the laboratory and in the field. He found that transgenerational plasticity to temperature prevailed in *M. laciniatus*, i.e., the parental environment had a significant effect on the offspring phenotype, which could be detected in offspring growing in different experimental field sites and years. The magnitude of transgenerational plasticity, however, varied among the different lineages studied, showing the challenges to unveiling epigenetic and genetic contributions to plant adaptation (Fig. 1). Changes in CG methylation appeared to mediate transgenerational plasticity to leaf damage in the sister species *M. guttatus* (Colicchio et al., 2018). Small RNAs (srRNA), which can be transmitted to the germline, could also contribute to the transmission of epigenetic marks between parents and offspring (Colicchio et al., 2020).

Teresa Boquete studies the role of epigenetic regulation in bryophytes in response to stress. Bryophytes rely heavily on asexual reproduction (Frey and Kürschner, 2011) and are common dwellers of highly stressful habitats, which makes them ideal models to study the importance of epigenetics in clonal plants. Using a reduced representation bisulfite sequencing technique (epiGBS; van Gurp et al., 2016), Boquete studied changes in DNA methylation in response to heavy metal exposure in two terrestrial mosses, *Scopelophila cataractae* and *Ceratodon purpureus*, clonally propagated in the laboratory for multiple generations. In general, the levels of genomic DNA methylation were very low in both species (<10% average methylation). She reported a series of differentially methylated positions (DMPs) between control and treated plants in the two species although the number of DMPs differed significantly between them. Thus, she provided evidence that abiotic stress induces a significant epigenetic response in bryophytes, and that the magnitude of this response is species-specific.

Koen Verhoeven studies the role of DNA methylation in plant response to stress and the transmission of environmental effects across generations in three species that can undergo asexual reproduction: black poplar (*Populus nigra*), dandelion (*Taraxacum officinale*), and duckweed (*Lemna minor*). Although these species have different asexual propagation mechanisms (i.e., hardwood cuttings, apomixis, and clonal propagation, respectively), they all escape the epigenetic reprogramming associated with germline development and genetic recombination associated with sexual reproduction, except for apomixis where reprogramming may happen during female gametogenesis (Verhoeven and Preite, 2014). Hence, environmentally induced epigenetic changes could be more straightforwardly transmitted across generations in these plants compared to sexually reproducing plants such as *A. thaliana*, in which epigenetic marks are partially reset during gametogenesis (Kawashima and Berger, 2014). Verhoeven's research included diverse experimental designs in which individuals of all three species were exposed to different stresses, both biotic and abiotic, and changes in the methylome were analyzed with epiGBS. Exciting preliminary results include (1) the methylome of asexual plant species is highly responsive to stress, especially in non-CG contexts, and this response is highly stress-specific; (2) exposure to stress induces DNA methylation changes that can be transmitted to progeny, although these changes are susceptible to revert back once the stress condition is no longer applied; (3) environmental effects can be detected in DNA methylation patterns even after 8–10 generations in duckweed and about two generations in dandelion which agrees with the hypothesis that

transmission of acquired DNA methylation is stronger in asexual compared to sexual plants; and (4) spontaneous epimutations happen in the CG context even in the absence of stress and are transmitted to subsequent generations and accumulate over time, resulting in significant DNA methylation divergence after a few generations.

FINAL REMARKS: THE IMPORTANCE OF EPIGENETIC REGULATION IN PLANTS

At the end of the symposium, we organized a short satellite discussion meeting in which we debated the role of epigenetics in plant evolution. This issue is well summarized in the question posed by Jablonka and Raz (2009, p. 162): “Given that epigenetic variations are often less stable than genetic variations, what evolutionary significance do they hold?” Current experimental evidence, including the latest results presented by the symposium speakers, supports that naturally occurring and stress-induced epigenetic variants can be related to phenotypic variation, can be transmitted to some extent to subsequent generations, and that epigenetic variation is subject to selection and can play a role in fast adaptive response (e.g., Schmid et al., 2018). However, current evidence also points out that the relationship among genetics, epigenetics, phenotype, and environment is highly complex. Hence, the significance of epigenetics in plant adaptation and evolution is probably species-, genotype-, and environment-specific.

During our satellite meeting discussion, we argued that even reversible epigenetic changes that aid individual plant survival may help populations to persist under environmental challenges and have an impact on the fate of populations or even species. We also argued that transmittable epigenetic marks could play a preponderant role in adaptation over a few generations. In that sense, epigenetics could lie in between acclimation (which happens at the level of the individual faced to environmental change) and evolution (which happens over multiple generations that modify allelic frequencies within populations in response to selection). Each epigenetic mark probably has its own scale along this timeline, for example, CHH methylation is probably mainly short term, CHG methylation could have a role over a few generations, and CG methylation a more long-term role. Further work along the two lines of research presented here and in species with diverse ecological and genomic features is needed to move the field forward and better quantify the impacts of plant epigenetics.

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AUTHOR CONTRIBUTIONS

Aline Muyle, Conchita Alonso, and M. Teresa Boquete organized the symposium at the Botany 2020 virtual conference; all authors contributed to manuscript writing, reviewing, and editing.

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