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OPINION

Transgenerational stress-adaption: an opportunity for ecological epigenetics

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Abstract In the recent years, there has been considerable interest to investigate the adaptive transgenerational plasticity of plants and how a "stress memory" can be transmitted to the following generation. Although, increasing evidence suggests that transgenerational adaptive responses have widespread ecological relevance, the underlying epigenetic processes have rarely been elucidated. On the other hand, model plant species have been deeply investigated in their genome-wide methylation landscape without connecting this to the ecological reality of the plant. What we need is the combination of an ecological understanding which plant species would benefit from transgenerational epigenetic stress-adaption in their natural habitat, combined with a deeper molecular analysis of non-model organisms. Only such interdisciplinary linkage in an ecological epigenetic study could unravel the full potential that epigenetics could play for the transgenerational stress-adaption of plants.

Keywords Cytosine methylation · Transgenerational stress-adaption · Epigenetic priming

General introduction

Asking for the composition of DNA, most people would reply by mentioning the four standard bases (A, T, G and C). Although modifications of these ordinary bases are known, like the methylated form of cytosine or 5-methyl-cytosine (m^5C), they are not accounted to the standard repertoire as they appear only in some organisms. The discovery of 5-methyl-cytosine has already been made more than 90 years ago (Johnson and Coghill 1925), several decades before the double helix structure of nucleic acids could be deciphered by the work of Franklin and Gosling (1953). Due to the focus on animal systems, 5-methyl-cytosine has always been considered as being an exceptional state or a "minor base" within eukaryotic genomes. What is less known today is the fact, that these early experiments demonstrated already the high abundance of 5-methyl-cytosine within plant tissues, and acknowledged this as a characteristic of flowering plants (Wyatt 1950; Vanyushin and Belozersky 1959). This reflects the vital role that DNA methylation plays as a gene regulatory mechanism for plants. Nowadays, epigenetics has become a popular field in biology and seems to undergo its second renaissance in plant ecology as an increasing number of studies focus on the ecological role of epigenetics (Schrey et al. 2013; Kilvitis et al. 2014; Herrera and Bazaga 2016; Alonso et al. 2016a; Richards et al. 2017; Herrera et al. 2017). Although the importance of epigenetic gene regulation has been widely recognized, its full contribution to an adaptive transgenerational response to environmental stresses, is not fully understood (Richards 2011; Gutzat and Mittelsten Scheid 2012; Viggiano and Pinto 2017). Within this manuscript, I would like to emphasize how our understanding about epigenetic stress-adaption is biased due to the focus on model-plant systems, and give incentives on how to choose suitable plant species and ecologically relevant environmental stress factors based on the plant´s natural history.

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The role of DNA methylation in plant evolution and phenotypic plasticity

Plants are known for their complex and sophisticated gene silencing machinery, including cytosine methylation, histone modifications and a vast variety of small RNAs (Law and Jacobsen 2010; Bej and Basak 2017). These processes are involved in tissue specific gene expression, embryogenesis, maternal and paternal imprinting and the control of transposable elements or viral sequences (Zilberman 2008). “Plants are considered as the masters of epigenetic regulation”, and the inheritance of epigenetic changes can be much easier implemented than for animals (Bej and Basak 2017). Somatic cells become part of the reproductive organs and plants do not eradicate epigenetic marks during the generational change so that epigenetic variations can be meiotically stable manifested (Heard and Martienssen 2014; Iwasaki and Paszkowski 2014a; Crisp et al. 2016).

The power of epigenetic changes is their non-permanent nature and their reversibility. Whereas genome recombination events and genetic mutations drive evolutionary processes in the long term, epigenetic modifications allow for a faster adaption to a changing environment. The reversible character of chromatin modifications and DNA methylations allows a rapid, but transitional adaption and, if necessary, a revision to the initial status. The evaluation of epigenetic variations in natural populations has become a topic of increasing interest and helps to elucidate what role such a dynamic methylation process plays in driving plant evolution (Richards et al. 2010; Herrera et al. 2016; Verhoeven et al. 2016; Niederhuth et al. 2016). Since epimutations show higher mutation rates than genetic mutations, they have a particular high potential for a dynamic evolutionary adaption (van der Graaf et al. 2015; Kronholm and Collins 2016). This inspired researchers to focus on epigenetic changes as the source of phenotypic plasticity for the explanation of adaptive plant phenotypes (Herman and Sultan 2011; Herrera and Bazaga 2013; Medrano et al. 2014; Colicchio et al. 2015). Especially facultative apomictic plants seem to benefit from the possibilities of an enhanced stress-adaption by epigenetic mechanisms (Rendina González et al. 2016; Wilschut et al. 2016; González et al. 2017). But what if such mechanisms are much more common among plants to adapt the offspring to a rapid changing environment? An epigenetic memory would allow to establish “transgenerational priming” and enables plants with an “adaptive transgenerational plasticity”. This has inspired many to look for possibilities to utilize this for adaptive agricultural breeding to produce seeds with local adaptions to extreme conditions (Bilichak and Kovalchuk 2016; Ramírez-Carrasco et al. 2017).

Transgenerational stress-adaption: the model species dilemma

Plants have a huge potential to utilize transgenerational epigenetic stress-adaption, where the environmental conditions experienced by the parental generation is inherited by the offspring. Plants do not have a sequestered germline compared to animals, but develop their germ cells relative late in their life cycle directly from meristematic cells and any epigenetic change acquired during vegetative growth could be transmitted to the offspring (Kinoshita and Jacobsen 2012).

Besides these good preconditions for epigenetic inheritance, some studies revealed surprisingly little consistency in methylation patterns following the stress treatments in plants (Eichten and Springer 2015). Other experiments suggest that environmental induced epigenetic changes are less important contributors to genome-wide heritable variations when looking at the persistence over several generations (Hagmann et al. 2015; Keller et al. 2016). But are these observations with agricultural crops or genetic model plants in artificial environments transferable to other land plants, which have evolved in different habitats? We should ask how representative these model plant systems are, that have been chosen for these studies. We need to add “ecological realism” to the used model species, to the experimental setup and to the entire study design, and do not perform experiments disentangled from the natural lifestyle of a plant (Richards et al. 2017). On the other hand, we should supplement ecological studies and designs with the molecular understanding of the epigenetic mechanisms and use the power of genome-wide transcriptional and methylation level analysis to gain the maximum potential to elucidate transgenerational stress-adaption in ecological-relevant epigenetic experiments (Bossdorf et al. 2008; Richards et al. 2017).

Many of the sophisticated studies on genome-wide DNA methylation, epigenome diversity, methylome analysis of native populations or even reviews about transgenerational epigenetics focus entirely on *A. thaliana* and its relatives as the most frequently used plant model system (Dowen et al. 2012; Seymour et al. 2014; Hagmann et al. 2015; Kawakatsu et al. 2016; Quadrana and Colot 2016). Despite the general acceptance as the standard genetic model species, it seems not to be the most representative system regarding *epigenetic* studies. In fact, considering genome-size and genome-wide methyl-cytosine content, *A. thaliana* appears to be an outlier (Alonso et al. 2015; Takuno et al. 2016; Niederhuth et al. 2016). While most plant species have global methyl-cytosine levels ranging from 10 to 40%, *A. thaliana* seems to be exceptional and shows only levels around 5% (Alonso et al. 2015; Richards et al. 2017; Viggiano and Pinto 2017). Even among plants with similar small genome and compared to close relatives (i.e., *Capsella rubella* or *A. lyrata*) the methyl-cytosine content of *A. thaliana* is unusual for an angiosperm and only undercut by non-vascular plants (Seymour et al. 2014; Takuno et al. 2016).

To be fair, methyl-cytosine content correlates with genome-size and has been mainly found at repetitive sequences (Alonso et al. 2015). The small genome of *A. thaliana* with its unusual low number of transposable elements (TEs) explains the low levels of DNA methylation. As good as such a small and compact genome with its low chromosome number had been in first place to establish this plant as a genetic model-system, it has become nowadays the major drawback on how findings with *A. thaliana* can be generalized for other angiosperms (Koenig and Weigel 2015; Richards et al. 2017). Its status as model plant was historically justified and has pioneered plant genetics for years, which paved the path for other plant systems. With its fast lifecycle and the unmatched availability of valuable genetic tools (e.g., methyltransferase mutants), it seems to be a convenient and popular system to study transgenerational effects and epigenetic regulation (Lang-Mladek et al. 2010; Slaughter et al. 2012; Luna et al. 2012; Rasmann et al. 2012; Wibowo et al. 2016). The trend goes even to an extension of these studies and the inclusion of natural ecotypes and accessions from various ecosystems (Dubin et al. 2015; Keller et al. 2016; Kawakatsu et al. 2016). However, as long as most of this work is done with *A. thaliana*, we should be critical about what we can infer and transfer to other plant systems.

Considering the plant ecology in transgenerational priming

An intriguing aspect of plant defense mechanisms where epigenetic processes could play a role is priming (Bruce et al. 2007). This describes a kind of stress-memory that allows to respond either faster or stronger to a reoccurring stimulus, when the plant has been previously "primed" (Hilker et al. 2016; Mauch-Mani et al. 2017). Priming is usually considered to occur within the same generation, but transgenerational effects are imaginable that extend to the following generation (Herman and Sultan 2011; Balmer et al. 2015). Although there is still limited experimental evidence for this, the potential that epigenetic memory could play for transgenerational stress-adaption has been discussed in various reviews (Gutzat and Mittelsten Scheid 2012; Holeski et al. 2012; Sahu et al. 2013; Crisp et al. 2016).

The versatility of epigenetic changes has been made responsible for some of the classical priming phenomena, as they allow a temporary adaption in a kind of "short-term memory" within a plant's life. If these adaptions are meiotically inherited, they would provide a "long-term memory" of stress-adaption or "transgenerational priming". But would it make sense that the progeny predicts the same kind of stress that the parents were primed for? Within a single generation, it could be beneficial to adapt to any fast appearing stress using a "short-term memory" so that the plant can respond much faster in case of a reoccurring event (e.g., herbivore attack, cold spell, drought or flooding within floodplain areas). But the ephemeral nature of herbivores for example make it difficult to predict their occurrence within the following generation, and it might be beneficial to "forget" this type of stress in the long term (Crisp et al. 2016). It seems that plants have even developed mechanisms which efficiently prevent the inheritance of certain epigenetic marks in the absence of stress, which has been at least shown for Arabidopsis (Iwasaki and Paszkowski 2014b; Wibowo et al. 2016). But this could be different for gradually increasing processes which happen slowly and do not require fast responses (e.g., soil salinization or the build up of toxic compounds, e.g., heavy metals). Whereas for the latter case a single plant generation would not benefit to be "primed", since these events are rather slow, a transgenerational adaption could make more sense here, as such an environmental change is rather predictable. Only if a stress could be reliably predicted for the offspring, a transgenerational stress-adaption in form of a "long-term memory" would have an evolutionary benefit. Changes in climate and soil properties are likely to affect the following generation as well, so that these stresses are good candidates to study transgenerational epigenetic stress-adaption. This does not necessarily mean that the offspring would be better adapted to these conditions physiologically (i.e., showing elevated salt tolerance), moreover a transgenerational aspect could be a simple avoidance strategy like an increase in seed dormancy, which allows to skip a season and wait for more favorable conditions to germinate. Still, this all depends on the individual life history of the plant and the dynamics of the natural habitat (Bräutigam et al. 2013; Schulz et al. 2014; Foust et al. 2016; Rendina González et al. 2016). It would make not much sense for the offspring to be preadapted to unfavorable soil conditions when the wind rolls the dice again where the seeds end up next.

Understanding the epigenetic machinery of plants

Several reports showed convincing examples of transgenerational priming and its ecological relevance for plants, but the underlying epigenetic mechanisms have been rarely investigated (Slaughter et al. 2012; Luna et al. 2012; Rasmann et al. 2012; Colicchio 2017). It seems that ecologists are intrigued by the possibilities of epigenetics, but content with the observation of phenotypes, without the need for further molecular analysis if (or if not) epigenetic changes are involved in these phenomena. There is a lot to gain for ecologically motivated studies, if they interlink observed phenotypes with epigenetic changes (Bossdorf and Zhang 2011; Richards et al. 2017). Thankfully, the recent years have shown that more and more studies were able to manage this interdisciplinary balancing act and combined ecological observations with epigenetic analysis (Foust et al. 2016; Rendina González et al. 2016; Trucchi et al. 2016; Alonso et al. 2016b, a; Richards et al. 2017; Herrera et al. 2017).

Plants have a very sophisticated silencing apparatus and make use of cytosine methylation at multiple sites, not only in CG dinucleotides like animals, but also in all other possible context at CHG and CHH positions (were H could be A, T or C) (Law and Jacobsen 2010). Methylation at CG or CHG context are considered as being symmetric, since they show mirrored methyl-cytosines on both complementary strands which can be easily sustained during DNA replication. In contrast, positions at the CHH sites are called asymmetric, since they are not mirrored on the complementary strand and would get lost during DNA replication. To be maintained during mitosis, these sites require a constant de novo methylation that uses mRNA as a guide to establish new methylation marks at respective genomic sequences in a process called RNA-directed DNA methylation (RdDM) (Dalakouras and Wassenegger 2013). This de novo methylation could change the expression of any gene, but not like a simple on-or-off switch; epigenetic processes work more like a dimmer and change expression levels gradually. Although epigenetic reprogramming is generally being considered to occur during the reproductive phase, distinct methylation changes happen during the vegetative growth stage. A lot of these informations come from the progressive silencing of transformation constructs in transgenic plants (Finnegan and McElroy 1994). Methylation changes are acquired surprisingly fast during vegetative growth, and showed for transgenic *Nicotiana attenuata* plants an increase in promoter methylation of up to 3% per day within a 15 day period (Weinhold et al. 2013). With such a rapid progression, the methylation levels increased finally by 78% (from 13.1 to 90.9%) within only 45 days of vegetative growth. As a consequence, the entire expression cassette showed a complete shutdown in gene expression, which was inherited to the offspring without discontinuation and no resetting or enhancement could be observed during generational transition (Weinhold et al. 2013). Although this example describes the methylation of a 35S promoter and the silencing of a heterologous transgene, it demonstrates how fast these changes can be acquired during vegetative growth. Experiments with reciprocal crosses could further demonstrate that the silenced allele was equally inherited by parental lines through both female as well as male gametes, which has interestingly also been observed in native populations (Herrera et al. 2013).

Suggestions for a simple experiment to investigate the epigenetic origin of transgenerational stress-adaption

A common approach in ecologically motivated studies is the manipulation of epigenetic marks by the application of demethylation agents (Herman and Sultan 2016). Despite the ease of application, this procedure has several deleterious effects on plant development and results in unwanted phenotypes.

An ecologically motivated experiment does not require mutants or certain cell lines, but only a near isogenic inbred-generation of plants as basis (Fig. 1). Here, any non-model plant system could be used as long as the natural life history of the plant has been considered for an appropriate trait analysis and the selection of a biotic or abiotic stress (Herman and Sultan 2016). Plants with short lifecycle or multiple generations per year are eligible, if their seeds are not dispersed, but rather drop on the ground and germinate in the same environment as the maternal generation. Compared to their genome-size, some plants seemed to have particular high levels in genome-wide DNA methylation and it could be worthwhile to preferentially select them for epigenetic studies. Among these, three millet species (*Pennisetum glaucum, Setaria italica, Eleusine coracana*) as well as sunflowers (*Helianthus annuus*), potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*) are worth mentioning (Alonso et al. 2015). Here, a further look at their wild relatives could be a good starting point to decide on a epigenetic model species.

More importantly, the type of stress needs to be selected based on the plant's natural life history (Bej and Basak 2017). Does the application of cold, heat or osmotic stress reflect the conditions of the plant's native growth habitat? Could it help the offspring to be preadapted to drought stress? Would a delay in the germination process be a possible trait to avoid unfavorable conditions? If seeds are dispersed by the wind or by animals, or have very long dormancy cycles, such pre-adaptations to temporally or spatially restricted conditions might be maladaptive. Is the appearance of a certain herbivore predictable (i.e., constant animal grazing), or is its lifecycles even tightly bound to the lifecycle of the host plant? Does the growth under competition result in phenotypic changes and could be used as alternative "stress" factor?

For the actual experiment, the plants would be divided into two groups, from which only one is exposed to the selected stress. The offspring of both groups would be used to screen for any transgenerational inherited trait (Fig. 1). Differences in gene expression could be analyzed by RNA-Seq (Colicchio et al. 2015) and used to select candidate genes which can be subsequently analyzed in their promoter methylation levels using bisulfite sequencing. Alternatively,

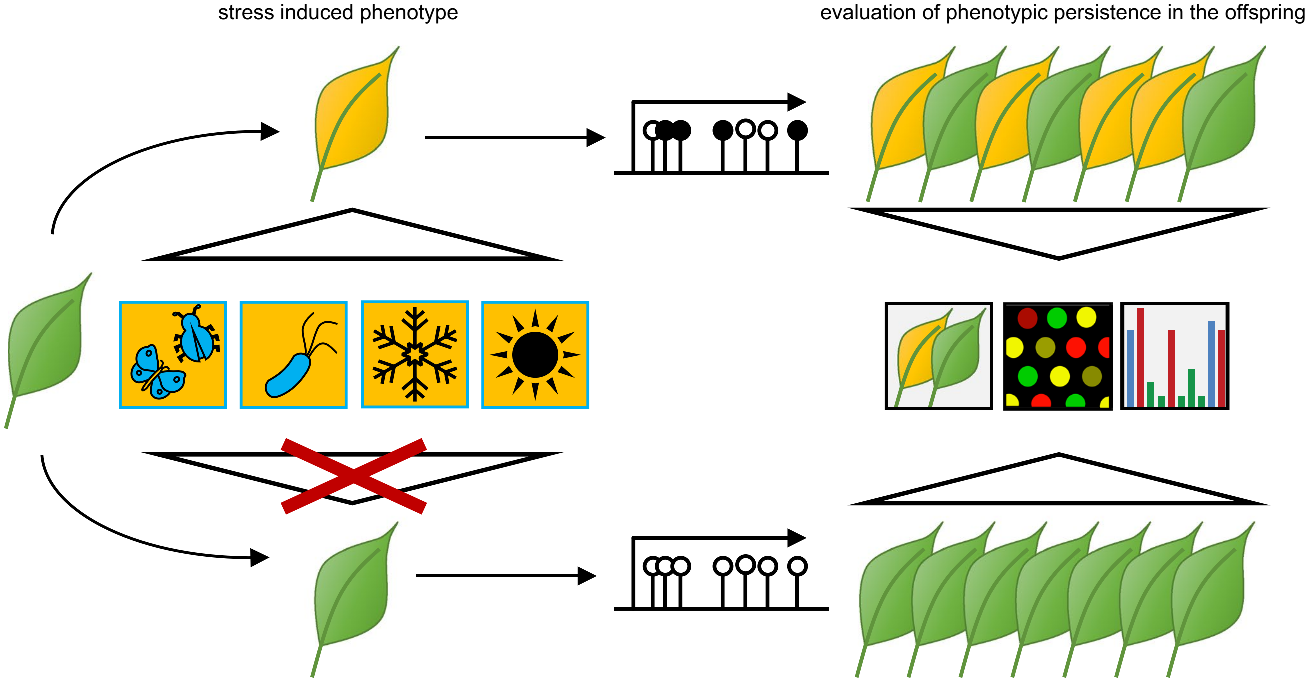

Fig. 1 Experimental setup to investigate the epigenetic origin of transgenerational stress-adaption in plants. A pool of near isogenic plants could be divided into control and stress receiving groups, and treated with either biotic or abiotic stresses. The offspring would be analyzed regarding persistence of a stress-induced phenotype. Candidate genes can be selected based on gene expression differences and analyzed for their epigenetic marks (e.g., cytosine methylation)

methylation-sensitive amplification polymorphism (MSAP), bisulfite-converted restriction site associated DNA sequencing (bsRADseq) or reference-free reduced representation bisulfite sequencing (epiGBS) have been successfully applied for genome-wide methylation analysis of non-model plants (Herrera and Bazaga 2011; Foust et al. 2016; Trucchi et al. 2016; Alonso et al. 2016b; van Gurp et al. 2016). This way, an ecologically motivated experiment which looks at transgenerational stress-adaption of a non-model plant could be connect to a subsequent molecular analysis to achieve a truly ecological epigenetic study as firmly suggested by Bossdorf and Richards (Bossdorf et al. 2008; Richards et al. 2017).

Conclusions

Regarding ecological epigenetics, the priority in the upcoming years should be the establishment of more realistic and plant life history-driven studies in combination with molecular tools for non-model species. So far, most studies lack the connection of the evaluation of epigenetic mechanisms combined with insights into the ecological or evolutionary background of the type of stress, which a plant perceives within the natural environments. We need true ecologically motivated experimental setups using suitable plant candidates and realistic environmental stimuli. Only then we could get full potential in finding and elucidating the role of epigenetic processes in transgenerational phenomena.

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