

The importance of forage legume epigenetics in the Anthropocene

Brady, J.* , Muir, J.P.* , Falk, A.†

* Texas A&M AgriLife Research; † Texas A&M University Kingsville

Key words: Anthropocene, forage legumes, genomes, genomics, epigenetics

Abstract. As climates change and economic as well as ecological pressures to produce more ruminant product on less land in the Anthropocene increase, the importance of using cutting-edge methods for forage legume improvement grows. The immediate return rate and turnaround on investment is likely greater for multi-species incorporation into grasslands but focusing on intraspecific variability and diversity could also contribute. The role of forage legumes in cultivated pasture and rangeland biodiversity as well as stability depends on genetics as well as management. Their epigenetics, however, may become increasingly important as climatic extremes and ecological pressures grow due to human-induced factors. Unfortunately, in many cases, forage legume epigenetics remain a black box. As we deal with the need for increasingly diverse forage legume species, should we be planning for this future in which genetic selection and improvement must also factor in epigenetic impacts in diverse edaphoclimatic and management systems?

Introduction

Studies of epigenetics involve changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself. Put another way, epigenetic changes to forage legume genomes can alter phenotypic outcomes without changing the underlying DNA sequence (Goldberg *et al.* 2007). The impacts of epigenetic systems can be mitotically and/or meiotically heritable, and they can influence plant phenotype for generations to come. The processes responsible for epigenetic changes are entirely natural, although they are now oftentimes initiated in response to man-made perturbations of the plant environment. Many forage breeders may not consider epigenetic impacts on their breeding program, while others may routinely use epibreeding as part of their breeding toolkit. For those not familiar with epigenetics, this short piece may serve as an initiation. Even if there's no plan to begin epibreeding, a basic idea of how phenotype can be influenced by many factors beyond gene content and DNA sequence can inform forage breeding program decisions.

One might wonder why a consideration of epigenetics would be important to plant breeders. In a simple case, if a dominant, single gene trait is incorporated into a plant, and that plant does not produce the expected phenotype, even though the same genotype has been consistent in phenotype for several previous years, frustration or curiosity might lead to an examination of what has happened. Also, in the case of crops with an extremely narrow genetic base due to limited breeding germplasm, a plant breeder might use epigenetics as a source of *de novo* phenotypic variation. One might also wonder why epigenetics and chromatin remodeling are necessary at all for a functional genome. The reasons may be reduced to two main factors: genomic organization and additional control of gene expression/DNA sequence mobility.

Chromosome 1 in the model legume *Medicago truncatula* is about 50 Mbp in length (Tang *et al.* 2014). If the DNA was placed in a straight line, it would be about 17 μm in length, as would its homolog. That single chromosome is about 7 times longer than the width of the nucleus (Carotenuto *et al.* 2019), where it shares space with 7 other chromosomes and their homologs. In order to efficiently package DNA into a small nuclear volume, eukaryotes wrap their DNA around 8 histone proteins in an open conformation called euchromatin. Euchromatin allows access to transcription factors required for active gene expression. Genomic regions that are not immediately required for gene expression are condensed into a tightly packed conformation called heterochromatin that prevents transcription factor access and takes up less nuclear volume. Most DNA is highly condensed and heterochromatic during cell division.

Beyond decreasing the nuclear volume occupied by DNA, epigenetic factors provide a means of controlling the expression of genes not immediately necessary (e.g., some genes required for germination are not required after successful germination), or regulating expression by environmental cues (Miryeganeh 2021). Additionally, many plant genomes are largely composed of transposable elements (TEs) and preventing further expansion by condensing TE DNA into heterochromatin provides genomic stability (Klein and Anderson 2022).

Mechanisms of Epigenetics

The transition from euchromatin to heterochromatin is reversible. Chromatin remodeling involves several mechanisms including methylation of DNA and multiple modifications of histone proteins that promote either attraction or repulsion of histone proteins from one another (Fig. 1). By tightly packaging histone proteins together, access to DNA by transcription factors is limited, preventing gene expression. Regardless of efforts to incorporate important genes for a given trait, if those genes are in heterochromatic chromosomal regions, they will not be expressed.

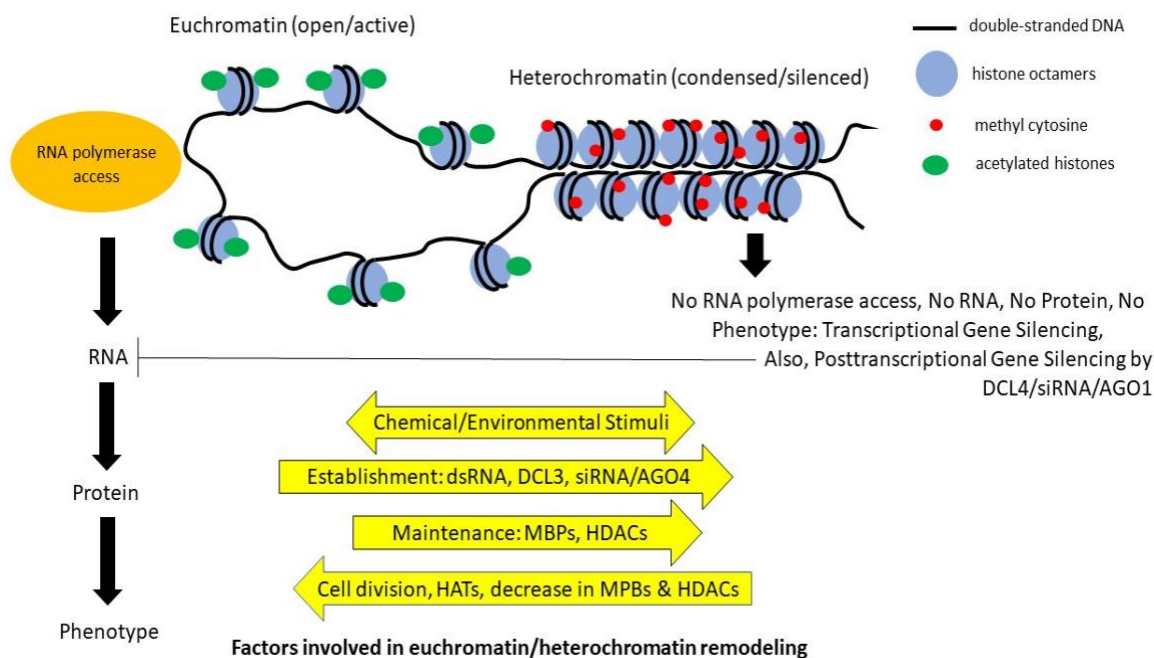


Figure 1. Abbreviated euchromatin/heterochromatin remodelling mechanisms and impacts on phenotypic expression of traits.

The epigenetic processes limiting expression of a given locus involve double-stranded RNAs for establishment, in a mechanism termed RNA interference (RNAi). RNAi initiates when a double-stranded RNA (dsRNA) is detected and processed into short 21- and 24-nucleotide short interfering RNAs (siRNAs) by DICER-LIKE endonucleases DCL4 and DCL3, respectively. Those 21- and 24-nt siRNAs are loaded onto ARGONAUTE 1 and ARGONAUTE 4 proteins that degrade RNA transcripts [post-transcriptional gene silencing (PTGS)] and initiate RNA-directed DNA methylation (RdDM) of the complementary genetic locus, respectively. RdDM requires dsRNA and siRNAs to initiate and maintain condensed heterochromatin. RdDM directs methylation of DNA that occurs on cytosine residues in CG, CHG, and CHH motifs (where H can be A, T, or C).

DNA methylation of CHH motifs is lost upon DNA replication during cell division without dsRNA/siRNA presence, but CG and CHG methylation is maintained by METHYL-BINDING PROTEINS (MBPs) that also recruit HISTONE DEACETYLASES (HDACs) to deacetylate specific lysine residues on histones H3 and H4, while HISTONE METHYLTRANSFERASES methylate H3, allowing histones to crowd even more tightly together and establish transcriptional gene silencing (TGS) in condensed, inaccessible heterochromatin.

Heterochromatin can be restored to euchromatin through cell division, in which CHH methylation of DNA is lost. Decreases in expression of the heterochromatin maintenance proteins also promote a transition from heterochromatin to euchromatin, reviewed in (Dalakouras and Vlachostergios 2021).

Chromatin remodelling is common to all eukaryotes. Many C motifs are methylated in plant genomes, with 50% of CG, 30% of CHG, and 3% of CHH motifs methylated in the legume *Phaseolus vulgaris* (Crampton *et al.* 2016) as an example. Much of that methylated and silenced DNA occurs in TEs.

Inducers and Outcomes of Epigenetic Modification

Euchromatin/heterochromatin remodelling impacts plant phenotype by providing an additional level of gene regulation, turning genes on/off due to environmental stimuli. Some examples of epigenetic impacts in

forage legumes (and other systems) and how anthropogenic inputs contribute are listed (Table 1). These include long-known plant environmental responses, such as the impact of vernalization on flowering in some

Table 1. Selected factors impacting epigenetic gene regulation in plants and other systems.

Stimulus	System	Reference	Impact
Biotic/abiotic			
Cold temperature (vernalization)	<i>Arabidopsis</i>	(Hepworth and Dean 2015)	Altered flowering, germination
Temperature (heat stress)	<i>Glycine max</i>	(Hossain <i>et al.</i> 2017)	Decreased DNA methylation
Drought	<i>Medicago</i>	(Ventouris <i>et al.</i> 2020)	Decreased DNA methylation
	<i>Pisum</i>	(Labra <i>et al.</i> 2002)	Increased DNA methylation
Repetitive heat, cold, salt stress	<i>Arabidopsis</i>	(Singh <i>et al.</i> 2014)	HDAC-mediated pathogen resistance
Herbivory	<i>Viola cazorlensis</i>	(Herrera and Bazaga 2011)	Differential methylation
Anthropogenic factors			
Air pollution	Mouse	(Park <i>et al.</i> 2022)	Downregulates cytoprotective gene expression
PFAs	Human children	(Xu <i>et al.</i> 2022)	Increased DNA methylation
Pesticides	Human	(Bianchi <i>et al.</i> 2020)	Compromised embryo development, other effects
Toxic metals from mining, fertilizers (e.g., Pb, Cd, Zn)	<i>Zea mays</i>	(Shafiq <i>et al.</i> 2020)	HDAC/MET up/down regulation
β -aminobutyric acid	<i>Solanum</i>	(Meller <i>et al.</i> 2018)	Increased resistance to fungal infection

species, to anthropogenic inputs such as polyfluoroalkyl substances (PFAs), and other chemicals/pharmaceuticals in our waste streams.

Considering Epigenetics in a Plant Breeding Program

Epigenetic changes to genomes should not be seen as a negative. In forage legumes and other crops with a narrow genetic base, it is possible to use inhibitors of the epigenome such as 5-azacytidine that inhibits DNA methylation, trichostatin A or sulfamethazine to inhibit histone deacetylation/or methylation, respectively. These treatments will create transient epialleles that can change phenotype. One thing to keep in mind, global hypomethylation of DNA will promote expression of previously silenced genes until the chemical treatment ends, then the epigenome will return to something close to its original state. However, TEs that mobilized during the hypomethylation will have created lasting genetic changes by moving from one genomic locus to another (DNA TEs) or by having been copied and pasted into novel genomic loci (retrotransposons). A TE insertion into a new locus represents a permanent change in DNA sequence and will be subject to the forces of breeder selection and/or population genetics. For breeders intent on exploiting and studying epigenetics, bisulfite DNA sequencing provides a method to study changes in DNA methylation across a genome.

In the face of rapidly changing weather patterns and long-standing as well as novel abiotic/biotic stresses occurring in breeding nurseries, tracking environmental conditions from previous years and conducting selection in multiple environments can become increasingly important. If a shuttle breeding season location is exceedingly wet and humid, drought tolerance phenotypes in the following year may reflect not only the genetic capacity of the cultivar, but a temporary epigenetic shift in phenotype away from drought tolerance. In such a situation, a breeder may be inadvertently selecting epialleles that are masking genetically controlled traits. Since the number of stresses impacting epigenetics is diverse, knowledge of how abiotic/biotic stresses compare from year to year can inform selection in wildly differing years. Additionally, seed increase with drastically different climate, latitude or management from the targeted growing region may lead to unexpected phenotypic changes in subsequent years. In other words, part of the genotype x environment x management interaction that confounds plant selection is due to epigenetics. From a practical standpoint, one way to minimize G x E x M interactions is to breed plants with environments/latitudes/management practices closely resembling near-future growing conditions.

Given the specific epigenetic response of some plants to environmental stress, it is also possible to epigenetically “prime” plants for that stress. If the planting location is almost certain to endure drought stress, producing seed under similar conditions can provide a more adapted epigenetic state prior to planting.

Genetics, Epigenetics versus Multi-species Mixtures

As climate trends, variability and extremes become more of an issue during the Anthropocene, the importance of epigenetics, as a tool available to forage legume breeders, may become an increasingly relevant issue to greater legume incorporation into grasslands. We know that, as species diversity increases, managed grasslands, whether cultivated, rangeland, or native prairie, tend to be more stable, resilient and productive vis-à-vis simpler swards (Richwine 2021; Taraborelli *et al.* 2022). Is the same true with epigenetic traits within legume species? A broader issue is whether grassland science should invest limited research resources in epigenetics (or breeding in general) instead of designing polycultures incorporating multiple legume species that return greater and more immediate return on investment.

Conclusions and/or Implications

Leaving aside the discussion of where forage legume research needs to invest—in intraspecific breeding programs or interspecific grassland ecosystems designs—as the Anthropocene advances, the mechanisms of how epigenetics modifies plant phenotype are likely to remain outside the concern of most forage breeders. Even plant breeders with highly integrated genomics programs seldom assess the epigenome as a routine practice. However, the impacts of epigenetics on plant phenotype as a result of changing environments and anthropogenic inputs should be a consideration for more forage breeders. In situations where a crop has a narrow genetic base, epigenetic modifications can be utilized to produce novel variation. As weather becomes more extreme, air quality degrades, infrastructure degrades, and inadvertent chemical/heavy metal exposure from water systems increases, it is wise to be mindful of epigenetics as a source of short- and long-term phenotypic variation in breeding programs that may at times mask the underlying genetics of plants.

References

- Bianchi, S., S. A. Nottola, D. Torge, M. G. Palmerini, S. Necozone and Macchiarelli, G. 2020. Association between female reproductive health and Mancozeb: Systematic review of experimental models. *International Journal of Environmental Research and Public Health* **17**(7): 2580.
- Carotenuto, G., I. Sciascia, L. Oddi, V. Volpe and Genre, A. 2019. Size matters: three methods for estimating nuclear size in mycorrhizal roots of *Medicago truncatula* by image analysis. *BMC Plant Biol* **19**(1): 180.
- Crampton, M., V. R. Sripathi, K. Hossain and Kalavacharla, V. 2016. Analyses of methylomes derived from Meso-American common bean (*Phaseolus vulgaris* L.) using MeDIP-Seq and whole genome sodium bisulfite-sequencing. *Front Plant Sci* **7**: 447.
- Dalakouras, A. and Vlachostergios, D. 2021. Epigenetic approaches to crop breeding: current status and perspectives. *J Exp Bot* **72**(15): 5356-5371.
- Goldberg, A. D., C. D. Allis and Bernstein, E. 2007. Epigenetics: A landscape takes shape. *Cell* **128**(4): 635-638.
- Hepworth, J. and Dean, C. 2015. Flowering locus C's lessons: Conserved chromatin switches underpinning developmental timing and adaptation. *Plant Physiol* **168**(4): 1237-1245.
- Herrera, C. M. and Bazaga, P. 2011. Untangling individual variation in natural populations: ecological, genetic and epigenetic correlates of long-term inequality in herbivory. *Molecular Ecology* **20**(8): 1675-1688.
- Hossain, M. S., T. Kawakatsu, K. D. Kim, N. Zhang, C. T. Nguyen, S. M. Khan, J. M. Batek, T. Joshi, J. Schmutz, J. Grimwood, R. J. Schmitz, D. Xu, S. A. Jackson, J. R. Ecker and Stacey, G. 2017. Divergent cytosine DNA methylation patterns in single-cell, soybean root hairs. *New Phytologist* **214**(2): 808-819.
- Klein, S. P. and Anderson, S.N. 2022. The evolution and function of transposons in epigenetic regulation in response to the environment. *Current Opinion in Plant Biology* **69**: 102277.
- Labra, M., A. Ghiani, S. Citterio, S. Sgorbati, F. Sala, C. Vannini, M. Ruffini-Castiglione and Bracale, M. 2002. Analysis of cytosine methylation pattern in response to water deficit in pea root tips. *Plant Biology* **4**(6): 694-699.
- Meller, B., D. Kuźnicki, M. Arasimowicz-Jelonek, J. Deckert and Floryszak-Wieczorek, J. 2018. BABA-primed histone modifications in potato for intergenerational resistance to *Phytophthora infestans*. *Frontiers in Plant Science* **9**.
- Miryeganeh, M. 2021. Plants' Epigenetic Mechanisms and Abiotic Stress. *Genes* **12**(8): 1106.
- Park, B., N. R. London Jr., A. Tharakan, P. Rengasamy, S. Rajagopalan, S. Biswal, J. M. Pinto and Ramanathan Jr., M. 2022. Particulate matter air pollution exposure disrupts the Nrf2 pathway in sinonasal epithelium via epigenetic alterations in a murine model. *International Forum of Allergy & Rhinology* **12**(11): 1424-1427.
- Richwine, J. D. 2021. Interseeding Native Warm-Season Grass Pastures. PhD diss., University of Tennessee, 2021.: https://trace.tennessee.edu/utk_graddiss/6597.
- Shafiq, S., A. Ali, Y. Sajjad, Q. Zeb, M. Shahzad, A. R. Khan, R. Nazir and Widemann, E. 2020. The interplay between toxic and essential metals for their uptake and translocation is likely governed by DNA methylation and histone deacetylation in maize. *International Journal of Molecular Sciences* **21**(18): 6959.
- Singh, P., S. Yekondi, P.-W. Chen, C.-H. Tsai, C.-W. Yu, K. Wu and Zimmerli, L. 2014. Environmental history modulates *Arabidopsis* pattern-triggered immunity in a HISTONE ACETYLTRANSFERASE1-dependent manner. *The Plant Cell* **26**(6): 2676-2688.
- Tang, H., V. Krishnakumar, S. Bidwell, B. Rosen, A. Chan, S. Zhou, L. Gentzbittel, K. L. Childs, M. Yandell, H. Gundlach, K. F. X. Mayer, D. C. Schwartz and Town, C.D. 2014. An improved genome release (version Mt4.0) for the model legume *Medicago truncatula*. *BMC Genomics* **15**(1): 312.

- Taraborelli, P., N. Carrasco, M. Malaspina, M. C. Domínguez, A. Belaus, A. López, A. S. Scavone, A. Barbera and Zamora, M.Y. 2022. Evaluation of agrobiodiversity and its trophic interactions as an indicator of sustainability in productive systems. *Open Journal of Environmental Biology* **7**(1): 006-013.
- Ventouris, Y. E., E. Tani, E. V. Avramidou, E. M. Abraham, S. N. Chorianopoulou, D. N. Vlachostergios, G. Papadopoulos and Kapazoglou, A. 2020. Recurrent water deficit and epigenetic memory in *Medicago sativa* L. varieties. *Applied Sciences* **10**(9): 3110.
- Xu, Y., C. H. Lindh, T. Fletcher, K. Jakobsson and Engström, K. 2022. Perfluoroalkyl substances influence DNA methylation in school-age children highly exposed through drinking water contaminated from firefighting foam: a cohort study in Ronneby, Sweden. *Environmental Epigenetics* **8**(1).