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DEVELOPMENTAL TIMING OF DAYLENGTH DEPENDENT CONDITIONAL LETHALITY IN *msh1/hda6* DOUBLE MUTANT *ARABIDOPSIS THALIANA*

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ABSTRACT

The mutation of the protein MSH1, responsible for organellar genome stability, induces genome wide epigenetic reprogramming. A histone deacetylase HDA6 is part of the RdDM pathway, which carries out these *msh1* mutant associated methylation changes, and also regulates circadian clock components. The mutation of *HDA6* on an *msh1* mutant background is conditionally lethal, as double mutants cannot be recovered in short daylength (12-hr) but are recovered in long daylength (16-hr) conditions. To determine the developmental timing of *msh1/hda6* conditional lethality, a series of transfers between daylength conditions were conducted. Double mutant *msh1/hda6* plants were transferred between 12- and 16-hr daylength at varying developmental stages. The persistence of the double mutant in 12-hr conditions showed that daylength-associated embryo lethality is unique to first generation *msh1*/*hda6* interaction. Heterozygous plants segregating for *msh1* and *hda6* were shifted from 12- to 16-hr daylength during flowering, revealing the ability to recover double mutant progeny during late-stage seed development. The results of these experiments suggest embryogenesis as a critical developmental stage for *msh1/hda6* conditional lethality, revealing the dynamics of environmental cues impacting chromatin remodeling.

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Chapter 1

Introduction

As sessile organisms, plants possess complex environmental response mechanisms to promote adaptation (Danchin et al., 2011). One form of non-genetic environmental response is epigenetic modification (Mirouze & Paszkowski, 2011). Epigenetic modifications can include changes in chromatin structure that are mediated through DNA methylation and histone acetylation. DNA methylation is the addition of methyl groups to cytosine bases in the DNA. Histone acetylation refers to the addition of acetyl groups on histone tails. Both DNA methylation and histone acetylation can change the condensation or accessibility of DNA and influence transcriptional activity and transcript processing (MacDonald & Roskams, 2009).

The manipulation of the MUTS HOMOLOG 1 (MSH1) protein serves as a model system for induced epigenetic reprogramming. *MSH1* is a nuclear gene that encodes a mitochondrial and plastid targeted protein responsible for organellar genome stability (Kundariya et al., 2022). *MSH1* disruption leads to genome-wide DNA methylation repatterning (Yang et al., 2020). The Mackenzie Laboratory studies the various epigenetic states that arise from MSH1 perturbation. Grafting *msh1* mutant rootstocks to wild-type scion leads to progeny with enhanced growth vigor (Kundariya et al., 2020; Virdi et al., 2015), making MSH1 manipulation of interest for crop yield improvement (Raju et al., 2018; Yang et al., 2015).

MSH1 depletion in plastids influences several developmental pathways that control flowering time, growth rate, abiotic stress response, and leaf morphology (Virdi et al., 2016; Xu et al., 2012; Yang et al., 2020). A central pathway in *msh1* effects is circadian clock regulation.

The *msh1* mutant undergoes differential gene expression for circadian rhythm specific loci compared to wild-type plants (Shao et al., 2017) and displays markedly more pronounced phenotype alteration under short day, 12-hr, growth conditions.

In plants, the RNA-directed DNA methylation (RdDM) pathway is responsible for *de novo* changes in DNA methylation that occur during *msh1*-triggered reprogramming (Kundariya et al., 2020, 2022; Yang et al., 2020). A component of the RdDM pathway that regulates circadian clock behavior and epigenetic machinery is *HISTONE DEACETYLASE 6* (*HDA6*). (Liu et al., 2012; Xiong et al., 2022). HDA6 participates in gene silencing (Aufsatz et al., 2007; Earley et al., 2010), nucleolar dominance (Pontes et al., 2007), drought tolerance (Kim et al., 2017), light-dependent chromatin compaction (Tessadori et al., 2009), and circadian rhythm regulation (Hung et al., 2019; Wang et al., 2013). HDA6 also enables DNA methylation by removing acetyl groups from histones, allowing the methyltransferase MET1 to methylate target loci (To et al., 2011). Disruption of HDA6 function leads to a decrease in global DNA methylation, although locus dependent variability has been observed (To et al., 2011). This combination of circadian clock regulation and methylation repatterning prompted us to investigate the potential for HDA6 interaction with MSH1.

Studies by Yang et al. (2020) first revealed HDA6 as essential for viability of the *msh1* mutant (Yang et al., 2020). Segregation of the *hda6* mutant in an *msh1* background showed that double mutant *msh1/hda6* progeny were not recovered under 12-hr daylength conditions. The observed double mutant lethality suggests that HDA6 functions are essential for the viability of the *msh1* mutant state. The previous study also showed evidence of skewing in favor of *msh1*(-/-)/*HDA6*(+/+) progeny, suggesting that successful gametes required a functional copy of HDA6 in

the mutant *msh1* background (Yang et al., 2020). The same pattern was true for fixed *hda6* and segregating *msh1* crosses similarly conducted under 12-hr daylength.

Identical crosses conducted in 16-hr daylength conditions successfully recovered *msh1/hda6* double mutant progeny at near-expected frequencies (Dopp et al., unpublished). This observation indicates that the double mutant lethality is daylength-dependent. The difference in double mutant recovery between 12- and 16-hr daylengths establishes a conditionally lethal phenomenon, with *msh1* mutant viability reliant on HDA6 function.

The goal of the current experiments was to determine the developmental timing of *msh1/hda6* conditional lethality, and the behavior of the double mutant once recovered. Data from these studies support a model of gametophytic influence of the *msh1/hda6* double mutant and provides evidence that the critical developmental window of epistatic interactions between the two genes occurs between pollination to embryogenesis.

Chapter 2

Methods

Experiment 1: Double mutant transfers

Plant materials and growth conditions

Four genotypes of *Arabidopsis thaliana* Col-0 plants were used in this study, *msh1/hda6* double mutant, *msh1* single mutant, *hda6* single mutant, and wild type (WT). The mutants were obtained from previous experiments conducted by the Mackenzie Lab. Parental populations were grown in 16-hr daylength conditions. Seeds were sown on a peat-vermiculite mixture and stratified for two days at 4°C. The plants were then moved to one of two growth chambers: non-permissive (12-hr) daylength or permissive (16-hr) daylength. Both growth chambers were set at 22°C and 120-150 μmol/m^2s light.

Daylength transfers

To test for a critical developmental time point in *msh1* reliance on HDA6 function, plants randomly arranged in trays were transferred from (i) 12-hr daylength to 16-hr daylength, or (ii) 16-hr daylength to 12-hr daylength (Fig. 1a). Each transfer group included six *msh1/hda6,* three *msh1*, three *hda6,* and three WT plants. Experimental groups included control (non-transferred), bolting (transferred at initiation of flower stalk growth), and flowering. Within the flowering group, four developmental stages were distinguished: mature silique, juvenile silique, open flower, and bud. Mature siliques were harvested, measured, and cleared in 50% ethanol solution to visualize seeds. The number of non-aborted seeds was recorded.

Data analysis

Seed density per silique was calculated as the number of normally developing seeds divided by silique length (mm). An ANOVA test compared the effect of genotype and treatment

on seed density. After a significant ANOVA result, Tukey's HSD Test for multiple comparisons was performed to determine statistically significant genotype and treatment groups.

Experiment 2: Double heterozygous shifts

Plant materials and growth conditions

Heterozygous *msh1*(+/-)/*hda6*(+/-) *Arabidopsis thaliana* Col-0 plants were generated from a cross of the two single mutant parents. Seeds were plated on 0.5 M Murashige and Skoog medium (Sigma, USA) with 1% (w/v) agar. The plates were stratified for two days in 4℃ and then placed in non-permissive (12-hr) daylength growth conditions. The seedlings were transferred to soil after 7 days.

Daylength shifts

At the stage where 10-12 siliques had developed in non-permissive (12-hr) daylength conditions, stems were marked above the highest developed silique and plants shifted to permissive (16-hr) daylength conditions (Fig. 5a). Mature siliques were collected prior to shattering and seeds were grouped based on treatment.

Genotype analysis

Seeds were surface sterilized with 100% ethanol, washed with 10% bleach, and rinsed 3x with distilled water. Seeds were plated on 0.5 M Murashige and Skoog medium (Sigma, USA) with 1% (m/v) agar. Seedling tissue was collected after 1-2 weeks. DNA was extracted using a DNA buffer containing 1 M Tris, 5 M NaCl, 0.5 M EDTA, 20x SDS, and dd-H_2O. To determine seedling genotype, PCR was performed with *HDA6* primers F: (5' GAT TCT GAG TGA GAG ACG GAG 3') and RP (5' AGC CAT ACG GAT CCG GTG AGG 3'), and *MSH1* primers: SAIL-RP: (5' GCT TTC CAT CGG CTA GGT TAG 3') and : SAIL-LP: (5' ACG GAA AAA

GTT CTT TCC AGG 3') to detect wild-type, and SAIL-RP and SAIL-LB: (5' TAG CAT CTG AAT TTC ATA ACC AAT CT 3') to detect *msh1* mutant. Amplification reactions were conducted in a 21 µL reaction volume, with 10µL distilled water, 9µL Taq red master mix, 0.5 µL forward primer, 0.5 µL reverse primer, and 1µL DNA. PCR conditions were 5 min at 95℃ for initial denaturation, 33 cycles of 30 sec at 95℃ for denaturation, 30 sec at 55°C for annealing, and 40 sec at 72℃ for extension. *HDA6* PCR products were fractionated by gel electrophoresis at 70 V for 70 min in a 2% (w/v) agarose gel, in 1 x TAE buffer. *MSH1* PCR products were fractionated by gel electrophoresis at 100 V for 40 min in a 1% (w/v) agarose gel in 1 x TAE buffer.

Chapter 3

Results

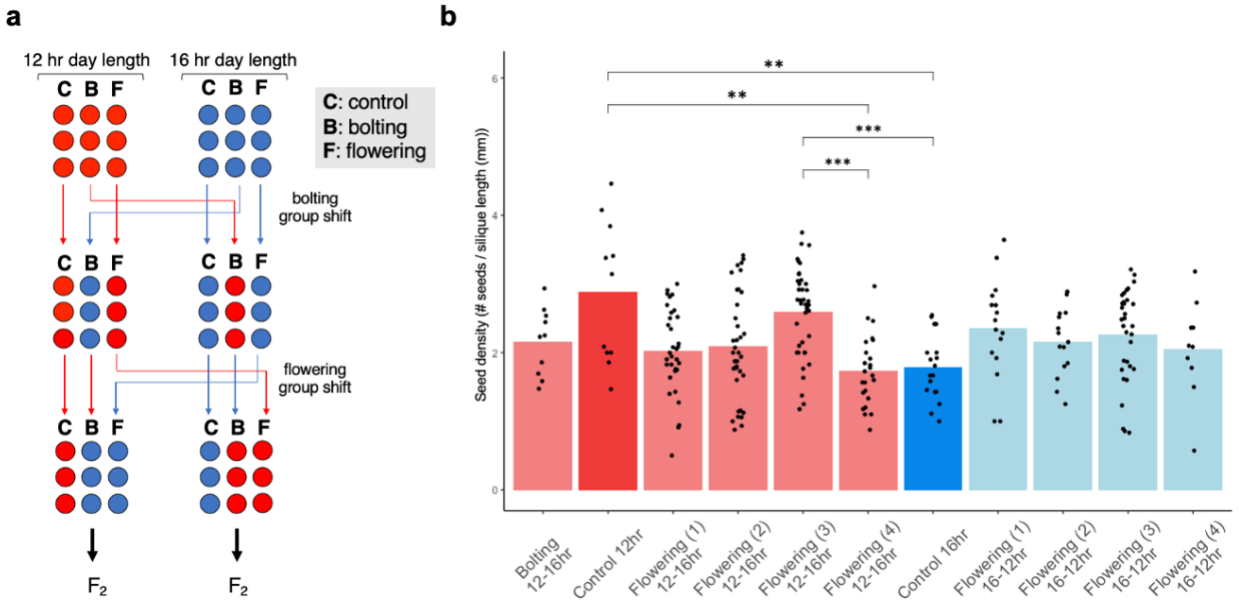

Figure 1: *msh1/hda6* double mutant daylength transfers between 12- and 16-hr daylength conditions at bolting and flowering.

(a) *msh1/hda6* daylength transfer experimental scheme**.** Seeds were germinated in either 12-hr daylength (red) or 16-hr daylength (blue). Genotypes included *msh1/hda6*, *msh1*, *hda6*, and WT. Three plants per genotype were transferred at bolting and three more at flowering (six for *msh1/hda6*). Plants were self-pollinated for F2 seeds. **(b)** Silique seed density results from *msh1/hda6* transferred between 12- and 16-hr daylength. Average seed density of siliques transferred at bolting (16-12hr transfer lost to disease), flowering, and non-transferred controls is shown. Transfers are colored as 12-hr controls (red), 12-16hr transfers (light red), 16-hr control (blue), 16-12hr transfers (light blue). Seed density measured as (total number of non-mutant seeds / silique length (mm)). Four flowering stages were distinguished: mature silique (4), juvenile silique (3), open flower (2), and bud (1). Tests for significance were performed using Tukey's HSD Test after a significant one-way ANOVA. Significance levels * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001.

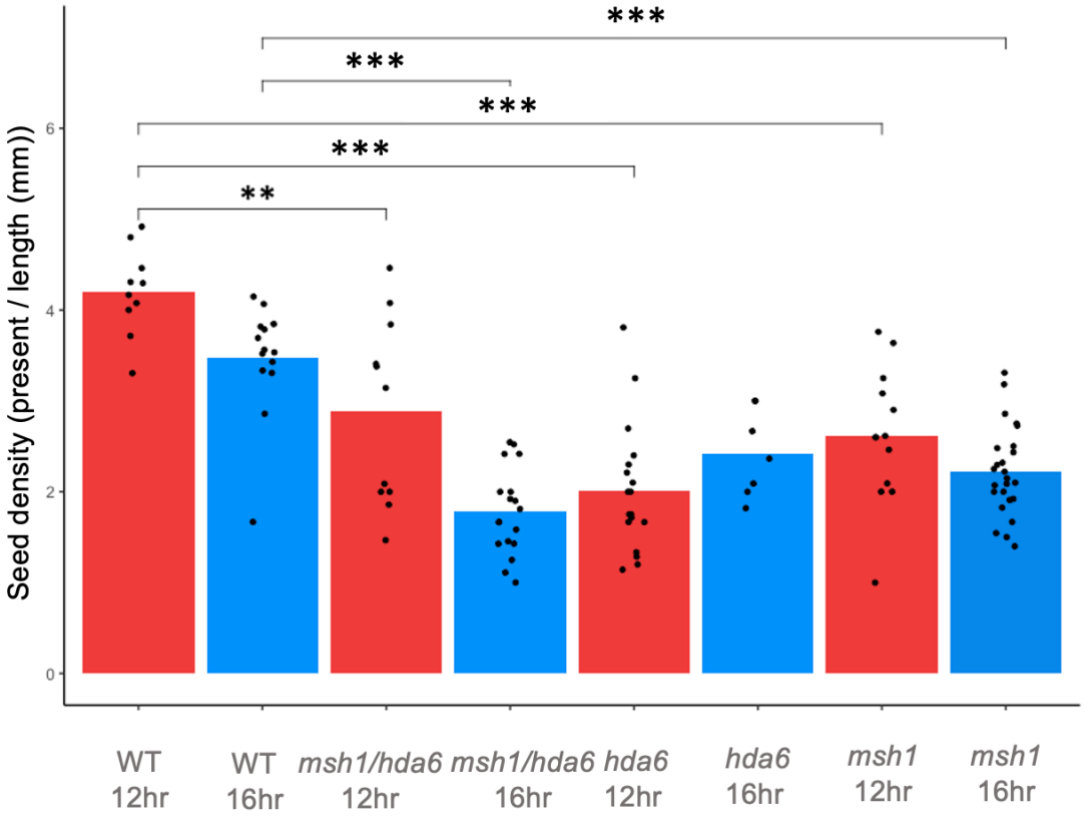

Figure 2: Non-transferred siliques.

Average seed density of WT, *msh1/hda6*, *hda6*, and *msh1* non-transferred control siliques. Controls are colored as 12-hr (red) and 16-hr (blue). Seed density measured as (total number of non-mutant seeds / silique length (mm)). Tests for significance were performed using Tukey's HSD Test after a significant one-way ANOVA. Significance levels * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001.

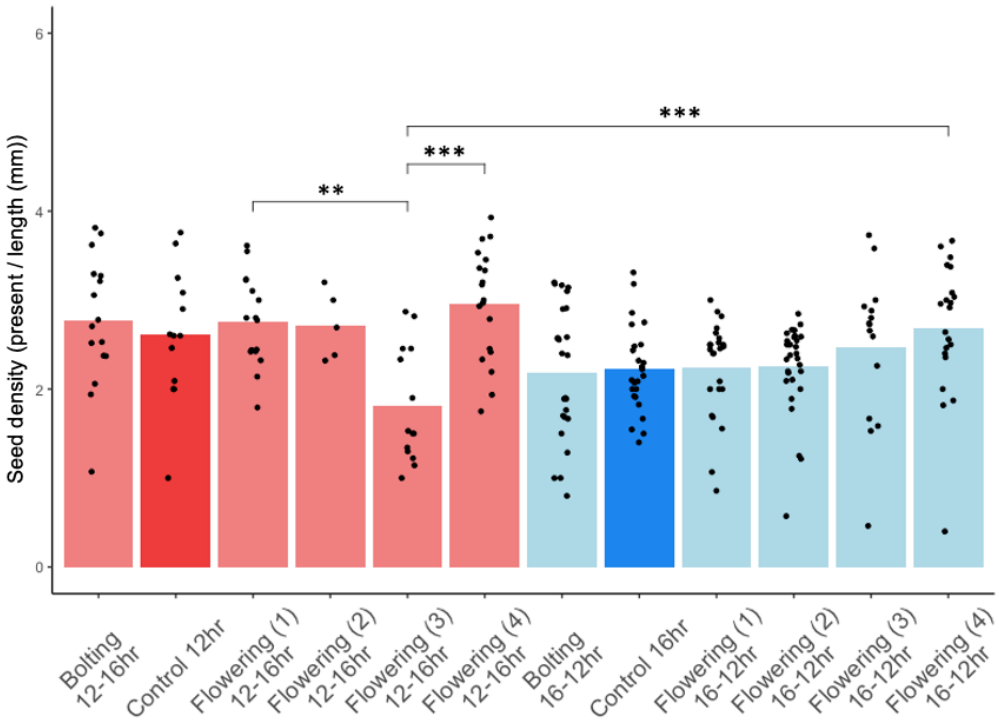

Figure 3: *msh1* single mutant transferred siliques.

Average seed density of *msh1* single mutant siliques transferred at bolting, flowering, and non-transferred controls is shown. Transfers are colored as 12-hr controls (red), 12-16hr transfers (light red), 16-hr control (blue), 16-12hr transfers (light blue). Seed density measured as (total number of non-mutant seeds / silique length (mm)). Four flowering stages were distinguished: mature silique (4), juvenile silique (3), open flower (2), and bud (1). Tests for significance were performed using Tukey's HSD Test after a significant one-way ANOVA. Significance levels * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001.

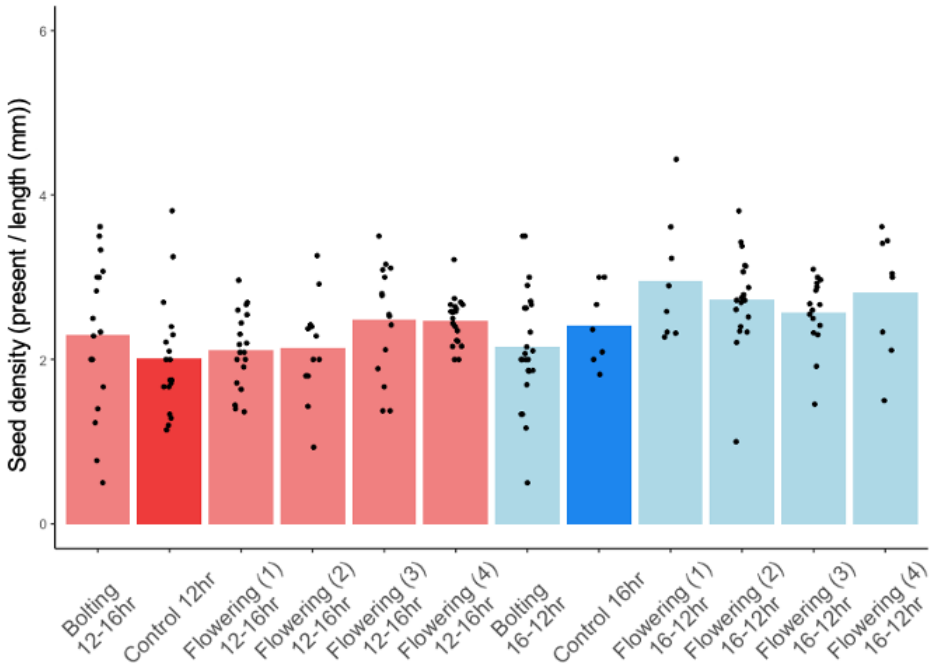

Figure 4: *hda6* single mutant transferred siliques.

Average seed density of *hda6* single mutant siliques transferred at bolting, flowering, and non-transferred controls is shown. Transfers are colored as 12-hr controls (red), 12-16hr transfers (light red), 16-hr control (blue), 16-12hr transfers (light blue). Seed density measured as (total number of non-mutant seeds / silique length (mm)). Four flowering stages were distinguished: mature silique (4), juvenile silique (3), open flower (2), and bud (1). Tests for significance were performed using Tukey's HSD Test after a significant one-way ANOVA. No transfer group comparisons were significant.

Transferring between permissive and non-permissive daylengths does not affect *msh1/hda6* seed abortion rate.

Initial experiments to determine the developmental timepoint of conditional lethality in *msh1/hda6* plants involved daylength shifts and calculation of resulting silique seed density. For the double mutant *msh1/hda6* plants, permissive (16-hr) non-transferred siliques had a significantly lower seed density than non-permissive (12-hr) non-transferred siliques (Fig 1b). The wildtype plants had a similar pattern of a lower mean seed density for the 16-hr non-transferred siliques, but this comparison was not significant. This observation provided an estimate of circadian influence on seed set. Four *msh1/hda6* transfer groups differed in seed

density: non-transferred 12-hr and transferred at flowering (mature silique) from 12- to 16-hr; non-transferred 12-hr and non-transferred 16-hr; transferred at flowering (juvenile silique) from 12- to 16-hr and transferred at flowering (mature silique) from 12- to 16-hr; transferred at flowering (juvenile silique) from 12- to 16- hr and flowering (mature silique) from 12- to 16-hr (Fig 1b). The *msh1/hda6* double mutants shifted from 16-hr daylength to 12-hr daylength at bolting were lost to disease.

Comparison of the transfers of single mutants *msh1* and *hda6* for silique seed density, indicated that loss of HDA6 eliminated or slightly reversed the circadian effect on seed density, while loss of *MSH1* resulted in overall lower seed density but continued to trend to lower seed density at 16-hr conditions (Figs. 3, 4). There was no significant difference in *hda6* single mutant controls or transfer groups (Fig. 4). This effect of *hda6* is likely due to loss of circadian clock regulation (Hung et al., 2019).

For the *msh1/hda6* transfers, there was no significant difference in seed abortion rate that would reflect a lethal developmental window when transferred to non-permissive conditions. This result, contrasted with earlier demonstration of first-generation *msh1/hda6* lethality at 12-hr daylength (Yang et al., 2020), implies that the breakdown in embryogenesis occurs at a defined interval restricted to first generation. Also, the seed density of *msh1/hda6* 12-16hr bolting transfer resembled the 16-hr control (Fig. 1b). Consequently, experiments were designed to focus on the timing of this first-generation lethality window.

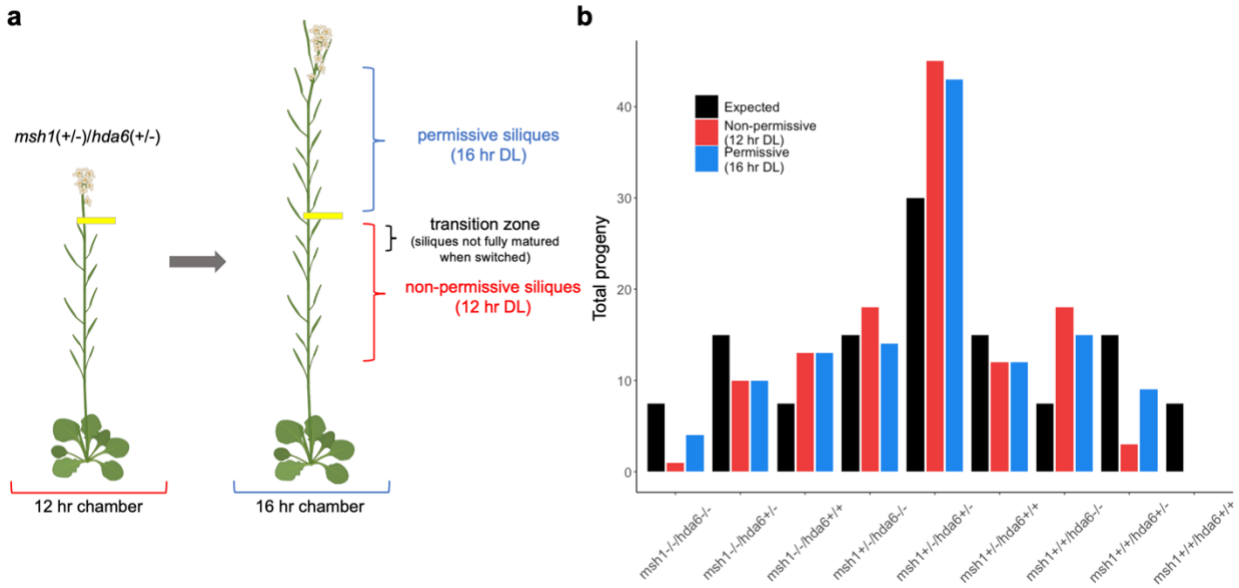

Figure 5: Heterozygous segregating *msh1*(+/-)/*hda6*(+/-) shifted between daylength conditions during flowering.

(a) The experimental scheme for shifting segregating heterozygous plants from non-permissive to permissive conditions. Five *msh1*(+/-)/*hda6*(+/-) were grown in 12-hr daylength conditions until halfway through flowering. Tape was placed above the elongated siliques, then the plant was transferred to 16-hr daylength conditions. The transition zone is defined by siliques not fully matured when the plant was shifted to permissive conditions. **(b)** Genotype distribution of F2 progeny from each daylength condition in (a). The expected distribution (black) is the distribution of an unlinked dihybrid cross.

Table 1: Segregation analysis of F2 progeny of four heterozygous *msh1*(+/-)/*hda6*(+/-) shifted from non-permissive to permissive daylength during flowering.

	CTRL	Plant 1		Plant 2		Plant 3		Plant 4			Total		
	***			*			**	***			***	***	
Progeny Genotype	12hr	12hr	16hr	12hr	16hr	12hr	16hr	12hr	16hr	**Expected**	12hr	16hr	**Expected**
msh1$^{+/+}$/*hda6*$^{+/+}$	1	0	0	0	0	0	0	0	0	1.875	0	0	7.5
msh1$^{+/-}$/*hda6*$^{+/+}$	1	7	4	3	4	1	2	1	2	3.75	12	12	15
msh1$^{-/-}$/*hda6*$^{+/+}$	12	2	2	4	2	4	4	3	5	1.875	13	13	7.5
msh1$^{+/+}$/*hda6*$^{+/-}$	0	1	2	0	1	0	5	2	1	3.75	3	9	15
msh1$^{+/-}$/*hda6*$^{+/-}$	6	1	4	14	13	10	7	10	9	7.5	45	43	30
msh1$^{-/-}$/*hda6*$^{+/-}$	4	1	4	1	1	6	3	2	2	3.75	10	10	15
msh1$^{+/+}$/*hda6*$^{-/-}$	5	3	1	3	3	4	7	8	4	1.875	18	15	7.5
msh1$^{+/-}$/*hda6*$^{-/-}$	3	5	3	5	5	4	2	4	4	3.75	18	14	15
msh1$^{-/-}$/*hda6*$^{-/-}$	0	0	0	0	1	1	0	0	3	1.875	1	4	7.5
Total	30	30	30	30	30	30	30	30	30	30	120	120	120

Significance levels (chi-squared test): *p-value< 0.05, **p-value < 0.01, ***p-value < 0.001.

Shifting heterozygous *msh1*(+/-)/*hda6*(+/-) to permissive daylength during flowering is sufficient for double mutant recovery in the F2 generation.

To investigate the effect of transfer timing on recovery of *msh1/hda6* progeny, heterozygous plants segregating for both *msh1* and *hda6* were shifted from non-permissive (12-hr daylength) to permissive (16-hr daylength) conditions halfway through flowering (Fig. 5). Siliques that matured fully in permissive conditions recovered four *msh1/hda6* double mutants, whereas siliques that matured in non-permissive conditions recovered one (Table 1). The distributions of genotypes were skewed significantly from the expected distribution in three of the groups. All plants showed some degree of skewing. Total counts for F2 progeny from both 12-hr and 16-hr shifted populations skewed significantly from the expected distribution (Table

1). The observation of a single viable double mutant in the non-permissive condition (12-hr) group was interpreted to reflect a single underdeveloped seed within a silique that, during shifting, remained receptive to the daylength effects.

Chapter 4

Discussion

The results of these experiments, taken together, are consistent with embryogenesis as a critical developmental stage in which circadian-driven conditional lethality occurs in the double mutant *msh1/hda6* in *Arabidopsis thaliana*. The *msh1/hda6* double mutant from 16-hr daylength parents was unaffected in creating progeny when germinated in 12-hr daylength, providing evidence that once *msh1/hda6* double mutants are created, the circadian-driven conditional lethality does not affect future generation embryogenesis. The recovery of double mutant *msh1/hda6* progeny from heterozygous *msh1*(+/-)/*hda6*(+/-) parents, when the siliques are allowed to fully develop in permissive conditions, provides evidence to support embryogenesis as the critical developmental stage for *msh1/hda6* conditional lethality.

The transfer of established *msh1/hda6* double mutant plants between permissive and non-permissive daylength conditions did not reveal the developmental stage of epistasis. This observation clarifies that 12-hr associated embryo lethality represents a condition unique to first-generation mutant MSH1-HDA6 interaction. The lower seed density that is observed in the 16-hr daylength *msh1/hda6* group can be explained by a phenomenon previously described in Arabidopsis, where wild type plants grown in longer days display decreased seed density as a result of larger seed size (Yu et al., 2023). This was observed in all genotypes except *hda6* single mutant, which displayed lower seed density for 12-hr control siliques, likely due to a loss of circadian clock regulation. Seed density as a metric to evaluate seed abortion in a daylength shifting experimental setup is therefore not appropriate for revealing the stage of epistasis, but it was sufficient in this study to measure circadian clock effects during reproduction. From this

experiment, it is reasonable to conclude that the developmental stage at which the double mutant effects are epistatic precedes bolting and is during reproduction in the parental generation.

When the heterozygous *msh1*(+/-)/*hda6*(+/-) parent generation was shifted from non-permissive (12-hr) to permissive conditions (16-hr) during reproduction, the recovery of the double mutant reveals the stage of epistatic interaction between *MSH1* and *HDA6*. The siliques from the two daylength conditions only slightly differed in genotype distributions but skewed away from the expected unlinked dihybrid cross distribution, in favor gametes with wild type *MSH1* allele, *HDA6* allele, or both. This was expected because gametes with the mutant copy of both *msh1* and *hda6* are less likely to contribute to the next generation, whereas gametes with a functional *MSH1* and/or *HDA6* are viable (Yang et al., 2020). In this experiment, some genotypes may have also been underrepresented due to low replicate numbers attributable to issues with disease and time limitations. The expectation of a skew in non-permissive conditions comes from previous self-pollination experiments of *msh1*(-/-)/*hda6*(+/-) plants that failed to recover double mutants in non-permissive conditions and strongly skewed towards the *HDA6* wild type allele (Yang et al., 2020). For siliques matured in permissive conditions, the genotype distributions were expected to follow a dihybrid cross distribution. A previous segregation analysis of *msh1*(+/-)/*hda6*(-/-) in 16-hr daylength found that the *MSH1* genotype segregated in the expected 1:2:1 ratio (Dopp et al., unpublished).

Four *msh1*(-/-)/*hda6*(-/-) progeny were recovered among 16-hr daylength siliques, confirming that the shift to permissive conditions in first-generation segregation permitted development of double mutant embryos (Table 1). Given previous experiments that established 12-hr daylength as non-permissive, any recovery of *msh1/hda6* progeny from a parent germinated at 12-hr conditions but later transferred to 16-hr daylength permissive conditions

identifies a developmental window for conditional lethality. Since shifting occurred during early silique formation, this window can be narrowed to embryogenesis. This model would align with what is known of HDA6 in the regulation of embryogenesis-associated components (Tanaka et al., 2008).

One *msh1*(-/-)/*hda6*(-/-) seed was recovered in the non-permissive (12-hr) group. This unexpected observation may be the consequence of a slight maturity variation among embryos. It is possible that a delayed maturity seed remained responsive to permissive daylength signals within a population insensitive to the shift (Table 1). Siliques located within the transition zone, collected after the shift to permissive conditions, were not fully matured when transferred (Fig. 5a). Seeds from these siliques were still in an early stage still susceptible to permissive signals that permit double mutant recovery.

Therefore, a permissive daylength signal during seed embryogenesis is sufficient to allow for *msh1/hda6* double mutant development, shown by *msh1*(-/-)/*hda6*(-/-) recovery in siliques moved to 16-hr daylength conditions during maturation. The data suggest that the developmental stage at which HDA6 function is critical for *msh1*-derived stress response signaling is at some point during embryogenesis. This implicates HDA6 as a possible gatekeeper in the environmental stress signaling from sensory plastid, via *msh1*, to the nucleus. MSH1 depletion results in a genome-wide methylation repatterning (Kundariya et al., 2022), and so the concurrent disruption of HDA6 in uncontrolled (12-hr) daylength conditions does not allow for the programmed methylation stress response to occur, leading to breakdown in the progeny (Yang et al., 2020).

These findings are important in determining the role of HDA6 function in association with the RdDM process that results from MSH1 depletion. Agricultural improvements resulting

from the epigenetic states of the *msh1* mutants can be further refined, as the mechanistic interaction between MSH1 and chromatin remodelers, such as HDA6, is still an active area of research. Overall, these experiments display a direct interaction between environmental sensing and chromatin remodeling within a limited developmental window in Arabidopsis.

BIBLIOGRAPHY

Aufsatz, W., Stoiber, T., Rakic, B., & Naumann, K. (2007). Arabidopsis histone deacetylase 6: A green link to RNA silencing. *Oncogene*, *26*(37), Article 37. https://doi.org/10.1038/sj.onc.1210615

Danchin, É., Charmantier, A., Champagne, F. A., Mesoudi, A., Pujol, B., & Blanchet, S. (2011). Beyond DNA: Integrating inclusive inheritance into an extended theory of evolution. *Nature Reviews Genetics*, *12*(7), Article 7. https://doi.org/10.1038/nrg3028

Earley, K. W., Pontvianne, F., Wierzbicki, A. T., Blevins, T., Tucker, S., Costa-Nunes, P., Pontes, O., & Pikaard, C. S. (2010). Mechanisms of HDA6-mediated rRNA gene silencing: Suppression of intergenic Pol II transcription and differential effects on maintenance versus siRNA-directed cytosine methylation. *Genes & Development*, *24*(11), 1119–1132. https://doi.org/10.1101/gad.1914110

Hung, F.-Y., Chen, F.-F., Li, C., Chen, C., Chen, J.-H., Cui, Y., & Wu, K. (2019). The LDL1/2-HDA6 Histone Modification Complex Interacts With TOC1 and Regulates the Core Circadian Clock Components in Arabidopsis. *Frontiers in Plant Science*, *10*. https://www.frontiersin.org/articles/10.3389/fpls.2019.00233

Imaizumi, T., Auge, G., & Donohue, K. (2017). Photoperiod throughout the maternal life cycle, not photoperiod during seed imbibition, influences germination in Arabidopsis thaliana. *American Journal of Botany*, *104*(4), 516–526. https://doi.org/10.3732/ajb.1600389

Kim, J.-M., To, T. K., Matsui, A., Tanoi, K., Kobayashi, N. I., Matsuda, F., Habu, Y., Ogawa, D., Sakamoto, T., Matsunaga, S., Bashir, K., Rasheed, S., Ando, M., Takeda, H., Kawaura, K., Kusano, M., Fukushima, A., Endo, T. A., Kuromori, T., … Seki, M.

(2017). Acetate-mediated novel survival strategy against drought in plants. *Nature Plants*, *3*(7), Article 7. https://doi.org/10.1038/nplants.2017.97

Kundariya, H., Sanchez, R., Yang, X., Hafner, A., & Mackenzie, S. A. (2022). Methylome decoding of RdDM-mediated reprogramming effects in the Arabidopsis MSH1 system. *Genome Biology*, *23*(1), 167. https://doi.org/10.1186/s13059-022-02731-w

Kundariya, H., Yang, X., Morton, K., Sanchez, R., Axtell, M. J., Hutton, S. F., Fromm, M., & Mackenzie, S. A. (2020). MSH1-induced heritable enhanced growth vigor through grafting is associated with the RdDM pathway in plants. *Nature Communications*, *11*(1), 5343. https://doi.org/10.1038/s41467-020-19140-x

Liu, X., Yu, C.-W., Duan, J., Luo, M., Wang, K., Tian, G., Cui, Y., & Wu, K. (2012). HDA6 Directly Interacts with DNA Methyltransferase MET1 and Maintains Transposable Element Silencing in Arabidopsis1[W][OA]. *Plant Physiology*, *158*(1), 119–129. https://doi.org/10.1104/pp.111.184275

MacDonald, J. L., & Roskams, A. J. (2009). Epigenetic regulation of nervous system development by DNA methylation and histone deacetylation. *Progress in Neurobiology*, *88*(3), 170–183. https://doi.org/10.1016/j.pneurobio.2009.04.002

Mirouze, M., & Paszkowski, J. (2011). Epigenetic contribution to stress adaptation in plants. *Current Opinion in Plant Biology*, *14*(3), 267–274. https://doi.org/10.1016/j.pbi.2011.03.004

Pontes, O., Lawrence, R. J., Silva, M., Preuss, S., Costa-Nunes, P., Earley, K., Neves, N., Viegas, W., & Pikaard, C. S. (2007). Postembryonic Establishment of Megabase-Scale Gene Silencing in Nucleolar Dominance. *PLOS ONE*, *2*(11), e1157. https://doi.org/10.1371/journal.pone.0001157

Raju, S. K. K., Shao, M.-R., Sanchez, R., Xu, Y.-Z., Sandhu, A., Graef, G., & Mackenzie, S. (2018). An epigenetic breeding system in soybean for increased yield and stability. *Plant Biotechnology Journal*, *16*(11), 1836–1847. https://doi.org/10.1111/pbi.12919

Shao, M.-R., Kumar Kenchanmane Raju, S., Laurie, J. D., Sanchez, R., & Mackenzie, S. A. (2017). Stress-responsive pathways and small RNA changes distinguish variable developmental phenotypes caused by MSH1 loss. *BMC Plant Biology*, *17*(1), 47. https://doi.org/10.1186/s12870-017-0996-4

Tanaka, M., Kikuchi, A., & Kamada, H. (2008). The Arabidopsis histone deacetylases HDA6 and HDA19 contribute to the repression of embryonic properties after germination. *Plant Physiology*, *146*(1), 149–161. https://doi.org/10.1104/pp.107.111674

Tessadori, F., Zanten, M. van, Pavlova, P., Clifton, R., Pontvianne, F., Snoek, L. B., Millenaar, F. F., Schulkes, R. K., Driel, R. van, Voesenek, L. A. C. J., Spillane, C., Pikaard, C. S., Fransz, P., & Peeters, A. J. M. (2009). PHYTOCHROME B and HISTONE DEACETYLASE 6 Control Light-Induced Chromatin Compaction in Arabidopsis thaliana. *PLOS Genetics*, *5*(9), e1000638. https://doi.org/10.1371/journal.pgen.1000638

To, T. K., Kim, J.-M., Matsui, A., Kurihara, Y., Morosawa, T., Ishida, J., Tanaka, M., Endo, T., Kakutani, T., Toyoda, T., Kimura, H., Yokoyama, S., Shinozaki, K., & Seki, M. (2011). Arabidopsis HDA6 Regulates Locus-Directed Heterochromatin Silencing in Cooperation with MET1. *PLOS Genetics*, *7*(4), e1002055. https://doi.org/10.1371/journal.pgen.1002055

Virdi, K. S., Laurie, J. D., Xu, Y.-Z., Yu, J., Shao, M.-R., Sanchez, R., Kundariya, H., Wang, D., Riethoven, J.-J. M., Wamboldt, Y., Arrieta-Montiel, M. P., Shedge, V., & Mackenzie, S. A. (2015). Arabidopsis MSH1 mutation alters the epigenome and produces heritable

changes in plant growth. *Nature Communications*, *6*(1), 6386. https://doi.org/10.1038/ncomms7386

Virdi, K. S., Wamboldt, Y., Kundariya, H., Laurie, J. D., Keren, I., Kumar, K. R. S., Block, A., Basset, G., Luebker, S., Elowsky, C., Day, P. M., Roose, J. L., Bricker, T. M., Elthon, T., & Mackenzie, S. A. (2016). MSH1 Is a Plant Organellar DNA Binding and Thylakoid Protein under Precise Spatial Regulation to Alter Development. *Molecular Plant*, *9*(2), 245–260. https://doi.org/10.1016/j.molp.2015.10.011

Wang, L., Kim, J., & Somers, D. E. (2013). Transcriptional corepressor TOPLESS complexes with pseudoresponse regulator proteins and histone deacetylases to regulate circadian transcription. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(2), 761–766. Scopus. https://doi.org/10.1073/pnas.1215010110

Xiong, L., Zhou, W., & Mas, P. (2022). Illuminating the Arabidopsis circadian epigenome: Dynamics of histone acetylation and deacetylation. *Current Opinion in Plant Biology*, *69*, 102268. https://doi.org/10.1016/j.pbi.2022.102268

Xu, Y.-Z., Santamaria, R. de la R., Virdi, K. S., Arrieta-Montiel, M. P., Razvi, F., Li, S., Ren, G., Yu, B., Alexander, D., Guo, L., Feng, X., Dweikat, I. M., Clemente, T. E., & Mackenzie, S. A. (2012). The Chloroplast Triggers Developmental Reprogramming When MUTS HOMOLOG1 Is Suppressed in Plants. *Plant Physiology*, *159*(2), 710–720. https://doi.org/10.1104/pp.112.196055

Yang, X., Kundariya, H., Xu, Y.-Z., Sandhu, A., Yu, J., Hutton, S. F., Zhang, M., & Mackenzie, S. A. (2015). MutS HOMOLOG1-derived epigenetic breeding potential in tomato. *Plant Physiology*, *168*(1), 222–232. https://doi.org/10.1104/pp.15.00075

Yang, X., Sanchez, R., Kundariya, H., Maher, T., Dopp, I., Schwegel, R., Virdi, K., Axtell, M. J., & Mackenzie, S. A. (2020). Segregation of an MSH1 RNAi transgene produces heritable non-genetic memory in association with methylome reprogramming. *Nature Communications*, *11*(1), 2214. https://doi.org/10.1038/s41467-020-16036-8

Yu, B., He, X., Tang, Y., Chen, Z., Zhou, L., Li, X., Zhang, C., Huang, X., Yang, Y., Zhang, W., Kong, F., Miao, Y., Hou, X., & Hu, Y. (2023). Photoperiod controls plant seed size in a CONSTANS-dependent manner. *Nature Plants*, *9*(2), Article 2. https://doi.org/10.1038/s41477-023-01350-y

ACADEMIC VITA
John Howard

EDUCATION

Pennsylvania State University
Schreyer Honors College | Presidential Leadership Academy
Bachelor of Science, Biology *Class of 2023*

WORK EXPERIENCE

Honors Undergraduate Researcher Sept 2021 – May 2023
Mackenzie Lab, Pennsylvania State University

- Conducting independent thesis research in *Arabidopsis thaliana* development, studying day length and epigenetic reprogramming.
- Designed and executed two stress growth trials managing 300+ *Arabidopsis thaliana* plants, including collected seed organization.
- Programmed bioinformatic pipeline in R analyzing differential epigenetic gene activation using whole genome sequencing data.
- Acquired $2,700 in research grants to support my thesis research from university funding agencies.
- Assisted in lab work of graduate researchers, including molecular biology techniques and methylome analysis, 15-20 hr/week.

Visiting Undergraduate Research Fellow Mar 2022 - July 2022
University of Bucharest & Pennsylvania State University

- Analyzed inorganic nutrient pollution concentrations through field sampling in the Danube River, predicting algal bloom threat.
- Modeled annual nutrient emissions of Brăila, RO, using wastewater treatment plant efficiency metrics and government data.
- Member of an American and Romanian undergraduate research team. Mentored teammates through oral presentation design and delivery, helping the development of English second language skills.
- Presented findings to members of the U.S. Embassy to Romania, local environmental officials, and university faculty.

National Science Foundation Undergraduate Researcher May 2021 - Aug 2021
Environmental Epigenetics Lab, Florida International University

- Researched epigenetic heat stress tolerance in corals as part of a larger FIU coral study in Bonaire, Dutch Caribbean.
- Analyzed chlorophyll density for over 300 coral fragment samples using pixel coloration and spectrophotometric measurements.
- Informed restoration efforts of replanting coral reefs on specific genotypic selection of corals to withstand ocean heat waves.
- Delivered individual oral presentation at FIU and the Council of Undergraduate Research NSF Conference in October 2021.

Data Analytics Summer Intern May 2020 - Aug 2020

Center for Nonprofit Management, Kellogg School, Northwestern University

- Analyzed participant demographic data and generated Excel portfolios for executive education end of year reports.
- Executed program management for LEAD Summer Business Institute providing technical support and faculty transition to virtual format for minority student leadership program.

LEADERSHIP EXPERIENCE

Presidential Leadership Academy May 2020 – May 2023

- One of 30 students selected to participate in leadership development facilitated by University President Emeritus Eric Barron.
- Four-year commitment including coursework on critical university issues and engaging with senior university leadership and major donors. Travel to US destinations for exposure to complex social, political, economic, and humanitarian issues.
- Learned decision making frameworks and applied through debates in diverse classroom settings and university case studies.

Pi Kappa Phi Fraternity Executive Board Nov 2020 - May 2022

- Standards Board Chairman leading a 90 member chapter through the pandemic and change in fraternity compliance leadership.
- Led initiative to create an internal judiciary process for brotherhood violations and formed our academic mentoring program.

Penn State Men's Club Volleyball Nov 2019 - May 2023

- General member practicing multiple times per week and traveled out of state to regional and national tournaments.