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# An Epigenetic Role for Disrupted Paternal Gene Expression in Postzygotic Seed Abortion in *Arabidopsis* Interspecific Hybrids

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## ABSTRACT

**Interspecific hybrids often increase the levels of heterozygosity and hybrid vigor, but some interspecific hybrid seeds are aborted shortly after fertilization. The mechanism behind this postzygotic seed abortion is poorly understood. Here, we report genome-wide analysis of allelic expression changes in developing siliques and seeds in three F1 interspecific crosses between *Arabidopsis thaliana* (Col, L*er*, or C24) and *Arabidopsis arenosa*. The majority of maternally expressed genes (MEGs) were shared among all three F1 interspecific crosses, whereas ~90% of 272 paternally expressed genes (PEGs) were found only in one or two F1 crosses, suggesting a role for disrupted paternal gene expression in seed abortion that varies in different crosses. Consistent with this notion, 12 PEGs in the infertile interspecific hybrids matched MEGs in fertile intraspecific hybrids. This disruption of PEGs in the interspecific hybrids was consistent with the upregulation of the genes in the paternal-excess interploidy cross (2X6) between a diploid mother and a hexaploid father, leading to the seed abortion. Moreover, a subset of PEGs in the interspecific crosses were also upregulated in the intraspecific hybrid *met1*XWT or *mea*XWT, in which the mutant of *MET1* (*DNA METHYLTRANSFERASE1*) or *MEDEA*, a Polycomb Repressive Complex2 gene, was used as the maternal parent. These data suggest that maternal epigenetic factors and paternal gene expression play important roles in the postzygotic seed abortion in interspecific hybrids or neo-allopolyploids.**

**Keywords:** epigenetics, imprinting, paternal gene expression, polyploidy, hybrid incompatibility, seed development

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## INTRODUCTION

Estimates indicate that ~10% of animal and ~25% of plant species hybridize with at least one other species (Mallet, 2007). Some plant species such as wild sunflower (Rieseberg et al., 2003) and animal species such as *Heliconius* butterflies (Mavarez et al., 2006) exist as hybrids. However, many hybrids have reduced viability and fertility, a phenomenon known as hybrid incompatibility or hybrid necrosis (Bomblies and Weigel, 2007). The Bateson-Dobzhansky-Muller model suggests that the hybrid incompatibility is caused by interactions between the genes that have functionally diverged in the respective hybridizing species (Bateson, 1909; Dobzhansky, 1936; Muller, 1942). These incompatibilities appear concurrently with speciation or consequently after species divergence. Indeed, postzygotic hybrid incompatibility has been associated with species-specific genes (Barbash et al., 2003; Brideau et al., 2006; Tang and Presgraves, 2009) and heterochromatin formation (Ferree

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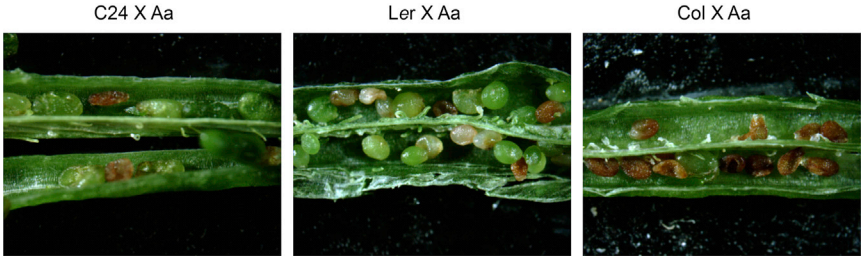


**Figure 1. Interspecific Seed Abortion Phenotypes.**
Seeds in siliques (mature stage) of three F1 interspecific hybrids or neo-allotetraploids between *A. arenosa* (Aa) and *A. thaliana* Columbia-0 (Col), Landsberg *erecta*, (L*er*), or C24 (C24) to illustrate abortive seed phenotype.

and Barbash, 2009) in *Drosophila*, nuclear and mitochondrial genome interactions in yeast (Lee et al., 2008), and evolution of duplicate genes into incompatibility genes in plants (Bomblies et al., 2007; Long et al., 2008; Bikard et al., 2009). Alternatively, the ''genomic imbalance'' model suggests association of incompatibility with disruption of maternal and paternal genome dosage and imprinted genes (Johnston and Hanneman, 1982; Haig and Graham, 1991; Scott et al., 1998). The incompatibility is also predicted to be associated with ''genome shock'' through restructuring of the genome by transposon activation and/or chromosomal rearrangements (McClintock, 1984). However, mechanisms for hybrid incompatibility remain largely elusive.

*Arabidopsis* interspecific hybrids provide a powerful model for studying postzygotic hybrid incompatibility. Seed abortion is frequently observed in the interspecific hybrids between *Arabidopsis thaliana* (At, maternal) and *Arabidopsis arenosa* (Aa, paternal), which is characterized by the failure of endosperm cellulization and embryo lethality (Bushell et al., 2003). In *Arabidopsis*, endosperm proliferates as a syncytium until heart stage (5–6 days after pollination [DAP]), followed by cellularization from the micropylar direction (Scott et al., 1998). During this process, if the endosperm fails to develop properly and is unable to provide nutrients for embryo, seeds are aborted. This often occurs between globular and heart stages at 4–5 DAP (Bushell et al., 2003). As a result, 90% or more of seeds produced in crosses between *A. thaliana* and *A. arenosa* are aborted (Comai et al., 2000; Bushell et al., 2003).

At the molecular level, seed abortion in these interspecific hybrids is accompanied by reactivation of silenced alleles such as paternal expression of heterochromatic element *ATHILA* and altered expression of *Polycomb Repressive Complex2* (*PRC2*) genes, including *PHERES1* (*PHE1*), *MEIDOS*, and *MEDEA* (*MEA*) (Josefsson et al., 2006). Mutations of *FIS2*-associated *PRC2* genes including *fis2*, *medea*, and *phe1* lead to endosperm overgrowth and seed abortion (Chaudhury et al., 1997; Kohler et al., 2003; Makarevich et al., 2008), which is similar to the phenotype observed in the F1 interspecific seeds. The FIS2–PRC2 complex controls imprinting status through repressing paternal *MEA* and maternal *PHE1*. PHE1 interacts with other type I MADS-box proteins to form a complex that represses early cellularization in endosperm (Makarevich et al., 2008). Thus, disruption of imprinting through PRC2-mediated repression can also cause seed abortion.

To uncover new genes and factors responsible for postzygotic hybrid incompatibility, we performed RNA sequencing (RNA-seq) analysis of allele-specific expression changes in three F1 interspecific hybrids or neo-allotetraploids formed between *A. arenosa* and three *A. thaliana* ecotypes (Col, L*er*, and C24). First, allelic expression data were compared among three F1 allotetraploids. Second, frequencies of maternal and paternal alleles in F1 allotetraploids were comparatively analyzed to test for the possibility of maternal- and paternal-biased expression during seed development. Third, maternal- and paternal-biased expression was compared with microarray data of reciprocal interploidy crosses and crosses with *MET1* and *MEDEA* mutants. The data linked seed abortion with disrupted paternal gene expression in interspecific hybrids. Expression disruption of paternally expressed genes (PEGs) is associated with paternal ploidy levels and maternal DNA methylation and MEDEA, which may modify chromatin of PEGs in seeds. A subset of maternally expressed genes (MEGs) and PEGs from RNA-seq analysis was validated by RT–PCR and sequencing of allelic transcript fragments that contain single-nucleotide polymorphisms (SNPs) between *A. thaliana* and *A. arenosa* genes.

## RESULTS

### Allelic Expression Changes in Different Interspecific Hybrids during Early Seed Development

It was observed that different ecotypes of *A. thaliana* used as maternal parent resulted in different levels of seed viability, ~1.6% in ColXAa (Bushell et al., 2003), 5%–13% in L*er*XAa (Comai et al., 2000; Bushell et al., 2003), and up to ~50% in C24XAa (all parents are tetraploids) (Bushell et al., 2003) (Figure 1). These data indicate that the seed fertility in interspecific hybrids or allopolyploids varies among maternal genotypes.

To test whether these differences were associated with gene expression changes, RNA-seq analysis was performed in developing siliques in three F1 interspecific hybrids derived from the crosses between *A. arenosa* and *A. thaliana* Col, L*er*, or C24 (Figure 1 and Supplemental Table 1). Because most immature seeds were aborted during early stages of development in these interspecific hybrids, we used siliques at 4 DAP for RNA-seq and gene expression analysis.

Imprinting plays an important role in the development of sexually reproducing organisms, including humans and flowering plants (Moore and Haig, 1991; Ferguson-Smith, 2011). In plants most imprinted genes are expressed in the endosperm (Berger and Chaudhury, 2009; Raissig et al., 2011). A previous study showed expression disruption of some imprinted genes during seed development in *Arabidopsis* allotetraploids (Josefsson et al., 2006), suggesting the possibility of disrupted expression of imprinted genes in these interspecific hybrids (Burkart-Waco