Parental Dialectic: Epigenetic Conversations in Endosperm

Souraya Khouider[1] and Mary Gehring[1,2*]

[1]Whitehead Institute for Biomedical Research, Cambridge MA 02142
[2]Dept. of Biology, Massachusetts Institute of Technology, Cambridge MA 02139
[*]Correspondence to: MG (mgehring@wi.mit.edu)

Abstract
Endosperm is a major evolutionary innovation of flowering plants, and its proper development critically impacts seed growth and viability. Epigenetic regulators have a key function in parental control of endosperm development. Notably, epigenetic regulation of parental genome dosage is a major determinant of seed development success, and disruption of this balance can produce inviable seed, as observed in some interploidy and interspecific crosses. These postzygotic reproduction barriers are also a potent driver of speciation. The molecular machinery and regulatory architecture governing endosperm development is proposed to have evolved under parental conflict. In this review, we emphasize parental conflict as a dialectic conflict and discuss recent findings about the epigenetic molecular machinery that mediates parental conflict in the endosperm.

Introduction

Reproduction in flowering plants is characterized by a double fertilization event where the haploid egg cell and the diploid central cell are both fertilized by haploid sperm cells, to produce the diploid embryo and the triploid endosperm, respectively (Figure 1). The endosperm is a transient nutritive tissue that interfaces with the embryo to support its growth and seedling germination and establishment [1]. Endosperm function is essential for seed viability. Most flowering plants have a triploid endosperm, composed of 2 maternal and 1 paternal genomes (2m:1p). Disruption of this genomic balance can result in a dysfunctional endosperm and abnormal seed development, although the sensitivity of the endosperm to parental ploidy deviations varies widely, even within a species [2–7]. These findings led to the concept of Endosperm Balance Number (EBN), where an effective ploidy value is assigned to a species based on its performance in crosses; effective ploidy might or might not correspond to actual ploidy. EBN thus defines the optimal maternal to paternal genome ratio that permits proper endosperm development [8–11]. Inter-ploidy or intra-specific hybrids where parental EBN is mismatched exhibit numerous phenotypes such as changes in seed morphology, hybrid seed inviability (HSI), or breakdown of postzygotic hybridization barriers [5,12–15]. At the molecular level, alterations to balanced effective ploidy and HSI are correlated with whole-genome transcriptional changes at imprinted and non-imprinted genes [16–19].

Genomic imprinting is an epigenetic phenomenon that results in the preferential expression of genes from the maternal or paternal allele. In plants, imprinting predominantly occurs in the endosperm and its establishment at least partly relies on modification of epigenetic profiles prior to fertilization, in the central cell and sperm cells (Figure 1) [20–24]. Imprinting is essential for proper seed development, and changes in expression of imprinted genes can impact seed growth or lead to seed abortion. Several hundred imprinted genes have been reported in plants, and although imprinting status is partially conserved across species, significant variations exist even within a species [25–38]. Distinct hypotheses have been proposed to explain the evolutionary forces that drive imprinting evolution [39]. Among those, the predominant parental conflict or kinship theory could be used to explain both EBN and imprinting evolution [40–47**]. This theory proposes that paternal and maternal parents have distinct interest in their offspring: fitness of the paternal parent is enhanced if maternal resources preferentially accumulate in his sired offspring (and not in unrelated maternal half-siblings), whereas fitness of the maternal parent is enhanced if her resources are equally distributed to

all her offspring. Parental conflict in endosperm could be molecularly mediated by the antagonistic effect of imprinted genes, and the parent-of-origin effect of genetic and epigenetic factors involved in imprinting establishment and maintenance of the EBN. The prediction is that paternally- and maternally-expressed imprinted genes (PEGs and MEGs) are selected to maximize the fitness of the paternal or maternal parent by promoting or repressing endosperm growth, respectively [42,43,48]. In this review, we explore recent findings on genetic and epigenetic regulation of seed development. We discuss molecular mechanisms behind parent-of-origin effects on parental fitness, as well as the dynamic balance between fitness optimization and speciation, emphasizing the dialectical notion of parental conflict in endosperm.

Parental conflict as a struggle for parental fitness

Imprinted gene expression relies on the establishment and maintenance of epigenetic asymmetry between parental alleles. Histone and DNA methylation are major determinants of the expression of MEGs and PEGs. Although various epigenetic profiles have been described, MEGs expression is generally associated with active DNA demethylation of the maternal allele in the central cell, prior to fertilization, whereas PEGs expression is associated with DNA methylation of the expressed paternal allele and active DNA demethylation and histone methylation-dependent silencing of the maternal alleles (Figure 1) [20–23,49–54].

As a major molecular outcome of parental conflict, imprinting is associated with presumed parental fitness traits, and several imprinted genes have been reported to be important for endosperm development and seed growth [22,55]. We highlight a few recent examples to illustrate the molecular mechanisms and pathways underlying parental control of endosperm development and seed size, which emphasize the role of epigenetic regulation and gene imprinting in the antagonist struggle of maternal and paternal parents after fertilization for their own fitness in the seed. Many such examples have been identified for MEGs, including the recent characterization of *EIN2*, an ethylene signal transduction gene that is imprinted in both *Arabidopsis thaliana* and maize [26–28,30,56]. Arabidopsis *EIN2* imprinting status depends on DNA methylation through the activity of the DNA demethylase (DME) prior to fertilization (Figure 1). *EIN2* negatively regulates seed size through temporal control of endosperm cellularization, providing a case study on how epigenetic imprinting regulation supports parental conflict [57]. In rice, the long non-coding RNA *MISSEN* is a MEG whose overexpression leads to increased endosperm proliferation and larger seeds, whereas reduced expression causes smaller seeds and endosperm deformity. *MISSEN* RNA is thought to mediate these effects by interaction with a helicase protein that in turns interacts with cytoskeletal proteins important for endosperm cellularization [58]. PEGs have also been proposed to mediate seed size in both maize and Arabidopsis through the regulation of essentials physiological processes such as auxin biosynthesis [59,60]. Nevertheless, fewer PEGs have been characterized as having a direct impact on seed development in diploid crosses [20,61,62]. A recent study in maize highlights a direct role for the quantitative PEG *Ded1*, which positively regulates kernel size in a dosage-dependent manner. *Ded1* encodes a MYB transcription factor that activates numerous genes during early endosperm development, including other imprinted genes. *ded1* mutants exhibit an incompletely-developed basal endosperm transfer layer, the region of the kernel important for maternal nutrient transfer [63**]. Of course not all, or even most, genes that affect seed development and size will be imprinted, but their regulation might still be tied to other imprinted genes or the imprinting machinery. *IKU2*, which encodes a leucine-rich repeat receptor-like kinase, is one such example. *IKU2* is expressed in early endosperm development and promotes endosperm proliferation. Mutation of *IKU2* in Arabidopsis or the cereal *Brachypodium* causes early endosperm cellularization and smaller seeds/grains [64,65*]. Recently, Wu *et al.* demonstrated that the FIS-PRC2 histone H3K27me3 methyltransferase complex, which is itself imprinted and maintains imprinting (Figure 1), methylates and silences *AtIKU2*, preventing further endosperm proliferation. In *Brachypodium*, *IKU2* is not methylated by the PRC2 complex, which is correlated with continued proliferation of cereal endosperm until maturity [65*].

Parental conflict: the need for an agreement

Parental fitness optimization also includes endosperm genomic balance. Disruption of EBN can have a drastic effect on seed development, leading to the impairment of endosperm development, embryo arrest, and seed abortion. This phenomenon, known as triploid block, is observed in interploidy hybridization (Figure 2) [13,14,66–69]. In species with nuclear-type endosperm development (a period of coenocytic endosperm development followed by cellularization), antagonistic effects are observed on endosperm cellularization timing, where paternal or maternal genome excess results in delayed or precocious endosperm cellularization, respectively [5,70,71]. Molecular mechanisms underlying triploid block have been elucidated by identifying factors that suppress triploid seed abortion, particularly in the context of paternal genomic excess, and implicate metabolic processes, genomic imprinting, and epigenetic regulation. Insights on triploid block and relevant metabolic pathways, including flavonoid biosynthesis, auxin homeostasis, and abscisic acid metabolism, can be found here [60,61,72–81].

Multiple epigenetic regulators and imprinted genes contribute to EBN and prevent successful seed development in conditions of paternal genome excess (Table 1) [16,17,19,61,82–86]. Although paternal inheritance of mutations in individual PEGs partially suppresses paternal excess seed abortion [61], normalization of PEG expression does not seem to be required for interploidy seed viability [19], suggesting multiple pathways independently contribute to EBN. Recently, chemically-induced epimutagenesis using 5-azacytidine, which causes DNA hypomethylation, suggests that additional epigenetically-regulated factors are involved in setting EBN [87*], supporting the long-standing link between parental control of seed development and DNA methylation [88,89]. These observations raises the question of how the molecular machinery mediates parental control of seed development. Multiple studies suggest a role for small RNAs and *de novo* DNA methylation, although the exact mechanism and site of action remains unclear. Expression of the antiviral protein RTL1, which suppresses small RNA biogenesis, under a pollen vegetative cell promoter, but not under a sperm cell promoter, paternally suppresses paternal excess seed abortion [90]. Interestingly, antagonistic parent-of-origin effects of *NRPD1* have been described [91–93*]. NRPD1 is the largest subunit of RNA Pol IV, a key component of the *de novo* DNA methylation pathway and an important factor for gene dosage control in endosperm. Disruption of *NRPD1* in paternal excess interploidy crosses has opposite effects depending of the parental origin of the mutation [19,93*]; maternal *NRPD1* promotes paternal excess seed viability, whereas paternal *NRPD1* restricts it. This observation is consistent with the parent-of-origin dependent effect of *NRPD1* on gene expression in diploid seeds, where maternal and paternal *NRPD1* regulate a partially distinct set of genes in endosperm, with some of them being antagonistically regulated [93*]. These observations support the hypothesis that epigenetic gene regulatory networks involved in parental control of seed development are evolving under parental conflict.

Parental conflict as a source of speciation

Features of the triploid block response are also frequently observed in hybrid seed inviability (HSI) resulting from interspecific hybridization. HSI is associated with gene misregulation, impaired imprinted gene expression, and defective endosperm development, although the molecular basis underlying this phenomenon can differ from that observed in intraspecific interploidy crosses. An increasing number of studies suggest that HSI evolves rapidly and is likely to be a major driver of speciation [18,44,94–105]. The impact of parental conflict in shaping endosperm-based reproductive barriers can vary based on species breeding strategies. The level of parental conflict is proposed to be higher in outbreeding species as suggested

by the weak inbreeder/strong outbreeder (WISO) hypothesis [106]. This model is supported by experimental evidence in several species and predicts that crossbreeding between outbreeders and selfers is likely to result in seed inviability due to dosage imbalance in the endosperm [15,44,107–111].

Endosperm functional and molecular heterogeneity plays an important role in seed development [112,113]. The endosperm chalazal region interfaces with maternal tissue to mediate nutrient transfer, making it a key factor in endosperm development [114]. Recent studies show that closely related *Mimulus* species exhibit differences in effective ploidy, which has been proposed to account for the severity of the seed inviability phenotype of *Mimulus* hybrids. This HSI phenotype is associated with abnormal chalazal haustorium development in a reciprocal parent-of-origin dependent manner, suggesting that this region is an important mediator of parental conflict [47**]. Interestingly, the chalazal endosperm region is also involved in the regulation of endosperm cellularization, and displays a very specific pattern of imprinted gene expression, with upregulation of PEGs [112,115]. Imprinting heterogeneity among endosperm domains is likely regulated by epigenetic mechanisms, since many epigenetic regulators are differentially expressed in the chalazal endosperm [112]. Investigating this question further will provide new insight on the epigenetic mechanisms underlying parental conflict.

The emergence of the endosperm is a key evolutionary innovation associated with angiosperms (flowering plants). Given its determinant function in HSI, endosperm has been proposed to be a critical contributor to angiosperm diversification, particularly through the rapid evolution of endosperm-dependent postzygotic reproduction barriers under parental conflict [103]. Nevertheless, a better comprehension of the underlying evolutionary and molecular mechanisms of endosperm development is necessary to enhance our understanding of parental conflict. Little is known about endosperm in Angiosperm lineages that predate the divergence of monocots and eudicots. Among these lineages, *Nymphaea* (water lily) seeds are characterized by a diploid endosperm (1m:1p). A recent study on these species revealed that endosperm hypomethylation and MEGs are an ancestral condition for endosperm, with PEGs likely emerging later in response to the doubling of maternal contribution in endosperm [116].

Together, these findings support the idea that biological mechanisms of endosperm development evolve under parental conflict and might be a powerful driver of angiosperm speciation and diversification.

Conclusion

Several scenarios have been proposed to explain the evolutionary forces that drive imprinting evolution in the context of seed development. These different theories are likely to coexist to explain the selective pressures that apply to such innovations in relation to their environments [117,118]. In this context, parental conflict provides an essential theorical evolutionary framework to understand how biological processes regulating seed development evolve. As a result of such a conflict, the molecular machinery underpinning seed development is subjected to an evolutionary arms race to ensure parental control. The characterization of genetic factors that are able to independently mediate both maternal and paternal control over seed development – like RNA Pol IV – pave the way for a better comprehension of the mechanistic complexity and the diversity of the molecular infrastructure underlying parental conflict.

Yet, the language of ‘conflict’ may obscure the vital need for compromise for both parents, without which, the viability of the offspring is in peril, negatively impacting parental fitness (e.g., triploid block). Thus, it might be informative to approach parental conflict as a dialectical conflict that embodies both a conflict and compromise component, and underlines the dynamic balance that must be struck by the parents in the interest of their offspring (Figure 2). When striving to improve their own fitness, each parent must avoid

upsetting the balance too much, which could impact offspring viability. From the species perspective, the breakdown of this compromise becomes a powerful driver of speciation, supported by postzygotic reproductive barrier mechanisms, that leads to rapid evolution of flowering plants. This phenomenon is likely to be a determinant factor in the astonishing diversification that makes angiosperms such an evolutionarily fruitful lineage. This implies that the parental conflict, conceived as a dialectical conflict, does not need to be resolved, since it contains in its very nature the elements that enable it to overcome its contradictions.

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Table 1: Epigenetic regulators that reduce or enhance endosperm effective ploidy in Arabidopsis

Gene	Molecular Function	Imprinted?	Effect
♀*MEA*, *FIS2*	H3K27 methylation, PRC2	MEGs	Reduce EBN
♀*NRPD1*	Subunit of RNA Pol IV	weak PEG	Reduce EBN
♂*NRPD1*	Subunit of RNA Pol IV	weak PEG	Increase EBN
♂*SUVH7*	Histone methylation	PEG	Increase EBN
♂*PKR2*	Chromatin remodeler	PEG	Increase EBN
♀,♂*DRM2*	DNA methyltransferase	No	Increase EBN
♂MET1	DNA methyltransferase	No	Increase EBN

Figure Legends

Figure 1: Epigenetic mechanisms of imprinting regulation. DNA and histone methylation are key epigenetic marks involved in imprinting regulation. Maternally expressed imprinted genes expression relies on DME-dependent active DNA demethylation of the maternal allele and silencing of the paternal allele through DNA methylation (1) or PRC2-dependent H3K27me3 histone methylation (2). DME-dependent demethylation can also prime the maternal allele in the central cell, which is then activated in the endosperm by transcription factors - here WRKY10 (3). Paternally expressed imprinted gene expression is usually associated with PRC2-dependent H3K27me3 silencing of the actively demethylated maternal allele while the paternal allele is protected from H3K27me3 methylation by DNA methylation (4) or other processes (5). These example epigenetic mechanisms are not mutually exclusive and generally coexist to regulate imprinting. Example genes are referenced here [57,62,91,119-126].

Figure 2: Parental genome dosage affects seed size and viability. Balanced parental genome dosage (middle panel) is essential for proper endosperm development. Factors improving maternal or paternal fitness restrict or promote seed growth, respectively, resulting in the production of smaller or larger seeds. In maternal and paternal excess genome crosses (left and right panels), genome dosage balance is upset, affecting seed viability. Precocious endosperm cellularization observed in maternal excess crosses results in small and shrunken inviable seeds, while paternal excess crosses delay endosperm cellularization, inducing abnormal seed enlargement that leads to abortion. Importantly, these phenotypes exist on a continuum.

Literature highlights

**Dai et al., 2022
Ded1 is a quantitative PEG that encodes a R2R3-MYB transcription factor expressed during early endosperm development. This study provides the first example of a PEG having a direct functional role in maize kernel development.

*Huc et al., 2022
This study demonstrates that 5-azacytidine-induced hypomethylation transiently bypasses the seed abortion phenotype associated with triploid block. Rescue is associated with genome wide CG-hypomethylation and restoration of normal PEGs expression levels. Interestingly, this chemically-induced hypomethylation also results in a partial breakdown of interspecies hybridization barriers between *Capsella* species.

*Satyaki et al., 2022
By using a multi-omic approach in *Arabidopsis thaliana*, this study highlights the differential effect on endosperm small RNA production, DNA methylation and gene expression regulation by maternal and paternal RNA Pol IV activity. They described an antagonistic parent-of-origin effect of Pol IV, and propose that Pol IV is part of a regulatory network evolving under parental conflict.

**Sandstedt and Sweigart, 2022
By performing reciprocal crosses between three *Mimulus* genus species, this work reveals a variable hybrid seed inviability severity in line with species divergence in effective ploidy. They also show that the chalazal haustorium exhibits differences between hybrid seeds that differ in effective ploidy, suggesting that the chalazal haustorium is a prime site for parental conflict.

*Wu et al 2022
This work examines the function of *IKU2* across several dicot and monocot species. *IKU2* regulates endosperm proliferation. Its repression by PRC2 in dicots but not monocots may explain differences in the extent of endosperm proliferation in these groups.

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