Kin Conflict in Seed Development: An Interdependent but Fractious Collective

Kin conflict in seed development: an interdependent but fractious collective

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ABSTRACT: Seeds are complex structures that unite diploid maternal tissues with filial tissues that may be haploid (gametophyte), diploid (embryo), or triploid (endosperm). These different tissues are subject to distinct, sometimes conflicting, selective forces with respect to control of seed size. The theory of kin conflict does not distinguish between the 'interests' of genes expressed in gametophytes before fertilization and the same genes expressed in embryos or endosperms after fertilization. Maternal tissues are predicted to favor smaller seeds than filial tissues, and filial genes of maternal origin are predicted to favor smaller seeds than filial genes of paternal origin. Consistent with these predictions, seed size is determined by an interplay between growth of maternal integuments, limiting seed size, and of filial endosperm, promoting larger seeds. Within endosperm, genes of paternal origin favor delayed cellularization of endosperm and larger seeds whereas genes of maternal origin favor early cellularization and smaller seeds. The ratio of maternal and paternal gene products in endosperm contributes to the failure of crosses between different ploidy levels of the same species and crosses between species. Small interfering RNAs (siRNAs) have been shown to inhibit the expression of complementary gene sequences. Within seeds, maternally-expressed siRNAs are predicted to associate with growth-enhancing genes and to be expressed before and after fertilization. By contrast, siRNAs associated with growth-inhibiting genes are expected to be expressed in male gametophytes before fertilization but not in endosperm after fertilization.

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INTRODUCTION

> "The seed will have to be viewed not as a totally harmonious unit, but as an interdependent but sometimes contentious assemblage of relatives." (Queller 1983)

A common mode of thinking sees parents as willing to make any sacrifice for offspring because offspring are parents' chance of genetic posterity. But this rosy picture ignores an inconvenient truth. Natural selection maximizes a parent's number of surviving offspring not the survival of individual offspring. Parents face an evolutionary tradeoff between investing less in each of a greater number of offspring or more in each of fewer offspring (Smith & Fretwell 1974).

Two ecotypes[1] of *Arabidopsis* illustrate the trade-off between offspring size and number: Cvi produces 30% fewer seeds than L*er* but Cvi's seeds are 80% heavier (Alonso-Bianca et al. 1999). Therefore, total seed weight per plant is more similar between ecotypes than either seed size or seed number. Similarly, overexpression of *KLU* increases *Arabidopsis* seed size without increasing total seed weight, because seeds per fruit and per plant are correspondingly reduced (Adamski et al. 2009). Thus, the size-versus-number trade-off may be expressed within individual fruits.

Mothers are selected to distribute resources among offspring to maximize the number of surviving offspring but offspring are selected to favor themselves over sibs. Therefore, offspring will attempt to acquire more resources from mothers than mothers have been selected to supply. This phenotypic conflict will typically be resolved with the production of larger but fewer offspring than would maximize maternal fitness (Trivers 1974; Godfray 1995).

A brief flurry of activity in the 1980s applied concepts of parent-offspring conflict to problems in seed development. Different tissues within ovules[2] (**Figure 1**) were shown to favor different trade-offs between seed size and seed number. Maternal tissues were predicted to abort a subset of seeds that

[1] **Ecotype**: The equivalent of an inbred line, propagated by self fertilization.

[2] **Ovule**: The maternal multicellular structure that develops into a seed after fertilization.

contained embryos of low quality or were surplus to the number mothers could provision, and to constrain the growth of seeds that were provisioned. Conversely, filial tissues were predicted to express adaptations to evade abortion and promote seed growth (Westoby & Rice 1982; Queller 1983, 1989; Haig & Westoby 1988).

The unusual genetic make-up of endosperm[3], triploid with two maternal genomes and one paternal genome (2m:1p), was a particular focus of attention. When endosperm was compared to an embryo[4], its extra maternal genome was interpreted as reducing filial demands and conferring greater maternal control (Westoby & Rice 1982) whereas, when endosperm was compared to a female gametophyte[5], its extra paternal genome was interpreted as favoring more aggressive acquisition of resources (Queller 1983). The parental-conflict theory of the evolution of genomic imprinting emerged from consideration of this contrast (Haig & Westoby 1989).

Control of seed size

Genetic data on seed size come mostly from *Arabidopsis* and the major grain cereals. These taxa may be atypical because parent-offspring conflict should be attenuated in predominantly self-fertilizing *A. thaliana* (de Jong et al. 2011) and because grain cereals have been subject to intense artificial selection to increase seed yields.

Arabidopsis seed volume is determined early, during rapid expansion of the central vacuole of syncytial endosperm. After endosperm cellularizes, the

[3] **Endosperm**: Product of the fertilization of the central cell of the female gametophyte by one of the two sperm released by a pollen tube. The central cell usually contains two haploid nuclei both of which fuse with the sperm nucleus to form the triploid primary endosperm nucleus.

[4] **Embryo**: Product of the fertilization of the egg nucleus of the female gametophyte by one of the two sperm released by a pollen tube.

[5] **Female gametophyte**: The multicellular haploid plant enclosed within an ovule. Fertilization of its egg cell gives rise to an embryo. Fertilization of its central cell gives rise to endosperm.

embryo expands, crushing the endosperm and accumulating food reserves, but there is little further increase in seed volume. Thus, seed maturation is associated with a rapid early increase in fresh weight, accompanied by a slower, but steady, increase in dry weight (Mansfield & Briarty 1992; Brown et al. 1999; Baud et al. 2002). Seed size in grasses is also determined by early endosperm expansion followed by deposition of food reserves but, unlike *Arabidopsis*, embryos remain relatively small and reserves are deposited in persistent endosperm (Olsen 2004).

During seed expansion of *Brassica napus*, invertase[6] splits sucrose into hexose sugars, doubling osmotic strength. The osmotically-driven influx of water is conjectured to facilitate rapid expansion of the central endosperm vacuole (Morley-Smith et al. 2008). In mechanical terms, endosperm turgor exerts tension on integuments[7], with the stiffness of integument cell walls determining the compression exerted on endosperm. Seed volume would then reflect, in part, how integuments respond to tension and endosperm to compression. *Mn1* encodes the invertase expressed in basal endosperm transfer cells[8] of maize during endosperm expansion (Kang et al. 2010). Loss of *Mn1* activity leads to endosperms with fewer and smaller cells (Vilhar et al. 2002). The causes of growth retardation are unknown but reduced turgor may contribute.

Endosperm expansion and integument growth interact to determine seed volume. *Arabidopsis* mutations with maternal effects on seed size commonly affect the proliferation or elongation of integument cells (Garcia et al. 2005; Schruff et al. 2006; FitzGerald et al. 2008; Hughes et al. 2008; Li et al. 2008; Adamski et al. 2009; Ohto et al. 2009) whereas mutations with filial effects on seed size commonly affect the timing of endosperm cellularization (Garcia et al. 2003; Luo et al. 2005; Kang et al. 2008; Zhou et al. 2009; Wang et al. 2010).

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[6] **Invertase**: Enzyme that catalyzes hydrolysis of sucrose to fructose and glucose.

[7] **Integument**: A maternal diploid tissue surrounding an ovule. Integuments give rise to the seed coat. Most angiosperms possess an inner and outer integument.

[8] **Transfer cells**: Cells with elaborate wall ingrowths that dramatically increase the cell membrane's surface area. Transfer cells are generally believed to play a role in secretion and/or absorption.

Endosperm and integument growth are coupled. Maternal *ttg*2 mutations cause reduced elongation of integuments and, as a filial response, precocious cellularization of endosperm whereas filial *haiku* mutations cause reduced proliferation of endosperm and, as a maternal response, reduced elongation of integuments (Garcia et al. 2005; Berger et al. 2006). The emerging picture, of maternal constraints on filial growth, is broadly consistent with kin-conflict theory (Westoby & Rice 1982; Queller 1983).

GENOMIC IMPRINTING IN ENDOSPERM

The genetic basis of parent–offspring conflict is subtle. If a sporophyte[9] is heterozygous for alleles at a locus expressed in integuments, then all ovules will exhibit the same pattern of expression, independent of which allele is inherited by the enclosed filial tissues. On the other hand, if the locus is expressed in filial tissues (female gametophytes, embryos, or endosperms), then the effects of maternal alleles will segregate among ovules depending on which allele a particular tissue inherits. Therefore, maternal alleles expressed in sporophytic tissues are selected to maximize the aggregate fitness of all embryos whereas maternal alleles expressed in filial tissues are selected to favor embryos with their copies over embryos without. A similar argument applies to paternal alleles expressed in filial tissues, but with a twist. Filial tissues of different seeds on a maternal sporophyte are less likely to share genes of paternal origin (patrigenes) than genes of maternal origin (matrigenes) when mothers produce offspring with multiple fathers. Therefore, patrigenes favor larger seeds than favored by matrigenes because competition among patrigenes is less constrained by costs to sibs (Haig & Westoby 1989; the matrigene/patrigene terminology was proposed by Queller 2003).

Maternal genes (expressed in maternal sporophytes) and matrigenes (expressed in filial tissues) are subject to different selective forces because alleles segregate at meiosis. Paternal genes (expressed in fathers) and patrigenes (expressed in filial tissues) similarly favor different outcomes. 'Parental-conflict' is a convenient label for conflict between matrigenes and patrigenes but is a

[9] **Sporophyte**: The diploid plant that produces haploid spores by meiosis.

misnomer if interpreted to mean that this is the same as conflict between maternal and paternal genes.

Matrigenes descend from an allele present in a megaspore[10] and benefit from the same outcomes whether expressed in female gametophytes before fertilization or from maternally-derived copies in embryos or endosperms after fertilization. Patrigenes descend from an allele present in a microspore[11] and benefit from the same outcomes whether expressed in male gametophytes or from paternally-derived copies in embryos or endosperms. A 'gametophytic' effect could be explained by persistence of transcripts from before fertilization or by parent-specific expression after fertilization (Curtis & Grossniklaus 2008). This makes a big difference if one's interest is mechanism, but no difference if one's interest is function[12]. It is the same gene, with the same interests, whether it is transcribed in female gametophytes before fertilization or from maternally-derived copies after fertilization. Kin-conflict theory distinguishes between gene expression before and after meiosis but not between gametophytic expression before fertilization and imprinted expression after fertilization.

Whether matrigenes and patrigenes pursue divergent goals in embryos and endosperms depends on genomic imprinting. Imprinted expression allows a gene to adopt different matrigenic and patrigenic roles whereas unimprinted expression forces an evolutionary compromise. Kin-conflict theory predicts maternally-expressed genes (MEGs) should reduce filial demands on mothers whereas paternally-expressed genes (PEGs) should increase filial demands, with the effects of unimprinted genes somewhere in the middle (Haig & Westoby 1989, 1991). In a completely self-fertilizing plant, matrigenes, patrigenes, and parental genes favor the same seed size.

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[10] **Megaspore**: A product of female meiosis. Megaspores produce female gametophytes by mitosis.

[11] **Microspore**: A product of male meiosis. Microspores produce male gametophytes (pollen) by mitosis.

[12] **Function**: 'Function of X' is used in this review as shorthand for 'reason why natural selection favors and maintains X.'

cis, *trans*, and *cum* effects

Gene expression is commonly viewed as jointly determined by *cis* effects, intrinsic to an expressed sequence's haplotype, and *trans* effects, exerted by the products of other genes (**Figure 2**). Epigenetic modifications in *cis* and *trans* are subject to different selective forces when meiosis intervenes between the establishment of a heritable modification and its effect on gene expression.

Suppose a parent is heterozygous for alleles at a locus that modifies imprints in *cis*. An imprint's effect on gene expression will be experienced only by offspring who inherit the allele responsible for the imprint. Therefore, epigenetic effects in *cis* will evolve to favor offspring who inherit the allele responsible for an effect at the expense of siblings who inherit alternative alleles (**Figure 3a**).

Suppose, instead, that the parent is heterozygous at a *trans*-acting locus that modifies imprints on both alleles at a second locus that is expressed in offspring. All of the parent's offspring will exhibit the same pattern of expression, independent of which allele at the *trans*-acting locus a particular offspring inherits (**Figure 3b**). If, however, alleles at the imprinted locus are differentially modified by the *trans*-acting locus, then these *cis* effects will segregate among offspring independently of alleles at the *trans*-acting locus (**Figure 3c**; assuming the loci are unlinked). In either case, *trans*-acting loci will evolve to favor parental interests.

Mammals establish imprints by *trans*-acting genes expressed in parental germ cells before meiosis. Therefore, conflict is possible between 'imprinting' genes expressed in parents and 'imprinted' genes expressed in offspring, with imprinted loci selected to re-interpret in *cis* parental instructions received in *trans*. Conflict similarly exists between imprinted and unimprinted loci expressed in offspring (Burt & Trivers 1998). For these reasons, natural selection at imprinted loci is expected to favor *cis* control. This may explain why imprinted loci in mammals often occur in *cis*-regulated clusters (Verona et al. 2003).

Angiosperms establish imprints in haploid gametophytes after meiosis. Therefore, *trans* effects of an imprinting locus are experienced by single alleles at imprinted loci. Conflict is absent because imprintor and imprintee are inherited

by the same set of offspring. Therefore, natural selection does not favor *cis* over *trans* control at imprinted loci. This may explain why mammal-like clusters of imprinted loci have not been found in plants (Luo et al. 2011; Wolff et al. 2011; but see Zhang et al. 2011).

A third set of effects are subject to different selective forces from *cis* and *trans* effects. I will call these *cum* effects for want of a better Latin preposition. A *cum* effect, or '*trans*-homolog interaction' (Hollick 2012), is an effect of a sequence on the expression of another copy of the same sequence (**Figure 2**). Genes with *cum* effects can respond to their own dosage and 'detect' whether their copies are inherited from the other parent, creating opportunities for 'collusion' between matrigenic and patrigenic alleles. Processes by which genes recognize their own copies have been called greenbeard effects after a genetically-encoded tag (the eponymous green beard) that allows altruists to recognize and cooperate with each other (Haig 1996; West & Gardner 2010). Newly-arisen *cum* effects can function as intragenomic green beards although the reliability of self-recognition decays as mutation creates variants that retain the tag but are no longer self. Existing models of kin conflict during seed development have not considered *cum* effects.

DNA methylation

Maternally-derived genomes of *Arabidopsis* endosperm are extensively demethylated relative to the paternally-derived genome of endosperm and the genomes of embryos and vegetative cells (Gehring et al. 2009; Hsieh et al. 2009). Differential methylation is established by coordinated expression of *DME*, a gene that encodes a DNA demethylase, and *MET1*, a gene that encodes a DNA methyltransferase. *DME* is expressed in central cells but neither sperm nor endosperm (Choi et al. 2002; Gehring et al. 2006; Schoft et al. 2011). *MET1* is expressed in sperm but not central cells (Xiao et al. 2006; Jullien et al. 2012). Therefore, at the time of fertilization, central cells are relatively demethylated whereas sperm are relatively methylated and this difference between maternal and paternal genomes is maintained after fertilization (Köhler et al. 2012). The methylated paternal genome and demethylated maternal genomes of *Arabidopsis*

endosperm contrast with the methylated maternal genome and demethylated paternal genome of mouse zygotes (Wu & Zhang 2012).

DME is expressed in central cells but not sperm cells whereas *MET1* is expressed in sperm cells but not central cells. Therefore, kin-conflict theory predicts *DME* should reduce endosperm proliferation whereas *MET1* should promote endosperm growth. Consistent with these predictions, seeds with maternal *dme* mutations have enlarged endosperm (Choi et al. 2002) whereas seeds with paternal *met1* mutations produce small, precociously cellularized, endosperm (Xiao et al. 2006). A simple story in which *DME* and *MET1* combine to establish different epigenetic states of maternal and paternal chromosomes cannot explain all imprinting in *Arabidopsis* endosperm because imprinted expression of *MEA* and a large class of small-interfering RNAs is maintained in the presence of maternal *dme* and paternal *met1* mutations (Mosher et al. 2011; Wöhrmann et al. 2012).

Rice endosperm does not undergo global demethylation nor have orthologs of *DME* been detected in the genomes of rice or other monocots (Zemach et al. 2010). Differentially-methylated regions of maize endosperm are maternally hypomethylated and paternally methylated but differential methylation is more localized than in *Arabidopsis* (Lauria et al. 2004).

Polycomb group proteins

Polycomb Repressive Complex 2 (PRC2) catalyzes trimethylation of lysine 27 residues on histone H3. H3K27me3 is frequently associated with transcriptional repression of associated DNA. Three subunits of PRC2 (MEA, FIS2, FIE) exhibit matrigenic expression in *Arabidopsis* endosperm (Rodrigues et al. 2010).

MEA is expressed in female gametophytes and from maternally-derived alleles in post-fertilization endosperm (Vielle-Calzada et al. 1999). A 200-bp sequence is necessary and sufficient for imprinted expression of *MEA* (Wöhrmann et al. 2012). MEA protein binds directly to *MEA* promoters and reduces transcription of maternal *MEA* alleles in endosperm. As a consequence of the absence of functional MEA, *mea* mutations are associated with dramatic upregulation of their own mRNA (Baroux et al. 2006). MEA also acts as part of

PRC2 to repress paternal *MEA* transcription in endosperm (Gehring et al. 2006; Jullien et al. 2006).

FIS2 is expressed in central cells before fertilization and from maternally-derived alleles in endosperm after fertilization (Luo et al. 2000). Therefore, *FIS2* and *MEA* have similar patterns of expression but, unlike *MEA*, imprinting of *FIS2* depends on DNA methylation (Jullien et al. 2006)

FIE is expressed exclusively from maternal alleles in early endosperm but from maternal and paternal alleles in later endosperm (Yadegari et al. 2000; Luo et al. 2000). Despite this phase of biallelic expression, patrigenic *FIE* does not rescue the endosperm overproliferation and seed abortion caused by matrigenic *fie* mutations (Vinkenoog et al. 2000). *fie* mutations are associated with reduced accumulation of their own transcripts in contrast to the effects of *mea* mutations that increase *mea* transcripts (Baroux et al. 2006). Patrigenic *fie* mutations cause patrigenic expression of *MEA* in endosperm (Jullien et al. 2006).

Seeds with matrigenic *mea*, *fis2*, or *fie* mutations initiate endosperm development without fertilization (Chaudhury et al. 1997; Luo et al. 1999, 2000) and exhibit prolonged proliferation and delayed cellularization of endosperm if central cells are fertilized (Grossniklaus et al. 1998; Kiyosue et al. 1999; Köhler & Makarevich 2006; Guitton & Berger 2011). Thus, wildtype PRC2 prevents endosperm proliferation before fertilization and accelerates differentiation of endosperm after fertilization (Ingouff et al. 2005*a*). These functions are consistent with predictions that MEGs will be associated with restraints on endosperm growth.

PRC2 genes of *Arabidopsis* and grasses lack one-to-one correspondence because of a complex history of gene duplication. The genomes of rice and maize lack obvious orthologs of *MEA* and *FIS2* (Spillane et al. 2007; Chen et al. 2009), but possess two *FIE*-related genes, at least one of which is maternally-expressed in endosperm (Springer et al. 2002; Danilevskaya 2003; Luo et al. 2009; Dickinson et al. 2012). Unlike *fie* mutations of *Arabidopsis*, disruption of *FIE1* in rice does not cause endosperm to develop without fertilization (Luo et al. 2009). Thus, the expression and function of imprinted genes is not conserved.

Three papers on molecular evolution of *MEA* collectively found stronger selection for new variants in outcrossing *Arabidopsis lyrata* than in self-fertilizing

A. thaliana, with evidence of balancing selection at the *MEA* promoter of *A. lyrata* (Kawabe et al. 2007; Spillane et al. 2007; Miyake et al. 2009). McVean and Hurst (1997) claimed the kin-conflict hypothesis entails an evolutionary 'arms race' that should leave a signature of accelerated evolution in the sequence of imprinted genes. However, an explicit model of the joint evolution of oppositely imprinted loci with antagonistic effects found a stable equilibrium at which there was no further change in expression levels (Wilkins & Haig 2001). Red Queen dynamics (running as fast as you can to stay in place) are not an inevitable outcome of kin-conflict.

Other imprinted loci

Deep sequencing of mRNA from reciprocal crosses between divergent strains of *Arabidopsis* (Gehring et al. 2011; Hsieh et al. 2011; Wolff et al. 2011), rice (Luo et al. 2011), and maize (Waters et al. 2011; Zhang et al. 2011) has identified numerous loci preferentially expressed from maternal or paternal alleles during seed development. These screens have substantially increased the number of candidate imprinted loci. Lists from different screens, even within the same species, show minimal overlap (Pignatta & Gehring 2012).

Little is known about the phenotypic effects of most imprinted genes. *FWA* and *AGL36*, for example, are MEGs expressed in endosperm, but their loss of function lacks an obvious phenotype in endosperm (Kinoshita et al. 2004; Lippman et al. 2004; Shirzadi et al. 2011). Therefore, whether these genes' functions support the kin-conflict hypothesis cannot be evaluated. Three imprinted loci with endosperm phenotypes are considered below. *PHE1* (*Arabidopsis*) is a PEG that exhibits a phenotype consistent with the kin-conflict hypothesis. *FH5* (*Arabidopsis*) and *Meg1* (maize) are MEGs whose phenotypes have been considered inconsistent with the hypothesis.

PHE1 is expressed from paternal alleles in syncytial endosperm. Maternal alleles are normally repressed by PRC2 but are reactivated in *mea* endosperms (Köhler et al. 2005; Makarevich et al. 2006, 2008; Villar et al. 2009). *mea* seeds abort after prolonged nuclear proliferation of endosperm, but this phenotype is partially rescued by reduced expression of *PHE1*. The rescued seeds are larger

than normal (Köhler et al. 2003). These data are compatible with a model in which paternally-expressed *PHE1* promotes nuclear proliferation in endosperm and larger seed size, but its expression is attenuated by maternally-expressed *MEA* (Schubert & Goodrich 2003). Another MEG (*At1g59930*) encodes a truncated PHE1-related protein that may act as a PHE1 antagonist (Hsieh et al. 2011).

FH5 is expressed from maternal alleles in chalazal endosperm (FitzGerald et al. 2009) with cellularization delayed in *fh5* seeds (Ingouff et al. 2005*b*). This phenotype has been interpreted as contradicting the kin-conflict hypothesis (Jullien & Berger 2010) but appears consistent with a model in which conflict is mediated by antagonism between endosperm proliferation (promoted by PEGs such as *PHE1*) and differentiation (promoted by MEGs such as *FH5*).

Meg1 is expressed from maternal alleles in just-cellularized endosperm but from both alleles at later stages of development (Gutiérrez-Marcos et al. 2004). Its expression is necessary and sufficient for formation of endosperm transfer cells. Knockdown of *Meg1* is associated with reduced seed weight whereas increased *Meg1* is associated with a dosage-sensitive increase in seed weight (Costa et al. 2012). Therefore, *Meg1* has been considered to contradict the parental-conflict hypothesis (Ikeda 2012; Jiang & Köhler 2012; Li & Berger 2012).

Effects of *Meg1* during the early phase of imprinted expression and the later phase of biallelic expression are entangled in Costa et al.'s (2012) study. Significantly, imprinted *Meg1* transgenes increase seed weight when inherited from fathers. Thus, at least some of *Meg1*'s effects on seed weight result from its biallelic expression, but the parental-conflict hypothesis addresses only effects of imprinted expression. Differentiation of transfer cells is determined early in syncytial development (Costa et al. 2003). Therefore, the hypothesis must address this aspect of phenotype. Imprinted expression of *Meg1* could be viewed as another expression of tension between MEG-promoted differentiation and PEG-promoted proliferation. This interpretation remains to be tested.

GENOMIC IMPRINTING IN EMBRYOS

Kin-conflict theory predicts imprinting of genes in endosperm but is equivocal about imprinting of genes in embryos. Seed volume is determined by maternal–endosperm interactions before food reserves are deposited, but the metabolic load on mothers, and competition among sibs, may be influenced by sink strengths during the filling phase. Whether embryos are passive observers or active participants in conflict over seed weight will depend on details of seed development and physiology.

A screen for imprinted loci in rice identified 262 candidates in endosperm but only three in embryos (Luo et al. 2011). Similar studies in maize (Waters et al. 2011) and *Arabidopsis* (Gehring et al. 2011) detected few imprinted genes in embryos. One gene (*mee1*) has been shown to be maternally-expressed in early embryos and endosperm of maize (Jahnke & Scholten 2009).

The paternal genome of *Arabidopsis* embryos is variously reported to be inactive during early embryogenesis (Autran et al. 2011) or to be active in two-celled embryos (Nodine & Bartel 2012). The latter report found little carry-over of mRNA from gametophytes in early embryos, but detected 77 transcripts with significant matrigenic bias and 44 transcripts with significant patrigenic bias, suggesting short-lived imprinting effects.

SSP mRNAs carried by *Arabidopsis* sperm are translated in zygotes and regulate the first asymmetric cell division that establishes apical (embryo) and basal (suspensor) cell fates (Bayer et al. 2009). *SSP* acquired its current function sometime after a whole-genome duplication in an ancestor of the Brassicaceae (Liu & Adams 2010). mRNAs transcribed in male gametophytes and delivered to zygotes via sperm are subject to the same selective forces as PEGs transcribed in embryos after fertilization.

INTERPLOIDY CROSSES

Failure of crosses between diploids ($2x$) and their own autotetraploids ($4x$) posed a puzzle for plant breeders. Viable seed was produced when diploids were crossed with diploids (embryo $2x$, endosperm $3x$) or tetraploids with tetraploids (embryo $4x$, endosperm $6x$), but endosperm development was grossly perturbed

when diploids were crossed with tetraploids. Embryos from interploidy crosses were triploid (3*x*), intermediate between viable 2*x* and 4*x* embryos, and endosperms were 4*x* or 5*x*, intermediate between viable 3*x* and 6*x* endosperms. So why did the crosses fail? Various hypotheses to explain 'triploid block' invoked requirements for particular ploidy ratios of seed coat, embryo, and endosperm, but these hypotheses were *ad hoc* attempts to fit the data without theoretical reasons why ploidy ratios should make a difference (Haig & Westoby 1991).

An elegant study in maize solved this puzzle by showing that normal endosperm development requires two maternal genomes for each paternal genome. Central cells of *ig* female gametophytes contain variable numbers of supernumerary nuclei. Fertilization of *ig* central cells by 1*x* or 2*x* pollen creates endosperms with a range of maternal and paternal ploidies. Most combinations abort. Normal endosperms are either 3*x* with two maternal and one paternal genome (2m:1p) or 6*x* (4m:2p). Significantly, 6*x* endosperms abort (5m:1p) or are well-formed (4m:2p) depending on the parental-genome ratio (Lin 1984).

Haig and Westoby (1991) proposed that seed phenotypes in crosses between 2*x* and 4*x* parents were explicable in terms of antagonistic actions of maternal and paternal genomes, with MEGs restraining and PEGs promoting endosperm growth. When mothers are 4*x* and fathers 2*x*, endosperms are 5*x* (4m:1p) and seeds are typically small with prematurely cellularized endosperm. We called this 'maternal excess.' In the reciprocal cross, endosperms are 4*x* and seeds often full-sized but shrivelled because endosperm fails to cellularize. We called this 'paternal excess.'

Viable seed are produced in interploidy crosses involving *Arabidopsis* accessions L*er* and C24 (Scott et al. 1998). Seeds with 4m:1p endosperms are lighter than normal, with slow mitosis in endosperm, whereas seeds with 2m:2p endosperms are heavier than normal, with accelerated endosperm mitosis. A plausible explanation why 2m:2p endosperms are viable in *Arabidopsis thaliana*, but non-viable in outcrossing relatives of *Arabidopsis* (Stoute et al. 2012) and most other species, is that conflict between matrigenes and patrigenes is attenuated in *A. thaliana* because of high rates of self-fertilization (Scott et al. 1998). In this view, imbalance in 2m:2p endosperms of outcrossing species causes seed

abortion but the underlying growth enhancement of paternal excess is uncovered in self-fertilizing *Arabidopsis* where imbalance is less severe.

Maternal sporophytic factors contribute to seed abortion in interploidy crosses. Fewer plump seed are produced in interploidy crosses using 4*x* Col rather than 4*x* L*er* as father (paternal effect) and 2*x* Col rather than 2*x* L*er* as mother (maternal effect). Recombinant inbred lines between Col and L*er* mapped the maternal effect to a QTL containing *TTG2*. Seed lethality in crosses with 4*x* Col fathers was rescued if 2*x* mothers were *ttg2* homozygotes (Dilkes et al. 2008).

Most seeds abort in interploidy crosses in maize: 2m:2p endosperms show prolonged proliferation with delayed onset of endoreduplication and fail to form a transfer cell layer (Charlton et al. 1995; Leblanc et al. 2002; Pennington et al. 2008); 4m:1p endosperms show reduced mitotic proliferation with premature onset of endoreduplication (Leblanc et al. 2002; Pennington et al. 2008). Maternal excess causes early silencing of genes associated with endosperm proliferation and early activation of genes associated with grain filling. Paternal excess has opposite effects (Li & Dickinson 2010).

These studies suggest maternal genomes of endosperm promote early differentiation with foreshortened nuclear proliferation and paternal genomes promote delayed differentiation with prolonged nuclear proliferation. Thus, the marginal effect of matrigenic expression would be to reduce seed size and the marginal effect of patrigenic expression to increase seed size. These effects are sometimes obscured by abortion of seeds with strongly perturbed development. Factors expressed in sporophytic tissues, with balanced maternal to paternal ratios, contribute to failure of seeds with imbalanced ratios in filial tissues.

INTERSPECIFIC CROSSES

Failure of crosses between species sometimes resemble failure of crosses between different ploidies within species, with features of maternal excess observed in one direction of a cross and features of paternal excess in the reciprocal cross (Johnstone et al. 1980; Bushell et al. 2003; Gutiérrez-Marcos et al. 2003; Köhler & Kradolfer 2011).

Success or failure of crosses between *Avena* species could be predicted by assigning each parent an Activating Value (AV) for its sperm nuclei and an equal Response Value (RV) for each nucleus of the central cell (Nishiyama & Yabuno 1978, 1979). Species with similar AVs crossed readily but development was perturbed in crosses between species with markedly different AVs. Normal endosperm development required an Activation Index (AI) = AV/2RV in the range 0.3 to 0.8; seeds were small and inviable if the AI was less than 0.2; small but viable for AIs between 0.2 and 0.3; but shrivelled and empty for AIs greater than 0.8.

A similar scheme was proposed to explain crosses between *Solanum* species. Each species was assigned an Endosperm Balance Number (EBN) determined by its behavior in crosses to a reference species. Normal endosperm required a 2:1 ratio of maternal to paternal EBNs (Johnstone et al. 1980). Failed crosses could sometimes be rescued by changing the ploidy of one of the parents to bring the ratio closer to 2:1 (Johnston & Hanneman 1982).

Haig and Westoby (1991) proposed that divergent expression of imprinted genes between species could result in developmental incompatibilities in hybrid endosperm because the balance of maternal and paternal products is perturbed. If divergence at multiple loci were haphazard, then increasing the ploidy of one parent might correct the ratio of products for some loci but exacerbate the problem at other loci. Therefore, we were puzzled why crossing behavior could be summarized by a single number (EBN or AI). Such indices suggested the effects of multiple imprinted loci aligned on a single developmental axis.

Mating system provides a possible solution to this puzzle. Conflict between matrigenes and patrigenes becomes less intense as single paternity of a mother's offspring becomes more common (Kondoh & Higashi 2000). Therefore, divergence in mating system could explain why multiple imprinted loci exhibit coordinated effects in hybrid seeds. A literature review of crosses between outcrossing and self-fertilizing plants found features of paternal excess when outcrossers were fathers but maternal excess when outcrossers were mothers (Brandvain & Haig 2005).

Endosperms exhibit severe paternal excess when *Arabidopsis thaliana* is crossed as seed parent to *A. arenosa*, but the cross succeeds when 4*x A. thaliana* is

substituted as seed parent. In reciprocal backcrosses of the latter hybrids to 4*x A. thaliana*, genomes of *A. arenosa* promoted early endosperm cellularization when contributed maternally but delayed endosperm cellularization when contributed paternally. Therefore, outcrossing *A. arenosa* was inferred to express greater "genomic strength" than self-fertilizing *A. thaliana* (Bushell et al. 2003) consistent with expectations from the divergence in mating system. Studies of hybrid *Arabidopsis* endosperm have shown extensive disruption of gene expression, including loss of imprinting of *PHE1* and *MEA* (Josefsson et al. 2006; Walia et al. 2009).

CONFLICTS AND PSEUDO-CONFLICTS

Two kinds of answer can be given to the question why a flower is red. The first explains redness in terms of mechanism: genes of the anthocyanin pathway are activated in petals. The second explains redness in terms of function: red flowers attract hummingbirds that move pollen from flower to flower. Different senses of 'why' are addressed by the two answers and few would argue that the truth of one negates the other. Unfortunately, explanations of mechanism and function are frequently confused when discussing the 'why' of genomic imprinting.

The 'maternal-offspring coadaptation' hypothesis explains the function of imprinted expression in terms of coordination rather than conflict (Curley et al. 2004; Swaney et al. 2007; Keverne & Curley 2008). The hypothesis is supported by a model of an epistatic interaction between an unimprinted gene in mothers and its imprinted or unimprinted copies in offspring (transgenerational *cum* effect). In this model, homozygous mothers benefit from inactivation of the paternal allele of their heterozygous offspring (Wolf & Hager 2006). At the time of writing, none of the imprinted genes claimed to support the hypothesis have been shown to conform to the model's rather specific assumptions (Haig 2013).

The 'differential-dosage' hypothesis proposes that many mechanisms, not just imprinted expression, cause parental effects in seed development (Dilkes & Comai 2004). Undoubtedly, parental effects exist that do not involve imprinted genes but the kin-conflict hypothesis neither denies their existence nor purports to explain them, whereas the differential-dosage hypothesis strives to

understand mechanisms of seed development, but does not purport to explain why parent-specific expression evolved. Presentation of these hypotheses as competitors confuses a hypothesis about mechanisms with one about functions.

The 'genome-defense' hypothesis proposes that DNA methylation and RNA interference have evolved to control the spread of genomic parasites and that these mechanisms are the means whereby genes acquire imprinted expression. The hypothesis has two separable components. The first posits that inactivation of selfish genetic elements is an important function, perhaps the primary function, of epigenetic gene silencing (Matzke et al. 1996; Köhler & Weinhofer-Molisch 2010). The second posits that some genes have acquired imprinted expression because of their proximity to, or resemblance of, sequences subject to processes of genome defense (Slotkin & Martienssen 2007; Gehring et al. 2009). Thus, an hypothesis about the function of epigenetic mechanisms is combined with an hypothesis about the mechanism of imprinted expression. Neither hypothesis directly challenges the kin-conflict hypothesis which addresses the function of imprinted expression.

Mechanisms of gene silencing may have evolved for reasons of defense but have been employed by other genes to achieve parent-specific expression because of conflict (Gehring et al. 2009; Köhler & Weinhofer-Molisch 2010). Male and female germ lines will possess different vulnerabilities to transposable elements (TEs) and different repertoires of host defense. Sex-specific adaptations of TEs, sex-specific counter-adaptations of hosts, and collateral effects of these adaptations provide a source of parent-specific variation in gene expression on which natural selection can act to mediate kin conflict (Haig 2012).

Explanations of mechanism do not obviate the need for explanations of function (why we observe this mechanism and not others) or lack of function. If the insertion of a transposable element confers imprinted expression upon an allele in *cis*, then the initially-rare imprinted allele becomes established in the population in one of two ways. Either imprinted expression conferred a benefit that caused the allele to sweep to high frequency under natural selection or imprinted expression had minimal effects on fitness and the allele drifted to high frequency by random processes. In the first case, the reasons why an imprinted allele is favored and maintained by natural selection relative to unimprinted

alleles comprise the function of imprinted expression. In the second case, imprinted expression does not have a function but is a side-effect of defense mechanisms (Haig & Trivers 1995).

What is the evolutionary history of genomic imprinting? What are the mechanisms of imprinting and what are the functions of these mechanisms? Why have some sequences, but not others, evolved to use these mechanisms to achieve imprinted expression? These are all important questions. Much needless argument would be avoided by paying close attention to the questions addressed by each hypothesis. 'Conflict' and 'coadaptation' hypotheses both purport to explain the function of imprinted gene expression. 'Differential dosage' and 'genome defense' are sometimes presented as rivals of 'conflict' but, for the most part, address different questions.

FUNCTIONS OF siRNAS

siRNAs and transposable elements

Small-interfering RNAs (siRNAs) target DNA methylation and histone modification to complementary DNA sequences and, by this means, exert *trans* or *cum* effects on gene expression. siRNAs complementary to TEs are believed to function in host defense (Lisch & Slotkin 2011). Most of the machinery that synthesizes siRNAs acts in *trans* and should evolve to promote host fitness and reduce TE activity, but how do the sequences recognized by this machinery evolve in *cis* and *cum*? The question whether an siRNA transcribed from a TE's own sequence is an adaptation of the host or the parasite is not simple.

Natural selection after insertion of a TE selects for variants that enhance fitness of the haplotype on which the TE resides but acts of insertion select for transposition-competent TEs. Therefore, the lineage of a recently-inserted TE will have been subject to both forms of selection. The footprints left by a mobile TE as it wanders through the genome are either erased by selective elimination of costly insertions or remain as domesticated remnants subject to degradation of their ability to transpose (Haig 2012). siRNA-encoding sequences of domesticated TEs can be considered host adaptations if they are selectively maintained because of their ability to silence their host sequence or related TEs.

siRNAs could also be considered adaptations of TEs if self-restrained TEs spread more readily than unrestrained TEs.

Self-restraint of TEs is considered unlikely to evolve in outbred plants but more likely to evolve in selfing plants (Charlesworth & Langley 1986). Insertions often reduce fitness. TEs of outcrossing plants that generate new insertions soon segregate away from their costly progeny, just as less-deleterious insertions segregate away from their more-costly brethren. By contrast, the recent copies of an active TE of a self-fertilizing plant are yoked together for many generations before parting company after rare outcrossing. An overly-active TE would foul its own nest. For this reason, TEs may evolve to be less virulent in selfing plants.

Once a TE is homozygous because of selfing, it is transmitted to all of a sporophyte's progeny. Propagation of new insertions might be facilitated by restricting transposition to occasions on which the TE is rendered heterozygous by outcrossing. Dosage-sensitive responses to its own *cum*-acting siRNA might allow a canny TE to 'recognize,' and act appropriately toward, its own copies on other chromosomes.

Arabidopsis has been proposed to use strategic bursts of TE activity to immunize its genome against proliferation of TEs. In this scenario, TEs are activated in cells that do not contribute to future generations; siRNAs are generated against the active TEs and then exported to nuclei of the germ lineage to silence endogenous TEs. On the male side, TEs are activated in vegetative nuclei of pollen grains and 21-nt siRNAs exported to sperm nuclei (Slotkin et al. 2009). On the female side, TEs are activated in endosperm and 24-nt siRNAs exported to embryos (Hsieh et al. 2009; Mosher & Melnyck 2010). The route by which siRNAs move from endosperm to embryo in the latter hypothesis is unclear because plasmodesmata[13] are absent between embryo and endosperm (Mansfield & Briarty 1991; Molnar et al. 2010).

siRNAs and kin conflict

Imbalances between 21 nt siRNAs contributed by sperm and TEs of central cells, and between 24 nt siRNAs contributed by central cells and TEs of sperm, have

[13] **Plasmodesmata**: Narrow cytoplasmic connections between neighboring cells.

been proposed to underlie failures of interploidy and interspecific crosses (Martienssen 2010; also see Josefsson et al. 2006). These effects could also be explained by siRNAs directly targeting genes involved in endosperm growth and differentiation. siRNAs transcribed in male and female gametophytes are subject to the same selective forces as MEGs and PEGs. Because siRNAs reduce expression of the genes from which they are transcribed, matrigenically-expressed siRNAs are predicted to accumulate in genes whose expression increases endosperm growth and patrigenically-expressed siRNAs are predicted to accumulate in genes whose expression inhibits endosperm growth.

A highly-diverse and abundant class of 24-nt siRNAs are expressed in *Arabidopsis* female gametophytes before fertilization and from maternal chromosomes of endosperm after fertilization (Mosher et al. 2009). Many of these siRNAs are not associated with TEs and mutations that abolish their expression do not reactivate TEs (Mosher 2010; Mosher & Melnyk 2010). In reciprocal crosses between 2*x* and 4*x* *Arabidopis*, 24-nt siRNAs are increased in maternal-excess endosperms but decreased in paternal-excess endosperms, and appear to promote precocious cellularization by targeting genes that promote proliferation (Lu et al. 2012). These observations are consistent with kin-conflict predictions. On the other hand, maternal mutations that abolish expression of 24-nt siRNAs do not have marked effects on growth (Mosher et al. 2009).

Pollen expresses both 21-nt and 24-nt siRNAs (Calarco et al. 2012). 21-nt siRNAs mediate post-transcriptional repression (Mosher 2010) and might target matrigenic mRNAs in early endosperm. 24-nt siRNAs target the promoters of MEGs in pollen and possibly contribute to the silencing of paternal alleles in endosperm (Calarco et al. 2012). Unlike the maternally-expressed siRNAs that are conjectured to target growth enhancers, paternally-expressed siRNAs that target growth inhibitors are not expressed in post-fertilization endosperm. This makes sense. Post-fertilization expression of paternal siRNAs would result in inactivation of maternal growth-inhibitor alleles in self-fertilized (or otherwise homozygous) endosperms whereas the strategic patrigenic response to homozygosity is greater growth inhibition.

Evolution of imprinted siRNAs

A *trans*-acting siRNA matches, but is unlinked to, its target whereas a *cum*-acting siRNA is directly encoded by its target. Both kinds of maternally-expressed siRNAs are predicted to target growth enhancers. The initial match of a *trans*-acting siRNA to a target is fortuitous, with natural selection sifting siRNAs with appropriate targets from siRNAs with inappropriate targets, whereas a *cum*-acting siRNA necessarily matches itself. siRNA-mediated inhibition may be evolutionarily stable if a *trans*-acting siRNA matches a functionally conserved region of its target gene, but *trans*-acting siRNAs that match genic regions that can evolve to evade the match (e.g., via synonymous base changes) will have evolutionarily transient effects.

Imprinted siRNAs that target their host gene have unusual evolutionary properties. This section can only sketch the complex interactions among alleles. My tentative conclusions will need to be validated with more formal models. Consider two alleles at a growth-enhancing locus of an outcrossing plant: *A'* contains an siRNA-generating sequence (siDNA) and is sensitive to the encoded siRNA; *A* lacks siDNA and is insensitive to the siRNA of *A'*. The alleles are otherwise equivalent with the expression of *A* an evolutionary compromise between a lower level favored as a matrigene and higher level favored as a patrigene. *A'*, when rare, will usually be inherited from one parent, not both, and be expressed at higher levels as a patrigene than as a matrigene because it is only in the latter role that its siRNA is expressed (**Figure 4**). Therefore, *A'* will increase in frequency when rare because it imposes lesser demands on mothers as a matrigene.

As *A'* increases in frequency, endosperms will often inherit *A'* from both parents. As a result, patrigenic *A'* encounters siRNA transcribed from matrigenic *A'* and both alleles are repressed. Patrigenic *A'* 'learns' that its seed contains an *A'A'* embryo rather than an *AA'* embryo (expected relatedness to own embryo is doubled) and that at least 50% of sibling embryos on the maternal sporophyte carry matrigenic *A'* (expected relatedness to other embryos more than doubled). Therefore, the optimal trade-off for patrigenic *A'* shifts toward smaller seeds and

more efficient use of maternal resources. Strategic use of this information would favor less production of growth enhancer, precisely the effect of the siRNA.

A′ performs a neat trick. It is associated with highest levels of growth enhancer when a patrigene in single dose (*AAA′*), intermediate levels when a matrigene in double dose (*A′A′A*), and lowest levels in triple dose (*A′A′A′*). Triploid endosperm may provide favorable stoichiometry for matrigenic silencing of patrigenes. In an *A′A′* diploid endosperm, siRNA transcribed from a single allele would have two targets to silence whereas, in an *A′A′A′* triploid endosperm, siRNA transcribed from two alleles has three targets to silence.

Repression of patrigenic *A′* in *A′A′A′* endosperms is a serendipitous effect that kicks-in as *A′* increases in frequency in outcrossing populations. By contrast, in plants that can self-fertilize, selfed seeds will contain *A′A′A′* endosperms, even when *A′* is rare. The siRNA causes patrigenic *A′* to be expressed at lower levels in self-fertilized than outcrossed seeds, increasing the genic fitness of *A′* and individual fitness of mothers (de Jong et al. 2005).

The growth-inhibitory effects of *A′* are likely to be evolutionarily transient because selection favors replacement of *A′* by *A″*, an allele that retains the siRNA but is relatively insensitive to its effects. *A″* shifts expression toward the level before invasion of *A′*. In a sense, nothing has changed except the genome has acquired an additional siRNA to which the host gene is relatively insensitive (**Figure 4**). The system is primed for the introduction of another siRNA. This iterative process could explain the great diversity of maternally-expressed siRNAs, their rapid evolutionary turnover, and the mildness of their effects (Mosher et al. 2009; Ma et al. 2010).

The imprinted siRNA acts as a 'green beard' when *A* and *A′* are the only alleles, causing patrigenic *A′* to be less demanding in the presence of matrigenic *A′*. The siRNA ceases to be a reliable marker of 'self' once *A″* also produces siRNA. Matrigenic *A″* induces patrigenic *A′* to reduce demand, but does not reciprocate when matrigenic and patrigenic roles are reversed.

Previous models of the evolution of imprinted expression have considered alleles with expression x_m as a matrigene and x_p as a patrigene. These models found that the unbeatable strategy at a growth-enhancing locus is $x_m = 0$, $x_p = X_p$, where X_p is the level of expression optimal for a rare allele in its patrigenic role

(Haig 1997; Wilkins & Haig 2001). The models assumed x_m and x_p were constant properties of an allele, determined in *cis*. Effects in *cum* violate this assumption and allow sophisticated strategies in which an allele changes its expression conditional on the identity of the other allele in its nucleus, and in which one allele can 'trick' its partner to reduce expression. This richer set of strategies appears to facilitate lower levels of overt conflict, with production of growth enhancer maintained below X_p.

ORIGIN AND SIGNIFICANCE OF ENDOSPERM

Segregation of parental alleles at meiosis and sharing of offspring by mothers and fathers create genetic conflicts over the optimal size of seeds. Maternal genes are predicted to favor the smallest seeds but greatest seed number, with matrigenes, then patrigenes, favoring progressively larger but fewer seeds. The expected number of surviving offspring declines as seed size increases above the maternal optimum (Haig 1992).

Triploid endosperm has been proposed to allow greater maternal control of the distribution of resources among seeds (Westoby & Rice 1982). Matrigenes of endosperm have a couple of intrinsic advantages over patrigenes. First, female gametophytes contribute more cytoplasm to early endosperm than do male gametophytes. Second, each matrigene is present in two copies for each copy of a patrigene. Matrigenes are therefore expected to exert more phenotypic power than patrigenes in endosperm. Maternally-expressed siRNAs may allow seed size to be maintained closer to the maternal optimum than would be otherwise possible. Growth-enhancing genes that incorporate imprinted siRNAs are predicted to exhibit imprinted expression when heterozygous but unimprinted expression when homozygous and to be expressed at lower levels than would conventionally imprinted genes.

The twin sperm of *Arabidopsis* appear to be functionally interchangeable (Ingouff et al. 2009). It seems reasonable to suppose that the sperm nucleus that first fertilized proto-endosperm of ancestral angiosperms contained the same epigenetic signature as the sperm nucleus that fertilized the egg. By contrast, egg and central cell nuclei of modern angiosperms are epigenetically distinct (Moll et

al. 2008). The much-debated question of the gametophytic versus embryonic origin of endosperm (Friedman 2001) may miss the mark if proto-endosperm was paternally embryonic but maternally gametophytic.

FUTURE ISSUES

1. Considerable progress has been made in understanding the genetic control of seed size once a commitment is made to provisioning a seed. However, many plants produce, and have fertilized, many more ovules than they can provision. Relatively little is known about the genetic control of adaptive seed abortion.
2. The evolution of siRNAs affecting seed size is expected to differ for siRNAs that target their own host sequence (*cum* effect) and those that target sequences at a different locus (*trans* effect). The relative importance of *cum* and *trans* effects needs to be addressed.
3. siRNAs with *cum* effects are subject to different selective forces in self-fertilizing and outcrossing plants. It will be important to determine whether observations in self-fertilizing *Arabidopsis thaliana* are also typical of outcrossing plants.
4. The identification of the targets of imprinted siRNAs will test their proposed role in kin conflict.

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Figure 1: (a) Before fertilization, the diploid ovule contains a haploid female gametophyte. A male gametophyte (pollen tube) grows to the ovule and releases two sperm. One sperm nucleus fuses with the egg nucleus to produce a zygote. The other sperm fuses with two haploid nuclei of the central cell to produce a triploid primary endosperm nucleus. (b) After fertilization, ovules are known as seeds. A seed consists of a diploid seed coat derived from the maternal integuments, a diploid embryo derived from the zygote, and a triploid endosperm derived from the primary endosperm nucleus. In the figures, a tissue containing *x* maternal genomes and *y* paternal genomes is labelled (*x*m:*y*p). The maternal sporophyte and seed coat are labelled with asterisks (1m*:1p*) to indicate that these are maternal and paternal genomes of the generation preceding the embryo and endosperm.

Figure 2: Expression of *A* is determined by a mixture of *cis*, *trans*, and *cum* effects. *cis* effects are mediated by linked sites on the same chromosome and therefore affect only the linked copy of *A*. *trans* effects are mediated by gene products (proteins or non-coding RNAs) and affect copies of *A* on all homologous chromosomes. A *cum* effect is an effect of *A* on its own copies on other chromosomes.

Figure 3: (a) A modifier (yellow) causes an epigenetic modification (red) in *cis*. The modifier and modification are inherited by the same set of offspring. (b) A modifier (yellow) causes an epigenetic modification (red) in *trans*. Offspring with and without the modifier inherit the modification. (c) A modifier (yellow) causes an allele-specific epigenetic modification (red) in *trans*. The modifier and modification segregate independently to offspring

Figure 4: The 'siRNA ratchet' at a growth-enhancing locus. A population fixed for an unimprinted allele *A* (red, top left) is invaded by *A′*, a version of *A* that incorporates a maternally-expressed siRNA (green, center). *A′* is in turn displaced by *A″*, a version of *A′* that is relatively insensitive to its own siRNA (yellow, bottom right). The triploid endosperm contains two maternal alleles (upper and middle alleles) and one paternal allele (lower allele). The main

diagonal represents homozygous endosperms and the off-diagonal elements heterozygous endosperms.