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CURRENT PERSPECTIVE ESSAY

A Differential Dosage Hypothesis for Parental Effects in Seed Development

Parent-of-origin effects generate phenotypes that depend on the direction of a cross. This phenomenon occurs frequently during angiosperm seed development, where maternal influence is most common (Alleman and Doctor, 2000). Various genetic mechanisms can contribute to parent-of-origin effects during seed development, including (1) the disproportionate maternal contribution to the endosperm, (2) plastidic and cytoplasmic inheritance, (3) expression of genes in the gametophytes and gametes, and (4) differential expression of parental alleles in the developing seed. In addition, the maternal sporophyte influences seed development by providing nutrients and other resources to the seed (Lynch and Walsh, 1998; Alleman and Doctor, 2000). Thus, parent-of-origin effects can be due to genetic or environmental differences between the parents attributable to processes acting at several distinct stages of development (Figure 1). Many parent-of-origin effects have been proposed to result from transcriptional imprinting, the differential expression of an allele when transmitted through the pollen or egg germline. Indeed, many genes are differentially expressed during seed development (Vielle-Calzada et al., 2000; Baroux et al., 2001; Weijers et al., 2001; Guo et al., 2003, 2004). A minority of imprinted genes are subject to the complete silencing of one parental allele, a condition we refer to as binary imprinting. The apparent contrast between the two categories of imprinted genes, differentially imprinted versus binary, creates difficulties in understanding the evolutionary and mechanistic relationship between the two fates. An example of such difficulties is that binary imprinting of growth-regulating genes is considered the sole stable outcome of conflict of interest between parents (Haig and Westoby, 1991, 1989; Haig, 1997; Wilkins and Haig, 2003), yet conflict of interest could potentially explain cases of differential imprinting. In this essay, we discuss how both types of imprinting are forms of dosage regulation and suggest that parent-of-origin phenomena can most easily be understood when considered in the light of a differential dosage hypothesis.

Imprinting is the most often discussed mechanism for parent-of-origin effects (Walbot and Evans, 2003; Gehring et al., 2004). Imprinting at a single locus was first demonstrated in any organism at the *R* gene of maize (Kermicle, 1970). Paternal transmission of *R* results in a stippled-red expression as a result of stochastic silencing of the *R* gene in a parent-of-origin–dependent manner. The behavior of *R* highlights one of the difficulties in classifying imprinting: silencing can be complete in one cell and absent in another within the same individual. Endosperms inheriting paternally transmitted *R* lack anthocyanin pigmentation in some aleurone cells but accumulate anthocyanins in others. Developmentally and spatially regulated suppression of gene expression is by no means specific to *R*. In plants, genes can be binarily imprinted in the endosperm and biallelically expressed in the embryo (Kinoshita et al., 1999).

Preaccumulated mRNA can be provided to the products of fertilization by either sporophytes or gametophytes. A robust demonstration of imprinting during early seed development is only possible via an in situ analysis of nascent RNA, which localizes transcripts to the chromosome. This type of evidence is becoming common in the mammalian literature but has only been reported for one higher-plant gene, *MEDEA* (*MEA*; Vielle-Calzada et al., 1999). Nonetheless, it seems probable that a number of genes are imprinted whose mRNA accumulates in seeds in a parent-dependent manner, including Arabidopsis *FWA* (Kinoshita et al., 2004) and maize *Fie1* (Danilevskaya et al., 2003). Many genes with phenotypes and mRNA accumulation patterns similar to *MEA*, however, are not completely or selectively imprinted in the seed. These include genes that mediate seed phenotypes due to a requirement for expression in the gametophytes (Yadegari et al., 2000; Xiao et al., 2003). Thus, before differential expression can be interpreted as the result of imprinting, differential transcription in the zygote or endosperm must be demonstrated.

An attractive mathematical model, parental conflict, proposes that imprinting is driven by conflicts of interest over resource allocation. Imprinting arises when half-siblings from multiple pollen parents compete with each other on the same seed parent. Imprinting of growth regulators can further the interest of plurigamous mothers via equal growth of all their progeny, whereas the interest of competing fathers is best served by preferential treatment of their progeny at the expense of maternal half-sibs (Wilkins and Haig, 2003). The parental conflict model predicts that imprinting is advantageous when genes expressed from the paternal chromosomes increase resource demand while those expressed from maternal chromosomes limit demand from the developing seed. It predicts that loss-of-function alleles at uniparentally expressed genes will have stereotypical and reciprocal effects on seed development dependent on the type of parental transmission that allows expression. The failure of endosperm to cellularize and the runaway cell proliferation in mutants of paternally silent genes, such as *MEA* and the *FIS*-class genes, could result in increased resource demand in these seeds and therefore are consistent with the parental conflict hypothesis. The reciprocal effects of interploidy crosses in Arabidopsis on seed size, endosperm cellularization, and cell number (Scott et al., 1998) and the altered kinetics of cell cycle parameters in interploidy crosses of maize (Leblanc et al., 2002) could derive from imprinted loci

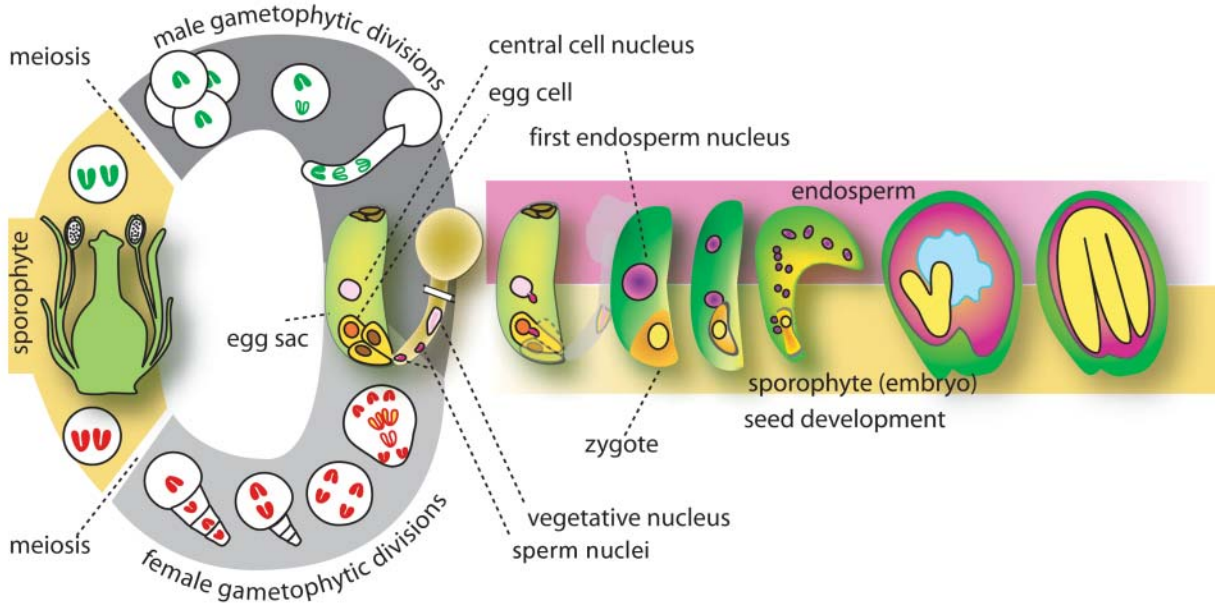

Figure 1. Life Cycle of Angiosperms.

The seed parent sporophyte on the left is separated from zygote and endosperm by the gametophytic generations (gray tracks) that produce the gametes via cell cycles. Parent-of-origin effects can originate during different developmental phases, including the gametophytic generations. The alternation of sexual and nonsexual organismal forms, double fertilization involving asymmetric gametes, and the different developmental speed of zygote and endosperm may influence dosage sensitivity to produce parent-of-origin effects. Elements of this figure were adapted from Satina et al. (1938) and Brown et al. (2003).

encoding resource demand modulators. Unfortunately, we know of no published studies of the effects of these manipulations on resource allocation.

A host of other parent-of-origin effects are difficult to reconcile with the parental conflict hypothesis. For example, the imprinting of genes without apparent strong effects on fitness (Hurst and McVean, 1998; Wilkins and Haig, 2003), some effects of chromosomal abnormalities on maize endosperm (Birchler, 1993), and the effects of uniparental disomy on human and mouse embryogenesis (Hurst and McVean, 1998) are incompatible with parental conflict causing these parent-dependent phenomena. Furthermore, maternally determined seed failure and gametophyte hypertrophy phenotypes reminiscent of *mea* are caused by loss-of-function alleles at other *FIS*-class genes that are specifically expressed in the female gametophyte but not imprinted in the developing seed (Vinkenoog et al., 2000; Yadegari et al., 2000). Other nonimprinted and biallelically expressed genes cause parent-dependent seed failure due to a requirement in the gametophyte stage (Ebel et al., 2004). Even the *mea* mutants show signs of runaway proliferation before fertilization (Kiyosue et al., 1999), demonstrating that the female gametophyte is abnormal in *mea* plants and suggesting that imprinting may not be the only mechanism responsible for parent-dependent seed failure in *mea*. Population genetic models suggest that although imprinting is more likely to arise under the conditions described for parental conflict, imprinted alleles may become fixed in a population if they arise at any dosage-dependent viability locus regardless of resource allocation, parental interests, and conflict (Spencer, 2000). These limitations and exceptions to the predictions of the parental conflict model, along with the multitude of modes for differential contributions by parents to offspring, suggest that a more general model that could be applied to differential effects would be useful.

THE DIFFERENTIAL DOSAGE HYPOTHESIS: A MORE GENERAL MODEL

Haig (1997) demonstrated that the complete silencing of one allele is predicted to be both the optimal and most stable outcome of parental conflict, a prediction which Haig and coworkers call ''the loudest voice prevails principle'' (Wilkins and Haig, 2003). The first two equations of this article state the assumption that the reproductive value of an individual ''is a function…of the amount of gene product produced….'' In other words, binary imprinted genes encode dosage-sensitive regulators of the ''reproductive output'' phenotype (Haig, 1997). The principal tenet of the differential-dosage hypothesis is that differential contributions to the developing seed of any dosage-sensitive regulator will result in a parent-of-origin effect. Differential contribution can occur by any mechanism, be it preaccumulated mRNA and protein or

CURRENT PERSPECTIVE ESSAY

chromosomal imprinting, but the complete suppression of one parental allele is not required.

According to Birchler et al. (2001), dosage sensitivity arises at regulatory pathways because the ''stoichiometric relationship of the components of regulatory complexes affects target gene expression.'' In other words, genes that cause dosage effects are expected to encode the subunits of macromolecular complexes, and a decrease in one component affects the function and assembly of the whole complex (Veitia, 2002). All the components of the complex need not be gene products. Indeed, genes encoding chromatin components are often dosage sensitive (Henikoff, 1979), and one of the components of this complex is genomic DNA and specific protein binding sites (Veitia, 2002, 2003). The dosage balance model was initially used to interpret the effects of aneuploidy on development (Bridges, 1922; Birchler, 1993; Birchler et al., 2001). It was proposed to explain heterosis (Birchler et al., 2003) as well as instability in neoallopolyploids and interspecific hybrids (Osborn et al., 2003; Riddle and Birchler, 2003). Recently, a demonstration of this principle was provided in yeast validating the balance hypothesis, which states that deleterious effects arise from an imbalance in the concentrations of subunits, for example, of multiprotein complexes (Veitia, 2002; Papp et al., 2003).

The balance hypothesis applies well to the dosage-sensitivity interpretation of parent-of-origin effects in interploidy crosses. Whole genome duplication does not change the stoichiometry of gene products, but a cross between individuals of different ploidy would result in a change of any component with parentally skewed expression. Dosage-sensitive regulators differentially contributed by one parent would be out of balance with matched components, altering downstream phenotypes in a dosage- and parent-dependent manner. For example, in a cross between a diploid seed parent and tetraploid pollen parent, the megagametophytically expressed chromatin modulating proteins, such as FIE or FIS2 in Arabidopsis, would be deficient relative to biparentally contributed chromatin. The sigmoidal transcriptional response of promoters to regulators can amplify the effect of failure in a dosage-sensitive transcriptional regulator (Veitia, 2002, 2003).

The parental conflict model, arguably, describes a subset of phenomena covered by the more general dosage model. Under the parental conflict model, as in the dosage model, the output phenotype is sensitive to gene dosage and subject to selective forces. Imprinting results in a change in gene dose. Binary imprinting, for example, reduces gene dosage from three to two or one in the endosperm and from two to one in the zygote and is a form of dosage regulation (Beaudet and Jiang, 2002). For these genes, the differential dosage hypothesis can be tested by altering the number of active alleles in the products of fertilization as, by definition, a silent allele has an output of zero and does not contribute to a dosage series. This approach has already been taken for binary imprinted genes in mouse, where a decrease in the dosage of the expressed allele allowed embryos to develop from gynogenetic fusions (Kono et al., 2004).

If the parental conflict and differential dosage hypotheses are so similar, why focus on dosage? Mainly because of one important difference: because the parental conflict model predicts binary imprinting, its a priori acceptance rejects a role for differential biallelic expression. The parental conflict model finds that differential expression will always be an evolutionarily unstable state and only exist in the absence of parental conflict or as a step toward binary imprinting (Wilkins and Haig, 2003). Yet, differential expression is widespread during seed development in maize and Arabidopsis (Baroux et al., 2001; Weijers et al., 2001; Guo et al., 2003, 2004). Thus, a more inclusive model explicitly based on dosage is needed.

To summarize, by examining the assumptions of the parental conflict and dosage balance models, a more general model emerges for parent-of-origin effects mediated by differential gene expression: the differential dosage hypothesis. This model allows differential contributions from binary imprinted genes but also from asymmetrically contributed factors from the gametophytes or differentially expressed genes in the fertilization product (Figure 1).

REINTERPRETATION OF PARENT-OF-ORIGIN EFFECTS BY DIFFERENTIAL DOSAGE

Experiments that support the parental conflict model demonstrated that the ploidy of the central cell, relative to the pollen sperm nucleus, determined crossing success in maize (Lin, 1984). Data from other plants are consistent with this interpretation (Esen and Soost, 1973; Haig and Westoby, 1991; Scott et al., 1998). By manipulating gametic ploidy, Lin (1984) demonstrated that seeds fail when the ratio of parental genomes in the endosperm is far from the normal ratio of two maternal to one paternal dose. When endosperm death is avoided using resilient species or less extreme genome ratios, however, dosage-sensitive parent-of-origin effects affecting endosperm development are visible. Paternal excess results in abnormal endosperm proliferation, whereas maternal excess results in early endoreduplication and differentiation (Charlton et al., 1995; Matzk et al., 2000; Leblanc et al., 2002).

Interestingly, reducing CG methylation phenocopies interploidy crosses in Arabidopsis. Hypomethylation of the maternal or paternal genome resembles paternal or maternal excess, respectively, as if methylation-dependent epigenetic marks modulated endosperm development (Adams et al., 2000). Similar endosperm hyperproliferation phenotypes display parent-of-origin effects in mutants of polycomb-complex subunits, which are either paternally imprinted or gametophytically expressed (Kiyosue et al., 1999; Vinkenoog et al., 2000). Because these mutant phenotypes are also sensitive to changes in DNA methylation and chromatin remodeling (Vielle-Calzada et al., 1999; Vinkenoog et al., 2000; Yadegari et al., 2000), it is attractive to interpret the effects of interploidy crosses as stemming from the action of loci imprinted by DNA methylation. Gametophytically expressed genes, such as *FIE*, are also sensitive to CG methylation, indicating that DNA

methylation affects seed development by means other than imprinting.

In addition to the persistent imprinting observed at selected loci, widespread suppression of many paternal genes occurs during early seed development (Vielle-Calzada et al., 2000; Weijers et al., 2001; Guo et al., 2003). As development progresses, this suppression is partially relaxed, resulting in maternally skewed, biallelic expression in the endosperm (Weijers et al., 2001; Guo et al., 2003). Transgenes follow a pattern similar to that of endogenous genes (Vielle-Calzada et al., 2000; Baroux et al., 2001; Weijers et al., 2001), displaying either absolute imprinting and differential expression or a reduction in expression from alleles contributed from the pollen parent. Differential expression of paternal alleles has also been demonstrated in maize endosperm, although twice as many mRNAs are preferentially expressed from the maternal allele (Guo et al., 2003). A dosage-sensitive phenotype effected by any of these gene products, imprinted or not, would result in parent-of-origin effects.

The dosage sensitivity of endosperm development is also observed during interspecific hybridization. In incompatible crosses between Solanum species, seed failure can be bypassed by embryo culture or by changing the dosage of either of the parental genomes. Thus, incompatibility is not intrinsic and is dosage sensitive (Carputo et al., 1999). The results of interspecific crosses of varying ploidy can be predicted by calculating an effective endosperm dose for the gametes of each species, the so-called endosperm balance number. This theory, also called polar nuclei activation (Nishiyama and Yabuno, 1978), predicts successes and failures observed during the mating of related taxa of oat, potato, and other species: crosses involving parents with the same endosperm balance number are most fertile regardless of ploidy. The endosperm balance number also predicts the failure of intraspecific interploidy crosses, as parental contributions to the endosperm are again out of balance. Although the endosperm balance number concept has been useful for breeding, it has been underutilized for elucidating the molecular basis of parent-of-origin effects. Yet, the endosperm balance number is compatible with the molecular model proposed here in which differentially contributed dosage-sensitive components interact to produce a viable endosperm.

Though the molecular basis of endosperm balance number is obscure, the experimental potential of the maize and Arabidopsis models should facilitate its elucidation. Maize, when used as the egg parent, can make fertile hybrids with the apomictic relative *Tripsacum dactyloides*. The highest fertility results when using diploid maize and tetraploid tripsacum (Kindiger and Beckett, 1992). *A. thaliana* can be crossed with *A. lyrata*, *A. arenosa*, and the allopolyploid *A. suecica*. The latter of these three crosses has been better studied for effects on seed development. Crossing diploid *A. thaliana* as the egg parent to either allotetraploid *A. suecica* or hexaploid *A. thaliana* has lethal consequences. Crosses between tetraploid *A. thaliana* and allotetraploid *A. suecica* are reminiscent of paternal excess crosses between diploid and tetraploid *A. thaliana* (Scott et al., 1998; Bushell et al., 2003). Consistent with these observations, when counts of endosperm nuclei are used to calculate an endosperm balance number, the tetraploid *A. arenosa* or *A. suecica* have approximately three times the endosperm balance number of diploid *A. thaliana.* Remarkably, the ploidy-dependent suppression of the postzygotic hybridization barrier in Arabidopsis and maize implicates an endosperm balance number-like mechanism in regulating species barriers.

It is tempting to interpret the similarities between interploidy crosses (Lin, 1984; Charlton et al., 1995; Scott et al., 1998) and interspecific hybridization (Carputo et al., 1999; Bushell et al., 2003) as caused by the action of a few imprinted genes critical for the proliferation of the endosperm. Such an explanation has already been suggested for these phenomena and the evolution of apomixis (Haig and Westoby, 1991). We prefer the differential dosage hypothesis, which makes starkly different predictions, namely that the similarities between imprinting, interploidy, and interspecies effects derive from the shared dosage sensitivity of the molecular mechanisms producing parent-of-origin effects. Allelic variation at any differentially expressed dosage-sensitive gene could result in parent-of-origin effects. Whereas only a handful of binarily imprinted genes have been identified, hundreds of genes are differentially expressed. If the incidence of dosage-sensitive fitness regulators in maize is comparable to that of yeast (Papp et al., 2003), 30 of the 600 genes differentially expressed in maize endosperm (Guo et al., 2003) would affect viability in a parent-of-origin–dependent manner. Therefore, we expect that the allelic diversity affecting differences in endosperm balance number and the fertility of interploidy crosses would derive from these dosage-sensitive genes, including but not limited to imprinted genes.

PARENT-OF-ORIGIN EFFECTS OCCUR IN A DEVELOPMENTAL CONTEXT

Dosage regulation, including imprinting, is prominent during seed development. Understanding angiosperm seed evolution and development should help elucidate the importance of dosage. A critical feature is that the zygote and endosperm are produced by the sexual union of two asymmetric gametes produced by the haploid gametophytes: pollen and egg sac. The gametophytes are sexual individuals, separated from the sporophytes by the processes of meiosis and fertilization. The path to sex cell production during the gametophytic cell cycles requires the maintenance of a germline. Any epigenetic state representing a sporophytic interest must persist through the gametophyte generations (Figure 1) to result in imprinting in the seed (Drews and Yadegari, 2002). Mechanistic insights into parent-of-origin effects must consider how the alternation of generations influences epigenesis. Such influences are exemplified by the loss of epigenetic imprints in gametophytes deficient in the DNA methyltransferase MET1 (Saze et al., 2003) and the derepression of epigenetically silenced *MEA* by DME (Choi et al., 2002).

CURRENT PERSPECTIVE ESSAY

Another implication of gamete development is that asymmetric expression in the zygote and endosperm is associated with structural differences in the nuclear genomes of the gametes. Plant sperm have highly condensed chromatin, as compared with the egg and central cell (Mogensen, 1982; Scholten et al., 2002). Consequently, there is a potential imbalance of regulatory factors contributed by the two parents to the products of fertilization. The male parent contributes a small compact nucleus, whereas the female parent contributes an active and decondensed genome, much cytoplasm, and many RNAs. It follows that the female might also contribute factors necessary for remodeling and unpackaging sperm chromatin and that development might impose a strict timing requirement on this phase. Interestingly, the zygote and endosperm develop at different rates: the zygote develops slowly while the endosperm rushes into a series of cell divisions, engaging in four rounds of mitosis before the embryo divides once (Boisnard-Lorig et al., 2001; Brown et al., 2003).

Asymmetric parental contributions during sexual reproduction have long been known to cause problems. Dissimilar paternal and maternal types, such as may be created by different copy numbers of heterochromatic elements, could create an impediment to hybridization. If, for example, a critical level of repressor is needed to suppress a locus, a maternal deficiency of repressor would result in locus activation, similar to hybrid dysgenesis in animals. Zygotic induction, the dosage-sensitive activation of lethal genes (prophages) in the male genome after conjugation with a female bacterium deficient in repressor activity, was the first description of such a phenomenon (Jacob, 1966). The possibility of a mechanism similar to zygotic induction should be considered in eukaryotic sex. The more rapid the developmental pace, the sooner remodeling must be complete and the greater the reliance on preexisting resources rather than the de novo synthesis of required factors. That is, early entry of the fertilized central cell into proliferative cell cycles might underlie endosperm sensitivity (Boisnard-Lorig et al., 2001; Olsen, 2004). Consistent with this hypothesis, certain chromosomal regions are only critical to the development of maize endosperm before the first endosperm mitosis (Birchler, 1993).

The disparity between cytoplasmic and nucleoplasmic contributions of the pollen sperm nucleus and of the egg or central cell is underscored by reports of parent-of-origin effects for regulators of small RNAs important for genome defense (Slotkin et al., 2003), the gametophytic apportionment of proteins with epigenetic activity (Ohad et al., 1999; Yadegari et al., 2000), and the accumulation of histone variants in sperm chromatin (Ueda et al., 2000). Allelic differences in these factors would exhibit parent-of-origin effects with a gametophytic inheritance pattern similar to imprinting.

CONCLUSION

The study of parent-of-origin effects during seed development has focused on the on–off state conferred by binary genetic imprinting. However, both gametophytic and differential expression of parental alleles in seed tissues are common. A dosage-sensitive regulatory model, in which the dose of a gene product determines the phenotype, is sufficient to describe parent-of-origin effects arising from differential parental contribution from preaccumulated pools of gene product, differential expression in the zygote or endosperm, and the complete and specific suppression of one parent's allele. This differential dosage hypothesis not only includes the common differential expression derived from imprinting, but can also account for the influence of gametophytic expression and binary imprinting. The hypothesis of dosage sensitivity can be tested in model organisms such as maize and Arabidopsis. We suggest that dosage sensitivity will be found both in the case of imprinted genes and, independently from imprinting, for epigenetic regulators. Such dosage sensitivity may explain the similarity between imprinting and the parent-of-origin effects of interploidy and interspecific crosses.

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