

Gametic imprinting in maize in relation to the angiosperm life cycle

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Summary

Differences in the activity of maternally and paternally derived genomes in maize endosperm have been observed at three levels of genetic manipulation. When the balance of entire chromosome sets departs from the standard ratio of two of maternal origin to one of paternal origin, development is impaired, often leading to seed failure. At the level of individual chromosomes, absence of a paternal representative for 8 of the 19 chromosome arms tested causes a marked reduction in kernel size. Replacement of the missing arms by ones of maternal origin does not complement this defect. At the gene level, some alleles of *R* confer solid coloration on the aleurone layer when transmitted maternally but patchy coloration (mottled) when transmitted *via* pollen. In contrast with the endosperm, no effect of parentage on *R*

phenotype has been detected in embryonic and seedling tissues. Furthermore, gynogenetic and androgenetic haploid plants are viable in maize and are similar in appearance. The detection of parental effects in the endosperm, but not the embryo, points to the few cell divisions of the gametophytes as a critical stage in imprinting. Chromosomally based epigenetic variation originating at this stage would be reflected as imprinting effects. A separate fertilization establishes a line of genetic descent in the embryo that appears to be relatively free of imprinted genes.

Key words: gametic imprinting, maize, endosperm, double fertilization.

Introduction

Fertilization events in flowering plants occur in pairs. Two sperm of a pollen grain fertilize two target cells within an ovule. One target, the egg, unites with one sperm to constitute the zygote. The other target of the multicellular female gametophyte or 'embryo sac' in the ovule is a centrally located cell. It is binucleate in most species and represents the maternal progenitor of the endosperm, a terminal tissue that nourishes the developing embryo. The presence of double fertilization provides a dual opportunity to detect imprinting. Furthermore, a difference in imprinting in the two products could serve to delimit crucial imprinting events to particular stages of the life cycle.

The pair of sperm in one pollen grain descend from a single product of meiosis. So too do cells of the embryo sac in most species. Thus the nuclei participating in a given double fertilization event typically have the same genotype. The two products of fusion nevertheless follow divergent developmental paths. Is gametic imprinting involved in this differentiation? That is, is the egg imprinted differently than the central cell relative to the sperm? And do such differences reflect chromosomally based differentiative events? These questions and the possible adaptive significance of double fertilization and triploidy of the resulting endosperm are considered after reviewing the evidence

concerning imprinting in maize embryos and endosperm.

Manipulations involving entire chromosome sets

Embryonic potential of gametophyte cells

Haploid plants occur sporadically in seed-propagated populations of maize. Most are maternal but a minority possess the nuclear characteristics of the paternal parent only (Chase, 1969). Inbred lines differ in the frequency of gynogenesis and, in crosses between lines, the frequency depends on the paternal as well as the maternal parent (Chase, 1952; Coe, 1959). In the Mendelian variant *indeterminate gametophyte*, gynogenesis is elevated several fold and androgenesis by about two orders of magnitude (Kermicle, 1969). The inheritance of gynogenetic and androgenetic frequencies suggests that the embryogenic potential of cells in the haploid gametophytes is inhibited in wild-type stocks. The fact that androgenetic plants can be derived through *in vitro* culture of immature anthers (Nitch *et al.* 1982) supports this view.

Although the totipotency of haploid gametophyte cells demonstrates an absence of vital genes in maize that are expressed only following transmission by one sex, it does not exclude the possibility of imprinting involving nonvital genes. Gynogenetic haploid maize plants regularly are smaller and deviate in characteristic

ways from their diploid counterparts (Chase, 1964). Androgenetic haploids have similar features. A comparison with gynogenetic haploids from the same strain would be necessary to detect quantitative differences due to imprinting. The viability of maternal and paternal maize haploids contrasts with the requirement of a nucleus from each parent for successful embryogenesis in the mouse (McGrath and Solter, 1984; Surani *et al.* 1984).

A critical balance of parental genomes in the endosperm

Gynogenetic or androgenetic development of maize endosperm has not been reported despite extensive use of this tissue for genetic investigations. Even when the embryo is maternally haploid, the endosperm contains a paternal chromosome complement (Sarker and Coe, 1966; Chase, 1969). Positive evidence indicating imprinting was obtained by varying the ratio of maternally and paternally derived chromosome sets. Normally, two nuclei of the embryo sac's central cell fuse with a sperm nucleus to constitute the triploid endosperm nucleus (Fig. 1). If genes of maternal origin introduced *via* nuclei of the central cell are imprinted differently than their counterparts in the sperm nucleus, a balance of two chromosome sets of maternal origin to one of paternal origin could be essential for normal endosperm growth. That is, if imprinted genes are dosage sensitive, departure from the standard ratio of two of maternal origin to one of paternal origin (designated 2:1) would be abnormal.

In embryo sacs inheriting the *indeterminate gametophyte* (*ig*) mutation, mitotic divisions are asynchronous in the developing embryo sac and the total number of cells and nuclei produced is not fixed (Kermicle, 1971; Lin, 1978, 1981). Accessory central cells frequently are present. In the primary central cell, the number of nuclei (so-called polar nuclei) varies from one to many. Following pollination with a chromosomally marked male, Lin (1984) found the ploidy level of endosperms

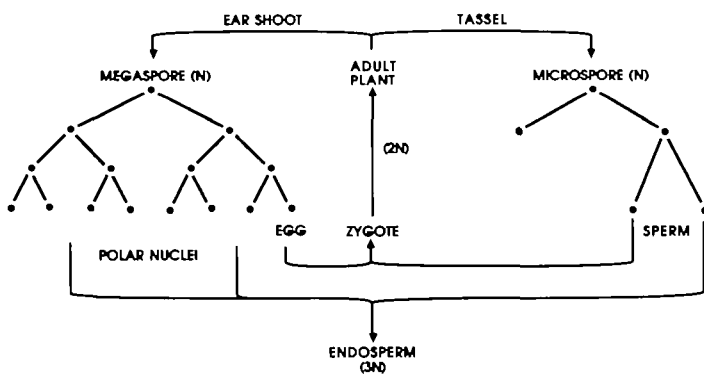


Fig. 1. Schematic representation of the maize life cycle. Cell lineages are detailed for the haploid stages (gametophytes), beginning with the products of meiosis (megaspore and microspore) and extending through to the cells and nuclei that participate in double fertilization. The mature maize kernel is composed of the embryo (*germ*), the endosperm (starchy portion) and remnants of the ovule and ovary wall (hull or pericarp).

to range from diploid through octaploid, of which one chromosome set regularly was paternal. Of these seven endosperm ploidy classes, only the triploid developed fully. The tetraploid class was subnormal whereas the remaining classes resulted in abortive kernels. Included in the abortive classes are those with diploid endosperm, whose genotype is identical to the accompanying embryo.

In maize, like many other angiosperm species, seeds regularly abort when plants in diploid strains are pollinated with tetraploid plants, due to failure of the resulting tetraploid endosperm. In contrast, about 15% of the seeds are full-sized following pollination of diploid *ig ig* with wild-type tetraploid stocks. Lin (1975, 1984) demonstrated that the endosperm in full-sized seed from this cross were hexaploid (4:2). As the ratio departed from 4:2, development became progressively more abnormal, consistent with the idea of a crucial balance of maternal and paternal genomes. He notes further that normalcy of the hexaploid endosperm class resulting from diploid *ig* × tetraploid *Ig* crosses is inconsistent with prior interpretations, which attribute seed failure following crosses involving parents of different ploidy levels to an imbalance of chromosome sets between the maternal, endosperm and embryo components of the seed. For crosses involving parents of the same ploidy level, this ratio is 2:3:2. For diploid *ig* × tetraploid *Ig* the ratio is 2:6:3. Lin attributes seed failure following standard diploid and tetraploid intercrosses to an imbalance of imprinted genomes within the endosperm.

The concept of a prescribed balance of maternal to paternal genomes within the endosperm for successful seed development has been extended to include interspecific crossing relations, most notably in *Avena* and in *Solanum* (Nishiyama and Yabuno, 1978; Johnston *et al.* 1980). In *Solanum*, species that cross successfully are assigned the same index value ('endosperm balance number') and polyploid counterparts a proportionate multiple of the index. Index values based on the success or failure of crosses were internally consistent and have been used to predict the success of subsequent crosses (Johnston and Hanneman, 1982).

Manipulations involving chromosome arms

Changes in dosage balance from 2:1

In principle, imprinting effects detected at the whole genome level might be assigned to particular chromosome regions using segmental duplications and deletions. A general means of constructing duplications and deletions up to the size of entire chromosome arms derives from a unique behavior of maize's accessory, or B, chromosome. B chromosomes nondisjoin at the second microspore division resulting in one sperm with two B chromosomes and one with none. The same behavior pertains when any of the 20 chromosome arms of the ten A chromosomes becomes coupled with the B centromere through translocation. Beginning with the work of Roman (1947), a set of A–B translocations has

been established involving 19 of the 20 A chromosome arms and encompassing from 80 to 85 % of the genome.

Duplication of a pollen-contributed chromosome arm, giving a 2:2 parental constitution in the endosperm, has in no case been reported to impair endosperm growth. Kernels with a 4:1 ratio of a particular segment are produced when a duplication-carrying sperm combines with the egg and the resulting plant is used as maternal parent in crosses with standard diploid. Only in the case of a translocation involving the long arm of chromosome 5 in a particular genetic background has this combination been reported to be subnormal (Beckett, 1983). These observations are consistent with the fact that endosperm containing 4:1 chromosome combinations associated with primary trisomy of the maternal parent are noticeably under-sized only for chromosome 1 (Birchler and Hart, 1987). The near absence of detectable effects of dosage imbalances at the level of individual chromosome arms implies that the effect of whole genome imbalance reflects the cumulative action of genes located on different chromosome arms.

Paternally required chromosome arms

A-B translocations also provide a systematic means of producing endosperm that is deficient for a paternal chromosome arm. Endosperm derived from fusions involving the deficient class of sperm regularly is subnormal for 8 of the 19 chromosome arms for which A-B translocations are available. Reductions in total kernel mass range down to one half. In a given case, subnormal kernels could result from a dosage imbalance between genes in the disomic and trisomic regions due to an ordinary effect of aneuploidy. However, the three instances investigated in detail demonstrate an absolute requirement for a paternal form of the region in question.

For the long arm of chromosome 10, Lin (1982) discovered that extra copies transmitted through the ovule did not compensate for absence of a sperm-derived copy. Specifically the 4:0 combination was reduced to the same extent as 2:0. In contrast, the 2:2 combination proved equivalent to standard 2:1 although its chromosome composition was identical to 4:0. Using a series of 38 such translocations between 10L and B, Lin identified three regions near the centromere that contributed to the effect. In parallel analyses involving chromosome arm 1L, Birchler (1979) found the 4:0 combination also to be reduced whereas 2:2 and 4:1 were normal. In this case, kernels belonging to the 4:0 class were still smaller than 2:0. Birchler and Hart (1987) found extra maternal doses of chromosome arms other than the paternally deficient one could accentuate reduction of the 2:0 endosperm class. Because this effect was restricted to those arms that themselves give small kernels in 2:0 combination, these workers suggest that the maternally transmitted copies may not be silent for the paternally required genes, but have opposite effects.

Deletions generated by means of A-B translocations are not transmitted to progeny. Because a general

means for producing female transmitted deficiencies currently is not available for maize, the possibility of maternally required segments remains largely untested. A number of variant kernel phenotypes associated with maternal parentage have been reported (Schwartz, 1965; Kermicle, 1978). They are candidates for genes involved in preferential maternal expression, but only in the case to be discussed in the next section have tests been performed to equalize dosage input from the two parents.

Imprinting of the R gene

Imprinting in maize was first recognized as an exceptional pattern of anthocyanin pigmentation in the endosperm's outer layer, the aleurone. When colored aleurone strains carrying certain red color (*R*) alleles are used as female parent in crosses by colorless (*r/r*), full-colored kernels (*R R/r* aleurone) are produced. Aleurone of *r r/R* genotype produced in the reciprocal cross is pigmented irregularly (mottled), rather than uniformly colored. The evidence demonstrating that the difference in *R* phenotypes reflects *R* parentage rather than dosage has been reviewed previously (Kermicle, 1978). It will suffice here to enumerate the salient features of the findings.

(1) When two copies of *R* were introduced through pollen, producing *r r /R R* endosperm, aleurone pigmentation was mottled, like *r r/R*.

(2) Introducing a second copy of *r* through the pollen produced uniformly colored *R R/r r* aleurone, like *R R/r*. Thus *r r/R R* and *R R/r r* have different phenotypes (Kermicle, 1970).

(3) The effect of the recessive *r* allele used for these test did not differ from a chromosomal deletion for the *R* locus.

(4) Not all *R* alleles behave in the above manner. Some confer uniform pigmentation when transmitted *via* both sexes and some respond in a dosage-dependent rather than a transmission-dependent manner.

(5) Full-colored (*R R/r*) and mottled (*r r/R*) F_2 kernels occur on the same ear in the proportion expected based on an absence of conventional maternal effects.

(6) Chimeric kernels composed of uniformly colored and colorless areas result when the *R* alleles of *R R/r* aleurone are lost during kernel development (see Fig. 2 for details). An absence of mottled sectors on such kernels indicates that a single dose of maternal *R* confers uniform coloration. The sharp boundary between colored and colorless regions suggests autonomy in expression of the two phenotypes at the level of individual cells.

(7) Kernel mosaics composed of uniform and mottled result when the maternal *R* alleles of *R R/R* are similarly lost (Fig. 2), demonstrating a chromosomal basis for the difference between the two phenotypes.

(8) When the trans modifier *mdr⁻* (*maternal depression of R*) is introduced together with *R* through ovules, the resulting *R R/r; mdr⁻ mdr⁻/mdr⁺* kernels are mottled. Wild-type *mdr⁺* evidently is necessary to imprint maternal *R* to a strong level of action.

Kernel Offspring of the Cross :





Endosperm genotype	Endosperm phenotype	Seedling phenotype
+ R-g', + R-g'/r-g; Ac		green
+ R-g', + R-g'/R-r'; Ac		red
Ds R-g', Ds R-g'/r-g; Ac		green
Ds R-g', Ds R-g'/R-r'; Ac		red

Fig. 2. Cross designed to test autonomy of maternally and paternally derived *R*-locus phenotypes using mosaic kernels. *Ds* designates a transposable element that breaks chromosomes in the presence of *Ac* (McClintock, 1951). With *Ds* inserted between *R* and the centromere of chromosome 10, sectors of *R* loss occur during endosperm development. *R-r* designates colored seed and red seedlings; *R-g*, colored seed and green seedlings; and *r-g*, colorless seed and green seedlings. First row: full-colored seed conferred by maternal *R-g'* carried in wild-type chromosomes. Second row: full-colored phenotype illustrating dominance of maternal *R-g'* to paternal *R-r'*. Third row: full colored background with colorless sectors resulting from loss of the maternal *R-g'* chromosomes. Last row: full-colored background with mottled sectors (illustrated by stippling), revealing action of paternal *R-r'* following loss of the maternal *R-g'* chromosomes. Two investigators classified kernels belonging to rows three and four with 90 and 93 % accuracy, despite the fact that sectors on some kernels were small and irregular.

(9) Using a class of mottling *R* alleles that confers pigmentation on embryonic and seedling as well as aleurone tissues, a difference attributable to parentage was detected in the aleurone but not in the scutellum, mesocotyl, or seedling roots. The restriction of detectable imprinting to the endosperm points to particular cell division cycles of the female gametophyte as the stage at which *R* imprinting occurs (Brink *et al.* 1970).

Imprinting in relation to embryo-endosperm differentiation

The products of double fertilization follow divergent development paths and are differentially imprinted. Is this relationship coincidental or might imprinting be a consequence of normal differentiative events? If the two are related causally, timing of critical imprinting and differentiative steps is expected to be coincident. The nature of the angiosperm life cycle (Fig. 1) allows the critical stages to be delimited to a few cell divisions.

On the male side, the sperm are sister cells. In some

species, the two sperm differ in abundance of mitochondria and plastids, with evidence of nonrandom fertilization of the egg and central cell (Russell, 1985). Sperm dimorphism has not been reported in maize but preferential fertilization involving the B chromosome does occur. Following nondisjunction in the division of the generative cell to produce sperm, the sperm receiving the B chromosomes preferentially fertilizes the egg (Roman, 1948). If one mitotic pole were destined to develop into the sperm that fertilizes the egg, the nondisjoining Bs should be directed to that pole. However, when two nondisjoining elements are present, Carlson (1969) found that they assort independently, indicating that either product could fertilize the egg. He concludes that B chromosomes actively influence the process of fertilization (Carlson, 1988).

In contrast, the maternal progenitors of the embryo and endosperm, respectively, egg and central cell, differ conspicuously. The central cell is much larger and is binucleate. After fusion with a sperm, the primary endosperm nucleus undergoes rapid mitotic divisions producing a multinuclear coenocyte having 128 to 256 nuclei at the 6- to 8-cell stage of the embryo. Division products of the zygote are cellular and differential from the beginning. The divergent developmental paths of embryo and endosperm evidently are prestaged in the mature embryo sac as differences between the egg and central cell. Differences that are chromosomally based and propagate mitotically would contribute to imprinting differences relative to the nuclear potential of sperm. According to this view, imprinted genes are representative of changes in cell heredity that occur during differentiation.

The binuclear condition of the central cell is unlikely to be a critical factor in differentiation of this cell as a progenitor of the endosperm. In Onagraceae such as the evening primrose, the functional meiotic product undergoes two rather than three cycles of mitotic divisions. One of the four products differentiates into a uninucleate central cell. Because it has the same gene content as the egg, the resulting endosperm and embryo have identical genotypes. The same is true of the abortive diploid endosperm exceptions in maize that result from action of the *ig* gene (Lin, 1984). Conversely, triploid embryos such as those resulting from diploid intercrosses with tetraploid can undergo normal embryogenesis and subsequent development when explanted onto artificial medium in order to avert adverse effects of the dysfunctional endosperm produced in these crosses. We know of no evidence indicating that the developmental path of embryo and endosperm is dependent on ploidy level *per se*.

Imprinting and gene action in the endosperm

Gametic imprinting acting in conjunction with an unequal contribution of chromosome sets from the parents constitutes a unique feature of gene action in the endosperm. Plant embryologists have long attributed rapid development of the endosperm to a

special type of hybridity (Brink and Cooper, 1947). This hybridity does not result simply from favorable combinations of allelic genes, since the same heterozygous combinations exist in the embryo. Furthermore, the sort of vigor described pertains to pure-breeding stocks where homozygosity prevails. We believe it likely that the 'hybridity' results from a unique combination of differentially activated genes received from the sperm and central cell, which complement to direct development along a certain path. Because this interaction involves epistatic combinations of epigenetic variation, it has been termed epihybridity.

As a consequence of imprinting and triploidy of the endosperm, four effective gene dosage levels are possible under normal circumstances. A gene may be turned off when received from both parents, turned on only following pollen transmission (one effective dose), turned on only following ovule transmission (two effective doses), or turned on when received from both parents (three doses). Were the endosperm diploid, imprinting would provide only three effective dosage levels. Thus triploidy could be a means of fine-tuning effective gene dosage levels. Another possibility has recently been presented by Haig and Westoby (1989). They view imprinting acting in conjunction with unequal ploidy contribution from the two parents as a means of optimizing the distribution of maternal resources.

In the preceding discussion, the two nuclei of the central cell have been assumed to be equivalent. One derives from each group of four nuclei positioned at opposite ends of the developing embryo sac. Each group of four descends from the initial division of the functional meiotic product. Although the two nuclei of the central cell frequently look similar, they need not be equivalent functionally. Indeed a difference in size sometimes is discernible at the level of light microscope investigations, with the larger one typically originating from the same group as the egg (Maheshwari, 1950). Thus, the possibility that the two nuclei are imprinted relative to one another should not be dismissed. According to this view, the two-way complementation between sperm and polar nuclei described in the preceding paragraph should be extended to a three-way complementation.

Imprinting in relation to double fertilization

As a repository of storage materials for use in nourishing the developing embryo and seedling, the role of the endosperm in seed development is not in doubt. What continues to evoke discussion is the functional significance, if any, of its peculiar origin. In particular, why should tissues that have the same qualitative gene content be separately fertilized? We suggest that double fertilization is not redundant since it preserves the epigenetic differences in progenitor nuclei, differences that prestage development of the endosperm and embryo.

It is instructive to consider alternatives to double

fertilization and the endosperm that are found in specialized *taxa*. In the Trapaceae and Orchidaceae, where endosperm is absent or virtually so, haustoria differentiate from the embryo's suspensor and function as absorptive structures. A pseudoembryo sac differentiates from nucellar (maternal) tissue and serves as a substitute for the endosperm in the Podostemaceae. In apomictic species, where the embryo develops without fertilization, the endosperm in some groups differentiates from the central cell of the embryo sac without fertilization. Despite this variety of alternatives within the angiosperms, double fertilization remains the norm. Concerning the embryo sac, one authority writes, 'The treasuring of a structure of such strange character, and the equally strange double fertilization associated with it... can only mean that it confers unique advantages, and advantages of a kind that can be foregone at some peril' (Heslop-Harrison, 1983). And then, 'The significance of the double fertilization itself and the consequent triploidy of the endosperm remain obscure, but the fact that its function, too, is rarely surrendered – and then only in a few advanced and specialized families such as the Orchidaceae – leaves little doubt that it also is a closely guarded and valuable part of the general angiospermic inheritance'. We consider it particularly telling that the majority of apomicts, while dispensing with a fertilized embryo, have retained a fertilized endosperm.

Particularly with annual plants growing in temperate zones, time is of the essence in completing the reproductive cycle during the span of a single growing season (Stebbins, 1976). After a period of vegetative growth, meristems are transformed to produce reproductive structures. Seed development involves distinct stages: first an ovule composed primarily of maternal tissue, next the young developing seed in which the endosperm is the primary component, and then the advanced seed stage with its prominent embryo. The multiple stages presumably represent not only an economic use of resources but also serve to minimize the post-flowering time span. In particular, critical differentiative events involving the first two stages occur in the embryo sac before flowering. Epigenetic differences between the egg and central cell nuclei are established that persist following double fertilization. Sperm and central cell nuclei that are differentially potentiated confer epistatic hybridity on the endosperm, involving complementary combinations of activated genes at different dosage levels. In the accompanying embryo, differences referable to egg and sperm nuclei appear to be lacking, thereby obviating the need for reversal of imprinting effects in the developing plant.

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