

ORIGIN OF THE FITTEST AND SURVIVAL OF THE FITTEST: RELATING FEMALE GAMETOPHYTE DEVELOPMENT TO ENDOSPERM GENETICS

William E. Friedman,^{1*} Eric N. Madrid,* and Joseph H. Williamst

*Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado 80309, U.S.A.; and †Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, Tennessee 37996, U.S.A.

For more than a century, most biologists have viewed the structural diversity of angiosperm female gametophytes as trivial variants of the reproductive process. However, analysis of variation among angiosperm female gametophytes from an evolutionary developmental perspective can provide new insights into patterns of reproductive innovation and evolution among flowering plants. The key is to link the developmental and structural diversity of angiosperm female gametophytes to evolutionary innovations (perhaps even adaptations) associated with endosperm genetics and ploidy. Selection has been hypothesized to favor endosperms with higher ploidy, higher heterozygosity, higher maternal-to-paternal genome ratios, and reduced opportunity for genetic (interparental and/or parent-offspring) conflict. We evaluate these hypotheses for the seven basic genetic types of endosperm known among flowering plants and interpret their relative importance when mating system is considered. We demonstrate that variation in female gametophyte developmental patterns represents the source material that ultimately creates variation in endosperm genetics. Evolutionary transitions in female gametophyte development are therefore a function of selection directly acting on the resultant phenotypes of endosperms. Thus, the relation between variation in female gametophyte development and variation in endosperm genetic constitution should be seen as one between the origin of structural novelties (origin of the fittest) and its downstream consequences on the relative fitness (survival of the fittest) of these novelties, as expressed in the biology of endosperm.

Keywords: female gametophyte, endosperm, fertilization, inclusive fitness theory, development, modularity, evolution.

Introduction

For more than a century, the intricate details of female gametophyte (embryo sac) development in flowering plants (monosporic vs. bisporic vs. tetrasporic; Polygonum-type vs. Allium-type vs. Fritillaria-type vs. Oenothera-type and so on) have been the bane of plant biologists (fig. 1). Much of this justifiably negative reaction to understanding the diversity of female gametophyte development and structure can be traced to a highly typological approach to the categorization of embryo sacs that has dominated flowering-plant embryological work. At the end of the day, most plant biologists respond to the details of embryology by accepting that there are well-established differences among angiosperm female gametophytes and rejecting that these differences are interesting.

Analysis of the diversity of angiosperm female gametophytes from an evolutionary developmental (evo-devo) perspective (as opposed to a strictly typological one) can be biologically meaningful. The key is to understand female gametophyte diversity (i.e., differences in basic structure) based on general principles of developmental biology and to link this diversity to critical aspects of flowering-plant reproductive biology, such as the fertilization process, and evolution-

ary innovations (perhaps even adaptations) associated with endosperm genetics and ploidy. Our goal, in presenting a developmentally based model of angiosperm female gametophyte evolution, is to reintegrate this important organismic generation into the broader reproductive biology of flowering plants and attempt to move the field of plant embryology beyond its historically typological roots.

In this article, we first examine correlations between patterns of female gametophyte development and the highly variable genetic constructs of endosperm among flowering plants. We show that the known diversity of female gametophyte development and mature structure among angiosperms is tightly linked to and constrained by the biology of endosperm, specifically, endosperm ploidy, maternal-to-paternal genomic ratios, degree of heterozygosity, and genetic relatedness to its compatriot embryo relative to its relatedness to other embryos on the maternal sporophyte.

We argue that the female gametophytes of all angiosperms are fundamentally modular entities (sensu Friedman and Williams 2003); in other words, they are composed of iteratively expressed units. We then go on to demonstrate that variation in mature angiosperm female gametophyte structure is the result of three basic types of developmental modifications or themes: (1) relative timing of the establishment of female gametophyte modules (during or after megasporogenesis), (2) early ontogenetic events that determine the number of developmental modules initiated (one, two, or four),

¹ Author for correspondence; e-mail: ned@colorado.edu.

Type	Megaspороgenesis			Megagametogenesis			
	Megaspore mother cell	Division I	Division II	Division III	Division IV	Division V	Mature embryo sac
Monosporic 8-nucleate Polygonum type							
Monosporic 4-nucleate Oenothera type							
Bisporic 8-nucleate Allium type							
Tetrasporic 16-nucleate Peperomia type							
Tetrasporic 16-nucleate Penaea type							
Tetrasporic 16-nucleate Drusa type							
Tetrasporic 8-nucleate Fritillaria type							
Tetrasporic 8-nucleate Plumbagella type							
Tetrasporic 8-nucleate Plumbago type							
Tetrasporic 8-nucleate Adoxa type							

Fig. 1 The famous diagram showing the basic types of female gametophyte development and structural diversity from Maheshwari (1950). Maheshwari was not the first to publish such a diagrammatic representation of angiosperm female gametophyte diversity (see, e.g., Chiarugi 1927; Schnarf 1929), nor was he the last (see, e.g., Gifford and Foster 1987; Haig 1990). This diagram and its intellectual descendants show the basic variation in megasporogenesis, meiotic and mitotic division patterns, and mature structure in angiosperms. Various portions of the diagram, in modified form, are used to examine the developmental evolution of angiosperm female gametophytes throughout this article.

and (3) ontogenetic events that result in developmental deviations from the basic (and plesiomorphic) female gametophyte developmental module. In so doing, we hope to illuminate the connection between the origin of structural variation in the angiosperm female gametophyte and the selective forces that have allowed novel endosperm genetic constructs to persist.

Connecting the Female Gametophyte Central Cell to Endosperm Genetics

Angiosperm female gametophytes are ontogenetically and structurally highly variable (fig. 1). They may be monosporic, bisporic, or tetrasporic in origin. The number of synergids in

the adult gametophyte ranges from zero to three (Maheshwari 1950; Friedman 2006a, 2006b), while antipodal number varies widely (zero to many). The number of nuclei contributed to the central cell ranges from one (Yoshida 1962; Galati 1985; Williams and Friedman 2002) to 14 (Johnson 1914; Maheshwari 1950). For the purposes of our analysis, variation in ontogeny and adult female gametophyte structure is biologically significant only if it has a downstream effect on the genetic constitution of endosperm.

Endosperm in flowering plants is initiated by the fertilization of the central cell of the female gametophyte during the process of double fertilization. Consequently, there is a direct relation between the genetic constitution of endosperm and the antecedent genetic constitution of the central cell (Palser 1975). Among the innumerable variants of angiosperm female gametophyte development and structure (see Haig 1990 and Johri et al. 1992 for excellent discussions), there appear to be just seven basic types of central cell genetic constitution and hence only seven genetic constructs or types of endosperm (table 1). For the purpose of cross-referencing the genetic constitutions of central cells and endosperms to the embryological literature, we have also circumscribed the classical names of embryo sac types that produce the seven genetic kinds of central cells and endosperms (table 1).

Monosporic diploid endosperms are produced by female gametophytes initiated by a single megaspore that mature a central cell with a single haploid nucleus. The phylogenetically widespread and common monosporic triploid endosperm is typically formed from Polygonum-type female gametophytes but may also be initiated upon fertilization in the recently discovered Amborella-type (nine-nucleate, eight-celled female gametophyte with three synergids; Friedman 2006a, 2006b). Monosporic triploid endosperms are derived from the fertilization of a central cell that contains two polar nuclei that are genetically identical to each other and to the egg nucleus.

All bisporic angiosperm female gametophytes produce bisporic triploid endosperms. The two haploid nuclei of the central cell are derived from the two megaspores from meiosis II of one of the dyads. As such, their coefficient of relatedness (r) is defined

as $r = 1 - q$, where q is the frequency of second-division segregation (Bulmer 1986; Haig 1986).

Tetrasporic triploid endosperms are initiated from a central cell with two polar nuclei that are lineal descendants of each dyad from meiosis I. As such, their coefficient of relatedness is defined as $r = q/2$, assuming that the egg is the mitotic sister nucleus of the micropylar polar nucleus, as expected (Bulmer 1986; Haig 1986). Tetrasporic pentaploid endosperms arise from a number of different types of female gametophytes. These female gametophytes are always initiated by four megaspore nuclei within a coenomegaspore, and the central cell contains a lineal descendant of each of the megaspores (this is assumed to be the case in those embryo sacs that form restitution nuclei).

Tetrasporic nonaploid (9N) and decapentaploid (15N) endosperms are confined to the genus *Peperomia* (Piperaceae; Johnson 1900, 1914). Tetrasporic nonaploid endosperms are formed by the fertilization of a central cell with four pairs of nuclei that are each mitotically descended from the original four megaspores that initiate embryo sac development. Tetrasporic decapentaploid endosperms are derived from a central cell that contains two nuclei genetically identical to the egg cell and three sets of four nuclei derived from the three megaspores not associated with the production of the egg (fig. 1; table 1).

The Basics of Endosperm Genetics: Heterozygosity, Ploidy, and Relatedness

There is good reason to carefully analyze the genetic constructs of the various entities found within a flowering-plant seed (female gametophyte, embryo, endosperm, and maternal sporophyte). Nearly three-quarters of a century ago, a rich, but sporadic, theoretical literature began to examine the genetic and evolutionary implications of a sexually formed, genetically biparental embryo-nourishing tissue, as found only in angiosperms. Beginning with the pioneering papers of Brink and Cooper (1940, 1947), three basic theories have been developed to explain the variable “consequences” of the

Table 1

The Seven Basic Genetic Constructs of Endosperms, the Mature Female Gametophyte Types That Produce These Endosperms, and Central Cell Genetic Constitution

Endosperm construct	Correlated female gametophytes	Central cell nuclear contents ^a
Monosporic 2N	Oenothera-type, Nuphar-type	M1
Monosporic 3N	Polygonum-type, Amborella-type	2(M1)
Bisporic 3N	Allium-type, Endymion-type, Drusa-type ^b	M1, M2
Tetrasporic 3N	Adoxa-type, Drusa-type ^b	M1, M3, or M4
Tetrasporic 5N	Penaea-type, Plumbago-type Fritillaria-type, Plumbagella-type	M1, M2, M3, M4
Tetrasporic 9N	Peperomia-type	2(M1), 2(M2), 2(M3), 2(M4)
Tetrasporic 15N	Peperomia-type	2(M1), 4(M2), 4(M3), 4(M4)

^a M1: female nucleus genetically identical to the egg nucleus; M2: female nucleus sister to M1 and derived from meiosis II of dyad I; M3: female nucleus derived from meiosis II of dyad II; M4: female nucleus sister to M3 and derived from meiosis II of dyad II.

^b For the Drusa-type female gametophyte, the chalazal polar nucleus may be derived either from the same meiotic dyad as the egg nucleus is derived from or from the other dyad. In the first case, the genetics of endosperm are the same as for bisporic 3N; in the second case, the genetics are the same as for tetrasporic 3N.

evolutionary origin and diversification of a sexually formed embryo-nourishing tissue, namely, endosperm.

Endosperm Heterozygosity

Brink and Cooper (1940, 1947) were the first of many (Stebbins 1976; Tiffney 1981; Takhtajan 1991; Donoghue and Scheiner 1992) to suggest that heterosis (essentially heterozygosity) creates in endosperm a more vigorous embryo-nourishing tissue than the haploid (hence hemizygous) embryo-nourishing female gametophytes of nonflowering plants. This theory (hereafter the “heterozygosity hypothesis”) leads to the prediction that further increases in the heterozygosity of bisexual endosperm should also be advantageous (Brink and Cooper 1940, 1947; Stebbins 1976; Tiffney 1981; Takhtajan 1991; Donoghue and Scheiner 1992).

Endosperm heterozygosity, defined as the average probability of having two or more different alleles per locus, is expected to increase as the probability of including both maternal alleles in the endosperm increases. This probability depends on the pattern of megasporogenesis and whether mitotic derivatives of one, two, or all four meiotic products (megaspore nuclei) are incorporated into the central cell that initiates an endosperm upon fertilization (table 1).

The egg cell and central cell of all monosporic female gametophytes are composed of lineal mitotic descendants of a single megaspore, such that endosperm heterozygosity is the same as embryo heterozygosity. The two polar nuclei of bisporic female gametophytes are the lineal mitotic descendants of two megaspores derived from the same dyad; hence, both maternal alleles will be represented at endosperm loci

that have been affected by crossing over between nonsister chromatids (table 1). In contrast, the two polar nuclei of the central cell of the tetrasporic Adoxa-type female gametophyte are derived from separate dyads; hence, both maternal alleles will be represented in endosperm for loci that have not been affected by crossing over between nonsister chromatids. In both of these cases, the probability that the two polar nuclei represent one or both maternal alleles depends on the second-division segregation rate: with no crossing over, alleles are always identical by descent in bisporic female gametophytes and are always nonidentical in tetrasporic Adoxa-type female gametophytes, whereas with maximum recombination, both maternal alleles will be represented two-thirds of the time in both cases (Fincham 1994). Among other tetrasporic female gametophytes (those that produce pentaploid or higher endosperms), the central cell contains lineal mitotic descendants of all four megaspores. Therefore, the central cell always includes both alleles, and before fertilization, its heterozygosity is equivalent to that of the maternal genotype (table 1).

We have calculated expected endosperm heterozygosity for monosporic, bisporic, and tetrasporic female gametophytes (table 2; appendix). Our results indicate that in a randomly mating population, heterozygosity at any single locus in an endosperm derived from a monosporic female gametophyte (H) is less than or equal to that of an endosperm formed from a bisporic female gametophyte ($H + qH/2$), where q is the frequency of second-division segregation. Heterozygosity of triploid endosperms derived from bisporic female gametophytes is less than or equal to heterozygosity of triploid endosperms derived from tetrasporic female gametophytes ($3H/2 - qH/4$), which in turn is always less than or equal to the heterozygosity

Table 2

Seven Basic Genetic Constructs of Endosperms among Flowering Plants

Endosperm type	M : P	H	r_{ms-ent}	$r_{ent-ce} : r_{ent-oe}$		
				Outcross, unrelated fathers	Outcross, same father	Self-fertilization
Monosporic 2N	1 : 1	H	1/2	4	2	3/2
Monosporic 3N	2 : 1	H	1/2	3	2	3/2
Bisporic 3N ^a	2 : 1	$H + qH/2$	1/2 to 1	$3 - q$	$2 - 2q/3$	$3/2 - q/3$
Tetrasporic 3N ^a	2 : 1	$3H/2 - qH/4$	1 to 1/2	$2 + q/2$	$4/3 + q/3$	$7/6 + q/6$
Tetrasporic 5N ^b	4 : 1	$3H/2$	1	3/2	6/5	11/10
Tetrasporic 9N	8 : 1	$3H/2$	1	5/4	10/9	19/18
Tetrasporic 15N	14 : 1	$3H/2$	1	1	29/30	29/30
Perisperm ^c	2 : 0	H_0	1	1	1	1

Note. Shown are ploidy, maternal-to-paternal genomic ratios (M : P), heterozygosity, and ratios of coefficients of relatedness of endosperm to its own embryo versus other embryos under conditions of outcrossing to unrelated pollen donors, outcrossing to the same pollen donor, and self-fertilization. H = expected heterozygosity of embryo-nourishing tissue, expressed as a function of theoretical expected heterozygosity in a randomly mating population; H_0 represents observed heterozygosity of sporophytes (as represented by perisperm); for derivations, see appendix. r_{ms-ent} = relatedness of maternal sporophyte to its embryo-nourishing tissue (ent), assuming nonimprinted gene expression with additive effects (see Queller 1989); $r_{ent-ce} : r_{ent-oe}$ = ratio of relatedness of ent to its compatriot embryo (ce) and that to another embryo (oe) on the same maternal sporophyte, assuming nonimprinted gene expression with additive effects (see Queller 1989). q = frequency of second-division segregation.

^a For the Drusa-type female gametophyte, the chalazal polar nucleus may be derived either from the same meiotic dyad as the egg nucleus is derived from or from the other dyad. In the first case, the genetics of endosperm are the same as for bisporic 3N; in the second case, the genetics are the same as for tetrasporic 3N.

^b This includes pentaploid endosperms derived from female gametophytes with restitution nuclei (e.g., Plumbagella-type and Fritillaria-type).

^c For comparative purposes, we have included calculations for the maternal sporophyte-derived tissue, perisperm.

of all other endosperms derived from tetrasporic female gametophytes ($3H/2$). Thus, according to the tenets of the heterozygosity (heterosis) hypothesis, endosperms derived from tetrasporic female gametophytes should be favored over those derived from bisporic female gametophytes, and endosperms derived from both tetrasporic and bisporic female gametophytes should be favored over endosperms derived from monosporic female gametophytes (but see Haig 1986 for a discussion of potential evolutionarily unstable genetic conflict among megaspores in female gametophytes that are bisporic or tetrasporic).

Endosperm Ploidy

A second theory concerned with endosperm origin and evolution suggests that higher levels of ploidy should benefit the embryo-nourishing function of endosperm when, for example, pentaploid endosperms are compared with triploid endosperms or any endosperm is compared with the haploid female gametophytes of nonflowering plants (Stebbins 1974, 1976). The gist of this argument is that higher ploidy will enable higher rates of gene transcription in support of the active physiological role of endosperm. There is certainly widespread evidence to support the concept that higher ploidy levels (e.g., through endopolyploidy or endoreduplication) are correlated with physiologically active regions of the plant body (D'Amato 1984; Galbraith et al. 1991). Thus, the prediction of this theory (hereafter the "ploidy hypothesis") is that evolutionary transitions to higher endosperm ploidy should be favored.

The vast majority of flowering-plant species produce female gametophytes with diploid central cells that contain two haploid polar nuclei or the product of their fusion, the secondary nucleus. However, the central cell does vary among different lineages of angiosperms, from haploid to decatetraploid (14N). Thus, endosperm ploidy among flowering plants ranges from diploid to decapentaploid (in *Peperomia*; Johnson 1914). Pentaploid endosperms derived from the fertilization of tetraploid central cells can be found in diverse angiosperm clades (e.g., *Penaea*, *Plumbago*, *Plumbagella*, and *Fritillaria*; Stephens 1909; Maheshwari 1950; Haig 1990; Johri et al. 1992). Although we have not performed an explicit phylogenetically based comparative analysis of endosperm ploidy, it is interesting to note that two of the most ancient lineages of angiosperms (Nymphaeales and Austrobaileyales) have recently been shown to produce diploid endosperm derived from the fertilization of a haploid central cell (Williams and Friedman 2002, 2004; Friedman and Williams 2003; Friedman et al. 2003). Given the straightforward predictions of the ploidy hypothesis, it would be well worth investigating whether evolutionary transitions to female gametophyte forms that produce higher-ploidy endosperms are largely (NB, but not always, e.g., Onagraceae; Ishikawa 1918; Tobe and Raven 1986) irreversible.

Endosperm Relatedness

A third body of literature that has been developed to help understand the unique genetic constitution of endosperm derives from inclusive-fitness theory. These analyses suggest that the original integration of a paternal genome into the embryo-nourishing tissue of flowering plants had a number

of profound effects on how maternal sporophytic resources might be allocated to seeds and their constituent embryos.

Inclusive-fitness analyses of angiosperm reproduction (Charnov 1979; Cook 1981; Westoby and Rice 1982; Queller 1983, 1989, 1994; Willson and Burley 1983; Law and Cannings 1984; Bulmer 1986; Haig 1986; Haig and Westoby 1989a, 1989b; Friedman 1995; Härdling and Nilsson 1999, 2001) indicate that the origin and subsequent evolution of a heterozygous and polyploid endosperm can be viewed as the outcome of (1) conflict between male and female parents over the investment of nutrients in the embryo-nourishing tissues of seeds of a single maternal sporophyte (interparental or intersexual conflict) and/or (2) conflict among sibling embryos for resources from the maternal sporophyte (kin or parent-offspring conflict).

Both interparental conflict and kin conflict hypotheses (hereafter "conflict hypotheses") assume that resources available for the production of seeds by a maternal sporophyte are limiting and that, as a consequence, a subset of embryos/seeds on a given plant will abort or be underprovisioned. Central to these ideas is the supposition that changes in the relatedness of the embryo-nourishing tissue to its own embryo, the maternal and paternal sporophytes, and other embryos and embryo-nourishing tissues on a single maternal sporophyte can affect the relative "aggressiveness" or "selfishness" of an embryo-nourishing tissue to procure nutrients on behalf of its own embryo.

A maternal sporophyte is equally related to all of her progeny (assuming that the maternal sporophyte is equally related, or unrelated, to the paternal sporophytes). Therefore, her fitness is maximized when the subset of embryos that are themselves most fit are successfully reared, while less-fit embryos are aborted. Charnov (1979) was the first to recognize that the evolutionary origin of a second fertilization event and its insertion of a paternal genome into the embryo-nourishing tissue had the effect of increasing the relatedness of the embryo-nourishing tissue to its own (same seed) embryo, when compared with its relatedness to other embryos, as well as decreasing its relatedness to the maternal sporophyte. Irrespective of whether interparental conflict or parent-offspring conflict drives the system, the maternal sporophyte should favor increases in its genomic and allelic contributions to its proxy embryo-nourishing tissue, the endosperm, to decrease the selfish behavior of this genetically biparental entity. These evolutionary transitions can be accomplished by increasing the number of maternal genomes contributed to the central cell (in essence, more maternal nuclei) and/or by increasing the number of megaspore genomes represented in the central cell through polysporic initiation of the female gametophyte (e.g., transitions from monosporic to bisporic, from monosporic to tetrasporic, or from bisporic to tetrasporic). Any and all changes in the maternal genetic contribution to the central cell will, of course, be manifest in the genetic constitution of the subsequently formed endosperm.

To analyze genetic conflict among parents, offspring, and endosperms on a single maternal sporophyte, Westoby and Rice (1982), Queller (1983, 1989, 1994), Willson and Burley (1983), Bulmer (1986), Haig (1986, 1987), and Haig and Westoby (1988, 1989a, 1989b) pioneered the use of relatedness ratios for flowering plant seeds. Their analyses derive from the basic construct of "Hamilton's rule," which circumscribes

the conditions under which natural selection will favor the evolution of altruistic behavior. In essence, the fitness cost (c) of an altruistic behavior by an individual (multiplied by its relatedness to itself, $r_{a,a}$) must be less than the benefit to a relative (b) multiplied by the relatedness of the altruist to the beneficiary ($r_{a,b}$),

$$r_{a,b}b > r_{a,a}c. \quad (1)$$

Thus, the benefit-to-cost ratio must be greater than the inverse of the relatedness ratio for natural selection to favor the expression of an altruistic behavior,

$$\frac{b}{c} > \frac{r_{a,a}}{r_{a,b}}. \quad (2)$$

In the case of endosperm and its nourishing relationship with its compatriot embryo, Hamilton's rule can be extended (Queller 1989, 1994) to take into account that the "actor" in a flowering plant seed is the individual endosperm, whose acquisition of nutrients from the maternal sporophyte affects the fitness of the embryo in its own seed as well as the fitness of embryos in other seeds. Under conditions of resource limitation, additional provisioning of an endosperm for its compatriot embryo (ce) will come at the cost of resources that another endosperm could have acquired from the maternal sporophyte for its embryo (other embryo [oe]). Thus, relatedness (r) in the standard equation (2) becomes the relatedness of the actor (embryo-nourishing tissue, endosperm) to the embryo whose fitness it affects. This leads to

$$\frac{b}{c} > \frac{r_{\text{ent-ce}}}{r_{\text{ent-oe}}}, \quad (3)$$

where $r_{\text{ent-ce}}$ is the relatedness of an embryo-nourishing tissue (ent), endosperm, to its compatriot embryo (within the same seed) and $r_{\text{ent-oe}}$ is the relatedness of the same endosperm to another embryo on the same maternal sporophyte.

A larger relatedness ratio ($r_{\text{ent-ce}}/r_{\text{ent-oe}}$) value is indicative of a higher degree of relatedness of an endosperm to its compatriot embryo relative to its relatedness to other embryos on a maternal sporophyte. The larger the relatedness ratio ($r_{\text{ent-ce}}/r_{\text{ent-oe}}$), the greater the conflict between maternal and paternal interests (interparental conflict) and/or between maternal parent and offspring interests (parent-offspring conflict)—and the more selfish or aggressive an endosperm is predicted to be in garnering resources for its own embryo at a cost to the fitness of other embryos and, ultimately, to the maternal sporophyte. In essence, relatedness ratios ($r_{\text{ent-ce}}/r_{\text{ent-oe}}$) reveal a tipping point (threshold) at which the benefit-to-cost ratio (from the perspective of an individual endosperm and its compatriot embryo) will favor the termination of maternal sporophyte investment into an endosperm (cost to the endosperm and its compatriot embryo), with consequent supplemental investment into another seed or seeds (benefit to related endosperm and its compatriot embryo).

The original relatedness ratio analyses for angiosperm seeds (Westoby and Rice 1982; Queller 1983, 1989, 1994; Willson and Burley 1983; Bulmer 1986; Haig 1986, 1987; Haig and Westoby 1988, 1989a, 1989b) were typically calcu-

lated for triploid endosperms derived from monosporic female gametophytes. We have extended these analyses to all seven types of endosperm genetic constructs found among extant flowering plants (table 2). We have also examined the effects on relatedness ratios of seeds sired by pollen derived from a single paternal sporophyte unrelated to the maternal sporophyte and under conditions of self-fertilization (table 2).

As revealed in table 2, evolutionary transitions to polysporous, as well as increases in the maternal genomic contribution to the central cell (and endosperm), serve to increase the relatedness of the endosperm to the maternal sporophyte (and vice versa) and decrease the ratio of the relatedness of the endosperm (embryo-nourishing tissue) to its own compatriot embryo ($r_{\text{ent-ce}}$) versus its relatedness to other embryos ($r_{\text{ent-oe}}$) on the maternal sporophyte ($r_{\text{ent-ce}} : r_{\text{ent-oe}}$). The effect of such changes in endosperm genetics is to decrease the selfish or aggressive behavior of an individual endosperm and increasingly coalign the resource allocation strategy of an endosperm with that of the maternal sporophyte (decreased parent-offspring and/or interparental conflict). Conflict over resource allocation strategy disappears when the relatedness ratio $r_{\text{ent-ce}} : r_{\text{ent-oe}}$ is 1.0, a condition found only in the tetrasporic decapentaploid endosperm of *Peperomia hispidula*, or when the maternal sporophyte substitutes her own tissue (perisperm) for an endosperm to nourish her offspring. Interestingly, if not enigmatically, *Peperomia* species have a minimally developed endosperm and use perisperm as the major embryo-nourishing tissue within a seed.

Effects of Biparental Inbreeding and Self-Fertilization

The relative magnitude of effects predicted by the heterozygosity and conflict hypotheses changes when populations are less than completely outbreeding (table 2). Our calculations show that the relatedness ratio $r_{\text{ent-ce}} : r_{\text{ent-oe}}$ decreases for any type of endosperm genetic construct when two seeds are sired by pollen from the same paternal sporophyte and further decreases when self-fertilization occurs. Consequently, under conditions of inbreeding, genetic conflict is diminished (lower relatedness ratios), ploidy predictions are unaltered, and the fitness benefits of heterozygosity assume greater importance (as an arbiter of deleterious effects of inbreeding). However, under long-term inbreeding, the benefits of increased heterozygosity and of reduced conflict are small, because there is little allelic variation left in the population. Thus, the advantages of evolutionary transitions to bisporous and tetrasporous should be greatest in highly outcrossing populations that are undergoing transitions to inbreeding or that typically experience periodic bouts of inbreeding. Bisporous and tetrasporous provide both a buffer from the effects of inbreeding and an escape from the effects of conflict.

Predictions of Homoplasmy

Although the heterozygosity hypothesis, ploidy hypothesis, and conflict hypotheses are essentially independent explanations of the potential fitness consequences of changes in endosperm genetic constructs, each of these theories is coaligned in the predictions it makes about trends that should emerge in the evolutionary history of female gametophyte development and downstream endosperm genetics. Endosperm hybrid vigor

(heterozygosity) increases in parallel with transitions from monospority to bispority to tetraspority, just as the relative relatedness of an endosperm to its own embryo versus other embryos decreases.

An important caveat to bear in mind is that the predictions of the heterozygosity hypothesis, ploidy hypothesis, and conflict hypotheses do not operate within a vacuum. Predicted trends need not occur inevitably. However, if patterns of increasing heterozygosity, increasing ploidy, and decreasing relative relatedness of the endosperm to its own embryo versus other embryos are selectively favored, considerable homoplasy should be apparent in endosperm genetic constructs among flowering plants. Hence, we predict that homoplasy should also be apparent in mature female gametophyte structure as well as underlying patterns of development.

Relating Female Gametophyte Evo-Devo to Endosperm Genetics: Heterochrony and the Modular Nature of the Angiosperm Female Gametophyte

Variation in the genetic constitution of the target of the second fertilization event (the central cell) directly affects the genetic constitution of the subsequently formed endosperm. The question is, how do innovations in female gametophyte development create new genetic constructs in the central cell?

Developmental analyses of four-nucleate, four-celled female gametophytes in the ancient angiosperm lineages Nymphaeales and Austrobaileyales led to the insight that the female gametophytes of most, if not all, flowering plants are likely to be fundamentally modular entities (Friedman and Williams 2003, 2004) composed of quartets of nuclei (*sensu* Favre-Duchartre 1977; Battaglia 1989; Haig 1990). The basic, and plesiomorphic, angiosperm female gametophyte developmental module proceeds through three critical ontogenetic stages: (1) positioning of a single nucleus within a developmentally autonomous cytoplasmic domain of the female gametophyte, (2) two free-nuclear mitoses to yield four nuclei within that domain, and (3) partitioning of three uninucleate cells adjacent to the pole such that the fourth nucleus is confined to the central cell of the female gametophyte (fig. 2; Friedman and Williams 2003). We wish to be explicit as to what we mean by the term “module” in its various biological manifestations. The “morphological” sense of a module makes

reference to a static adult structure (terminal ontogenetic stage). A “developmental” module, in this case, refers to the compartmentalized ontogenetic events within a cytoplasmically autonomous domain of the female gametophyte.

Variation in mature angiosperm female gametophyte structure results from developmental differences in one or more of three basic aspects of ontogeny: (1) early ontogenetic events that determine the number of developmental modules initiated (one, two, or four) in a female gametophyte, (2) relative timing of the establishment of female gametophyte modules (after or during megasporogenesis), and (3) ontogenetic events that result in developmental deviations from the plesiomorphic female gametophyte developmental module.

Evolution of Module Number in Angiosperm Female Gametophytes: Ploidy and Conflict Consequences

The key to evolutionary transitions between angiosperm female gametophytes with different numbers of developmental (and hence, morphological) modules lies in the modification of early developmental events to position nuclei within one, two, or four cytoplasmic and developmentally autonomous domains (Friedman and Williams 2003). In Nymphaeales and Austrobaileyales (fig. 3; Schisandra-type), a single modular domain is established by the functional megaspore nucleus at the micropylar pole of the female gametophyte. The chalazal region of the female gametophyte remains “unfilled” throughout ontogeny. Subsequent cellularization yields a three-celled egg apparatus, while the fourth nucleus is contributed to the central cell (fig. 3; Friedman and Williams 2003).

In angiosperms with a Polygonum-type female gametophyte (fig. 3), the uninucleate functional megaspore divides mitotically to produce two daughter nuclei that migrate to opposite poles (domains). Each of these nuclei initiates an independent developmental module that produces four free nuclei (for a total of eight free nuclei). At the eight-nucleate stage, cytokinesis partitions three nuclei into three cells at each pole, while the remaining free nucleus from each of the two modular quartets is contributed to the common cytoplasm of the central cell. Thus, after cellularization, the Polygonum-type female gametophyte is seven-celled and eight-nucleate. Likewise, early developmental establishment of four cytoplasmic domains



Fig. 2 The plesiomorphic angiosperm developmental module. A single nucleus within a cytoplasmically autonomous region of a female gametophyte undergoes two free-nuclear divisions to yield four free nuclei. Three of these nuclei are partitioned into uninucleate cells, and the fourth nucleus is contributed to the common cytoplasm of the central cell, where it will contribute to the second fertilization event to initiate endosperm. The yellow background indicates the time of module establishment, green indicates a two-nucleate module, blue indicates a four-nucleate module, and red indicates a mature cellularized module.

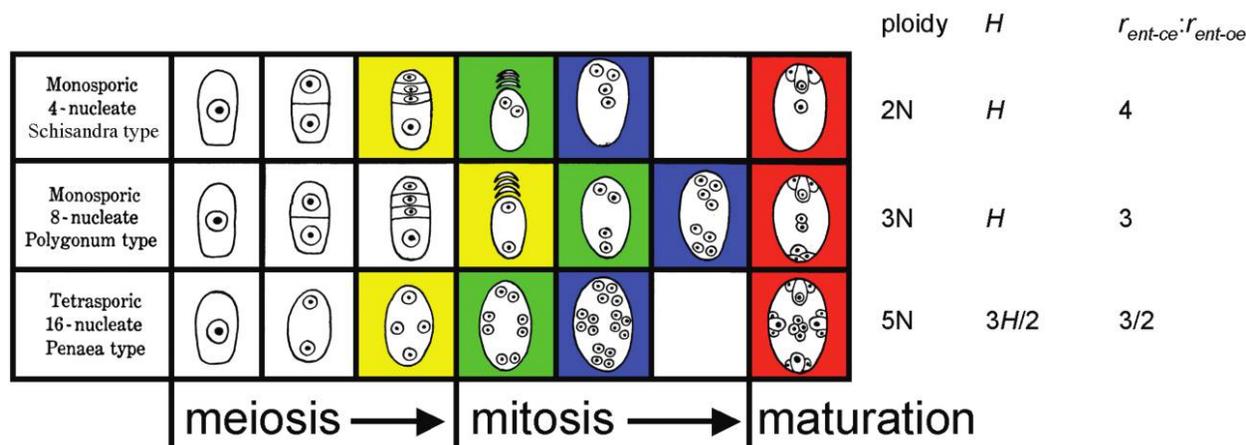


Fig. 3 An example of how changes in module number in an angiosperm female gametophyte can affect the mature structure and nuclear contents of the central cell. In these three cases, a plesiomorphic module ontogeny is apparent. In the Schisandra-type (Nuphar-type) female gametophyte, a single developmental module is initiated and yields a three-celled egg apparatus and a uninucleate central cell (hence, diploid endosperm). In the Polygonum-type, two developmental modules are established at opposite poles of the female gametophyte. The result is a three-celled egg apparatus (derived from the micropylar module), a set of three antipodals (derived from the chalazal module), and a binucleate central cell (hence, triploid endosperm) with a nuclear contribution from each of the two modules. In the Penaea-type, four developmental modules are initiated, and a four-nucleate central cell forms (hence, pentaploid endosperm). As previously discussed (Friedman and Williams 2003), initiation of “extra” modules in the female gametophyte has the effect of increasing the ploidy of endosperm. In addition, the ratio of endosperm relatedness to its own embryo compared with other embryos ($r_{ent-ce} : r_{ent-oe}$) is diminished with increased nuclear contributions to the endosperm by the female gametophyte. This ratio is shown for panmictic outcrossing only (calculations for other mating systems can be found in table 2). The yellow background indicates the time of module establishment, green indicates two-nucleate modules, blue indicates four-nucleate modules, and red indicates mature cellularized modules. The micropylar pole is up, and the chalazal pole is down.

can yield a 16-nucleate (e.g., *Gunnera* and *Penaea*; Stephens 1909; Samuels 1912; Arias and Williams forthcoming) structure consisting of four modules (fig. 3; Penaea-type).

We have speculated (Friedman and Williams 2004) that differences in patterns of nuclear positioning and cytoplasmic regionalization at early ontogenetic stages may hold the key to understanding and predicting the number of modules that are initiated in angiosperm female gametophytes. In essence, the number of modules initiated can be viewed as establishing the body plan (bauplan) of the embryo sac.

To demonstrate how changes in module number can directly affect the genetic constitution of endosperm, we have compared the ontogenies of Schisandra-type (Yoshida 1962; Friedman et al. 2003), also referred to as the Nuphar-type (Williams and Friedman 2002; one plesiomorphic developmental module), Polygonum-type (two plesiomorphic developmental modules), and Penaea-type (four plesiomorphic developmental modules) female gametophytes (fig. 3). Since each plesiomorphic embryo sac module (fig. 2) contributes a single haploid polar nucleus to the central cell, changes in the number of modules initiated directly alter the ploidy of the resulting central cell and endosperm. In addition to modified endosperm ploidy, increases in the relative maternal genomic contributions to the central cell and endosperm also drive the critical ratio $r_{ent-ce} : r_{ent-oe}$ (under conditions of outcrossing) from 4, in the case of a diploid endosperm, to $3/2$, in the case of a pentaploid endosperm in angiosperms with a Penaea-type female gametophyte. As discussed above, the closer this ratio is to 1, the more closely aligned the behavior of an endosperm should be to that which maximizes the reproductive

fitness of the maternal sporophyte in terms of which progeny should be provisioned and which should be aborted (under resource-limited conditions). Finally heterozygosity in endosperms derived from four-module female gametophytes is higher than that in endosperms derived from two modules. This is a by-product of the correlated tetrasporic condition, which ensures that both maternal alleles at each locus will be incorporated into the endosperm.

Assuming that the predictions of the endosperm ploidy hypothesis, heterozygosity hypothesis, and conflict hypotheses are correct, increases in the number of female gametophyte developmental modules should have been favored over the course of angiosperm evolutionary history. The benefits of higher module number should be manifest in trends toward increased endosperm ploidy, increased maternal-to-paternal genomic ratios, increased relatedness of the maternal sporophyte to the endosperms contained within its seeds, and diminished conflict through decreased ratios of relatedness of endosperm to its compatriot embryo versus other embryos (hence, a less “selfish” endosperm).

Relative Timing of Module Establishment: Heterochronic Effects and Genetic Outcomes

The overwhelming majority of angiosperm species produce female gametophytes that are monosporic and initiate two developmental modules (the Polygonum-type). The establishment of module identity in these female gametophytes occurs after megasporogenesis and the first free-nuclear mitotic division of the embryo sac (fig. 4, Polygonum-type, yellow box).

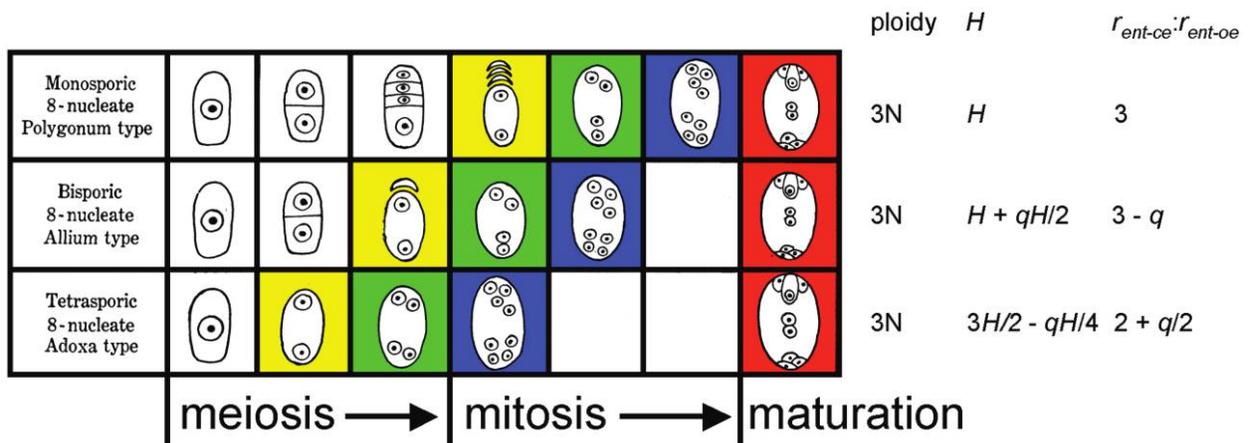


Fig. 4 An example of how heterochronic changes in relative timing of module establishment in an angiosperm female gametophyte can affect the genetic relationships of the nuclear contents of the central cell. In these three cases, a plesiomorphic module ontogeny is apparent, and a two-module, seven-celled, eight-nucleate female gametophyte is formed. The result of pushing module initiation to the end of meiosis II (Allium-type) or meiosis I (Adoxa-type) is to decrease the ratio of endosperm relatedness to its own embryo over that with other embryos ($r_{ent-ce} : r_{ent-oe}$; shown for panmictic outcrossing only; calculations for other breeding systems can be found in table 2) and increase the heterozygosity of endosperm. In these three examples, central cell ploidy and endosperm ploidy are unchanged. The yellow background indicates the time of module establishment, green indicates two-nucleate modules, blue indicates four-nucleate modules, and red indicates mature cellularized modules. The micropylar pole is up, and the chalazal pole is down.

Thus, all nuclei within mature monosporic two-module female gametophytes are derived from a single megaspore (the functional megaspore) and are mitotic relatives; the egg and the two polar nuclei are genetically identical.

In contrast to Polygonum-type female gametophytes, all other types of angiosperm female gametophytes establish developmental modules during or at the completion of megasporogenesis. Monosporic one-module female gametophytes initiate their single developmental module at the end of meiosis II from a cytoplasmic domain that contains the single functional megaspore nucleus (fig. 3, Schisandra-type, yellow box). All bisporic and tetrasporic embryo sacs, with the exception of Adoxa-type female gametophytes, establish the basic number of modules at the end of meiosis II (figs. 4, 5). In Adoxa-type embryo sacs, the two developmental modules are established at the end of meiosis I (fig. 4, yellow box).

Figure 4 shows three ontogenetic paths that result in mature (two-module) seven-celled, eight-nucleate female gametophytes. In essence, these three types of female gametophytes are structurally identical at maturity. What differs in each case is the point of establishment of the two modules (fig. 4, yellow boxes) relative to the process of megasporogenesis and free-nuclear mitotic development of the female gametophyte. As a consequence of heterochronic shifts in module initiation, the genetic relatednesses of the polar nuclei to each other and to the egg cell differ. In the Polygonum-type embryo sac (fig. 4), the polar nuclei are genetically identical. In the Allium-type female gametophyte (fig. 4), the two polar nuclei are derived from meiosis II of a single dyad (and hence are related by $1 - q$, where q is the frequency of second-division segregation; table 1). In the Adoxa-type female gametophyte (fig. 4), the two polar nuclei are derived from separate dyads and are related by $q/2$ (table 1). The relatedness of the polar nuclei to each other and to the egg will be strongly influenced

by the second-division segregation rate, which is maximal when genes are far from the centromere.

Overall, changes in the developmental timing of module establishment (fig. 4; table 2) have profound consequences for both levels of endosperm heterozygosity (higher in bisporic than in monosporic and higher in tetrasporic than in bisporic) and ratios of relatedness of an endosperm to its own embryo to relatedness with other embryos on a maternal sporophyte (polyspory drives down the selfishness of an endosperm and diminishes parent-offspring and interparental conflict). Thus, both the heterozygosity hypothesis and the conflict hypotheses predict evolutionary developmental trends toward earlier establishment of modules and the possibility of considerable homoplasy in these transitions among flowering plants.

Effects of Developmental Changes to Plesiomorphic Module Ontogeny

The plesiomorphic angiosperm module is initiated with a single nucleus within a cytoplasmically autonomous zone of the female gametophyte. Two free-nuclear divisions ensue, and maturation of the module is accomplished through the partitioning of three of the four nuclei into uninucleate, parietally positioned cells. The fourth nucleus of a plesiomorphic module is contributed to the common cytoplasm of the central cell (fig. 2). Considerable structural diversity among angiosperm female gametophytes can be attributed to developmental deviations from this plesiomorphic pattern. These modifications fall into three distinct classes: early ontogenetic changes that affect the establishment of modules, heterochronic modifications that lead to the maturation of juvenilized modules, and heterotopic modifications that alter cell fate in the mature module such that additional nuclei are contributed to the central cell (and, ultimately, the endosperm).

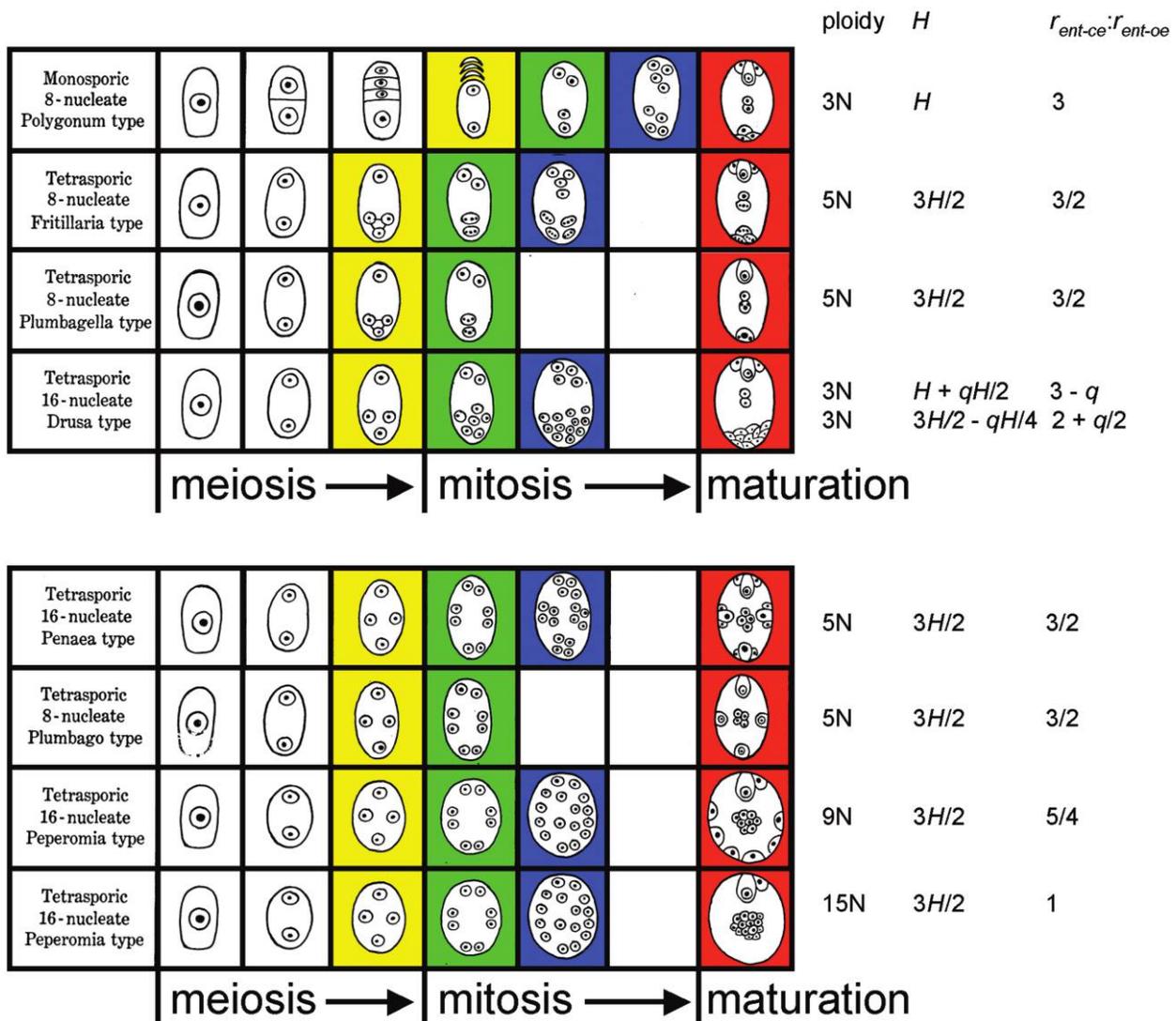


Fig. 5 Examples of how changes in the plesiomorphic module development in an angiosperm female gametophyte can affect the mature structure and nuclear contents of the central cell. In the top panel, the Polygonum-type female gametophyte (with plesiomorphic modules) is compared with other types of two-module female gametophytes with apomorphic module developmental patterns. Both Fritillaria- and Plumbagella-type female gametophytes initiate a chalazal module with three haploid nuclei that produce two triploid restitution nuclei (early ontogenetic modification). In addition, Plumbagella-type female gametophytes cellularize at the two-nucleate stage of module ontogeny (paedomorphosis). The bottom panel shows apomorphic module ontogenies in female gametophytes with four modules. The developmental and genetic consequences of the apomorphic modules are shown for panmictic outcrossing only (calculations for other breeding systems can be found in table 2). The yellow background indicates the time of module establishment, green indicates two-nucleate modules, blue indicates four-nucleate modules, and red indicates mature cellularized modules. The micropylar pole is up, and the chalazal pole is down.

Among the most enigmatic and remarkable female gametophyte types are those in which three haploid nuclei enter into a common division that results in the production of two triploid nuclei, the so-called restitution nuclei. This pattern of development is found in Fritillaria- and Plumbagella-type female gametophytes and is almost certainly highly homoplasious among angiosperms (found in Piperaceae, Tamaricaceae, Liliaceae, Plumbaginaceae, Asteraceae, and Cornaceae; Maheshwari 1950; Haig 1990; Johri et al. 1992). Female gametophytes that form triploid restitution nuclei initiate two developmental

modules from a tetrasporic coenomegaspore (fig. 5, Fritillaria-type and Plumbagella-type, yellow boxes). However, the chalazal module contains three nuclei instead of the usual one nucleus (thus, an early modification of module patterning). The transition to the two-nucleate stage of module ontogeny involves a mitotic division of the single-nucleate micropylar module and the formation of two triploid restitution nuclei in the chalazal module. The net result of this apomorphic ontogeny is the production of a triploid polar nucleus in the chalazal module, increased central cell and endosperm ploidy, and decreased

relative relatedness of an endosperm to its own embryo versus other embryos on a maternal sporophyte (diminished conflict).

Heterochronic truncation of module ontogeny can be found in two types of female gametophytes, the Plumbagella-type and the Plumbago-type (fig. 5). In these female gametophytes, each developmental module yields two (Plumbagella-type) or four (Plumbago-type) two-nucleate modules. Cellularization of each two-nucleate module creates a single parietal cell, with the other nucleus being contributed to the central cell. These pedomorphic modules do not alter the ploidy, heterozygosity, or relative relatedness values involved in reproduction relative to their presumed ancestral types (Fritillaria-type in the case of Plumbagella-type, Penaea-type in the case of Plumbago-type).

Ontogenetic modifications late in module development can have a profound effect on the genetics of endosperm. This is most apparent in the Piperaceae, where different species of *Peperomia* have been reported to contribute as many as 14 nuclei to the central cell and produce a decapentaploid endosperm (Johnson 1914). *Peperomia*-types of female gametophytes are tetrasporic and initiate four developmental modules that yield 16 total nuclei (fig. 5). Rather than partition three nuclei into uninucleate cells and contribute the fourth nucleus to the central cell, in certain *Peperomia* species, these modules alter cell/nucleus position (and hence fate) and contribute two free nuclei per module to the central cell (nonaploid endosperm). In the most extreme case (*Peperomia hispidula*), three of the four developmental modules contribute all four nuclei to the central cell, while the egg-producing module contributes two nuclei to the central cell and produces an egg and a single synergid.

The major consequence of heterotopic changes that alter cell/nucleus position and fate within modules in *Peperomia* is a significant increase in the ploidy of the resultant endosperm, which, in turn, drives down the relative relatedness (selfishness) of an endosperm to its own embryo as compared with its relatedness to other embryos ($r_{\text{ent-ce}} : r_{\text{ent-oe}}$). Altered developmental identity of what were ancestrally somatic female gametophyte nuclei to gametic female gametophyte (central cell) nuclei results in increased ploidy and decreased relatedness ratios but does not change endosperm heterozygosity relative to the presumed ancestral female gametophyte type (Penaea-type). As noted above, endosperm in *Peperomia* plays only a minor role in nourishing the embryo; perisperm (maternal sporophyte tissue) serves as the principal embryo-nourishing tissue.

Why Are Monosporic Triploid Endosperms So Common among Flowering Plants?

The heterozygosity, ploidy, and conflict hypotheses each predict that evolutionary transitions to higher levels of endosperm heterozygosity and ploidy and diminished levels of genetic conflict should be selectively favored. Our analyses of the underlying female gametophyte developmental patterns that generate changes in central cell genetic constitution demonstrate how such variation in endosperm genetic profiles can evolve. Therefore, bisporic and tetrasporic higher-ploidy endosperms (with their correlated female gametophytes) might

be expected to be common among angiosperms. Thus, it is reasonable to ask why well over 80% (Palser 1975; this is likely to be a conservative estimate) of extant angiosperm species produce a monosporic triploid endosperm with relatively low levels of heterozygosity and ploidy and relatively high levels of interparental and/or parent-offspring conflict. To counter this seeming paradox, it is essential to identify when, in the evolutionary history of a clade, a character evolved, as well as the number of times a type of character transition occurred (homoplasy).

The plesiomorphic condition for the angiosperm female gametophyte is unresolved (Friedman 2006b). Nevertheless, the Polygonum-type female gametophyte evolved no later than the common ancestor of monocots, eudicots, and eumagnoliids (Williams and Friedman 2004), roughly 12 million years after the origin of flowering plants, and may have been present in the common ancestor of all angiosperms (Friedman 2006b). As such, monosporic triploid endosperm retains the benefit of position in that the clade defined by its origin (all angiosperms or all angiosperms except *Amborella*, Nymphaeales, and Austrobaileyales) includes no less than 99% of the quarter-million extant angiosperm species. Explicit examination of character transitions among angiosperms indicates that if a Polygonum-type female gametophyte was present in the common ancestor of all angiosperms, triploid monosporic endosperm evolved only once in the entire history of flowering plants. If the Schisandra-type (Nuphar-type) female gametophyte that yields a monosporic diploid endosperm is plesiomorphic for flowering plants, monosporic triploid endosperm will have evolved only twice (once in the ancestor of *Amborella* and once in the common ancestor of monocots, eudicots, and eumagnoliids). Interestingly, such a homoplastic transition from diploid to triploid endosperms is predicted by the ploidy and conflict hypotheses previously discussed.

Homoplasy has long been viewed as evidence of potential adaptation or the product of natural selection (Wake 1991; Armbruster 1996). While the total number of angiosperm species with bisporic and tetrasporic higher-ploidy endosperms is relatively modest, it is clear that transitions away from the monosporic condition have occurred many times. To date, evolutionary transitions from polyspority to monospority (except for pseudomonosporic cases) or from higher endosperm ploidy to lower endosperm ploidy (with the exception of the Onagraceae) appear to be exceedingly rare. From this perspective, the many homoplastic origins of bisporic and tetrasporic female gametophytes and higher-ploidy central cells and, importantly, the unidirectional nature of these evolutionary transitions, should be taken as strong evidence that the predictions of the heterozygosity, ploidy, and conflict hypotheses have been borne out over the course of flowering plant evolutionary history.

The “Origin of the Fittest” and the “Survival of the Fittest”: The Relation between Female Gametophyte Development and Endosperm Genetics

Edward Cope, the late nineteenth-century neo-Lamarckian, first coined the phrase “origin of the fittest” to describe his focus on the then mysterious source of variation on which natural selection was hypothesized to act (Cope 1887). It is,

however, an apt phrase to use in the context of our analysis. The relation between female gametophyte development (and its variation) and endosperm (and its genetic constitution) should be seen as one between the origin of structural novelty (variation in mature female gametophyte construction) and its downstream consequences on the relative fitness (survival) of these novelties, as expressed in the biology of endosperm.

For more than a century, biologists have viewed the structural diversity of angiosperm female gametophytes as trivial variants of the reproductive process. We hope that we have demonstrated that this diversity is the direct result of developmental evolution that is tightly linked to the genetic constitution of endosperm. Variation in female gametophyte developmental patterns represents the raw material that ultimately generates variation in endosperm genetics. Variation in endosperm genetic patterns presents a diversity of functional phenotypes that are subject to natural selection. Thus, culling among the possible variants in female gametophyte development will be a function not of selection directly acting on the phenotypes of female gametophytes but rather of selection on the resultant phenotypes of endosperms.

After 130 million years of angiosperm evolution and diversification, we can discern seven basic genetic types of endosperm: monosporic diploid, monosporic triploid, bisporic triploid,

tetrasporic triploid, tetrasporic pentaploid, tetrasporic nonaploid, and tetrasporic decapentaploid. Each of these distinctive types of endosperm has arisen through developmental modifications of female gametophyte ontogeny. In many cases, there is compelling evidence for the homoplastic origins of a particular mature female gametophyte type and its linked endosperm genetic type (e.g., *Fritillaria*-type and tetrasporic pentaploid endosperm). In several cases, the evolution of different apomorphic female gametophyte ontogenies (e.g., *Fritillaria*-type and *Penaea*-type) has led to identical endosperm genetic constructs (e.g., tetrasporic pentaploid). In any case, we hope that we have shown that embedded in figures such as those of Maheshwari (1950; fig. 1) is a wealth of information waiting to be connected to a world of developmental biology, genetic theory, and the amazing diversity of plant mating systems.

Acknowledgments

We thank Pamela Diggle, Paula Rudall, and an anonymous reviewer for excellent suggestions for the improvement of this manuscript. This work was supported by grants from the National Science Foundation to W. E. Friedman (IOB-0446191) and J. H. Williams (DEB-0640792).

Appendix

Expected Endosperm Heterozygosity

Table A1 shows the gamete (diploid central cell and haploid sperm) frequencies in a population and the probabilities of all six possible genotypic classes that can arise when the central cell is polyploid and derived from more than one megaspore. The diploid central cell frequencies are written as the product of haploid egg and sperm gamete frequencies in the previous generation (here, generation $n - 1$) and/or the second-division segregation rate, q , in the present generation, n . This assumes Hardy-Weinberg (HW) fertilization probabilities in generation $n - 1$. In the case of inbreeding, one should substitute observed sporophyte genotypic frequencies for the HW theoretical frequencies given in the central cell gamete frequency column. Diploid central cell gamete frequencies are equivalent to female sporophytic genotypic frequencies in the case of five-ploid or higher tetrasporic endosperms because all four megaspores are represented in the central cell. However, in bisporic 3N and tetrasporic 3N endosperms, only two megaspores are represented; hence, average central cell heterozygosity will always be reduced from the maximum possible because of recombination.

If a population is randomly mating, then one can assume that the haploid sperm and egg allele frequencies in generation $n - 1$ are the same as haploid sperm allele frequencies in the current generation, n , and thus, $x_{n-1} = x_n$ and $y_{n-1} = y_n$. For bisporic 3N and tetrasporic 3N endosperms, table A1 shows that only two of the six classes are homozygous, so the probability of heterozygosity can be written as one minus the probability that the two alleles in a dyad (bisporic 3N),

or in separate dyads (tetrasporic 3N), are the same. Then from table A1, for bisporic triploid endosperm,

$$H = 1 - [x^3 + x^2y(1 - q)] - [y^3 + y^2x(1 - q)], \quad (\text{A1})$$

$$H = 1 - x^3 - y^3 - xy(1 - q), \quad (\text{A2})$$

$$H = 2xy + qxy, \quad (\text{A3})$$

which is equivalent to

$$H = H_e + \frac{qH_e}{2}, \quad (\text{A4})$$

where H_e is the expected HW heterozygosity of embryos and $0 \leq q \leq 2/3$.

In some tetrasporic female gametophytes (*Adoxa*-type), only two nuclei are contributed to the central cell and to endosperm (tetrasporic 3N). These two maternal nuclei apparently derive from different dyads. From table A1, for tetrasporic triploid endosperm,

$$H = 1 - \left[x^3 + x^2y\left(\frac{q}{2}\right) \right] - \left[y^3 + y^2x\left(\frac{q}{2}\right) \right], \quad (\text{A5})$$

$$H = 1 - x^3 - y^3 - xy\left(\frac{q}{2}\right), \quad (\text{A6})$$

$$H = 3xy - xy\left(\frac{q}{2}\right), \quad (\text{A7})$$

which is equivalent to

$$H = \frac{3H_e}{2} - \frac{qH_e}{4}, \tag{A8}$$

$$H = \frac{3H_e}{2}. \tag{A11}$$

where H_e and q are as in equation (A4).

For the cases when all four megaspores are represented in the central cell, recombination does not affect central cell heterozygosity, and thus,

$$H = 1 - x^3 - y^3, \tag{A9}$$

$$H = 3xy, \tag{A10}$$

Expected endosperm heterozygosity in endosperms derived from monosporic female gametophytes is equivalent to that of the embryo. Thus, heterozygosity of monosporic (diploid and triploid) endosperm is equivalent to

$$H = 2xy = H_e. \tag{A12}$$

Table A1

Expected Heterozygosity of Endosperm Derived from Polysporic Female Gametophytes

Central cell genotypes	Sperm genotypes			
	Genotype A ₁ (frequency = x_n)		Genotype A ₂ (frequency = y_n)	
	Endosperm class	Frequency	Endosperm class	Frequency
A ₁ A ₁ ^a	A ₁ A ₁ A ₁	$(x_{n-1}^2 + x_{n-1}y_{n-1}S_{\text{hom}})x_n$	<u>A₁A₁A₂</u>	$(x_{n-1}^2 + x_{n-1}y_{n-1}S_{\text{hom}})y_n$
A ₁ A ₂ ^b	<u>A₁A₂A₁</u>	$2x_{n-1}y_{n-1}S_{\text{het}}(x_n)$	<u>A₁A₂A₂</u>	$2x_{n-1}y_{n-1}S_{\text{het}}(y_n)$
A ₂ A ₂ ^c	<u>A₂A₂A₁</u>	$(y_{n-1}^2 + x_{n-1}y_{n-1}S_{\text{hom}})x_n$	A ₂ A ₂ A ₂	$(y_{n-1}^2 + x_{n-1}y_{n-1}S_{\text{hom}})y_n$

Note. The genotypic frequencies of six endosperm classes are the products of sperm and central cell genotypic frequencies. Here, x and y are the frequencies of alleles A₁ and A₂, respectively, from generation n or $n - 1$. Central cells (endosperm precursor cells) are diploid, and their genotypic frequencies are determined by egg/sperm allele frequencies in generation $n - 1$ and the second-division segregation rate, q . For convenience, the generic term “ S ” is used to designate a fraction of central cell genotypes that arise from second-division segregation of sporophytic alleles. For bisporic 3N endosperm, $S_{\text{het}} = q$ and $S_{\text{hom}} = (1 - q)$; for tetrasporic 3N endosperm, $S_{\text{het}} = 1 - q/2$ and $S_{\text{hom}} = q/2$; and for all other tetrasporic endosperms, $S_{\text{het}} = 1$ and $S_{\text{hom}} = 0$. Heterozygous endosperm classes are indicated by underlining.

^a Frequency = $x_{n-1}^2 + x_{n-1}y_{n-1}S_{\text{hom}}$.

^b Frequency = $2x_{n-1}y_{n-1}S_{\text{het}}$.

^c Frequency = $y_{n-1}^2 + x_{n-1}y_{n-1}S_{\text{hom}}$.

Literature Cited

Arias T, JH Williams Forthcoming Embryology of *Manekia naranjoana* (Piperaceae) and the origin of tetrasporic, sixteen-nucleate female gametophytes in Piperales. Am J Bot.

Armbruster WS 1996 Exaptation, adaptation, and homplasy: evolution of ecological traits in *Dalechampia* vines. Pages 227–243 in MJ Sanderson, L Hufford, eds. Homoplasy: the recurrence of similarity in evolution. Academic Press, New York.

Battaglia E 1989 The evolution of the female gametophyte of angiosperms: an interpretive key. Ann Bot (Rome) 47:7–144.

Brink RA, DC Cooper 1940 Double fertilization and development of the seed in angiosperms. Bot Gaz 102:1–25.

——— 1947 The endosperm in seed development. Bot Rev 13:423–541.

Bulmer MG 1986 Genetic models of endosperm evolution in higher plants. Pages 743–763 in S Karlin, E Nevo, eds. Evolutionary process and theory. Academic Press, New York.

Charnov EL 1979 Simultaneous hermaphroditism and sexual selection. Proc Natl Acad Sci USA 76:2480–2484.

Chiarugi A 1927 Il gametofito femminile delle angiosperme nei suoi vari tipi di costruzione e di sviluppo. Nuovo G Bot Ital (NS) 34: 1–133.

Cook RE 1981 Plant parenthood. Nat Hist 90:30–35.

Cope ED 1887 The origin of the fittest: essays on evolution. Appleton, New York.

D’Amato FD 1984 Role of polyploidy in reproductive organs and tissues. Pages 519–543 in BM Johri, ed. Embryology of angiosperms. Springer, New York.

Donoghue MJ, SM Scheiner 1992 The evolution of endosperm: a phylogenetic account. Pages 356–389 in R Wyatt, ed. Ecology and evolution of plant reproduction. Chapman & Hall, New York.

Favre-Duchartre M 1977 Eight interpretations of the embryo sac. Phytomorphology 27:407–418.

Fincham JRS 1994 Genetic analysis. Blackwell, London.

Friedman WE 1995 Organismal duplication, inclusive fitness theory, and altruism: understanding the evolution of endosperm and the angiosperm reproductive syndrome. Proc Natl Acad Sci USA 92: 3913–3917.

——— 2006a Embryological evidence for developmental lability during early angiosperm evolution. Nature 441:337–340.

——— 2006b Sex among the flowers. Natl Hist 115:48–53.

Friedman WE, WN Gallup, JH Williams 2003 Female gametophyte development in *Kadsura*: implications for Schisandraceae, Austrobaileales, and the early evolution of flowering plants. Int J Plant Sci 164(suppl):S293–S305.

Friedman WE, JH Williams 2003 Modularity of the angiosperm female gametophyte and its bearing on the early evolution of endosperm in flowering plants. Evolution 57:216–230.

- 2004 Developmental evolution of the sexual process in ancient flowering plant lineages. *Plant Cell* 16(suppl):S119–S132.
- Galati BG 1985 Estudios embriológicos en *Cabomba australis* (Nymphaeaceae). I. La esporogenesis y las generaciones sexuadas. *Bol Soc Argent Bot* 24:29–47.
- Galbraith DW, KR Harkins, S Knapp 1991 Systemic endopolyploidy in *Arabidopsis thaliana*. *Plant Physiol* 96:985–989.
- Gifford EM, AS Foster 1987 Morphology and evolution of vascular plants. WH Freeman, New York.
- Haig D 1986 Conflict among megaspores. *J Theor Biol* 123:471–480.
- 1987 Kin conflict in seed plants. *Trends Ecol Evol* 2:337–340.
- 1990 New perspectives on the angiosperm female gametophyte. *Bot Rev* 56:236–274.
- Haig D, M Westoby 1988 Inclusive fitness, seed resources, and maternal care. Pages 60–79 in J Lovett Doust, L Lovett Doust, eds. *Plant reproductive ecology*. Oxford University Press, Oxford.
- 1989a Parent-specific gene expression and the triploid endosperm. *Am Nat* 134:147–155.
- 1989b Selective forces in the emergence of the seed habit. *Biol J Linn Soc* 38:215–238.
- Hårdling R, P Nilsson 1999 Parent-offspring conflict and the evolution of seed provisioning in angiosperms. *Oikos* 84:27–34.
- 2001 A model of triploid endosperm evolution driven by parent-offspring conflict. *Oikos* 92:417–423.
- Ishikawa M 1918 Studies on the embryo sac and fertilization in *Oenothera*. *Ann Bot* 32:277–317.
- Johnson DS 1900 The embryo sac of *Peperomia pellucida*. *Proc Am Assoc Adv Sci* 49:279–280.
- 1914 Studies of the development of the Piperaceae. II. The structure and seed-development of *Peperomia hispida*. *Am J Bot* 1:357–397.
- Johri BM, KB Ambegaokar, PS Srivastava 1992 Comparative embryology of angiosperms. McGraw-Hill, New York.
- Law R, C Cannings 1984 Genetic analysis of conflicts arising during development of seeds in the angiospermophyta. *Proc R Soc B* 221:53–70.
- Maheshwari P 1950 An introduction to the embryology of angiosperms. McGraw-Hill, New York.
- Palser BG 1975 The bases of angiosperm phylogeny: embryology. *Ann Mo Bot Gard* 62:621–646.
- Queller DC 1983 Kin selection and conflict in seed maturation. *J Theor Biol* 100:153–172.
- 1989 Inclusive fitness in a nutshell. *Oxf Surv Evol Biol* 6:73–109.
- 1994 Male-female conflict and parent-offspring conflict. *Am Nat* 144(suppl):S84–S99.
- Samuels J 1912 Études sur le développement du sac embryonnaire et sur la fécondation du *Gunnera macrophylla* Bl. *Arch Zellforsch* 8:52–120.
- Schnarf K 1929 Embryologie der angiospermen. Borntraeger, Berlin.
- Stebbins GL 1974 Flowering plants: evolution above the species level. Harvard University Press, Cambridge, MA.
- 1976 Seeds, seedlings, and the origin of angiosperms. Pages 300–311 in CB Beck, ed. *Origin and early evolution of angiosperms*. Columbia University Press, New York.
- Stephens EL 1909 The embryo sac and embryo of certain Penaeaceae. *Ann Bot* 23:363–378.
- Takhtajan A 1991 Evolutionary trends in flowering plants. Columbia University Press, New York.
- Tiffney BH 1981 Diversity and major events in the evolution of land plants. Pages 193–230 in KJ Niklas, ed. *Paleobotany, paleoecology, and evolution*. Praeger, New York.
- Tobe H, PH Raven 1986 A comparative study of the embryology of *Ludwigia* (Onagraceae): characteristics, variation, and relationships. *Ann Mo Bot Gard* 73:768–787.
- Wake DB 1991 Homoplasmy: the result of natural selection, or evidence of design limitations? *Am Nat* 138:543–567.
- Westoby M, B Rice 1982 Evolution of the seed plants and inclusive fitness of plant tissues. *Evolution* 36:713–724.
- Williams JH, WE Friedman 2002 Identification of diploid endosperm in an early angiosperm lineage. *Nature* 415:522–526.
- 2004 The four-celled female gametophyte of *Illicium* (Illiciaceae; Austrobaileyales): implications for understanding the origin and early evolution of monocots, eumagnoliids, and eudicots. *Am J Bot* 91:332–351.
- Willson MF, N Burley 1983 Mate choice in plants: tactics, mechanisms, and consequences. Princeton University Press, Princeton, NJ.
- Yoshida O 1962 Embryologische Studien über *Schisandra chinensis* Bailey. *J Coll Arts Sci Chiba Univ* 3:459–462.