

## Chapter 9

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**Parent-specific gene expression and the triploid endosperm**

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The development of the placental habit is one of the most remarkable examples of parallel evolution in the plant and animal kingdoms.

Harper, Lovell & Moore 1970.

The previous chapter discussed various hypotheses about which characters are responsible for the evolutionary success of angiosperms. Most angiosperms differ from most gymnosperms in having much more rapid seed development. The rapid development of angiosperm seeds is associated with extreme reduction of the female gametophyte and the process of double fertilization, which produces a unique angiosperm tissue called endosperm. This chapter presents a new hypothesis to explain the unusual genetic composition of endosperm. The next chapter will discuss the abbreviated development of the angiosperm female gametophyte.

**I. TRIPLOID ENDOSPERM**

The endosperm of flowering plants is a tissue that acquires resources from the maternal sporophyte and is in turn digested by the developing embryo in its own seed. In this respect the endosperm takes the role filled by the female gametophyte in gymnosperms. Endosperm is a tissue unique to angiosperms. At fertilization, a male gametophyte (pollen tube) releases two sperm into the female gametophyte. One sperm fuses with the egg to form a diploid zygote, and the other sperm fuses with two female-gametophyte nuclei (known as polar nuclei) to form the triploid primary endosperm nucleus. Therefore, the endosperm, which develops from this triploid nucleus, has an unusual genetic composition. Each nucleus contains one copy of the paternal contribution to the associated embryo and two copies of the maternal contribution. A minority of flowering plant species have endosperms of different genetic composition, but almost all share the characteristic that more of the endosperm genome is derived

from the maternal sporophyte than from the paternal sporophyte. In this chapter, "mother" and "father" will be synonymous to the maternal and paternal sporophyte respectively.

Double fertilization and the formation of endosperm are often considered the major features that distinguish flowering plants from other seed plants. Because of this importance, many authors have suggested adaptive explanations for the unusual genetic constitution of endosperm. Heterozygote vigor (Jones 1918; Brink & Cooper 1940) can explain the presence of a paternal contribution, but not why the maternal contribution should be doubled. Polyploid vigor (Stebbins 1974) can explain the advantage of triploid over diploid endosperm, but not the weighting toward the maternal contribution. In other words, the polyploid-vigor explanation does not explain why genome doubling should occur in the female gametophyte before fertilization, particularly given that endosperm cells often increase their ploidy by other means after fertilization (D'Amato 1984).

Recent hypotheses have treated the endosperm as a participant in conflicts of interest between mother, father, and offspring. Conflict arises because sibling offspring on the same mother tend to compete with each other. Each offspring's prospects of survival and eventual reproduction are sensitive to the quantity of resources it obtains from the mother, and when some offspring obtain more resources, others tend to obtain less. Because the mother is equally related to (has an equal genetic investment in) each of her offspring, her inclusive fitness is greatest when all nonaborted offspring are provisioned equally (Smith & Fretwell 1974, Trivers 1974). However, each offspring is more closely related to itself than to its siblings. For this reason, individual offspring would benefit from some redistribution of the mother's resources toward themselves and away from their sibs. In this sense, there is a conflict of interest over resource allocation between each offspring and the mother. The fact that offspring are more closely related to themselves than to their sibs arises in part because they carry genes from different fathers. To this extent, the conflict of interest between a mother and each offspring is also a manifestation of conflict between the mother and the fathers over

resource allocation.

Charnov (1979) suggested that double fertilization functioned to shift the genetic interests of the resource-acquiring tissue toward the father's or embryo's interests and away from the mother's interests. The relevant coefficients of kinship were calculated by Westoby & Rice (1982), Queller (1983), and Willson & Burley (1983). These authors argued that the endosperm's interests should in some sense be intermediate between those of the mother and the embryo and between those of the mother and the father. In consequence, the endosperm should behave differently from both the embryo and the mother with respect to acquiring resources at the expense of siblings.

In contrast, Law & Cannings (1984) developed an explicit genetic model not based on kinship coefficients and concluded that "the addition of a second polar nucleus (identical to the first) makes no difference to the fitness of maternal sporophyte or embryo sac" (p. 67). Queller (1984) presented a different genetic model, in which the behavior of triploid endosperm was consistent with predictions based on kinship coefficients. The difference between the two models has two basic causes. First, Law & Cannings only considered alleles that were dominant or recessive or had threshold effects. Queller also considered alleles with additive effects. Second, and more important, in Law & Cannings' model the costs associated with overconsumption by some endosperms were experienced solely by underconsuming endosperms within the same brood. In Queller's model, the cost of overconsumption in the current brood was experienced as reduced resources for offspring in subsequent broods. Therefore, the cost was experienced by both underconsuming and overconsuming genotypes.

I present a model for the evolution of triploid endosperm from a diploid "endosperm" containing a single genome from each parent. This is best understood as a purely formal device to show that the double maternal dose can have a significant effect on gene expression. However, demonstrating a selective advantage for maternal doubling in the context of such a diploid "endosperm" may have phylogenetic implications. The resource-acquiring tissue in gymnosperms is the female gametophyte. Triploid endosperm

differs from a female gametophyte in two respects. A paternal genome is added, and so is a second maternal genome identical to the first. Logically, the addition of a male genome to a gametophytic nucleus other than the egg (double fertilization) could have preceded doubling of the maternal genome, or vice versa. My model indirectly suggests that double fertilization is more likely to have preceded maternal doubling than the other way around.

It should be emphasized, however, that my model does not directly address the phylogenetic issue. Rather, it addresses the question arising from the literature: why should a triploid endosperm behave differently from a tissue with a single maternally derived genome, considering that the triploid endosperm's genotype is qualitatively (though not quantitatively) identical?

## II. PARENT-SPECIFIC GENE EXPRESSION

I propose a mechanism whereby the maternal-loading in the 2:1 endosperm could generate a tissue that behaves differently with respect to resource acquisition than would a hypothetical 1:1 endosperm. The mechanism I propose requires that an allele has different expression depending on its parent of origin. This contrasts with the traditional assumption that an allele's expression is independent of whether the allele is derived from the mother or the father.

Recent advances in mouse embryology and molecular biology have demonstrated that the traditional assumption must be rejected in at least some cases. Maternal and paternal genomes are both necessary for normal development in mice, and this is believed to account for the absence of parthenogenesis in mammals (Surani 1987). Mouse embryos formed from two male pronuclei have larger trophoblasts than do normal embryos or embryos formed from two female pronuclei (Barton, Surani & Norris 1984). The trophoblast is the offspring tissue directly involved in nutrient transfer from the mother. Mouse neonates with both copies of chromosome 11 derived from their mother are smaller than normal litter mates whereas neonates with two paternal copies of chromosome 11 are larger than normal litter mates (Cattanach &

Kirk 1985). These observations are consistent with greater activity by paternally derived alleles in acquiring resources. In addition, differences have been detected in methylation and gene expression between maternally and paternally derived chromosomes (Reik et al. 1987; Sapienza et al. 1987; Swain, Stewart & Leder 1987). I refer to all cases in which an allele has a different phenotypic effect depending on its parent of origin as parent-specific gene expression (PSGE). Evidence for PSGE in endosperm will be discussed in a later section.

Why should natural selection favor alleles that are more active in acquiring resources from the mother when paternally derived than when maternally derived? If other offspring of the mother sometimes have different fathers, there is an asymmetry between the interests of an offspring's alleles that are derived from its father and the alleles that are derived from its mother. A paternally derived allele will be absent from maternal half-sibs, but a maternally derived allele will be present in 50% of these half-sibs. Paternally derived alleles should be selected to acquire more resources than maternally derived alleles, because resources acquired by the offspring should tend to reduce a mother's expectation of reproductive success through other offspring. This reduction imposes greater costs on the overall transmission of maternal alleles than of paternal alleles.

Of course, it is not always the case that alleles expressed in offspring can influence the amount of resources acquired from a parent. In most oviparous animals, egg size and egg contents are determined before alleles expressed in offspring can have any effect. The requirements for PSGE are met only for organisms in which the offspring genotype is active while the mother is supplying resources. The obvious example occurs in viviparous species, such as eutherian mammals. In contrast, there is no scope for the evolution of PSGE in pelagic-spawning fish, because fertilization is external and there is no subsequent parental care. These considerations might explain the distribution of parthenogenesis among vertebrates. Parthenogenesis occurs in all major groups of vertebrates except mammals. Surani (1987) and others have suggested that parthenogenesis is impossible in mammals because both a paternal and a maternal genome are

required for normal development. PSGE may be absent from vertebrate groups with parthenogenetic members.

### III. PARENT-SPECIFIC GENE EXPRESSION IN ANGIOSPERMS

The necessary conditions for PSGE are met in flowering plants, though here the resource-acquiring tissue is often triploid endosperm rather than a derivative of the diploid zygote. In this section I speculate as to how selection for PSGE might favor a triploid endosperm over a hypothetical diploid produced by double fertilization.

My hypothesis supposes that natural selection is acting on the level of transcription of genes. A gene can be considered to consist of a transcribed coding sequence and nontranscribed control sequences that determine the level of transcription. In effect, I consider cases in which selection operates on the control sequences, without affecting the coding sequence.

First, let us consider selection in a diploid resource-acquiring tissue that is genetically identical to its associated embryo. Consider a gene encoding a protein that acquires resources from the mother. Assume that initially there is no PSGE, that all copies of this gene are initially transcribed at some level  $x$  that is independent of parental origin. The total transcription of genes at this locus in the diploid tissue will be  $2x$ . This level of transcription is subject to natural selection, and therefore, under the assumption of no PSGE,  $2x$  is expected to be optimal from the perspective of an allele that is as often maternally derived as paternally derived.

However, from the perspective of paternal allele expressed in offspring, the optimal level of transcription would be greater than  $2x$ . It would be greater for the reason outlined above: briefly, the other individuals that would suffer deleterious effects from an individual offspring acquiring more resources would be less likely to carry the offspring's paternal allele than the offspring's maternal allele. Let the optimal level of transcription for a paternally derived allele be  $2x$  plus some amount  $y$ . By the same token, the optimal level of transcription for a maternally derived allele would be  $2x$  minus some amount  $z$ . In other words, for alleles of this type and in the absence of

PSGE, the achieved level of transcription,  $x$ , should be a compromise between the different levels that are optimal from the perspectives of paternally derived and maternally derived alleles.

Now, suppose that PSGE is possible, thus allowing mutant alleles that are transcribed at one level when maternally derived but at a different level when paternally derived. Specifically, consider a mutant that is transcribed at level  $x$  when maternally derived but at level  $x + y$  when paternally derived. The new allele would initially be present in heterozygous diploid endosperms with total transcription  $2x$  when the allele is maternally derived but with total transcription  $2x + y$  when the allele is paternally derived. Such a mutant would be able to invade a population fixed for transcription level  $x$  because, from the perspective of a paternally derived allele,  $2x + y$  is a better level of transcription than  $2x$ . By a similar argument, an allele that has transcription level  $x - z$  when maternally derived but transcription level  $x$  when paternally derived could also invade a population fixed for transcription level  $x$ .

Thus, given that PSGE is practicable in a biochemical sense, we would expect populations fixed for transcription level  $x$  to be invaded by alleles with higher transcription when paternally derived, with lower transcription when maternally derived, or with both. At what transcription levels would this selective process be expected to stop?

Clearly, the optimal transcription level for the paternally derived allele at a locus depends on the number of transcripts produced by the maternally derived allele and vice versa. As the transcription of paternally derived alleles increases under the influence of natural selection, the optimal transcription level of maternally derived alleles decreases. Provided that transcription of the paternally derived allele is not constrained below  $2x - z$ , the evolutionarily stable state will be zero transcription of the maternally derived allele. All transcription at the locus should be of the paternally derived allele. Therefore, the model predicts the existence of a group of loci (referred to here as class-A loci) with paternal expression only. Remember that the argument thus far deals only with loci that

encode proteins directly involved in acquiring resources from the mother. All class-A loci would be of this type.

My argument aims to explain how it could come about that a triploid resource-acquiring tissue would behave differently from a diploid one, even though no different alleles were present. The model thus far, which considers only class-A loci, cannot explain differences in expression at these loci between triploid and diploid resource-acquiring tissues. This is because the triploid tissue differs only in containing an extra dose of the maternally derived allele; and because maternally derived class-A alleles are predicted to have zero expression, the extra dose would make no difference.

However, the addition of an extra maternal dose would affect the expression of class-A loci, if two additional assumptions are made: (1) there are other loci, essential to offspring fitness, that do not acquire resources from the mother and are not subject to selection for PSGE (class-B loci); and (2) the level of expression of class-A loci is not independent of the level of expression of class-B loci. Specifically, I assume that high levels of expression of class-B loci reduce the expression of class-A loci. This would result from competition between loci for nucleotides or polymerases during transcription or from competition between transcripts for amino acids, ribosomes, or tRNA's at the stage of translation into protein.

Given these assumptions, the effect of doubling the maternal dose in triploid endosperm is to reduce the expression of class-A loci. Let the total transcription at all class-A loci be  $P$  and the total transcription at all class-B loci be  $2Q$ . Therefore, the total transcription of a diploid resource-acquiring nucleus is  $P + 2Q$ , and the proportion of transcripts that come from class-A loci is  $P/(P + 2Q)$ . Now compare the diploid tissue to a triploid endosperm. Transcription at class-B loci is now  $3Q$  and the proportion of class-A transcripts is  $P/(P + 3Q)$ . Provided that  $Q$  is substantial relative to  $P$ , the effect should be to reduce transcription (or translation) of class-A loci.

In summary, a triploid resource-acquiring tissue could be expected to transcribe resource-acquiring loci at a lower level than a diploid resource-acquiring tissue, given selection for



PSGE and some competition for transcription between resource-acquiring (class-A) and other (class-B) loci. In this sense, a triploid tissue would behave in a manner closer to the genetic interests of the maternal sporophyte and the female gametophyte. This, in turn, could explain why natural selection might produce doubling of the maternal genetic dose in endosperm.

#### IV. TESTABLE FEATURES OF THE HYPOTHESIS

The hypothesis outlined above relies on the standard assumptions of evolutionary theory, that natural selection acts on the phenotypic consequences of alleles expressing themselves in particular tissues and that the direction of selection can be predicted by considering how different phenotypic characteristics of a tissue would affect the frequency of the responsible alleles in future generations. Beyond this generality, the hypothesis makes some more particular assumptions or predictions, and it is open to falsification by testing these. There are two major assumptions.

1. Biochemical mechanisms exist by means of which PSGE could be brought into existence, if natural selection favored it.
2. Resources available for gene expression are restricted at the level of whole genomes, such that increased expression of one set of loci implies decreased expression of some other set.

The major prediction of the hypothesis is that there exists a class of loci for which paternally derived alleles are considerably more strongly expressed than maternally derived alleles. These loci are predicted to encode proteins responsible for acquiring resources from the mother for the offspring, and the parent-specific effect should be found in the offspring tissue that acquires resources.

#### V. SOME SUPPORTING EVIDENCE

Parent-specific gene expression has been reported from maize endosperm (Kermicle 1970; Lin 1982, 1984). Endosperms that lack a paternal copy of the long arm of chromosome 10 produce small kernels. Kernel size is not restored by adding extra maternal doses of 10L (Lin 1982). This is direct evidence that there exist gene loci on 10L that are preferentially expressed when

paternally derived.

In a series of elegant crosses, Lin (1984) produced maize endosperms with either one or two paternal genomes and from one to eight maternal genomes. The only combinations that produced normal endosperms were two maternal genomes to one paternal genome and four maternal genomes to two paternal genomes (2m:1p and 4m:2p). This suggested that a ratio of two female genomes to one male genome is necessary for normal endosperm function. Two classes of tetraploid endosperm were produced. Endosperms with 3m:1p produced small kernels, whereas those with 2m:2p were aborted. For endosperms with 3m:1p, my hypothesis predicts that the extra dose of maternal class-B genes should further dilute the resource-acquiring activity of class-A paternal genes. Thus, the small size of these endosperms is consistent with my hypothesis. Similarly, my hypothesis (in its simplest form) would predict that endosperms with 2m:2p would be abnormally large, because of increased resource-acquiring activity by class-A paternal genes and reduced expression of class-B genes. However, these endosperms were aborted. Such abortions could be explained, within the framework of my hypothesis, either by an imbalance between expression of class-A and class-B genes or by the maternal sporophyte actively aborting ovules that express unusually high resource-acquiring activity. There are still some puzzling features in Lin's data. Though endosperms with 3m:1p were subnormal, those with 6m:2p and 5m:2p and the same or smaller deviation from a 2:1 ratio were aborted.

Lin's evidence is especially convincing because he was able to study endosperms that had different maternal:paternal ratios but which were all interacting with the same maternal sporophyte. Other evidence comes from interspecific crosses in which seeds abort because of endosperm failure. In some cases, viable hybrids can be produced by altering the ploidy of one of the parents. This evidence usually cannot separate the effects of different maternal contributions to endosperm from effects due to different maternal sporophytes.

In crosses among *Solanum* (Solanaceae) species, the ploidy changes needed to produce viable seed can be understood in a simpler way than by having special rules for each cross. Johnston

et al. (1980) assigned species an endosperm balance number (EBN), which described their behavior in crosses. Crosses were viable between species with the same EBN, but crosses between species with different EBN's showed abnormal endosperm development. However, if the ploidy of the male or female parent was adjusted to generate a 1:1 ratio of parental EBN's, normal endosperm development could be obtained. A similar coefficient can explain interspecific crossing rules in oats (Nishiyama & Yabuno 1978).

Two closely related diploids, *Solanum commersonii* (1 EBN) and *S. chacoense* (2 EBN), are normally intersterile. Nevertheless, Ehlenfeldt & Hanneman (1988) were able to produce a small number of diploid  $F_1$  hybrids from a large number of interspecific pollinations (see their paper for the probable origin of these hybrids). Thus, the  $F_1$  hybrids were heterozygous for those alleles determining the different EBN's of the parent species. Furthermore, the meiotic products of the hybrids would contain different combinations of the parental alleles. Crosses using the hybrids would be expected to produce neither consistent development nor consistent failure of endosperm but varying proportions of each. When hybrids were used as pollen parents in crosses with *S. chacoense* (2 EBN) about 50% of seeds aborted and the viable seeds were of small to average size. When *S. chacoense* was the pollen parent, most seeds aborted but viable seeds were of average to large size. In crosses of hybrids with *S. commersonii* (1 EBN), the outcomes were reversed: viable seeds were average to large with the  $F_1$  as pollen parent, but small to average in the reciprocal cross. Taken together, the results are consistent with a positive relationship between seed size and relative paternal activity, except that seeds are aborted when paternal activity is either very high or very low.

The interpretation of *Solanum* crosses in terms of species-specific EBN's would be interpreted by my model as follows. Over evolutionary time, different species evolve to have male genomes with different levels of activity in resource acquisition. However, within each species, this paternal activity comes into balance with the effects of the double maternal contribution. Since endosperms resulting from crosses between species often do not have the appropriate balance between paternal and maternal

activity, defective endosperms can result. However, endosperm balance can be restored by appropriate changes in the ploidy of one of the parents. These features indicate that although different species maintain a similar balance between paternal activity and the effects of the maternal double contribution, this balance may be achieved at different levels of paternal activity.

Kihara & Nishiyama (1932) studied endosperm development in reciprocal crosses between diploid and hexaploid *Avena*. When the diploid was used as seed parent and the hexaploid used as pollen parent, endosperms were 2m:3p. The initial development of endosperm was more rapid than that of controls, but soon acquired a degenerate appearance. Seed set was good and yielded large kernels. However, these kernels were shrivelled, empty, and none germinated. When the diploid was used as pollen parent, the endosperm was 6m:1p. Endosperm development was slower than controls. Seed set was poor and yielded small kernels. However, these kernels were plump and germinated well.

Nishiyama & Inomata (1966) subsequently studied endosperm development in reciprocal crosses between *Brassica chinensis* and its autotetraploid, *B. pekinensis*. Crosses between diploid parents and crosses between tetraploid parents were used as controls. These crosses produced viable seed, with 2m:1p and 4m:2p endosperms respectively. When a diploid was used as the seed parent and a tetraploid used as the pollen parent, the endosperm was 2m:2p and its development was accelerated during early stages. In the reciprocal cross, the endosperm was 4m:1p and its development was retarded during early stages. Later stages of endosperm development were abortive in both crosses, and neither cross produced viable seed.

These results are consistent with paternally derived genes promoting more rapid endosperm development than maternally derived genes. Endosperms of *Avena* and *Brassica* normally undergo several free-nuclear divisions before wall formation. The same patterns of endosperm development, in reciprocal crosses and controls, were observed in both genera. If the parent with higher ploidy was used as the pollen parent, free-nuclear divisions were more rapid than occurred in controls, but wall formation was

inhibited and the endosperm degenerated. If the parent with lower ploidy was used as pollen parent, free-nuclear divisions were slower than those of controls, but wall formation began earlier than in controls (Kihara & Nishiyama 1932; Nishiyama & Inomata 1966). These similarities are particularly significant because *Avena* is a monocot and *Brassica* is a dicot. Perhaps paternally derived genes are active in the free-nuclear divisions and early expansion of the endosperm, whereas maternally derived genes are responsible for bringing this expansion to a halt by cell formation.

## VI. APOMIXIS IN ANGIOSPERMS

Parent-specific effects are thought to be the major obstacle to the evolution of parthenogenesis in mammals, because both a maternal and a paternal genome are necessary for normal embryonic development (Surani 1987). In this chapter, I have argued that parent-specific effects are also expected during seed development, yet apomixis is well-known in angiosperms. The probable reason for this difference is that the trophoblast and embryo of mammals are derivatives of a single fertilization, but the endosperm and embryo of angiosperms are products of separate fertilizations. For example, both maternal and paternal genomes are necessary for normal endosperm development in maize (see above), but maternal haploid embryos and paternal haploid embryos are viable in this species, provided that the polar nuclei are fertilized to give a normal 2m:1p endosperm (Sarkar & Coe 1966; Chase 1969; Kermicle 1969).

Apomixis is the obligate mode of reproduction in many angiosperms. Asexual embryos can arise from a somatic cell of the ovule or from an unreduced female gametophyte but, in most cases, asexual embryos cannot complete development unless the polar nuclei are fertilized to produce an endosperm (Lakshmanan & Ambegaokar 1984; Nogler 1984). This is called *pseudogamous apomixis*. Not all apomicts require fertilization of the polar nuclei. In most apomictic Asteraceae, the embryo and endosperm both develop without fertilization. This is called *autonomous apomixis* and has only a sporadic occurrence outside the Asteraceae (Nogler 1984).

In Chapter 6 (III.F), I suggested that the apparent absence of apomixis in gymnosperms could be explained if there was parent-specific gene expression in gymnosperm embryos. Under this hypothesis, gymnosperms differ from angiosperms because there are several embryos in most gymnosperm seeds (with identical maternal genomes but different paternal genomes) whereas there is usually only one embryo per angiosperm seed. Therefore, parent-specific gene expression is absent from angiosperm embryos because there is no conflict between embryos within ovules and embryos cannot influence resource distribution among ovules (unlike endosperms).

## VII. SYNOPSIS

A characteristic feature of flowering plants is endosperm, a tissue with more doses of maternally derived than paternally derived genes. Endosperm is responsible for acquiring resources for offspring from mother plants. Recent hypotheses about the unusual genetic composition of endosperm have argued that because endosperm is more closely related to other offspring of the same mother than is the embryo, natural selection would cause the endosperm to be less vigorous than the embryo in acquiring provisions from the mother. However, since endosperm contains qualitatively the same alleles as the embryo with which it is associated (for monosporic gametophytes, which are the great majority), it has not been clear how extra maternally derived alleles could actually reduce the vigor with which a tissue sought to acquire resources. In this chapter, I have proposed a mechanism by which this could happen.

At loci that encode proteins directly responsible for acquiring resources from the mother, parent-specific gene expression (PSGE) would be expected to arise, with strong expression of the paternally derived allele and little expression of the maternally derived allele. At these class-A loci, adding an extra copy of the maternally derived allele could not reduce the vigor with which the offspring sought to acquire resources from the mother. However, it can be assumed that there exist other class-B loci, which are important to tissue functioning but not directly involved with acquiring resources from the mother. I assume that total gene expression across the whole genome is

restricted, such that increased expression at class-B loci has the effect of decreasing expression at class-A loci. Given these assumptions, I show that adding extra maternally derived alleles at all loci would moderate gene expression at class-A loci.

Parent-specific gene expression is therefore capable of explaining in general terms how the second maternal genome in endosperm nuclei might affect the endosperm's resource-acquiring behavior. The hypothesis also implies that triploid endosperms might have evolved from an immediate ancestor with one maternal and one paternal genome, rather than from an ancestor with two maternal genomes to which an extra paternal genome was added. Several aspects of the hypothesis are testable, and some supporting evidence already exists.

## Chapter 10

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**New perspectives on the angiosperm female gametophyte**

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**I. Abstract**

This review builds upon previous classifications of angiosperm female gametophytes but offers two new perspectives. Firstly, the course of development is compared to an algorithm: a predetermined set of rules that produces a mature female gametophyte. This analogy allows hypotheses to be developed as to what change in the "developmental program" is responsible for variant forms of development. Secondly, the review recognizes that the four meiotic products of a megaspore mother cell have different genetic constitutions and may have conflicting interests. In most cases, only one member of a megaspore tetrad gives rise to a functional egg. This megaspore is called the germinal spore. The other members of the tetrad are called somatic spores. Somatic spores do not give rise to functional eggs and, therefore, cannot leave direct genetic descendants.

Non-monosporic embryo sacs are genetic chimeras containing derivatives of more than one megaspore nucleus. Conflict may arise within such embryo sacs between the derivatives of the germinal megaspore nucleus and the derivatives of somatic megaspore nuclei. "Antipodal eggs" and chalazal "strike" are interpreted as evidence of this conflict. The behavior of somatic spores and their derivatives is often variable for different embryo sacs produced by the same sporophyte. This has created difficulties for existing classifications of embryo sac "types" because more than one type is sometimes recognized within a species. A new classification of developmental algorithms is presented that emphasizes the fate of the germinal spore and its derivatives.

**II. Introduction**

The life cycle of seed plants alternates between diploid (sporophyte) and haploid (gametophyte) generations. The obvious, vegetative generation is the sporophyte. The female gametophyte



remains enclosed within the ovule and is nutritionally dependent on the sporophyte. After fertilization, the ovule develops into a seed. Thus, an ovule/seed contains a number of genetically distinct tissues (maternal sporophyte, female gametophyte, embryo, endosperm). Seed development has traditionally been viewed as a harmonious process in which all tissues cooperate to produce a mature seed. However, the alternative kin-conflict interpretation recognizes that different tissues may have conflicting interests (Charnov 1979; Queller 1983, 1984; Haig 1986, 1987; Westoby & Rice 1982; Willson & Burley 1983).

The contrast between the traditional (non-conflict) and kin-conflict interpretations is illustrated by debate about the function of the hypostase. The hypostase is a structure that develops between the chalazal vasculature of an ovule (if present) and the antipodal end of the embryo sac. Bouman (1984) listed several possible functions. These included the proposal that the hypostase acts to restrain growth of the embryo sac. Bhatnagar & Bhatnagar (1986) dismissed this suggestion out of hand -- "as if [the] embryo sac was a wayward or cancerous structure. We cannot recall any other system in plants in which a cell or tissue needed to be surrounded by a special structure to merely stop its growth". This rejection reflects the traditional interpretation. However, maternal tissues (such as the hypostase) and offspring tissues are genetically distinct, and their relationship is not comparable to that between genetically identical tissues. Briggs et al. (1987) were inspired by the kin-conflict interpretation to compare the development of aborting and non-aborting ovules of *Pisum sativum*. A hypostase only developed in aborting ovules. They concluded that "the hypostase is the main instrument by which the mother plant imposes abortion on individual offspring".

The aim of this chapter is to review the diversity of female gametophyte development in angiosperms from the perspective of kin-conflict. Embryologists have traditionally classified this diversity into a number of discrete "types", each named after the first genus in which it was correctly described. This classification has been useful in ordering a large body of data but it has two major weaknesses. Firstly, types are often seen as

abstract ideals of which actual gametophytes are imperfect representations. Thus, the *Drusa* type is described as 16-nucleate, even though its physical manifestations almost never have this many nuclei. Secondly, some types have evolved independently in a number of groups. Therefore, types are not phylogenetic units, yet embryologists often present "phylogenies" that derive one type from another without reference to the types' taxonomic distribution. To give a recent example, Herr (1984) presents a "phylogeny" in which developmental variants within the genus *Tamarix* have three independent derivations from an angiosperm archetype. If the phylogeny is taken at face value, members of the same genus are more distantly related than members of different orders. Despite these reservations, this paper will often refer to "types" for reasons of convenience. I will use the classification of embryo sac types that is presented in Fig. 4.1 of Willemse & van Went (1984). I will use Dahlgren's (1980) classification of dicotyledons and Dahlgren, Clifford & Yeo's (1985) classification of monocotyledons.

### III. Preliminary Considerations

#### A. DEVELOPMENT AS AN ALGORITHM

Female gametophyte development must reflect some underlying sequence of gene expression. Presumably each stage and each cell type is characterized by the activity of a specific set of loci. From this perspective, the genetic control of development consists of the switching-on and switching-off of particular sets of loci at appropriate stages of development. Thus, development can be seen as a genetically controlled algorithm that produces a mature embryo sac from a megaspore mother cell. The algorithm requires that a complex set of "instructions" be executed in a precise order, and that this order be specified in the instructions. In many respects, the algorithm can be compared to a computer program, though the instructions are coded as a string of bases (A,G,C,T) on chromosomes rather than as a string of binary digits (0,1) in memory.

The analogy to a computer program is useful. Programmers usually adopt a hierarchical approach to simplify the writing of complex computer algorithms. A task is broken down into a series

of sub-tasks; sub-tasks are divided into sub-sub-tasks; and so on, until the problem is reduced to a set of simple primary processes. The resulting algorithm consists of a master program that calls nested subroutines in an appropriate sequence. By analogy, stage-specific or cell-specific patterns of gene expression can be likened to subroutines. A different set of (regulatory) loci would correspond to the master program. The analogy is not exact, particularly where branching in the algorithm is concerned. A program may have alternative pathways but only one path is taken at any one time. In contrast, nuclei replicate during development. All nuclei share a common set of loci, and in this sense carry a common genetic program. However, different nuclei take different pathways in the algorithm. Moreover, nuclei can interact in their gene expression. It is as if several computers are running in parallel, providing input to each other's programs.

The importance of the hierarchical perspective is that an algorithm can be understood at different levels. At present, little is known about gene expression during gametophyte development but I believe useful statements can still be made about the upper levels of the developmental algorithm. To return to our analogy, a program is usually written in a higher level language and its programmer need have no knowledge of how the program is implemented in machine language, at the level of binary digits. Sometimes a programmer may merely specify the algorithm's subroutine structure and delegate the writing of subroutines to other workers.

### *1. The Polygonum algorithm*

The Polygonum type (monosporic, eight-nucleate) is the commonest pattern of development among angiosperms, and will serve as a useful starting point for discussing development. In this type, the two meiotic divisions of the megaspore mother cell produce four megaspores, three of which degenerate. After meiosis, the functional megaspore undergoes three mitotic divisions. The sister nuclei of the first division become separated by a large vacuole, and after two further divisions produce a micropylar and a chalazal quartet of nuclei. Cell walls form after the final

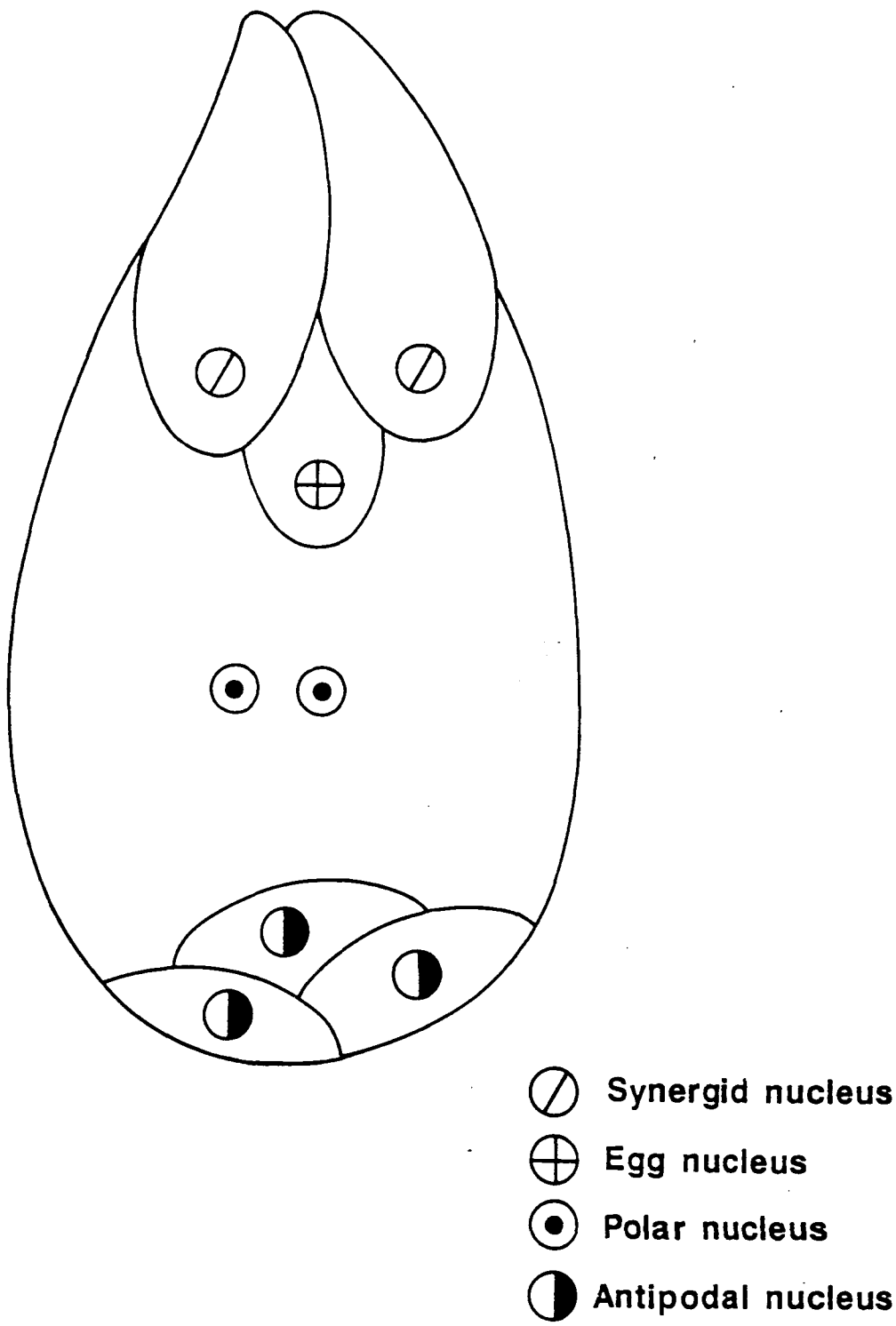
division. Each quartet forms three cells and contributes a polar nucleus to the central cell. The three cells at the micropylar pole form an egg apparatus: two synergids and an egg. The synergid nuclei are sisters, as are the egg and a polar nucleus. At fertilization, a pollen tube penetrates one synergid and releases two sperm. One sperm fuses with the egg to form the zygote and the other fuses with the polar nuclei to form the primary endosperm nucleus. The three cells at the chalazal pole are known as antipodal cells (Cass, Peteya & Robertson 1985, 1986; Russell 1979; Willemse & van Went 1984; Fig. 1).

Polygonum-type development can be represented by an algorithm with four subroutines (Fig. 2). Each subroutine represents a coordinated pattern of gene expression and the four subroutines are assumed to act sequentially during development.

- (A) MEIOSIS (both divisions followed by cytokinesis)
- (B) FREE-NUCLEAR DIVISIONS (three mitotic cycles)
- (C) CELL FORMATION (cell walls are formed on the persistent spindles of the final free nuclear mitosis)
- (D) DIFFERENTIATION
  - (D<sub>1</sub>) If a cell lies at the micropylar pole it helps form an egg apparatus. Different sets of loci are activated in the synergids (D<sub>1a</sub>) and the egg (D<sub>1b</sub>).
  - (D<sub>2</sub>) If a nucleus lies in the central cell, it behaves as a polar nucleus.
  - (D<sub>3</sub>) If a cell lies at the chalazal pole, it develops as an antipodal cell.

Meiosis results in a tetrad of four megaspores. Usually only one megaspore organizes a gametophyte and the other three megaspores degenerate. Kapil & Bhatnagar (1981) discussed the mechanisms whereby only one megaspore continues development. The functional megaspore usually occupies a nutritionally more advantageous position in the ovule. Non-functional megaspores are often surrounded by a callose wall that isolates them from maternal

Figure 10.1. Schematic diagram of a Polygonum-type gametophyte.  
The micropylar end is uppermost.



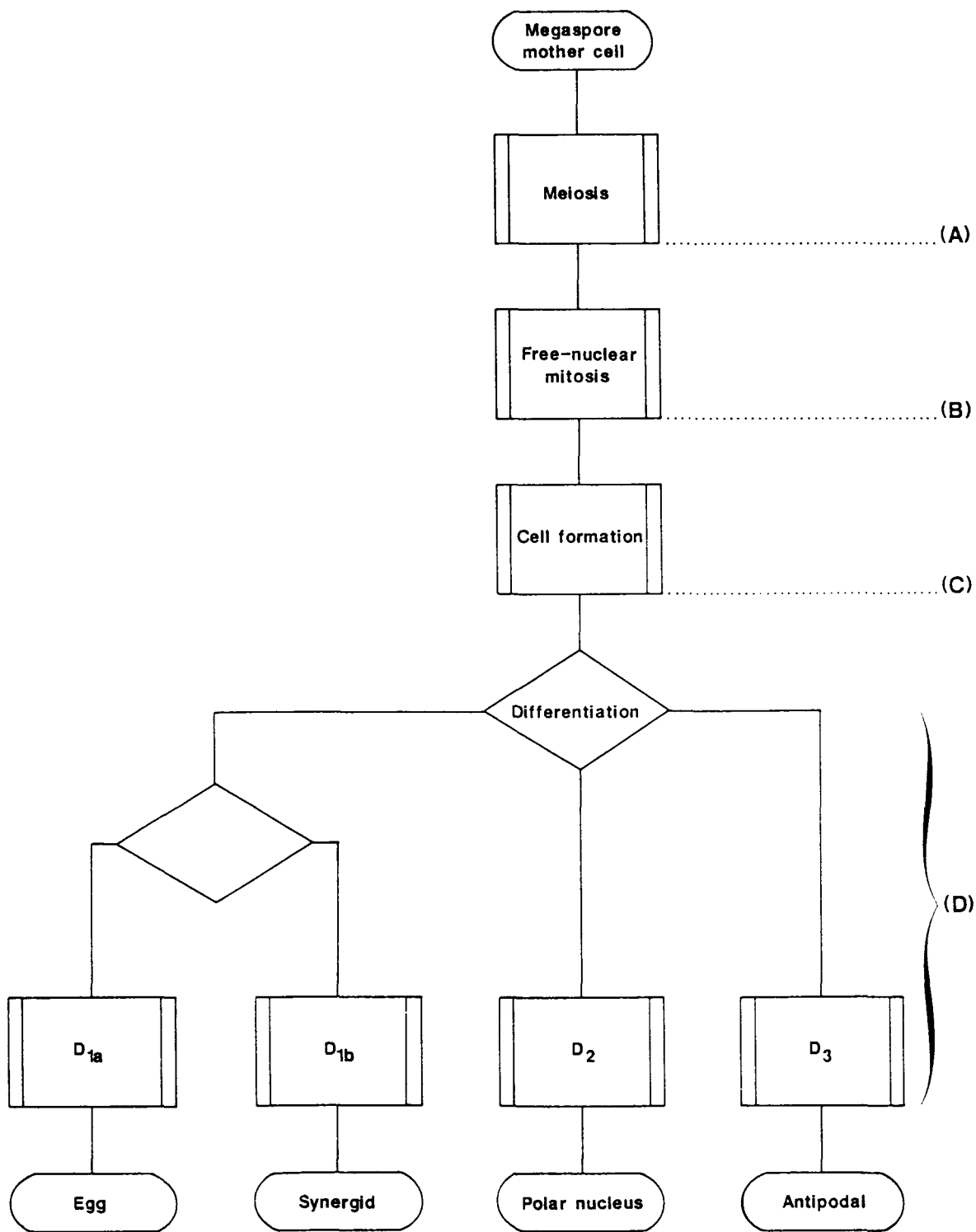


Figure 10.2. Flowchart of the Polygonum-type algorithm.

nutrient supplies, whereas callose deposition around the functional megaspore is usually absent or short-lived. In some species, the functional megaspore contains more plastids and mitochondria than non-functional megaspores. This might be a mechanism that determines the position of the functional megaspore. Alternatively, the functional megaspore may already be determined and the asymmetric distribution of organelles may reflect adaptations by organellar genes to be included in the functional megaspore (see Cosmides & Tooby 1981; Eberhard 1980).

Haig (1986) referred to the functional megaspore as a *germinal spore* and the non-functional megaspores as *somatic spores*. This was by analogy to the zoological distinction between germ-line and soma. The four members of a tetrad have distinct genotypes but only the germinal spore can leave direct genetic descendants. Haig argued that the maternal sporophyte suppresses somatic spores by isolating them from nutrient supplies and that all spores would continue development if they were not suppressed. In support of this contention he cited Schnarf's (1929, p. 108) list of species from 53 families in which the gametophyte may develop from more than one position in the tetrad.

Single-locus mutations are natural experiments in which one part of a system is varied while other parts are held constant. As such, they are particularly useful in determining the structure of developmental algorithms. Below I discuss three mutant alleles that affect species with Polygonum-type algorithms. These are the *dyad* allele (*dy*) of *Datura* (Satina & Blakeslee 1935), the *male sterile* allele (*ms<sub>1</sub>*) of soybean (Kennell & Horner 1985), and the *indeterminate gametophyte* allele (*ig*) of maize (Kermicle 1971). The first two alleles have their primary effect during meiosis whereas the third allele has its primary effect during the free nuclear divisions. All three alleles also have effects on the development of male gametophytes.

The *dyad* allele of *Datura* suppresses meiosis II. As a result, meiosis produces a dyad of two diploid nuclei rather than a tetrad of four haploid nuclei. Subsequent development is normal, producing an eight-nucleate gametophyte that conforms to

the Polygonum type except that all nuclei are diploid (Satina & Blakeslee 1935). Therefore, subroutines B, C and D are expressed independently of ploidy.

The  $ms_1$  allele of soybean (Kennell & Horner 1985) provides evidence that all megaspore nuclei will continue development if not suppressed. In  $ms_1/ms_1$  homozygotes, cytokinesis is completely or partially absent after meiosis I and meiosis II. As a result, all four megaspore nuclei occur within a single cell, and all four nuclei continue development. The  $ms_1$  gene does not affect the number of mitotic divisions following meiosis but the absence of walls between megaspore nuclei allows nuclei to come into contact and occasionally to fuse. This contributes to variation in the number of nuclei in mature embryo sacs. Despite this variation the relative positions of antipodal cells, polar nuclei and egg apparatuses are unaffected. Ovules have been observed with up to four egg cells, but the ratio of egg cells to synergids remains 1:2. Similarly, up to 12 antipodal cells have been recorded within an ovule and the central cell nucleus is often abnormally large, suggesting the fusion of more than two polar nuclei.

Three observations can be made about the preceding example. Firstly, the full developmental algorithm was expressed despite a change in the number of nuclei participating. In terms of the algorithm model,  $ms_1$  causes a change within meiosis but all other parts of the algorithm remain unchanged. Therefore, each megaspore nucleus underwent three free nuclear divisions, followed by cell formation and differentiation. Secondly,  $ms_1$  has recessive inheritance, suggesting that the meiotic divisions are controlled by the sporophyte genome. Thirdly, the fusion of megaspore nuclei is an unselected epiphenomenon of the failure of cytokinesis. This is relevant because nuclear fusions are a feature of several non-Polygonum types of development.

The *indeterminate gametophyte* (*ig*) allele of maize causes major disturbances in gametophyte development. The primary cause of these effects appears to occur during the free nuclear divisions. Nuclei sometimes divide asynchronously and there are more than three free nuclear divisions in at least some gametophytes. The number of free nuclear mitoses cannot be



determined by a simple count of gametophyte nuclei because normal development in maize is characterized by secondary proliferation of antipodal cells and early degeneration of the synergids (Lin 1978, 1981).

Disturbances during the free nuclear divisions are probably the cause of the other effects of *ig*. Cell formation and differentiation are affected because the gametophyte contains extra nuclei and their spatial relationships are disturbed. The cells closest to the chalaza are usually uninucleate and develop as antipodal cells. Gametophytes sometimes contain more than one central cell. The central cell closest to the micropyle is always the largest and usually contains two or more polar nuclei. Genetic evidence suggests that up to eight polar nuclei may fuse with the second male nucleus to initiate endosperm. Central cells closer to the chalaza are usually uninucleate (Lin 1978, 1981, 1984). My interpretation of the extra central cells is that cell formation results in more than three uninucleate cells near the chalazal pole. Sometimes some of these cells are sufficiently displaced towards the micropyle to miss out on the cytoplasmic determinants of antipodal differentiation or to receive the cytoplasmic determinants of central cell differentiation. Lin (1978) observed gametophytes with up to five micropylar cells which were often not clearly recognizable as either eggs or synergids. Kermicle (1971) obtained seeds with twin, triplet and quadruplet embryos formed by the fertilization of multiple eggs in the same gametophyte.

*Ig/ig* heterozygotes produce about half as many abnormal seeds as *ig/ig* homozygotes (Kermicle 1971). This suggests that *Ig* has gametophytic expression and that genetic control of development passes to the gametophyte after meiosis. Of course, more than one genome may influence development at a given stage. The sporophyte surrounds the gametophyte and controls its access to resources. Sporophytic gene products could also persist in the cytoplasm and have their phenotypic effect at a later stage.

I do not know of any well characterized mutations affecting cell formation and differentiation, but some of the possible changes can be discussed. Cell formation takes place on the mitotic spindles of the final free nuclear mitosis. In *Hordeum*,

each quartet possesses two cell plates but is able to segregate nuclei into four cells (including the central cell) because one of the cell plates branches to produce a third wall (see Cass et al. 1985, 1986). In *Ranunculus*, each quartet forms two cell plates on the spindles of the final free nuclear mitosis. A third cell plate appears to form without branching and without the aid of a mitotic spindle (Bhandari & Chitralkha 1989). Simple changes in the geometry of wall formation could change the number of cells formed. In several members of the Asteraceae, only two antipodal cells are formed, one of them binucleate (Howe 1975; Newcomb 1973).

Differentiation requires that specific loci be activated in response to positional information supplied by cytoplasmic inclusions or by reference to the surrounding nucellus. We do not know whether positional information is provided by a simple gradient; by separate cytoplasmic determinants for antipodal cells, central cells and egg apparatuses; or whether one path of differentiation is a "default option" that is followed in the absence of determinants. Mutations could change the distribution of determinants or change the developmental response to the determinants.

The algorithm presented in Figure 2 is intended as a working hypothesis and will undoubtedly be modified in response to additional evidence, particularly from the study of developmental mutants in agricultural species.

## 2. *Non-Polygonum* algorithms

The different developmental programs of angiosperm female gametophytes must all be derived from some common ancestral program. (Such will be the case whether angiosperms as a taxon are monophyletic, polyphyletic or paraphyletic.) Thus, the different forms of development will have evolved by modifications of a pre-existing genetic program, and all developmental algorithms should show evidence of this common ancestry. The *Polygonum* type is the only type reported from almost 80% of families and the predominant type in several other families (Palser 1975). In those families where the *Polygonum* type occurs with other types, it seems reasonable to assume that the other

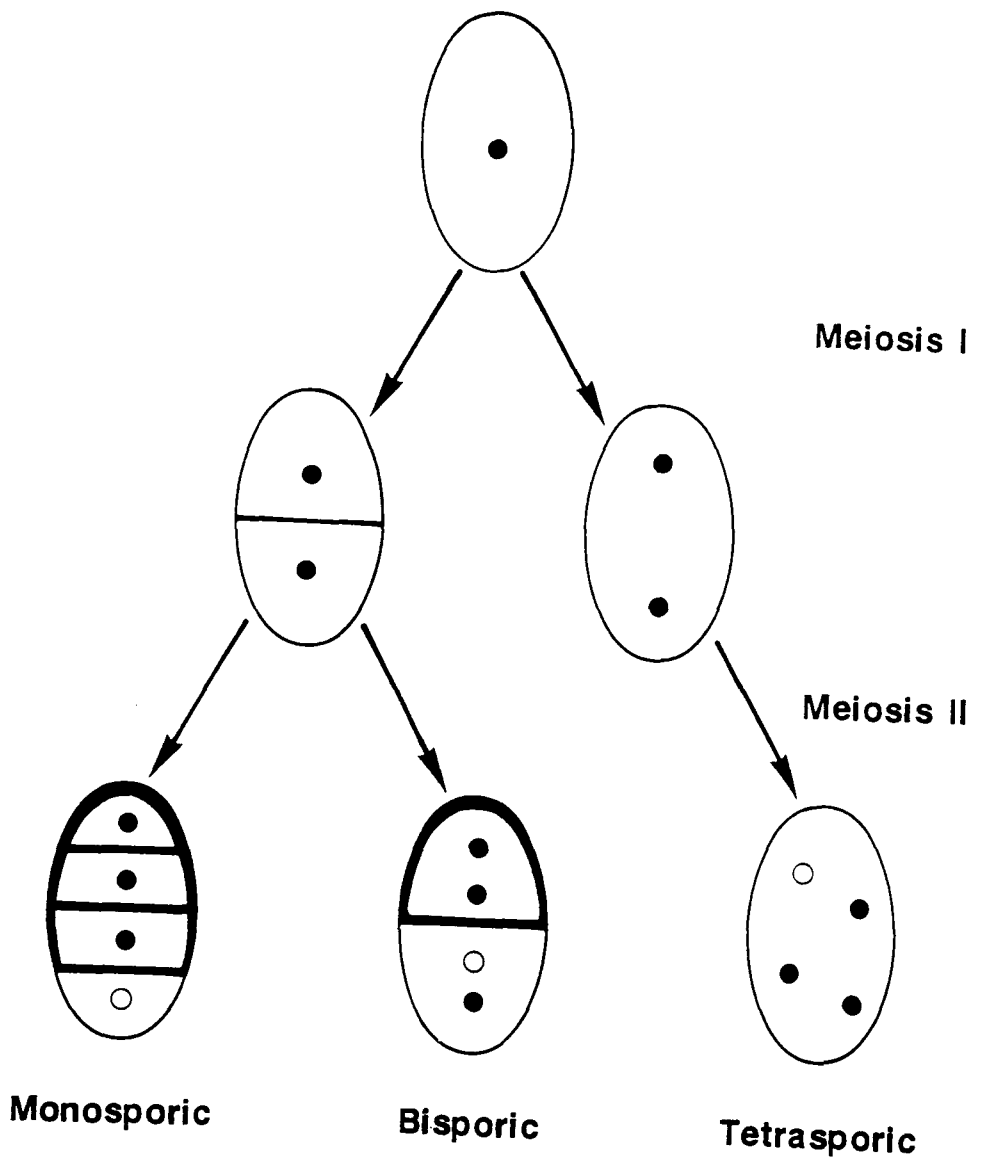
types are derived from the Polygonum type. However, in families that lack the Polygonum type the situation is less clear, as the family might have a non-Polygonum ancestor from which families with the Polygonum type are also derived. In either case, the variant algorithms should be related to, but not necessarily derived from, the Polygonum type.

Embryo sacs are usually classified as either monosporic, bisporic or tetrasporic. In monosporic development, cytokinesis follows both meiotic divisions of the megaspore mother cell. Thus, the four megaspore nuclei lie in individual cells. Three cells degenerate and the female gametophyte is organised from the mitotic products of the fourth cell. In bisporic development, cytokinesis follows the first but not the second meiotic division. Therefore, the tetrad consists of two cells each containing two megaspore nuclei. The embryo sac is organized from the mitotic products of one of these cells. In tetrasporic development neither division is followed by cytokinesis and the embryo sac may include derivatives of all four megaspore nuclei (Figure 3).

Monosporic, bisporic and tetrasporic development have been defined in two different ways (Nagendran 1974). The definition used in this review, classifies development by the number of megaspore nuclei present in the initial cell of the embryo sac. This is determined by the presence or absence of cell walls after meiosis. The alternative definition classifies gametophytes by the number of spores that contribute nuclei to the mature embryo sac. The definitions are not equivalent because a spore may be included in the initial cell of an embryo sac but degenerate without further division (see Section III; C).

Bisporic and tetrasporic development result from the failure of cytokinesis after one or both of the meiotic divisions. The simple failure of cytokinesis may result in an embryo sac of greatly altered final appearance (cf. the  $ms_1$  allele in soybeans). An algorithmic model of development allows one to observe actual bisporic and tetrasporic embryo sacs and ask which of their unusual features are direct consequences of the failure of cytokinesis, and which are consequences of additional developmental changes. Although the algorithm analogy may be new,

**Figure 10.3.** During meiosis the megaspore mother cell nucleus divides twice. If cytokinesis fails after meiosis II, development is bisporic. If cytokinesis fails after both divisions, development is tetrasporic. Cells in heavy outline usually degenerate. The micropylar end is uppermost.



this approach is implicit in earlier reviews of embryo sac development. My treatment departs from previous reviews in accepting that different megaspores and their derivatives may have different genetic interests, and that this may explain some features of embryo sac organization.

Bisporic and tetrasporic embryo sacs are chimeras, composed of more than one genetic individual and, as such, are ambiguous structures. One could consider the embryo sac to be homologous to a single monosporic gametophyte, or one could consider each genetic individual within the embryo sac to be the homologue of a monosporic gametophyte. Which perspective is more helpful will depend on the degree of integration among the individuals within an embryo sac. The first perspective may be preferable if these individuals express different parts of the developmental algorithm and cooperate for the benefit of a single egg. The second perspective is preferable if the genetic interests of individuals conflict and gene expression in one nucleus of the embryo sac conflicts with gene expression in other nuclei. For these reasons, I use the neutral term *embryo sac* to refer to the cell that is formed after meiosis as well as its subsequent derivatives, and restrict the term *gametophyte* to monosporic embryo sacs.

The different genetic individuals in non-monosporic embryo sacs are each derived from a megaspore nucleus. If only one megaspore nucleus gives rise to a functional egg, a distinction can be made between germinal and somatic megaspore nuclei. The germinal megaspore nucleus is usually the nucleus closest to the micropyle.

Developmental algorithms can be classified as monosporic, bisporic or tetrasporic depending on whether cytokinesis occurs after meiosis I and II. Algorithms can also be classified on the basis of the number of free nuclear divisions that intervene between meiosis and cell formation. Algorithms with three mitotic divisions (e.g., the *Polygonum* type) will be referred to as three-phasic algorithms. Algorithms with only two mitotic divisions will be referred to as two-phasic algorithms. A small minority of angiosperms have one-phasic algorithms. This terminology follows that of Fagerlind (1944). Female gametophytes

of angiosperms have many fewer free nuclear divisions than the female gametophytes of other seed plants. Battaglia (1951) has suggested that the angiosperm gametophyte evolved by a gradual reduction in the number of free nuclear mitoses. The formation of two-phasic and one-phasic algorithms would be a continuation of this process.

#### B. THE ALLELE AS UNIT OF SELECTION

The central premise of the kin-conflict interpretation is that, for a character to evolve by natural selection, the character must promote the replication and transmission of those alleles that determine the character. Thus, the allele rather than the individual is seen as the unit of natural selection. This is not simple reductionism because "the allele" must be understood in the collective sense of all copies of the same gene. The qualification is important because an allele can increase in numbers even if its phenotypic effect is to reduce the reproductive success of some of its bearers. The necessary condition is that the allele causes a greater increase in the reproductive success of other bearers.

In practice, one does not need to know the fate of all copies of an allele to predict whether the allele will increase or decrease in numbers. This is because gene expression in one bearer usually only affects the reproductive success of a subset of all bearers. Therefore, natural selection can be predicted from the fate of the allele within the smaller group of interacting individuals. In many cases, the "group" is the individual and natural selection can be described as acting on individuals. In other cases, the interacting unit is a group of relatives. Such cases are usually described in terms of kin selection. Of course any prediction must allow for variance in the allele's fate among groups.

Genes expressed in female gametophytes can plausibly affect the reproductive success of current or future giblings (male and female gametophytes with the same sporophyte parent) and the embryos produced by the female gametophytes. Effects on the reproductive success of other individuals should be weak or non-existent. Within gibships, gametophytes may interact directly or

by making competing demands on the maternal sporophyte.

Therefore, the appropriate group for studying natural selection appears to be a sporophyte and its gametophyte progeny. Such a family possesses either one or two alleles at a locus, depending on whether the sporophyte is homozygous or heterozygous at that locus. Because gametophytes are haploid, the phenotypic effects of alleles expressed in gametophytes are not complicated by dominance relationships. An allele that is expressed in female gametophytes can increase its numbers in one of two ways.

(1) *Fecundity selection*: The allele improves the viability of the gametophyte in which it is expressed. The effect is to increase the total number of successful offspring produced by the sporophyte. Such adaptations are predicted under both the traditional and kin-conflict interpretations.

(2) *Gametic drive*: The allele promotes its own inclusion in successful offspring at the expense of alternative alleles in heterozygous sporophytes. Gametic drive includes all situations where one of the alleles at a heterozygous locus receives a disproportionate share of the sporophyte's reproductive resources. An ovule usually produces a single egg. Thus, only one of the two alleles at a heterozygous locus can be transmitted via each ovule. Gametic drive may be expressed as competition among individuals within ovules to produce this egg, or as competition for maternal resources among individuals in different ovules. In this context, an "individual" corresponds to the distinct haploid genotype that originates in a spore. Such "adaptations" need not increase the reproductive success of a sporophyte.

The distinction between fecundity selection and gametic drive can be illustrated by considering the necessary conditions for a mutant allele to invade a population fixed for another allele. A mutant allele is initially rare and found only in heterozygous sporophytes and their gametophyte offspring. On the other hand, an established allele occurs predominantly in homozygous sporophytes and their progeny. Thus, the criterion for a new allele to invade a population is that the number of offspring

carrying the allele in heterozygous families should be greater than half the number of offspring produced by sporophytes without the allele. Fecundity selection corresponds to the simple case where heterozygotes produce more offspring than normal homozygotes. Gametic drive includes all other cases where a mutant is able to invade because it is represented in more than half of a heterozygote's offspring. Gametic drive is mathematically analogous to meiotic drive (e.g., Wright 1969) but I use a different term to emphasize that the allele may have its effect after meiosis.

So far, I have only considered the relative fitness of a gametic drive mutant when it is rare. The full genetic model is complex because, as a mutant becomes common, some sporophytes will be homozygous for the mutant. The commoner the mutant becomes, the greater the proportion of its copies in homozygous sporophytes. Therefore, fitnesses are frequency dependent because selection differs in heterozygous and homozygous families. Models of meiotic drive predict that an allele's equilibrium frequency will depend on mating system, the relative fitnesses of the homozygous and heterozygous genotypes, and the departure from equal division of resources in heterozygotes (see Wright 1969). For some values of these parameters, a gametic drive allele will eliminate the established allele. In this case, the character determined by the allele will have evolved by gametic drive, but gametic drive will not be detected because the population consists solely of homozygotes.

In this review, I will not be concerned with the details of formal genetic models. Most established patterns of development must be evolutionarily stable to most commonly occurring mutants. If a character is fixed within a population, it is probable that the alleles determining the character became established because they satisfied the invasion criterion and the other conditions necessary for fixation. A major aim of this review is to identify characters that are most easily understood as the product of gametic drive. My approach will be comparative rather than rigorously genetic. Thus, if a derived character often occurs in *Drusa*-type embryo sacs but rarely occurs in *Polygonum*-type gametophytes, one can ask why the *Polygonum* algorithm is stable



with respect to this character whereas the Drusa algorithm is not.

Haploid genetic individuals originate from spores. The extent to which two individuals have interests in common will be determined by the probability that their spores share the same allele. Genetically, there are three types of relationships between the spores produced by a single sporophyte: the spores can be derived from different spore mother cells; the spores can be derived from different products of the first meiotic division; or the spores can be sister products of the second meiotic division. The probability of shared alleles differs for these three relationships (<sup>see</sup> ~~Figure 3~~; Haig 1986).

### C. STRIKE, SABOTAGE AND ANTIPODAL EGGS

At cell formation an egg apparatus is organized from a micropylar quartet of nuclei. This quartet is produced by two free nuclear divisions of the primary micropylar nucleus. In three-phasic development, the primary micropylar nucleus is a product of the first free nuclear mitosis. In two-phasic development, it is the germinal megaspore nucleus. Chalazal nuclei usually divide at the same time as micropylar nuclei but there are often fewer than two full division cycles. A reduced number of divisions of chalazal nuclei is referred to as "strike".

Most bisporic and tetrasporic embryo sacs are two-phasic. Therefore, the primary micropylar nucleus is a germinal megaspore nucleus and chalazal nuclei are derivatives of somatic megaspore nuclei. Strike appears to be particularly common in such embryo sacs where chalazal nuclei differ genetically from micropylar nuclei. Admittedly, this is a subjective impression and adequate data do not exist for a quantitative analysis. In extreme cases of strike, the somatic megaspore nuclei degenerate without further division. As a result, every functional nucleus is a derivative of a single spore. Harling (1950) described such embryo sacs as pseudomonosporic.

If the association between strike and non-monosporic development is real, two types of explanation can be suggested.

1. Sabotage:-- This was the explanation of strike suggested in a previous article (Haig 1986). I proposed that embryo sacs compete

to avoid abortion, and that functional antipodal cells promote an embryo sac's chances of being provisioned. Therefore, if an allele prevented normal antipodal function, the allele could invade if other embryo sacs (with the allele in their micropylar nuclei) benefited from the reduced competitiveness of those embryo sacs in which the allele was expressed (in their chalazal nuclei). This hypothesis is weakened because antipodal cells in many monosporic taxa degenerate before they could have a significant nutritive function (Willemse & van Went 1984). I now believe that suppression of chalazal nuclei is a more important factor in explaining strike.

2. Suppression:-- Chalazal nuclei may be suppressed, either by the micropylar spore and its derivatives or by the maternal sporophyte. Suppression of chalazal nuclei would be adaptive for micropylar nuclei if chalazal nuclei were involved in "sabotage" or if chalazal nuclei formed "antipodal eggs" that competed with the micropylar egg for fertilization. The simplest mechanism of suppression would be to starve chalazal nuclei by isolating them from resources necessary for division.

"Sabotage" may appear far-fetched, but I present it as a theoretical possibility. "Antipodal eggs" are reported in the literature. Rutishauser (1969) has remarked on an apparent association between antipodal eggs and embryo sacs with bisporic or tetrasporic development. The kin-conflict interpretation offers a causal explanation for this association. Suppose that a mutant allele expressed in the chalazal quartet causes the quartet to develop an egg apparatus ( $D_1$ ) in the place of antipodal cells ( $D_3$ ). Such a mutant allele might be able to invade if chalazal eggs are occasionally fertilized in the place of micropylar eggs carrying the alternative allele. There would be no such selective advantage for a similar allele expressed in the chalazal quartet of a monosporic gametophyte.

The degree of strike in non-monosporic embryo sacs is often variable among the gametophyte progeny of a single sporophyte. In addition, chalazal nuclei often show abortive or irregular divisions. These observations are compatible with chalazal nuclei attempting to express a more complete developmental program, but being suppressed. In most taxa with strike there is no evidence

that antipodal eggs are ever formed or fertilized. Therefore, if chalazal spores attempt to form antipodal eggs they are always suppressed. One possibility is that antipodal eggs were formed at some stage in the past and that suppression evolved in response.

These arguments can be expressed in more explicitly genetic terms. I will illustrate with an extended example. Bisporic embryo sacs contain derivatives of two megaspore nuclei. In the monosporic ancestors of bisporic species, the antipodal subroutine ( $D_3$ ) was expressed in the derivatives of germinal spores. Presumably the antipodal cells contributed to the reproductive success of their gametophyte's egg, and this pattern of gene expression was evolutionarily stable to invasion by alternative alleles. However, once development became bisporic, these alleles would be expressed in the derivatives of a somatic spore for the benefit of the germinal spore in the embryo sac. Whether this pattern of gene expression would still be evolutionarily stable should depend on the probability that the somatic and germinal spore carry the same allele.

The two spores in a bisporic embryo sac are usually sisters of the second meiotic division. Therefore, at any given locus, their genes will have been attached to the same centromere at anaphase I. If the recombination frequency between a locus and its centromere is  $r$ , the probability that genes in the two spores are identical by descent from the megaspore mother cell will be  $(1 - 2r)$ . This probability can range between 0 and 1, because recombination frequencies can lie between 0 and 0.5 (see Haig 1986, where I use a parameter  $c$  that is equivalent to  $2r$ ).

Suppose that  $r = 0$ . For such a locus, the germinal and somatic spores will always carry the same allele. If gene expression was evolutionarily stable in a monosporic gametophyte, it will also be stable in a bisporic embryo sac. On the other hand, suppose that  $r = 0.5$ . For this locus, a gene expressed in antipodal nuclei will only be expressed in those embryo sacs in which the germinal spore carries a copy of the other maternal gene. Most loci will lie somewhere between these extremes. Thus, whether an allele is able to invade may depend on the recombination frequency between its locus and the centromere. By corollary, different loci in the same nucleus may be subject to

divergent natural selection.

I will consider the invasion criteria for a "sabotage" allele expressed in the chalazal spore and its derivatives. Suppose that the mutant allele interferes with normal antipodal function. The allele will initially be expressed in 50% of the embryo sacs produced by heterozygous sporophytes, and should reduce the reproductive success of the egg in those embryo sacs in which it is expressed. Clearly, if the somatic and germinal spore usually carry the same allele, the mutant cannot invade. However, if somatic and germinal spores usually carry different alleles, the mutant might be able to invade. This would be the case if other embryo sacs on the same sporophyte benefit from the reduced nutrient demands of embryo sacs in which the mutant is expressed. Thus, the embryo sacs that would benefit from "sabotage" would usually carry the mutant allele in their germinal spore.

#### IV. Monosporic Development

Most monosporic gametophytes are assigned to the *Polygonum* type, but this single class conceals considerable diversity. Development at the chalazal pole is particularly variable. Antipodal cells may be ephemeral or may persist until after fertilization. In some species, there are fewer than three antipodal cells because of division failures, nuclear fusions or because more than one nucleus is included in a cell. Other species show secondary proliferation of antipodal cells, or a failure of cell formation so that the antipodal nuclei remain free in the chalazal end of the embryo sac (for a review of the *Polygonum* type see Maheshwari 1950).

The major variant form of monosporic development is the two-phasic *Oenothera* type. After meiosis, the functional megaspore undergoes two mitotic divisions to form a micropylar quartet. The quartet organises an egg apparatus and a single polar nucleus (Fig. 4b). The *Oenothera* type could have arisen from a *Polygonum* algorithm by the loss of a single free nuclear mitosis. The type is restricted to the *Onagraceae* where it has been found in all members investigated. Therefore, its derivation from a *Polygonum*-type ancestor must remain speculative. By the definitions used in

this review, monosporic two-phasic development also occurs in the Limnocharitaceae, but this family will be discussed under bisporic development (Section V; B) because this is the usual interpretation of its development.

All nuclei of a monosporic gametophyte possess the same set of alleles. Thus, each gametophyte corresponds to a genetic individual and kin-conflict will be expressed between gametophytes but not within gametophytes. The kin-conflict interpretation predicts that all four members of a megaspore tetrad should attempt to form a functional gametophyte. However, in most monosporic angiosperms, only one megaspore (the germinal spore) organises an embryo sac, and the other three (somatic) spores degenerate. This is compatible with all spores attempting to form a functional gametophyte if the degeneration of somatic spores is imposed by the maternal sporophyte, rather than by gene expression in the somatic spores themselves. Haig (1986) interpreted the callose wall that surrounds the tetrad as the mechanism by which the sporophyte aborts somatic spores. Callose deposition is usually heavier around the degenerating megaspores.

Of particular interest are species where all four members of a tetrad continue development without suppression. In such cases the megaspores often form haustoria (Rutishauser 1969), presumably to compete for privileged access to nutrients or pollen tubes. Rutishauser (1969) gives *Potentilla heptaphylla*, *Sedum sempervivum* and *Rosularia pallida* as examples. In *Sedum chrysanthum* (= *R. pallida*) several megaspores, from more than one tetrad, form haustoria that penetrate the nucellus. One megaspore is able to grow in advance of the others and forms a vesicle at the base of the micropyle. This megaspore then develops into the functional gametophyte (Subramanyam 1967).

The Loranthaceae provide a related example. Members of this family do not produce ovules. A mature fruit contains a single embryo, but an ovary may contain several gametophytes. Therefore, these gametophytes compete to form the successful embryo. The gametophytes form micropylar extensions containing the egg apparatus that grow up the style to meet pollen tubes growing down. After fertilization the zygote grows back down the style and enters the developing endosperm (Bhatnagar & Johri 1983).

This extraordinary behavior is explicable in terms of gametic drive. Consider a hypothetical mistletoe in which female gametophytes remained in the ovary and waited for fertilization. A new allele that caused its bearers to grow to a higher position in the style would on average be fertilized more often and earlier. This would be a great advantage if pollen is sometimes limiting or if early fertilization is important in competition among embryos. The pattern of development found in the Loranthaceae would be the result of many such mutations at many loci. The prothallial tubes of the chlamydosperm *Welwitschia mirabilis* show similar behavior to the micropylar extensions of the Loranthaceae, probably because of convergent evolution due to similar gametic drive (Haig 1987).

#### V. Bisporic Development

In bisporic development, cytokinesis is absent after meiosis II. The embryo sac is initiated from a binucleate cell (dyad) that contains two megaspore nuclei. The dyad nuclei divide twice to form eight nuclei, and the mature embryo sac is organized in the same manner as the Polygonum type (Fig. 4d). Most commonly, the embryo sac develops from the chalazal dyad, in which case the embryo sac is assigned to the Allium type. If the embryo sac develops from the micropylar dyad, it is referred to the Endymion type. As I have argued for the somatic spores in monosporic development, I believe the failure of one or other dyad to develop is due to suppression by the maternal sporophyte, rather than to the effect of genes expressed in the degenerating dyad. Therefore, I do not place great significance on the distinction between Allium and Endymion types.

The major secondary sources for the occurrence of bisporic embryo sacs are Maheshwari (1955) and Davis (1966). Palser (1975) collated data from Davis and more recent sources. She found that the Allium type had been reported from 45 families and was the most common non-Polygonum type. In most of these families, the Polygonum type also occurred, suggesting that bisporic development must have evolved in several independent lineages. Maheshwari's review attempted to eliminate doubtful cases. He found bisporic development to be "an established feature" in

representatives of 30 families, but characteristic of only the "Podostemaceae, Butomaceae (except *Butomus*), Alismaceae [sic], and the sub-family Viscoideae of the Loranthaceae." In those families where bisporic development coexists with monosporic development it seems reasonable to conclude that bisporic development is the derived condition. However, in those families that lack close monosporic relatives, the derivation of bisporic development from a *Polygonum* type ancestor must be less certain. In the classification used in this review, bisporic development is characteristic of three families: Viscaceae, Alismataceae and Podostemaceae. Development in the Limnocharitaceae is usually considered to be bisporic, though I classify it as monosporic. These four families will be considered later in this section.

Bisporic development usually results from the failure of wall formation after meiosis II. In some cases an ephemeral wall is formed but in other cases a wall is entirely absent (Rutishauser 1969). If this were the only change to the *Polygonum* algorithm, one would expect bisporic embryo sacs to be 16-nucleate. However, the *Allium* type has only eight nuclei because one free nuclear mitosis has been eliminated. Thus, its algorithm is two-phasic and resembles the *Oenothera* type, except that cytokinesis is absent after meiosis II.

This creates a problem because bisporic development has several independent origins but the loss of cytokinesis is (almost) always accompanied by the loss of a free nuclear mitosis. Two hypotheses can be suggested for the derivation of a two-phasic bisporic (*Allium*) algorithm from a three-phasic monosporic (*Polygonum*) algorithm. One hypothesis is that bisporic embryo sacs are initially three-phasic and 16-nucleate, but there are strong selective pressures to reduce this number of nuclei. The hypothesis is weakened by the lack of any reports of bisporic embryo sacs with three-phasic development.

Bisporic embryo sacs with more than eight nuclei may have been classified as tetrasporic because every embryologist "knows" that bisporic embryo sacs have only eight nuclei. In the Hamang population of *Polygonatum multiflorum*, Björnstad (1970) found that 62% of tetrads formed cell walls after both meiotic divisions, 27% of tetrads formed cell walls after the first

division only, and 11% formed cell walls after neither division. At a later stage of development 27% of embryo sacs had more than eight nuclei. Björnstad assumed that all monosporic embryo sacs were three-phasic but all non-monosporic embryo sacs were two-phasic. Therefore, she believed that embryo sacs with more than eight nuclei had to be tetrasporic. A hypothesis that is simpler, and more consistent with her data, is that all embryo sacs were three-phasic, and that embryo sacs with more than eight nuclei could have been either bisporic or tetrasporic. The Hamang population would then have a *Polygonum*-like algorithm, but with erratic wall-formation after meiosis. Other populations of the species had almost exclusively monosporic development. Thus, Björnstad's study could be considered to provide evidence for a transitory three-phasic, bisporic intermediate in the evolution of the *Allium* type.

The alternative hypothesis is that the loss of cytokinesis after meiosis II and the reduction to two post-meiotic divisions are caused by the same mutation. This could be achieved if the developmental algorithm "skips" meiosis II and its associated cytokinesis. Thus, the first free nuclear division would substitute for meiosis II and there would be only two mitotic divisions. Chromosome behavior is essentially similar in meiosis II and mitosis, and the same set of genes appear to control both types of division (Grallert & Sipiczki 1989). Therefore, such a mutation is not completely implausible. In this scenario, mutations causing three-phasic bisporic development could also arise (as in *Polygonatum multiflorum*), but such mutations would be eliminated by natural selection.

#### A. VISCACEAE

Development in the Viscaceae is bisporic. The two megaspore nuclei of a dyad undergo two free nuclear divisions to produce an 8-nucleate embryo sac. As in the Loranthaceae, the Viscaceae lack ovules. Each ovary usually contains two or more embryo sacs but only one produces an embryo in the mature "seed" (Bhandari & Vohra 1983). The Viscaceae lack a chalaza and micropyle. Therefore, I will use "upper" to refer to the end of the embryo sac closest to the stigma. At the 4-nucleate stage in *Korthasella*



*dacrydii* and some other species, the lower end of the embryo sac bends upward and grows within the ovary wall until it assumes a higher position than the original upper end of the embryo sac. After the final mitotic division, the egg apparatus organizes in the new upper end (Maheshwari 1955; Rutishauser 1935). It is tempting to see this reversal of polarity as evidence of kin-conflict. However, similar behavior is reported in monosporic members of the Balanophoraceae (Arekal & Shivamurthy 1978). The family Viscaceae is thought to have its closest affinities with either the Loranthaceae or the Santalaceae, both families with members possessing Polygonum type algorithms (Bhandari & Vohra 1983).

#### B. ALISMATACEAE AND LIMNOCHARITACEAE

Dahlgren et al. (1985) classify the Alismataceae and Limnocharitaceae as closely related families in the order Alismatales. Maheshwari (1955) considered bisporic development to be characteristic of both families. The Alismatales also contains the Aponogetonaceae, Hydrocharitaceae and Butomaceae: all families with Polygonum type development (Davis 1966; Roper 1952).

In the Alismataceae, cytokinesis is absent after meiosis II and the embryo sac develops from the chalazal dyad. The megaspore nucleus at the micropylar end of the chalazal dyad divides twice to produce a micropylar quartet. After cell formation this quartet contributes an egg apparatus and a polar nucleus to the embryo sac. The other megaspore nucleus usually divides only once. One of the resulting nuclei appears to function as a polar nucleus. The other nucleus either remains within the central cell or becomes separated by a delicate cell wall. This nucleus has a degenerate appearance in several illustrations. Thus, the mature embryo sac contains six nuclei (Fig. 4e). On rare occasions, one or both of the chalazal nuclei divide to produce a 7- or 8-nucleate embryo sac (Dahlgren 1928, 1934; Johri 1935a, 1935b, 1935c, 1936b; Maheshwari & Singh 1943).

In the Limnocharitaceae, cytokinesis follows meiosis II and the two megaspore nuclei of the chalazal dyad are separated by a delicate membrane. The megaspore nucleus closest to the chalaza

usually degenerates soon after meiosis but persists as a dark-staining blob. The remaining megaspore nucleus of the chalazal dyad divides twice to form a micropylar quartet. After cell formation, the "embryo sac" contains an egg apparatus and polar nucleus, all derived from a single megaspore, and a chalazal cell containing a degenerated megaspore nucleus (Fig. 4c). On rare occasions, the chalazal nucleus may divide once or twice so that the "embryo sac" has more than five nuclei. These additional nuclei always remain separated from the rest of the embryo sac by the "delicate membrane" and never contribute a second polar nucleus (Johri 1936a, 1938a, 1938b).

Development in the Limnocharitaceae and the Alismataceae is often described as conforming to a reduced *Allium* type because embryo sacs are considered to be bisporic but both families show a reduced number of divisions at the chalazal end ("strike"). This description is probably satisfactory for the Alismataceae but is misleading for the Limnocharitaceae. In this family, cytokinesis follows meiosis II and only one spore contributes functional nuclei to the embryo sac. Such development should be described as monosporic or, at least, pseudomonosporic. In many respects the algorithm resembles that observed in *Oenothera* (Roper 1952). The occasional divisions of the chalazal spore would then correspond to "attempts" by a suppressed spore to express the full developmental algorithm.

The developmental algorithms in the two families appear to be related. Therefore, it is possible that two-phasic bisporic development (as in the Alismataceae) was derived from two-phasic monosporic development (as in the Limnocharitaceae) by the failure of cytokinesis after meiosis II. The reverse sequence is also possible, but this would require the *de novo* origin of cytokinesis after meiosis II.

#### C. PODOSTEMACEAE

The Podostemaceae is a family of highly specialized aquatics. Corner (1976, p. 44) suggests that the family has affinities with the Piperaceae, whereas Herr (1984) argues that the family is derived from the Crassulaceae. Dahlgren (1980) places the family in its own superorder with no obvious affinities to other

superorders. The Podostemaceae lack double fertilization and do not form endosperm, among other unusual features. All members of the Podostemaceae lack cytokinesis after meiosis II and are thus classified as bisporic. Development may be two-phasic or one-phasic (Battaglia 1971). Two-phasic development is more common and presumably ancestral to one-phasic development.

Two-phasic development is called the Apinagia type. Embryo sacs develop from the chalazal dyad. After meiosis II, the two megaspore nuclei of the chalazal dyad become separated by a central vacuole. The chalazal megaspore nucleus rapidly degenerates and is absent from the mature embryo sac or persists as a pycnotic blob. The remaining megaspore nucleus divides twice, followed by cell formation. Three cells form an egg apparatus. The fourth cell occupies the chalazal end of the embryo sac and degenerates prior to or during fertilization (Battaglia 1971; Fig. 4f). Battaglia (1971) believed that this cell was an antipodal cell, on the basis of its early degeneration and the absence of fertilization by the second male nucleus. Nagendran, Arekal & Subramanyam (1977) identified the nucleus as a polar nucleus because it is the sister of the egg nucleus.

One-phasic development is known from four species of *Polypleurum* and *Hydrobryopsis sessilis* (Arekal & Nagendran 1976; Mukkada 1964; Nagendran et al. 1977). The two nuclei of the chalazal dyad do not become separated by a central vacuole. Both megaspore nuclei divide once and the mature embryo sac contains four cells (Fig. 4n). Two interpretations of the embryo sac have been proposed: (1) the chalazal megaspore nucleus gives rise to two antipodal cells and the other megaspore nucleus forms an egg and one synergid (Battaglia 1971; Mukkada 1964); (2) one megaspore nucleus (usually the chalazal nucleus) forms two synergids and the other megaspore nucleus forms an egg and polar nucleus (Nagendran et al. 1977).

A third interpretation is possible if the different genetic individuals within the embryo sac are viewed separately. Each megaspore nucleus produces a two-celled unit consisting of an egg and synergid, and there are two such units in the embryo sac. From this perspective a number of observations can be made (all

figs. are in the references cited). The configuration of the units with respect to each other is variable. The units are usually arranged at right angles to each other, however, either the chalazal or the micropylar unit can be aligned with the long axis of the ovule (e.g., Arekal & Nagendran 1976, figs. 2b, 2e). In other ovules, the units may form a row of four cells (Arekal & Nagendran 1976, fig. 2f) or the units may be obliquely placed (Mukkada 1964, fig. 12; Nagendran et al. 1977, fig. 3f). One of the megaspore nuclei may fail to divide (Mukkada, fig. 11) or the nucleus may divide without cytokinesis. Binucleate cells have been observed in both the micropylar and the chalazal position (Arekal & Nagendran 1976, fig. 2c; Nagendran et al. 1977, figs. 3g-i). Taken together these observations suggest that the two units may be basically autonomous.

In summary, I propose that embryo sacs with one-phasic development can be interpreted as containing two two-celled gametophytes each consisting of an egg and one synergid. Ultrastructural studies should decide among the different interpretations.

## VI. Tetrasporic Development

Tetrasporic development is defined by the absence of cytokinesis after both meiotic divisions. Thus, all four megaspores lie within a single cell, the coenomegaspore. Fagerlind (1944) reviewed the various types of tetrasporic embryo sacs. He classified embryo sacs as three-phasic, two-phasic or one-phasic depending on whether there were three, two or one divisions of the micropylar megaspore nucleus. He further classified gametophytes by the configuration of nuclei within the coenomegaspore and by the presence or absence of nuclear fusions in the coenomegaspore.

The configuration and behavior of megaspore nuclei is of prime importance to the final organization of the embryo sac. This is because megaspore fusions will affect the number of nuclei in the mature embryo sac, and because the configuration of megaspore nuclei will influence the number of micropylar and chalazal quartets. In monosporic lineages, megaspore nuclei are not subject to selection on their behavior in a coenomegaspore.

Therefore, if tetrasporic development originates from a mutation in a monosporic algorithm, the initial configuration and behavior of megaspore nuclei is likely to be determined by idiosyncratic features of the lineage such as the shape of the megaspore mother cell. Some patterns of behavior would be compatible with the formation of a viable gametophyte whereas others would not. For example, the micropylar megaspore must remain unfused if the egg is to be haploid. If the initial configuration of megaspore nuclei was such that micropylar fusions were common, tetrasporic development would be unlikely to become established. Thus, some monosporic lineages may be more likely than others to give rise to tetrasporic descendants.

Once tetrasporic development becomes established, natural selection should stabilize favorable patterns of megaspore behavior. Three patterns are potentially viable: (1) the four megaspore nuclei remain unfused; (2) the three chalazal nuclei fuse so that the "tetrad" consists of a haploid nucleus at the micropylar pole and a triploid nucleus at the chalazal pole; (3) two megaspore nuclei fuse and two remain unfused. These three possibilities will be encountered in the following discussion. Three-phasic, two-phasic and one-phasic development will be considered in turn. Two-phasic development will be considered under three subsections: two-poled embryo sacs, four-poled embryo sacs, and Piperaceae. One-phasic development will be considered under two subsections: the Adoxa type, and Plumbaginaceae.

#### A. THREE-PHASIC ALGORITHMS

Three-phasic development is known from only one tetrasporic species. In *Chrysanthemum balsamita*, all four megaspore nuclei share a common cytoplasm, but the three nuclei closest to the chalaza degenerate without further division. The remaining megaspore nucleus divides three times to produce an eight-nucleate gametophyte that organizes an egg apparatus, two polar nuclei and three antipodal cells (Fagerlind 1939a; Harling 1951a). Development resembles the Polygonum type, except that the three degenerating megaspore nuclei occur in the same cell as the germinal megaspore nucleus. The suppression of somatic megaspore nuclei is probably enforced by a different mechanism than occurs

in the *Polygonum* type.

## B. TWO-PHASIC ALGORITHMS

### 1. Two-poled embryo sacs

As a rule in two-poled, two-phasic, tetrasporic development, a single megaspore nucleus occurs at the micropylar pole of the embryo sac, and the other three megaspore nuclei are located towards the chalazal pole. The micropylar megaspore nucleus divides twice to produce two synergids, an egg and a polar nucleus. The behavior of chalazal megaspore nuclei is variable. Two kinds of behavior are relatively common. In the *Drusa* type, the three chalazal nuclei remain unfused, whereas in the *Fritillaria* type, the nuclei fuse to form a triploid chalazal nucleus. The third possibility, fusion of only two nuclei, is less common and referred to as the *Chrysanthemum cinerariaefolium* type.

In the idealized form of the *Drusa* type, all spore nuclei divide twice to produce a 16-nucleate gametophyte. The 12 chalazal nuclei then organize 11 antipodals and a single polar nucleus (Fig. 4g). In monosporic (and bisporic) development, the chalazal quartet organizes three cells and contributes a polar nucleus to the central cell. Therefore, one might expect the three chalazal quartets of the *Drusa* type to produce nine antipodal cells and three polar nuclei. The expectation does not necessarily follow because the "polar nucleus" of one quartet might become included in the antipodal cell of another quartet resulting in a binucleate antipodal cell. This would depend on the geometric arrangement of cell plates during cell formation. These speculations are largely academic because few gametophytes actually produce three chalazal quartets. The *Drusa* type has been described from members of at least seven families, including the *Convallariaceae*, *Apiaceae*, *Ulmaceae* and *Asteraceae* (Davis 1966).

The occurrence of the *Fritillaria* type has been reviewed by Maheshwari (1946a). The type is relatively common in the *Piperaceae*, *Tamaricaceae*, *Limoniaceae* and *Liliaceae* but has only an occasional occurrence in other families. The *Fritillaria* type is defined by the fusion of the three chalazal megaspore nuclei. The nuclei usually remain unfused until the first free nuclear

division when their spindles unite to form a large common spindle. After this division, the embryo sac contains two haploid (micropylar) nuclei and two triploid (chalazal) nuclei. In some taxa, the megaspore nuclei fuse before the first mitotic division resulting in a secondarily two-nucleate stage. The two mitotic divisions of the micropylar megaspore nucleus produce four nuclei that organize a three-celled egg apparatus and a polar nucleus. If the chalazal nuclei also divided twice, the mature embryo sac would be eight-nucleate (Fig. 4h). In reality, however, the number of chalazal divisions varies because of "strike". In some representatives of the *Fritillaria* type, all triploid nuclei degenerate and the only functional nuclei of the mature embryo sac are derivatives of the micropylar megaspore nucleus. Such pseudomonosporic embryo sacs have been described in the *Liliaceae* (*Tulipa maximoviczii*: Romanov 1939; Fig. 4i), *Berberidaceae* (*Caulophyllum robustum*: Mauritzon 1936) and *Limoniaceae* (*Armeria bupleuroides*, *Statice sinuata*: Fagerlind 1938, 1939b).

I will consider two-poled, two-phasic, tetrasporic development in the *Ulmaceae*, *Asteraceae*, *Tamaricaceae*, *Limnanthaceae* and *Uvulariaceae*. Embryological studies of *Ulmus* and *Tamarix* have concluded that one-phasic development and two-phasic development coexist among the gametophyte progeny of individual sporophytes (Hjelmqvist & Grazi 1965; Walker 1950). I find the evidence for one-phasic development unconvincing (Section VI; C.1) and will only describe the accepted course of two-phasic development. The purpose of considering such a large number of examples is to show not only the similarities between the conventionally recognized types but also variation within these types.

In *Ulmus* (*Ulmaceae*), the four megaspore nuclei adopt a (1+3) configuration, but the three chalazal nuclei do not fuse. Therefore, development conforms to the *Drusa* type. The micropylar spore divides twice to produce an egg, two synergids and a polar nucleus. Gametophytes with 16 nuclei are rare because of chalazal "strike". The number of antipodal nuclei may vary among the gametophyte progeny of a single sporophyte, as may the number of polar nuclei. Walker (1950) figures gametophytes with three nuclei in the central cell (her figs. 15, 22) as well as

gametophytes with only two central cell nuclei.

Of particular interest are reports of antipodal eggs and embryos in *Ulmus americana* (Shattuck 1905) and *Ulmus glabra* (Ekdahl 1941). Shattuck and Ekdahl both observed egg-like cells among the antipodal cells and recorded antipodal and micropylar embryos in the same gametophyte. Both authors figured gametophytes with two chalazal embryos and gametophytes with two micropylar embryos. Although Walker (1950) did not find any examples of polyembryony, she did observe gametophytes of *Ulmus pumila* with six or eight micropylar cells and only two chalazal cells. This suggests the possibility of more than one micropylar egg apparatus.

Harling (1950, 1951a, 1951b) studied gametophyte development in the Anthemideae and Astereae of the Asteraceae. Both subfamilies contained monosporic, bisporic and tetrasporic members. Most tetrasporic species conformed to the Drusa type, though there was considerable variation in development among these species. Cell walls between spores were either ephemeral or absent. Some gametophytes were 16-nucleate (e.g., *Chrysanthemum viscidohirtum*) but most had fewer than 16 nuclei because of chalazal "strike". The number of chalazal divisions was often variable within species. In extreme cases, such as *Anthemis altissima*, the chalazal spores degenerated without dividing so that the mature gametophyte contained four nuclei, all derived from the micropylar spore. In *Anthemis altissima*, the three chalazal nuclei sometimes appeared to fuse and this could be interpreted as evidence of the Fritillaria type. No cases of antipodal eggs were reported.

Development has a somewhat different course in *Chrysanthemum cinerariaefolium*. In some ovules, the two central nuclei of the tetrad fuse to form a diploid nucleus. If all nuclei divided twice, the gametophyte would be 12-nucleate, but this is rarely observed because of division strikes by the diploid nucleus or by the haploid chalazal nucleus. In other ovules, the two central nuclei do not fuse but lie side by side (Harling 1951a; Martinoli 1939). Harling (1951a) observed the occasional fusion of the two central megaspore nuclei in ovules of *C. vulgare* and the occasional fusion of the two megaspore nuclei nearest the chalaza



in ovules of *C. parthenium*. In most ovules of these species, the megaspore nuclei remain unfused. Fritillaria type development, with fusion and subsequent division of the three chalazal nuclei, is also found in the Asteraceae among members of *Rudbeckia* (Fagerlind 1939b; Maheshwari 1946a).

The occurrence of monosporic, bisporic and tetrasporic development within the Asteraceae raises the question of how the underlying algorithms are related. One pathway would be from three-phasic monosporic development to two-phasic bisporic development, followed by the loss of cytokinesis after meiosis I to give two-phasic tetrasporic development. As an alternative, a direct derivation of two-phasic tetrasporic development from three-phasic monosporic development would require the loss of cytokinesis after both meiotic divisions (the *ms<sub>1</sub>* mutant of soybeans has this effect), as well as the loss of a free nuclear mitosis. These effects could be achieved by a developmental "short-cut" that goes directly from the end of meiosis I (before cytokinesis) to the first free nuclear division, which substitutes for normal meiosis II. This hypothesis is admittedly *ad hoc*.

Development in the Tamaricaceae is also highly variable. In *Tamarix*, the three chalazal nuclei usually fuse and undergo two mitotic divisions to produce three triploid antipodals and a triploid polar nucleus (Fritillaria type) but there is considerable variation within species. In a minority of ovules the chalazal nuclei do not fuse, or two nuclei fuse but one remains unfused. The Fritillaria, Drusa and Chrysanthemum cinerariaefolium types have all been described from the one species (Hjelmqvist & Grazi 1965; Johri & Kak 1954).

Limnanthaceae and Uvulariaceae are characterized by chalazal strike. In *Limnanthes douglasii*, the chalazal dyad nucleus degenerates without dividing. After meiosis II, the embryo sac contains two megaspore nuclei and the degenerated dyad nucleus. The micropylar megaspore nucleus divides twice and organizes the egg apparatus and a polar nucleus. The other megaspore nucleus usually does not divide and occupies the position of a polar nucleus (Fig. 4j). There is considerable variation among embryo sacs because of occasional divisions of this nucleus (Fagerlind

1939a; Mathur 1956). In *Clintonia* (Uvulariaceae) four megaspore nuclei are formed but the three chalazal nuclei degenerate without fusion or subsequent division. Thus, the mature embryo sac contains an egg apparatus and a single polar nucleus, all derived from the micropylar megaspore nucleus (Björnstad 1970; Smith 1911; Fig. 4k).

This brief review of two-poled, two-phasic, tetrasporic development exposes some of the weaknesses of the type concept. The behavior of chalazal nuclei is often variable within species. Thus, in *Tamarix*, the *Fritillaria*, *Drusa* and *Chrysanthemum cinerariaefolium* types all occur among the gametophyte progeny of individual sporophytes. A more helpful viewpoint would be to recognize that *Tamarix* has a single developmental algorithm but that the behavior of chalazal spores is variable. Harling's (1950, 1951a, 1951b) descriptions of tetrasporic development in the Asteraceae also provide examples of highly variable behavior of chalazal nuclei among the gametophyte progeny of individual sporophytes. Such variable behavior does not appear to be a feature of monosporic development. This is explicable once it is realized that the chalazal nuclei of tetrasporic gametophytes are the derivatives of somatic megaspore nuclei. Two factors are probably at work. (1) The behavior of chalazal megaspore nuclei is initially variable because megaspore nuclei have not previously been subject to natural selection in a coenomegaspore. (2) Once tetrasporic development becomes established, there is only weak selection on chalazal nuclei to behave in a regular manner for the benefit of the germinal spore. In fact, there may be conflicting selective pressures. The derivatives of somatic megaspore nuclei may be selected to produce antipodal eggs, and the maternal sporophyte or the derivatives of germinal spores may be selected to suppress antipodal eggs.

In contrast to the variable behavior of chalazal megaspore nuclei, the behavior of the micropylar megaspore nucleus is remarkably uniform. In all embryo sacs discussed in this section the micropylar nucleus divides twice and produces a three-celled egg apparatus and a polar nucleus.

## 2. Four-poled embryo sacs

In four-poled, tetrasporic embryo sacs, the megaspore nuclei remain unfused. At the tetrad stage, the nuclei adopt a cross-shaped or tetrahedral configuration in which they are more evenly spaced than in the (1+3) arrangement of the Drusa type. After two mitotic divisions, each spore forms a peripheral group of three cells and contributes a polar nucleus to the central cell. As a consequence four peripheral groups, each derived from a single megaspore nucleus, can be easily distinguished. This type of development is classified as the Penaea type (Fig. 41). The peripheral groups usually resemble an egg apparatus, even when they occupy a non-micropylar position. In the Penaeaceae, the position of the groups is variable. "Indeed, when (as quite often occurs) two groups are equidistant from the apex of the sac or, as has been several times observed, three groups are thus placed, it is impossible to say before fertilization which is to function as the egg-apparatus" (Stephens 1909; her references to figures deleted).

The Penaea type is characteristic of the Penaeaceae (Stephens 1909; Tobe & Raven 1984), and has been reported from several members of the Malpighiaceae and Euphorbiaceae. Members of the Malpighiaceae are either bisporic (Allium type) or tetrasporic (Penaea type). In tetrasporic members of this family, the peripheral groups usually lack a distinct organisation into eggs and synergids. Most investigated species reproduce by nucellar embryos rather than by embryos derived from a zygote (Subba Rao 1940). In the Euphorbiaceae, non-monosporic development is known from three genera: *Acalypha*, *Euphorbia* and *Mallotus*. The Penaea type occurs in some members of all three genera (Kapil 1960; Rao 1970). *Acalypha* will be considered in greater detail.

All members of *Acalypha* are tetrasporic. The Penaea type is found in a number of species, including *A. brachystacha* (Kapil 1960). In this species, the peripheral groups resemble egg apparatuses but only the micropylar group has been observed to produce an embryo. The other three groups degenerate before fertilization. A different type of development has been described in *A. indica* and a number of other species (Johri & Kapil 1953;

Kapil 1960; Maheshwari & Johri 1941). In this type, each quartet forms a peripheral group of two cells and contributes two polar nuclei to the central cell (Fig. 4m). A third type has been described from *A. lanceolata*. The gametophyte is similar to that of *A. indica*, but only the micropylar quartet organizes a peripheral group. The remaining 14 nuclei fuse in the central cell (Thathachar 1952). Morphologically, the two-celled peripheral groups resemble an egg and a synergid, but it is not known whether the cells are sisters, or whether each is the sister of a polar nucleus. The embryo sac of *A. indica* could be derived from the Penaea type by a partial failure of cell formation in each peripheral group; the embryo sac of *A. lanceolata* could be derived from the Acalypha indica type by the complete failure of cell formation in three of the peripheral groups.

### 3. Piperaceae

Most members of the Piperaceae belong to one of two very large genera, *Piper* and *Peperomia*. In most classifications, these genera are placed in separate subfamilies if not in separate families.

Development in the Piperaceae is two-phasic and tetrasporic. In *Piper* and related genera, the three chalazal megaspore nuclei fuse to form a triploid nucleus. After two mitotic divisions, the embryo sac contains a haploid egg apparatus and upper polar nucleus, derived from the micropylar megaspore nucleus, and a triploid lower polar nucleus and three triploid antipodal cells. Thus, the embryo sac conforms to the traditional definition of the Fritillaria type. In at least some species of *Piper*, the antipodal cells proliferate to form a tissue that may exceed 100 cells (Johnson 1902, 1910; Kanta 1962).

Fagerlind (1939a) reported a variant form of development in an unidentified species of *Piper*. After the completion of meiosis, two of the chalazal megaspore nuclei degenerated. As a consequence, only the micropylar megaspore nucleus and its sister nucleus contributed to further development. This could be described as pseudobisporic development.

Embryo sacs of *Peperomia* are 16-nucleate. Johnson (1900)

investigated *Peperomia pellucida*. He reported that each spore nucleus divided twice to produce four nuclei. For each quartet, two nuclei were located in peripheral cells and two remained in the central cell. He interpreted the peripheral cells of the micropylar quartet as an egg and synergid. Thus, the mature embryo sac was composed of a two-celled egg apparatus, six other peripheral cells, and eight nuclei that fused in the central cell. Johnson (1914) later described a different type of development in *P. hispidula*. Only two peripheral cells were formed, the egg and synergid. The remaining 14 nuclei fused in the central cell.

Brown (1908) and Fisher (1914) studied a further ten species of *Peperomia*. In general, their observations supported Johnson's interpretation of development in *P. pellucida*. Brown concluded that the egg was the sister of a central cell nucleus, as was the single synergid. Fisher observed some variation in the number of lateral cells and central cell nuclei. Fagerlind (1939c) reinvestigated *P. pellucida* and found that approximately 50% of gametophytes formed an egg and one synergid, and the other 50% formed an egg and two synergids. The number of lateral cells and central cell nuclei also varied among embryo sacs.

All these studies identified synergids by their position next to the egg, but these cells showed few other features of synergids. Fagerlind (1939c) observed that the pollen tube entered the embryo sac directly, rather than via a synergid. He remarked that the egg and synergid did not always occur in such intimate contact as in other angiosperms and suggested that this could explain the unusual behavior of the pollen-tube. Fagerlind also observed that synergids and lateral cells often divided to form two-celled structures that resembled embryos.

More recently, Soviet researchers have reinvestigated *P. blanda*, one of the species studied by Fisher (1914). They found that the egg apparatus is always three-celled, and that the pollen-tube enters the embryo sac through one of the synergids, which promptly degenerates. They believe that previous researchers had mistaken post-fertilization stages for unfertilized embryo sacs (Nikiticheva, Yakovlev & Plyushch 1981; Bannikova & Plyushch, pers. comm.). It is possible that a three-

celled egg apparatus is the rule in *Peperomia* or that there is some variation within the genus. Until some consensus is achieved, it would be premature to speculate further.

In *Peperomia*, the central cell nuclei fuse to produce a highly polyploid nucleus. After fertilization this nucleus divides several times to produce endosperm. It is generally assumed that the second male nucleus participates in the fusion of central cell nuclei to form the primary endosperm nucleus. To the best of my knowledge, this second fertilization has never been observed. Fagerlind (1939c) provides the best evidence. He observed embryo sacs containing 18 nuclei, of which two lay in the egg cell, but he could not distinguish the second male nucleus from the other nuclei in the central cell. Double fertilization has been accepted as a fact even though the objective evidence presently available does not justify this confidence.

This review has not been concerned with post-fertilization phenomena, but the unusual organisation of the seed in *Piper* and *Peperomia* deserves comment. In the mature seed, embryo and endosperm occupy little more space than the unfertilized embryo sac. Most of the seed is occupied by a maternal tissue known as perisperm. This tissue is derived from the nucellus and contains the food reserves of the seed. The endosperm appears to function in the transfer of nutrients from the perisperm to the germinating embryo. Similar seeds are found in the Saururaceae, Nymphaeaceae and Hydatellaceae (Dahlgren et al. 1985).

The embryo sacs of *Acalypha indica* and *Gunnera* have sometimes been assigned to the *Peperomia* type. *Acalypha indica* has been discussed in the previous section. In *Gunnera*, the four megaspore nuclei divide twice to produce 16 nuclei. These are organized as a three-celled egg apparatus, seven nuclei in the central cell, and six antipodal cells (Modilewski 1908; Samuels 1912). *Gunnera* is an isolated genus of uncertain affinities (Philipson 1987).

## C. ONE-PHASIC ALGORITHMS

1. *Adoxa* types

The one-phasic *Adoxa* type was once thought to be the most common form of tetrasporic development. In the ideal conception of this type, the four megaspore nuclei divide once to produce an eight-nucleate embryo sac that is organised in the same manner as the *Polygonum* type (Fig. 4a). However, many species that were originally described as conforming to the *Adoxa* type have subsequently been found to follow the *Fritillaria* or *Polygonum* types (Fagerlind 1939a; Maheshwari 1946b).

The kin-conflict interpretation predicts that the ideal conception of the *Adoxa* type should be evolutionarily unstable. This is because the egg apparatus is derived from two megaspore nuclei. Thus, the synergids are derived from one megaspore nucleus, and the egg from another. Synergids are thought to play an essential role in fertilization, but they do not leave direct descendants. At some heterozygous loci, an allele that is expressed in synergids would be more likely to be present in the egg of another ovule on the same sporophyte than in the egg of the ovule in which it is expressed. A mutant allele that interfered with synergid function could reduce fertilization in those ovules in which it occurred in the synergids. Other ovules - with the mutant allele in their egg but the alternative allele in their synergids - might benefit from reduced competition for resources. The kin-conflict interpretation, therefore, predicts that *Adoxa*-type algorithms should be vulnerable to invasion by gametic drive mutants that interfere with synergid function. Such mutants could invade even though they might have disastrous effects on sporophyte fitness. From an evolutionary perspective, these negative effects should contribute to low persistence of the lineage and the rarity of the *Adoxa* type.

According to Maheshwari (1946b), the *Adoxa* type only occurs with certainty in four genera (*Adoxa*, *Sambucus*, *Erythronium* and *Tulipa*). Pollen grains of *Adoxa* resemble those of *Sambucus* (Erdtman 1966). Therefore, the occurrence of the *Adoxa* type in these genera may reflect a single evolutionary origin. Fagerlind (1938) gives the most accessible account of development in *Adoxa moschatellina*. In his figures, the derivatives of the micropylar

and chalazal megaspore nuclei are smaller than the derivatives of the two central megaspore nuclei and have a degenerate appearance. The *Adoxa* gametophyte does not appear to have functional synergids. On the other hand, all nuclei in the female gametophyte of *Sambucus ebulus* appear functional (Fagerlind 1939a).

*Erythronium* and *Tulipa* both belong to the Liliaceae. Most members of these genera have two-phasic, tetrasporic development (F. Smith 1955; Romanov 1959). Cooper (1939) described one-phasic development in *Erythronium albidum*. The embryo sac was organized into an egg apparatus, three antipodal cells and two polar nuclei. One-phasic development was confirmed by haploid chromosome counts in the antipodal cells whereas triploid counts would be expected if development conformed to the two-phasic *Fritillaria* type. Haque (1951) described early stages of development (before cell formation) in *E. americanum* which suggested that development was usually one-phasic. One-phasic embryo sacs, similar to those of *E. albidum*, have been observed in *Tulipa ostrovskiana* and *T. kolpakovskiana* (Romanov 1959).

Some unusual forms of one-phasic development have been described in the *Eriostemon* section of *Tulipa*. In *T. sylvestris*, the four megaspore nuclei gather at the micropylar pole and divide once to give rise to a group of six cells and two free nuclei (Bambacioni-Mezzetti 1931; Fig. 4p). In *T. tetraphylla*, three megaspore nuclei gather at the micropylar end and one at the chalaza. After one division, there are five micropylar cells, two free nuclei and a single chalazal cell which soon degenerates (Romanov 1938). The conventional interpretation of these embryo sacs is that the micropylar groups contain a single egg and several synergids. The kin-conflict interpretation predicts that the concentration of cells at the micropylar end reflects competition among different genetic individuals for fertilization, and the micropylar group should contain more than one egg.

Apart from the species described above, in which one-phasic development is considered to be the rule, the *Adoxa* type is also claimed to occur sporadically in a number of species with normally two-phasic development (Maheshwari 1946b). I believe



these claims are questionable. The coexistence of two-phasic and one-phasic development requires that different numbers of free nuclear divisions take place in different ovules of the same sporophyte. Moreover, I have argued above that Adoxa-type algorithms should be evolutionarily unstable. If these theoretical objections were countered by strong empirical evidence, the claims should be accepted but the evidence for Adoxa-type development is weak.

*Ulmus* and *Tamarix* will provide examples of genera in which the Adoxa type reputedly occurs in some ovules. Two-phasic development in *Ulmus* roughly conforms to the Drusa type. Two lines of evidence are used to support the occasional occurrence of the Adoxa type. Firstly, some mature embryo sacs have only eight nuclei. Secondly, some tetrads have a (2+2) rather than a (1+3) arrangement of megaspores. This evidence is unconvincing. Walker (1950) reported mature embryo sacs with any number from eight to 16 nuclei. She assigned embryo sacs with nine or more nuclei to the Drusa type, but embryo sacs with eight nuclei to the Adoxa type. Among Drusa-type embryo sacs, variation in the number of nuclei was explained by "strike", but "strike" was not invoked to explain embryo sacs with eight nuclei. The second line of evidence is equally unconvincing. To conclude that tetrads with a (2+2) arrangement will divide only once whereas tetrads with a (1+3) arrangement will divide twice requires a leap of faith. This is particularly true when Walker describes occasional embryo sacs with six to eight cells at the micropylar pole. Such embryo sacs must be two-phasic, as they have more than eight nuclei in total, but their organization suggests that two or three megaspores may have been located at the micropylar pole.

In *Tamarix*, the Fritillaria type is the commonest form of development, but the *Chrysanthemum cinerariaefolium* and Drusa types are reported to occur in some ovules (Hjelmqvist & Grazi 1965; Johri & Kak 1954). These two-phasic types are characterized by a (1+3) arrangement of megaspores accompanied by the fusion of 3, 2 or none of the chalazal megaspores. As I have already argued, variable fusions of somatic megaspores are compatible with a single developmental algorithm. However, the Adoxa type has also been reported and this requires a change in the number

of free nuclear divisions. As in *Ulmus*, the evidence is primarily based on a (2+2) arrangement of megaspores at the tetrad stage. Mature embryo sacs of the *Fritillaria* and *Adoxa* types have the same arrangement of nuclei and are difficult to distinguish unless the critical developmental stages are seen.

In summary, the kin-conflict interpretation predicts that the *Adoxa* type should be evolutionarily unstable and, in fact, the type does have a very restricted occurrence. In *Adoxa*, the synergids are possibly non-functional and, in *Tulipa* some species show unusual forms of development with many cells at the micropylar pole. A few species in *Erythronium*, *Tulipa* and *Sambucus* appear to conform to the traditional conception of the type. The *Adoxa* type is claimed to be a variant form in some genera, but the evidence for these claims is inconclusive.

## 2. *Plumbaginaceae*

One-phasic tetrasporic development is characteristic of the *Plumbaginaceae* (excluding *Limoniaceae*), but its course is very different from the *Adoxa* type. In *Plumbago*, *Vogelia* and *Ceratostigma*, development conforms to the *Plumbago* type. There is no cytokinesis after either meiotic division, and the four megaspores adopt a cruciform configuration. Each megaspore divides once, and the sister nuclei are separated by cell walls. The mature embryo sac consists of a micropylar cell, a chalazal cell, two lateral cells, and four polar nuclei that fuse in the central cell (Boyes & Battaglia 1951; Haupt 1934; Mathur & Khan 1941; Fig. 4q). In *Plumbagella micrantha*, the megaspore nuclei adopt a (1+3) configuration. The three chalazal nuclei fuse to form a secondary two-nucleate stage. The two nuclei divide once and are separated by cell walls. Thus, the mature embryo sac consists of a haploid micropylar cell, a triploid chalazal cell, and two polar nuclei (one haploid, one triploid) in the central cell (Boyes 1939; Russell & Cass 1988; Fig. 4r). This type of development is known as the *Plumbagella* type. In about 5% of ovules, only two of the chalazal nuclei fuse, and the mature gametophyte contains six nuclei (Boyes 1939).

The most distinctive feature of the *Plumbago* and *Plumbagella* types is that a single cell takes the place of the three-celled

egg apparatus. This micropylar cell functions as both egg and synergid. Its wall contains a filiform apparatus (Cass 1972) through which the pollen tube enters the embryo sac (Russell 1982). In other families, the pollen tube enters the embryo sac through the filiform apparatus of a synergid.

A single-celled egg apparatus (an egg/synergid) has only been found in the Plumbaginaceae. Therefore, it appears likely that this structure evolved only once. The Limoniaceae, the other family belonging to the Plumbaginales (Dahlgren 1980), has two-phasic development. All investigated members of this family possess *Fritillaria*-type development except for an unidentified member of *Statice* that has the *Penaea* type (Fagerlind 1938, 1944).

The most likely scenario is that the *Plumbagella* algorithm was derived from a *Fritillaria* algorithm, because these types share fusion of the chalazal megaspore nuclei. The principal difference between them is the loss of a free nuclear mitosis from the *Plumbagella* type. As a consequence, there are only four nuclei in the embryo sac at cell formation and only two mitotic spindles on which cell walls can form. Cell formation therefore produces a three-celled embryo sac. The normal process of differentiation would result in the single chalazal cell developing as an antipodal cell and the two nuclei in the central cell developing as polar nuclei. In the *Fritillaria* algorithm, egg-specific and synergid-specific loci are expressed in different cells. In the *Plumbagella* algorithm, these loci are expressed in the single micropylar cell. We do not know enough about the differentiation of eggs and synergids in normal egg apparatuses to tell whether the expression of both sets of loci in the one cell requires changes within the differentiation subroutine (D) or whether this is an epiphenomenon of a three-celled embryo sac.

The *Plumbago* algorithm could be derived from the *Plumbagella* algorithm by non-fusion of the chalazal megaspore nuclei. Alternatively, the *Plumbago* algorithm could be derived from the *Penaea* type, and the *Plumbagella* algorithm be derived from the *Plumbago* type. This scenario seems less likely because the *Fritillaria* type is more widely distributed than the *Penaea* type,

both within the Plumbaginales and among angiosperms in general. Boyes (1939) has suggested that the Plumbago and Plumbagella types are independently derived from the Penaea and Fritillaria types respectively, but this would require a double origin of egg/synergids.

#### VII. A Classification of Developmental Algorithms

Previous classifications of the angiosperm embryo sac have implicitly treated bisporic and tetrasporic embryo sacs as "individuals", directly comparable to a monosporic gametophyte. The perspective presented in this review considers such embryo sacs to be "groups" of related individuals, where the genetic "individual" is a megaspore nucleus and its derivatives.

Two kinds of genetic individual are found in non-monosporic embryo sacs. The "germinal individual" consists of the germinal megaspore nucleus and its derivatives. One of these derivatives is the egg nucleus. A "somatic individual" consists of a somatic megaspore nucleus and its derivatives. As this review has shown, the distinction between germinal and somatic individuals is sometimes ambiguous because more than one megaspore nucleus within a tetrad may attempt to form an egg apparatus. In this section, I will use "germinal spore" to refer to that megaspore or megaspore nucleus giving rise to the micropylar egg apparatus.

The most widely used classification of embryo sacs (Maheshwari 1950) emphasizes differences in the behavior of somatic individuals. This behavior is often variable within the progeny of individual sporophytes causing more than one type to be recognized within a species. I propose that embryo sacs be classified by the behavior of the germinal spore and its derivatives. If necessary, a brief description can be appended describing somatic spore behavior. I choose to de-emphasize differences in somatic spore behavior because variable behavior within species suggests that such variation does not reflect important differences in the underlying developmental algorithm. Moreover, the kin-conflict interpretation suggests reasons why germinal spore behavior should be less variable than somatic spore behavior.

## A. GERMINAL SPORES AND THEIR DERIVATIVES

Below I present a classification of developmental algorithms based on the fate of the germinal spore and its derivatives. The classification uses the number of free nuclear divisions of the germinal spore and the number of megaspore nuclei present in the initial cell of the embryo sac. It should be emphasized that this is an artificial classification. Members of the same genus belong to different types and members of different families belong to the same type. Somatic spores and their derivatives are discussed in the next section.

**Three-phasic algorithms**

The germinal spore divides three times to produce eight nuclei. These nuclei organize a micropylar egg apparatus, two polar nuclei and (usually) three antipodal cells.

(i) *Three-phasic monosporic*

The Polygonum type: found in the majority of angiosperms.

(ii) *Three-phasic bisporic*

No known examples.

(iii) *Three-phasic tetrasporic*

*Chrysanthemum balsamita*.

**Two-phasic algorithms**

In types (iv), (v) and (vii) the germinal spore divides twice to produce four nuclei. These nuclei organize a three-celled egg apparatus and a polar nucleus. Such algorithms can be derived from a three-phasic algorithm by the loss of a free nuclear mitosis. Type (vi) is basically similar except the fourth nucleus does not function as a polar nucleus.

(iv) *Two-phasic monosporic*

Onagraceae and Limnocharitaceae.

(v) *Two-phasic bisporic*

The *Allium/Endymion* type: found in several families including the Viscaceae and Alismataceae.

(vi) *Two-phasic bisporic (Apinagia type)*

Most members of the Podostemaceae.

(vii) *Two-phasic tetrasporic*

Widespread: examples include *Drusa*, *Penaea*, *Fritillaria*, *Limnanthes*, *Clintonia*, *Gunnera* and *Peperomia*. *Peperomia* is included in this list on the basis of Nikiticheva et al. (1981).

(viii) *Two-phasic tetrasporic (Acalypha indica type)*

The germinal spore produces an egg, one synergid and two polar nuclei. Occurs in *Acalypha indica*, *A. lanceolata* and a few related species.

**One-phasic algorithms**

The germinal spore divides once to produce two nuclei.

(ix) *One-phasic monosporic*

None known.

(x) *One-phasic bisporic (Polypleurum type)*

I interpret the derivatives of the germinal spore as an egg and a synergid. The type occurs in a few members of the Podostemaceae.

(xi) *One-phasic tetrasporic (Adoxa type)*

The derivatives of the germinal spore are an egg and a polar nucleus. The functions of synergids are served by the derivatives of a somatic spore.

(xii) *One-phasic tetrasporic (Eriostemonas type)*

The derivatives of the germinal spore are two micropylar cells. Occurs in the *Eriostemonas* section of *Tulipa*.

(xiii) *One-phasic tetrasporic (Plumbago/Plumbagella type)*

The derivatives of the germinal spore are a polar nucleus and an egg/synergid. Occurs in the Plumbaginaceae.

Plumbago and Plumbagella types differ in the behavior of somatic spores.

## B. SOMATIC SPORES AND THEIR DERIVATIVES

Somatic spores show a wide range of behaviors. I will not attempt a formal classification but will discuss some of the alternatives. If somatic spores are excluded from the embryo sac, they are usually suppressed by the maternal sporophyte and do not develop further. This is the usual fate of somatic spores in monosporic development and of the non-functional dyad in bisporic development. Suppression is less common when somatic spores are found within the embryo sac. Bisporic and tetrasporic embryo sacs contain derivatives of one or more somatic spores as well as derivatives of the germinal spore. The two extremes of a spectrum of behaviors can be specified. (1) The genetic difference between germinal and somatic spores has no effect: chalazal nuclei derived from somatic spores express all the functions of antipodals. This has been the traditional assumption. (2) Somatic spores express or attempt to express the developmental algorithm of a germinal spore. "Antipodal eggs" are the obvious example.

Classification is difficult because the behavior of somatic spores is often variable among the embryo sacs produced by a single sporophyte. Embryo sacs may vary in the fusion or non-fusion of megaspore nuclei and in the degree of chalazal strike. Such variability contrasts with the comparatively uniform behavior of germinal spores in the same embryo sacs. The more variable behavior of somatic spores may be the result of (1) an originally monosporic algorithm being expressed under different circumstances, (2) relaxed selection for uniform behavior because chalazal nuclei are no longer genetically identical to the egg, or (3) conflict between genes expressed in somatic spores and

genes expressed in the germinal spore or maternal sporophyte.

### VIII. Evolutionary Implications

A striking generalization can be made. Monosporic gametophytes are three-phasic, but bisporic and tetrasporic embryo sacs are two-phasic or occasionally one-phasic. Exceptions are rare: two-phasic monosporic gametophytes are known from the Onagraceae and Limncharitaceae; three-phasic tetrasporic embryo sacs occur in *Chrysanthemum balsamita*. These exceptions show that the relationship cannot be explained by some absolute developmental constraint.

The association between number of megaspore nuclei and number of mitotic divisions is possibly explained by the relative cost of different types of female gametophyte. A three-phasic monosporic gametophyte contains eight nuclei, whereas a three-phasic bisporic embryo sac would contain 16 nuclei and a three-phasic tetrasporic embryo sac would contain 32 nuclei. Every extra division doubles the number of nuclei in the female gametophyte (barring "strike"), and correspondingly increases the cost of a prefertilization ovule. Prefertilization costs are incurred whether or not the ovule is fertilized. Therefore, there is a selective pressure to shift costs of seed production from before fertilization until after fertilization (see Chapter 7). This explanation begs the question why reduction has not proceeded further. That is, it does not explain why there are so few two-phasic monosporic gametophytes, and why most bisporic and tetrasporic embryo sacs are two-phasic rather than one-phasic.

Monosporic gametophytes will be considered first. The antipodal nuclei of the eight-nucleate *Polygonum* type are often ephemeral. Therefore, three-phasic development appears to be maintained because the other five nuclei have essential functions, and because five nuclei require three division cycles. An egg nucleus is required for syngamy and the synergids appear to be involved in the fertilization process. Only one of the synergids, the degenerating synergid, is penetrated by a pollen tube. Gametophytes may have two synergids because of constraints of the developmental algorithm, or because the persistent synergid also has some essential function. The two polar nuclei



are involved in the triple fusion with the second sperm to form the primary endosperm nucleus.

In at least some monosporic taxa, two polar nuclei are essential, because a 2:1 ratio of maternal to paternal genomes is required for normal endosperm function and because diploid endosperms formed by the fertilization of a single polar nucleus are abortive. Chapter 9 discusses reasons why this requirement may have evolved. If a 2:1 ratio is a general requirement of monosporic gametophytes, it could provide a major obstacle to changes in the developmental algorithm that cause changes in the number of polar nuclei. The two-phasic monosporic gametophytes of the Onagraceae and Limncharitaceae have only a single polar nucleus. Either the ancestors of these families did not have a 2:1 requirement, or the requirement was circumvented in some unknown manner.

In bisporic development, two mitotic cycles are sufficient to produce an egg, two synergids and two polar nuclei. One polar nucleus is a derivative of the germinal spore and the other polar nucleus is a derivative of a somatic spore. Thus, a 2:1 ratio is maintained in endosperm, but the two polar nuclei are not genetically identical. The bisporic Podostemaceae are a special case. Endosperm is not formed. Thus, there is no requirement for two polar nuclei. Embryo sacs of the two-phasic Apinagia type are pseudomonosporic, and probably contain only three "essential" nuclei, two synergid nuclei and the egg nucleus. The one-phasic Polypleurum type has an egg and a single synergid (interpretation adopted in this review).

Most tetrasporic algorithms are two-phasic, although one mitotic cycle would be sufficient to produce an egg apparatus and two polar nuclei from four megaspore nuclei. I have argued above (Section VI; C.1) that one-phasic tetrasporic algorithms of the Adoxa type are evolutionarily unstable because the synergid nuclei and egg nucleus are derivatives of different megaspore nuclei. The one-phasic Adoxa and Eriostemonas types appear to have very limited occurrence. The Plumbaginaceae are also one-phasic. In this family, a one-phasic algorithm is made possible because a single cell assumes the separate functions of the egg and synergids. The other derivative of the germinal spore is a

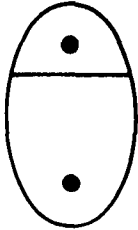

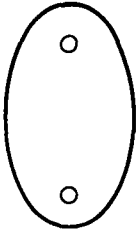
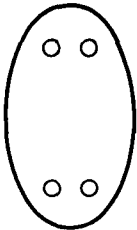
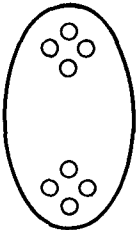
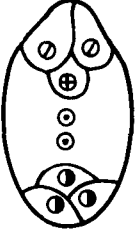
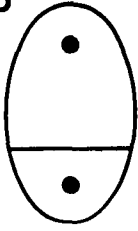



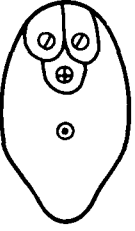
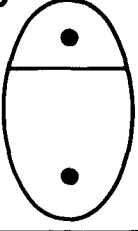

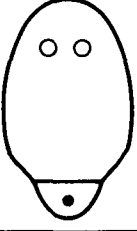
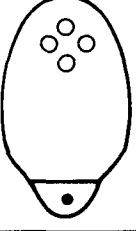
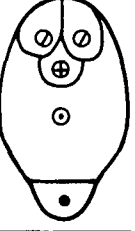
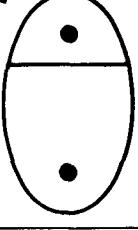

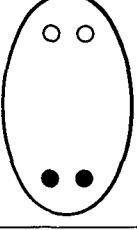
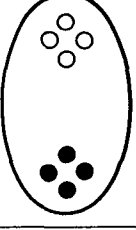
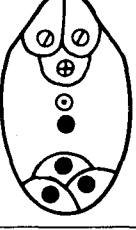
polar nucleus.

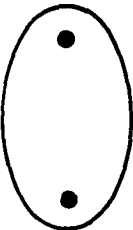
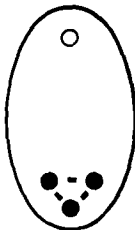



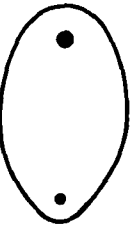
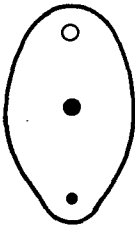
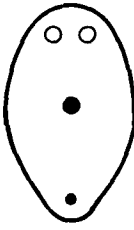




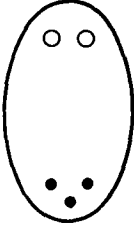


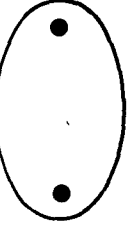
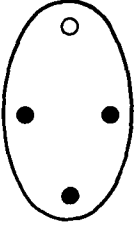
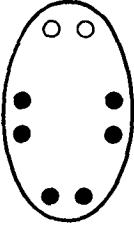
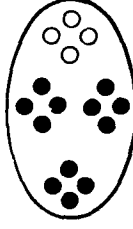
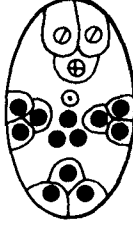
Endosperm ploidy is variable among species with tetrasporic development. Pseudomonosporic embryo sacs have a single polar nucleus and presumably form a diploid endosperm. Several of the traditionally-recognized types of embryo sac form pentaploid endosperms (e.g. *Fritillaria*, *Penaea*, *Plumbago*, *Plumbagella* types). Drusa embryo sacs are conventionally assumed to contain two polar nuclei but the number appears to be variable. *Peperomia* embryo sacs reputedly have up to 14 polar nuclei. It is unclear how these embryo sacs could be derived from a monosporic gametophyte with a 2:1 requirement in endosperm.

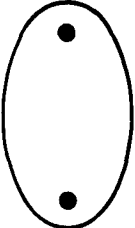

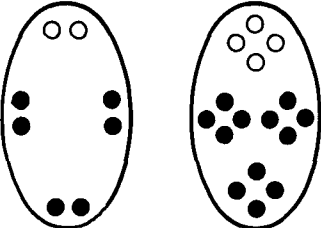
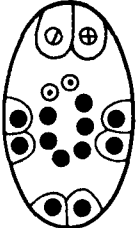
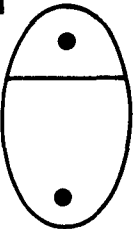

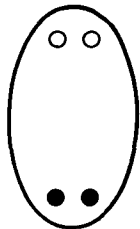
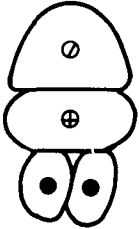
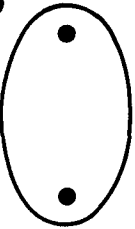
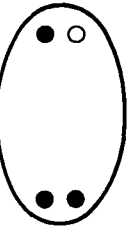
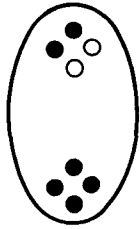
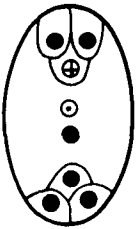
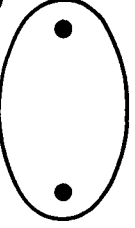

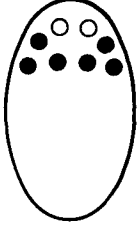

The Asteraceae is one family that contains monosporic, bisporic and tetrasporic species. There is circumstantial evidence that a paternal genome is not necessary for normal endosperm function in this family. Apomixis in angiosperms is usually pseudogamous. That is, the polar nuclei must be fertilized to produce an endosperm, even though the embryo can develop without fertilization (Nogler 1984). Pseudogamy can be understood as a requirement for paternally-imprinted genes during normal endosperm development (see Chapter 9). In the Asteraceae, apomictic seeds can develop without fertilization of the polar nuclei (Nogler 1984), suggesting that a specific ratio of maternal and paternal genomes is not required in endosperm.

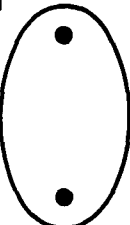


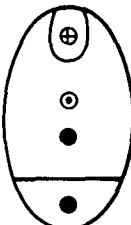
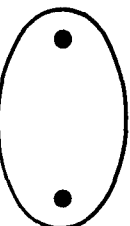


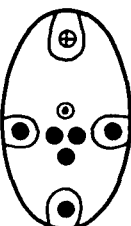
Female gametophytes of angiosperms undergo many fewer mitotic divisions than female gametophytes of gymnosperms. The reduced nature of angiosperm gametophytes is possibly related to the abandonment, by angiosperms, of simple polyembryony as a mechanism of developmental selection within ovules and its replacement by developmental selection among ovules (see Chapter 6). The reduction appears to have reached a limit (with a few exceptions) at three mitotic divisions in monosporic development and at two mitotic divisions in bisporic and tetrasporic development. Three-phasic monosporic algorithms have a wide distribution among angiosperms. Does this distribution reflect a shared three-phasic ancestry for all angiosperms, or could a number of lineages with related many-phasic algorithms have converged on the same minimum number of divisions and a similar organization of the embryo sac?

Figure 10.4. Schematic diagram of the different types of embryo sac development discussed in the text. From the left, the columns represent meiosis I, meiosis II, the free nuclear divisions, and the mature embryo sac after cell formation and differentiation. The fifth column from the left gives the identification used in the text. The rightmost column gives the classification of algorithm types presented in Section VII. In the mature embryo sac, the derivatives of the germinal megaspore nucleus are given symbols that identify synergid, egg, polar and antipodal nuclei. The interpretations are those adopted in the text. Open circles are nuclei that are not interpreted. The derivatives of somatic megaspore nuclei are represented by solid circles.

Melosis I	Melosis II	Free nuclear divisions			Mature embryo sac	"Type" or example	Algorithm type
a 						"Polygonum type"	Three-phasic monosporic
b 						"Oenothera type"	Two-phasic monosporic
c 						<i>Limnocharis</i> spp.	
d 						"Allium type"	Two-phasic bisporic

Melosis I	Melosis II	Free nuclear divisions		Mature embryo sac	"Type" or example	Algorithm type
i 					<i>Tulipa maximovicii</i>	Two-phasic tetrasporic
j 					<i>Limnanthes douglasii</i>	
k 					<i>Clintonia</i> spp.	
l 					"Penaea type"	

Meiosis I	Meiosis II	Free nuclear divisions	Mature embryo sac	"Type" or example	Algorithm type
m 				<i>Acalypha indica</i>	Two-phasic tetrasporic ( <i>A.indica</i> type)
n 				"Polypleurum type"	One-phasic bisporic (Polypleurum type)
o 				"Adoxa type"	One-phasic tetrasporic (Adoxa type)
p 				<i>Tulipa sylvestris</i>	One-phasic tetrasporic ( <i>Eriostemon</i> type)

Meiosis I	Meiosis II	Free nuclear divisions	Mature embryo sac	"Type" or example	Algorithm type
q 				<i>Plumbagella micrantha</i>	One-phasic tetrasporic  (Plumbago/ Plumbagella type)
r 				"Plumbago type"	



Degenerating megaspore or dyad  
(Meiosis II)



Somatic megaspore nucleus or  
derivative (after Meiosis I)



Degenerating nucleus



Fusion of somatic megaspore nuclei



Germinal megaspore nucleus  
or derivative

In mature embryo sac:



Synergid nucleus



Egg nucleus



Polar nucleus



Antipodal nucleus