FROM ECOLOGY AND EVOLUTION OF PLANT REPRODUCTION, ed. Robert Wyatt N.Y. Chapman & Hall, 1992

14

The Evolution of Endosperm: A Phylogenetic Account

Michael J. Donoghue and Samuel M. Scheiner

University of Arizona
Northern Illinois University

The seeds of flowering plants differ in many ways from those of other seed plants, and any explanation of the origin of angiosperms must account for the evolution of these differences. Prominent among the characteristic features of the angiosperm seed is the development of endosperm tissue from the product of a second fertilization. A variety of explanations have been proposed for the evolution of this unique tissue. It has been suggested, for example, that the greater genetic relatedness of the endosperm to the embryo (relative to the female gametophyte) provides a better environment for embryo growth (Sargant, 1900; Sporne, 1975). The most detailed treatment of the problem was provided by Brink and Cooper (1940, 1947), who supported the widespread view that increased vigor resulting from the union of genetically distinct nuclei results in a superior nurse tissue for the developing embryo. Stebbins (1976) proposed that endosperm is "doubly equipped for rapid growth" in that heterozygosity yields hybrid vigor and triploidy provides a larger number of RNA templates, making possible an increased rate of protein synthesis.

Recent interest in this problem was sparked by Charnov's (1979) suggestion that intersexual and/or kin selection might account for the evolution of double fertilization and endosperm. Explanations involving kin selection have now been elaborated (or extended to other aspects of seed biology) by many authors, including Cook (1981), Westoby and Rice (1982), Queller (1983, 1984, 1989), Willson and Burley (1983), Law and Cannings (1984), Bulmer (1986), Mazer (1987), Haig and Westoby (1988, 1989a,b), Uma Shaanker et al. (1988), and Haig (1990). As applied to seeds, such arguments are meant to explain or predict the way(s) in which selection will act to alter the resource allocation of tissues based on their genetic relatedness. In general, it is argued that a typical triploid endosperm will be selected to garner fewer resources (i.e., be less aggressive) than its associated embryo, but should be selected to garner more resources than its female gametophyte.

In an outstanding review of the subject, Queller (1989) noted a significant shortcoming of arguments based on inclusive fitness: they do not actually account for the origin of new tissues, nor for their maintenance for a long enough period of time that kin selection could have a significant impact on patterns of resource allocation (also see Bulmer, 1986). Our aim is to focus directly on explanations for the origin and persistence of events in the development of the angiosperm seed. Accordingly, our arguments can be viewed as complementary to kin selection hypotheses. We attempt to explain the origin and maintenance of seed tissues, whereas kin selection arguments properly apply to the subsequent modification of these tissues with respect to allocation. Nevertheless, we also will advance the stronger claim that arguments based on intersexual or kin conflict are not necessary to explain what we know about the behavior of seed tissues. In arguing this point, our intention is not to take issue with kin selection in general, nor with kin selection as applied to plants or even to seed tissues. Instead, we contend that it is possible to identify explanations that are at least as simple, and that it is therefore unnecessary to invoke kin selection to explain either the origin *or* the subsequent modification of seed tissues.

Our analysis is consciously historical in that we begin by inferring a chronicle of evolutionary events based on recent phylogenetic analyses and then provide a narrative account for this sequence of changes (O'Hara, 1988; Donoghue, 1989; Brooks and McLennan, 1991). Consideration of factors involved in the origin of angiosperms suggests that selection for rapid reproduction and increased rate of development played a critical role (either directly or indirectly) in the evolution of seed tissues. We will argue that double fertilization was an incidental outcome of changes related directly to more rapid reproduction, and that polyploidy was selected to increase the growth rate and storage capacity of the resulting tissue. Similar arguments are also applied to the evolution of the female gametophyte within angiosperms. Tests of these hypotheses depend critically on obtaining better data on the relative and absolute timing of developmental events, and we hope to encourage more detailed analyses along these lines.

A Chronicle of Events

The analysis below relies directly on the phylogenetic results summarized in Figure 14.1, which are derived primarily from the cladistic studies of extant and extinct lines of seed plants conducted by Crane (1985, 1988) and Doyle and Donoghue (1986, 1992; Donoghue and Doyle, 1989a). Based mainly on morphological features, these studies concluded that an-

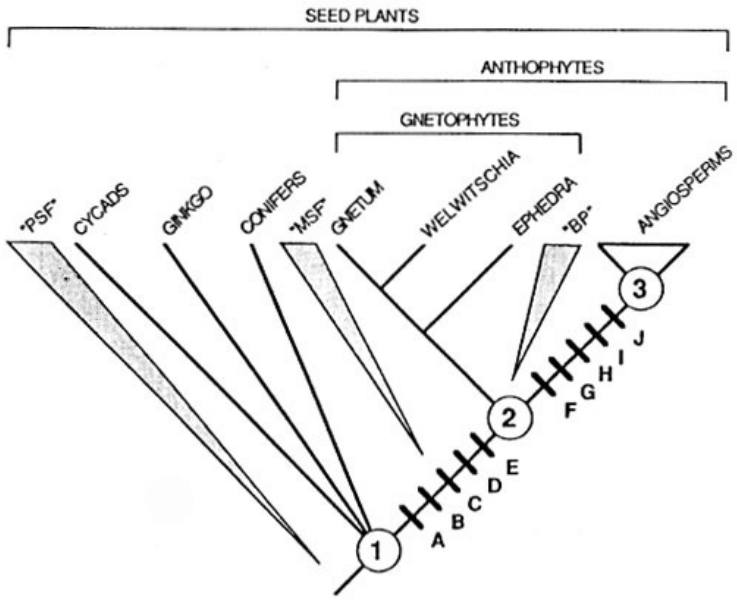

Figure 14.1. A phylogeny of relevant seed plants based on morphological and molecular evidence (references in text). Parsimony optimizations of relevant features at the internal nodes labeled 1, 2, and 3 are based upon the seven recent groups, for which the most complete data are available. The relative positions of various fossil taxa (presumably paraphyletic groups) are shown by shaded triangles: "PSF," various Paleozoic "seed ferns"; "MSF," various Mesozoic "seed ferns," including *Caytonia*; "BP," Bennettitales and *Pentoxylon*. Selected evolutionary changes between nodes 1 and 2: (A) more rapid reproduction; (B) smaller seeds/gametophytes; (C) siphonogamy/nonmotile sperm; (D) retention of the ventral canal nucleus; and (E) double fertilization. Selected evolutionary changes between nodes 2 and 3: (F) further reduction of the female gametophyte; (G) partial alveolarization; (H) loss of archegonia; (I) addition of a second female gametophyte nucleus/triploidy; and (J) other specializations of male and female gametophytes. See text for discussion.

giosperms are monophyletic and belong to a clade including the extant Gnetales and the extinct groups Bennettitales and *Pentoxylon*. The relatives of this anthophyte clade are various "seed ferns" of Mesozoic age. The monophyly of Gnetales is very well supported, as is the conclusion that *Welwitschia* and *Gnetum* are more closely related to each other than

either is to *Ephedra*. Relationships among the other major lines of extant seed plants (ginkgos, cycads, conifers) are still uncertain (Doyle and Donoghue, 1992).

These basic phylogenetic results are consistent with morphological analyses of extant seed plant groups alone (Loconte and Stevenson, 1990), as well as with analyses of ribosomal RNA (Zimmer et al., 1989; Hamby and Zimmer, 1991) and nucleotide sequences of the chloroplast gene *rbc*L (M. Chase and R. Olmstead, personal communication). A combined analysis of morphological and ribosomal data yielded the same results (Doyle, Donoghue, and Zimmer, unpublished).

The sequence of character changes leading to endosperm can be determined by parsimoniously reconstructing (optimizing) the states at the internal nodes (presumed common ancestors) labeled 1, 2, and 3 in Figure 14.1. This approach was highlighted by Donoghue (1989) in reference to the evolution of endosperm and other characters, and was also employed recently by Friedman (1990a,b). Here we present a more detailed account of the basic conclusions. Before proceeding, however, it must be noted that most embryological features are unknown for the relevant fossil groups, and for this reason these characters were generally not included in the phylogenetic studies involving fossils. Unfortunately, missing data on fossils also prevent us from obtaining a more complete assessment of the states at the several nodes of interest, or the exact sequence of changes along particular branches. Instead, our reconstructions are based only on extant groups, for which more or less complete data are available. Except as noted below, basic information on seed development was obtained from Maheshwari and Singh (1967), Singh (1978), Foster and Gifford (1974), Sporne (1974, 1975), and Johri (1984). In *Ephedra*, we have relied primarily on the studies of Friedman (1990a,b), who also provided a thorough summary of previous literature.

Changes between Nodes 1 and 2

Cycads and ginkgos (represented by the extant species *Ginkgo biloba*) are critical in reconstructing the condition of the seed at node 1 in Figure 14.1 (representing the ancestor of modern seed plants). In both groups the megaspore mother cell (megasporocyte) divides by meiosis to produce a linear tetrad of spores. Three of the haploid products abort, leaving only the cell at the chalazal end of the ovule (monosporic development). This cell enlarges and undergoes a series of mitotic divisions without the formation of cell walls between nuclei, which results in a large number of free nuclei (e.g., more than 3000 nuclei in some cycads and over 8000 in some ginkgo plants). This phase is followed by "alveolarization," wherein cell walls are laid down between nuclei in a characteristic centripetal pattern.

The differentiation of (1–)2–6 archegonia at the micropylar end of the gametophyte begins with the division of an archegonial initial, resulting in a primary neck cell and a central cell. The primary neck cell gives rise to a four-celled neck, and the central cell divides to form the ventral canal nucleus and the egg nucleus. In ginkgo and most cycads a wall usually forms between the egg and the ventral canal nucleus, and, except under very rare circumstances (Bryan and Evans, 1957), the ventral canal nucleus soon disintegrates.

In both cycads and ginkgo two large multiflagellate sperms are differentiated after the last mitotic division in the male gametophyte. The sperm are released into the archegonial chamber, where usually only one of the two enters an archegonium and fertilizes an egg. Rarely, the second sperm enters the same archegonium, but it soon degenerates. Simple polyembryony (i.e., the development of embryos from more than one archegonium) is common in both groups, but typically only one embryo survives to seed maturity. Although it is possible for the two sperm from one male gametophyte to fertilize eggs in different archegonia, multiple embryos probably more often involve sperm from different male gametophytes. The two cotyledons remain inside the seed on germination and obtain nutrients for the developing seedling. A rather long time period is required to complete sexual reproduction in cycads and ginkgos, and we assume that was also true of the first seed plants. In ginkgo, for example, there may be as many as 5 months between pollination and fertilization, and maturation of the embryo may require another 8 months.

The phylogenetic relationship of conifers to other extant groups is still not well resolved (Doyle and Donoghue, 1992), and their bearing on the condition at node 1 is therefore uncertain. We have based our assessment at node 1 primarily on cycads and ginkgo rather than conifers. In most respects conifer seed development is similar to cycads and ginkgo, and where there are differences it appears that the condition in conifers is derived. Thus, the sperms of conifers lack flagellae and are delivered by the pollen tube directly to the archegonium (siphonogamy). In comparison with cycads and ginkgo, the female gametophyte is generally reduced. Molecular data sets, and those morphological analyses including fossil groups, favor the view that these derived traits of conifers evolved independently in anthophytes (everything from node 2 up in Fig. 14.1; see references above). On the other hand, the analysis of extant seed plant groups by Loconte and Stevenson (1990) concluded that conifers are directly united with anthophytes, implying that reduction of the gametophyte and siphonogamy are homologous in the two groups. However, even if this phylogenetic conclusion were correct, these features still appear to have evolved independently when fossils (e.g., early conifers) are taken into account (Donoghue et al., 1989; Doyle and Donoghue, 1992). It should also be noted that a connection between conifers and anthophytes would not change our basic conclusions on character evolution. If Loconte and Stevenson (1990) are correct, an additional node would be inserted between the base of the seed plants and the anthophytes, and the shift to siphonogamy and reduction of the gametophyte would simply be interpreted as antedating the origin of anthophytes.

The states present at node 2 in Figure 14.1 (the common ancestor of anthophytes) are probably best inferred by reference to extant Gnetales. Based on relationships within Gnetales, and on comparison with cycads, ginkgo, and conifers, the basal condition in Gnetales is best estimated by the condition in *Ephedra* (Donoghue, 1989; Friedman, 1990a,b). Many embryological features of *Welwitschia* and *Gnetum* (e.g., tetrasporic development, partial alveolarization) apparently evolved within Gnetales (Doyle and Donoghue, 1986). Although there is little direct information on seed development in the relevant fossil groups (*Caytonia*, Bennettitales, *Pentoxylon*), the seeds of these plants were smaller than those found in modern cycads or ginkgo, and we presume that their female gametophytes were correspondingly reduced (Harris, 1951, 1964; Stewart, 1983; Tiffney, 1986).

Initial stages in the development of the female gametophyte of *Ephedra* are similar to those in cycads, ginkgo, and conifers (Land, 1904; Maheshwari, 1935; Lehmann-Baerts, 1967). Meiosis results in a linear tetrad of megaspores, of which three degenerate, leaving the chalazal cell to undergo a series of free-nuclear divisions. Alveolarization results in a cellular gametophyte, with generally from 250 to 512 nuclei present at the time of wall formation. From 1–3(–12) archegonia are differentiated at the micropylar end of the gametophyte. Each archegonial initial divides to produce a primary neck cell and a central cell; in turn, the neck cell divides to produce a multicellular, multitiered neck, while the central cell (following pollination) divides to form the ventral canal nucleus and the egg nucleus. Most importantly, a wall is not formed between these two nuclei and the ventral canal nucleus is generally present at the time of fertilization (Friedman, 1990a,b).

In *Ephedra* a binucleate sperm cell is produced by each male gametophyte. In contrast to cycads and ginkgo, the sperm are nonmotile and are delivered directly to the archegonium, where both nuclei are discharged into the egg cell. One of the sperm nuclei fuses with the egg nucleus to form the zygote, and it appears that the second sperm often fuses with the persistent ventral canal nucleus (Land, 1907; Herzfeld, 1922; Maheshwari, 1935; Khan, 1940, 1943; Mulay, 1941; Narang, 1955; Moussel, 1978; Friedman, 1990a,b). The earlier reports are reviewed by Friedman (1990a,b), who has provided the most complete documentation of the process. In addition to demonstrating that this second fertilization is a regular event in *Ephedra*, Friedman (1990a,b) confirmed that the additional fusion occurs

when the ventral canal nucleus is displaced away from the vacuolate apex of the egg cytoplasm toward the chalazal end of the archegonium, where it enters a distinctive column of cytoplasm rich in organelles (also see Moussel, 1977). Similar displacement of the ventral canal nucleus also seems to be correlated with anomalous egg-like behavior in cycads (Bryan and Evans, 1957) and conifers (Murrill, 1900; Hutchinson, 1915).

The fate of the diploid product of the second fertilization is not yet clear (Friedman, 1990a,b, and personal communication). It may undergo a limited number of mitotic divisions (Kahn, 1940, 1943), but there is no evidence of development of a second embryo or of a nutritive tissue, nor for the fusion of additional nuclei. Double fertilization has also been reported in *Gnetum* (Waterkeyn, 1954; Vasil, 1959; Martens, 1971) and *Welwitschia* (Pearson, 1909, 1929). In these plants additional free nuclei are present in the vicinity of the egg at the time of fertilization owing to incomplete alveolarization.

Embryo development in *Ephedra* begins with a series of free-nuclear divisions, with around eight nuclei produced before cell walls are laid down. Precocious cleavage polyembryony, wherein any or all of the first tier of eight cells independently develop into an embryo, occurs frequently (as in conifers). Simple polyembryony may also occur, but in either case only one embryo usually survives in each mature seed. As in cycads and ginkgo, two cotyledons are produced, and the female gametophyte serves as the nutritive tissue. The overall length of time required to complete the sexual cycle is considerably reduced in Gnetales as compared to cycads and ginkgo. For example, the time from pollination to fertilization may be as little as 10–36 hr in *Ephedra* (Land, 1970; Moussel, 1977).

Assessment of the states present at nodes 1 and 2 in Figure 14.1 allows us to infer a set of changes along the intervening branch, although it is not possible to determine the exact order in which these occurred. It appears that there was a shift to a more rapid sexual cycle and a reduction in overall size of the seed and female gametophyte. There also appears to have been a switch from haustorial male gametophyte development and production of swimming sperm to siphonogamous delivery of nonmotile sperm directly to the archegonium. Finally, we support previous arguments that double fertilization evolved between nodes 1 and 2, associated with the delivery of both sperm nuclei to the egg and the persistence of the ventral canal nucleus until the time of fertilization (Donoghue, 1989; Friedman, 1990a,b).

Changes between Nodes 2 and 3

Node 3 in Figure 14.1 represents the common ancestor of angiosperms. Within angiosperms there are a number of different patterns of female gametophyte development (for recent reviews see Battaglia, 1989; Haig, 1990). For the reasons given below, we agree with most other authors (e.g., Maheshwari, 1950; Davis, 1966; Takhtajan, 1969; Stebbins, 1974; Palser, 1975; Cronquist, 1988; Battaglia, 1989; Haig, 1990) that the ancestral form of female gametophyte development in angiosperms was probably most like the *Polygonum* type.

The development of the *Polygonum*-type embryo sac begins as it does in other seed plants (except *Gnetum* and *Welwitschia*): the megaspore mother cell divides by meiosis to produce a linear tetrad, of which three products abort and the remaining chalazal spore undergoes free-nuclear mitotic divisions. The mature female gametophyte consists of only eight nuclei in seven cells. Cell walls are formed so as to delimit three antipodal cells at the chalazal end of the gametophyte, two synergids and an egg cell at the micropylar end (the egg apparatus), and a large central cell containing two free polar nuclei (one the sister nucleus of the egg and the other the sister of an antipodal: Brink and Cooper, 1947). The egg in angiosperms is a free cell (i.e., an archegonium is not differentiated).

Two sperms are produced by the angiosperm male gametophyte and delivered directly to the female gametophyte by the pollen tube. One sperm nucleus fuses with the egg to form the zygote (which develops directly into the embryo), while the second sperm nucleus fuses with the two polar nuclei to form a triploid primary endosperm nucleus. In most cases the polar nuclei fuse prior to fertilization, but in others the three nuclei fuse more or less simultaneously (Davis, 1966). In either case, the primary endosperm nucleus then undergoes a series of mitotic divisions to become triploid endosperm (Vijayaraghavan and Prabhakar, 1984). In some cases this involves a series of free-nuclear divisions before cell walls are formed (nuclear development), whereas in other cases cell walls are laid down from the start (cellular development); "helobial" development, found among monocots, is an intermediate condition. Phylogenies of angiosperms support the view that cellular development is ancestral (Donoghue and Doyle, 1989b).

The view that the *Polygonum* type is ancestral has mainly been based on the observation that it is the most common and widespread type within angiosperms. Our own assessment is based first of all on outgroup comparison (Maddison et al., 1984). Although the *Polygonum* type is unknown outside of angiosperms, there are elements of this developmental program that are found in other seed plants, suggesting that other embryo sac types were derived within angiosperms. In particular, outgroup comparison implies that monosporic development, with mitotic divisions of the chalazal nucleus, is the primitive condition in angiosperms. Within angiosperms only the *Polygonum* type fits this description. The *Oenothera* type is monosporic, but its development is derived in that it is the micropylar nucleus that undergoes divisions. Other gametophyte types are bi- or tetrasporic.

Comparison with other seed plants also favors the *Polygonum* type on the grounds that it entails the largest number of mitotic divisions. The *Polygonum* type is said to be three-phasic, because a series of three divisions result in the mature eight-nucleate condition. By contrast, with only very rare exceptions, other types are two- or one-phasic (Battaglia, 1989; Haig, 1990).

Phylogenetic analyses within angiosperms also support the hypothesis that the *Polygonum* type is primitive. Optimization of an embryo sac development character on the most parsimonious trees of Donoghue and Doyle (1989b) unequivocally assigns the *Polygonum* type to the basal node. Indeed, among primary lines of angiosperms (e.g., Magnoliales, Laurales, Winterales, tricolpates, and paleoherbs, all sensu Donoghue and Doyle, 1989b), other types of development are very rare, so that almost any rooting would find the *Polygonum* type to be ancestral. Although the *Fritillaria* and *Peperomia* types (both tetrasporic and two-phasic) are found in Piperaceae, these appear to have been derived within Piperales (because the *Polygonum* type is found in Saururaceae). Therefore, even if the root were placed among paleoherbs (as suggested especially by ribosomal RNA sequences: Zimmer et al., 1989; Hamby and Zimmer, 1991), it would still be most parsimonious to conclude that the *Polygonum* type was ancestral in angiosperms.

We conclude from the foregoing considerations that between nodes 2 and 3 in Figure 14.1 there must have been a substantial reduction in size of the female gametophyte, a switch from complete to partial alveolarization (such that two free nuclei remain in the central cell at maturity), and the complete loss of archegonia. Most importantly, there was a change in the source of the nutritive tissue, from the haploid female gametophyte to triploid endosperm, which required the fusion of a second female gametophyte nucleus (the second polar nucleus). There were also significant changes in the structure and function of the angiosperm female gametophyte, such as specialization of the synergids for their role in fertilization (Willemse and van Went, 1984; van Went and Willemse, 1984). Related changes appear to have occurred in the angiosperm male gametophyte, including reduction of the number of divisions and especially derivation of the "male germ unit," which entailed the physical connection of the two sperm cells and their association with the vegetative nucleus (Dumas et al., 1984; Russell, 1991).

A Narrative Account

Having inferred a series of evolutionary events leading to endosperm, we now consider the possible causes of these changes. We recognize that this involves considerable speculation, perhaps more than many readers would condone. Our aim is to try to formulate a model that is consistent with what we know about phylogeny, and to provide an alternative to kin selection models (which are themselves highly speculative). In doing so, we hope to identify and encourage critical tests of these alternative explanations.

Changes between Nodes 1 and 2

We suggest that the changes between nodes 1 and 2 (more rapid reproduction, decreased seed size, siphonogamy and nonmotile sperm, double fertilization) were directly or indirectly related to the spread of seasonally drier, less equable, and generally less predictable climates during the Mesozoic (e.g., Parrish et al., 1986), and to the shift in anthophytes to growth in disturbed (e.g., riparian) habitats. These factors may have selected directly for increased speed of reproduction and possibly smaller seeds, and perhaps indirectly for an earlier onset of alveolarization and differentiation of the female gametophyte.

The origin of siphonogamy and nonmotile sperms might also have been related to life in drier habitats and selection for more rapid reproduction. Haustorial male gametophyte growth in cycads and ginkgo is a slow process (generally requiring months), and fertilization is dependent on the presence of liquid in the archegonial chamber. It is noteworthy that siphonogamy and nonmotile sperm probably evolved independently within conifers, also perhaps as an adaptation to drier climates (Doyle and Donoghue, 1986, 1992).

We agree with several previous suggestions that double fertilization, when it first originated, was an accidental consequence of the delivery of both male gametes directly to the archegonium and the presence of an additional female gametophyte nucleus at that time (see Meeuse, 1963, 1986; Donoghue, 1989; Friedman, 1990a,b). The persistence of the second nucleus, the sister nucleus of the egg, may have been an effect of selection for more rapid reproduction (i.e., the ventral canal nucleus simply had not disintegrated by the time of fertilization). This was probably largely a function of a shortening of the life cycle, especially the time between pollination and fertilization. Fusion of the second sperm with the ventral canal nucleus may simply have been a consequence of the tendency for compatible nuclei to undergo fusion, given the opportunity to do so (Khan, 1943).

This interpretation is consistent with the observation that fusion evidently occurs whenever sperm are delivered directly to the archegonium and there is an additional female nucleus present at the time of fertilization. Double fertilization is also found, for example, in *Welwitschia* and *Gnetum*, where

both conditions hold. Moreover, when both of these conditions are not met, double fertilization has not been reported. Although conifers are siphonogamous, free female nuclei are usually not present at the time of fertilization. The few instances of double fertilization reported in conifers seem always to be associated with abnormal persistence of the ventral canal nucleus (Land, 1902; Nichols, 1910; Hutchinson, 1915; Allen, 1946).

Changes between Nodes 2 and 3

Many of the changes between nodes 2 and 3 in Figure 14.1 (further reduction of the female gametophyte, partial alveolarization, loss of archegonia) probably resulted from continued selection for rapid growth and reproduction. Paleoecological data suggest that the first angiosperms evolved in marginal, unpredictable habitats, where they were probably selected for an accelerated life cycle (Doyle and Hickey, 1976; Doyle, 1978, 1984; Hickey and Doyle, 1977; Crane, 1987; also see Cornet, 1986). Although it could be argued that each characteristic of the angiosperm seed and gametophyte was independently selected, other traits of angiosperms (e.g., the carpel) appear to be paedomorphic and may have originated through progenesis (Takhtajan, 1969; Doyle, 1978). This entire suite of characters might best be accounted for by continuation of a general progenetic shift that began earlier in the anthophyte line, in which case the angiosperm ovule can be interpreted as "underdeveloped" at the time of fertilization.

Why did a new tissue largely replace the nutritive function of the female gametophyte, and why was another female gametophyte nucleus added? In some nonangiospermous seed plants (cycads, ginkgo) the female gametophyte is already fully developed at the time of fertilization; in others (conifers, gnetophytes), the gametophyte is usually well developed (generally having reached mature size), although some provisioning occurs after fertilization (Haig and Westoby, 1989b). Because of the presumed shift in the timing of fertilization in the first angiosperms, the gametophyte would have been considerably reduced in size during early embryo development. This would have set the stage for the evolution of a nutritive tissue other than the gametophyte (Queller, 1983). We suggest that the diploid product of the second fertilization was selected over the haploid gametophyte, owing both to heterotic effects (Brink and Cooper, 1940, 1947) and to the effects of increased ploidy on cell size, tissue growth rate, and nutrient storage capacity (see below). The fusion of an additional female nucleus may have further enhanced the rate of growth and storage capacity of the nutritive tissue.

The switch from gametophyte to endosperm set the stage for further modifications. First, double fertilization became a highly dependable event. This presumably meant ensuring a favorable position for the second female nucleus within the egg cytoplasm (cf. *Ephedra*: Friedman, 1990a,b), which would also facilitate the subsequent growth of the product of the second fertilization. Second, with the evolution of a new nutritive tissue, the female gametophyte was selected for specialized functions during the earliest stages of development. Thus, the egg apparatus (the synergids in particular) appears to have evolved to facilitate the movement of sperms during fertilization. In addition, the central cell became specialized for nutrient storage and transfer very early in embryo development (Willemse and van Went, 1984), and the timing of fusion of the polar nuclei shifted. These observations contradict the common assumption that the angiosperm female gametophyte is a functionless vestige. Finally, there were significant changes in the structure of the male gametophyte, especially the evolution of the male germ unit, which presumably increased the effectiveness of sperm delivery and ensured simultaneous transmission of the two gametes (Russell, 1991).

Possible Objections

The new and probably most controversial element in this explanation concerns the impact of increasing the amount of DNA. An increase in DNA content is generally correlated with an increase in total cell volume, presumably due to mechanisms that maintain a more or less fixed ratio of nucleus to cytoplasm volume (e.g., Price, 1976; D'Amato, 1977; Cavalier-Smith, 1978, 1985a,b,c). In turn, cellular demand for metabolites and metabolic rate might rise through an increase in the rate of transcription and translation owing to the availability of additional DNA templates. Although an increase in the volume of DNA also tends to slow down the rate of cell division (Nagl, 1974; Nagl and Ehrendorfer, 1975; Price and Bachmann, 1976; Cavalier-Smith, 1978), this is not necessarily the case when DNA content is increased by polyploidy. Indeed, polyploids apparently do not show an increase in the DNA synthesis period (Troy and Wimber, 1968), and there are cases in which somatic cell cycle time is significantly shorter in polyploids in comparison to related diploid species or to diploid tissues in the same species (Bennett, 1973, and references therein). Cavalier-Smith (1985b) suggested a mechanism to explain such observations: multiplication rate in polyploids may not be greatly impacted by DNA amount because cell volume is increased without a corresponding decrease in gene concentration.

Based on these considerations, we propose that increasing the amount of DNA by polyploidy would have had the effect of increasing the rate at which a resulting tissue could fill a given volume in comparison to the haploid gametophyte tissue (also see Grime and Mowforth, 1982; Grime et al., 1985). This follows if polyploid cells are larger but divide at about the same or at a greater rate. Furthermore, we reason that the maximum

rate of volume growth might be achieved with some intermediate amount of DNA per cell. Haploid cells would be smaller, so that the overall growth of the tissue might be retarded, even if cell division rates were somewhat higher. In contrast, much larger amounts of DNA may slow cell division rate, such that the rate of growth would be reduced.

In view of the evident relationship between genome size and cell division rate, Cavalier-Smith (1978) actually made the opposite suggestion, namely that selection for rapid seed development should favor haploidy of endosperm cells. However, we believe that this expectation fails to take into account the contribution of increased cell size to filling rate, and the observation that polyploidy may not have a significant impact on cell division rate. In any case, this difference of opinion highlights that the critical factor in our account is the net effect of the interaction of cell size and division rate on volume increase. Although the evidently rapid development of endosperm (e.g., Brink and Cooper, 1940, 1947) is certainly consistent with our scenario, it is clear that detailed quantitative comparisons are needed to evaluate critically the interaction of cell size and division rate on the growth of seed tissues.

The argument just given concerns the rate of growth of the nutritive tissue, but there are perhaps more important factors that must be considered, especially the function of endosperm as a storehouse of energy and nutrients to be utilized during later stages of embryo growth and on seed germination. We suggest that an increase in the amount of DNA and in cell size would also have resulted in a more efficient storage tissue (cf. Cavalier-Smith, 1978). In the early stages of endosperm development, the need for rapid growth may have imposed limits on the increase in cell size, as suggested above. During later stages of development, however, as cell division rate declines, there appear to be distinct advantages to large cell size and increased metabolic activity. For lipid storage, the same vacuole volume can be accommodated in a smaller number of larger cells, with fewer resources tied up in such things as cell membranes, cell walls, and organelles. Likewise, in the case of carbohydrates stored in thick cell walls, larger cells are less costly than an equivalent volume of smaller cells. Increase in cell volume and activity seems to have been achieved primarily through endoreduplication, wherein chromosome replication occurs without mitotic reduction. It is well known that mature endosperm cells generate very large volumes of DNA through this process (D'Amato, 1984) and obtain very large sizes (Vijayaraghavan and Prabhakar, 1984; Jacobsen, 1984). We also note that other plant tissues specialized for rapid nutrient uptake and transfer (e.g., the tapetum in anthers) are characterized by high DNA content because of endopolyploidy (Kapil and Tawari, 1978).

According to our account, the dual requirements of rapid growth and nutrient storage are accomplished by two separate mechanisms affecting the amount of DNA: fusion of nuclei early in development and endopolyploidy later on. Greatly increasing the amount of DNA by endopolyploidy at an early stage might constrain growth rate by slowing the rate of cell division, whereas fusion of a very large number of nuclei would be necessary to increase DNA content to the levels achieved through endopolyploidy later in development.

Aside from concerns about the role of nucleotypic effects, our account may be seen as incomplete for several reasons. One might ask, for example, why there should have been fusion of a male with a female nucleus (rather than two female nuclei, for example), if increased vigor is simply a function of the amount of DNA? In response, it should first be noted that we have not rejected the arguments of Brink and Cooper (1940, 1947) regarding the benefits of heterozygosity. We have simply added another argument based on nucleotypic effects. We think that both played a role in the selection of polyploid endosperm over the female gametophyte. Second, based on the phylogenetic arguments above, double fertilization probably originated in anthophytes prior to the divergence of the angiosperm line. If not, it would have been very likely to occur early in angiosperm evolution owing to siphonogamy and the probable presence of a free female nucleus at the time of fertilization. Thus, we would argue that retention and modification of the product of the second fertilization would have been the path of least resistance, simply because this product was already available.

Another problem concerns the identity of the added nucleus: why was this a second female gametophyte nucleus rather than some other nucleus? For that matter, why not modify a portion of the developing embryo, the nucellus, or the integumentary tissue? Although available evidence cannot rule out the possibility that double fertilization in the angiosperm line yielded a triploid product directly, there is no precedent for an initial fusion of two female nuclei in related plants (except perhaps in *Welwitschia* and *Gnetum*, which represent a highly specialized line within Gnetales). If we therefore assume an initially diploid product, we would argue that the fusion of a second female nucleus would have been the most likely outcome. Fusion of an additional male nucleus would require the presence in the embryo sac of additional male gametes. This would entail the production of additional sperm by increasing the number of mitotic divisions in the development of the male gametophyte. Production of additional sperm is known to occur in some seed plants (especially some conifers: Willson and Burley, 1983), but not in Gnetales or among potentially basal angiosperms (Maheshwari, 1950). Fusion of a diploid nucleus of sporophyte origin seems unlikely in view of the fact that any such nuclei would already have been walled off. Elaboration of other seed tissues as a nutritive tissue would

entail a change in the developmental program of the relevant cells, which would already be committed to different functions. Of course, in some cases with angiosperms, nucellar tissue has been modified for a nutritive function (perisperm), which indicates that this is possible and perhaps an option that was easier than reinstating the nutritive role of the gametophyte.

A third problem arises from concerns that underlie kin conflict arguments (discussed below). Rapid early growth and increased storage/transfer capacity could be viewed as increasing the "aggressiveness" of the endosperm. This is seen as conflicting with the interests of the sporophyte, which is viewed as partitioning a fixed (or significantly limited) quantity of nutrients among its offspring. We believe that the strength of this conflict may have been exaggerated, and that evidence presented in favor of conflict is consistent with alternative explanations. The development of invasive haustoria (Masand and Kapil, 1966) may not reflect conflict; instead, such structures may simply have been selected for their role in garnering nutrients for the rapidly developing embryo. The observation that such haustoria are most often formed by embryos and endosperms (Queller, 1983) may simply reflect the fact that these tissues are undergoing growth at the appropriate time and are not already committed to other functions (as opposed to the gametophyte or adjacent sporophyte tissues). Likewise, the role of hypostase tissue, found in only some angiosperm seeds, is still disputed (see Masand and Kapil, 1966; Haig and Westoby, 1988, and references therein).

In this regard, it is important to consider relevant aspects of source-sink relationships in plants, in particular the finding that there is not a fixed pool of photosynthate resource. Seeds, particularly endosperm tissues, tend to be rich in lipids relative to other plant tissues, providing a high-energy source of carbon for embryo and seedling development (Mooney, 1972; Levin, 1974; Vijayaraghavan and Prabhakar, 1984; Jacobsen, 1984). Thus, the developing endosperm is a sink for carbon and energy. The increased demand for photosynthates by the endosperm sink can result in an increased rate of photosynthesis in source tissues (Sweet and Wareing, 1966; Evans and Rawson, 1970; Geiger, 1976; Watson and Casper, 1984). Embryos with different photosynthate sources, such as those in different fruits, would not be affected. Furthermore, if the sporophyte is limited by some other resource, such as nitrogen, a more "aggressive" endosperm can increase carbon and energy resources for its associated embryo without decreasing the amount available to the sporophyte. Indeed, it is often nitrogen, not photosynthate, that limits sporophyte growth (see Tilman, 1984, and references therein).

Even though the pool of photosynthate may not be limited on a whole plant basis, for a single sink (e.g., a seed or fruit) there is still a limit to the amount of photosynthate that can be supplied per unit time (Garrish and Lee, 1989). In cycads, ginkgo, and conifers, seed development may take many months. Even with the lower photosynthetic rates characteristic of conifers (Mooney, 1972), there is ample time to supply the developing seed with sufficient photosynthate. If the amount of time for seed development were significantly limited, however, then the rate of photosynthesis might become a limiting factor. Under these circumstances a polyploid tissue might even have been selected so as to increase the carbon and energy sink and, thereby, increase photosynthetic rate.

Explanations Involving Kin Selection

The application of kin selection arguments to the evolution of endosperm has been carefully reviewed elsewhere (e.g., Queller, 1989), and it is not our intention to provide a detailed critique. Instead, we wish to highlight several general features that are relevant in evaluating the role of kin selection and then to contrast aspects of previous scenarios with our own. In making comparisons with other explanations, our aim is not to give an exhaustive account of previous arguments, but rather to begin to identify important differences and critical tests. More generally, we hope to convince the reader that explanations such as ours, which do not invoke kin selection, warrant renewed attention.

General Considerations

Kin selection explanations are largely elaborations of two models presented by Charnov (1979). In the first model, double fertilization makes the endosperm more similar genetically to its associated embryo than to embryos in other seeds borne by the same sporophyte and, therefore, a stronger competitor for nutrients for its embryo. In Charnov's second model, double fertilization permits the male gametophyte to play a role in garnering resources for its offspring. The increased competition for nutrients is viewed in this case as a conflict of interest between the male and female parents as to how the seed should be provisioned. Both models assume resource trade-offs leading to conflict over the distribution of investment (Trivers, 1974): when more resources are directed to one embryo, other embryos on the parent sporophyte suffer a cost, either by a direct reduction in resources or indirectly through effects on the survival of the sporophyte. In general, provisioning by tissues within the seed is selected to increase at a cost to other seeds on the sporophyte to the extent that the cost–benefit ratio is less than the coefficient of relatedness (r) with other seeds (Hamilton, 1964). When embryos borne on a given sporophyte are sired by different fathers and are therefore half-sibs ($r = \frac{1}{4}$), the embryo should

favor its seed more than the endosperm will, the endosperm more than the female gametophyte, and the female gametophyte more than the parent sporophyte.

Several assumptions of published kin selection models may limit their relevance in accounting for the evolution of endosperm. Kin selection models generally have assumed (for simplicity) the existence of a single locus that controls the nutrient-garnering ability ("aggressiveness") of the embryo, endosperm, gametophyte, and parent sporophyte (Queller, 1984; Law and Cannings, 1984; Bulmer, 1986). There is as yet no direct evidence for the existence of such a locus, and we interpret several studies as casting doubt on this assumption (Nakamura and Stanton, 1989; Schwaegerle and Levin, 1990). Haig and Westoby (1989b) reviewed evidence for parent-specific gene expression in maize and postulated the existence of a second set of loci (epistatic with the first) that would yield these genotypic effects. Multilocus models may give the same qualitative outcome as single-locus models, but this will depend on details of the genetic system.

In addition, genetic models are not entirely robust to changes in assumptions regarding the degree of relatedness and the timing of the effects of selfishness. Models that result in strong kin selection effects are based on outcrossing plants that store significant amounts of energy, so that the immediate effects of selfishness are not felt by contemporary embryos. The outcome predicted on the basis of simple calculations of relatedness may not be obtained when selfishness affects contemporary seeds that may be more closely related than half-sibs (compare Queller, 1984; Law and Cannings, 1984). Queller (1989) provided a thorough analysis of this point and concluded that the predicted rank-order of aggressiveness (embryo>endosperm>gametophyte>mother) is generally upheld. However, he also identified several conditions under which it is not; in general, when overconsumption affects siblings nonrandomly. Even if the rank order held, however, the predicted strength of selection would differ depending on the level of outcrossing and the life form of the plants under consideration.

The fossil record of angiosperms suggests that they were colonizing plants at first, living primarily in disturbed environments, where they were strongly selected for rapid maturation and reproduction (Stebbins, 1974; Doyle and Hickey, 1976). The first angiosperms were probably insect-pollinated (most likely by beetles and/or flies: Crepet and Friis, 1987) and may have produced flowers with multiple ovules. Under these circumstances, inbreeding might have been common and seeds in a single fruit might frequently have been full-sibs. Moreover, it may well have been contemporary siblings that would have felt the effects of an overconsuming embryo. If so, the likelihood that kin selection played an important role in the evolution of endosperm is decreased.

It is also important to note that most such models assume that the fitness of a seed is a concave monotonically increasing function of the amount of resource that it garners. The optimal resource allocation for a given seed vis-à-vis the parent sporophyte exists at the inflection point of the fitness function, where more benefit to the sporophyte is gained by allocating additional resources to a different seed. These assumptions may be inappropriate for several reasons. First, if increased demand by the seed increases the available pool of resources, as described above, then an optimal allocation point may not exist. Second, even if an optimal resource allocation exists, seeds may be constrained below the optimal level, perhaps by a limit on the rate of development (e.g., Benner and Bazzaz, 1985). Finally, kin selection models (as standardly formulated) assume that the critical determinant of fitness is the amount of resource, but if a particular size or shape of seed enhances dispersal or germination (or some other critical attribute), then increasing the amount of resource obtained could actually decrease fitness. Our own model, in contrast, focuses directly on rate of development and storage efficiency of the nutritive tissue as critical factors. In this connection it is noteworthy that weedy angiosperms in ephemeral environments typically produce small seeds (presumably as an adaptation for rapid development and/or dispersal: Salisbury, 1942; Harper et al., 1970; Levin, 1974), and that the early angiosperms also produced small seeds (Tiffney, 1986).

Scenarios, Predictions, and Tests

Kin selection arguments have sometimes been accompanied by specific scenarios for the evolution of double fertilization and endosperm. In this section we briefly consider several of these (Westoby and Rice, 1982; Queller, 1983; Willson and Burley, 1983) in order to highlight ways in which our own account differs. Predictions based on these alternatives suggest a number of tests.

Westoby and Rice (1982) proposed that selection for deferment of maternal investment in offspring, which would allow a better assessment of offspring quality, was a driving force in the evolution of endosperm. The first evolutionary step was a switch in the timing of fertilization, such that it occurred prior to extensive development of the female gametophyte. This allowed maternal investment after fertilization and placed the embryo in a position to garner resources aggressively. The evolution of endosperm was a response on the part of the sporophyte to interpose a less aggressive tissue between it and the embryo.

Westoby and Rice (1982) implied that precocious fertilization arose as an adaptation for deferment of investment. According to our explanation (and others: e.g., Takhtajan, 1976; Queller, 1983), this switch was an effect

of selection for rapid maturation and was therefore not itself an adaptation (sensu Gould and Vrba, 1982; Greene, 1986; Coddington, 1988). We agree that precocious fertilization set the stage for the evolution of endosperm, but we believe, with Queller (1983), that it did so by placing the product of double fertilization on nearly equal footing with the much-reduced gametophyte. Under these circumstances, the polyploid tissue would have been favored by selection because of its increased growth rate and ability to garner and store nutrients. Whereas Westoby and Rice (1982) would predict a decrease in "aggressiveness" with the addition of a second maternal nucleus, we argue that the triploid tissue would have been more aggressive.

According to Westoby and Rice (1982), alternative hypotheses do not explain the observation that endosperm is found only where maternal investment is deferred until after fertilization or the fact that endosperm contains more doses of maternal than paternal genes. Regarding the correlation, reduction of the female gametophyte (and deferment of maternal investment) created the need for a rapidly growing storage tissue, setting the stage for the evolution of endosperm. According to our hypothesis, triploidy was selected for its effects on vigor and an extra maternal genome was added because it was readily available, whereas other possibilities were not (see above).

Queller (1983) recognized that reduction of the female gametophyte, brought about by selection to reproduce quickly, might permit double fertilization to occur and would put the gametophyte and the product of the second fertilization on nearly equal footing. Kin selection enters his argument in explaining the presumably gradual switch in the source of the nutritive tissue. The role of the (diploid) "endosperm" would increase because it would be selected to garner more resources for its embryo. This increase would be countered by a concomitant reduction in the gametophyte, because it would presumably be advantageous to the sporophyte to maintain a fixed level of resource allocation. Triploidy is seen as a possible maternal strategy to reduce the aggressiveness of the endosperm, but its appearance was not integrated by Queller (1983) into the sequence of events.

This scenario is generally consistent with our own except for one important point. In our view, the switch to endosperm came about because it grew more rapidly and yielded a better storage tissue. Any further reduction in the size of the gametophyte occurred because there were limits on seed size and only the most efficient storage tissue was retained. Furthermore, the presence of a new nutritive tissue meant that the gametophyte could become increasingly specialized for its role in fertilization. We hypothesize that the addition of a second female gametophyte nucleus was

favored because it further increased the endosperm's growth rate and storage capacity.

Queller (1983) made several predictions based on kin selection, including (1) metabolic activity should be highest in endosperm, which should predominate over the female gametophyte in nourishing the embryo; (2) when endosperm is lost, its function should be taken over by the embryo; and (3) there should be reduced conflict in self-fertilizing or apomictic species. The first prediction is consistent with our hypothesis, but we assume that increased metabolic activity resulted from the nucleotypic effects of polyploidy, not from single locus genotypic effects. Our argument also implies that triploidy should increase metabolic activity, not decrease it, as predicted by most kin selection models.

In support of the second prediction, Queller (1983) noted that most angiosperms store seed reserves either in the endosperm or in the embryo, rather than in the gametophyte or maternal tissue. However, as he also noted, the evidence is equivocal. Thus, loss of endosperm function in the Caryophyllidae was accompanied by the evolution of perisperm, which originates from the nucellus (parent spropophyte), while in grasses, antipodal cells (of gametophytic origin) may play a nutritional role early in development (cf. Willemse and van Went, 1984). Queller (1983) observed that endosperm has been lost in Orchidaceae, Trapaceae, and Podostemaceae, and that in these cases there has been compensation in the form of absorptive suspensors (proembryo tissue). As we suggested above, haustoria do not provide unambiguous evidence of conflict. In any case, Queller (1983) also indicated that there may be compensation by the parental sporophyte in these plants.

Regarding the third prediction, Queller (1983) cited a comparison of an apomictic and a sexual species of *Taraxacum* (Cooper and Brink, 1949). In support of decreased conflict, he noted that apomicts averaged fewer endosperm cells at a given embryo size, but we find these data difficult to interpret. Cooper and Brink (1949) showed that in the absence of normal fertilization the development of the embryo and endosperm are uncoupled, but it is not clear that this resulted from selection for diminished conflict. The number of endosperm cells varies enormously during early stages of embryo growth, but apomictic endosperms apparently grow more rapidly at later stages and reach larger sizes. This is consistent with our account, as the apomicts also seem to have a larger genome size in this case.

Willson and Burley (1983) summarized a variety of kin selection arguments, but they reemphasized Charnov's (1979) idea that the evolution of double fertilization reflected male–female conflict. They argued that selection for polyploidy is due to "gene level" effects (not "individual level" effects), because polyploidy changes the number of loci but does not affect relatedness. The possible effects of polyploidy on nucleus and cell volume,

growth rate, and RNA synthesis were discussed by Willson and Burley (1983), but these factors were not explicitly incorporated into a model for the origin of double fertilization and endosperm. Finally, based on the observation that in many modern angiosperms fusion of maternal nuclei occurs before arrival of the sperm, they suggested that the evolution of the fusion of polar nuclei might have preceded double fertilization.

In our model, double fertilization is seen as an incidental by-product of selection for rapid reproduction, continued reduction of the female gametophyte, and siphonogamy, not the outcome of male–female conflict. In contrast to Willson and Burley (1983) we argue that polyploidy was favored because of its nucleotypic, not genotypic, effects. Seed plant phylogenies, and the evidence from *Ephedra* reviewed above, indicate that double fertilization evolved before the addition of a second female nucleus, and we therefore interpret the precocious fusion of polar nuclei as having been derived within the angiosperm line.

From the foregoing comparisons it should be clear that even when kin selection is invoked, it is envisioned to have played a rather limited role. Many of the critical events in these scenarios, such as the reduction of the female gametophyte, the origin of double fertilization, and the initial persistence of the product of double fertilization, are simply not explained by kin selection. Because much of our account deals with these events, many of our suggestions are not at odds with kin selection arguments. In other words, if there are disagreements about the mechanisms we have proposed for these events, these should not be interpreted as disagreements over the role of kin selection.

There do appear to be a few critical differences between our account and kin selection accounts—differences that actually concern the role of kin selection. In several of the scenarios presented above, kin selection is thought to have been critical in the transition from dominance of the female gametophyte to dominance of polyploid endosperm. A diploid tissue derived from a second fertilization should be selected to garner more resources, and the gametophyte responds by garnering less, thereby maintaining the same overall level of support on the part of the sporophyte. Our alternative is that the polyploid cell line was selected because it grew faster than the haploid cell line and made a more efficient storage tissue. The gametophyte was then reduced in favor of the more efficient tissue and became increasingly specialized for fertilization. Our account identifies an immediate outcome on which selection could act, whereas kin selection arguments do not (Queller, 1989).

A second difference concerns the consequences of adding a second female gametophyte nucleus. Some kin selection arguments interpret this as the sporophyte's way of making a less aggressive nutritive tissue, which implies that the triploid endosperm should show a decrease in metabolic

activity and growth rate. This has been a major difficulty, because the endosperm contains qualitatively the same alleles as the embryo. To explain how a decrease might come about, Haig and Westoby (1989b) invoked parent-specific gene expression coupled with competition for limiting factors (e.g., nucleotides) in polyploid cells (see Queller, 1989). In contrast, we propose that the triploid tissue would have had a higher metabolic level, growth rate, and storage capacity (also see Bulmer, 1986). Here, it will be critical to make quantitative comparisons of the growth rates of diploid versus triploid endosperms, making use of naturally occurring variants or experimentally manipulated tissues. Broad phylogenetic comparisons (e.g., comparing Onagraceae, which have diploid endosperms, to related plants with triploid endosperm) might not be very revealing, as a variety of factors may have subsequently influenced endosperm growth. Because our suggestions concern the immediate consequences of an increase in ploidy, comparisons of tissues within species or among close relatives are more appropriate. Additional comparisons of sexual and apomictic plants, along the lines reported by Cooper and Brink (1949), might be especially useful.

These considerations highlight a more fundamental difference between our account and kin selection models. Kin selection arguments assume the existence of one or more genes that control the nutrient garnering ability of the endosperm. While we assume that there are genes that are expressed in the endosperm, which influence endosperm structure and function, it remains to be seen whether there are genes that have the particular effects required by kin selection models. It would obviously be of great significance in evaluating the alternatives if genes of this sort were discovered. In our view the amount of DNA can have overriding and more immediate effects upon which selection might act. As outlined above, evidence already exists for such effects, but quantitative studies of tissue growth are needed to evaluate our proposal further.

Finally, a point of departure between our hypothesis and the male–female conflict models (Charnov, 1979; Willson and Burley, 1983) concerns whether or not double fertilization arose as an adaptive strategy. An assumption of male–female conflict is that some immediate advantage existed for those organisms in which double fertilization first occurred. In contrast, we favor the view that double fertilization was an accident and did not confer any immediate advantage. We predict that double fertilization will occur automatically whenever one or more free female nuclei are present in the vicinity of the egg at the time that sperm are delivered (if both sperm are viable). Friedman's (1990a,b) studies of double fertilization in *Ephedra* provide support for this position, but the movement of the ventral canal nucleus to a favorable position within the egg cytoplasm requires further attention. In particular, we suppose that this movement occurred (at least initially) as a consequence of other changes during the development of the

cell (e.g., movement of the egg nucleus/zygote). If there were significant variation in the occurrence of double fertilization in *Ephedra* and if it were possible to determine whether it had taken place, this might provide a means of testing whether there are any immediate advantages associated with double fertilization. Clearly, we need to know more about the fate of the product of double fertilization in *Ephedra*. In any case, Friedman's (1990a,b) observations suggest that double fertilization may be largely under maternal control, which conflicts with the view that it is a male strategy.

Diversity within Angiosperms

The account presented above points to the importance of selection for increased rate of development, the role of chance in relation to fusion of nuclei, and the benefits of increased DNA content in connection with tissue growth rate and storage capacity. These same factors could also explain the origin and maintenance of much of the embryological variation seen within angiosperms, some of which has been interpreted as the outcome of kin selection (e.g., Haig, 1990).

Based on our best understanding of angiosperm phylogeny, modifications of *Polygonum*-type development are known to have originated many times independently and to occur sporadically throughout angiosperms, but the regular occurrence of derived types is phylogenetically quite limited and evidently nonrandom in distribution. In particular, bisporic and tetrasporic types appear to us to have evolved most often in aquatic plants (e.g., Podostemaceae), in parasitic plants (e.g., Loranthaceae), and in plants that are otherwise highly reduced or grow in extreme environments (e.g., *Adoxa*). Better phylogenetic tests of the sequence of evolutionary events are needed, but these correlations suggest that selection to speed up sexual reproduction might have driven the reduction and elimination of steps in the development of the female gametophyte and endosperm.

The occurrence of bisporic and tetrasporic types in Liliaceae (sensu lato) appears to be an exception to the idea that modifications of female gametophyte development are related to selection for rapid reproduction. In some geophytic groups, however, the time available to complete early reproductive events may be highly constrained, especially where these take place just before the onset of cold weather and dormancy. There may also be other factors involved, which foster selection for derived types. For example, it has been suggested (Geeta Bharathan, personal communication) that the large genome sizes characteristic of many of these monocots (Grime and Mowforth, 1982) may slow the rate of cell division, and that this might have selected for the elimination of developmental events (e.g., cell wall formation).

The fusion of additional female nuclei in some angiosperms (resulting in endosperm of higher ploidy) and changes from a bipolar to tetrapolar arrangement of nuclei in the embryo sac (e.g., the *Peperomia* type in Piperaceae) might have originated as an outcome of shifts in the timing of mitotic divisions in relation to cell size, cell shape, and the timing of vacuole formation. Such shifts may have altered the proximity of female gametophyte nuclei (and hence their propensity to undergo fusion) and the orientation of divisions. Although the fusion of additional nuclei may have originated as an accidental consequence of other developmental changes, endosperms of higher ploidy may have been selected for their effects on growth rate and storage capacity.

The arguments just presented to account for changes within angiosperms obviously closely parallel our arguments for the origin of endosperm. Selection to speed up reproduction may have resulted in reduction of the female gametophyte in some lines of angiosperms and elimination of developmental steps. For example, elimination of wall formation after meiosis and maintenance (and subsequent division) of all four products of meiosis would result in the tetrasporic pattern of development. These changes may have brought about the fusion of additional female gametophyte nuclei, especially when accompanied by reduction of the ovule and changes in embryo sac shape. As noted above, the evolution of much higher ploidy levels may have been constrained by the negative effects of very large amounts of DNA on cell division rate, with further changes in cell size and storage capacity being brought about instead by endoreduplication later in development.

Our reactions to kin selection arguments that have been applied to the evolution of the angiosperm female gametophyte also parallel the arguments given above. First, it is important to note that kin selection explanations have not been given for the basic changes in embryo sac development described above (Haig, 1990). For example, no explanation has been provided for the shift from monosporic to bisporic or tetrasporic embryo sacs or for the shift occurring in particular habitats or plant groups. Instead, kin selection has been viewed as effecting subsequent changes in derived embryo sacs, where there might have been conflict among genetically distinct nuclei. Haig (1990) suggested, for example, that the elimination in some tetrasporic embryo sacs of some or all of the chalazal divisions leading to the antipodals (a phenomenon known as "strike") may have been the result of selection to minimize competition for fertilization between these nuclei and the egg. He postulated that if the antipodals were retained this would have allowed the production of "antipodal eggs" and that suppression of divisions was selected as a mechanism to avoid this.

As Haig (1990) himself noted, there is no evidence for this scenario. In particular, antipodal eggs are not found in tetrasporic groups, so that the supposed problem to which "strike" is the solution might never have existed. Furthermore, we can provide a simpler explanation that does not involve kin selection, which is consistent with our observations on the distribution and possible causes of tetrasporic development. Given that antipodals are expendable (as they seem to be in most groups), and given that selection for rapid reproduction was intense, it stands to reason that the divisions giving rise to these cells might have been omitted. Again, our explanation highlights timing and efficiency.

Conclusions

Previous discussions of the evolution of endosperm have focused on mechanisms such as heterosis or kin selection and have argued from supposed selection pressures to a sequence of evolutionary events (e.g., Haig and Westoby, 1989b). In contrast, we inferred a chronicle of evolutionary events by reference to a hypothesis of seed plant relationships and then considered the possible causes of the hypothesized character state changes (O'Hara, 1988). This approach helps to focus attention on how changes inside the seed might have been related to environmental and morphological changes associated with the origin of angiosperms and to ecological shifts within angiosperms. Moreover, analysis of evolutionary changes in a phylogenetic context focuses attention on interrelationships among changes and possible common causes. For example, a single cause (viz. selection for more rapid reproduction) may have been responsible for siphonogamy, reduction of the female gametophyte, and, incidentally, double fertilization. Likewise, selection within angiosperms for rapid reproduction may have driven the evolution of bisporic and tetrasporic embryo sacs.

A phylogenetic perspective also suggests how one change may have set the stage for another (Donoghue, 1989). Thus, with double fertilization already established, further reduction in the size of the gametophyte apparently set the stage for the elaboration of a polyploid nutritive tissue. In this light, double fertilization can be seen as an exaptation (Gould and Vrba, 1982; Greene, 1986; Baum and Larson, 1991), its regular occurrence having preceded the function with which it later became associated (Donoghue, 1989). Even changes that are spatially or historically separated from one another may have had a common underlying cause. For example, it may be that a polyploid nutritive tissue, endoreduplication, and fusion of additional nuclei in the development of some angiosperm female gametophytes were all selected as a function of the beneficial effects of increasing the amount of DNA per cell on tissue growth rate and/or storage capacity.

We have argued that the available evidence is consistent with an explanation for the origin of endosperm that does not require intersexual or kin selection. Indeed, conflict arguments are unable to explain some of the changes that appear to have been critical in the evolution of endosperm (e.g., selection for rapid reproduction), and they fail to account for the origin and persistence of the tissues on which kin selection is supposed to have acted. The mechanisms we have identified (e.g., incidental fusion of nuclei; benefits associated with increased DNA) are meant to explain the origin and maintenance of seed tissues. Kin selection might have operated to shift the allocation of resources in these tissues, but, as we have suggested, there may be even simpler explanations. The same sorts of arguments also apply to changes within angiosperms. Kin conflict arguments have not been advanced for the origin of derived embryo sac types, whereas we can provide explanations for these shifts in terms of development time, as well as for the minor changes to which kin selection arguments have been applied.

Most importantly, our analysis focuses attention on the need for more concrete information on the timing of developmental events. In the past, emphasis has been placed primarily on describing the fusion of various nuclei and on the theoretical consequences of the existence of different genotypes within the seed. Our account points to the possibly overriding effects of the volume of DNA present in cells and its impact on growth rates. The limited information available on rates of development is consistent with our arguments, but much more data of this sort are needed in order to choose among competing hypotheses.

Additional Evidence on Post-Fertilization in Ephedra

Friedman (1992) recently confirmed earlier suggestions (Kahn, 1943) that the product of the second fertilization event in *Ephedra* can undergo several mitotic divisions. The four nuclei produced by two sets of free-nuclear divisions are diploid, and there is no evidence that they function in nutrition of the embryo. Therefore, it appears that triploidy and nutritive function are derived features of angiosperms.

According to Friedman (1992) the development of the second fertilization product in *Ephedra* is "fundamentally similar" to free-nuclear endosperm development in angiosperms, which he presumed to be the primitive condition (following Stebbins, 1974). However, as he noted, free-nuclear development in angiosperms is far more extensive than it is in *Ephedra*. Furthermore, in the case of free-nuclear development in angiosperms cell walls are usually not formed until the embryo is differentiated. Finally, Friedman's (1992) assumption that free-nuclear endosperm development is primitive in angiosperms is questionable: it is not supported by phylo-

genetic analyses of angiosperms (Donoghue and Doyle, 1989a), which imply that cellular development is ancestral. If cellular endosperm development is primitive in angiosperms, then the transition to endosperm must have involved a change in the timing of cell wall formation.

Acknowledgments

We thank Larry Venable and his Plant Population Ecology seminar of 1985 for stimulating our interest in this subject, and L. Aviles, B. Baldwin, G. Bharathan, J. Doyle, M. Folsom, W. Friedman, B. Knox, D. Lloyd, S. Mazer, D. Queller, and R. Wyatt for thoughtful comments on the ideas presented here. David Queller provided a very helpful review of the manuscript.

Literature Cited

Allen, G.S. 1946. Embryogeny and development of the apical meristems of *Pseudotsuga*. I. Fertilization and early embryogeny. Am. J. Bot. **33**:666–677.

Battaglia, E. 1989. The evolution of the female gametophyte of angiosperms: An interpretative key. (Embryological questions: 14). Ann. Bot. (Rome) **47**:7–144.

Baum D.A., and A. Larson. 1991. Adaptation reviewed: A phylogenetic methodology for studying character macroevolution. Syst. Zool. **40**:1–18.

Benner, B.L., and F.A. Bazzaz. 1985. Response of the annual *Abutilon theophrasti* Medic. (Malvaceae) to timing of nutrient availability. Am. J. Bot. **72**:320–323.

Bennett, M.D. 1973. The duration of meiosis. *In* M. Balls and F.S. Billett, eds., The Cell Cycle in Development and Differentiation. Cambridge University Press, Cambridge, pp. 111–131.

Brink, R.A., and D.C. Cooper. 1940. Double fertilization and development of the seed in angiosperms. Bot. Gaz. **102**:1–25.

Brink, R.A., and D.C. Cooper. 1947. The endosperm in seed development. Bot. Rev. **13**:423–541.

Brooks, D.R., and D.A. McLennan. 1991. Phylogeny, Ecology, and Behavior. University of Chicago Press, Chicago.

Bryan, G.S., and R.I. Evans. 1957. Types of development from the central nucleus of *Zamia umbrosa*. Am. J. Bot. **44**:404–415.

Bulmer, M.G. 1986. Genetic models of endosperm evolution in higher plants. *In* S. Karlin and E. Nevo, eds., Evolutionary Process and Theory. Academic Press, New York, pp. 743–763.

Cavalier-Smith, T. 1978. Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. J. Cell Sci. **34**:247–278.

Cavalier-Smith, T. 1985a. Introduction: The evolutionary significance of genome size. *In* T. Cavalier-Smith, ed., The Evolution of Genome Size. John Wiley, New York, pp. 1–36.

Cavalier-Smith, T. 1985b. Cell volume and the evolution of eukaryotic genome size. *In* T. Cavalier-Smith, ed., The Evolution of Genome Size. John Wiley, New York, pp. 105–184.

Cavalier-Smith, T. 1985c. DNA replication and the evolution of genome size. *In* T. Cavalier-Smith, ed., The Evolution of Genome Size. John Wiley, New York, pp. 211–251.

Charnov, E.L. 1979. Simultaneous hermaphroditism and sexual selection. Proc. Natl. Acad. Sci. U.S.A. 76:2480–2484.

Coddington, J. 1988. Cladistic tests of adaptational hypotheses. Cladistics **4**:3–22.

Cook, R.E. 1981. Plant parenthood. Nat. Hist. **90**:30–35.

Cooper, D.C., and R.A. Brink. 1949. The endosperm-embryo relationship in an autonomous apomict, *Taraxacum officinale*. Bot. Gaz. **111**:139–153.

Cornet, B. 1986. The leaf venation and reproductive structures of a Late Triassic angiosperm, *Sanmiguelia lewisii*. Evol. Theory **7**:231–309.

Crane, P.R. 1985. Phylogenetic analysis of seed plants and the origin of angiosperms. Ann. Missouri Bot. Gard. **72**:716–793.

Crane, P.R. 1987. Vegetational consequences of the angiosperm diversification. *In* E.M. Friis, W.G. Chaloner, and P.R. Crane, eds., The Origins of Angiosperms and Their Biological Consequences. Cambridge University Press, Cambridge, pp. 107–144.

Crane, P.R. 1988. Major clades and relationships in the "higher" gymnosperms. *In* C.B. Beck, ed., Origin and Evolution of Gymnosperms. Columbia University Press, New York, pp. 218–272.

Crepet, W.L., and E.M. Friis. 1987. The evolution of insect pollination in angiosperms. *In* E.M. Friis, W.G. Chaloner, and P.R. Crane, eds., The Origins of Angiosperms and Their Biological Consequences. Cambridge University Press, Cambridge, pp. 181–201.

Cronquist, A. 1988. The Evolution and Classification of Flowering Plants, 2nd ed. New York Botanical Garden, Bronx, NY.

D'Amato, F. 1977. Nuclear Cytology in Relation to Development. Cambridge University Press, Cambridge.

D'Amato, F. 1984. Role of polyploidy in reproductive organs and tissues. *In* B.M. Johri, ed., Embryology of Angiosperms. Springer-Verlag, Berlin, pp. 519–566.

Davis, G.L. 1966. Systematic Embryology of Angiosperms. John Wiley, New York.

Donoghue, M.J. 1989. Phylogenies and the analysis of evolutionary sequences, with examples from seed plants. Evolution **43**:1137–1156.

Donoghue, M.J., and J.A. Doyle. 1989a. Phylogenetic studies of seed plants and angiosperms based on morphological characters. *In* B. Fernholm, K. Bremer, and H. Jornvall, eds., The Hierarchy of Life. Elsevier, Amsterdam, pp. 181–193.

Donoghue, M.J., and J.A. Doyle. 1989b. Phylogenetic analysis of angiosperms and the relationships of Hamamelidae. *In* P. Crane and S. Blackmore, eds., Evolution, Systematics and Fossil History of the Hamamelidae, Vol. 1. Clarendon Press, Oxford, pp. 17–45.

Donoghue, M.J., J.A. Doyle, J. Gauthier, A.G. Kluge, and T. Rowe. 1989. The importance of fossils in phylogeny reconstruction. Annu. Rev. Ecol. Syst. **20**:431–460.

Doyle, J.A. 1978. Origin of angiosperms. Annu. Rev. Ecol. Syst. **9**:365–392.

Doyle, J.A. 1984. Evolutionary, geographic, and ecological aspects of the rise of angiosperms. *In* Proceedings of the 27th International Geological Congress, 2 (Moscow, 1984). VNU Science Press, Utrecht, pp. 23–33.

Doyle, J.A., and M.J. Donoghue. 1986. Seed plant phylogeny and the origin of angiosperms: An experimental cladistic approach. Bot. Rev. **52**:321–431.

Doyle, J.A., and M.J. Donoghue. 1992. Fossils and seed plants revisited. Brittonia 44:in press.

Doyle, J.A., and L.J. Hickey. 1976. Pollen and leaves from the mid-Cretaceous Potomac group and their bearing on early angiosperm evolution. *In* C.B. Beck, ed., Origin and Early Evolution of Angiosperms. Columbia University Press, New York, pp. 139–206.

Dumas, C., R.B. Knox, C.A. McConchie, and S.D. Russell. 1984. Emerging physiological concepts in fertilization. What's New Plant Phsiol. **15**:17–20.

Evans, L.T., and H.M. Rawson. 1970. Photosynthesis and respiration by the flag leaf and components of the ear during grain development in wheat. Aust. J. Biol. Sci. **23**:245–254.

Foster, A.S., and E.M. Gifford. 1974. Comparative Morphology of Vascular Plants, 2nd ed. W. H. Freeman, San Francisco.

Friedman, W.E. 1990a. Double fertilization in *Ephedra*, a nonflowering seed plant: Its bearing on the origin of angiosperms. Science **247**:951–954.

Friedman, W.E. 1990b. Sexual reproduction in *Ephedra nevadensis* (Ephedraceae): Further evidence of double fertilization in a nonflowering seed plant. Am. J. Bot. **77**:1582–1598.

Friedman, W.E. 1992. Evidence of a pre-angiosperm origin of endosperm: Implications for the evolution of flowering plants. Science **225**:336–339.

Garrish, R.S., and T.D. Lee. 1989. Physiological integration in *Cassia fasciculata* Michx.: Inflorescence removal and defoliation experiments. Oecologia **81**:279–284.

Geiger, D.R. 1976. Effects of translocation and assimilate demand on photosynthesis. Can. J. Bot. **54**:2337–2345.

Gould, S.J., and E.S. Vrba. 1982. Exaptation—a missing term in the science of form. Paleobiology **8**:4–15.

Greene, H.W. 1986. Diet and arboreality in the monitor, *Varanus prasinus*, with comments on the study of adaptation. Fieldiana Zool. **31**:1–12.

Grime, J.P., and M.A. Mowforth. 1982. Variation in genome size—an ecological interpretation. Nature (London) **299**:151–153.

Grime, J.B., J.M.L. Shacklock, and S.R. Band. 1985. Nuclear DNA contents, shoot phenology and species co-existence in a limestone grassland community. New Phytol. **100**:435–445.

Haig, D. 1990. New perspectives on the angiosperm female gametophyte. Bot. Rev. **56**:236–274.

Haig, D., and M. Westoby. 1988. Inclusive fitness, seed resources, and maternal care. *In* J. Lovett Doust and L. Lovett Doust, ed., Plant Reproductive Ecology Patterns and Strategies. Oxford University Press, Oxford, pp. 60–79.

Haig, D., and M. Westoby. 1989a. Selective forces in the emergence of the seed habit. Biol. J. Linn. Soc. **38**:215–238.

Haig, D., and M. Westoby. 1989b. Parent-specific gene expression and the triploid endosperm. Am. Nat. **134**:147–155.

Hamby, R.K., and E.A. Zimmer. 1991. Ribosomal RNA as a phylogenetic tool in plant systematics. *In* P.S. Soltis, D.E. Soltis, and J.J. Doyle, eds., Molecular Systematics in Plants. Chapman & Hall, New York, pp. 50–91.

Hamilton, W.D. 1964. The genetical evolution of social behavior. J. Theor. Biol. **7**:1–52.

Harper, J.L., P.H. Lovell, and K.G. Moore. 1970. The shapes and sizes of seeds. Annu. Rev. Ecol. Syst. **1**:327–356.

Harris, T.M. 1951. The relationships of the Caytoniales. Phytomorphology **1**:29–39.

Harris, T.M. 1964. The Yorkshire Jurassic flora. II. Caytoniales, Cycadales & Pteridosperms. British Museum (Natural History), London.

Herzfeld, S. 1922. *Ephedra campylopoda* Mey. Morphologie der weiblichen Blute und Befruchtungvorgang. Denkschriften Acad. Wiss. Wein. **98**:243–268.

Hickey, L.J., and J.A. Doyle. 1977. Early Cretaceous fossil evidence for angiosperm evolution. Bot. Rev. **43**:3–104.

Hutchinson, A.H. 1915. Fertilization in *Abies balsamea*. Bot. Gaz. **60**:457–472.

Jacobsen, J.V. 1984. The seed: Germination. *In* B.M. Johri, ed., Embryology of Angiosperms. Springer-Verlag, Berlin, pp. 611–646.

Johri, B.M., ed. 1984. Embryology of Angiosperms. Springer-Verlag, Berlin.

Johri, B.M., and K.B. Ambegaokar. 1984. Embryology: Then and now. *In* B.M. Johri, ed., Embryology of Angiosperms. Springer-Verlag, Berlin, pp. 1–52.

Kapil, R.N., and S.C. Tawari. 1978. The integumentary tapetum. Bot. Rev. **44**:457–490.

Kahn, R. 1940. A note on "double fertilization" in *Ephedra foliata*. Curr. Sci. **9**:323–334.

Khan, R. 1943. Contributions to the morphology of *Ephedra foliata* Boiss. II. Fertilization and embryogeny. Proc. Natl. Acad. Sci. India **13**:357–375.

Land, W.J.G. 1902. A morphological study of *Thuja*. Bot. Gaz. **34**:249–259.

Land, W.J.G. 1904. Spermatogenesis and oogenesis in *Ephedra trifurca*. Bot. Gaz. **38**:1–18.

Land, W.J.G. 1907. Fertilization and embryogeny in *Ephedra trifurca*. Bot. Gaz. **44**:273–292.

Law, R., and C. Cannings. 1984. Genetic analysis of conflicts arising during development of seeds in the Angiospermophyta. Proc. R. Soc. London Ser. B **221**:53–70.

Lehmann-Baerts, M. 1967. Etude sur les Gnetales—XII. Ovule, gametophyte femelle et embryogenese chez *Ephedra distachya* L. Cellule **67**:53–87.

Levin, D.A. 1974. The oil content of seeds: An ecological perspective. Am. Nat. **108**:193–206.

Loconte, H., and D.W. Stevenson. 1990. Cladistics of the Spermatophyta. Brittonia **42**:197–211.

Maddison, W.P., M.J. Donoghue, and D.R. Maddison. 1984. Outgroup analysis and parsimony. Syst. Zool. **33**:83–103.

Maheshwari, P. 1935. Contributions to the morphology of *Ephedra foliata* Boiss. I: The development of the male and female gametophytes. Proc. Indian Acad. Sci. **1**:586–601.

Maheshwari, P. 1950. An Introduction to the Embryology of Angiosperms. McGraw-Hill, New York.

Maheshwari, P., and H. Singh. 1967. The female gametophyte of gymnosperms. Biol. Rev. **42**:88–130.

Martens, P. 1971. Les Gnetophytes (Handbuch der Pflanzenanatomie, Band 12, Teil 2). Gebrueder Borntraeger, Berlin.

Masand, P., and R.N. Kapil. 1966. Nutrition of the embryo sac and embryo—a morphological approach. Phytomorphology **16**:158–175.

Mazer, S.J. 1987. Maternal investment and male reproductive success in angiosperms: Parent-offspring conflict or sexual selection. Biol. J. Linn. Soc. **30**:115–133.

Meeuse, A.D.J. 1963. Some phylogenetic aspects of the process of double fertilization. Phytomorphology **13**:136–144.

Meeuse, A.D.H. 1986. Again: Double fertilization and the mono- versus pleiophyletic evolution of angiosperms. Phytomorphology **36**:17–21.

Mooney, H.A. 1972. The carbon balance of plants. Annu. Rev. Ecol. Syst. **3**:315–346.

Moussel, B. 1977. Structures protoplasmiques au sein de la cavite archegoniale et modalites de la proembryogenese chez l'*Ephedra distachya* L. Rev. Cytol. Biol. Veg. **40**:73–123.

Moussel, B. 1978. Double fertilization in the genus *Ephedra*. Phytomorphology **28**:336–345.

Mulay, B.N. 1941. The study of the female gametophyte and a trophophyte in *Ephedra foliata* Boiss. J. Univ. Bombay **10**:56–69.

Murrill, W.A. 1900. The development of the archegonium and fertilization in the hemlock spruce *Tsuga canadensis*. Ann. Bot. **14**:583–607.

Nagl, W. 1974. Role of heterochromatin in the control of cell cycle duration. Nature (London) **249**:53–54.

Nagl, W., and F. Ehrendorfer. 1975. DNA content, heterochromatin, mitotic index, and growth in perennial and annual Anthemidae (Asteraceae). Plant Syst. Evol. **123**:35–54.

Nakamura, R.R., and M.L. Stanton. 1989. Embryo growth and seed size in *Raphanus sativus*: Maternal and paternal effects *in vivo* and *in vitro*. Evolution **43**:1435–1443.

Narang, N. 1955. Contributions to the life history of *Ephedra campylopoda*. II. Fertilization and embryogeny. Proc. Indian Sci. Cong. **3**:224.

Nichols, G.E. 1910. A morphological study of *Juniperus communis* var. *depressa*. Beiheft Bot. Zentralb. **25**:201–241.

O'Hara, R.J. 1988. Homage to Clio, or, toward an historical philosophy for evolutionary biology. Syst. Zool. **37**:142–155.

Palser, B.F. 1975. The bases of angiosperm phylogeny: Embryology. Ann. Missouri Bot. Gard. **62**:621–646.

Parrish, J.M., J.T. Parrish, and A.M. Ziegler. 1986. Permian-Triassic paleogeography and paleoclimatology and implication for therapsid distribution. *In* N. Hotton, P.D. MacLean, J.J. Roth, and E.C. Roth, eds., The Ecology and Biology of Mammal-like Reptiles. Smithsonian Institution Press, Washington, D. C., pp. 109–131.

Pearson, H.H.W. 1909. Further observations on *Welwitschia*. Phil. Trans. R. Soc. London Ser. B **200**:331–402.

Pearson, H.H.W. 1929. *Gnetales*. Cambridge University Press, Cambridge.

Price, J.H. 1976. Evolution of DNA content in higher plants. Bot. Rev. **42**:27–52.

Price, H.J., and K. Bachmann. 1976. Mitotic cycle time and DNA in annual and perennial Microseridinae (Compositae, Cichoriodeae). Plant Syst. Evol. **126**:323–330.

Queller, D.C. 1983. Kin selection and conflict in seed maturation. J. Theor. Biol. **100**:153–172.

Queller, D.C. 1984. Models of kin selection on seed provisioning. Heredity **53**:151–165.

Queller, D.C. 1989. Inclusive fitness in a nutshell. *In* P.H. Harvey and L. Partridge, eds., Oxford Surveys in Evolutionary Biology, Vol. 6. Oxford University Press, pp. 73–109.

Russell, S.D. 1991. Isolation and characterization of sperm cells in flowering plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **42**:189–204.

Salisbury, E.J. 1942. The Reproductive Capacity of Plants. G. Bell and Sons, London.

Sargant, E. 1900. Recent work on the results of fertilization in angiosperms. Ann. Bot. **14**:689–712.

Schwaegerle, K.E., and D.A. Levin. 1990. Quantitative genetics of seed size variation in *Phlox*. Evol. Ecol. **4**:143–148.

Singh, H. 1978. Embryology of Gymnosperms (Handbuch der Pflanzenanatomie 10). Gebrueder Borntraeger, Berlin.

Sporne, K.R. 1974. The Morphology of Gymnosperms. Hutchinson, London.

Sporne, K.R. 1975. The Morphology of Angiosperms. St. Martin's Press, New York.

Stebbins, G.L. 1974. Flowering Plants: Evolution Above the Species Level. Harvard University Press, Cambridge, MA.

Stebbins, G.L. 1976. Seeds, seedlings, and the origin of angiosperms. *In* C.B. Beck, ed., Origin and Early Evolution of Angiosperms. Columbia University Press, New York, pp. 300–311.

Stewart, W.N. 1983. Paleobotany and the Evolution of Plants. Cambridge University Press, Cambridge.

Sweet, G.B., and P.E. Wareing. 1966. Role of plant growth in regulating photosynthesis. Nature (London) **210**:77–79.

Takhtajan, A. 1969. Flowering Plants—Origin and Dispersal. Edinburgh University Press, Edinburgh.

Takhtajan, A. 1976. Neoteny and the origin of flowering plants. *In* C.B. Beck, ed., Origin and Early Evolution of Angiosperms. Columbia University Press, New York, pp. 207–219.

Tiffney, B.H. 1986. Evolution of seed dispersal syndromes according to the fossil record. *In* D.R. Murray, ed., Seed Dispersal. Academic Press, Sydney, pp. 273–305.

Tilman, G.D. 1984. Plant dominance along an experimental nutrient gradient. Ecology **65**:1445–1453.

Trivers, R.L. 1974. Parent-offspring conflict. Am. Zool. **14**:249–264.

Troy, M.R., and D.E. Wimber. 1968. Evidence for a constancy of the DNA synthetic period between diploid-polyploid groups in plants. Exp. Cell Res. **53**:145–154.

Uma Shaanker, R., K.N. Ganeshaiah, and K.S. Bawa. 1988. Parent offspring conflict, sibling rivalry and brood size patterns in plants. Annu. Rev. Ecol. Syst. **19**:177–205.

van Went, J.L., and M.T.M. Willemse. 1984. Fertilization. *In* B.M. Johri, ed., Embryology of Angiosperms. Springer-Verlag, Berlin, pp. 273–318.

Vasil, V. 1959. Morphology and embryology of *Gnetum ula* Brongns. Phytomorphology **9**:167–215.

Vijayaraghavan, M.R., and K. Prabhakar. 1984. The endosperm. *In* B.M. Johri, ed., Embryology of Angiosperms. Springer-Verlag, Berlin, pp. 319–376.

Waterkeyn, L. 1954. Etude sur les Gnetales—I. Le strobile femelle, l'ovule et de grains de *Gnetum africanum* Welw. Cellule **56**:103–146.

Watson, M.A., and B.B. Casper. 1984. Morphogenetic constraints on patterns of carbon distribution in plants. Annu. Rev. Ecol. Syst. **15**:233–258.

Westoby, M., and B. Rice. 1982. Evolution of the seed plants and inclusive fitness of plant tissues. Evolution **36**:713–724.

Willemse, M.T.M., and J.L. van Went. 1984. The female gametophyte. *In* B.M. Johri, ed., Embryology of Angiosperms. Springer-Verlag, Berlin, pp. 159–196.

Willson, M.F., and N. Burley. 1983. Mate Choice in Plants. Princeton University Press, Princeton.

Zimmer, E.A., R.K. Hamby, M.L. Arnold, D.A. LeBlanc, and E.C. Theriot. 1989. Ribosomal RNA phylogenies and flowering plant evolution. *In* B. Fernholm, K. Bremer, and H. Jornvall, eds., The Hierarchy of Life. Elsevier, Amsterdam, pp. 205–214.