Abstract.—Recently, two areas of plant phylogeny have developed in ways that could not have been anticipated, even a few years ago. Among extant seed plants, new phylogenetic hypotheses suggest that Gnetales, a group of nonflowering seed plants widely hypothesized to be the closest extant relatives of angiosperms, may be less closely related to angiosperms than was believed. In addition, recent phylogenetic analyses of angiosperms have, for the first time, clearly identified the earliest lineages of flowering plants: Amborella, Nymphaeales, and a clade that includes Illiciales/Trimeniaceae/Austrobaileyaceae. Together, the new seed plant and angiosperm phylogenetic hypotheses have major implications for interpretation of homology and character evolution associated with the origin and early history of flowering plants. As an example of the complex and often unpredictable interplay of phylogenetic and comparative biology, we analyze the evolution of double fertilization, a process that forms a diploid embryo and a triploid endosperm, the embryo-nourishing tissue unique to flowering plants. We demonstrate how the new phylogenetic hypotheses for seed plants and angiosperms can significantly alter previous interpretations of evolutionary homology and firmly entrenched assumptions about what is synapomorphic of flowering plants. In the case of endosperm, a solution to the century-old question of its potential homology with an embryo or a female gametophyte (the haploid egg-producing generation within the life cycle of a seed plant) remains complex and elusive. Too little is known of the comparative reproductive biology of extant nonflowering seed plants (Gnetales, conifers, cycads, and Ginkgo) to analyze definitively the potential homology of endosperm with antecedent structures. Remarkably, the new angiosperm phylogenies reveal that a second fertilization event to yield a biparental endosperm, long assumed to be an important synapomorphy of flowering plants, cannot be conclusively resolved as ancestral for flowering plants. Although substantive progress has been made in the analysis of phylogenetic relationships of seed plants and angiosperms, these efforts have not been matched by comparable levels of activity in comparative biology. The consequence of inadequate comparative biological information in an age of phylogenetic biology is a severe limitation on the potential to reconstruct key evolutionary historical events.

Key words.—Amborella, angiosperms, double fertilization, endosperm, Gnetales, phylogeny, seed plants.

Along with the origins of vascular plants and seed plants, the origin of angiosperms represents one of the three most significant events in the 475 million-year evolutionary history of land plants. With more than 250,000 extant species (Crane et al. 1995), angiosperms are the largest and most diverse group of extant plants. Although ubiquitous and dominant in nearly every terrestrial ecosystem, angiosperms are also one of the most recent major groups of land plants to have evolved, with a fossil record extending back only to the early Cretaceous, more than 130 million years ago (Crane et al. 1995). Paradoxically, we know less about the origin and early evolutionary history of angiosperms than we do about many considerably older groups of land plants. Darwin’s “abominable mystery” of the origin and early evolution of flowering plants continues to challenge evolutionary biologists.

There have been three major obstacles to the reconstruction of historical events associated with the origin and early diversification of angiosperms. First, the macrofossil record of early flowering plants has shed little light on the question of which angiosperm lineages are truly most ancient. Fossils with affinities to diverse flowering plant lineages, including monocots (Crane et al. 1995), Platanaceae (Friis et al. 1994b), Ceratophyllaceae (Dilcher 1989), Nelumboaceae (Upchurch et al. 1994), Laurales (Upchurch et al. 1994), Winteraceae (Walker et al. 1983), and Chloranthaceae (Friis et al. 1986, 1994a) are all found in early Cretaceous floras. Second, for more than a century there has been considerable uncertainty about the identity of the closest seed plant relatives of angiosperms (Doyle 1998). Difficulty in establishing angiosperm outgroups (or immediate ancestors) has made assess-
ment of the homologies of important flowering plant features, from the carpel to the second integument of the ovule, problematic. Third, a number of conflicting hypotheses about the identity of the most basal (and potentially plesiomorphic) extant angiosperms competed for much of the twentieth century. During the last 25 years alone, everything from the Magnoliaceae (and allies), the traditional favorite (Takhtajan 1969; Dahlgren 1980; Cronquist 1981; Walker and Walker 1984; Donoghue and Doyle 1989a; Thorne 1992), to Ceratothyllium (Les 1988; Chase et al. 1993), Chloranthaceae/Piperaeae (Taylor and Hickey 1992; Nixon et al. 1994), Schisandraceae (Martin and Dowd 1986), monocots (Burger 1977, 1981), and Nymphaeaceae (Hamby and Zimmer 1992; Doyle et al. 1994) have been proposed as the earliest divergent lineage of angiosperms.

To decipher the origin and early evolutionary history of angiosperms, a clear formulation of the phylogenetic relationships of angiosperms to other groups of seed plants and the phylogenetic interrelationships of basal angiosperms is absolutely necessary (Friedman and Carmichael 1996). Once robust phylogenetic hypotheses have been established, critical interpretation of the comparative biological features of basal angiosperms and angiosperm outgroups can be used to infer and reconstruct the evolutionary history of a broad range of biological characters (Floyd et al. 1999). Thus, study of the origin of flowering plants, like the origin of any of organisms, relies on the interplay of phylogenetic and comparative biology.

The past two years have been a time of unprecedented turmoil, surprise, and advancement in our understanding of the phylogenetic relationships of plants. In particular, two areas of plant phylogy have developed in ways that could not have been anticipated, even a few years ago: the phylogenetic relationships of extant seed plants (and specifically hypotheses for the closest extant relatives of angiosperms) and the identification of the most basal extant angiosperm lineages.

THE NEW PHYLOGENIES AND THEIR IMPACT ON ANALYSIS OF CHARACTER EVOLUTION

Seed Plant Phylogeny: A Radical Change in Favored Hypotheses

During the last century, many seed plant lineages were proposed as the closest relatives of angiosperms. These include Gnetales, Bennettitales, Caytoniales, Pentoxylylales, and Glossopteridales (Doyle 1998). Beginning with the morphological cladistic studies of extant and extinct seed plants by Crane (1985) and Doyle and Donoghue (1986), a consensus emerged that angiosperms may be phylogenetically nested within a clade that contains several extinct lineages (Bennettitales, Pentoxylylales, Caytoniales) and extant monophyletic Gnetales (Éphedra, Gnetum, and Welwitschia; Crane 1985; Doyle and Donoghue 1986, 1992; Martin and Dowd 1986, 1991; Zimmer et al. 1989; Loconte and Stevenson 1990; Hamby and Zimmer 1992; Chase et al. 1993; Doyle et al. 1994; Rothwell and Serbet 1994; Doyle 1996, 1998; Stefanovic et al. 1998). Although the phylogenetic position of the Gnetales had been viewed as uncertain for much of the 20th century, morphological cladistic analyses, along with additional molecular phylogenetic analyses of extant seed plants consistently supported the status of the Gnetales as the most closely related extant seed plants to angiosperms (Fig. 1). By the mid-1990s, the issue of the relationship of angiosperms and Gnetales was widely perceived as resolved.

Although the hypothesis that Gnetales is closely related to angiosperms was consistently supported in phylogenetic analyses of the last 15 years, almost every molecular phylogenetic analysis of extant seed plants since 1996 (Goremykin et al. 1996; Chaw et al. 1997; Hansen et al. 1999; Qiu et al. 1999; Samigullin et al. 1999; Bowe et al. 2000; Chaw et al. 2000; Frohlich and Parker 2000; Sanderson et al. 2000) has failed to detect an affinity between the Gnetales and angiosperms. Instead, these recent molecular phylogenetic analyses report that Gnetales are most closely related to, or even nested within, conifers (Fig. 2). There may be good reason to suspect that the finding that Gnetales are nested within (paraphyletic) conifers is spurious (Donoghue and Doyle 2000; Sanderson et al. 2000) based on an unusual chloroplast DNA structural mutation (loss of one copy of the inverted repeat) shared by all conifers to the exclusion of other seed plants (including Gnetales; Raubeson and Jansen 1992). However, the hypothesis that Gnetales are closely related to conifers (among extant seed plants) appears to be supported by DNA sequence data and the discovery of two derived gene duplications in the chloroplast that are shared by Gnetales and conifers (Raubeson 1998, pers. comm.).

In itself, the close alliance between conifers and Gnetales might not have represented a radical change from earlier seed plant phylogenies, had conifers plus Gnetales been shown to be closely related to angiosperms (as originally suggested by...
Within the context of earlier anthophyte hypotheses (Gnetales most closely related to angiosperms among extant seed plants), homologous features of angiosperms and Gnetales must have been present in the common ancestor of a monophyletic subset of seed plants that included Gnetales, angiosperms, and extinct lineages such as Bennettitales. If extant gymnosperms are monophyletic, any feature of Gnetales and angiosperms that is presumed to be evolutionarily homologous must have been present in the common ancestor of all extant seed plants (Fig. 2).

If this hypothesis is correct, the most recent common ancestor of Gnetales and angiosperms is more ancient than is the case with the anthophyte hypothesis and is the common ancestor of all extant seed plants.

Given the reality that most seed plant lineages are extinct (only five major clades are extant) and the complexities and conflicting results of recent molecular sequence analyses (Bowe et al. 2000; Chaw et al. 2000; Sanderson et al. 2000), it may yet be some time before the final chapter is written on the interrelationships of cycads, Ginkgo, conifers, Gnetales and angiosperms. However, even if extant gymnosperms should ultimately be shown to be paraphyletic, the hypothesized sister group status of conifers and Gnetales indicates that the most restrictive clade that Gnetales could possibly share with angiosperms must also include the relatively ancient conifers.

Evolutionary Implications of the New Seed Plant Phylogenies

The newest seed plant phylogenetic hypotheses (Fig. 2) reveal that the paradigm of the late 20th century—that Gnetales are the closest living relatives of angiosperms—may well be incorrect. Gnetales may be most closely related to conifers, and the concept of a monophyletic extant gymnosperm clade that is the sister group to angiosperms is, for the time being, supported by a significant body of data (Bowe et al. 2000; Chaw et al. 2000; Frohlich and Parker 2000; Sanderson et al. 2000).

There are several aspects of the new seed plant phylogenies that bear directly upon the interpretation of character evolution associated with the origin of flowering plants. First, the close relationship of Gnetales and conifers suggests that it is appropriate to more critically examine the comparative biology of these two major seed plant lineages to determine whether some of their similar features reflect underlying homologies that, in the past, were not recognized. These features might range from aspects of wood anatomy to embryogeny (e.g., suspensor and proembryo organization, which are quite similar in Gnetales and Araucariaceae; Martens 1971) to sperm cell organization (Gnetales and some conifers are the only seed plants with binucleate sperm cells; Friedman 1991), female gametophyte ontogeny (conifers and Gnetales are the only seed plants in which substantial development of the female gametophyte occurs after fertilization; Friedman and Carmichael 1998), and the process of fertilization itself (as will be discussed).

Within the context of earlier anthophyte hypotheses (Gnetales most closely related to angiosperms among extant seed plants), homologous features of angiosperms and Gnetales must have been present in the common ancestor of a monophyletic subset of seed plants that included Gnetales, angiosperms, and extinct lineages such as Bennettitales. If extant gymnosperms are monophyletic, any feature of Gnetales and angiosperms that is presumed to be evolutionarily homologous must have been present in the common ancestor of all extant seed plants (if homologous) than had previously been assumed or they must represent homoplasies.

The Search for the Earliest Lineages of Extant Angiosperms Yields a Surprise Consensus

Recent analyses of angiosperm relationships is that whereas monocotyledonous flowering plants comprise a monophyletic group (monocots), the dicotyledonous flowering plants (dicots) are a paraphyletic assemblage that includes magnoliids and a large, nested monophyletic group, the eudicots (Fig. 3). Magnoliid lineages include diverse clades such as Aristolochiaceae, Chloranthaceae, Nymphaeales, Piperaceae, Illiciaceae, Winterales, Laurales, and Magnoliaceae.

Recent phylogenetic analyses of basal angiosperm interrelationships also call into question longstanding assumptions about which lineages constitute the earliest divergent (and potentially plesiomorphic) angiosperms. Although traditional views focused on the Magnoliaceae and close relatives (Takhtajan 1969; Dahlgren 1980, 1983; Cronquist 1981, 1988; Walker et al. 1983; Donoghue and Doyle 1989a,b; Thorne 1992), several alternative extant angiosperm groups have been favored in more recent phylogenetic analyses. These include the reduced aquatic Ceratophyllum (Les 1988; Les et al. 1991; Chase et al. 1993; Nixon et al. 1994), Chloranthaceae/Piperaceae (Taylor and Hickey 1992; Nixon et al. 1994), Nymphaeaceae (Hamby and Zimmer 1992; Doyle et al. 1994), Schisandraceae (Martin and Dowd 1991), Schisandraceae/Illiciaceae/Austrobaileyaceae (Soltis et al. 1997), monocots (Burger 1977, 1981), and Amborella (Soltis et al. 1997).

Beginning in 1999, a series of independent analyses (Mathews and Donoghue 1999; Parkinson et al. 1999; Qiu et al. 1999, 2000; Soltis et al. 1999; Graham and Olmstead 2000; Graham et al. 2000) produced an unanticipated consensus on the most basal extant lineages of angiosperms. These phylogenetic analyses indicate that monotypic Amborella is sister to all other angiosperms; that Nymphaeales (Nymphaeaceae plus Cabombaceae) is sister to all angiosperms exclusive of Amborella; and a clade that includes Illiciales (Illiciaceae plus Schisandraceae) plus Trimeniaceae plus Austrobaileyaceae is sister to the remaining angiosperms (Fig. 3). Together, Amborella, Nymphaeales, and the Illiciales/Trimeniaceae/Austrobaileyaceae clade represent a grade at the base of angiosperms (the “ANITA grade” of Qiu et al. 1999; hereafter, the “basal angiosperm grade” of Fig. 3).

Taken altogether, the current phylogenetic analyses strongly support the hypotheses (Fig. 3) that monocots are monophyletic, but dicots are paraphyletic; magnoliids are a paraphyletic basal assemblage of angiosperms in which monophyletic monocot and eudicot (having tricolpate pollen) clades are nested; and Amborella, Nymphaeales, and the Illiciales/Trimeniaceae/Austrobaileyaceae clade are basal to the common ancestor of monocots and eudicots. Although not formally noted in the literature, these phylogenetic analyses demonstrate that the common ancestor of monocots and eudicots is not the common ancestor of all extant angiosperms (Fig. 3). This simple fact, as will be seen, has major implications for assumptions about what is actually known about defining and synapomorphic features of flowering plants.

Evolutionary Implications of the New Angiosperm Phylogenies

For more than a century, biological features common to both monocots and “dicots” were assumed to be general (synapomorphic) features of all angiosperms. A key implication of the new angiosperm phylogenies (Fig. 3) is that those features of angiosperms assumed to be synapomorphic of angiosperms, but known only from monocots and eudicots (the vast majority of dicots), may not be flowering plant synapomorphies if they are absent from some or all of the most basal flowering plant lineages. The common ancestor of monocots and eudicots is not the common ancestor of all extant angiosperms (Fig. 3); in all recent phylogenetic analyses, three lineages of flowering plants (Amborella, Nymphaeales and Illiciales/Trimeniaceae/Austrobaileyaceae) are basal to the common ancestor of monocots and eudicots. Although flowers, carpels, microsporophylls with four sporangia (stamens), bitegmic ovules, a highly reduced female gametophyte (embryo sac), a three-celled male gametophyte, and endosperm have been documented in all major lineages of flowering plants and are known synapomorphies of angiosperms, there are some biological features whose representation in basal lineages of flowering plants remains undocumented.

The remainder of this paper will be devoted to a case study of the impact of the new seed plant and basal angiosperm phylogenetic hypotheses on the reconstruction of the evolutionary history of two of the most defining features of angiosperm biology: the process of double fertilization and the formation of an endosperm that nourishes the embryo. As will be seen, the continuous interplay of phylogenetic systematics and comparative biology can sometimes yield surprising insights into what is known and, perhaps even more importantly, what is unknown.

Fig. 3. Current hypothesis of relationships of extant basal angiosperms based on recent molecular phylogenetic analyses (Mathews and Donoghue 1999; Parkinson et al. 1999; Qiu et al. 1999, 2000; Soltis et al. 1999; Soltis et al. 1997), Schisandraceae (Martin and Dowd 1991), Schisandraceae/Illiciaceae/Austrobaileyaceae clade represent a grade at the base of angiosperms (the “ANITA grade” of Qiu et al. 1999; hereafter, the “basal angiosperm grade” of Fig. 3).
A CASE STUDY: THE EVOLUTION OF DOUBLE FERTILIZATION AND ENDOSPERM

Although determination of character distribution and its developmental underpinnings in basal angiosperms is critical to understanding the diversification of flowering plants (Doyle and Donoghue 1993; Friedman 1994), there have been few recent investigations of the reproductive biology of basal angiosperms. Except for important contributions on floral morphology among basal extant taxa (e.g., Endress 1987, 1994, 1995, 1997; Friis and Endress 1990; Tucker and Bourland 1994; Williamson and Schneider 1994; Friis 1996; Tucker and Douglas 1996; Endress and Igersheim 1997, 2000; Igersheim and Endress 1997), a few studies of embryology in basal taxa (Prakash et al. 1992; Chitrakaleka and Bhandari 1993; Toke et al. 1993; Heo and Tohe 1995; Rudall and Funnell 1997; Xuhan and Van Lammeren 1997; Floyd et al. 1999; Floyd and Friedman 2000; Tober et al. 2000), and recently described early Cretaceous angiosperms (Taylor and Hickey 1990; Crane et al. 1994; Friis et al. 1994a,b, 1999, 2000; Crane et al. 1995; Sun et al. 1998; Mohr and Friis 2000), the origin and diversification of many of the defining reproductive features of flowering plants remain unstudied. Most importantly, for the purposes of this paper, definitive information about double fertilization and endosperm development in basal angiosperms, two features of angiosperm biology long thought to have been central to the radiation of flowering plants (Stebbins 1976; Tiffney 1981), is virtually nonexistent.

Double Fertilization: A Synapomorphy of Angiosperms?

For a century, a double fertilization process that yields a diploid embryo and a triploid endosperm has been considered a defining feature of angiosperms. Although endosperm is known to be a ubiquitous feature of angiosperms, except where it has been secondarily lost (as in the Podostemaceae; Johri et al. 1992), the participation of two sperm from a single pollen tube in an act of double fertilization in angiosperms has been carefully documented in only a relatively small number of mostly derived monocots and eudicot taxa. The intellectual history of how double fertilization (to yield an embryo and a sexual endosperm) came to be thought of as a general and defining feature of flowering plants (Friedman 2001a) is fascinating.

The process of double fertilization in flowering plants was independently discovered in *Lilium* and *Fritillaria* by Navashin (1898) and Guignard (1899). Prior to this, it had been assumed that endosperm, the embryo-nourishing tissue of flowering plants, was a developmental product of the fused polar nuclei of the female gametophyte (embryo sac; Fig. 4). Guignard and Navashin documented that the endosperm of *Lilium* and *Fritillaria* originates from a fertilization of the two polar nuclei of the embryo sac by the second sperm of a pollen tube.

Within months of the announced discoveries of double fertilization in *Lilium* and *Fritillaria* (both members of the Liliaceae), a massive effort was underway to determine whether a second fertilization event to initiate endosperm was widespread among flowering plants. By 1900, double fertilization had been additionally documented in monocotyle-
dous orchids and diverse dicotyledonous taxa such as Ranunculaceae, Asteraceae, and Monotropaceae; by 1903, 16 angiosperm families were known to have a second fertilization event (Coulter and Chamberlain 1903). The determination that double fertilization could be found in both major groups of flowering plants, the monocots and the dicots, led Sargent (1900) to conclude that this unique reproductive process was likely to be a feature of all flowering plants. Less than two years after its discovery, the race to determine the extent of double fertilization among flowering plants was over, and the inference that double fertilization was a synapomorphy (using modern terminology) of angiosperms, was firmly established in the fields of comparative and evolutionary plant biology.

Modern research (mostly transmission electron microscopy) on double fertilization (specifically the second fertilization event) in angiosperms has involved the study of a very limited number of phylogenetically highly derived eudicot taxa that include *Gossypium* (Malvaceae) (Jensen and Fisher 1967), *Linum* (Linaceae; d’Alascio 1974), *Spinacia* (Chenopodiaceae; Wilms 1981), *Plumbago* (Plumbaginaceae; Russell and Cass 1981; Russell 1982), *Populus* (Salicaceae; Russell et al. 1990), *Glycine* (Fabaceae; Folsom and Cass 1992), and *Nicotiana* (Solanaceae; Yu et al. 1994) and the highly derived monocot taxa *Zea*, *Hordeum*, *Triticum*, and *Triticale* (all members of Poaceae; Cass and Jensen 1970; You and Jensen 1985; Hause and Schröder 1987; Mogensen 1988; Gao et al. 1992; Møl et al. 1994). Although the second fertilization process to initiate a triploid endosperm may vary among flowering plants (polar nuclei may fuse prior to or concurrent with the second fertilization event), descriptions are fundamentally similar in monosporic monocots and eudicots. This strongly suggests that the common ancestor of these two large angiosperm clades expressed a pattern of double fertilization in which a second sperm nucleus fused with the polar nuclei of the central cell of the embryo sac (Fig. 4).

In contrast with the study of derived angiosperms, there are just three reports of the fusion of a second sperm with the two polar nuclei (or their fusion product) of the embryo sac in angiosperm lineages that are not within the clade defined by the common ancestor of eudicots and monocots: *Brasenia* (Khanna 1965), *Nymphaea* (Khanna 1967), and *Illicium* (Hayashi 1963). A single drawing, but no micrograph, of a second fertilization event accompanied each publication and each of the original figures is reproduced in Figure 5. These drawings (Hayashi 1963; Khanna 1965, 1967) may accurately depict the initiation of a second fertilization event (i.e., proximity of a putative sperm nucleus and the fused polar nuclei) in angiosperms basal to the common ancestor of monocots and eudicots, however, they cannot be considered strong proof of double fertilization. This is especially so in light of an earlier report of reproduction for *Nymphaea* that indicated that “endosperm tissue [can form] without triple fusion” (Cook 1909). Equally intriguing is a suggestion for the basal monocot *Acorus* that a second fertilization event does not occur prior to the development of an embryo-nourishing endosperm (Buell 1938). In *Acorus*, the two polar nuclei were reported to fuse (in the absence of a sperm) and divide transversely to initiate a diploid endosperm. Unfor-
Unfortunately, no micrographs were published in support of this conclusion.

Even if second fertilization events are eventually conclusively documented in the Nymphaeales and the Illiciales/Trimeniaceae/Austrobaileyaceae clade, determination of whether this feature of endosperm initiation represents a synapomorphy of flowering plants may still depend on an assessment of the condition in *Amborella*. The fertilization process in *Amborella* has never been studied. If a second fertilization event is to be definitively documented in an angiosperm basal to the common ancestor of monocots and eudicots, micrographs of developmental events associated with a triploid fusion, as well as DNA quantitation of the putative fertilization product, will be essential.

Discovery that a second fertilization event (triple fusion) does not occur in some or all key basal angiosperm taxa would have significant, and unexpected, implications for understanding the evolutionary origin of endosperm. Although a second fertilization event has not been definitively documented in any basal angiosperm, the formation of an endosperm (assumed to be sexually produced) derived from the central cell of the embryo sac has been reported for all basal angiosperms (Johri et al. 1992). Thus, if a second fertilization event is ultimately proven to be absent in key basal angio-
sporocytes, and plesiomorphic endosperm is shown to be a strictly maternal tissue derived from the fusion of two polar nuclei, a new hypothesis for the origin of endosperm will involve (Figs. 4, 6): (1) An evolutionary transitional stage in which the fusion product of the two polar nuclei in the central cell of the female gametophyte directly proliferates into a strictly maternal endosperm tissue; and (2) A later stage of endosperm evolution in which additional participation of a sperm nucleus with the fusion of polar nuclei results in the origin of a hybrid (maternal/paternal) triploid endosperm, as occurs in monocots and eudicots. The inevitable consequence of such a discovery (if it were made) would be that, because the evolution of endosperm did not initially involve a fusion between a sperm and two polar nuclei, endosperm tissue is evolutionarily homologous with the female gametophyte. Importantly, even if double fertilization is eventually documented in all extant basal angiosperms, it is still possible that the endosperm of the ancestors of extant angiosperms (stem lineage angiosperms) was originally a diploid maternal structure and later became a triploid product of a sexual fusion with a sperm (Friedman 2001a,b).

From a theoretical perspective, if a sexually formed endosperm is not a synapomorphy of angiosperms, this would have a significant impact on hypotheses concerning the possible adaptive value or key innovation status (sensu Sanderson and Donoghue 1994) of a hybrid (biparental) embryo-nourishing tissue in flowering plants (see Brink and Cooper 1940; Stebbins 1976). Recent evolutionary analyses of the origin of endosperm based on the constructs of inclusive fitness theory (kin selection) have focused on potential fitness benefits of a maternal/paternal endosperm over a strictly maternal embryo-nourishing female gametophyte tissue in non-flowering seed plants (Charnov 1979; Westoby and Rice 1982; Queller 1983, 1989; Haig and Westoby 1988, 1989; Friedman 1995, 1998). These models for the functional replacement of the seed plant female gametophyte by an endosperm tissue for the purposes of embryo nourishment will need to be reexamined if it should be discovered that the earliest manifestation of endosperm was a strictly maternal (and not a biparental) tissue.

Fig. 5. Figures of double fertilization in (A) *Brasenia* (Cabombaceae, Nymphaeales); (B) *Nymphaea* (Nymphaeaceae, Nymphaeales); and (C) *Illicium* (Illiciaceae) reproduced from Khanna (1965), Khanna (1967), and Hayashi (1963), respectively. Labeling has been added: A, antipodals; E, egg; FPN, fused polar nuclei; PT, pollen tube; S1, first sperm; S2, second sperm.

Fig. 6. Hypothesis for the origin and early evolution of endosperm based on the assumption that basal angiosperms do not have a second fertilization event. Basal angiosperms would have a diploid maternal endosperm that represents a continuation of female gametophyte development derived from the fusion of the two polar nuclei of the central cell. At a later point, in the common ancestor of monocots and eudicots, the participation of a sperm in the initiation of a triploid biparental endosperm evolves. If it should turn out that basal angiosperms lack a second fertilization event, the homology of flowering plant endosperm would be with the female gametophyte of the life cycle.
In summary, although a triploid second fertilization event has long been considered a synapomorphy for flowering plants (and was essentially first reported as such by Sargent 1900), it has yet to be definitively characterized in any of the most basal angiosperms. This unanticipated realization (more than a century after it was assumed that double fertilization was a general feature of all angiosperms) is the direct result of the interplay of new phylogenetic hypotheses and analysis of the comparative biology of critical and diverse angiosperm taxa. This situation represents an enormous gap in our knowledge of the most basic aspects of fertilization biology that characterized the first angiosperms. It is essential that the developmental origin of endosperm be studied in basal taxa to unequivocally document or refute whether a triploid second fertilization event is a synapomorphic condition for flowering plants.

**Double Fertilization: A Synapomorphy of Seed Plants?**

The new seed plant phylogenetic hypotheses have clear implications that directly relate to the potential homology of endosperm to structures found in nonflowering seed plants. For much of the last century, two dramatically different hypotheses of endosperm homology have competed (Friedman 1998). One hypothesis holds that the endosperm of flowering plants is evolutionarily homologous with a supernumerary embryo whose normal development was transformed into an embryo-nourishing tissue (LeMonnier 1887; Sargent 1900; Haig and Westoby 1988, 1989; Queller 1989; Friedman 1992a). Others have argued (Strasburger 1900; Coulter 1911) that the fusion of male and female nuclei associated with the initiation of endosperm in angiosperms is not sexual in nature, but instead is a ‘‘vegetative fertilization’’ that serves to stimulate ‘‘belated’’ development of the embryo sac. As such, endosperm is viewed as a phase of female gametophyte development that is evolutionarily homologous with the female gametophytes of all other seed plants.

Recent research on the fertilization biology of members of the Gnetales appeared to have shed light on the homology of endosperm. Regular double fertilization events were documented in *Ephedra* and *Gnetum* (Friedman 1990; Carmichael and Friedman 1995). The product of the second fertilization event in Gnetales is a diploid supernumerary embryo (Friedman 1992a; Carmichael and Friedman 1996). Comparisons of the presumed plesiomorphic manifestations of double fertilization in angiosperms and Gnetales demonstrate key developmental and genetic similarities (Friedman 1995). In both groups, two sperm from a single pollen tube engage in separate fertilization events with an egg and its mitotic sister nucleus. The products of double fertilization in *Ephedra* (two embryos) are genetically identical (coefficient of relatedness = 1.0), as is the case with the two products of double fertilization in (monosporic) angiosperms (an embryo and an endosperm).

Explicit analyses of comparative developmental patterns and genetic constructs associated with double fertilization in Gnetales and angiosperms are congruent with the hypothesis that double fertilization in each of these lineages was derived from a common ancestor and is therefore homologous (Friedman 1994, 1995, 1998). Moreover, determination of character polarity and the potential for developmental transformation indicated that the ancestral double fertilization process was likely to have produced a normal embryo and a genetically identical supernumerary embryo (similar to the plesiomorphic condition in Gnetales). If the second fertilization event in Gnetales and angiosperms is homologous, it follows that endosperm is likely to represent a developmental transformation of an embryo (Friedman 1995).

The hypothesis that double fertilization events may be homologous in Gnetales and angiosperms rests upon two key assumptions: (1) double fertilization to produce an embryo and an endosperm was a feature of the common ancestor of flowering plants; and (2) a process of double fertilization was present in the common ancestor of Gnetales and angiosperms. Clearly, if endosperm is not formed from a second fertilization event in basal angiosperms, the hypothesis that double fertilization events in Gnetales and angiosperms are homologous must be rejected. This will be the case irrespective of the seed plant phylogeny that is favored (Gnetales closely related to angiosperms or Gnetales distantly related to angiosperms).

If double fertilization is eventually shown to be a feature of the common ancestor of extant angiosperms, the evaluation of its potential homology with the process in Gnetales is complex. Ultimately, however, ‘‘homologous features . . . in two or more organisms are those that can be traced back to the same feature (or state) in the common ancestor of those organisms’’ (Mayr 1969). Even if Gnetales were conclusively shown to be closely related to angiosperms, this would not imply that all of their shared features are necessarily evolutionarily homologous. For example, it has long been hypothesized that the vessel elements of angiosperms and Gnetales are homoplasmous based on the evidence that pitting patterns and perforation plates in these two lineages are too dissimilar to represent developmental transformations of an original vessel element in a common ancestor (Carlquist 1996).

Conversely, the hypotheses that Gnetales are closely related to conifers and that extant gymnosperms are the monophyletic sister group to angiosperms do not necessitate the conclusion that all shared biological features of Gnetales and angiosperms are homoplasmous, as has been asserted recently by Hansen et al. (1999), Chaw et al. (2000), and others (for an additional critique, see Axsmith et al. 1998). The general conclusion that ‘‘shared morphological characters [of the Gnetales and angiosperms] are convergent, rather than homologous’’ (part of the title of Hansen et al. 1999) is incorrect. Many, if not most, shared features of the Gnetales and angiosperms are evolutionarily homologous and represent either symplesiomorphic or synapomorphic features of seed plants. Gnetales and angiosperms share homologous roots, homologous leaves, homologous ovules, and homologous chloroplasts to name but a few obvious structures that were inherited from a common ancestor.

Within the context of the current phylogenetic hypotheses for extant seed plants (conifers sister to Gnetales and monophyletic extant gymnosperms sister to angiosperms), any structure or process hypothesized as homologous in Gnetales and angiosperms must either be a synapomorphy or symplesiomorphy of extant seed plants. For double fertilization, the
The issue of whether double fertilization in the Gnetales is evolutionarily homologous with the process in angiosperms requires careful analysis of the interplay of phylogeny and the often very limited knowledge of comparative biology. If extant gymnosperms are monophyletic, a finding of homology requires that double fertilization be a synapomorphy of extant seed plants; and barring any loss of this character in extant seed plant lineages, it would be predicted that conifers, cycads, and Ginkgo would display this developmental process. Although double fertilization may occur in both the Pinaceae and Cupressaceae (see above), there are no reports of double fertilization events in cycads or Ginkgo. However, several studies have documented developmental events that are consistent with the potential for double fertilization events in cycads and Ginkgo (reviewed in Friedman 1992b).

In cycads and Ginkgo, anomalous egglike development of the sister nucleus of the egg (this nucleus participates in the second fertilization event in Gnetales, angiosperms, and conifers) has been reported (Ikeno 1901; Chamberlain 1906, 1912; Sedgewick 1924; Bryan and Evans 1957). This suggests that the developmental conditions for a second fertilization event may well be present in these much understudied seed plants (Friedman 1992b).

Future documentation of a regular or occasional second fertilization event in all major lineages of extant seed plants would be congruent with the hypothesis that double fertilization evolved in a common ancestor of extant seed plants and is homologous throughout the seed plants (Fig. 7). However, if double fertilization is ultimately shown to be absent in cycads and Ginkgo and future phylogenetic analyses uphold the hypothesis of monophyletic extant gymnosperms, this would suggest that double fertilization in Gnetales and angiosperms is homoplasious.

One important possibility that has been overlooked in recent discussions of seed plant phylogeny (given some of the current uncertainties over the monophyly of extant gymnosperms) is the hypothesis that Gnetales, conifers, and angiosperms may comprise a monophyletic group to the exclusion of cycads and Ginkgo. Interestingly, if a conifer/Gnetales clade was ultimately shown to be sister to angiosperms (among extant seed plants), double fertilization and siphonogamy (the use of a pollen tube to conduct nonmotile sperm to an egg) might prove to be key synapomorphies of this clade (Fig. 7).
FIG. 7. Alternative hypotheses for the origin and early evolution of double fertilization based on the assumption that basal angiosperms, Gnetales, and some conifers have a second fertilization event. (A) Under this scenario, double fertilization is assumed to have evolved in the common ancestor of extant seed plants and is homologous in Gnetales, conifers, and angiosperms. Although double fertilization events have not been reported in *Ginkgo* or cycads, the potential for double fertilization events exists in these groups and will require further study. (B) Under this scenario, double fertilization is assumed to have evolved in the common ancestor of Gnetales and conifers and separately in the common ancestor of angiosperms. Although double fertilization is homoplasious (and represents an apomorphic tendency among seed plants) under this scenario, the underlying developmental preconditions that enable the homoplasious evolution of double fertilization events (the production of pairs of male and female nuclei that are capable of engaging in fusion processes) are a defining feature ("underlying synapomorphy," see text for discussion) of seed plants. (C) This scenario assumes that gymnosperms are paraphyletic, monophyletic conifers are sister to Gnetales (supported by sequence and indel data; L. Raubeson, pers. comm.), cycads are sister to *Ginkgo* (supported by some sequence data and indel data), and the conifer/Gnetales clade is sister to angiosperms. This hypothesis of relationship was originally recovered in the cladistic analysis of Hill and Crane (1982), but has not been supported in molecular analyses to date. A sister group relationship of a conifer/Gnetales clade to angiosperms (among extant seed plants) would support the hypothesis that double fertilization and siphonogamy are important synapomorphies of a conifer/Gnetales/angiosperm clade.

form pairs of both male and female nuclei that may participate in separate fertilization events (Fig. 7).

If the evolution of double fertilization is homoplasious among seed plant lineages, this phenomenon would stand as a premier example of an apomorphic tendency predicated upon what has been referred to as an "underlying synapomorphy" (Tuomikoski 1967; Sæther 1983; Brooks 1996), in essence, a case of parallelism that results from an underlying developmental constraint or bias (sensu Maynard Smith et al. 1985; Schwenk 1995; Wake 1996). The concept of an underlying synapomorphy can be broadly viewed as a developmental and evolutionary capacity to produce a specific apomorphic trait repeatedly within a clade. This developmental and evolutionary capacity (the underlying synapomorphy) to produce the apomorphic and homoplasious trait will have evolved once in the common ancestor of the clade that contains the various lineages exhibiting the homoplasious trait. In the case of seed plants, a system of fertilization biology that involves the production of two genetically identical sperm per pollen tube (a synapomorphy of seed plants) that are brought through a pollination process (another synapomorphy of seed plants) into proximity to an egg and its genetically identical sister nucleus represent the underlying synapomorphy. The potentially iterative (homoplasious) evolution of double fertilization events (involving pairs of sperm and pairs of female nuclei that can behave as gametes) in various seed plant lineages may be the end result of a system of fertilization biology that was established with the origin of the seed plant clade more than 360 million years ago.

The Search for the Homologue of Endosperm

If the process of double fertilization in angiosperms should ultimately prove to be homoplasious with all other manifestations of double fertilization in seed plants, the issue still remains that endosperm must have an evolutionary antecedent or homologue. For more than a century, only two hypotheses have been proposed for the origin of endosperm: it is either a homologue of the female gametophyte or a developmentally transformed embryo (Friedman 1998). If the endosperms of basal angiosperms are not the product of a second fertilization event, it must be concluded that endosperm is homologous with the female gametophyte and became a hybrid (biparental) structure subsequent to the origin of flowering plants (Friedman 2001a). However, if basal angiosperms are ultimately shown to have a sexually formed endosperm, double fertilization processes in nonflowering seed plants may yet provide critical insights into the origin
of the endosperm of flowering plants—even if double fertilization arose independently in angiosperms, Gnetales, and perhaps conifers. When double fertilization has been reported in nonflowering seed plants, the product has always been shown to be an embryo (Friedman 1992b). Indeed, it is hardly surprising that the product of a novel fertilization event would be a manifestation of the plesiomorphic product of a normal fertilization event (i.e., an embryo). This may well have been the starting point for the evolution of a second fertilization product in the angiosperms and their immediate ancestors. Analyses of the origin of endosperm based on the constructs of inclusive fitness theory (kin selection) have demonstrated that it is theoretically possible for a supernumerary embryo in the life cycle of a seed plant to acquire altruistic (nourishing) behavior through increases in the inclusive fitness of a compatriot embryo (Charnov 1979; Westoby and Rice 1982; Queller 1983, 1989; Haig and Westoby 1988, 1989; Friedman 1995, 1998).

In addition to theoretical constructs, there are new empirical developmental data that may shed light on the homology of endosperm. In vitro formed endosperm in Zea has been reported to exhibit a marked differentiation of two poles (Kranz et al. 1998). These endosperms (formed outside of the physical constraints of an ovule) typically form a globular region of densely cytoplasmic cells and a filamentous region of larger, more vacuolate cells. Kranz noted the embryo-like developmental properties of these endosperms. Additionally, recent studies of endosperm development in diverse basal angiosperm taxa (Floyd et al. 1999; Floyd and Friedman 2000) have revealed that plesiomorphic endosperm development in flowering plants is remarkably similar to the basic developmental patterns expressed by embryos. The early ontogenies of both embryos and endosperms in basal angiosperms involve unequal partitioning of the first cell, followed by differential patterns of development in chalazal and micropylar regions, or “domains.” In fact, those principles thought to govern embryo development in angiosperms (reviewed by Kaplan and Cooke 1997) appear to be equally applicable to the endosperms of basal angiosperms (Floyd and Friedman 2000).

Definitive resolution of the evolutionary origin and homology of endosperm remains, as it has always been, complex and difficult at best. Nevertheless, there is reason to believe that a solution may someday be at hand. The recent evidence of embryo-like features of endosperms in basal angiosperms suggests that comparative studies of molecular expression in the endosperms and embryos of basal angiosperms and the female gametophytes of nonflowering seed plants could prove quite valuable. If it could be shown that gene expression patterns associated with early pattern formation and morphogenesis in basal angiosperm endosperms and embryos were similar, these data could support a finding of homology of these two critical components of the angiosperm life cycle.

A comparative molecular developmental approach might also be fraught with pitfalls. Comparisons of gene expression patterns in endosperms of basal angiosperms and the female gametophytes of nonflowering seed plants might also reveal much in common. However, similarities in molecular expression patterns in endosperms and nonflowering seed plant female gametophytes could be interpreted as the result of functional constraints; the embryo-nourishing program of endosperm was likely to have been borrowed from the female gametophyte of the ancestors of angiosperms, irrespective of the morphological homology of endosperm (with an embryo or a female gametophyte). Ultimately, at the molecular level endosperm may have hybrid origins: a functional (physiological) program coopted from the previous embryo-nourishing component of the seed plant life cycle (the female gametophyte) and a structural (pattern level) program that was inherited from the antecedent morphological or life cycle homologue (either an embryo or a female gametophyte).

Conclusions

The questions of whether conifers are paraphyletic, gymnosperms are monophyletic, or even whether Amborella is, in fact, the sister taxon to all other flowering plants (see Barkman et al. 2000; Graham et al. 2000) will likely find definitive resolution in the coming years. Perhaps what is most important about the new, and in many ways, radically different hypotheses of seed plant and angiosperm relationships is that these hypotheses have the important effect of challenging old (and often static) concepts about character evolution and homology. Regardless of whether some or all of the new phylogenetic hypotheses for plants are shown to be robust or ephemeral, these hypotheses can and should directly stimulate new ways of thinking about the transformation of characters, as well as focused studies of the biology of phylogenetically critical and often overlooked lineages.

Clearly, substantive progress has been made in the determination of phylogenetic relationships of seed plants and angiosperms. Regrettably, these efforts have not been matched by coordinate levels of activity in comparative biology. Far too little is known about the most basic reproductive features of extant basal angiosperms and nonflowering seed plants; even less is known of the biology of critical extinct lineages. In the absence of a rich and detailed knowledge of the comparative biology of key lineages of plants, the utility of new insights into phylogenetic relationships remains limited. A commitment to comparative biology in all of its manifestations is now needed, from developmental morphology to cellular and molecular biology. Only when knowledge of phylogenetic relationships is joined with the powerful insights of comparative information will it be possible to make significant progress in reconstructing the evolutionary and developmental history of plants.

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