REFERENCES AND NOTES

1. P. W. Berman *et al.*, *Nature* **345**, 622 (1990).
2. D. Zagury *et al.*, *ibid.* **332**, 728 (1988).
3. M. Clerici *et al.*, *Eur. J. Immunol.* **21**, 1345 (1991).
4. R. R. Redfield *et al.*, *N. Engl. J. Med.* **324**, 1677 (1991).
5. T. J. Palker *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1932 (1988).
6. J. R. Rusche *et al.*, *ibid.*, p. 3198.
7. J. Goudsmit *et al.*, *ibid.*, p. 4478.
8. H. Takahashi *et al.*, *ibid.*, p. 3105. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
9. H. Takahashi, R. N. Germain, B. Moss, J. A. Berzofsky, *J. Exp. Med.* **171**, 571 (1990).
10. M. Clerici *et al.*, *Nature* **339**, 383 (1989).
11. M. Clerici *et al.*, *J. Immunol.* **146**, 2214 (1991).
12. G. Myers *et al.*, *Human Retroviruses and AIDS 1989* (Los Alamos National Laboratory, Los Alamos, NM, 1989).
13. G. J. LaRosa *et al.*, *Science* **249**, 932 (1990).
14. J. Albert *et al.*, *AIDS* **4**, 107 (1990).
15. P. L. Nara *et al.*, *J. Virol.* **64**, 3779 (1990).
16. H. Takahashi *et al.*, *Science* **246**, 118 (1989).
17. H. Takahashi *et al.*, *J. Exp. Med.* **170**, 2023 (1989).
18. Peptide analogs of 18MN (*16*) were synthesized by solid-phase peptide synthesis [J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis* (Pierce Chemical, Rockford, IL, 1984)] and purified by gel filtration and high-performance liquid chromatography (HPLC). We purified the peptides to single peaks on reverse-phase HPLC using C-18 columns and a buffer system of 0.1% trifluoroacetic acid–water and 0.1% trifluoroacetic acid–acetonitrile. Each peptide had the expected amino acid analysis.
19. Although precedent exists for a response to be due to a shorter peptide contaminant in a synthetic preparation [T. N. M. Schumacher, M. L. H. De Bruijn, L. N. Vernie, W. M. Kast, C. J. M. Melief *et al.*, *Nature* **350**, 703 (1991)] or for possible trimming by proteases once a peptide is bound to a class I MHC molecule [G. M. Van Bleek and S. G. Nathenson, *ibid.* **348**, 213 (1990); O. Rötzschke, K. Falk, K. Deres, H. Schild, M. Norda *et al.*, *ibid.*, p. 252; K. Falk, O. Rötzschke, S. Stevanovic, G. Jung, H.-G. Rammensee, *ibid.* **351**, 290 (1991)], the reciprocal nature of the recognition by different T cells, correlating with the chemical character of a single core residue, when the rest of the peptide, the presenting class I major histocompatibility complex (MHC) molecule, D^d, and the antigen-presenting cells and their processing machinery are held constant, could not simply be explained by arbitrary levels of peptide contaminants or differences in antigen processing.
20. H. Takahashi *et al.*, unpublished observations.
21. M. A. Alexander, C. A. Damico, K. M. Wieties, T. H. Hansen, J. M. Connolly, *J. Exp. Med.* **173**, 849 (1991).
22. B. D. Evavold and P. M. Allen, *Science* **252**, 1308 (1991).
23. S. Chakrabarti, M. Robert-Guroff, F. Wong-Staal, R. C. Gallo, B. Moss, *Nature* **320**, 535 (1986).
24. R. A. Houghten, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5131 (1985).
25. R. E. Phillips *et al.*, *Nature* **354**, 453 (1991).
26. We thank B. Moss and S. Merli for the generous gifts of recombinant vaccinia viruses and W. Biddison and J. Yewdell for critical reading of the manuscript and helpful suggestions. This work was supported in part by grants from the Ministry of Education, Culture, and Science and from the Ministry of Health, Japan.

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Evidence of a Pre-Angiosperm Origin of Endosperm: Implications for the Evolution of Flowering Plants

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The formation of a polyploid endosperm tissue has long been considered a unique and defining feature (autapomorphy) of angiosperms. Contemporaneous with the fertilization of an egg nucleus by a sperm nucleus in *Ephedra trifurca* (a nonflowering seed plant closely related to angiosperms), a second fertilization event has previously been shown to occur between a second sperm nucleus and the sister nucleus of the egg nucleus. Development of the second fertilization product is now shown to be fundamentally similar to that of endosperm in primitive flowering plants: both are characterized by an initial period of free nuclear proliferation followed by a process of cellularization. In *Ephedra*, however, the second fertilization product ultimately yields additional embryos. If double fertilization in *Ephedra* and angiosperms is evolutionarily homologous, it is likely that endosperm evolved from a supernumerary fertilization event that originally produced embryos into one that produced a specialized nonembryo tissue dedicated to the nourishment of the zygotic embryo.

"The unravelling of the history of the phylogenetic evolution of the process of endosperm formation should prove one of the most interesting developments in botany, and if accomplished will go far to solve the problem of the origin of Angiosperms." These words, written in 1907 by the botanist E. N. Thomas (*1*) are still true today. Despite considerable research into developmental, physiological, and ecological aspects of endosperm (*2*), relatively little is known of the sequence of evolutionary events that led to the origin and establishment of this distinctive feature of angiosperm reproduction.

Double fertilization and the associated formation of polyploid endosperm have long been considered unique and defining features of angiosperms (*3*). Recent studies, however, have established that a process of double fertilization also occurs in members of the genus *Ephedra* (*4–6*). These findings are significant in view of the critical phylogenetic position of *Ephedra*, which is a basal member of the most closely related extant group of seed plants (Gnetales) to angiosperms (*7*). Thus, the presence of double fertilization in *Ephedra* and angiosperms suggests that this important feature of reproduction may be evolutionarily homologous in both groups of seed plants, having been inherited from a common pre-angiosperm ancestor (*4, 5, 8*).

Although a pattern of double fertilization has been established in *Ephedra nevadensis* (*4, 5*) and *Ephedra trifurca* (*6*), the fate of the second fertilization product has remained uncertain. In this report, evidence is advanced that supernumerary embryos are formed from the fusion product of the second fertilization event in *E. trifurca*. These findings have profound implications for our interpretation of the evolutionary history of endosperm and the origin of angiosperms.

Ovules of *E. trifurca* were collected from naturally occurring populations near Tucson, Arizona, from 1987 through 1990.

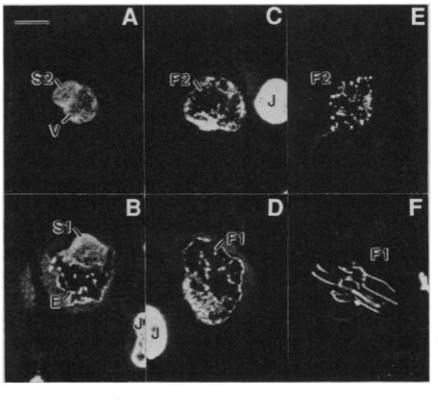

Fig. 1. Fluorescence views (stained with DAPI) of double fertilization and early post-fertilization free nuclear development in three individual egg cells of *E. trifurca*. Early stage of double fertilization: (**A**) second sperm nucleus (S2) and ventral canal nucleus (V) and (**B**) first sperm nucleus (S1) and egg nucleus (E). Fusion products of double fertilization: (**C**) second fertilization product (F2) and (**D**) first fertilization product (F1). Mitosis of fusion products of double fertilization: (**E**) second fertilization product and (**F**) first fertilization product. Jacket cell nuclei (J) in jacket cells adjacent to the egg cell are visible in several frames. Scale bar, 25 μm.

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Ovules were dissected, chemically preserved, and prepared for histological sectioning. Each ovule was serially sectioned to ensure that all nuclei within a specific archegonium could be accounted for and correctly interpreted. In total, more than 400 ovules were serially sectioned and analyzed. From these, more than 100 ovules containing archegonia (usually two or three) at stages of development between the time of pollination and the formation of cellular proembryos were examined.

At the time of fertilization in *E. trifurca*, egg cells are binucleate and contain an egg nucleus and a ventral canal nucleus. Following entry of two sperm nuclei (from a binucleate sperm cell) into the egg cell, one sperm nucleus migrates in a chalazal direction to fuse with the egg nucleus. Contact is established between the egg nucleus and first sperm nucleus (Figs. 1B and 2A), and these two nuclei begin a coordinated process of migration toward the base of the egg cell. At the same time, the ventral canal nucleus typically migrates from its initially apical position to a more central position within the egg cell cytoplasm, where it initiates a process of fusion with the second sperm nucleus (Figs. 1A and 2A).

At the end of the double fertilization process in *E. trifurca*, two diploid nuclei reside within the cytoplasm of the former egg cell (Figs. 1, C and D, and 2B). The zygote nucleus, derived from the fertilization of the egg nucleus by a sperm nucleus, is situated at the base of the former egg cell. A second nucleus, formed from the fusion of the ventral canal nucleus and the second sperm nucleus, is located approximately one-quarter to one-third of the distance from the apex of the former egg cell. Microspectrophotometric data indicate that the product of the second fertilization event in *E. trifurca* contains a 4C quantity of DNA (*6*), as does the fusion product of the egg nucleus and first sperm nucleus (*6*). Thus, the nucleus derived from the second fertilization event is similar, both genetically (*9*) and cell biologically, to the zygote nucleus that forms from the fusion of the first sperm nucleus and egg nucleus.

Light and fluorescence microscopy show that, in *E. trifurca*, each fertilization product enters into mitosis (Fig. 1, E and F). The synchronous mitotic divisions of each nucleus result in the production of two sets of daughter nuclei (four nucleate stage). One pair of nuclei (derived from the zygote nucleus) is situated at the base of the former egg cell and a second pair of nuclei (derived from the second fertilization product) is positioned within the apical half of the former egg cell (Fig. 2C). A second wave of mitotic activity is initiated by each of the four nuclei within the former egg cell to yield a total of eight nuclei (eight nucleate stage) (Fig. 2D). These nuclei are typically arranged in two groups within the former egg cell cytoplasm: a basal set of four nuclei (derived from the "true" zygote nucleus) and an apical set of four nuclei that are descendants of the second fertilization product.

Following the production of eight free nuclei (*10*) within the cytoplasm of the former egg cell in *Ephedra*, phragmoplasts develop around individual nuclei and spherical cell walls are produced. These cell walls separate each nucleus (and an associated region of cytoplasm) from the remaining cytoplasm of the former egg cell. As a consequence of this process of cellulariza-

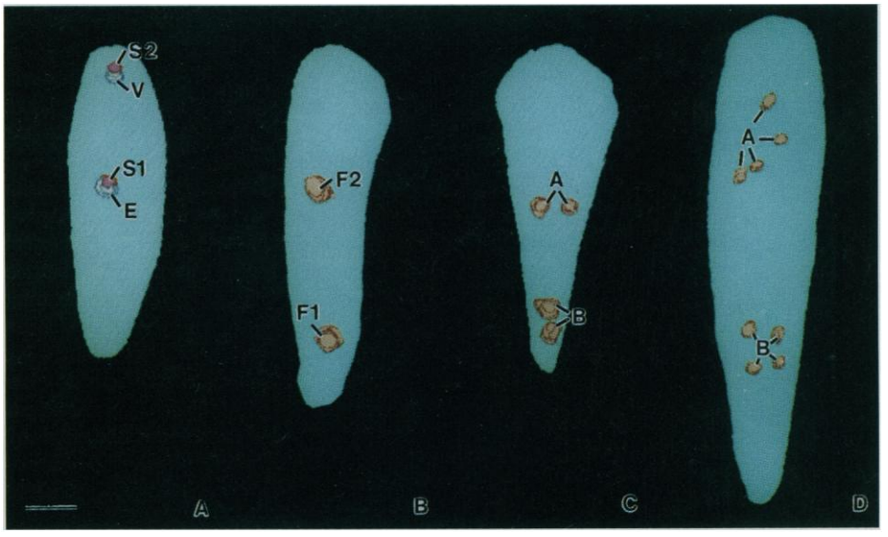

Fig. 2. Computer reconstructed images from serial sections of entire archegonia during double fertilization and free nuclear development. (**A**) Early stage of double fertilization and (**B**) two-nucleate stage. The apical nucleus is derived from the second fertilization event. The basal nucleus is derived from the first fertilization event; (**C**) four-nucleate stage and (**D**) eight-nucleate stage. Nuclei labeled (A) are derived from the second fertilization product. Nuclei labeled (B) are derived from the first fertilization product. Scale bar, 100 μm.

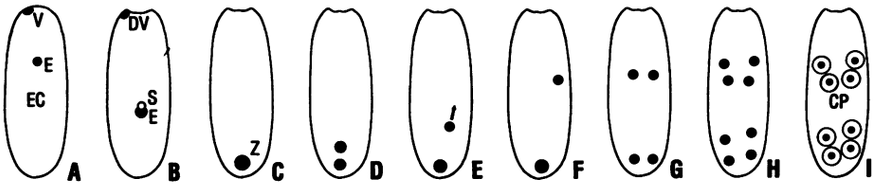

Fig. 3. Schematic of earlier hypothesis of proembryo development in *Ephedra*. (**A**) Binucleate egg cell (EC) before fertilization with egg nucleus (E) and ventral canal nucleus (V); (**B**) single fertilization of egg nucleus by a sperm nucleus (*S*) and the degeneration of the ventral canal nucleus (DV); (**C**) migration of zygote nucleus to base of former egg cell; (**D**) two nucleate stage of post-fertilization development following mitosis of zygote nucleus. Evidence of this single mitotic division was not found; (**E** to **F**) migration of nucleus to apical end of former egg cell to produce late two-nucleate stage; (**G**) four-nucleate stage of post-fertilization development following mitosis of two nuclei; (**H**) eight-nucleate stage of post-fertilization development following mitosis of four nuclei; and (**I**) cellularization of proembryo nuclei to produce eight cellular proembryos (CP).

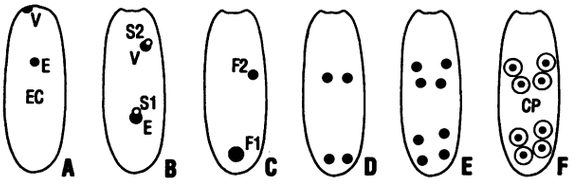

Fig. 4. Schematic of double fertilization and proembryo development in *E. trifurca* based on findings reported here. (**A**) Binucleate egg cell (EC) before fertilization with egg nucleus (E) and ventral canal nucleus (V); (**B** to **C**) Double fertilization of egg nucleus and ventral canal nucleus by two sperm nuclei (S1 and S2) to produce two fertilization products (F1 and F2) and the two-nucleate stage of post-fertilization development; (**D**) four-nucleate stage of post-fertilization development following mitosis of two nuclei; (**E**) eight-nucleate stage of post-fertilization development following mitosis of four nuclei; and (**F**) cellularization of individual proembryo nuclei to produce eight cellular proembryos (CP).

tion, eight individual (and genetically identical) potential embryos are formed (*9, 11*). Ultimately, only one embryo will survive during the maturation of the seed (*12*).

The present report of post-fertilization events in *E. trifurca* differs fundamentally from previous descriptions of embryogeny in a variety of *Ephedra* species. Earlier investigators of reproduction in *Ephedra*, unaware of a regular process of double fertilization, inferred that all of the free nuclei and resulting proembryos produced within an individual archegonium were derived from a single zygote nucleus through a series of three (as opposed to two, reported here) sets of mitotic divisions (Figs. 3 and 4) (*13–15*). It was postulated that a mitotic division of a single zygote nucleus produced two nuclei at the base of the former egg cell (*14, 16*). One of the two nuclei was thought to migrate subsequently to the micropylar (apical) end of the former egg cell (*14*) (Fig. 3, D to F). The reported positions of these two nuclei, after the hypothesized migration of one nucleus, appear to match those of the two fertilization products in *E. trifurca* prior to any mitotic activity (Figs. 2B and 4C). Following the two nucleate stage of development, earlier workers documented two "additional" sets of mitotic divisions to produce a total of eight free nuclei within the cytoplasm of the former egg cell (*13–15*) (Fig. 3, F to H). These divisions correspond to the only observed free nuclear divisions in *E. trifurca* after double fertilization (Fig. 4, C to E).

The two models of post-fertilization free nuclear development in *Ephedra* (Figs. 3 and 4) can be distinguished by the expected patterns of mitosis and proembryo formation: in particular, the presence or absence of a single mitotic figure at the outset of free nuclear development. In *E. trifurca*, 34 eggs were observed with two pairs of gametes in the process of fusion (double fertilization stage). Twenty-three fertilized egg cells contained two nuclei: a zygote nucleus located at the basal end of the egg cell and an additional nucleus in the apical half of the cell (two-nucleate stage) (*17*). Nine archegonia were found to contain mitotic figures of the two-to-four nucleate stage of post-fertilization development. Nine additional archegonia contained mitotic figures of the four-to-eight nucleate stage. Notably, however, archegonia with only a single mitotic figure (a single zygote nucleus) or two free nuclei situated at the base of the former egg cell were not observed. Thus, the critical first division in the old model of post-fertilization development (Fig. 3, C and D) was not found.

From a comparative viewpoint, the second fertilization product in *Ephedra* exhibits a developmental pattern fundamentally similar to that of endosperm in primitive groups (*2, 18*) of flowering plants ("free nuclear endosperm"). In *Ephedra*, the second fertilization product yields free nuclei that subsequently undergo a process of cellularization (to produce embryos). Among flowering plants with a free nuclear pattern of endosperm development, the primary endosperm nucleus (a triploid fertilization product formed from the fusion of a sperm nucleus with two polar nuclei) also initiates a process of free nuclear proliferation. This free nuclear period of development typically is followed by a process of cellularization (*2*). Although the extent of free nuclear development of endosperm is far more extensive (often hundreds of nuclei that later become cellular) than is the case with the derivatives of the second fertilization product in *Ephedra* (four nuclei that become cellular), the underlying pattern of free nuclear to cellular development is, in most critical aspects, identical.

The current data indicate that double fertilization (*4, 5, 19*) and a subsequent free nuclear to cellular proliferation of the second fertilization product can no longer be assumed to be autapomorphies of angiosperms. If double fertilization and free nuclear to cellular development of the second fertilization product are evolutionarily homologous (synapomorphous) in *Ephedra* and angiosperms, endosperm is likely to have evolved through an intermediate stage in which a diploid embryo initially resulted from the second fertilization event. This stage of evolutionary advancement would have been established in the common pre-angiosperm ancestor of *Ephedra* and flowering plants (Fig. 5) (*20*).

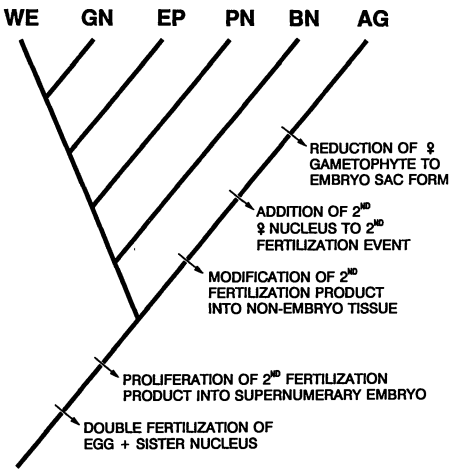

Fig. 5. Cladogram indicating the most parsimonious interpretation of the evolution of double fertilization and endosperm in angiosperms (AG) and their sister group [phylogenetic relationships based on Doyle and Donoghue (7)]. Double fertilization of an egg and its sister nucleus is a synapomorphy of angiosperms and their sister group [*Ephedra* (EP), *Gnetum* (GN), *Welwitschia* (WE); character states of fossil groups *Pentoxylon* (PN) and Bennettitales (BN) are unknown], as is the free nuclear to cellular proliferation of the second fertilization product. Autapomorphies of angiosperms are the amplification of development of the second fertilization product and its modification into a nonembryo endosperm tissue; the addition of a second female nucleus to the second fertilization event to yield a triploid fusion product; and the reduction of the female gametophyte to a simple embryo sac form. The order in which these three autapomorphies evolved is unresolved. Additional autapomorphies not indicated on the cladogram are the loss of a free nuclear stage of embryo development in angiosperms, and the modification of free nuclear to cellular embryo development to produce multiple embryos (*11*) in *Ephedra*.

An early study of embryogeny in *E. trifurca* indicates that the basal set of embryos (now shown to be derived from the "true" zygote nucleus) typically yields the embryo that ultimately survives within the seed (*13*), whereas the apical embryos (derived from the second fertilization product) tend to abort at earlier stages of development. This suggests an additional similarity between the second fertilization product in *Ephedra* and endosperm: both second fertilization products do not appear to produce viable progeny. However, endosperm (in primitive flowering plants) and supernumerary embryos in *Ephedra* are genetically identical, at the level of shared alleles, to the corresponding sister embryos derived from the first fertilization event. From this perspective, the second fertilization products in *Ephedra* and angiosperms can perpetuate their genes through the successful development of sibling embryos.

Although supernumerary embryos derived from a second fertilization event in the ancestors of angiosperms might not have survived to reproduce, the behavior of the second fertilization product could have assisted in the ultimate survivorship of its genetically identical kin without sacrifice to its own genetic survival. If "altruistic" behavior of the second fertilization product were selected for in the ancestors of angiosperms (*21*), a plausible outcome might have been the developmental modification of a supernumerary fertilization product (originally an embryo) into a specialized tissue (endosperm) dedicated to the nourishment of its genetically identical sister embryo.

The present findings are consistent with the hypothesis that the earliest angiosperms inherited the basic foundations of a process of double fertilization and the potential for subsequent development of this fusion product (Fig. 5). From this perspective, the only distinguishing features (autapomorphies) of angiosperms with respect to sexual reproduction are (i) the modification of development of the second fertilization product from supernumerary embryo pro-

duction to the formation of endosperm, (ii) the addition of a second female nucleus to the second fertilization event, and (iii) the reduction of the female gametophyte to the characteristic seven-celled, eight-nucleate angiosperm embryo sac form.

It is presently unresolved whether the addition of a second female nucleus to the second fertilization event occurred before or after the evolution of an embryo-nourishing tissue. However, it is likely that the extreme reduction of the female gametophyte (which in all nonflowering seed plants is responsible for the nourishment of the embryo) to the embryo sac form was predicated upon the prior evolution of a novel embryo-nourishing tissue. With the evolution of a process of double fertilization in the ancestors of flowering plants, and the subsequent modification of the second fusion product into a unique nonembryo nutritive tissue (endosperm), the pattern of sexual reproduction that is characteristic of angiosperms was established.

REFERENCES AND NOTES

1. E. N. Thomas, *Sci. Prog.* **1**, 420 (1907).
2. M. R. Vijayaraghavam and K. Prabhakar, in *Embryology of Angiosperms*, B. M. Johri, Ed. (Springer-Verlag, New York, 1984), pp. 319–376.
3. G. L. Stebbins, in *Origin and Early Evolution of Angiosperms*, C. B. Beck, Ed. (Columbia Univ. Press, New York, 1976), pp. 300–311; A. Cronquist, *The Evolution and Classification of Flowering Plants* (New York Botanical Garden, New York, 1988).
4. W. E. Friedman, *Science* **247**, 951 (1990).
5. ———, *Am. J. Bot.* **77**, 1582 (1990).
6. ———, *Protoplasma*, in press.
7. P. R. Crane, *Ann. Mo. Bot. Gard.* **72**, 716 (1985); J. A. Doyle and M. J. Donoghue, *Bot. Rev.* **52**, 321 (1986).
8. M. J. Donoghue, *Evolution* **43**, 1137 (1989).
9. The two sperm nuclei involved in double fertilization are likely to be from a single pollen tube, and hence genetically identical; the egg nucleus and ventral canal nucleus are derivatives of the mitotic division of the central cell nucleus. Thus, each fertilization product is genetically identical.
10. Instances were observed where fewer or more than eight free nuclei resulted from double fertilization in *E. trifurca*.
11. In most nonflowering seed plants, initial free nuclear development of the zygote is followed by cellularization to yield a single multicellular embryo. In *Ephedra*, however, a free nuclear to cellular pattern of embryogeny results in the production of multiple unicellular embryos and probably represents a modification of the sympleisiomorphic condition of embryo development.
12. In ovules where all the egg cells (typically three) are fertilized, 24 or more proembryos may theoretically be produced, only one of which will ultimately survive during maturation of the seed.
13. W. J. G. Land, *Bot. Gaz.* **44**, 273 (1907).
14. R. Khan, *Proc. Natl. Acad. Sci. India* **13**, 357 (1943).
15. N. Narang, *Proc. Indian Sci. Cong.* **3**, 224 (1955); M. Lehmann-Baerts, *Cellule* **67**, 53 (1967); B. Moussel, *Rev. Cytol. Biol. Veg.* **40**, 73 (1977); *Rev. Cytol. Biol. Veg. Bot.* **6**, 103 (1983).
16. No figures (photographic or camera-lucida) were published as evidence of the proposed initial division of a single zygote nucleus.
17. In six cases, a zygote nucleus was situated at the base of the egg cell, and two additional nuclei, the second sperm nucleus and ventral canal nucleus, remained separate within the apical portion of the egg cell. In six additional archegonia, the second sperm nucleus and ventral canal nucleus were in contact, but did not appear to be in the process of fusing. Thus, the second fertilization event, although almost always initiated, may not always be completed in *E. trifurca*.
18. G. L. Stebbins, *Flowering Plants, Evolution Above the Species Level* (Harvard Univ. Press, Cambridge, MA, 1974).
19. In both *Ephedra* and angiosperms, the second fertilization event involves a fusion of a sperm nucleus with the sister nucleus of the egg nucleus. In *Ephedra*, the ventral canal nucleus is the sister nucleus of the egg nucleus (*4–6*). In angiosperms with a primitive pattern of embryo sac development (*Polygonum* type) [M. F. Willson and N. Burley, *Mate Choice in Plants* (Princeton Univ. Press, Princeton, NJ, 1983)], one of the two polar nuclei with which the second sperm nucleus fuses is the sister nucleus of the egg nucleus [R. A. Brink and D. C. Cooper, *Bot. Rev.* **13**, 423 (1947)].
20. These findings are consistent with an hypothesis concerning the evolutionary origin and history of endosperm first proposed in 1900 [E. Sargant, *Ann. Bot.* **14**, 689 (1900)]. She suggested that subsequent to the establishment of a process of double fertilization in the ancestors of flowering plants, the second fertilization product originally yielded a supernumerary diploid embryo. Sargant hypothesized that endosperm later evolved through a modification of supernumerary embryo development into an aberrant non-embryo (endosperm) tissue.
21. Several papers have addressed theoretical aspects of kin selection and endosperm: E. L. Charnov, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2480 (1979); M. Westoby and B. Rice, *Evolution* **36**, 713 (1982); D. C. Queller, *J. Theor. Biol.* **100**, 153 (1983); *Heredity* **53**, 151 (1984). See also discussion by M. F. Willson and N. Burley [*Mate Choice in Plants* (Princeton Univ. Press, Princeton, NJ, 1983)].

22. I thank P. Diggle and J. Hamrick for critical reading of the manuscript, M. Donoghue for stimulating discussions at the outset of this research, T. Reagin and B. Yao for assistance with histological preparations, and S. Buchmann, M. Buchmann, R. Robichaux, S. Nelson, and M. Porter for logistical assistance with fieldwork. Supported by NSF research grant BSR 8818035.

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G_z-Mediated Hormonal Inhibition of Cyclic AMP Accumulation

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Hormones inhibit synthesis of adenosine 3′,5′-monophosphate (cAMP) in most cells via receptors coupled to pertussis toxin (PTX)–sensitive guanine nucleotide-binding (G) proteins. Mutationally activated α subunits of G_{i2} (α_{i2}) constitutively inhibit cAMP accumulation when transfected into cells. Cells have now been transfected with mutant α subunits of four other G proteins—G_z, a PTX-insensitive G protein of unknown function, and G_{i1}, G_{i3}, and G_o, which are PTX-sensitive. Mutant α_z, α_{i1}, and α_{i3} inhibited cAMP accumulation but α_o did not. Moreover, expression of wild-type α_z produced cells in which PTX did not block hormonal inhibition of cAMP accumulation. Thus, G_z can trigger an effector pathway in response to hormone receptors that ordinarily interact with PTX-sensitive G_i proteins.

HETEROTRIMERIC G PROTEINS receive biological signals from receptors for hormones and neurotransmitters and transduce them into regulation of effector enzymes and ion channels. Each G protein sorts signaling information in a distinctive pattern. Structural features of the α subunit allow it to receive incoming information from a limited subset of hormone receptors and to convey that information to a specific subset of effectors (*1*). The rapidly increasing number of new G proteins makes it desirable to identify the receptors and effector pathways associated with each.

Point mutations that replace key conserved amino acids in the α subunits of G_s (α_s) (*2*) and G_{i2} (α_{i2}) (*3, 4*) create G proteins that constitutively activate their downstream effector pathways—stimulation and inhibition of cAMP synthesis, respectively. Because these mutations replace amino acids that are conserved in all known α chains, cognate mutations in other α subunits should provide a general approach to determine whether an individual G protein can trigger a specific effector pathway. This approach is independent of receptor activation and receptor–G protein coupling. Thus, expression of mutationally activated α_{i2} in cultured cells constitutively inhibits cAMP accumulation (*4*), confirming one of the putative functions of G_{i2}. We have now extended this strategy to test whether four other G protein α chains inhibit cAMP accumulation.

Mutant α_{i1} and α_{i3} would be expected to mimic α_{i2} because these proteins resemble one another in primary structure (~85% identical amino acid sequences) and because all three members of the α_i family can open atrial potassium channels (*5*). The greater structural difference between α_o and the α_i subunits (~70% identical sequences) suggested that α_o (*6*) would not inhibit cAMP accumulation. The signaling function of G_z

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