closely related genes and the regulation of other genes modulated during acute inflammation. Finally, these results indicate that a single mediator, IL-1, can induce increases and decreases in the expression of genes encoding liver-derived plasma proteins that are affected during the acute-phase response.

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Lower Cretaceous Angiosperm Flowers: Fossil Evidence on Early Radiation of Dicotyledons

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Three-dimensionally preserved unisexual angiosperm flowers and inflorescences have been recovered from the Lower Cretaceous Patapsco Formation (Potomac Group) of eastern North America, in sediments palynologically dated as late Albian, approximately 100 million years old. In situ tricolpate pollen shows that the flowers were produced by some of the earliest higher (nonmagnoliid) dicotyledons, and the morphology of pollen, flowers, and inflorescences indicates a close relation to extant Platanaceae. Combined with architectural and cuticular features of associated leaves these floral remains suggest that *Platanus*-like plants with unisexual, probably insect-pollinated flowers were an important element in the mid-Cretaceous diversification of dicotyledonous flowering plants.

PALEOBOTANICAL AND STRATIGRAPHIC analyses during the last 30 years have documented a major radiation of flowering plants during the mid-Cretaceous (*1–3*). Between the Hauterivian and Cenomanian, unequivocal angiosperm pollen and leaves first appear in the fossil record and exhibit parallel patterns of increasing diversity, complexity, and abundance (*1–5*). Available evidence suggests that this reflects a major systematic and ecological radiation, during which many of the features of pollen morphology and leaf architecture that characterize extant flowering plants appeared for the first time (*5, 6*). Although detailed studies of fossil leaves and pollen have begun to clarify the systematic relations of mid-Cretaceous angiosperms (*3, 5–9*), many critical aspects of their structure and biology remain inaccessible from studies of leaves and pollen alone. Where available, mid-Cretaceous flowers supply unequivocal evidence of systematically important floral structure and provide an improved basis for interpreting pollination and other aspects of early angiosperm biology (*10, 11*).

Extant dicotyledons are divided into six subclasses: Magnoliidae, Hamamelidae, Caryophyllidae, Dilleniidae, Rosidae, and Asteridae. The higher (nonmagnoliid) dicotyledons include over 70 percent of extant angiosperm species (*12*), and triaperturate pollen diagnostic of this group first appears at very low concentrations in the Barremian-Aptian of Northern Gondwana (*13*), approximately 120 million years ago. During the Aptian and Albian, the variety and abundance of triaperturate pollen increases dramatically, and by the middle Cenomanian, approximately 25 million years later, many palynofloras include tricolpate, tricolporoidate, tricolporate, and triporate forms (*1–3*). This clear chronological pattern in the fossil pollen record has been interpreted as reflecting the initial major radiation of nonmagnoliid dicotyledons.

The Early Cretaceous plants that produced tricolpate pollen have not been identified, but studies of foliar remains suggest that they may have been early representatives of the Hamamelidae and Rosidae, represented by so-called platanoid and *Sapindopsis* leaves, respectively (*2, 5, 9*). The fossil flowers discussed here provide information on floral structure in this important group of flowering plants during the Early Cretaceous and predate previous reports of angiosperm flowers with in situ pollen by approximately 6 million years (*10*).

Fossil inflorescences and flowers were recovered by sieving from a grey clay collected at the West Brothers locality in the Patapsco Formation (Potomac Group) of Maryland. The associated palynoflora suggests a late Albian age, approximately 100 million years old (*2, 14*). The fossiliferous sediments are lenticular and interpreted as the fill of an abandoned channel (*15*). They contain wood fragments, conifer cones, seeds and shoots, and a variety of angiosperm fruits, seeds, and other reproductive structures. The leaf flora is dominated by *Sapindopsis* foliage, and the diversity of angiosperm reproductive structures recovered far exceeds that of the associated fossil leaves.

Staminate and pistillate flowers are clustered in separate, more or less spherical heads that are sessile on an elongated inflorescence axis. Although both kinds of inflorescence occur separately, the similar spherical heads and floral structure suggest that they were derived from closely related plants. Pistillate flowers consist of several membranous tepals surrounding five free carpels (Fig. 1A). The outer tepals are short, but the inner are longer and frequently extend to the apex of the carpels. Clumps of pollen identical to that preserved in the staminate flowers occur at the apex of some of the inner tepals. Stamens are not present, but the possibility that some of the inner tepals may be staminodes cannot be resolved with present material. The carpels are oblong, with an incompletely fused adaxial suture extending for most of their length.

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There is no clearly differentiated style or stigma but the carpel apex is swollen into two lobes. The number of ovules within each carpel is unknown.

Staminate flowers consist of several membranous tepals surrounding five stamens (Fig. 1B): carpels are not present. The stamens consist of a very short filament bearing elongated anthers. At the apex the connective is expanded into a short rounded cap. Pollen within the anthers (Fig. 1C) is similar to the dispersed pollen species *Tricolpites minutus* (Brenner) Dettmann (*1, 14*). The grains are small, semitectate, and microreticulate (Fig. 1D). They are prolate, 8 to 10 μm long, with three colpi extending for three-quarters of their length. Some specimens (Fig. 1D) show a slightly bowed area in the center of the colpus, perhaps indicating the presence of a poorly defined endoaperture, as is typical of *T. minutus* (*14*). Other grains show no external indication of an equatorial thinning.

The morphology of both staminate and pistillate inflorescences is similar to inflorescences reported from other localities in the Potomac Group (*5*) and from other Cretaceous floras (*16, 17*). The similarities between these Cretaceous inflorescences and those of the extant southeast Asian species *Platanus kerrii* have been noted (*17*), but detailed comparisons have been precluded by poor preservation of floral details in fossil material. The specimens now available from the Potomac Group provide information that strongly supports the proposed relation between these fossil inflorescences and extant Platanaceae. The similarities include unisexual flowers clustered into more or less spherical heads that are sessile along an elongated inflorescence axis, and tricolpate microreticulate pollen. Differences from extant Platanaceae include the regular occurrence of floral parts in fives, the very small pollen size, the presence of a well-developed perianth, and the absence of hairs around the base of each carpel. All of these features also occur in unequivocal Platanaceae from the Late Cretaceous and early Tertiary (*18*), and the only nonplatanaceous characteristic of the Early Cretaceous material is the presence of a probable rudimentary equatorial thinning in the colpi of some pollen grains.

Early Cretaceous and extant platanoids may have differed in their mode of pollination. Extant *Platanus* is wind-pollinated with pollen typically ranging from 16 to 22 μm in length (*18*). Pollen from the Early Cretaceous staminate flowers is typically 8 to 10 μm in length. This is smaller than the 11 to 16 μm reported for Late Cretaceous and early Tertiary Platanaceae (*18*) and well below the 20 to 40 μm size range characteristic of the pollen of anemophilous angiosperms (*19*). Taken together, the very small pollen size, the clumps of pollen on pistillate flowers, and the well-developed perianths suggest that these Early Cretaceous platanoids were probably insect-pollinated.

Further evidence of a platanaceous relation for the Early Cretaceous flowers and inflorescences is provided by the cuticles prepared from pistillate inflorescences. Details of hair bases, probable secretory structures, and epicuticular ornamentation are similar to those reported for rare palmately lobed platanoid leaves from the West Brothers locality (*9*) and thus support a connection between some Early Cretaceous tricolpate pollen and contemporaneous platanoid leaves.

During the mid-Cretaceous, palmately lobed platanoid leaves and pinnately lobed or compound *Sapindopsis* leaves exhibit similar, partially intergrading, patterns of venation and cuticular structure (*2, 5, 9, 20*). These two leaf types are interpreted as early representatives of extant Hamamelidae and Rosidae, respectively, and their similarities are thought to indicate a close relation between these two angiosperm subclasses (*2, 5, 20*). *Sapindopsis* leaves dominate the flora of the West Brothers locality and may be related to other reproductive structures that occur in our samples. Detailed interpretations of these reproductive structures are not possible with the specimens currently available, but, like the probable platanaceous inflorescences, they consist of sessile, more or less spherical heads containing numerous flowers with carpels in fives. The flowers and carpels are however larger, and in some features the cuticles prepared from inflorescences resemble those from associated *Sapindopsis* leaves (*9*). The available information therefore suggests that the reproductive structures of platanoid and *Sapindopsis* plants were similar, and this adds to the evidence that early representatives of the Hamamelidae and Rosidae are closely related.

Although it is clear that the unisexual flowers and inflorescences reported here are relatively advanced with respect to extant and fossil Magnoliidae, the abundance of platanoid and *Sapindopsis* leaves in the mid-Cretaceous floras suggests that these plants

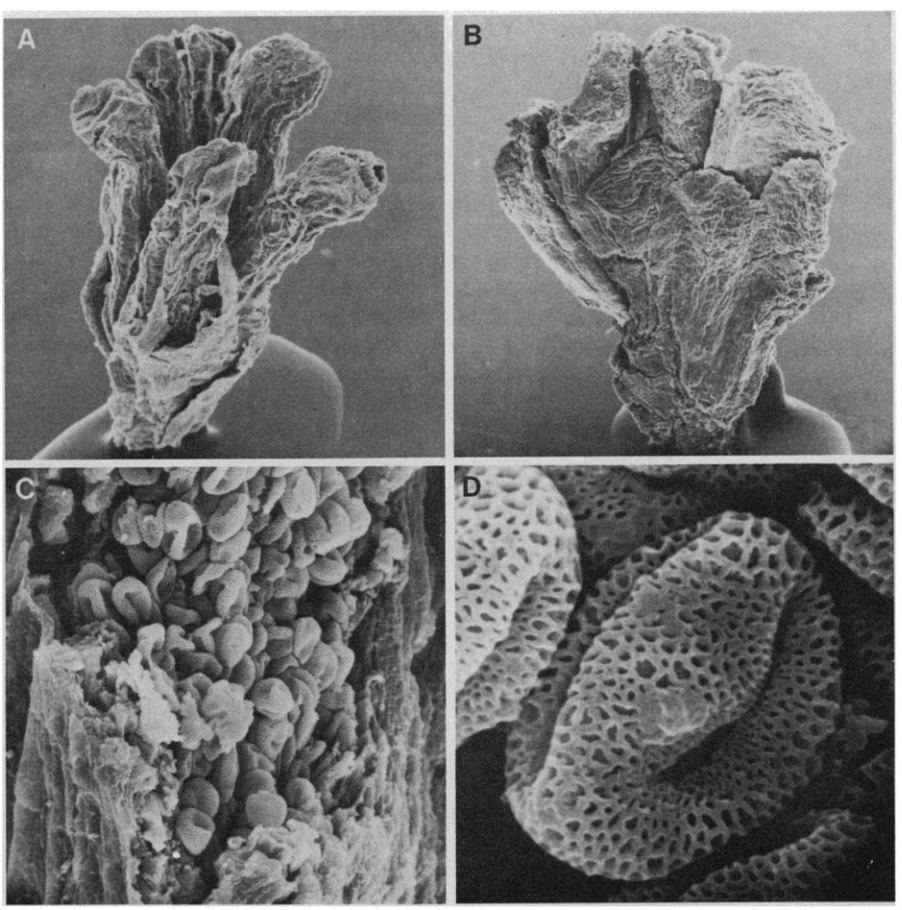

Fig. 1. Scanning electron micrographs of fossil flowers and pollen from the West Brothers locality (Lower Cretaceous, Potomac Group). (A) Pistillate flower with broken tepals showing five carpels (×73). (B) Staminate flower showing four stamens and three tepals (×73). (C) Detail of broken anther showing in situ tricolpate pollen (×882). (D) Tricolpate microreticulate pollen grain from the specimen in (C) (×6072).

played an important role in the initial divergence of the Hamamelidae and Rosidae. Early, primitive representatives of these subclasses were major elements in the primary radiation of the higher dicotyledons.

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Cloning of a cDNA for a T Cell–Specific Serine Protease from a Cytotoxic T Lymphocyte

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A new serine protease was encoded by a clone isolated from a murine cytotoxic T-lymphocyte complementary DNA library by an RNA-hybridization competition protocol. Complementary transcripts were detected in cytotoxic T lymphocytes, spleen cells from nude mice, a rat natural killer cell leukemia, and in two of eight T-helper clones (both cytotoxic), but not in normal mouse kidney, liver, spleen, or thymus, nor in several tested T- and B-cell tumors. T-cell activation with concanavalin A plus interleukin-2 induced spleen cells to express this gene with kinetics correlating with the acquisition of cytolytic capacity. The nucleotide sequence of this gene encoded an amino acid sequence of approximately 25,700 daltons, with 25 to 35 percent identity to members of the serine protease family. The active site "charge-relay" residues (His^{57}, Asp^{102}, and Ser^{195} of the chymotrypsin numbering system) are conserved, as well as the trypsin-specific Asp (position 189 in trypsin). A Southern blot analysis indicated that this gene is conserved in humans, mouse, and chicken. This serine protease may have a role in lymphocyte lysis and a "lytic cascade."

CYTOTOXIC T LYMPHOCYTES (CTL's) are a subset of antigen-induced T lymphocytes with the capacity to recognize, bind, and lyse target cells bearing the inducing antigen (*1*). Usually, the CTL "recognizes" a target cell bearing a nonself major histocompatibility (MHC) class I gene product (K or D in the mouse) or an ill-defined assemblage of antigens (often viral) plus a self-MHC class I gene product. Natural killer (NK) cells are constitutive cytotoxic cells that recognize, bind, and lyse a restricted set of target cells in an apparently MHC-unrestricted fashion. Target recognition by both active CTL's and NK cells is followed by a lysis mechanism that includes, in turn, a binding step, a lag interval, and unidirectional lysis events. Although many properties of CTL's have been characterized (*2*), the mechanisms mediating the cytolytic events are unclear. Several polypeptides released from killer cells and their cytoplasmic granules are thought to participate in the lytic event; these polypeptides include serine proteases, toxic lymphokines, and pore-forming "polyperforins" (*3, 4*).

To examine the activation and cytolytic mechanisms of CTL's, we used a natural history approach, searching for genes expressed preferentially in CTL's but absent in noncytolytic T cells. We report the isolation of a complementary DNA (cDNA) clone transcribed at high levels in CTL's but not in resting T lymphocytes. This cDNA clone encodes a previously unknown serine protease that probably has trypsin-like specificity and may play a role in cytolytic effector functions.

In order to isolate genes preferentially expressed in CTL's, we used an RNA-hybridization competition protocol (*5*) to identify sequences expressed in the CTL clone 1E4 but absent in the tumor cell line VL3. Briefly, the 1E4 cDNA library was screened with labeled 1E4 messenger RNA in the presence of excess VL3 RNA. The 1E4 cell line is a cloned cytotoxic, Lyt-2^+ T-lymphocyte line from the C57L mouse strain, lysing only Abelson virus–infected cells bearing syngeneic H-2^b class I molecules (*6*). The VL3 cell is a noncytolytic radiation virus (RadLV)–induced thymic lymphoma cell line from the C57BL/Ka (H-2^b) mouse with the surface phenotype of an immature intrathymic cell (Lyt-1^+, Lyt-2^+, GK 1.5^+) (*7*). To minimize the possible isolation of endogenous retroviral sequences, we used a CTL line from the relatively ecotropic retrovirus–deficient C57L mouse strain (*8*), for competition with RNA from the virus-producing VL3 line. We prepared a cDNA phage library from the CTL clone 1E4 (*9, 10*). This 1E4 cDNA phage library was screened with a mixture of an alkali-treated, ^{32}P-kinase–labeled 1E4 poly(A)–RNA probe and a several hundredfold excess of cold total VL3 RNA. The labeled sequences shared by VL3 and 1E4 cells would thus be diluted by competition for the probe, whereas sequences occurring only in 1E4 would not be diluted and would produce hybridization signals.

Using this RNA hybridization competition scheme, we screened 4.8×10^4 recombinant phage plaques of the 1E4 cDNA library (*11*). Initially, 70 positive phage plugs were picked and rescreened through two more rounds of RNA hybridization competition; this yielded 13 plaque-purified clones with reproducible signals. As an assay for the specificity of these clones, these putatively CTL-preferential clones were placed into an array, and replicates of the

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