evidence for the precise location of the sperm enzymes or lysins responsible has not been forthcoming. Even though the localization of the protease in the acrosomes of the sea urchin sperm had been reported (*19*), the accompanying fluorescent photomicrographs did not confirm this observation. Indeed, the fluorescence can be observed over the entire sperm nucleus. The biochemical work of Levine *et al.* (*8*) does show a good correlation between acrosomally reacted sperm (as judged by light microscopy) and activity of a trypsin-like protease. Levine *et al.* were able to show strong inhibition of fertilization of eggs with reacted sperm given preliminary treatment with either PMSF (phenylmethanesulfonyl fluoride) or DFP (diisopropyl fluorophosphate), two serine protease inhibitors. Their use of the well-known trypsin substrate BAEE, and our ferritin labeling with soybean trypsin inhibitor indicate that the protease in question is related to trypsin. Hoshi *et al.* (*20*) have shown inhibition of fertilization in sea urchins by chymostatin and TPCK (L-1-tosylamide-2-phenylethyl chloromethyl ketone), which are chymotrypsin-specific inhibitors, and NPGB (*p*-nitrophenyl-*p'*-guanidino-benzoate) which inhibits both trypsin and chymotrypsin; but trypsin-specific inhibitors were ineffective. Hoshi *et al.* believe that a chymotrypsin-like enzyme is involved in sperm penetration of the vitelline coat. However, since their work consisted of inseminating eggs in the presence of the inhibitors, it is difficult to evaluate whether the inhibitors were acting on the sperm, the eggs, or both. At present, both trypsin- and chymotrypsin-like enzymes in the acrosomes cannot be ruled out.

Our study leaves open the question of the role of such sperm enzymes in fertilization. The protease appears to be located at the tip of the acrosomal tubule indicating a possible role in the dissolution of the vitelline coat (*21*), but numerous experiments with SBTI [see (*22*) for review] show that fertilization does occur in the presence of some trypsin inhibitors. Another possibility, as previously pointed out (*8*), is that the enzyme may function in the acrosome reaction itself. This would not be surprising, as phospholipase activity has been reported in sea urchin sperm (*23*). If phospholipase mediates gamete membrane fusion as suggested (*23*), or acrosomal vesicle dehiscence in the initial stages of the acrosome reaction, a trypsin-like enzyme may activate the phospholipase (*22*), as can occur with mammalian pancreatic trypsin in the digestive tract (*24*). Another possibility for the role of the protease is the modification or deposition (or both) of bindin on the extruded acrosomal tubule. Now that the protease has been definitively localized on the acrosomal tubule of *S. purpuratus* sperm, the function of the enzyme can be further investigated.

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12. The mixtures were concentrated at ~4°C (ice bath), with stirring, under 3 atm of nitrogen, in a cylindrical plexiglass container with a PM 30 Diaflo ultrafiltration membrane (Amicon). This membrane retains molecules greater than 30,000 daltons.
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Intrafloral Pollen Differentiation in the New World Lecythidaceae, Subfamily Lecythidoideae

Abstract. *Floral biology, pollen germination, and scanning electron microscopy studies have indicated different types of pollen within the same flower in at least two species of New World Lecythidaceae, subfamily Lecythidoideae. Two types of pollen are produced in different parts of the androecium and serve different functions. One type attracts pollinators by providing a reward, and the other functions in fertilization. The former type has lost its ability to germinate, at least under in vitro conditions.*

The Lecythidaceae or Brazil nut family is pantropical in distribution (*1*) and consists of four subfamilies, 20 genera, and 278 species. However, in the New World subfamily Lecythidoideae, consisting of ten genera and about 206 species (*1*), an adaptive radiation of the androecium has occurred in response to animal pollen vectors.

In the Lecythidoideae, the structure of the androecia ranges from symmetrical with many stamens and no nectar to asymmetrical with fewer stamens and with nectar. The least complicated androecial type is found in *Gustavia*, *Grias*, and *Allantoma*, which have symmetrical androecia with many stamens that are fused at their bases into a ring (Fig. 1A). In *Couroupita*, *Lecythis*, *Corythophora*, *Bertholletia*, *Eschweilera*, and *Couratari*, the ring is prolonged on one side into a straplike structure that arches over the summit of the ovary (Fig. 1, B to E). This structure consists of the ligule, a stamen-free area adjacent to the staminal ring, and the hood, a distal portion with variously modified appendages. The hood may be open or tightly appressed to the summit of the ovary.

The intricate hood-ligule structure of asymmetrically flowered Lecythidoideae encourages pollination in the following ways: (i) nectar or pollen, which attract

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pollinators, is produced in the hood; (ii) the hood serves as a landing platform for pollinators; and (iii) the hood-ligule acts as a spring that forces the pollinator's back against the staminal ring and thereby ensures the deposition of the pollen in the proper position. In addition, in species with tightly appressed hoods, only insects with enough strength to force the hood open can enter the flower; the hood thus protects the flower's nectar and pollen from other insects. In all species of Lecythidoideae the style is situated in the center of the doughnut-shaped staminal ring (Fig. 1, A, B, and F).

Observations of pollination (*2*) indicate that flowers of species of Lecythidoideae with symmetrical androecia are visited by many different species of bee, whereas flowers having androecia with flat appressed hoods are usually visited by short-tongued bees that collect pollen but not nectar from the hood. Flowers with zygomorphic androecia with coiled appressed hoods are visited only by long-tongued bees that collect nectar from the apex of the coil, and at least one species with zygomorphic flowers and flat hoods, *Lecythis poiteaui* Berg, is visited by bats. In general, the more specialized the androecium the more specialized the pollinator that visits the flower.

Both *Lecythis pisonis* Cambess. and *Couroupita guianensis* Aubl. have zygomorphic androecia, flat hoods with antheriferous appendages, and no nectar. Their androecia differ in that the hood of *L. pisonis* is appressed to the staminal ring whereas that of *C. guianensis* is open.

Observations of the crown of *L. pisonis* made in southern Bahia, Brazil, have indicated that *Xylocopa frontalis* (Oliver) (*3*) is the only bee that enters its flowers (*4*). This bee lands on the hood and enters the androecium with its back and head against the staminal ring (Fig. 1F). It removes pollen from the anthers of the hood and places it among the hairs on its legs. At the same time pollen from the staminal ring is deposited on the back and head of the bee. When the bee visits another flower it carries staminal ring pollen in the proper position to be deposited on the stigma.

The petals and androecium of *L. pi-*

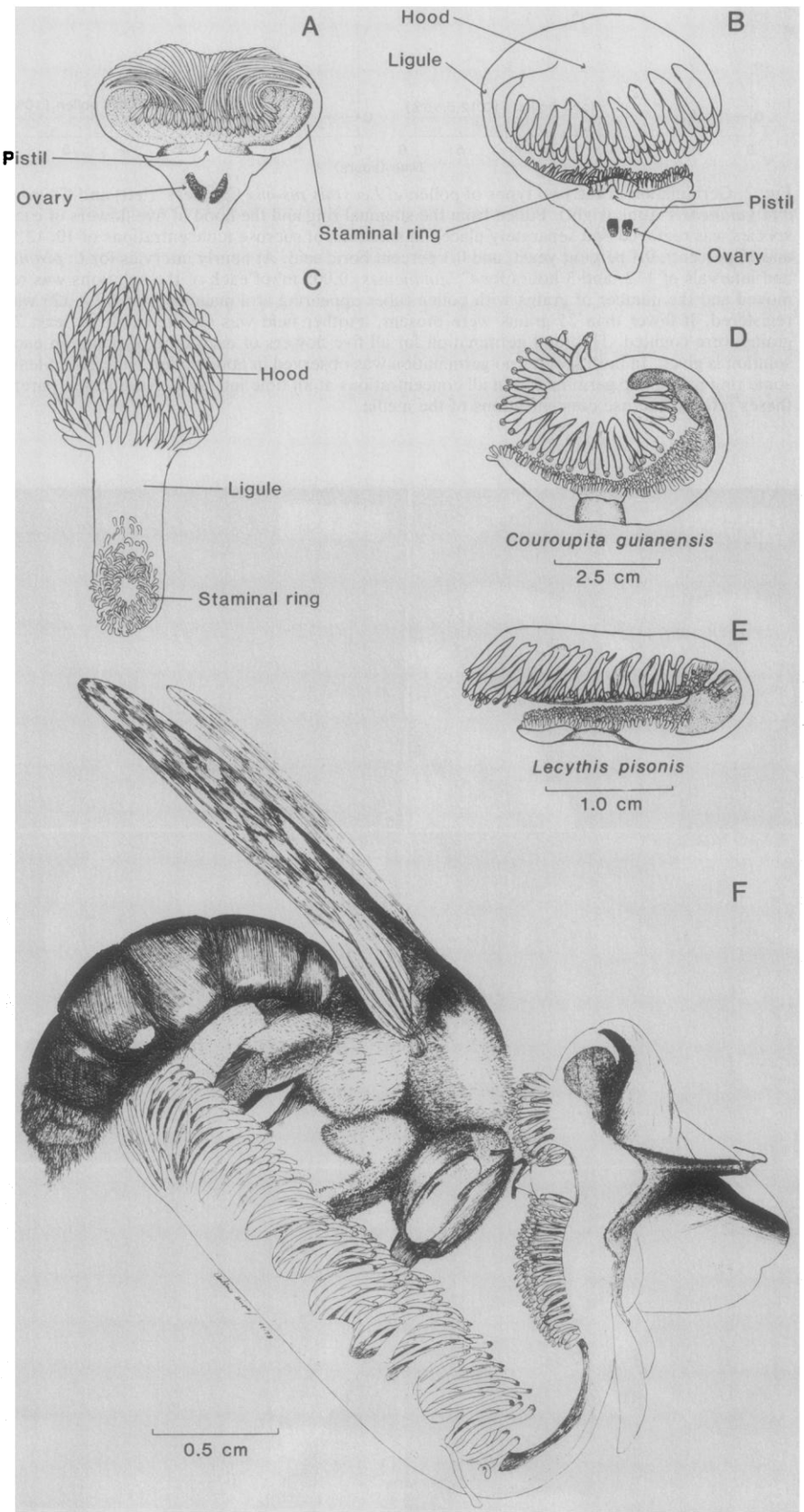


Fig. 1. Androecium types of New World Lecythidaceae subfamily Lecythidoideae. (A) Medial section of the symmetrical androecium of *Gustavia* showing the anthers fused at their bases into a ring. (B) Section of the asymmetrical androecium of *Lecythis* showing the ring expanded on one side into the straplike ligule and the expanded distal portion called the hood. Nectar is produced at the base of the sterile hood appendages. (C) Intact asymmetrical androecium removed from the ovary, opened up, and viewed from above. (D) Medial section of the androecium of *Couroupita guianensis* Aubl. The hood stamens bear anthers but no nectar. (E) Medial section of the androecium of *Lecythis pisoni* in which the hood stamens also bear anthers. (F) The carpenter bee *Xylocopa frontalis* collecting pollen from *Lecythis pisonis*. The head and back of the bee are forced by the androecium structure to touch the anthers of the staminal ring and the stigma thereby facilitating cross pollination when it visits another flower.

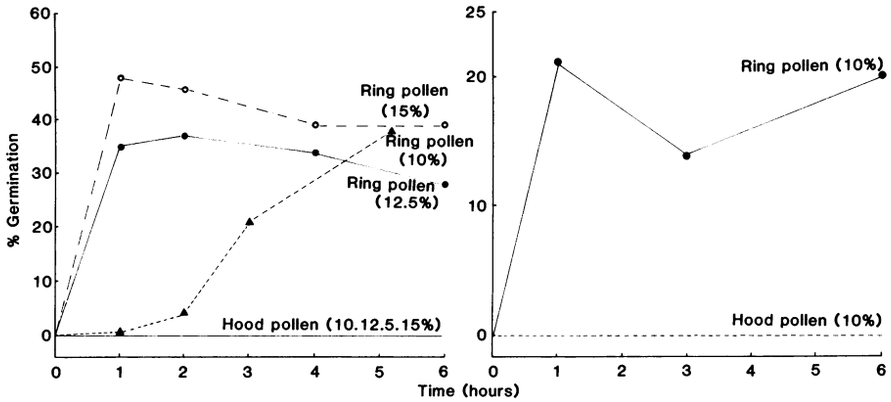
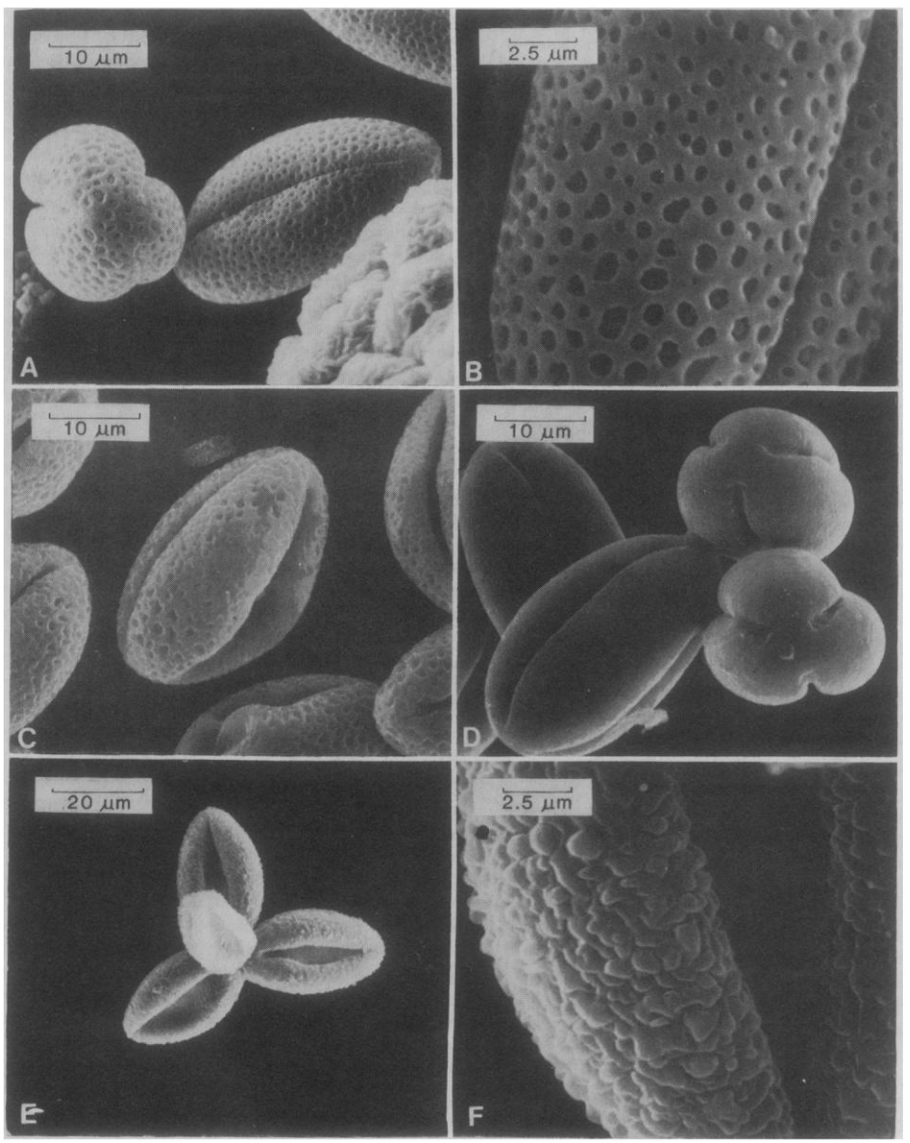


Fig. 2. Germination of the two types of pollen of *Lecythis pisonis* Cambess. (left) and *Couroupita guianensis* Aubl. (right). Pollen from the staminal ring and the hood of five flowers of each species was removed and separately placed in solutions of sucrose (concentrations of 10, 12.5, and 15 percent), 0.1 percent yeast, and 0.1 percent boric acid. At hourly intervals for *L. pisonis* and intervals of 1, 2, and 3 hours for *C. guianensis*, 0.005 ml of each of the solutions was removed and the number of grains with pollen tubes appearing at a magnification of × 125 was registered. If fewer than 25 grains were present, another field was selected until at least 25 grains were counted. The total germination for all five flowers of each type of pollen in each solution is given. In both species no germination was observed in hood pollen, whereas at least some ring pollen had germination in all concentrations at all time intervals. Numbers in parentheses refer to sucrose concentrations of the media.



sonis, upon opening at daybreak, are purple. By the next day their color is less intense, and 48 hours later, when they have fallen from the ovary, they are white. The pollen from both the staminal ring and the hood of freshly opened flowers is yellow. After 24 hours the remaining pollen of the hood has turned black, whereas that of the staminal ring is still yellow. This differential color change indicates a physiological difference between the two pollen types. Further evidence of pollen differentiation comes from studies of germination in vitro. Pollen from the hood of *L. pisonis* did not germinate under experimental conditions, whereas up to 48 percent of the grains from the staminal ring germinated (Fig. 2). Scanning electron microscopy of ring and hood pollen of *L. pisonis* indicates no readily distinguishable morphological differences between the two types of pollen (Fig. 3).

The dimorphic nature of the pollen of *Couroupita guianensis* was pointed out by Jacques (*5*). We have established that there is a physiological difference between the pollen types and that there are at least two species in two different genera with pollen differentiation. We studied the pollen of *C. guianensis* from trees cultivated in Ilhéus, Bahia. Although we have no pollination data for this species, the large size of the flower and its nocturnal opening (*6*) suggest that bats may be the pollinators. Pollen of *C. guianensis* was removed from the hood and ring and placed in the germination media. No grains from the hood were observed to germinate, whereas up to 21 percent of the ring pollen germinated (Fig. 2). In addition, scanning electron microscopy indicated morphological differences between the two types of pollen. The pollen of the hood tends to remain in tetrads (*7*) and is rugose, whereas that of the staminal ring is separate and smooth (Fig. 3).

Although these experiments do not conclusively demonstrate that hood pollen from the two species does not germinate under natural conditions, they do

Fig. 3. Photomicrographs of hood and staminal ring pollen of (A to C) *Lecythis pisonis* Cambess. (voucher specimen, Mori 11069) and (D to F) *Couroupita guianensis* Aubl. (voucher specimen, Silva & Hage 200). (A) Ring pollen of *L. pisonis*. (B) Surface of a grain of ring pollen of *L. pisonis*. (C) Hood pollen of *L. pisonis*. (D) Ring pollen of *C. guianensis*; note the separate grains and the smooth surface. (E) Tetrad of pollen from the hood of *C. guianensis*. (F) Surface of a grain of hood pollen of *C. guianensis*. Note that the two types of pollen of *L. pisonis* are indistinguishable, whereas those of *C. guianensis* are morphologically distinct.

suggest that there is a physiological difference in the two types of pollen. This suggestion is supported by the differential color change verified in the two types of pollen of *L. pisonis* and by the observation that the bee *X. frontalis* collects only hood pollen from *L. pisonis*. In addition, ring and hood pollen of *C. guianensis* are morphologically distinct. Consequently, we suggest that pollen differentiation in these species of Lecythidaceae has evolved in response to different functions, that of the hood to attract pollinators by providing them with a reward and that of staminal ring to function in fertilization.

Dimorphic pollen has been reported in a number of plant groups (*8*), especially where heterostyly and its associated diallelic incompatibility occurs (*9*), as in *Linum* (*10*), *Waltheria* (*11*), and Rubiaceae (*12*). In these genera, both pollen forms are normally fertile. Dimorphy has been reported in a number of homostylous angiosperms such as *Silene alba* (*13*), *Utricularia flexuosa* (*14*), *Cuscuta reflexa* (*15*), and *Urena lobata* (*16*). Ong and Rao (*8*), in a study of the dimorphic pollen of six nonheterostylous species, found only one, *Brexia madagascariensis* (Saxifragaceae), that showed significant differences in germination between pollen morphs. The smaller pollen form showed no germination in vitro. In the above examples in which heterostyly does occur (*9–12*), the pollen is not produced from different parts of the androecium, as it is in the two species of Lecythidaceae. Therefore, our findings differ from those previously reported. Furthermore, the physiological difference in vitro in the pollen of the two Lecythidoideae studied is established.

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Genesis of Petroleum Hydrocarbons in Marine Sediments

Abstract. *Distribution patterns of isopentane and normal pentane in marine sediments show a reversal in slope at a subsurface temperature of about 90°C. The data indicate that three types of reactions are involved in pentane formation: (i) biological origin at the sediment surface, (ii) low-temperature (< 90°C) chemical reactions yielding predominately secondary carbon structures, and (iii) high-temperature (> 90°C) cracking reactions at great depth yielding predominately straight carbon chains.*

Living organisms and Recent sediments contain a predominance of *n*-alkanes with odd-numbered carbon chains (*1–3*). For example, $C_{29}H_{60}$ composes 86 percent of the normal alkanes in apple wax, and over 90 percent of the normal alkanes in marine plants contain either $C_{15}H_{32}$, $C_{17}H_{36}$, $C_{19}H_{40}$, or $C_{21}H_{44}$. The biogenic origin of *n*-alkanes with odd-numbered chains causes these compounds to dominate mixtures of branched alkanes in surface sediments (*3*).

We have found that normal pentane is dominant over isopentane and neopentane in near-surface samples of Recent marine sediments. Also, the concentration of normal heptane always exceeds that of the methylhexanes. The evidence to date indicates that these *n*-alkanes, like the higher members, are of biological origin. They are believed to be biosynthesized in the organism or by low-temperature decarboxylation of even-numbered fatty acid chains (*4*). Normal pentane is present in concentrations of up to 5 ng per gram of sediment. The mole ratio of isopentane to normal pentane in these sediments is generally less than 1 (Table 1). Most of the samples listed in Table 1 were obtained at sediment depths of less than 25 m with a gravity or piston corer. Nine other samples from the U.S. Gulf Coast, six from the Gulf of Maine, and one from Walvis Bay contained normal pentane but no isopentane.

In many areas, this ratio changes from a value of less than 1 to greater than 1 in the first 500 m of sediments, indicating that isopentane is being formed in larger quantities than normal pentane by low-temperature (< 50°C) diagenetic chemical reactions (*5, 6*). The formation of isopentane by microbiological reactions is considered unlikely due to the rapid decrease in microbial activity in fine-grained sediments after the first few meters. (Most of these sediments are fine-grained clays or carbonates.)

In the Black Sea, the mole ratio of isopentane to normal pentane in the free gas state is about 1 at a sediment depth of 300 m. We previously determined values for the mole ratio of these pentanes in the first 1050 m of Black Sea sediments

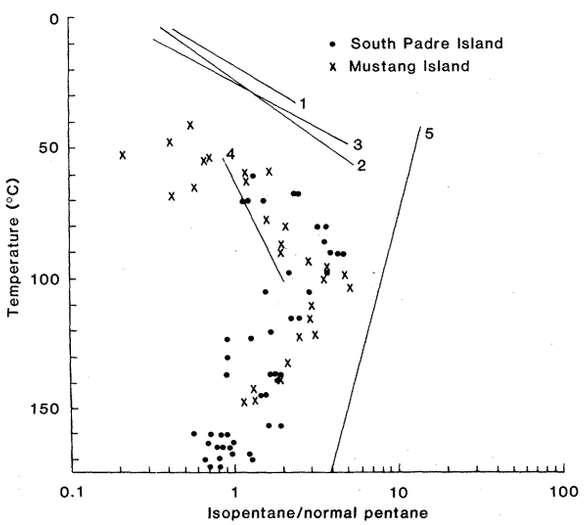

Fig. 1. Change in the mole ratio of isopentane to normal pentane with increasing subsurface sediment temperature. Lines 1, 2, and 3 represent Deep Sea Drilling Project holes 379, 380, and 381 in the Black Sea; line 4, the Paris Basin. Line 5 indicates the calculated equilibrium value.

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