the nucellus cells. We are currently trying to identify the mature ZmEA1 protein, which will then be used to study in vitro pollen tube guidance in maize. Nevertheless, our results bring potentially new insights into the fertilization process. It had been suggested in 1918 (*11*) that the filiform apparatus is required for pollen tube attraction, and in 1964, the existence of chemotropic substances, which are produced by the synergids, was postulated (*12*). Also, numerous secretory vesicles have been observed at the micropylar region of maize synergids but not the egg cell (*13*). Loss of pollen tube guidance via synergid ablation was recently substantiated in *Torenia fournieri* (*7*). Our data suggest that, at least in maize, not only the synergids but the whole egg apparatus could be involved in micropylar pollen tube guidance. Whether the egg cell is also involved in synthesizing a ZmEA1 precursor that is transported toward the synergids via the endoplasmic reticulum or whether the egg cell is capable of ZmEA1 secretion itself is a matter of further experimentation. Rapid down-regulation of the pollen tube guidance signal(s) is a major prerequisite to preventing polyspermy, and fertilization is first sensed by the synergids and egg cell, features of *ZmEA1* gene activity and protein.

The occurrence of *ZmEA1*-related genes in cereals, but not in dicotyledonous species, might be one explanation; wide crosses involving successful pollen tube guidance and fertilization are possible within genera of the Gramineae but not between species spanning wider taxonomic boundaries (*14*). In *Torenia fournieri*, pollen tubes of related species do not respond to the synergids attraction signal, which suggests species-specific short-range attractants (*2*). A similar observation was made conducting interspecific crosses using *Arabidopsis* and other Brassicaceaes where pollen tubes grew normally through the transmitting tissue but rarely arrived at the funiculus and did not enter the micropyle (*15*). Sequence identity between ZmEA1 from two maize inbred lines (A188 and H99) is 91.5% (*16*) and less than 45 and 43% between the maize and the two rice homologs (Fig. 1A). The low homology between EA1 proteins provides further support that specific short-range guidance signals may be involved in the species-barrier concept. The identification of further molecules involved in female gametophyte pollen tube guidance in maize and other plant species should not only help understanding of many outstanding issues in plant reproductive biology but may also be used for future plant breeding to overcome some of the current crossing barriers and to allow hybridization between plant genera that cannot be crossed today.

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A Brief History of Seed Size

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Improved phylogenies and the accumulation of broad comparative data sets have opened the way for phylogenetic analyses to trace trait evolution in major groups of organisms. We arrayed seed mass data for 12,987 species on the seed plant phylogeny and show the history of seed size from the emergence of the angiosperms through to the present day. The largest single contributor to the present-day spread of seed mass was the divergence between angiosperms and gymnosperms, whereas the widest divergence was between Celastraceae and Parnassiaceae. Wide divergences in seed size were more often associated with divergences in growth form than with divergences in dispersal syndrome or latitude. Cross-species studies and evolutionary theory are consistent with this evidence that growth form and seed size evolve in a coordinated manner.

Seed mass affects many aspects of plant ecology. Small-seeded species are able to produce more seeds for a given amount of energy than are large-seeded species (*1*, *2*), whereas large-seeded species have seedlings that are better able to tolerate many of the stresses encountered during seedling establishment (*3*). Seed mass is also correlated with the environmental conditions under which species establish (*4–6*) and with traits such as plant size, dispersal syndrome, plant life-span, and the ability to form a persistent seed bank (*7–13*). Present-day species have seed masses ranging over 11.5 orders of magnitude, from the dust-like seeds of orchids (some of which weigh just 0.0001 mg) to the 20-kg seeds of the double coconut (Fig. 1A). Improving our knowledge of the changes in seed mass that occurred as the angiosperms radiated out of the tropics, colonized a wide range of habitats, developed a range of growth forms and dispersal strategies, and became the most abundant and diverse group of plants on earth (*14–18*) will greatly enhance our understanding of the ecological history of plants.

Using newly developed software (*19*, *20*), we integrated a large seed mass data set with current best opinion for the phylogeny of seed plants. Our seed mass data set included 12,669 angiosperms and 318 gymnosperms. This is ~5% of all extant angiosperm and 38% of all extant gymnosperm species. The angiosperm species are from 3158 genera (22% of the global total) and 260 families (57%) and include representatives from all extant orders. The gymnosperm species are from 52 genera (63%) and 10 families (63%) (*21*).

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The resulting phylogeny has 2223 nodes (uncertain regions of the phylogeny were represented by polytomies), which we analyzed for divergences in seed size. The dense coverage of this data set allows us to locate and quantify the major divergences in seed mass.

We identified the largest divergence events and those that contributed most to the spread of seed mass across present-day species by using two indicators, width and contribution:

(1) Divergence width is a measure of the absolute size of divergences. Width was calculated as the standard deviation of $\log_{10}$ seed mass across the clades immediately descended from the focal node. We used standard deviation instead of the absolute difference in seed mass between daughter clades (i.e., phylogenetic independent contrasts), because the former can be applied to polytomies. The 20 widest divergences are listed in Table 1 and table S1. Frequency distributions of divergence size across all divergences and across dichotomous divergences are shown in Fig. 1, B and C.

(2) Contribution is a measure of how much a divergence contributed to the variation in $\log_{10}$ seed mass across all present-day species in the data set. Contribution takes into account the number of descendant species, the spread of seed mass among those species, and the width of the divergence in question and is analogous to a partitioning of sums of squares in an analysis of variance (22). The 20 largest contributions are listed in Table 1 and table S2.

Our analysis considers divergences rather than historical trait values of given nodes. Historical values cannot be accurately estimated from extant species, because descendant branches might have undergone parallel directional evolution and because unknown extinct branches may have had different trait values compared to surviving branches. Thus, we are highlighting major radiations in seed mass, not parts of the tree where there have been absolute changes in seed mass. Seed masses presented here are cross-species geometric means based on our sample of extant taxa in a clade. Calculations of divergence width and contribution size are based on estimated values at internal nodes using an iterative phylogenetic algorithm (22). However, we have been careful not to interpret these values as ancestral states.

Dates are presented to indicate the sequence and relative timing of major evolutionary events. Ages of major nodes were taken from the literature. These ages are derived from molecular data, calibrated on a single point in the fossil record. The ages of remaining nodes were calculated by interpolation from these "known" dates (22).

The divergence between angiosperms and gymnosperms is the most important, yet least well resolved, divergence in the tree. Molecular evidence generally supports the view that the extant gymnosperms are monophyletic (23, 24), but there is still some doubt about this (25). Under the monophyletic gymnosperm hypothesis, there is a 59-fold divergence in seed mass between angiosperms (small-seeded) and gymnosperms (large-seeded), ~325 million years ago (Ma) (26) (Fig. 2, node A). This is the 54th widest divergence in the data set and contributes six times more to present-day variation in seed mass than the next highest contributing divergence (contribution = 0.43) (Fig. 1D and table S2). The alternative hypothesis, supported by morphological traits but not by molecular evidence (24, 27), is that Gnetales are the sister group of angiosperms. Under this hypothesis, the downward shift in seed mass associated with the divergence of angiosperms from Gnetales would be ~16-fold. Despite the uncertainty around this node, it is clear that the evolution of the angiosperms was associated with a major reduction in seed mass. The difference in seed mass between gymnosperms and angiosperms is also apparent in the fossil record. The earliest angiosperms have much smaller seeds (averaging ~1 mm³) than coeval gymnosperms (200 mm³) (17, 28, 29).

The lower costs of pollen capture, early abortion, and reduced gametophyte size associated with the angiosperm reproductive system might have allowed angiosperms to produce smaller seeds than was economically viable for gymnosperms (30). The ability of angiosperms to produce smaller seeds than gymnosperms is demonstrated in present-day species and in the fossil record. The smallest gymnosperm seeds in our data set (*Chamaecyparis pisifera*, 0.63 mg) are almost four orders of magnitude larger than the smallest angiosperm seeds (Fig. 1A), and
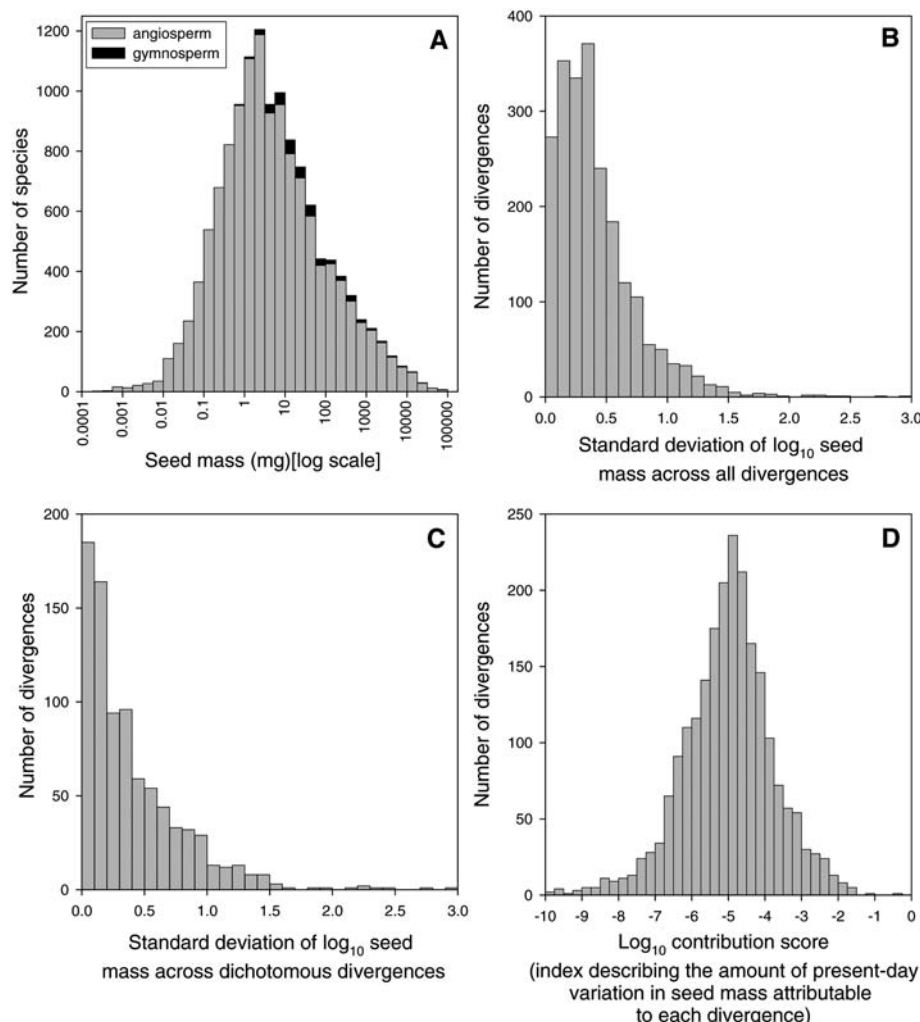
Fig. 1. Frequency distributions for seed mass, divergence size, and contribution scores. (**A**) Seed mass across 12,987 seed plant species. (**B**) Standard deviation of divergence in seed mass across all 2223 divergences. The low frequency of very small divergences results from the fact that the likelihood of all daughter nodes having the same value decreases steeply as the number of daughter nodes increases. (**C**) Standard deviation of divergence in seed mass across the 870 dichotomous divergences. (**D**) Contribution scores (22).

the smallest Cretaceous gymnosperm seeds are about two orders of magnitude larger than the smallest Cretaceous angiosperm seeds (28). The earliest divergences in the angiosperm lineage (estimated to have occurred 179 to 165 Ma) (14) were between clades with moderate-sized seeds, including *Amborella* (3.7 mg), Nymphaeaceae (water lilies, 5.9 mg), and Austrobaileyales (7.5 mg), and the remaining angiosperm clades.

Relationships among monocots, eudicots, and magnoliids are weakly resolved (31), but the sequence of these divergences does not substantially affect our results. An analysis with this node collapsed to a polytomy gave near-identical results to the analysis on the tree in Fig. 2 (nodes B and C).

Most monocots are herbaceous and have small-to-medium seeds (mean = 1.5 mg). However, the monocotyledon clade includes two notable divergences, associated with the origin of orchids (Orchidaceae) and the origin of palms (Arecaceae) (Fig. 3A). These are the only divergences in the whole tree that rank in the top 20 both on absolute width and on their contribution to present-day variation in seed mass. The 435-fold divergence between palms and other commelinids was the second-greatest contributor to seed mass variation in the whole tree (contribution = 0.07). This divergence in seed mass was associated with a shift from herbaceous to arborescent growth forms. The massive shift in seed size associated with the divergence between "dust-seeded" orchids and the rest of the Asparagales [a clade including Iridaceae (irises), Alliaceae (onions), and Asparagaceae] was also associated with a single change in life history: the evolution of mycotrophic seedlings. Because germinating orchid seeds depend entirely on fungi for nutrition, orchids can make seeds with almost no nutritive reserve. Seeds of most other taxa carry enough reserves to sustain a seedling until it can support itself through photosynthesis (32).

The magnoliids and Chloranthales span a wide variety of seed mass strategies (Magnoliales, 260 mg; Laurales, 470 mg; Canellales, 52 mg; Piperales, 0.64 mg; and Chloranthaceae, 1 mg), with no single divergence showing particular importance.

Most angiosperms are eudicots (Fig. 2). The earliest divergence in the eudicots separated Ranunculales (a clade including buttercups and poppies) from other eudicots. This divergence [estimated at 147 Ma (14)] was the ninth-

Table 1. The widest and most important divergences in seed mass. Clades involved in the 20 widest divergences in log$_{10}$ seed mass and the divergences that make the 20 largest contributions to present-day variation in seed mass. Details are given in tables S1 and S2 and in fig. S2. Semicolons separate the clades involved in the divergence of interest from their higher taxon.

Rank	Divergences
	Widest divergences
1	Celastraceae versus Parnassiaceae; Celastrales
2	*Corynocarpus* versus *Coriaria*; Cucurbitales
3	Malpighiaceae versus Elatinaceae; Malpighiales
4	Rhizophoraceae mangroves versus terrestrial species
5	Connaraceae versus Oxalidaceae; Oxalidales
6	*Simmondsia chinensis* versus some other Caryophyllales
7	Dipterocarpaceae versus Cistaceae; Malvales
8	Polytomy across three *Bassia* spp.; Amaranthaceae
9	Clusiaceae versus Hypericaceae; Malpighiales
10	Arecaceae versus Poales, Commelinales, and Zingiberales
11	Orchidaceae versus other Asparagales
12	Polytomy in Proteaceae
13	Polytomy in Cornales
14	Polytomy in Clusiaceae
15	Polytomy in Calycanthaceae
16	Polytomy in Bambusoideae and Poaceae
17	Juglandaceae versus Casuarinaceae and Betulaceae
18	Polytomy in Ochnaceae
19	Combretaceae versus Lythraceae and Onagraceae; Myrtales
20	Polytomy in Lythraceae
	Divergences making the largest contribution
1	Angiosperms versus gymnosperms
2	Arecaceae versus Poales, Commelinales, and Zingiberales
3	Polytomy at base of Euasterid 1
4	Detarieae versus some other Fabaceae; Fabaceae
5	Polytomy in Ericales
6	Polytomy near the base of rosids
7	Saxifragales versus Vitaceae and rosids
8	Polytomy in Papilionoideae
9	Ranunculales versus other eudicots
10	Asparagales versus commelinids
11	Polytomy in Myrtaceae
12	Oleaceae versus the rest of Lamiales
13	Orchidaceae versus other Asparagales
14	Polytomy at the base of core eudicots
15	Polytomy in Lamiales
16	Polytomy in Brassicaceae
17	Euasterid 1 versus Euasterid 2
18	Polytomy in Rosaceae
19	Polytomy across Fabales, Rosales, Cucurbitales, and Fagales
20	Poales versus Commelinales and Zingiberales
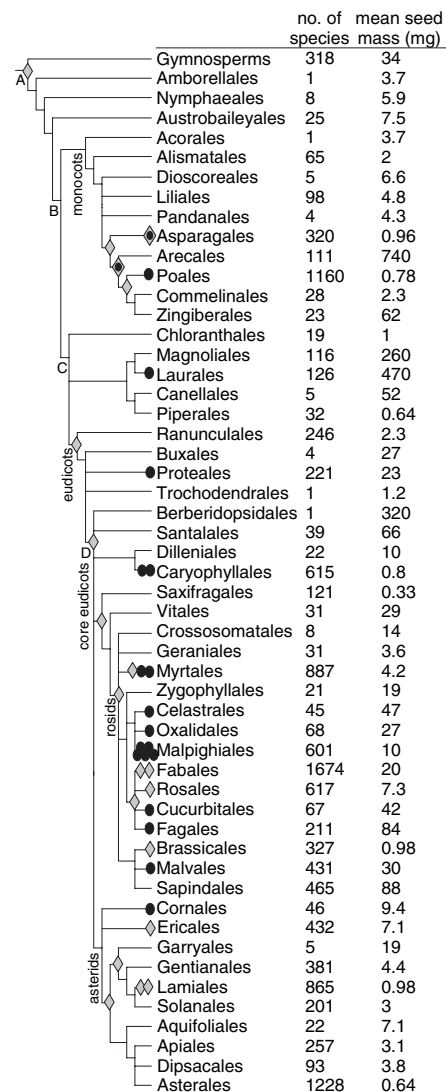
Fig. 2. Topology of the phylogenetic tree to order level, showing the location of the 20 widest divergences (black ellipses) and the 20 divergences making the largest contribution to present-day variation in seed mass (gray diamonds). Symbols at the branch tips represent changes within orders. The sample size and geometric mean seed mass presented for each order are for illustration only; all analyses were performed on species-level data. Some taxa have been omitted from this tree.

largest contributor to seed mass variation (contribution = 0.017). Ranunculales are predominantly herbaceous and have relatively small seeds (2.3 mg) compared to other eudicots.

There is uncertainty regarding the order and timing of the radiation at the base of the core eudicots, so it is represented in our tree by a five-way polytomy between Aextoxicaceae (320 mg), Dilleniaceae/Caryophyllales (0.86 mg), Saxifragales and the rosids (13 mg), Santalales (66 mg), and asterids (1.6 mg). Several of these clades became species-rich, and they range widely in seed mass, so the polytomy ranks as a high-contributing node (estimated at 129 Ma; contribution = 0.012) (Fig. 2, node D) (22). Soon after the core eudicots radiated, there was a divergence between the small-seeded, predominantly herbaceous Saxifragales (0.33 mg) and the larger-seeded rosids (14 mg) and Vitales (29 mg). This divergence was the seventh-greatest contributor to present-day variation in seed mass (contribution = 0.019).

Most present-day eudicots are rosids (represented by 5455 species in our data set) or asterids (3764 species). Wide divergences have been more frequent in the rosids (12 of the top 20) than in the asterids (one of the top 20). The greater dynamism in rosid seed-size evolution has been paralleled by their greater propensity to change growth form (33). Most of the wide divergences in the rosids were associated with a divergence in growth form (e.g., Celastraceae versus Parnassiaceae, Connaraceae versus Oxalidaceae, Clusiaceae versus Hypericaceae, Malpighiaceae versus Elatinaceae) (Table 1 and table S1). Five of the 20 widest divergences were in Malpighiales. Seven of the 20 biggest-contributing divergences were within the rosids, including a divergence across the base of rosids; a divergence near the base of Fabaceae; a polytomy across Fabales, Rosales, Cucurbitales, and Fagales; and polytomies at the base of Papilionoideae (Fabaceae), Myrtaceae, Brassicaceae, and Rosaceae (Fig. 2 and table S2).

The asterids had 5 of the 20 biggest-contributing divergences in seed size, but only 1 of the 20 widest divergences. That is, divergences in seed mass within the asterids have given rise to a number of species-rich groups with a broad spread in seed size, though the absolute width of the divergences has not been exceptional. The widest divergence in the asterids was between Cornaceae (49 mg), Loasaceae/Hydrangeaceae (0.53 mg), and Nyssaceae (390 mg). The biggest-contributing divergences were the divergence at the base of Euasterid 1 (between Icacinaceae, Garryaceae, and the remainder of Euasterid 1; contribution = 0.026), the divergence between Oleaceae and the remainder of the Lamiales (0.014), the divergence between Euasterids 1 and 2 (0.010), a polytomy across eight families within the Lamiales (0.012),

and a polytomy near the base of the Ericales (0.020) (Fig. 2 and table S2).

Divergences in seed size appear to have been relatively consistent through time, both in terms of magnitude (fig. S1) and in the association between divergences in seed mass and divergences in explanatory variables such as growth form (table S1). That is, we have not found evidence that any particular event or period during geological history made an especially large contribution to the radiation of seed size. Our ability to ask whether radiations in traits are concentrated at particular times throughout history will increase as the precision and accuracy of divergence time estimates improves.

The widest divergence in the history of seed mass was a 13,650-fold divergence between Parnassiaceae, a small-seeded clade of temperate herbs, and Celastraceae, a large-seeded clade of tropical trees, shrubs, and lianes (Fig. 3B). The second widest divergence (6780-fold) was between *Coriaria* (Coriariaceae) and *Corynocarpus* (Corynocarpaceae) (Table 1, table S1, and fig. S2). This divergence was not associated with a divergence in dispersal syndrome (both are bird-dispersed) or with biogeographic shift (*Coriaria* has a slightly more poleward distribution than *Corynocarpus*, but their geographic ranges are mainly overlapping). However, it was associated with a shift in growth form and successional status: *Coriaria* tend to be shrubby, early successional species, whereas *Corynocarpus* tend to be later successional trees (34). The third widest divergence in seed mass (2760-fold) was between Malpighiaceae (a clade of large-seeded trees, shrubs, and lianes from tropical or warm climates) and *Elatine californica* (California waterwort, a small-seeded herb of damp habitats in California).

The three widest divergences in seed size were all associated with divergences in growth form. This was also the case for the 5th to 10th widest divergences [Connaraceae versus Oxalidaceae; Simmondsiaceae versus other Caryophyllales; Dipterocarpaceae versus Cistaceae; across *Bassia hyssopifolia*, *B. latifolia*, and *B. longifolia*; Clusiaceae versus Hypericaceae; and palms (Arecaceae) versus other commelinid monocots (Fig. 3A and table S1)]. Larger seed masses were associated with larger growth forms in 9 of the 11 dichotomous divergences in Table 1 (table S1). This association was also seen in notable changes in growth form, such as the divergence between *Pandanus* (large-seeded pachycaul trees and shrubs) and *Freycinetia* (small-seeded lianes; this was the 23rd widest divergence) and the divergence between Bambusoideae (bamboos) and the smaller Ehrhartoideae and Pooideae (grasses; this was the 122nd widest divergence). There were associations with other variables, but
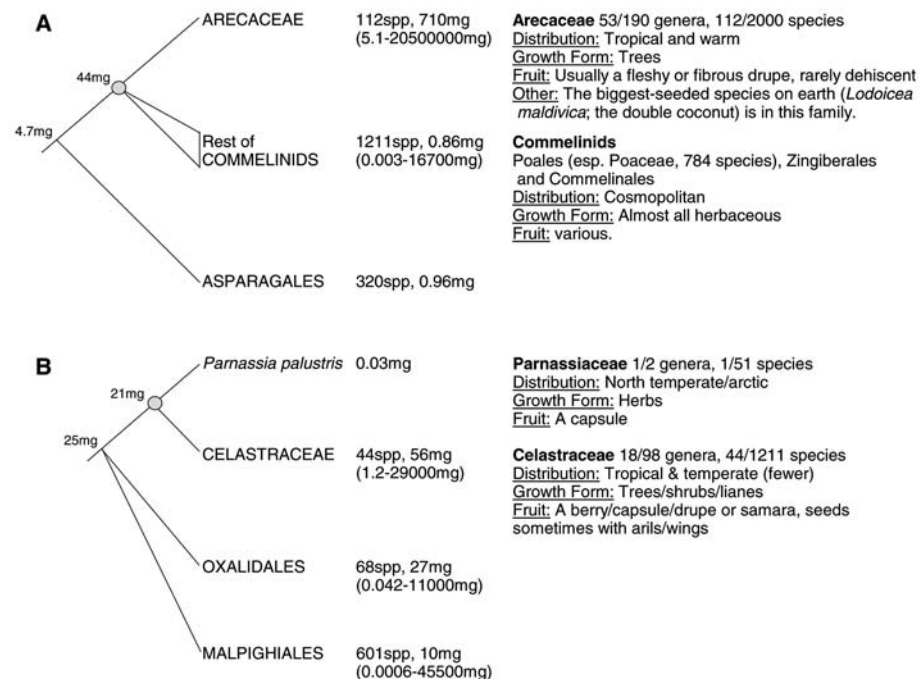
Fig. 3. Two important divergences in seed mass. (**A**) The divergence between palms and the remainder of the commelinid monocots. (**B**) The widest divergence in our tree. The text to the right shows the fraction of extant taxa in each group included in our data set and the distribution, growth form, and fruit morphology of each clade. Small numbers on interior nodes represent estimated internal means (22), and values given after clade names are geometric means taken across the tip nodes (species). Trees for the 20 widest divergences are given in fig. S2.

they were less consistent. Smaller seed mass was associated with more poleward distribution in 6 of the 11 dichotomous divergences and with a more tropical distribution in one clade. Larger seed mass was associated with biotic dispersal in 6 of the 11 dichotomous divergences and with abiotic dispersal in one divergence. An analysis across all of the divergences in the tree (based on independent contrasts) also showed that shifts in seed mass have been much more closely associated with shifts in growth form than with shifts in latitude or dispersal syndrome (*35*). Thus, our data are more consistent with Eriksson, Friis, and Lofgren's suggestion (*29*) that changes in seed mass during angiosperm evolution resulted primarily from changes in vegetation structure than with Tiffney's hypothesis (*17*) that changes in dispersal fauna (particularly the radiation of mammals across the Cretaceous-Tertiary boundary) allowed angiosperms to radiate into larger seed masses.

Two of the 11 top-ranking dichotomous divergences in seed mass were not associated with divergences in plant stature. One was the divergence between Juglandaceae and Casuarinaceae/Betulaceae, which was associated with a divergence between biotic and abiotic dispersal. The other was a divergence within Rhizophoraceae, between a small-seeded terrestrial habit and a large-seeded mangrove habit. A shift to a mangrove habit has generally been associated with increases in seed mass. The mangrove habit has evolved in seven families, five of them represented in our database. In four of these (Acanthaceae, Myrsinaceae, Meliaceae, and Rhizophoraceae, but not Combretaceae), mangroves have the largest seeds in the family.

The most consistent pattern we revealed was the association between changes in seed mass and changes in growth form. This result is in line with Charnov's life history theory for mammals (*36*). In Charnov's treatment, offspring size is coordinated with size at adulthood, because larger offspring offset the low survivorship to adulthood that would otherwise be a consequence of longer juvenile periods. This result is also consistent with cross-species studies showing that growth form is the strongest correlate of seed size (*9, 10*). A recent compilation of data for 2113 species from around the world (*7*) showed a highly significant positive relationship between seed mass and plant height ($R^2 = 0.35$). Of course, there is still great variation in seed mass for a given plant size. Some of this variation can be attributed to differences in dispersal syndrome, some to biogeography, and more variation is undoubtedly attributable to factors that we have not considered here.

The synthesis of robust phylogenies with global trait data sets holds great promise for elucidating the ecological and evolutionary history of seed plants and of other major groups of organisms.

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A Molecular Phylogeny for Bats Illuminates Biogeography and the Fossil Record

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Bats make up more than 20% of extant mammals, yet their evolutionary history is largely unknown because of a limited fossil record and conflicting or incomplete phylogenies. Here, we present a highly resolved molecular phylogeny for all extant bat families. Our results support the hypothesis that megabats are nested among four major microbat lineages, which originated in the early Eocene [52 to 50 million years ago (Mya)], coincident with a significant global rise in temperature, increase in plant diversity and abundance, and the zenith of Tertiary insect diversity. Our data suggest that bats originated in Laurasia, possibly in North America, and that three of the major microbat lineages are Laurasian in origin, whereas the fourth is Gondwanan. Combining principles of ghost lineage analysis with molecular divergence dates, we estimate that the bat fossil record underestimates (unrepresented basal branch length, UBBL) first occurrences by, on average, 73% and that the sum of missing fossil history is 61%.

Bats are a unique and enigmatic group of mammals that account for ~1,100 species (*1*). They are the only mammals to have achieved true self-powered flight, are found throughout the globe, and play a major ecological role as pollinators and insect predators (*2*). Although