as diverse as the sources of aerosols, with some aerosols warming the planet (such as black carbon) and others cooling the planet (such as sulfate) (*1*). The net effect of anthropogenic aerosols is to cool the planet (*1*), however, so projected changes in emissions will tend to reduce the aerosol radiative forcing (*36*–*42*). These cuts in aerosols will not only cause an increase in temperatures (*43*) but will also cause a decrease in the uptake of carbon by the land and the ocean. This may preferentially affect the more aggressive carbon policies, because these also will result in the fastest decrease in aerosol emissions (*36*) (SOM). For high–carbon emission cases, changes in emissions should not be large enough to cause significant impacts due to changing aerosol production. However, for the low-emission pathways, even lower emissions will need to be achieved than previously estimated, because of the impact of the aerosol indirect effect on carbon uptake. Although there are many uncertainties in estimating future mitigation costs, it is clear that lower targets for CO_2 concentrations correspond to greater costs. The relationship between cost and targets is highly nonlinear (Fig. 2) (*36*), with costs rising rapidly in a kind of "cliff" as CO_2 targets decrease. Because it is generally not accounted for, the aerosol indirect effect on CO_2 uptake, mediated by biogeochemical cycles as described here, tends to shift the cost cliff toward higher costs for the same CO_2 level at 2100 as compared to when it is ignored (Fig. 2) (SOM). Therefore, achieving lower atmospheric CO_2 concentrations may be even costlier than previously estimated. The estimates provided here suggest that more detailed studies on the effect of aerosols on biogeochemical cycles are important for understanding future climate.

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Acknowledgments: This work was supported by grants from NSF (078369, 0832782, 0932946, 1021613, 1049033), NASA (NNG06G127G), and the Atkinson Center for Sustainable Future. Discussions with C. Goodale, P. Hess, and D. Miller contributed to this paper. Comments by R. Scanza and two anonymous reviewers improved the paper.

Supporting Online Material

www.sciencemag.org/cgi/content/full/334/6057/794/DC1
SOM Text
Tables S1 and S2

22 April 2011; accepted 19 September 2011
10.1126/science.1207374

Recent Synchronous Radiation of a Living Fossil

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Modern survivors of previously more diverse lineages are regarded as living fossils, particularly when characterized by morphological stasis. Cycads are often cited as a classic example, reaching their greatest diversity during the Jurassic–Cretaceous (199.6 to 65.5 million years ago) then dwindling to their present diversity of ~300 species as flowering plants rose to dominance. Using fossil-calibrated molecular phylogenies, we show that cycads underwent a near synchronous global rediversification beginning in the late Miocene, followed by a slowdown toward the Recent. Although the cycad lineage is ancient, our timetrees indicate that living cycad species are not much older than ~12 million years. These data reject the hypothesized role of dinosaurs in generating extant diversity and the designation of today's cycad species as living fossils.

Living fossils and evolutionary relicts are surviving representatives of once diverse or abundant groups. They are noteworthy because they originated tens or even hundreds of millions of years ago yet have persisted with little morphological change. Well-known examples include the coelacanth, the horseshoe crab, the *Ginkgo* tree, and the cycads (Cycadophyta). Fossils indicate the cycads originated before the mid-Permian and reached their peak morphologically, geographically, and in taxic diversity in the Jurassic–Cretaceous (*1*–*4*). Their subsequent decline has been attributed to competition with flowering plants (*5*, *6*) and also to the loss of dinosaurs as dispersal agents (*3*); however, numerical analyses testing a coradiation between dinosaurs and cycads are inconclusive (*7*).

Fossil-calibrated phylogenies (timetrees) were used to test whether living cycads are relics or

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whether their morphological conservation might mask more recent diversification events. To estimate the ages of living cycad species, we sampled the nuclear gene *PHYTOCHROME P* (*PHYP*) from two-thirds of living cycads [199 of the ~300 recognized species (*8*)] using proportional sampling within the large genera (*9*). Our sampling included all of the 11 currently recognized genera [including *Chigua*, which is nested within *Zamia* (*10*, *11*)]. We also assembled plastid data matrices from published *rbcL* and *matK* sequences (Table 1 and table S1). These matrices had fewer taxa but allowed us to test the ages estimated from the *PHYP* data. Topologies were inferred from single and combined gene regions, and the divergence times between the extant lineages were estimated by subjecting the trees to relaxed molecular clock analysis with penalized likelihood and to strict molecular clock analysis with the Langley-Fitch method (*12*); Bayesian searches for topologies and divergence times were conducted by using an uncorrelated lognormal relaxed clock (*13*). The fossil record was used to assign minimum age constraints on three internal nodes and to provide a fixed age constraint for the divergence time between the cycads and their outgroups. The use of a fixed age constraint, coupled with the incompleteness of the fossil record, means that the inferred ages underestimate the true divergence times.

The timetree derived from the *PHYP* data was used to assess changes in diversification rates within genera by using the gamma (γ) statistic (*14*) and per-million-year diversification rates (*15*). To account for the effect of undersampling, we also calculated the rates assuming all the missing taxa had originated in the last time bin.

Our phylogenetic analysis did not produce any surprises topologically: The relationships inferred from the *PHYP* data (and from the combined *PHYP*, *rbcL*, and *matK* data) are consistent with well-supported nodes resolved in previously published trees (*10*, *11*, *16*, *17*). Although the remarkably short terminal branches may raise doubts over the validity of the defined cycad species, reproductive, morphological, and geographical evidence strongly support their specific status (*9*, *18*–*20*).

Unexpectedly, the timetrees indicate that all extant species (except for those in monotypic genera) derive from recent divergence events that occurred no later than the late Miocene to the Pliocene (Fig. 1 and Table 1). Initiation of species diversification occurred in a very short ~5 million year (My) timeframe for all of the large genera—that is, *Cycas*, *Encephalartos*, *Macrozamia*, *Zamia*, and *Ceratozamia* (Fig. 1). Subsequently, all of these genera show significant declines in diversification rate, dropping to almost zero in the past ~2 My (Fig. 2). Even when we use our conservative approach to account for the undersampling of extant species, we find that the rates peaked early in the radiation of each of these genera (table S7).

We also detected the signal of a recent and near-synchronous global radiation using different methods, genes, and gene combinations (Table 1, tables S2 to S5, and figs. S1, S4, S5, S8, and S9). It is robust to topological and branch-length uncertainty and to uniform, correlated, or uncorrelated rates across the tree (tables S3 and S5)—at most, the minimum timeframe for the radiation varies from the late Miocene–Pliocene to the mid- to late Miocene. Accounting for the incompleteness of the fossil record yields median (50%) crown group age estimates for the genera in the late Miocene to earliest Pliocene [11.6 to 5.2 million years ago (Ma)] and maximum (95%) estimates in the early to mid-Miocene (20.7 to 9.2 Ma) (tables S4 and S5).

The late Cenozoic radiation reported here is consistent with the young ages for *Encephalartos* and *Cycas* species (~10 Ma) inferred from *rbcL* substitution rates (*16*) and with a gymnosperm *matK* and 18*S* ribosomal DNA (rDNA) timetree that includes a much smaller sampling of cycads (3 to 6 species per genus) (*21*), although the median age estimates from this latter study extend as far back as the early Miocene. These slightly older age estimates may have resulted from differences in how key fossil calibrations were applied (*9*). Recent divergences have also been hypothesized within many of the living genera on the basis of the low genetic diversity characteristic of con-generic species (table S8) (*18*, *22*, *23*). Last, our findings are consistent with data from some highly specialized insect pollinators of cycads (weevils), in which low interspecific divergence among mitochondrial DNA sequences is also suggestive of recent diversification (*24*).

The cycad timetree is remarkable for its long branches subtending the late Cenozoic radiations (Fig. 1). These suggest "phylogenetic fuses," in which the origin of a clade is decoupled from its later evolutionary explosion (*25*). This hypothesis requires the assumption that the long fuse (branch) represents a period of low diversity. Alternatively, the long branches may result from considerable extinction, and this is consistent with at least three lines of evidence. First, fossil data indicate that cycads were diverse in the Mesozoic, but with extinctions occurring toward the end of the Mesozoic (*1*, *5*, *6*). Second, a birth-death model used in the Bayesian analyses (*9*) yielded a high ratio of extinction to speciation (relative death rate, 0.97). Last, numerical simulations show that long fuses may result from mass extinctions (*26*). However, we cannot currently address the exact role of extinction in shaping the cycad timetree because of a limited understanding of the Cenozoic cycad fossil record and our current inability to extract accurate data on extinction patterns from molecular phylogenies (*27*). Thus, we do not know whether Cenozoic cycad diversity remained low until the late Cenozoic radiations reported here, or whether substantial early to mid-Cenozoic diversity existed but was affected by major Cenozoic extinctions.

The near-simultaneous initiation of diversification of six of the living cycad genera across the globe (in Australia, Africa, Southeast Asia, and Central to tropical South America) indicates a single trigger may have been responsible. During the late Miocene, the global climate shifted as the world's landmasses largely assumed their current positions (*28*). This closed the last of the equatorial seaways that had allowed warm tropical water to circulate the globe, leading to a transition from globally warm, equable climates to present-day cooler, more seasonal climates (*29*). The majority of cycad species live in tropical or subtropical climates in regions of predominantly summer rainfall (*2*). Thus, it is possible that cycad diversification was largely driven by the global climate change that increased the geographic extent of those subtropical and tropical biomes that became marked by seasonality. Nonetheless, despite their recent success almost two-thirds of cycads are on the International Union for Conservation of Nature (IUCN) Red List of Threatened Plants (~62% of cycads are threatened—the highest value of any plant group) (*30*). Thus, their relatively recent radiation does not appear to have buffered them from high extinction risk or the threat of becoming victims of a human-induced sixth mass extinction (*31*).

Table 1. Congruence of crown group ages from nuclear and/or plastid markers. Ages are shown only for genera with more than five species. PL, penalized likelihood; BI, Bayesian inference; na, not applicable because only one species was sampled from the crown group for the genus. A dash indicates a marker was not used in that analysis (*9*).

	Nuclear PL	Nuclear BI	Nuclear plus plastid PL (missing data)	Nuclear plus plastid PL (fully sampled)	Plastid PL (fully sampled)
Number of taxa: *PHYP*	199	199	199	20	—
Number of taxa: *matK*	—	—	34	20	20
Number of taxa: *rbcL*	—	—	59	20	20
Age (Ma): *Cycas*	9.77	12.80	8.17	8.68	9.46
Age (Ma): *Encephalartos*	9.21	11.37	8.49	10.25	7.99
Age (Ma): *Macrozamia*	5.36	7.48	5.43	3.33	4.83
Age (Ma): *Zamia*	4.77	11.25	5.77	na	na
Age (Ma): *Ceratozamia*	4.37	11.48	4.40	na	na

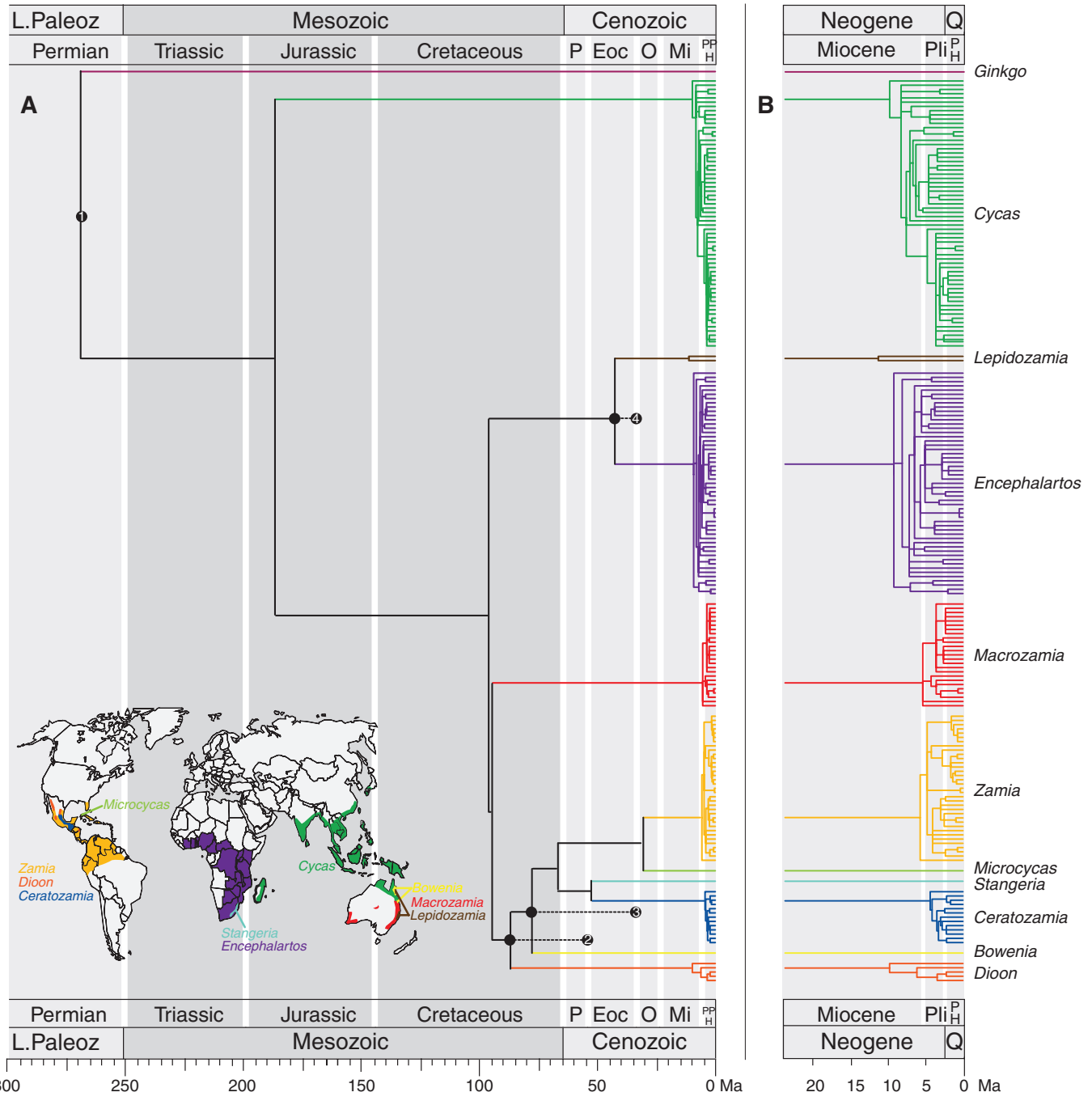

Fig. 1. Cycad timetree inferred from *PHYP* data assuming a relaxed molecular clock (*12*), and map showing geographic distribution of genera. (**A**) Timetree and (inset) distribution of genera. Numbered circles mark the ages of fossil constraints, and unnumbered circles mark the inferred ages of the constrained nodes (*9*). Geographic distributions were obtained from (*2*). (**B**) Enlarged view of timetree from (A) focusing on the Miocene–Recent. L. Paleoz, Late Paleozoic; P, Paleocene; Eoc, Eocene; O, Oligocene; Mi, Miocene; PPH, Pleistocene–Pliocene–Holocene; Q, Quaternary; Pli, Pliocene; PH, Pleistocene–Holocene.

Given their ancient origins, it is remarkable that virtually all cycad species-level diversity is due to recent speciation events. Groups of somewhat less ancient plants that also radiate later in their histories include the Pinaceae, Ephedraceae, Nymphaeales, and Chloranthaceae (*32*–*35*), although diversification within these groups was not as synchronous and occurred earlier than the cycad diversification, during the Oligocene–Miocene. However, independently evolved lineages of succulents also show an increased rate of diversification approximately contemporaneous with the cycad radiations, most likely triggered by the increased aridity that was correlated with the shift to increased seasonality (*36*). The possibility of concurrent bursts of speciation across the plant tree of life is an intriguing pattern that warrants closer assessment.

The fossil-calibrated molecular phylogenies of the cycads presented here reject the prevailing hypothesis that extant species are relictual living fossils (*2*, *4*, *6*), whose current diversity was established through interactions in the deep past with the dinosaurs (*3*). Their recent radiation suggests that coevolution of living cycads and their insect pollinators should be examined over a substantially shorter time period (*37*–*39*) and may explain low levels of genetic diversity that have been observed within cycad species (*40*, *41*).

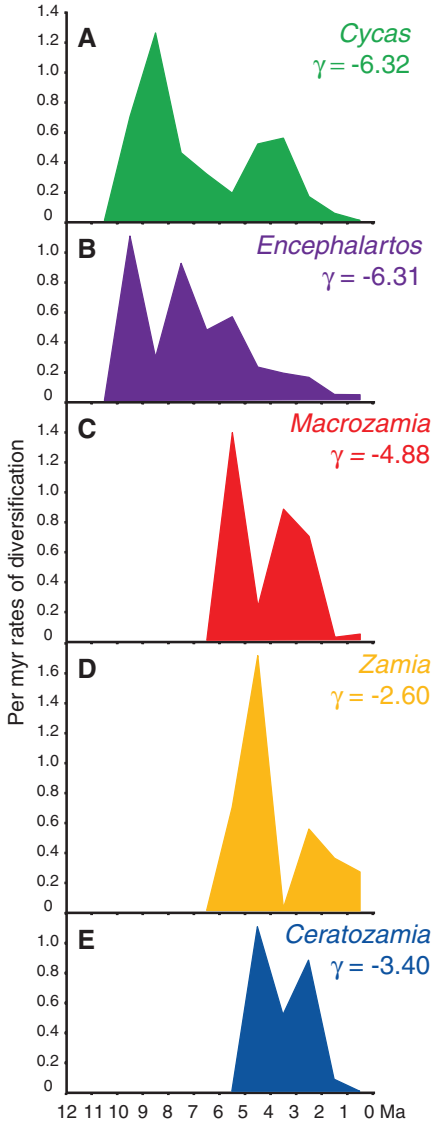

Fig. 2. Per-million-year diversification rates and γ values for the cycad genera. (**A**) *Cycas*, (**B**) *Encephalartos*, (**C**) *Macrozamia*, (**D**) *Zamia*, and (**E**) *Ceratozamia*. Rates and γ values are shown only for genera with more than five species. All γ values shown are significant, indicating decreasing diversification rates. The time of initiation of the genus-level radiations depends on the analysis (the penalized likelihood analysis is shown here); see Table 1 for alternative possibilities.

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Acknowledgments: This work was supported by NSF's Assembling the Tree of Life program (grant EF-0629890 to S.M.). The data reported in this paper are tabulated in the supporting online material and archived in GenBank (JN655891 to JN656096) and TreeBase (11891). We thank M. Beilstein, M. Clements, and K. Schellenberg for discussions and technical assistance; A. Vo for help with cloning and sequencing; J. Hilton for information regarding fossil ages; S. Ho and M. Sanderson for advice on divergence time analyses; D. Stevenson for cycad tissue; and the reviewers for suggestions and comments.

Supporting Online Material

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Materials and Methods
Figs. S1 to S13
Tables S1 to S8
References (*42*–*76*)

16 June 2011; accepted 27 September 2011
Published online 20 October 2011;
10.1126/science.1209926

Global DNA Demethylation During Mouse Erythropoiesis in Vivo

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In the mammalian genome, 5′-CpG-3′ dinucleotides are frequently methylated, correlating with transcriptional silencing. Genome-wide demethylation is thought to occur only twice during development, in primordial germ cells and in the pre-implantation embryo. These demethylation events are followed by de novo methylation, setting up a pattern inherited throughout development and modified only at tissue-specific loci. We studied DNA methylation in differentiating mouse erythroblasts in vivo by using genomic-scale reduced representation bisulfite sequencing (RRBS). Demethylation at the erythroid-specific β-globin locus was coincident with global DNA demethylation at most genomic elements. Global demethylation was continuous throughout differentiation and required rapid DNA replication. Hence, DNA demethylation can occur globally during somatic cell differentiation, providing an experimental model for its study in development and disease.

The formation of enucleated red cells, or erythropoiesis, first occurs in the murine fetal liver between embryonic day 11 (E11) and E15 and is dependent on the hormone erythropoietin (Epo). We labeled mouse fetal liver with the cell surface markers CD71 and Ter119 and identified six subsets of cells, S0 to S5, which form a sequence of increasingly mature erythroid cells (Fig. 1A) (*1*). Subsets S1 to S5 contain only erythroid cells. S0 contains erythroid progenitors (70%), which we further enriched (>95%) by negative selection for cells expressing the cell surface markers CD41, Mac-1, and Gr-1 (*1*). We recently found that transition of erythroid

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Science **334** (6057), 796-799. [doi: 10.1126/science.1209926]
originally published online October 20, 2011

Editor's Summary

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.