

Recent synchronous radiation of a living fossil

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Modern survivors of once 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 (199.6-65.5 Ma), 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 re-diversification beginning in the late Miocene, followed by a slowdown toward the Recent. Although the cycad lineage is ancient, our timetree indicates that living cycad species are not much older than ~12 Ma. 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 significant 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) — the oldest extant lineage of seed plants. Cycad fossils, dated to 271-266 million years ago (*1*), indicate they originated before the mid-Permian. They apparently reached their peak morphologically, geographically and in taxic diversity in the Jurassic-Cretaceous (*2-4*). Their subsequent decline has been attributed to competition with flowering plants (*1, 5, 6*) and also to the loss of dinosaurs as dispersal agents (*7*). Interactions with dinosaurs have also been proposed as a mechanism for generating the extinct and the relictual extant species diversity (*3, 7*).

Fossil-calibrated phylogenies (timetrees) were used to test whether living cycads are relicts or 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 (*PHYYP*) 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 (Tables 1 and S1). These matrices had fewer taxa, but they allowed us to test the results of ages estimated from the *PHYYP* 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 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. Note that 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 *PHYYP* data was used to assess changes in diversification rates within genera using the gamma (γ) statistic (14) and per-myr 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. Despite this very conservative approach, the results support the conclusion that after radiating, diversification rates in the genera decreased (Table S7).

Our phylogenetic analysis did not produce any surprises topologically: the relationships inferred from the *PHYYP* data (and from the combined *PHYYP*, *rbcL*, and *matK* data) are consistent with well-supported nodes resolved in previously published trees (10, 11, 16, 17). While 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 (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 myr 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 last ~ 2 Ma (Fig. 2). Even when we use our conservative approach for accounting for the undersampling of extant species, we find that the rates peaked early in the radiation of each of these genera (Table S7).

The signal of a recent and near-synchronous global radiation is also detected using different methods, genes and gene combinations (Tables 1, S2-5, Figs. S1, S4, S5, S8, S9). It is robust to topological and branch length uncertainty and to uniform, correlated or uncorrelated rates across the tree (Tables S3, S4) – 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 mid- to late Miocene (12.2-6.4 Ma), and maximum (95%) estimates in the early to mid-Miocene (23.9-9.2 Ma) (Tables S4, S5).

The late Cenozoic radiation reported here is consistent with the young ages for *Encephalartos* and *Cycas* species (~ 10 Ma) inferred from *rbcL* mutation rates (16), and with a gymnosperm *matK* and 18S rRNA timetree that includes a much smaller sampling of cycads (3-6 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 based on the low genetic diversity characteristic of con-generic species (e.g., 18, 22, 23 and see also Table S8). Finally, our findings are consistent with data from some highly specialized insect pollinators of cycads (weevils), where low inter-specific 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”, where 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). Finally, 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 due to a limited understanding of the Cenozoic cycad fossil record, and to 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 impacted by major Cenozoic extinctions.

The near-simultaneous initiation of diversification of six of the living cycad genera across the globe (in Australia, Africa, south-east Asia, and central 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 shift 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 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, and the threat of becoming victims of a human-induced sixth mass extinction (31).

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 co-evolution of living cycads and their insect pollinators should be examined over a significantly shorter time period (37-39), and it may explain low levels of genetic diversity that have been observed within cycad species (40, 41).

References and notes

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Supporting Online Material

Materials and Methods

References (42-75)

Figs. S1-S13

Tables S1-S8

Figure Legends

Fig. 1. Cycad chronogram inferred from *PHYC* data assuming a relaxed molecular clock (12), and map showing geographic distribution of genera. (A) Chronogram and distribution (inset) of genera.

Numbered circles mark the ages of fossil constraints and unnumbered circles mark the inferred ages of the constrained node (9). Geographic distributions obtained from (2). **(B)** Enlarged view of chronogram from A focusing on the Miocene-Recent. Abbreviations: L, Paleoz, Late Paleozoic; P, Paleocene; Eoc, Eocene; O, Oligocene; Mi, Miocene; PPH, Pleistocene-Pliocene-Holocene; Q, Quaternary; Pli, Pliocene; PH, Pleistocene-Holocene.

Fig. 2. Diversification rates per myr 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 5 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.

Table 1: Congruence of crown group ages from nuclear and/or plastid markers. Ages are shown only for genera with more than 5 species. “PL” =Penalized Likelihood; “BI”=Bayesian Inference mean age; “na” = not applicable because only one species was sampled from the crown group for the genus; “—“ = marker not used in that analysis (9).

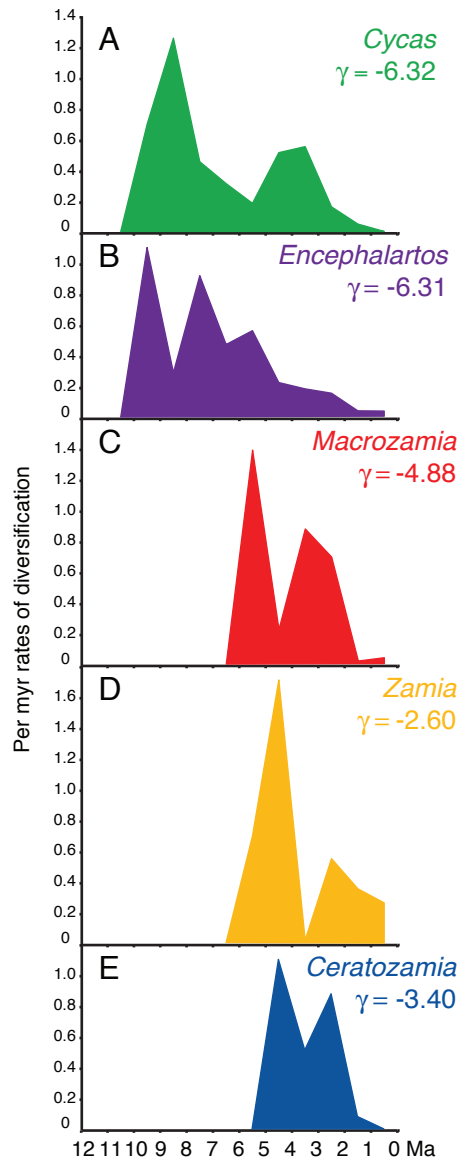


Figure 2. Nagalingum et al.

Table 1: Congruence of crown group ages from nuclear and/or plastid markers.

Ages are shown only for genera with more than 5 species. “PL” =Penalized Likelihood; “BI”=Bayesian Inference mean age; “na” = not applicable because only one species was sampled from the crown group for the genus; “—” = marker not used in that analysis (9).

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