Gene duplications and phylogenomic conflict underlie major pulses of phenotypic evolution in gymnosperms

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Abstract

Inferring the intrinsic and extrinsic drivers of species diversification and phenotypic disparity across the Tree of Life is a major challenge in evolutionary biology. In green plants, polyploidy (or whole-genome duplication, WGD) is known to play a major role in microevolution and speciation[1], but the extent to which WGD has shaped macroevolutionary patterns of diversification and phenotypic innovation across plant phylogeny remains an open question. Here we examine the relationship of various facets of genomic evolution—including gene and genome duplication, genome size, and chromosome number—with macroevolutionary patterns of phenotypic innovation, species diversification, and climatic occupancy in gymnosperms. We show that genomic changes, such as WGD and genome-size shifts, underlie the origins of most major extant gymnosperm clades, and notably our results support an ancestral WGD in the gymnosperm lineage. Spikes of gene duplication typically coincide with major spikes of phenotypic innovation, while increased rates of phenotypic evolution are typically found at nodes with high gene-tree conflict, representing historic population-level dynamics during speciation. Most shifts in gymnosperm diversification since the rise of angiosperms are decoupled from putative WGDs and instead are associated with increased rates of climatic occupancy evolution, particularly in cooler and/or more arid climatic conditions, suggesting that ecological opportunity, especially in the later Cenozoic, and environmental heterogeneity have driven a resurgence of gymnosperm diversification. Our study provides critical insight on the processes underlying diversification and phenotypic evolution in gymnosperms, with important broader implications for the major drivers of both micro- and macroevolution in plants.

Main Text

Connecting microevolutionary processes with emergent macroevolutionary patterns of phenotypic disparity and species diversification is one of the grand challenges of evolutionary biology. In green plants, polyploidy is a common phenomenon in many lineages[1,2] that has long been considered a major evolutionary force[3,4], unique in its ability to generate manifold phenotypic novelties at the population level and in its potential to drive long-term evolutionary trends. However, despite its prevalence and clear microevolutionary importance, its macroevolutionary significance remains unclear and, in major clades such as gymnosperms, understudied. For example, analyses of the relationship between WGD and diversification have produced conflicting results[5,6,7,8], and no studies to our knowledge have examined whether spikes in gene duplication tend to directly coincide with bursts of phenotypic novelty on a macroevolutionary scale, although clearly gene duplications have facilitated the origins of numerous key traits in plants[9,10,11]. It is possible that other processes—e.g., major fluctuations in population size during rapid speciation events, in concert with extrinsic factors such as climate change or ecological opportunity—may contribute more fundamentally to major phases of morphological innovation and diversification[12,13]. More realistically, however, these various phenomena may interact in complex ways to shape macroevolutionary patterns in plants and, more broadly, across the Tree of Life, and detailed case studies are needed to disentangle their relative contributions to the origins of phenotypic and taxonomic diversity.

Here we examine potential drivers of phenotypic innovation and species diversification in seed plants (=*Spermatophyta*[14]), with a focus on *Acrogymnospermae*[14], the most ancient subclade of living spermatophytes, including all extant gymnosperms (conifers, cycads, Gnetales, and *Ginkgo biloba*) as well now-extinct groups such as Cordaitales[15]. For the past ~300 million years, seed plants have occupied center stage in terrestrial ecosystems[16], constituting the most dominant plant clade in terms of both biomass and diversity and forming myriad ecological relationships with other organisms across the Tree of Life, such as bacteria, fungi, insects, and vertebrates[17]. Reconstructing the diversification of seed plants is therefore essential for a better understanding of the history of terrestrial ecosystems across the latter half of the Phanerozoic. Many aspects of seed plant phylogeny remain poorly known or contentious[18], including the phylogenetic placements of numerous extinct lineages (e.g., Bennettitales and Caytoniales) with respect to living gymnosperms (*Acrogymnospermae*) and flowering plants (*Angiospermae*[14]). Nevertheless,

93 based on the extensive fossil record of seed plants[19] and phylogenetic studies of living
94 representatives *Acrogymnospermae*[20,21] (which comprise ca. 1100 species), extant gymnosperm
95 lineages clearly exhibit a complex history of ancient radiations, major extinctions, extraordinary
96 stasis, and recent diversification, but the correlates and causes of major phases of gymnosperm
97 evolution remain understudied. Given that recent studies[22–25] have challenged the traditional
98 notion[26,27] that WGD is rare or insignificant in gymnosperms, a focused investigation on the
99 prevalence and macroevolutionary significance of polyploidy in gymnosperms is timely.
100 We synthesize phylogenomic analyses, trait reconstructions, and examinations of
101 extensive comparative data on a comprehensive phylogeny of gymnosperms to understand major
102 processes that have shaped phenotypic innovation, species diversification, and climatic evolution
103 in this clade. Specifically, we analyze a novel transcriptome dataset including representatives of
104 nearly all extant genera of gymnosperms to identify hotspots of genomic conflict and gene
105 duplication across gymnosperm phylogeny, which we relate to reconstructed bursts of
106 phenotypic innovation and major shifts in species diversification and climatic evolution.
107

108 **Gymnosperm phylogenomics, polyploidy, and genome evolution**

109 Based on phylogenomic analysis of our transcriptome dataset (including 790 inferred orthologs
110 across 121 ingroup species, 77 of which were newly sequenced for this study; Supplementary
111 Table 1), we recovered a well-resolved and highly supported species tree for gymnosperms (Fig.
112 1). We then took a multifaceted approach for WGD inference to circumvent the limitations of
113 some methods and datatypes[28]. Using the species-tree phylogeny and the inferred orthogroups,
114 we performed gene-tree reconciliation to identify hotspots of gene duplication supporting WGD
115 (Fig. 1; Methods), and compared these results against synonymous substitution (*Ks*) plots
116 (Extended Data Figs. 1 and 2; Supplementary Fig. 1; data deposit), as well as reconstructions of
117 chromosome number (Supplementary Fig. 2) and genome size (Extended Data Fig. 3) across a
118 broader sampling of gymnosperm species from our supermatrix phylogeny (Methods). Particular
119 attention was paid to branches with previously inferred (and debated) instances of WGD in
120 gymnosperms, such as the gymnosperm root, Pinaceae, and Cupressaceae[22,29–32].
121 We inferred elevated levels of gene duplication (Fig. 1) in the branches subtending seed
122 plants (56.2 % = percentage of orthogroups showing a duplication), gymnosperms (41.7 %),
123 *Coniferae*[14] (including Gnetales, 18.8 %), Pinaceae (12.1 %), *Cupressophyta*[14] (i.e.,

124 Araucariaceae + Podocarpaceae + Sciadopityaceae + Taxaceae + Cupressaceae; 13.9 %),
125 Podocarpaceae (11.3 %), and Araucariaceae (10.1 %). Most of these branches also show at least
126 one of the following features: shared *Ks* peaks, inferred changes in chromosome number, or
127 extreme shifts in genome size (Fig. 1; Extended Data Figs. 1–3; Supplementary Fig. 1): seed
128 plants (shared *Ks* peak), *Acrogymospermae* (shared *Ks* peak), *Cupressophyta* (n = 7 to n = 11),
129 Pinaceae (shared *Ks* peak; n = 7 to n = 12), Podocarpaceae (n = 11 to n = 10; major C-value
130 decrease), and Araucariaceae (n = 11 to n = 13). Cycads and *Ginkgo* show clear *Ks* spikes around
131 0.8, previously thought to represent a potential WGD event unique to this clade[30], but these
132 paralog *Ks* peaks are deeper than the ortholog *Ks* peak for gymnosperms as a whole (Extended
133 Data Fig. 1), suggesting instead that these peaks represent a gymnosperm-wide WGD event. This
134 putative gymnosperm peak is also evident in *Ks* plots of conifers, but less prominently and at a
135 *Ks* range of around 1.0 to 1.5. We suggest these *Ks* differences between 'ginkads' and conifers
136 might be due to differences in rates of molecular evolution or differences in the timing of
137 diploidization in each lineage if they diverged prior to the diploidization of the polyploid
138 gymnosperm ancestor.
139 Ancestral chromosomal changes were also observed in Cycadaceae (n = 7 to n = 11),
140 Zamiaceae (n = 7 to n = 8), and Gnetaceae (n = 7 to n = 12), as well as within Cupressaceae,
141 Podocarpaceae, and the genera of Gnetales, due to more recent paleopolyploidy or
142 neopolyploidy[24,25,33] (Supplementary Fig. 2). Regarding genome size, rate shifts (inferred using
143 BAMM) and/or major 'jumps' (i.e., extreme changes in reconstructed ancestor to descendant C-
144 values) in genome size evolution (Methods) were inferred at the origins of several major clades,
145 including abrupt genome downsizing in Podocarpaceae (as noted above), Gnetales, and
146 Cycadaceae and rate shifts in Zamiaceae (excluding *Dioon*), Taxaceae + Cupressaceae, and
147 Ephedraceae; jumps or rate shifts were also inferred in numerous more recent clades (Extended
148 Data Fig. 3). See Supplementary Information for additional results and discussion of
149 gymnosperm genome evolution, including more detailed comparisons of our results with those of
150 previous studies on WGD in gymnosperms[22,29,30,32].
151

152 **Genomic correlates of diversification and phenotypic evolution**

153 Our diversification analysis (Supplementary Fig. 3), based our dated supermatrix phylogeny of
154 extant gymnosperms (including 82% species representation), recovered 17 major shifts, only two

155 of which correspond directly to branches with possible WGDs: *Cupressophyta* and Pinaceae
156 (Fig. 1). Seven other diversification shifts corresponded to major jumps or rate shifts in genome
157 size evolution or changes in chromosome number (Fig. 1, Extended Data Fig. 3, and
158 Supplementary Information). Although most diversification shifts (15/17) were not associated
159 with putative WGDs, it is possible that major extinctions have obscured instances of
160 diversification shifts associated with WGD in deep time. On the other hand, extinction of
161 depauperate branches separating a WGD event and a diversification shift could also lead to their
162 erroneous association. With these considerations in mind, we argue at least that WGD has played
163 a minimal role in driving the more geologically recent diversification shifts detected here. Our
164 results also align with recent studies showing an inconsistent relationship between WGD and
165 diversification[5,6,7]. We suggest that in cases where WGD is associated with diversification, it is a
166 product of WGD mediating changes in traits (such as key innovations[9]) or life history that
167 influence population structuring, with diversification only being a secondary consequence[7]. This
168 is exemplified by Pinaceae, where a possible ancestral WGD is associated with not only a shift in
169 diversification (Fig. 1) but also a major spike in morphological innovation (Fig. 2) and a rate
170 shift in climatic evolution (see below).

171 To examine the general relationship of gene duplications and genomic (gene-tree)
172 conflict with levels of phenotypic innovation and rates of phenotypic evolution, we analyzed a
173 newly compiled trait dataset for gymnosperms (available in the data deposit) comprising 148
174 phenotypic characters, with sampling matched to the transcriptome phylogeny (Supplementary
175 Information). Sub- and neo-functionalization of duplicate genes are considered the primary
176 means by which polyploidy can lead to phenotypic novelty[34,35], and therefore an examination of
177 the direct correspondence of gene duplication numbers to levels of phenotypic novelty at nodes
178 across gymnosperm phylogeny serves as a means of assessing the potential contribution of large-
179 scale (WGD) and small-scale genomic events to macroevolutionary patterns of phenotypic
180 evolution. Genomic conflict, on the other hand, records information about population-level
181 processes during speciation events—e.g., rapid population expansions or fragmentation, resulting
182 in incomplete lineage sorting (ILS), or introgression—that might also play a role in rapid
183 acquisition of phenotypic novelties[36]. Thus, these represent two different, albeit not mutually
184 exclusive, processes that might shape macroevolutionary patterns of morphological evolution in
185 plants. We reconstructed levels of phenotypic innovation or novelty (defined here simply as state

186 changes along a branch) and rates (state changes/time) for each branch in the phylogeny[13] and
187 plotted these against corresponding levels of gene duplication and gene-tree conflict (Figs. 2 and
188 3). Linear regression and permutation tests were also performed to evaluate the statistical
189 strength of these relationships (Methods).

190 We find a positive relationship between levels of phenotypic innovation and levels of
191 gene duplication (linear regression: $r^2 = 0.2038$, $p = 0.000$; permutation test: $p = 0.038$;
192 Supplementary Figs. 4 and 5), suggesting that gene duplications may have played a vital role in
193 the generation of phenotypic novelty in gymnosperms evident at a macroevolutionary scale (Fig.
194 2). Major examples include Araucariaceae, Cupressaceae, *Pinus*, Pinaceae, Podocarpaceae, and
195 Zamiaceae, which all show corresponding, elevated levels of gene duplication and phenotypic
196 innovation. There are also evident temporal patterns, with, for example, a noticeable increase in
197 duplications and phenotypic innovation near the present (Fig. 2). While causation between gene
198 duplication and phenotypic innovation cannot be demonstrated on the basis of these observed
199 patterns, this connection is plausible in cases of complex innovations and clearly deserves future
200 study given that gene duplications have long been considered to play a pivotal role in the origin
201 of evolutionary novelties[34]. It has been suggested that small-scale and large-scale (e.g., WGD)
202 duplication events might tend to have different immediate consequences and evolutionary
203 trajectories[35]. Some of the putative WGD events examined here seem most salient in terms of
204 morphological innovation, but the general correspondence (Supplementary Figs. 4 and 5)
205 suggests that perhaps both large- and small-scale duplication events have played an important
206 role in the generation of novel traits in gymnosperms. It is possible that some of the
207 phylogenetically deeper spikes in phenotypic innovation are exaggerated due to prevalent
208 extinction of stem lineages with intermediate morphologies. However, the sudden appearance of
209 major lineages with distinct morphologies is a common phenomenon across the Tree of Life[13],
210 and neofunctionalization of duplicate genes (and/or other results of gene or genome duplication)
211 may serve as an important driver of instances of radical and rapid phenotypic change. A better
212 integration of fossil and phylogenomic data might offer important insight into this question.

213 Although we observed a significant relationship between gene duplication and
214 phenotypic novelty, there are notable discrepancies, the most salient being Gnetales (Fig. 2).
215 However, this is perhaps not surprising given that Gnetales have experienced significant life
216 history changes and stands out among gymnosperms in having relatively small genomes (despite

217 at least recent WGD events[22,25]) and remarkably fast ("angiosperm-like") rates of molecular
218 evolution[37,38]. *Coniferae* (including Gnetales) also represents a major discrepancy, and the
219 exclusion of Gnetales from the phenotypic reconstructions results in an increase in the number of
220 inferred apomorphies for conifers (Supplementary Fig. 6). However, another explanation for the
221 low levels of innovation inferred for the *Coniferae* node might be the prevalence of traits shared
222 between conifers and *Ginkgo*. Although our species tree places *Ginkgo* sister to cycads (Fig. 1),
223 morphological phylogenetic analyses of seed plants[18] have typically placed *Ginkgo* with extinct
224 and extant conifers (rather than cycads), and among our gene trees (Fig. 1) the dominant
225 alternative topology for *Ginkgo* is sister to *Coniferae* (with 81 gene trees showing this alternative
226 topology with strong support). This finding suggests that the proposed gymnosperm-wide WGD
227 event might have involved reticulate evolution in the early history of *Acrogymnospermae*,
228 providing a partial explanation for this discrepancy. It is also noteworthy that several well-
229 documented gymnosperm polyploids (e.g., *Sequoia sempervirens*, *Fitzroya cupressoides*[25]) do
230 not show major phenotypic changes. However, these species both show polysomic inheritance[33]
231 with gene copies still functioning as alleles, thus constraining the ability of duplicate genes to
232 generate novel phenotypes via neofunctionalization or other processes.

233 In contrast to cumulative levels of phenotypic innovation, rates of phenotypic evolution
234 show a strong relationship with levels of gene-tree conflict (Fig. 3; linear regression: $r^2 = 0.2308$,
235 $p = 0.000$; Supplementary Fig. 7), with extreme values of conflict and rate in particular tending
236 to co-occur (permutation test: $p = 0.001$; Supplementary Fig. 8). This finding is in line with a
237 recent study[13] documenting a similar correspondence in multiple clades across the Tree of Life.
238 Figure 3 clearly shows that the branches with the highest rates of morphological evolution also
239 have extreme levels of gene-tree conflict. A noteworthy example is the Podocarpaceae subclade
240 including *Phyllocladus* and *Parasitaxus* (Fig. 3), which is characterized by extensive conflict as
241 well as remarkable shifts in phenotype (e.g., the acquisition of phylloclades) and life history
242 (e.g., parasitism) in several of the constituent lineages.

243 If gene-tree conflict reflects a signature of historical population-level processes such as
244 ILS[39], the observed relationship between conflict and phenotypic rates suggests that, at
245 macroevolutionary scales, many instances of rapid morphological evolution in gymnosperms are
246 a product of episodic and dramatic population perturbations associated with rapid speciation
247 events. Given the life history characteristics of (most) gymnosperms—including large genome

248 sizes, long time to reproduction, and, in some species, exceptional longevity[40,41]—instances of
249 population reduction or isolation should theoretically be essential for instances of rapid
250 phenotypic evolution. Thus, areas of gymnosperm phylogeny with elevated conflict and
251 morphological rates perhaps reflect the rapid fragmentation of broad, variable ancestral
252 populations. As discussed below, this type of process might explain the prevalence of young
253 conifer and cycad species with highly restricted distributions today. We also note that the
254 processes outlined above are not mutually exclusive putative drivers of phenotypic evolution,
255 given that gene duplications might serve as a source of novelties sorted by population-level
256 processes and that WGD can theoretically result in the rapid establishment of small,
257 reproductively isolated populations[9,42], which could result in the rapid fixation of polymorphisms
258 via genetic drift.
259

260 **Temporal patterns of climatic occupancy evolution and species diversification**

261 Reconstructions of climatic variables on the dated supermatrix phylogeny—focusing on mean
262 annual temperature (Bio 1), mean annual precipitation (Bio 12), and principal components one
263 and two of the 19 WorldClim bioclimatic layers—revealed that rate shifts and jumps in climatic
264 occupancy are concentrated in the last ~70 Myr (and particularly the last ~25 Myr;
265 Supplementary Figs. 9–14). Additionally, many of the branches (10/17) with a diversification
266 shift also included a shift in the rate of climatic evolution (Fig. 4), with four additional
267 diversification shifts separated from a climatic shift by only one or two nodes (i.e., 14/17
268 diversification shifts are directly or strongly associated with climatic shifts; Fig. 4). Except for
269 two of the concerted shifts (in the branches subtending the cycad genera *Ceratozamia* and
270 *Zamia*), all others involved shifts associated with cooler and/or drier climatic conditions (Fig. 4
271 and Supplementary Figs. 9–14). Undoubtedly, there have been many deeper bursts of
272 diversification and shifts in climatic occupancy in gymnosperms that are not evident in our
273 phylogeny due to extinction[19]; this renders the inference of diversification dynamics across the
274 depth of gymnosperm history a major, if not insurmountable, challenge without extensive data
275 from the fossil record[43]. Nevertheless, considering an extensive body of research concerning
276 gymnosperm vs. angiosperm functional morphology and competitive dynamics today[44] and
277 patterns in the fossil record[45], we might predict many gymnosperms (and especially conifers) to

thrive with the expansion of cooler and more arid conditions after late Eocene climatic deterioration and particularly after the mid-Miocene[46].

The global ecological ascendance of angiosperms, from the early Cretaceous to early Cenozoic, was a complex and relatively protracted process, with gymnosperms showing heterogeneous patterns of decline, in terms of abundance and diversity, across different lineages, ecological guilds, geographic areas, and points in time[45,47]. It was not until the early Cenozoic, perhaps in part facilitated by major ecological upheaval at the K-Pg boundary, that angiosperms came to dominate (or co-dominate) nearly all geographic areas and vegetation types[47]. Numerous attributes of angiosperms may underpin their macroevolutionary success, including their reproductive innovations and ecological associations[48], proclivity for polyploidy[32,49,50], and faster rates of molecular evolution[37]. However, their functional morphology and correspondingly elevated growth rates have been highlighted as particularly important in the context of competition with gymnosperms[44]. The early Cenozoic was a time of warm, equable climate—conditions well suited to rapid growth strategies of many angiosperms, facilitated by broad leaves and a more efficient vascular system including vessels. Gymnosperms, with narrow tracheids and (generally) smaller, more poorly vascularized evergreen leaves, may have struggled to compete with angiosperms across much of the landscape of the early Cenozoic given the growth-rate limitations imposed by their less efficient vascular systems[44]. Exceptions that prove the rule include *Gnetum* and some members of Podocarpaceae that independently evolved angiosperm-like vasculature and/or broader leaves, allowing them to compete in wet tropical conditions where gymnosperms (especially conifers) are otherwise largely excluded[51].

However, the same basic traits (and consequent slow-growth strategies) that exclude most gymnosperms from habitats that are more favorable to angiosperm plant growth allow many to persist in more extreme, stressful habitats[52]. The expansion of cool and arid conditions after the Eocene and especially after the mid-Miocene therefore may have created many new opportunities for gymnosperm expansion and diversification. This hypothesis is consistent with our results showing many gymnosperm lineages shifting into cooler and/or more arid conditions after the late Eocene (Supplementary Figs. 9–14). Diversification shifts associated with cool/arid transitions that occurred prior to the late Eocene (e.g., *Pinus* subsection *Ponderosae*, ~56 Ma; the *Juniperus-Cupressus* clade, ~70 Ma; the *Podocarpus-Retrophyllum* clade, ~68 Ma) might represent competitive displacement by angiosperms and/or expansion into upland or montane

habitats[53]. Future biogeographic analyses integrating fossil evidence might shed light on the geographic context of these coordinated climatic-diversification shifts. In general, our results challenge suggestions that recent climatic cooling precipitated macroevolutionary declines in conifers[54] (Supplementary Information). The Northern and Southern Hemispheres, however, clearly do show distinct patterns with respect to the extinction and turnover of conifer diversity during the Cenozoic[21], and numerous extant gymnosperm species (especially cycads) are at risk of extinction due to pressures including habitat destruction[55,56].

While climatic shifts have played a significant role in the Cenozoic history of gymnosperms, many major climatic shifts are not associated with changes in diversification (Fig. 4 and Supplementary Figs. 9–14), suggesting that climatic evolution might be an important but insufficient vehicle for diversification. Ecological factors (e.g., environmental heterogeneity related to elevation or soil type) and/or traits (e.g., dispersal strategies) that more directly control population size and spatial structuring are likely necessary concomitant factors for driving diversification patterns in gymnosperms, and more broadly in green plants. This finding is consistent with the observation that many recent gymnosperm radiations (e.g., multiples lineages of cycads, Pinaceae, and Cupressaceae) include numerous species with insular/restricted distributions[57]. The small distributions of many gymnosperm species have typically been interpreted as relictual[40], but clearly many of these species stem from recent, Neogene radiations given their remarkably short molecular branch lengths[20,21,58]. Aside from these numerous recent radiations, however, extensive data from the fossil record show that many extant gymnosperms—e.g., *Ginkgo biloba*, members of Taxaceae (including *Cephalotaxus*), and the "taxodioid" Cupressaceae—are clearly relics of historically more diverse and broadly distributed groups.

Macroevolutionary synthesis

Our analyses provide an unprecedented and multifaceted view of genome evolution in gymnosperms, with broad implications for how different attributes of genome evolution might both drive and signal major phases of phenotypic evolution in plants. Using multiple lines of evidence, we show that polyploidy and dynamic shifts in chromosome number and genome size are common occurrences across the phylogenetic breadth of gymnosperms. We also provide compelling evidence for a WGD event in the direct ancestor of gymnosperms, a possibility that

has been suggested previously but with limited and conflicting evidence[59]. Furthermore, we provide one of the first direct documentations of a general relationship between levels of gene duplication and phenotypic innovation at a macroevolutionary scale. Gene duplications have long been emphasized as a fundamental source of novelty, but their evolutionary outcomes have typically been studied at a microevolutionary scale or with respect to particular traits or nodes of interest[9,10,11] (but see Walden et al.[8]). The broader patterns documented here provide a framework of promising areas of gymnosperm phylogeny in which to investigate connections between duplications or gene-family expansions and phenotypic novelties.

Contrary to the association of duplications and innovation, we do not observe a consistent relationship between polyploidy and diversification—consistent with other recent studies also showing a sporadic association[5,6,7]. This implies that, across the euphyllophyte radiation, WGD is more fundamentally tied to phenotypic innovation, and that subsequent diversification is contingent on the nature of the traits acquired and the presence of ecological opportunity[9]. In gymnosperms, recent phases of diversification appear closely tied with shifts in climatic occupancy, suggesting that the expansion of cool and arid climates in the later Cenozoic created new ecological opportunities suited to the basic functional morphology of this clade. Finally, we show that elevated rates of phenotypic evolution appear most closely tied to phylogenetic regions with extensive gene-tree conflict—a signature of dynamic population processes during rapid speciation events. Collectively these threads create a nuanced picture of how different processes—including gene and genome duplication, population dynamics such as ILS and introgression, and ecological opportunity in the face of climate change—may interact to drive the emergence of novel morphologies and major radiations in plants.

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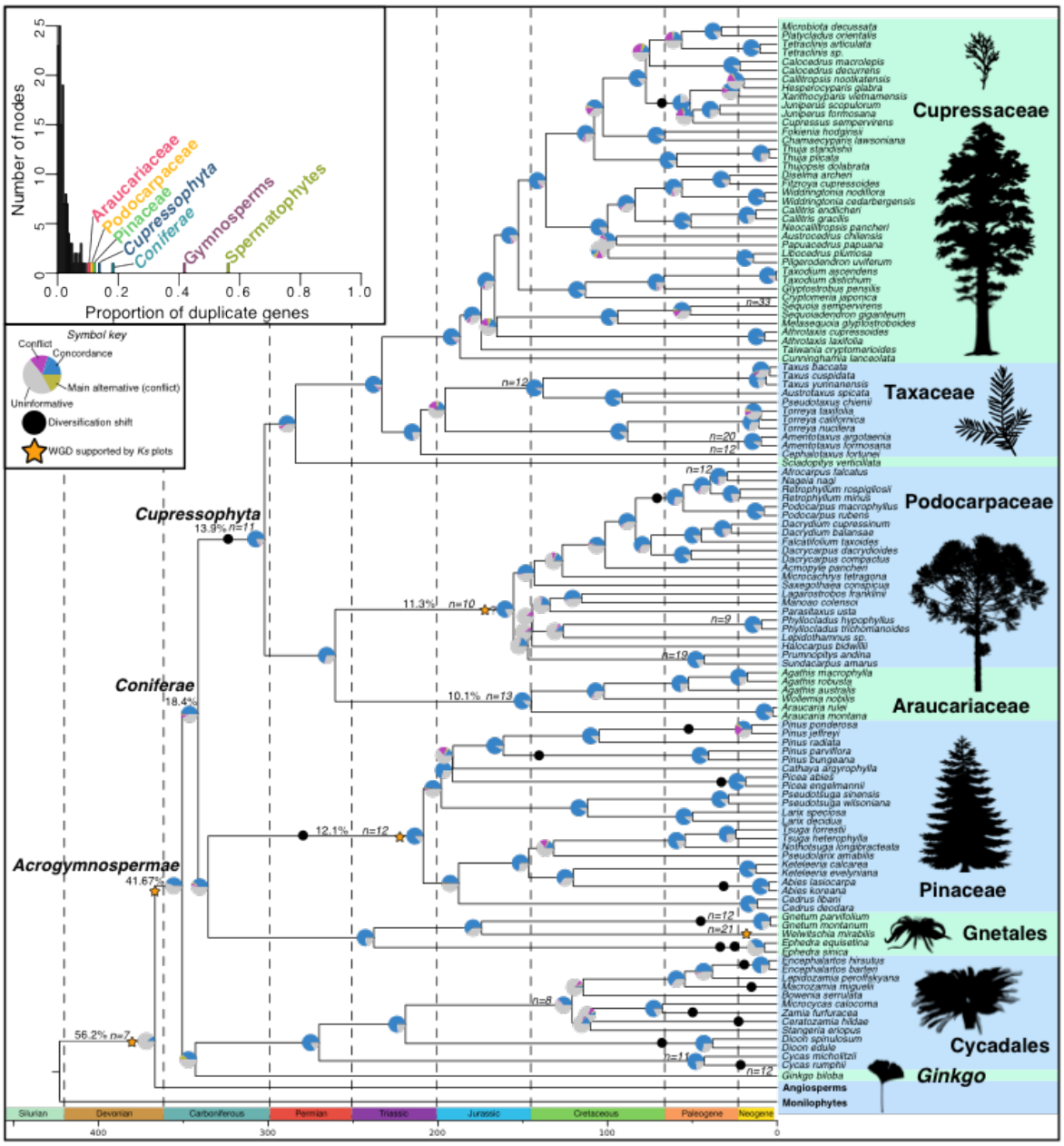
Author contributions. G.W.S., D.-Z.L., S.A.S., and T.-S.Y. conceived the study; X.-J.Q., Y.-Y.Y., Y.H., J.-B.Y., Z.-Y.Y., and H.M. and collected and prepared samples for transcriptome and plastome sequencing; G.W.S. generated the trait dataset, and compiled publically available data for the supermatrix and comparative analyses; G.W.S. conducted analyses with help from C.P.-F., S.A.S., and X.-J.Q; G.W.S, C.P.-F., P.S.S., D.E.S., S.A.S., and T.-S.Y. interpreted the results; G.W.S. wrote the manuscript, with contributions from C.P.-F., P.S.S., D.E.S., S.A.S., and T.-S.Y. All authors approved the manuscript.

523 **Figures**



524

525 **Figure 1**. **Transcriptome species tree showing major genomic events**. Species tree inferred
526 from the 790 gene trees using ASTRAL, scaled to time by fixing the nodes to the ages inferred in
527 the supermatrix dating analysis. Pie charts at each node show the proportion of gene trees in
528 concordance (blue), in conflict (chartreuse = dominant alternative; magenta = other conflicting
529 topologies), or without information for that particular bipartition (grey). Nodes with the highest
530 percentages of duplicate genes (indicative of WGD) are highlighted in the inset plot in the top

left corner; the percentages of duplicate genes inferred for these outlier nodes are also displayed on the tree. Orange stars indicated WGD events supported by *Ks* plots; uncertainty regarding the phylogenetic placement(s) of such events is indicated with a question mark. Changes in the haploid chromosome number (based on the ChromEvol analyses; Methods) are indicated on the phylogeny. Black circles indicate diversification rate shifts (inferred using BAMM; Methods). Plant silhouettes were obtained from PhyloPic (http://phylopic.org); credit is acknowledged for the silhouettes of *Araucaria angustifolia* (LeonardoG and T. Michael Keesey); *Zamites* (Robert Gay), which closely resembles the architecture of cycads; and *Ginkgo* (Joe Schneid and T. Michael Keesey). The *Taxus* silhouette was generated from a line drawing of *Taxus brevifolia* obtained from the website of Natural Resources Canada, Canadian Forest Service (https://tidcf.nrcan.gc.ca/en/trees/factsheet/288).

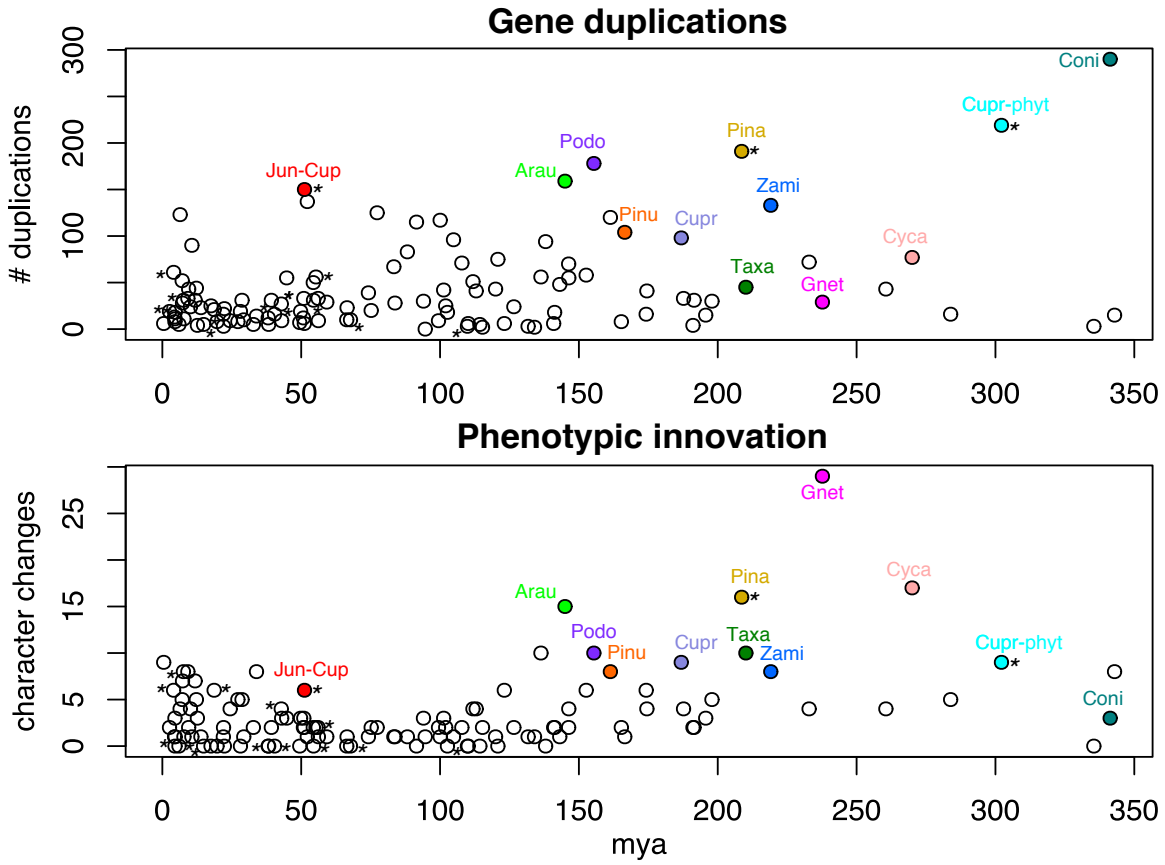


Figure 2. **Gene duplications vs. phenotypic innovation**. Plots juxtaposing levels of gene duplication and phenotypic innovation (i.e., the number of state changes) at corresponding

branches across gymnosperm phylogeny (n = 119). The x-axis represents time in millions of years. Nodes with an inferred diversification shift are marked with an asterisk.

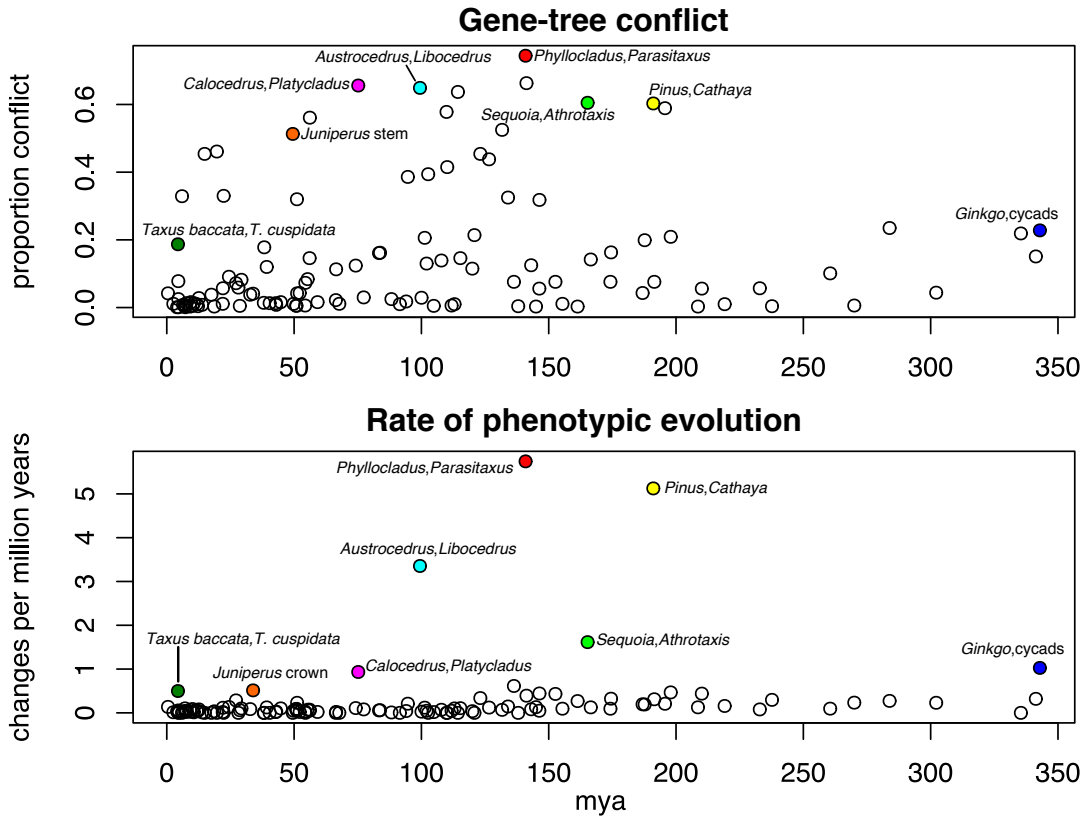
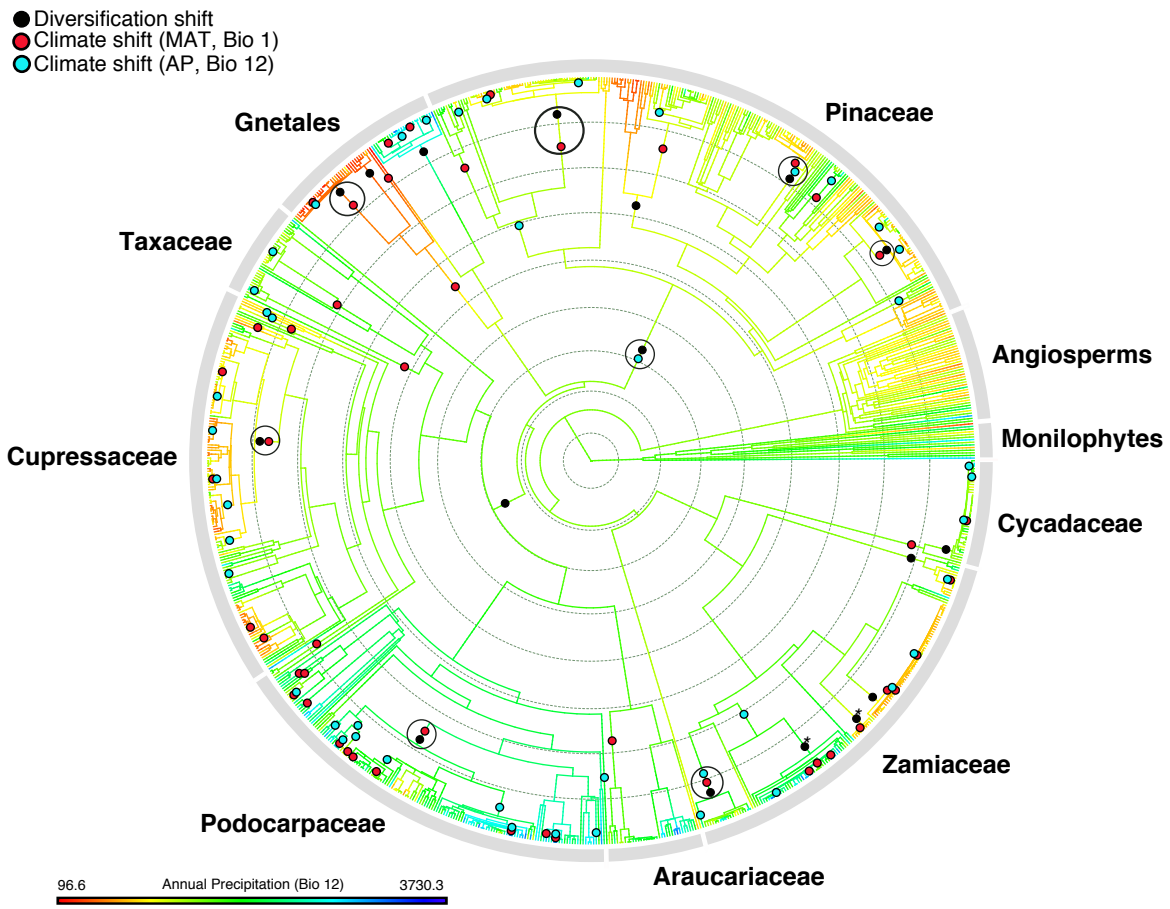


Figure 3. **Gene-tree conflict vs. phenotypic rates**. Plots juxtaposing rates of phenotypic evolution and levels of gene-tree conflict at corresponding branches across gymnosperm phylogeny (n = 119). Note that the conflict node highlighted as corresponding with the *Juniperus* rate node is its immediate parent, comprising *Cupressus sempervirens*, *Juniperus*. The x-axis represents time in millions of years.



559

560 **Figure 4**. **Climatic and diversification shifts across gymnosperms**. Ancestral reconstruction of
561 annual precipitation (Bio 12) on the supermatrix phylogeny, showing diversification rate shifts
562 (black circles) and shifts in rates of climatic evolution (Bio 1, mean annual temperature = red
563 circle; Bio 12, annual precipitation = cyan circle) inferred using BAMM. The diversification
564 shifts marked with asterisks (corresponding to *Macrozamia* and *Zamia*) show concerted rate
565 shifts with PC2 of the climatic variables, bringing the number of directly concerted
566 diversification and climate shifts to ten. See Supplementary Fig. 9 for species tip labels.

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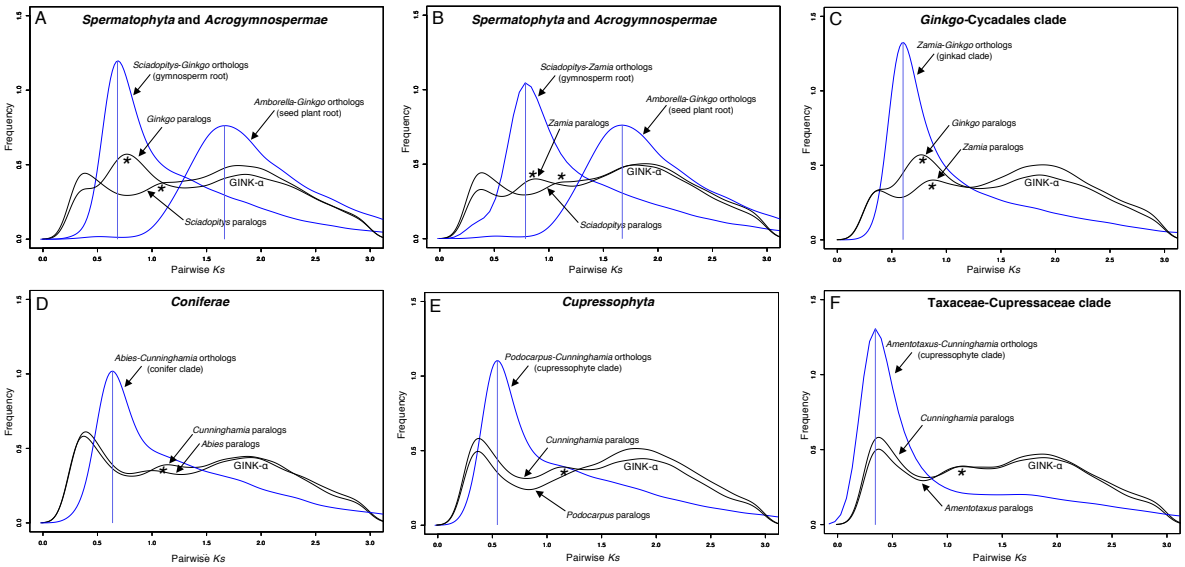
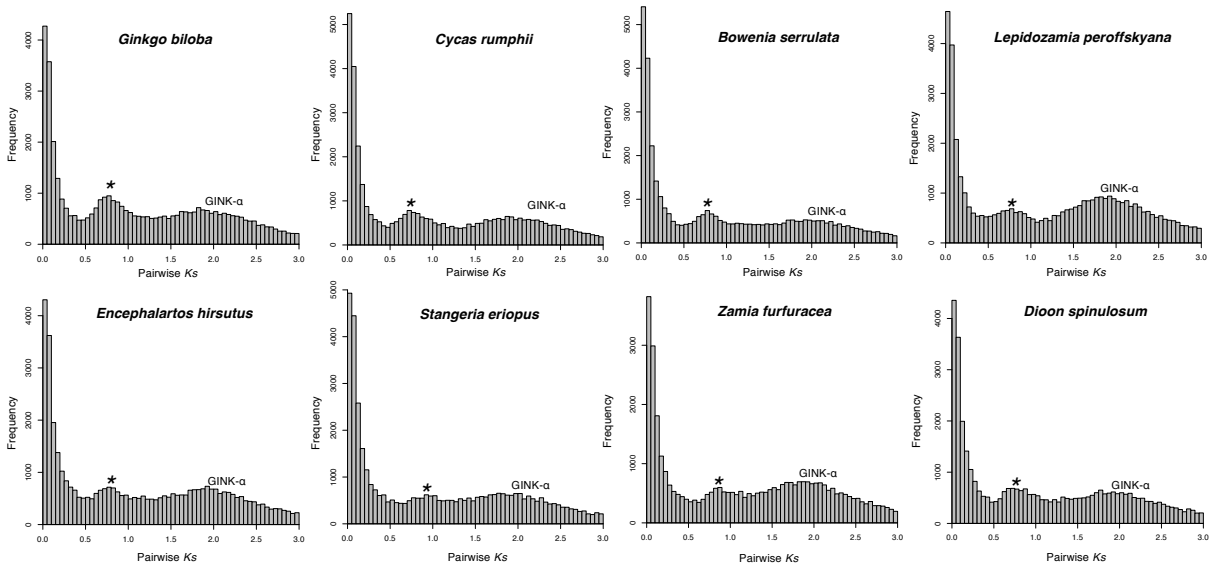


572

573 **Extended Data Figure 1. Plots of synonymous substitutions per site (*Ks*) for within-taxon**
574 **paralog pairs (black lines) and between-taxon ortholog pairs (blue lines).** The relative
575 positions of ortholog vs. paralog *Ks* spikes help clarify the phylogenetic positions of possible
576 WGD events. The seed plant WGD event (GINK-α) is labeled. *Ks* peaks corresponding to an
577 inferred WGD for gymnosperms are highlighted with an asterisk. The taxa compared capture the
578 root nodes of (A, B) seed plants and gymnosperms, (C) the 'ginkad' clade, (D) conifers, (E) the
579 cupressophyte clade, and (F) the Taxaceae-Cupressaceae clade. Ortholog and paralog *Ks* plots
580 were generated using the pipelines of Walker et al.[83] and Yang et al.[28], respectively.
581



582

583 **Extended Data Figure 2**. **Plots of synonymous substitutions per site (*Ks*) for within-taxon**
584 **paralog pairs of representatives of the 'ginkad' clade.** The seed plant WGD event (GINK-α)
585 is labeled. *Ks* peaks corresponding to an inferred WGD for gymnosperms are highlighted with an
586 asterisk.

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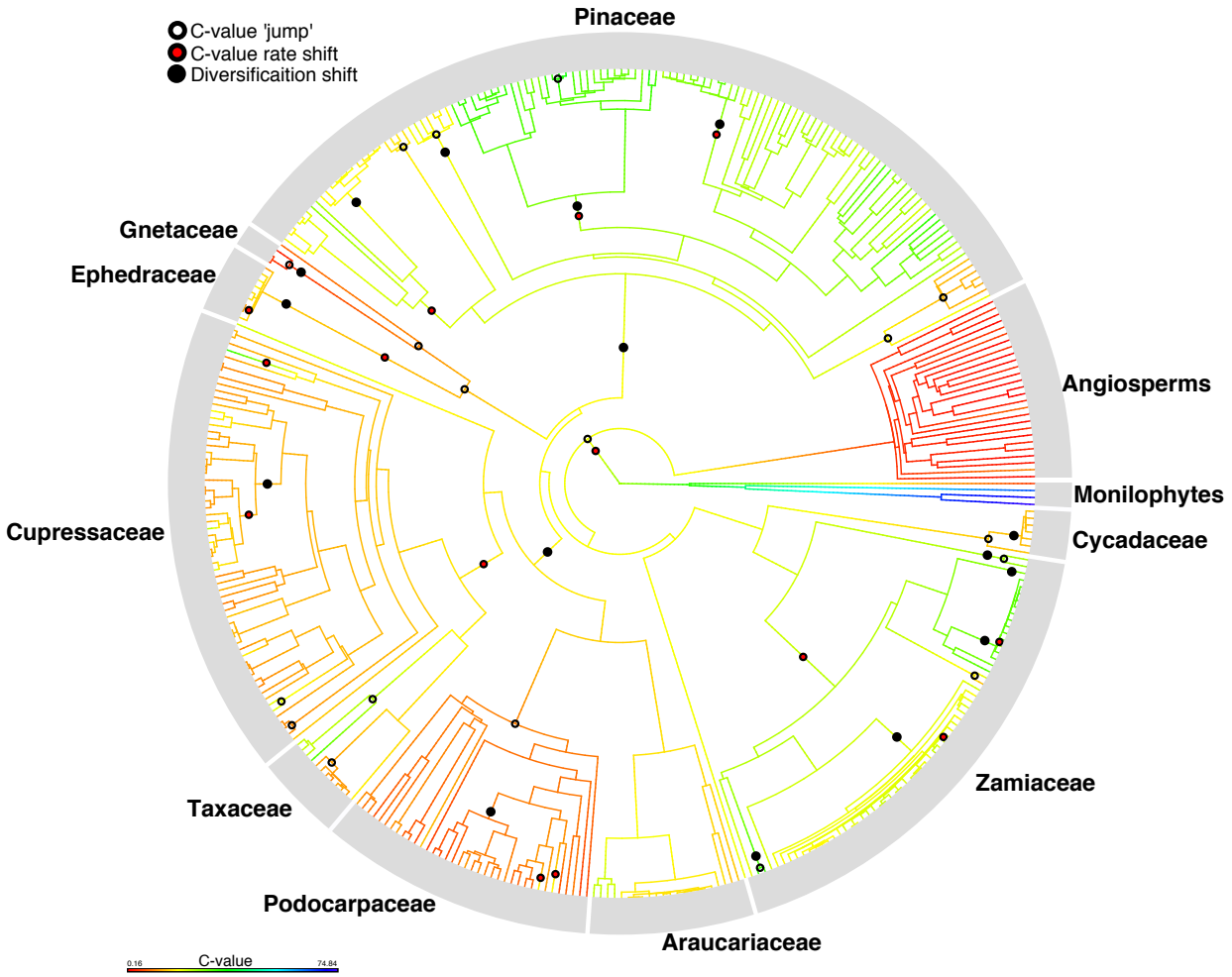

591

592 **Extended Data Figure 3**. **Genome size evolution in gymnosperms**. Ancestral reconstruction of
593 genome size (C-value) on the pruned supermatrix phylogeny, showing BAMM rate shifts (red
594 circles) and jumps (i.e., extreme differences in ancestor-descendent values; white circles) in
595 genome size evolution, as well as BAMM diversification shifts (larger black circles).
596 Supplementary Fig. 15 shows this figure with species tip labels.

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Methods

Sampling overview

We generated two major phylogenetic datasets. The first is a transcriptomic and genomic dataset comprising 144 species (including 121 ingroup species, representing 83 of the 85 gymnosperm genera recognized by the Kew checklist: https://wcsp.science.kew.org/home.do); this was used to resolve broad-scale phylogenetic relationships, characterize patterns of phylogenomic conflict, and infer hotspots of gene duplication. Representatives of angiosperms, monilophytes, and lycophytes were used as outgroups. In all, 77 transcriptomes were newly generated for this study; the remaining transcriptomes were obtained from the One Thousand Plants (1KP) Consortium (https://sites.google.com/a/ualberta.ca/onekp) and several genomes were obtained from the Phytozome database v9.1[60]. See Supplementary Table 1 for transcriptomic and genomic sampling details. The two missing gymnosperm genera, *Pectinopitys* and *Pherosphaera*, represent genera nested within Podocarpaceae and thus their absence should not impact the resolution of major relationships. The newly generated transcriptomes were deposited in the Sequence Read Archive (BioProject PRJNA726756). We also generated a phenotypic dataset of 148 traits for the seed plant species in the transcriptomic dataset to permit examination of the correspondence of different aspects of genomic and phenotypic evolution; the trait dataset is described below.

The other phylogenetic dataset is a plastid supermatrix, based on publicly available plastomes and plastid genes in NCBI (data deposit), as well as 32 plastid genomes newly generated through genome skimming (Supplementary Table 2). In total, this dataset includes 78 plastid genes across 1055 accessions, including 995 ingroup accessions. The ingroup names were checked against the Kew gymnosperm checklist (https://wcsp.science.kew.org/home.do) to resolve issues with synonymy. In total, 890 gymnosperm species are represented, comprising ca. 82% of the 1090 recognized gymnosperm species[56]; our sampling also includes numerous infraspecific taxa (i.e., varieties and subspecies; https://wcsp.science.kew.org/home.do).

The supermatrix dataset was used to generate a comprehensive dated phylogeny for gymnosperms for reconstructions of diversification shifts, climatic evolution, genome size evolution, and chromosome evolution. We used exclusively plastid data for two reasons: (1) plastid genes are more widely sequenced across gymnosperms, allowing for greater species representation; and (2) conflict within phylogenetic datasets can greatly (and negatively) impact

635 branch length estimation and downstream analyses such as divergence-time estimation[61]. While
636 studies show that the plastome is not without conflict[62], it clearly shows more minimal levels of
637 conflict compared to nuclear genes. Plastid relationships of gymnosperms, as shown by previous
638 studies[56,63] and our results here, largely agree with those of the nuclear genome, and thus a plastid
639 supermatrix tree should accurately capture major macroevolutionary patterns.
640
641 **Transcriptome and plastome sequencing and assembly**
642 To generate the new transcriptomes, total RNAs were isolated from young buds or leaves using
643 the Spectrum™ Plant Total RNA Kit (SIGMA-ALDRICH). Approximately 5 ug of total RNAs
644 were used to construct cDNA libraries (NEBNext Ultra Directional RNA Library Prep Kit for
645 Illumina, Illumina, San Diego, CA, USA). Transcriptome sequencing was performed using the
646 Illumina Hi-Seq 2000 platform. Paired-end reads of 150 bp were *de novo* assembled with Trinity
647 v2.4.0 (Grabherr et al., 2011) using default parameters. Transcriptomes from the 1KP project
648 were previously assembled (Wickett 2014; Leebens-Mack et al. 2019) using SOAPdenovo v1 (Li
649 et al. 2010), and amino acid sequences for three gymnosperm species derived from genome
650 annotations were downloaded from the Phytozome database v9.1[60]. All transcripts were
651 translated using TransDecoder v5.3.0[64], and then cd-hit-est[65] (-c 0.99 –n 5) was used to reduce
652 sequence redundancy in all CDS.
653 Plastid genomes were sequenced and assembled as follows. Total genomic DNA was
654 extracted from fresh leaves using the CTAB method[66]. Approximately one microgram of total
655 genomic DNA from each sample was used for ~400-bp library construction with the NEBNext
656 DNA Library Prep Kit (New England Biolabs, Ipswich, MA, USA) and Covaris-based
657 fragmentation. The Illumina MiSeq instrument (Illumina, San Diego, CA, USA) was used to
658 carry out 150-bp, paired-end sequencing. We assembled the plastomes using both the
659 GetOrganelle pipline[67] and SPAdes v3.13.0[68], the latter with the "careful"-option and kmers of
660 61, 81, 101, and 121. In order to validate the plastome assembly, we mapped all paired-end reads
661 to the consensus sequence of the assembled plastomes with the local-sensitive option of Bowtie
662 v2.3.2[69]. Plastid Genome Annotator[70] was used to perform plastome annotation, coupled with
663 manual correction in Geneious v8.0.2 (https://www.geneious.com/).
664
665

666 **Phylogenomic workflow**

667 **Homology and orthology inference.** We followed two major procedures for homology and
668 orthology inference in the transcriptomic dataset. First, we employed the hierarchical homology
669 inference approach of Walker et al.[71]. Given the size of our transcriptomic dataset (including 144
670 transcriptomes), performing an all-by-all BLAST search across all samples for initial homology
671 assessment would have been computationally prohibitive. The Walker et al.[71] approach
672 overcomes this problem by conducting all-by-all BLAST searches on subsets of the data (*tip*
673 *clustering*), which are then combined using a post-order (tip to root) tree traversal method (*node*
674 *clustering*). We divided our samples into 11 subgroups for tip clustering: **(1)** monilophytes and
675 lycophytes (7 samples), **(2)** angiosperms (16 samples), **(3)** cycads and *Ginkgo* (14 samples), **(4)**
676 Gnetales (5 samples), **(5)** Pinaceae (22 samples), **(6)** Podocarpaceae (23 samples), **(7)**
677 Araucariaceae (6 samples), **(8)** *Sciadopitys* and Taxaceae (12 samples), **(9)** 'basal' Cupressaceae
678 (11 samples), **(10)** cupressoid Cupressaceae (17 samples), and **(11)** callitroid Cupressaceae (11
679 samples); see Supplementary Table 1 for lists of species included in each grouping. For tip
680 clustering, for each subset, we conducted an all-by-all BLASTP search[72] with an e-value cutoff
681 of 10. The tip clusters were then sequentially combined in a post-order fashion (node-clustering)
682 using the scripts developed by Walker et al.[71] (https://github.com/jfwalker/Clustering).

683 For homolog tree and orthology inference from the combined clusters, we followed the
684 Yang and Smith[73] pipeline (scripts available at
685 https://bitbucket.org/yangya/phylogenomic_dataset_construction/src/master/), which we briefly
686 outline here. Fasta files were written from each cluster. The clusters were then aligned using
687 MAFFT v7[74] and cleaned using the phyx[75] program 'pxclsq' (retaining alignment columns with
688 at least 10% occupancy), after which homolog trees were built using FastTree v2 with the WAG
689 model[76]. The trees were then pruned of extreme long branches, monophyletic tips were masked
690 (i.e., only one sequence was retained of species/sample-specific alleles/paralogs/duplications),
691 and then long internal branches (representing deep paralogs) were pruned. Fasta files were then
692 written again from the resulting subtrees, and two more iterations of alignment, homolog tree
693 construction, and homolog tree pruning were conducted. A final stage of homolog tree
694 construction was then conducted using RAxML v8.2.12[77] to generate more robust homolog trees.
695 The resulting 1,992 homolog trees were then used for orthology inference and gene-duplication
696 mapping. For orthology inference, we used the 'RT' method of Yang and Smith[73], which extracts

697 ingroup clades and then cuts paralogs from root to tip. For this procedure, the three lycophyte
698 samples were designated as outgroups (and thus these were pruned and excluded from
699 subsequent analyses), and the minimum ingroup sampling was set at 100. This approach resulted
700 in a set of 790 orthologs. Nucleotide fasta files were then written from each extracted ortholog,
701 re-aligned using MAFFT, and then cleaned by removing alignment columns with under 25%
702 occupancy using 'pxclsq' in phyx.
703
704 **Coalescent species-tree inference.** Gene trees (from the 790 orthologs inferred above) were
705 constructed using RAxML, using the GTRGAMMA model and 100 bootstrap replicates. The
706 gene trees were then used for species-tree analysis in ASTRAL-III[78]. Prior to the ASTRAL
707 analysis, low supported branches (<10 BS) in the gene trees were collapsed using Newick
708 utilities[79], as this has been shown to increase accuracy[78]. ASTRAL was then run under default
709 settings, producing a 'species tree' tree with local posterior probability (LPP) support values for
710 the bipartitions and branch lengths in coalescent units (Supplementary Fig. 16). The resulting
711 phylogeny was used as a framework for various subsequent analyses, including conflict analysis,
712 analysis of gene duplications, inference of whole-genome duplications, morphological trait
713 reconstructions, and comparisons of phenotypic innovation and rates of phenotypic evolution
714 with levels of genomic conflict and gene duplications.
715
716 **Concatenation species-tree inference.** The individual gene/ortholog alignments were
717 concatenated using the phyx program 'pxcat'. We then inferred phylogenies from the
718 concatenated nucleotide alignment (comprising 1,444,037 bp) using IQ-TREE[80] with the
719 GTRGAMMA model and ultrafast bootstrapping (1000 replicates). Two analyses were
720 conducted: one unpartitioned, and the other with separate model parameters estimated for each
721 gene region[81] (Supplementary Fig. 17).
722
723 **Conflict analysis.** To examine patterns of gene-tree conflict across gymnosperm phylogeny, we
724 used the program PhyParts[82], which maps gene trees onto an input species tree and summarizes
725 the number of gene trees in concordance or conflict with each bipartition. When gene trees have
726 inadequate species sampling or bootstrap support (if a support threshold is specified) to speak to
727 a particular bipartition, those are considered uninformative. We use the python script

728 'phypartspiecharts.py'
729 (https://github.com/mossmatters/phyloscripts/tree/master/phypartspiecharts) to visualize the
730 PhyParts results, which are shown in Fig. 1. The levels of conflict calculated for each bipartition
731 were also plotted against corresponding levels of morphological innovation (i.e., the number of
732 new states appearing at each node; see below). We also used the program 'minority_report.py'
733 (https://github.com/mossmatters/phyloscripts/tree/master/minorityreport) to examine the number
734 of gene trees supporting alternative placements for particular lineages (namely, *Ginkgo*).
735
736 **Inference of whole-genome duplication**
737 We used a multi-faceted approach to examine genomic changes and infer putative WGDs across
738 gymnosperm phylogeny, employing gene-duplication mapping, plots of synonymous
739 substitutions per site (*Ks*), and reconstructions of chromosome number and genome size (C-
740 value). Gene duplications were mapped onto the species tree topology using the scripts
741 (https://bitbucket.org/blackrim/clustering/src/master/) and method of Yang et al.[28] This involved
742 extracting rooted orthogroups from the homolog trees (with lycophytes designated as the
743 outgroup), which were then mapped onto the species tree topology. We used two methods of
744 mapping: one maps duplications to the most recent common ancestor (MRCA) in the species
745 tree, with a bootstrap filter of 50% (Supplementary Fig. 18); the other imposes a topology filter
746 such that duplications are only mapped when the sister clade of the gene duplication node
747 contains at least a subset of the taxa present in that clade in the species tree[28] (Supplementary
748 Fig. 19).
749 *Ks* plots of within-taxon paralog pairs were generated for the newly sequenced
750 transcriptomes. First, CD-HIT (-c 0.99 –n 5) was used to reduce highly similar sequences, after
751 which an all-by-all BLASTP search was carried out within each taxon (e-value cutoff = 10). The
752 results were then filtered such that sequences with ten or more hits were removed (to avoid
753 overrepresentation of large gene families), as were hits with pident/nident less than 20/50%. *Ks*
754 values were then calculated from the CDS of the remaining paralog pairs, using the pipeline
755 https://github.com/tanghaibao/bio-pipeline/tree/master/synonymous_calculation, and plotted
756 using scripts from Yang et al.[28] We used between-taxon ortholog pairs to generate ortholog *Ks*
757 plots following the method of Walker et al.[83]; relative comparisons of ortholog and paralog *Ks*
758 peaks are useful for determining whether observed peaks are shared (occurred prior to

759 ortholog/lineage divergence) or more species- or clade-specific (occurred after ortholog/lineage
760 divergence). The ortholog and paralog *Ks* plots (Extended Data Fig. 1 and 2; data deposit) were
761 visually inspected to identify clade-specific shared peaks, which were compared against the
762 results from gene-duplication mapping, as well as the reconstructions of chromosome number
763 and genome size (outlined below). We also generated within-species paralog *Ks* plots using the
764 pipeline of Scott et al.[33] (https://github.com/nstenz/plot-ks) and these are also provided in the
765 data deposit.
766
767 **Supermatrix tree construction and dating**
768 To assemble a comprehensive species-level phylogeny of gymnosperms, we mined plastid gene
769 and genome sequences available on NCBI and combined these with genes extracted from the 32
770 newly sequenced and assembled plastomes. While the plastomes from NCBI were directly
771 downloaded, individual plastid genes were mined from NCBI using the program PyPhlawd[84].
772 Large-scale plastid gene clusters obtained using PyPhlawd were then combined with the
773 corresponding individual gene sequences derived from the plastomes.
774 The plastid genes were aligned individually using MAFFT v7[74], after which alignment
775 columns with under 25% occupancy were removed using the phyx function 'pxclsq'. The
776 alignments were then concatenated using the phyx function 'pxcat'. The final concatenated
777 matrix included 20.35% matrix occupancy (i.e., 79.65% missing data). Prior to phylogenetic
778 analysis, PartitionFinder v2[85] was used to determine the optimal model partitioning strategy for
779 the concatenated alignment. Phylogenetic analyses were conducted using RAxML-ng[86] with a
780 topological constraint enforcing major relationships to match the transcriptomic species tree
781 topology: ((*Ginkgo*,cycads),((Gnetales,Pinaceae),((Araucariaceae,Podocarpaceae),
782 (Sciadopityaceae, (Taxaceae,Cupressaceae))))). When unconstrained, the plastome tree only
783 differed from the constraint in the placement of Gnetales. Using RAxML-ng, we conducted 25
784 independent searches for the maximum likelihood tree ("raxml-ng --search1"), from which the
785 tree with the best likelihood score was selected for downstream analyses of diversification,
786 climate evolution, and genome evolution (chromosome number and cval), although all 25 trees
787 were dated in treePL[87] to obtain confidence intervals on node ages (see below). We also inferred
788 100 bootstrap trees to evaluate topological support for the supermatrix phylogeny.
789

790 **Divergence dating.** Given the scale of the supermatrix tree (1055 terminals), typical methods of
791 Bayesian divergence dating were unfeasible. Therefore, we used treePL[87] to generate a dated
792 phylogeny. We used extensive fossil evidence to inform our calibration scheme (see
793 Supplementary Information for detailed discussion of the fossils used, their justification, and
794 references). We conducted independent analyses on the 25 inferred ML trees using the same set
795 of calibrations as a means of obtaining confidence intervals for inferred node ages
796 (Supplementary Fig. 20). Cross-validation was performed for each analysis to determine the
797 optimal smoothing parameter. Node ages from the dated supermatrix tree were then applied as
798 fixed ages (also using treePL) to the corresponding nodes in the transcriptomic species tree. This
799 allowed us to place subsequent analyses involving the transcriptomic phylogeny (i.e.,
800 examinations of conflict and rates of morphological evolution) in a temporal context.
801 Given uncertainty in the placements of two calibrations (for the *Acmopyle* stem and the
802 *Keteleeria* stem; see Supplementary Methods), we also conducted a dating analysis on the best
803 ML tree excluding these calibrations to assess their impact on the ages inferred for major
804 relevant clades (i.e., Pinaceae, Podocarpaceae). The resulting ages for these clades fell within the
805 confidence intervals defined by the analyses of the 25 ML trees noted above (Supplementary Fig.
806 20), and therefore we proceeded with our initial analyses including these calibrations.
807
808 **Chromosome number and genome size reconstructions**
809 Chromosome counts were obtained from the Chromosome Count Database[49]
810 (http://ccdb.tau.ac.il; accessed 9 December 2019), and the parsed (haploid) counts were used for
811 subsequent analysis. In cases where several different counts were recorded for a given species,
812 the most frequently occurring count was used. Measurements of genome size (i.e., C-values, the
813 amount of DNA in the haploid genome) were obtained from the Plant DNA C-values Database[50]
814 (https://cvalues.science.kew.org; accessed 9 December 2019). The dated supermatrix phylogeny
815 was pruned to match the sampling in each dataset (chromosome counts and C-values), and these
816 two trees were used in the analyses outlined below. The chromosome count tree/dataset included
817 478 species, and the cval tree/dataset include 373 species. The chromosome count and C-value
818 datasets examined are available in the data deposit.
819 Reconstructions of ancestral chromosome number were implemented in ChromEvol
820 v2.0[88]. We examined the data under eight different available models, each including different

821 combinations of the rate parameters *gain* (ascending dysploidy), *loss* (descending dysploidy),
822 *duplication* (polyploidization), and *demi-duplication* (multiplication of the chromosome number
823 by a factor of 1.5), with the rates for each treated either as constant or linear. The model with the
824 lowest Akaike information criterion (AIC) score (i.e., 'LINEAR RATE DEMI EST') was used in
825 a more thorough analysis involving default settings and 100,000 simulations. We then visualized
826 changes in chromosome number across the tree using FigTree (Supplementary Fig. 2) and
827 compared changes against reconstructions of genome size and gene duplication.
828 Ancestral reconstructions of genome size (using C-values) were implemented in R[89] (R
829 Core Team 2020) using the 'fastAnc' function in phytools[90]. The C-value data were not
830 transformed prior to analysis as they conform to a more-or-less normal distribution. To identify
831 the most extreme changes in genome size (which we call C-value 'jumps'), we then calculated
832 the difference between each node's reconstructed value and that of its immediate parent node,
833 and selected branches in the 95th percentile of the distribution of parent-child C-value
834 differences. These are shown in Extended Data Fig. 3. We also examined rates of genome size
835 evolution using BAMM[91,92], using the same tree as above. The priors for the BAMM analysis
836 were determined using the function 'setBAMMpriors' in the BAMMtools package[93]. The
837 analysis was run for one billion generations using the phenotypic evolution model, with
838 convergence assessed using BAMMtools. Rate shifts from the maximum a posteriori probability
839 (MAP) shift configuration are shown in Extended Data Fig. 3.
840
841 **Species diversification analysis**
842 We used BAMM to detect diversification rate shifts across gymnosperm phylogeny, using the
843 dated supermatrix tree as input. The analysis employed the speciation-extinction model, with the
844 priors determined using the function 'setBAMMpriors' in BAMMtools. We also specified
845 sampling fractions specified for each family of *Acrogymnospermae*. This analysis was initially
846 run for 1 billion generations, but then was re-run for 2 billion generations in order to achieve
847 convergence. Rate shifts from the MAP shift configuration are shown in Fig. 1, 4, and
848 Supplementary Fig. 3.
849
850 **Reconstructions of climatic occupancy evolution**

Geographic occurrences of gymnosperms were pulled from GBIF (https://www.gbif.org; accessed 15 May 2020), and then compared against occurrences from iDigBio (https://www.idigbio.org/portal/search) to check for available data of the species missing from GBIF. Occurrences were then cleaned using the R package CoordinateCleaner[94], removing duplicate records as well as those with problematic coordinates (e.g., non-terrestrial, identical lat/lon, located within major cities or biodiversity institutions). Synonymy was reconciled using data from the Kew checklist (https://wcsp.science.kew.org/home.do). The occurrences were then used to extract climatic values from the 19 WorldClim bioclimatic variables, and means were calculated for each species for each climatic variable. We dropped tips from the dated supermatrix tree that were absent from the climatic dataset sampling, resulting in a matched sampling of 908 species for subsequent climatic analyses. We then conducted a principal component analysis in R, and principal components (PC) 1 and 2 were used in subsequent analyses of climatic evolution. We also conducted analyses of mean annual temperature (Bio1) and annual precipitation (Bio12), using the mean values for each species.

As with the genome size analyses, for the climate analysis we reconstructed major jumps and rate shifts in climatic evolution, involving separate analyses for PC1, PC2, Bio1, and Bio12. Jumps were determined by reconstructing ancestral climatic values (using the 'fastAnc' function in phytools), calculating the difference between each node and its immediate parent, and identifying those branches in the 95th percentile. Extreme climatic jumps for PC1, PC2, Bio1, and Bio12 are shown in Supplementary Figs. 13 and 14. BAMM analysis of rates of climatic occupancy evolution were also conducted on PC1, PC2, Bio1, and Bio12, with each run for 1 billion generations, employing the phenotypic evolution model, with priors determined using BAMMtools. Rate shifts from the MAP shift configuration for Bio1 and Bio 12 are shown in Fig. 4 and Supplementary Figs. 9 and 10; those of PC1 and PC2 are shown in Supplementary Figs. 11 and 12.

Although analysis of historical climatic information would be ideal for understanding the climatic context of previous diversification shifts in greater detail, this absence of reliable estimates spanning the depth of gymnosperm evolutionary history makes this, at the present, not feasible given the scope of our study. Thus, we only used current global climate data for our reconstructions. However, the use of historical climate data to investigate particular Cenozoic radiations of gymnosperms would be a promising avenue for future research.

882

883 **Morphological reconstructions and genomic comparisons**

884 A phenotypic dataset comprising 148 traits across the 137 seed plant species included in the
885 transcriptomic dataset was assembled to examine the correspondence of phenotypic and genomic
886 evolution (matrix available in the data deposit). Many of the traits examined were also included
887 in previous morphological datasets. However, the taxonomic sampling in some of these previous
888 datasets was limited; furthermore, many traits from clade-specific datasets needed to be modified
889 and reconciled with similar traits in other datasets in order to establish characters applicable
890 across all sampled taxa. The traits examined (and their corresponding states) are listed in
891 Supplementary Information, and we indicate how these relate to traits in previously published
892 datasets. The primary source datasets were those of Hart[95], Hilton and Bateman[96], Mao et al.[97],
893 Escapa and Catalano[98], Coiro and Pott[99], Herrera et al.[100,101], Gernandt et al.[102], and Andruchow-
894 Colombo et al.[103] See Supplementary Information for a complete list of references from which
895 phenotypic data were obtained. We note that while determining homology for character
896 delimitation in seed plants is a major challenge[104], we do not believe that instances of
897 misidentified homology (i.e., treating independently evolved structures as a single trait or
898 expression of a trait) should necessarily undermine our goals here, given that we are not using
899 this trait set to reconstruct phylogeny. Our goal is to identify state changes along branches, and
900 even in instances of conflated homology, e.g., with two distantly related lineages scored with the
901 same state for a particular trait despite independent origins of the relevant structures, a state
902 change should nevertheless be 'correctly' reconstructed and recorded for each branch.

903 Morphological reconstructions on the species-tree were performed using Maximum
904 Parsimony, following the procedure of Parins-Fukuchi et al.[13], briefly outlined here. Each trait
905 was reconstructed on the phylogeny, and then the number of state changes along each branch
906 was tallied—this is considered the degree or level of phenotypic 'innovation' or 'novelty' along
907 each branch. To determine rates of phenotypic evolution for each branch, we divided the number
908 of state changes by the duration of branch (in million years). We then plotted levels of
909 morphological innovation and rates of phenotypic evolution for each branch/node (with the x-
910 axis representing time or node age), and compared these against plots of the same nodes showing
911 their levels of gene duplication and gene-tree conflict (Figs. 2 and 3), allowing for visual
912 inspection of these patterns and their correspondence through time. Because Gnetales was

913 detected as a major outlier, with extreme levels of morphological innovation perhaps driven by
914 life history shifts, we also conducted morphological reconstructions excluding Gnetales to ensure
915 that the inclusion/placement of Gnetales was not significantly biasing reconstructions for other
916 near-by nodes (Supplementary Fig. 6).

917 On the basis of the visual correspondences observed from the plots noted above, we then
918 explicitly tested the relationships of (1) phenotypic innovation vs. gene duplication and (2)
919 phenotypic rates vs. gene tree conflict by conducting linear regression and permutation analyses
920 of each pair of variables. Linear regression was performed using the 'lm' function in R, treating
921 phenotypic innovation and rate as the dependent variables in the respective analyses. For the
922 examination of innovation vs. duplication, we conducted an analysis including all nodes
923 (Supplementary Fig. 21), as well as one excluding two major outlier nodes: Gnetales and
924 conifers (Supplementary Fig. 6). As noted in the main text, we believe exclusion of these outliers
925 is warranted given that (1) Gnetales has experienced major changes in genome size (i.e.,
926 reduction) and life history with generally elevated rates of molecular evolution that might erase
927 signatures of a relationship between gene duplication and morphological innovation, and (2) trait
928 reconstruction for conifers is complicated by its inclusion of Gnetales (a highly derived lineage)
929 and the prevalence of features shared among conifers and *Ginkgo*, which may have been
930 involved in a reticulation event early in the evolution of the conifer lineage based on patterns of
931 gene-tree conflict.

932 The permutation test, developed by Parins-Fukuchi et al.[13], identifies nodes with
933 significantly elevated values for a particular variable by resampling (with replacement) observed
934 values 1000 times and using the means from each replicate to create an empirical null
935 distribution. We executed this procedure for each of the four variables considered above
936 (duplication levels, conflict levels, innovation levels, and phenotypic rate), with nodes/values
937 falling in the 95th percentile deemed significant. Then, to examine the strength of the association
938 between (1) innovations and duplications and (2) rates and conflict, we determined whether the
939 number of nodes with significantly high values for both of the variables in question (e.g.,
940 innovations and duplications) departed from the expectation if the same number of nodes was
941 randomly sampled (Supplementary Figs. 5 and 8). This permutation test was designed
942 specifically to examine whether extreme/significant values (in, for example, conflict and rates)
943 tend to coincide, even if there is not a strict positive monotonic relationship between the two

variables across the spectrum of values. Finally, we also used this test to determine if diversification shifts tend to co-occur with significant/extreme levels of phenotypic innovation (Supplementary Fig. 22).

Data availability

The newly generated raw sequence data is available at the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under BioProject PRJNA726756 (transcriptomic samples) and PRJNA726638 (genome skimming samples). The newly assembled plastid genomes are also available at NCBI (https://www.ncbi.nlm.nih.gov); see Supplementary Table 2 for sample accession numbers. Sequence alignments, phylogenies, *Ks* plots, phenotypic trait data, and other data analyzed (chromosome counts, c-values) are available on figshare (10.6084/m9.figshare.14547354).

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