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PERSPECTIVE

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Ephedra as a gymnosperm evo-devo model lineage

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Abstract

Established model systems in the flowering plants have greatly advanced our understanding of plant developmental biology, facilitating in turn its investigation across diverse land plants. The reliance on a limited number of model organisms, however, constitutes a barrier for future progress in evolutionary developmental biology (evo-devo). In particular, a more thorough understanding of seed plant character evolution and of its genetic and developmental basis has been hampered in part by a lack of gymnosperm model systems, since most are trees with decades-long generation times. Guided by the premise that future model organisms should be selected based on their character diversity, rather than simply phylogenetic "position," we highlight biological questions of potential interest that can be addressed via comparative studies in *Ephedra* (Gnetales). In addition to having relatively small genomes and shorter generation times in comparison to most other gymnosperms, *Ephedra* are amenable to investigations on the evolution of the key reproductive seed plant innovations of pollination and seed dispersal, as well as on polyploidy, and adaptation to extreme environments.

KEYWORDS
fleshiness, gnetales, model system, pollination, polyploidy, seed dispersal

1 | INTRODUCTION

Plant biology has benefited greatly from in-depth investigations in a few predominant model systems, namely, the small and weedy mustard *Arabidopsis thaliana*. Candidate genes emerging from single model systems have fueled the investigation of plant development across other plants in an evolutionary context (Di Stilio et al., 2017). However, having to rely on a small number of model organisms presumed to represent whole lineages has been a limitation for the discipline of evolutionary developmental biology (evo-devo), underscoring the need for comparative studies in organisms chosen based on their character diversity, in addition to phylogenetic provenance (Jenner, 2006). Gymnosperms are particularly notorious for their paucity of model systems, the high interest in their evolution as the sister group to angiosperms having been described as diametrically opposed to their amenability as genetic systems (Wang et al., 2010). Here, we argue that *Ephedra* (Gnetales) is a prime gymnosperm lineage for comparative studies and point out the multiple reasons why we think it has potential as the next evo-devo model system.

The decline of the gymnosperms and rise of angiosperms is one of the most spectacular biotic turnovers to have affected terrestrial ecosystems. Gymnosperms are relatives of flowering plants that also have seeds yet lack flowers; the two clades jointly constitute the seed plants. In addition to the challenges posed by the majority

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of seed plants having gone extinct (Hilton & Bateman, 2006), our understanding of seed plant character evolution has been compounded by a lack of gymnosperm model systems among the extant taxa, since most are trees with decades-long generation times.

Conifers have managed to maintain a significant presence in modern ecosystems (e.g., boreal forests), including important timber trees and some of the most impressive terrestrial organisms such as the tallest tree (California Coastal Redwood, New Zealand Kauri) and the oldest living trees (Ancient Bristlecone pine). Due to their economic importance in silviculture, and in spite of their highly repetitive giga-genomes (20–30 giga base pairs [Gb]), the first gymnosperm reference genomes were in fact from conifer trees (Figure 1a), including spruces, pines and fir (De La Torre et al., 2014 and references therein; Neale et al., 2017; Stevens et al., 2016). Even though transgenic approaches are complicated by long generation times, promising avenues for functional studies in conifers include somatic embryogenesis, and the development of gene editing technologies (see Uddenberg et al., 2015 for a full discussion of conifer evo-devo model systems).

Within the other three major groups of extant gymnosperms, a sequenced draft genome is available for the monotypic tree *Ginkgo biloba* (Guan et al., 2016), while transcriptomic resources are available for cycads (Wickett et al., 2014). Gnetales include a reference genome for *Gnetum montanum* (Wan et al., 2018), and transcriptomic resources for *Welwitschia mirabilis* and *Ephedra sinica* (1 KP project, Wickett et al., 2014).

The gymnosperm order Gnetales (Kubitzki, 1990) is an unusual clade consisting of three disparate genera, *Ephedra*, *Gnetum* and *Welwitschia*, with a controversial

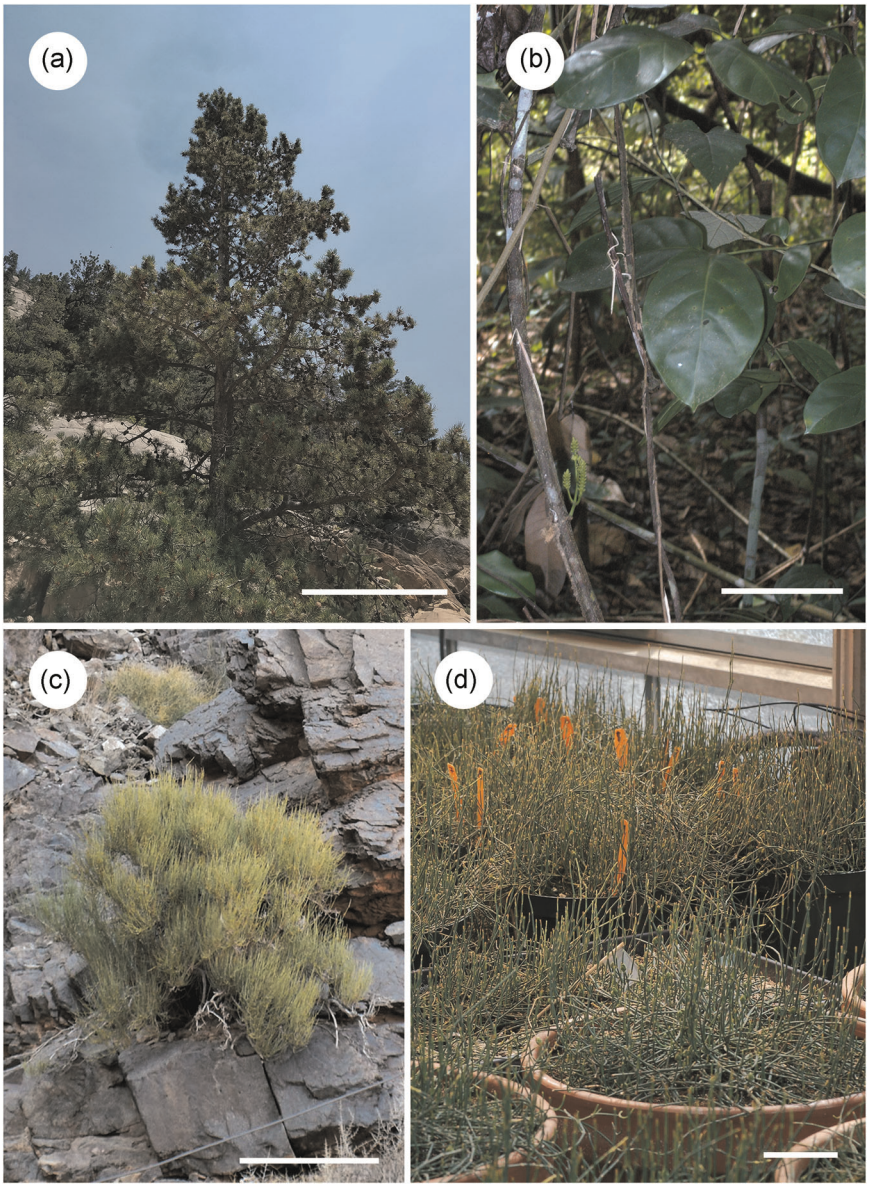

FIGURE 1 General plant habit of existing and proposed gymnosperm model systems. (a) *Pinus flexilis* (conifer, tree), (b) *Gnetum cuspidatum* (Gnetales, vine, photograph by J. Wen); (c) *Ephedra chilensis* (Gnetales, shrub); (d) *Ephedra monosperma* (semi-herbaceous shrub, University of California, Davis, *Ephedra* "farm," photograph by S. Little). Scale bars: a = 3 m, b = 3 cm, c = 75 cm, d = 5 cm [Color figure can be viewed at wileyonlinelibrary.com]

history (Mathews, 2009). Phylogenetic evidence supports Gnetales originating within conifers, and sister to Pinaceae (Leebens-Mack et al., 2019 and references therein; Ran et al., 2018). *Gnetum* (Gnetales, Figure 1b), consisting of tropical evergreen trees, shrubs and vines, has proven to be an informative gymnosperm model to investigate the evolution of flower development. *Gnetum* contributed to the reconstruction of a pre-angiosperm origin of the floral quartet model (Theißen & Saedler, 2001) in which MADS proteins initially interacted as homodimers before acquiring the ability to heterodimerize in the angiosperms, to regulate downstream events leading to reproductive development of cones or flowers, (Ruelens et al., 2017; Theißen et al., 2016, and references therein). Beyond its valuable contribution to developmental genetics of flower evolution, *Gnetum*'s limited distribution in tropical forests and peculiar habit (as an understory liana) highlights the need for additional, complementary gymnosperm model systems.

Ephedra (Ephedraceae, Figure 1c,d) is sister to the other two genera and comprises 54 species of shrubs, subshrubs or climbers, rarely small trees, with widespread distribution in arid and semi-arid regions worldwide (Ickert-Bond & Renner, 2016 and references therein). Members of the South American clade in particular, such as *Ephedra tweediana* and *Ephedra triandra*, are small diploid vine-like shrubs that trail on the ground or clamber over bushes or trees, with life cycles of 2–4 years that can potentially be shortened further by in vitro propagation and cloning from stolons (Lodha et al., 2014). *Ephedra monosperma* has been successfully cultivated at large scale for the production and investigation of its pollination droplets (Nepi et al., 2017, listed as *E. minuta*; Little et al., 2012; Figure 1d). *Ephedra* have genomes half the size of *Pinus* and diploids have the next smallest genome for a gymnosperm (8–9 Gb; Ickert-Bond et al., 2020), after *Gnetum* (2–4 Gb, Wan et al., 2018). *Ephedra* comes with a strong phylogenetic and ecological framework, unusually abundant polyploidization events for a gymnosperm (Ickert-Bond et al., 2020), and a rich diversity of pollination systems (Bolinder et al., 2016) and seed dispersal strategies tied to its evolutionary history (Fuster & Traveset, 2019; Hollander et al., 2010; Loera et al., 2015). Nevertheless, uncovering genes that underlie key innovations in this lineage will also require a strong genomic toolkit, including a solid reference genome, the ability to grow axenic cultures in the laboratory and a transformation protocol for functional studies, all in a feasible timeframe. Here, we outline promising research directions for *Ephedra*, as well as challenges and future steps to build a genetic toolkit for evo-devo studies.

1.1 | Research directions enabled by *Ephedra*

1.1.1 | Comparative morphology of independently-derived fleshy propagules in gymnosperms

Seed dispersal systems in gymnosperms range from those via wind (anemochory), aided by winged seeds (the wing formed from the ovule integument) in conifers such as Araucariaceae, Pinaceae, and Taxodiaceae, to those with obvious adaptations for animal dispersal (zoochory) in *Gingko*, cycads, yews, podocarps, junipers, and gnetophytes. Moreover, phylogenetic mapping uncovered three distinct types of fleshy propagules that have evolved repeatedly across at least six major gymnosperm clades (Cupressaceae, cycads, *Ginkgo*, Gnetales and Podocarpaceae; Leslie et al., 2017). Fleshy propagules are found in most Gnetales (except *Welwitschia* and one *Gnetum* species) but are not present in its presumed sister group (Pinaceae). Even though *Ginkgo* and all cycads have fleshy seeds, it is unclear whether fleshy propagules are ancestral for gymnosperms, partly because the majority of seed plants have gone extinct.

Fleshiness in gymnosperm reproductive structures associated with animal dispersal is an interesting aspect of their reproductive biology that appears to result from convergent evolution in the development of distinct organ types surrounding the seed (Figure 2). The fleshy envelope surrounding *Gnetum* ovules develops from bracts (modified leaves) at its base (Figure 2a), while a thin "sarcotesta" (outer layer of the seed coat) with a pungent smell is found in *Ginkgo* (Figure 2b). Fleshy structures in plum yews (Cephalotaxaceae, Figure 2c) develop on seed cone bracts, just like conifers, except that the bracts grow outward into a fleshy seed covering. Cycads are characterized by large seeds with a fleshy sarcotesta (e.g., in *Macrozamia*, Figure 2d), while podocarps have either fleshy bracts, or a single seed surrounded by a fleshy aril (Figure 2e). In yews (Taxaceae, Figure 2f), the fleshy structure surrounding the seed is interpreted as either a novel outgrowth at the base of the ovule (an aril) or a fleshy seed coat derived from the integument (a sarcotesta; Dörken et al., 2019). Young seed cones of conifers such as *Larix* (Pinaceae) are compound, with ovules found on ovuliferous scales subtended by red or purple seed cone bracts during the pollination phase (Figure 2g).

Fleshy bracts characterize the seed cones of 38 species of *Ephedra* dispersed by birds and lizards (Figures 3a, 3c, 3e,f, and 3h-j; Fuster & Traveset, 2019; Hollander et al., 2010; Loera et al., 2015). A few North American endemics have membranous or coriaceous

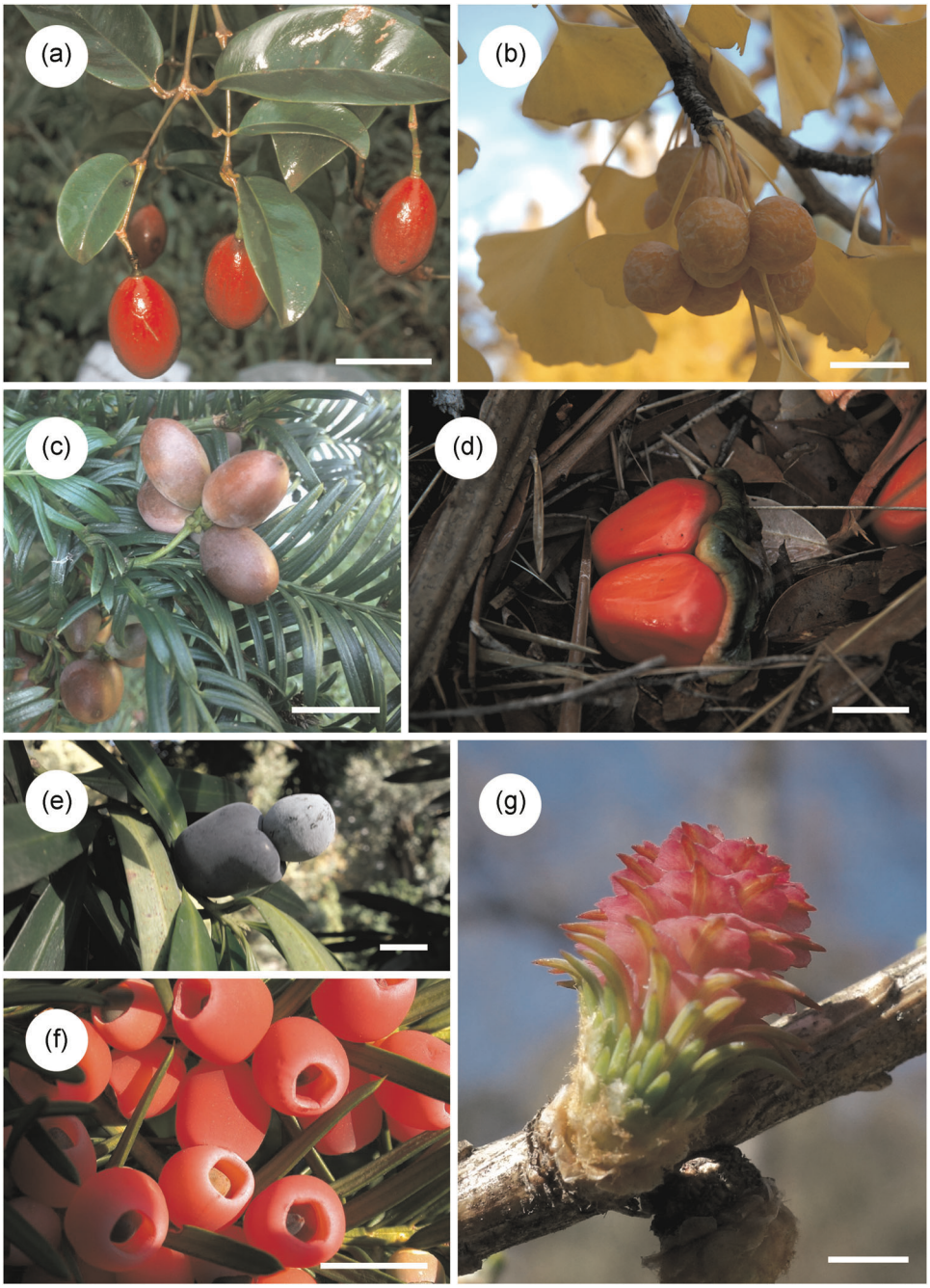

FIGURE 2 Examples of gymnosperm fleshy and/or colored reproductive structures. (a) *Gnetum urens*, fleshy, bright red seeds (from bracts surrounding the ovule). b) *Ginkgo biloba*, fleshy seeds (outer seed coat layer, or sarcotesta). (c) *Cephalotaxus sinensis*, fleshy seeds (seed cone bracts, outward-growing). (d) *Macrozamia fraseri*, fleshy bright red seeds (sarcotesta). (e) *Podocarpus neriifolius*, fleshy purple cone (aril). (f) *Taxus baccata*, fleshy red cone (aril or sarcotesta). (g) *Larix decidua*, bright red bracts of young seed cone at the pollination stage (photograph by N. Friedman, Arnold Arboretum). Photographs courtesy of D. Stevenson, except where otherwise indicated. Scale bars a = 40 mm, b–d = 20 mm, e and f = 10 mm, g = 5 mm [Color figure can be viewed at wileyonlinelibrary.com]

bracts lacking wings and are dispersed by seed-caching rodents (Figures 3b and 3g; Hollander et al., 2010), and fewer species have dry, winged bracts (Figure 3d) and are anemochorous (Stapf, 1889; Figure 4). Phylogenetic reconstructions suggest that seed cones with fleshy bracts are ancestral within *Ephedra*, while those with dry bracts have evolved multiple times (Ickert-Bond & Wojciechowski, 2004; Loera et al., 2015). *Ephedra* bracts are yellow to green at the pollination stage (Figure 3a), and species with future dry or fleshy bracts do not appear to differ significantly at this stage (Rydin et al., 2010; Figure 4). Certain species, like *Ephedra nevadensis*

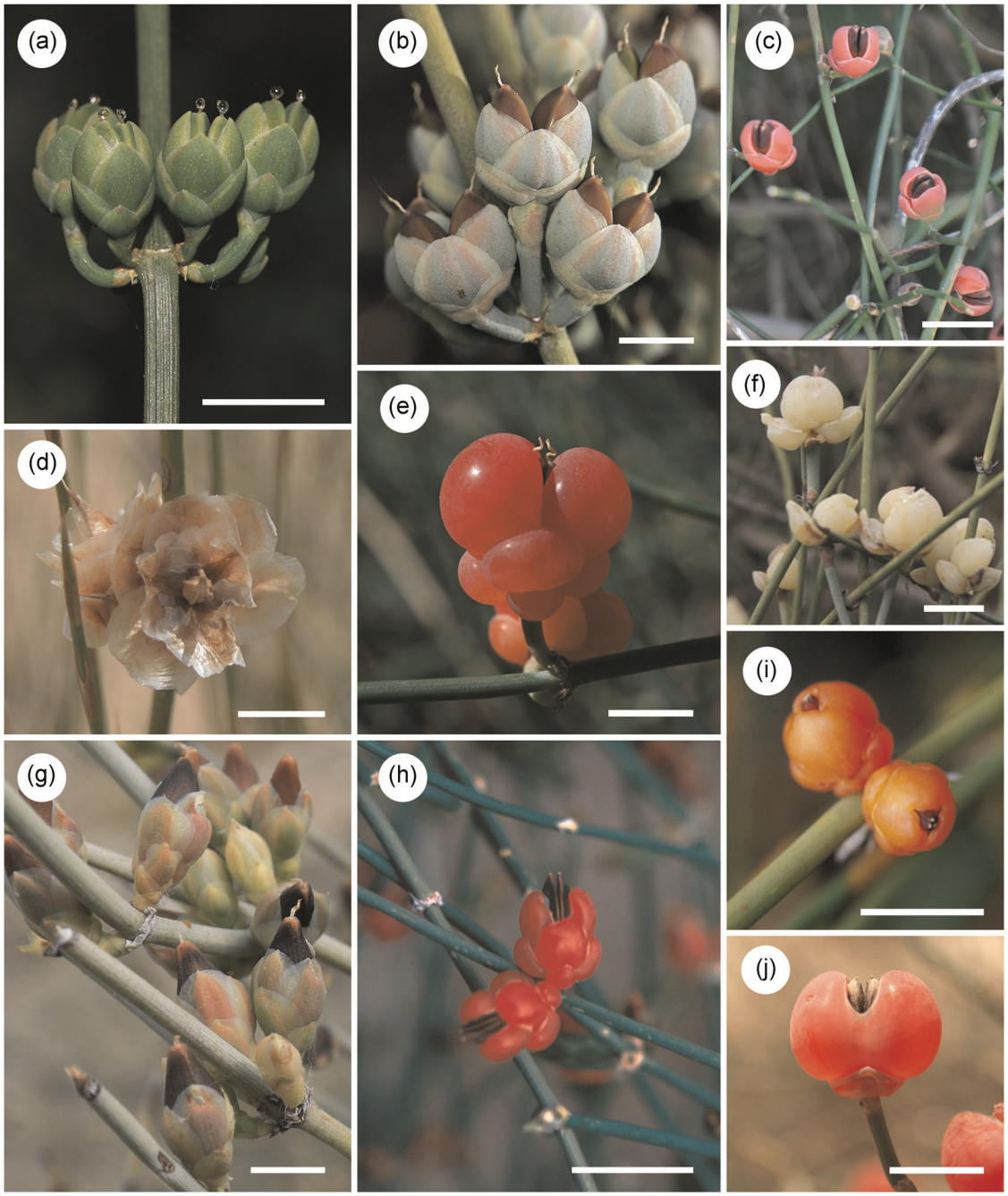

FIGURE 3 *Ephedra* seed cone diversity. (a) *Ephedra tweediana* green bracts at pollination stage, note pollination droplets at micropylar extensions (photograph by M. Bonifacino). (b) *Ephedra nevadensis* with coriaceous bracts (photograph by S. Matson). (c) *E. tweediana* with fleshy bracts. (d) *Ephedra trifurca* with dry winged bracts. (e) *Ephedra distachya* ssp. *helvetica* showing fleshy red bracts at maturity (photograph by E. Zippel). (f) *Ephedra coryi* with white fleshy mature bracts. (g) *Ephedra aspera* with coriaceous bracts. (h) *Ephedra triandra* with red fleshy bracts. (i) *Ephedra ochreata* with orange fleshy bracts. (j) *Ephedra aphylla* with red fleshy bracts (photograph by M. Hassan). Images by the authors, except where indicated. Scale bars: a–d, f–j = 10 mm, e = 5 mm [Color figure can be viewed at wileyonlinelibrary.com]

(Figure 3b), never turn red nor fleshy, in spite of having the developmental hallmarks of a fleshy *Ephedra* (S. Ickert-Bond, *pers. observation*). *Ephedra* is therefore amenable to addressing the evolution of development of fleshiness in gymnosperm seed dispersal structures.

1.1.2 | Evolution of diverse gymnosperm seed cone colors

The pollination syndromes of angiosperms and gymnosperms differ significantly: biotically-pollinated angiosperms

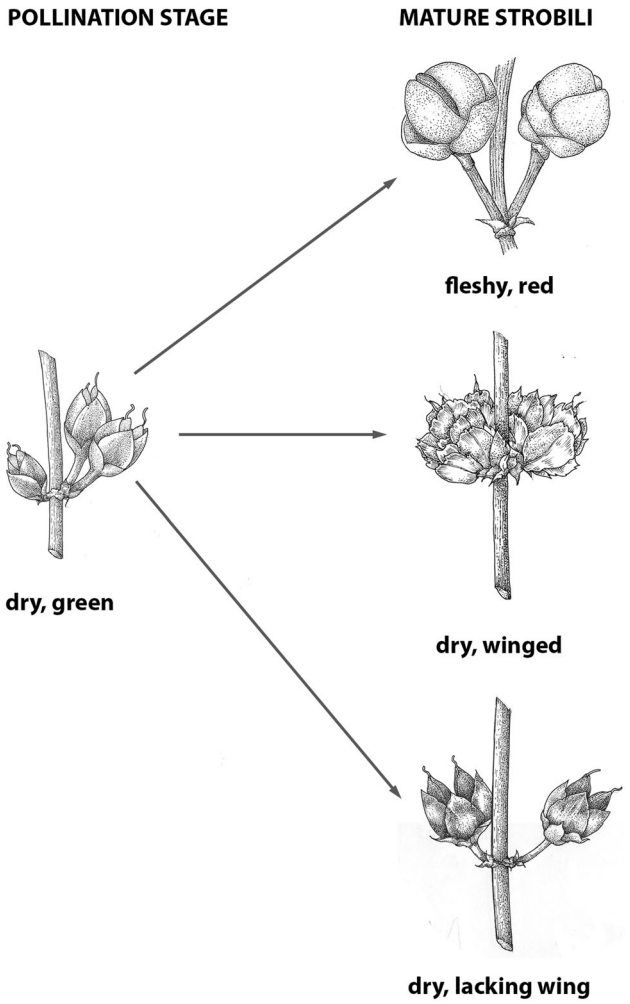

FIGURE 4 Three distinct types of mature *Ephedra* seed cones associated with different dispersal syndromes develop from similar, nonfleshy bracts at the pollination stage. Fleshy red bracts in *E. tweediana* are dispersed by birds, dry winged bracts in *Ephedra torreyana* are wind-dispersed, and dry coriaceous bracts lacking a wing found in several North American species (*E. nevadensis* shown) are dispersed by seed-caching rodents. Illustrations by Elaine Hultgren and Sandra Turico

produce a wide spectrum of color cues that attract pollinators, while gymnosperms are mostly wind-pollinated and much less colorful. Yet, a number of conifers produce vibrantly colored seed cones during seed dispersal (Figure 2f), or earlier during pollination (Figure 2g). These colors have been attributed to the presence of high levels of anthocyanin in the juvenile reproductive phase, as in young larch cones for example (Figure 2g), and their presence has been interpreted as either photoprotective, or as an aid in thermoregulation (Rudall, 2020). Given that the two main categories of plant pigments: carotenoids and flavonoids (e.g., anthocyanins), predate the origin of seed plants (Piatkowski et al., 2020), the evolution of flower color for pollinator attraction in angiosperm petals might thus have its origin in the protection of the developing reproductive structures of gymnosperms (Rudall, 2020). Fleshy *Ephedra* seed cones, while green at the pollination stage, range from red to orange and yellow at the seed dispersal stage (Figures 3c, 3e, and 3h-j), and a few species lack pigments altogether (e.g., *Ephedra coryi*, Figure 3f and *Ephedra foliata*). In other species, bract color is polymorphic such that white and red bracts are known from the same species (e.g., *Ephedra americana*, *Ephedra chilensis*, and *Ephedra frustillata*). Understanding how these pigmentation differences are achieved at the cellular, biochemical and genetic levels constitutes another promising research avenue, analogous to the rich field of angiosperm flower and fruit pigmentation. Different pigmentation patterns likely contribute to varying degrees of "attractiveness" to seed dispersers, resulting in different seed dispersal syndromes. Hence, *Ephedra* presents a unique opportunity to compare and contrast the developmental and evolutionary mechanisms underlying the evolution of color asoociated with increased reproductive success.

1.1.3 | Evo-devo of convergent fruit-like function in seed plants

Even though fruits are an angiosperm innovation, gymnosperms have analogous structures that function in seed dispersal, becoming fleshy and brightly colored (Figure 2). Since the genetic basis of fleshy angiosperm fruits is well characterized in crops such as tomatoes (The Tomato Genome Consortium et al., 2012), gymnosperm fleshy seed cones like those found in *Ephedra* make it possible to investigate whether orthologs of such angiosperm candidate genes might have been repurposed from analogous structures in a seed plant ancestor. More generally, seed plant-specific gene lineages involved in carpel development and their orthologs constitute a potential source of candidate genes (Pfannebecker et al., 2017). Similar gene functions could have been independently recruited in fruit-like structures across seed plants, and perhaps certain aspects of the developmental pathway were assembled from preexisting combinations of interacting genes predating the divergence of flowering plants and the clade containing conifers and Gnetales. Thus, gymnosperms can be informative about angiosperm reproductive innovations, not by representing the ancestral state, but rather by allowing the inference of ancestral states and the investigation of different hypotheses on fruit evolution and development.

Orthologs of floral MADS box genes are usual suspects in the development of fruit-like structures because they are involved in reproductive organ identity in both gymnosperms and angiosperms (Wellmer et al., 2014). In

the gymnosperms *Ginkgo* and *Taxus*, floral MADS box gene orthologs appear to have been recruited independently multiple times in the convergent evolution of fleshy structures (Lovisetto et al., 2012; Lovisetto et al., 2015). However, nuances occur depending on the developmental origin of the fleshy structure: for example, gymnosperm-specific "B-sister" genes play a role in *Ginkgo*, where fleshiness arises from the ovule integument, but not in yews, where it develops from the ovule's stalk (Lovisetto et al., 2013). *Ephedra* offers a fresh perspective on this topic, since its fleshy seed cones have a different developmental origin from the ones studied so far: from modified leaves (bracts), rather than from ovule-derived structures. Another interesting feature of this lineage is the combined presence of papillate cells on the bract epidermis, known from other plants to be under the control of the MYB transcription factor *MIXTA* (Di Stilio et al., 2009; Noda et al., 1994), and of stomata that are typically suppressed in the presence of conical cells (Rudall, 2020). More generally, *Ephedra* provides an opportunity to investigate whether orthologs of genes known to be involved in angiosperm fleshy fruits play a role in extant gymnosperms, and potentially had a gymnosperm precursor that was repurposed from analogous structures. Thus, *Ephedra* enables investigations into the evolution of development of fleshy seed dispersal structures in the seed plants.

1.1.4 | Rampant polyploidy without genome downsizing

Ancient as well as recent polyploidization events are known to impact angiosperm diversification (Van de Peer et al., 2017 and references therein), yet they appear to be rarer in gymnosperms. Ancient whole genome duplications have been found in conifers, *Ginkgo* and cycads, but not in gnetophytes (Wan et al., 2018 and references therein). The unusually high incidence of recent polyploidy events in *Ephedra* (Ickert-Bond et al., 2020; Wu et al., 2016) provides a unique opportunity to increase understanding of genome dynamics and rates of molecular evolution in gymnosperms, generally considered more conserved compared to angiosperms (De La Torre et al., 2017). The near-absence of genome downsizing following polyploidy in *Ephedra* is associated with the observation that genome size and chromosome number are strongly and positively correlated (Figure 5)—a correlation mostly lacking across other seed plants (Barker, 2013). Multiple lines of evidence, including cytological (i.e., chromosome size and structure), genome size stability (Hizume & Tominaga, 2016; Renner & Sousa dos Santos, 2019) and unbiased subgenome evolution of *Ephedra* polyploids (Wu et al., 2020), all point to a slower rate of diploidization than reported in most angiosperms (reviewed in Van de Peer et al., 2017). Characters that likely increase polyploid formation and survival in *Ephedra* include a relatively rapid generation time (compared to other gymnosperms, Figure 5), a higher rate of unreduced gamete formation (Leitch & Leitch, 2012; Ickert-Bond et al., 2003 and references therein), which is a main route to polyploid formation in angiosperms, and the ability to propagate vegetatively (Stapf, 1889). On the one hand, additional genome copies might promote higher tolerance to environmental conditions due to the higher gene content enabling neofunctionalization of duplicated genes, and higher heterozygosity (Roddy et al., 2020). On the other hand, the large genome hypothesis posits that large genomes may have reduced photosynthetic rates and restricted ecological distributions, due to the negative effects of genome size on metabolic efficiency (Knight, 2005). Whether or not genomic constraint has influenced speciation in *Ephedra* is an interesting avenue for future studies.

1.1.5 | Adaptation to extreme environments: attractiveness in the Anthropocene under a changing climate scenario

Ephedra is unique among gymnosperms in occupying a wide range of habitats from deserts to subtropical thorn scrub, and from below sea-level (in Death Valley, California [USA] or the Dead Sea area) to high elevation, where winter freezing is extreme (above 5000 m in the high Andes and Himalayas, Ickert-Bond & Renner, 2016).

The vasculature of dry habitat plants consists of narrow vessels (xylem) that likely provide conductive assurance by reducing embolisms (Carlquist, 2012), while species from extreme alpine arid environments are near-vesselless (Motomura et al., 2007). In fact, diversification in North and South American *Ephedra* is associated with the expansion of arid habitats since the Oligocene-Miocene (Loera et al., 2012), with speciation in South America associated with the uplift of the Andes between 10 and 2 mya (Loera et al., 2015). The availability of empty environmental niches after periods of extreme climate change would have selected for generalists with high dispersal ability to adapt to very specific environmental conditions, thus increasing the number of polyploids (Fawcett & Van de Peer, 2010). Hence, *Ephedra* is well positioned to investigate the genetic basis of adaptation to extreme environments (arid, hyperarid and high altitude), of special relevance in the face of global warming and increased desertification.

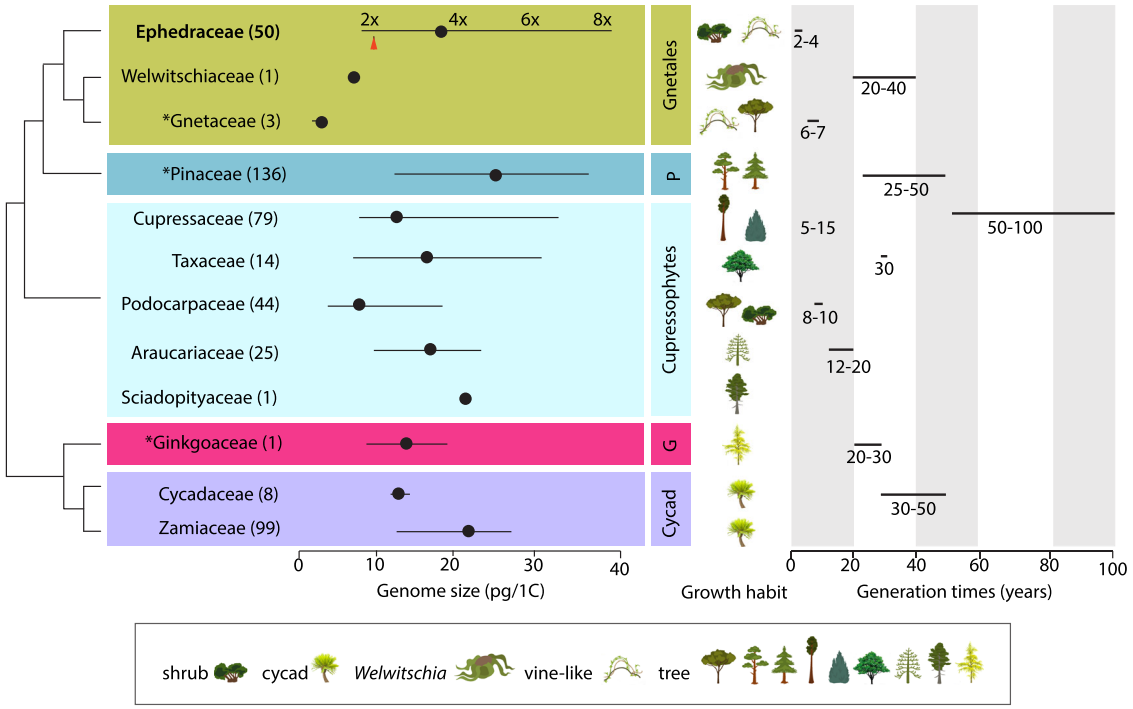

FIGURE 5 Genome size, growth habit, and generation times of gymnosperms. Phylogenetic relationships are shown schematically on the left (after Leebens-Mack et al., 2019). Mean (●) and range of genome sizes (1C-values) in each of the 12 accepted families (after Christenhusz et al., 2011), asterisks denote lineages with a sequenced reference genome. The number of taxa for which genome estimation values are known is shown in brackets. Observed 1C-values at each ploidy level (2x–8x) in 50 species of *Ephedra* show that diploid South American species (*Ephedra triandra* and *E. tweediana*) fall within the smaller end of the range (red arrowhead). Growth habit is represented with icons, and generation times are shown as approximate ranges (in years), where available (Burns & Honkala, 1965; Dallimore & Jackson, 1967; Di Salvatore et al., 2013; Wang et al., 2015; Whitmore, 1977 and references therein). Figure adapted from Ickert-Bond et al. (2020) [Color figure can be viewed at wileyonlinelibrary.com]

1.1.6 | Remaining challenges and future directions

A reference genome for *Ephedra* would be greatly beneficial in the search for candidate genes, to that end we propose sequencing one of the fleshy-bracted diploid South American species with smallest genomes, such as *Ephedra ochreata* (8.27 Gb, smallest genome in the New World), *E. tweediana* (8.7 Gb) or *E. triandra* (9.5 Gb), the latter two having the added advantage of being smaller shrubs or climbers with less secondary growth (Figure 5). Amongst the Asian species, *Ephedra regeliana* is a small, diploid subshrub with potential as a model species (8–15 cm, 7.91 Gb, Ickert-Bond et al., 2020). Even though the smallest of *Ephedra* genomes are still fairly massive, they can be tackled with long-read sequencing technologies that are more likely to span long repeat regions and produce a more continuous assembly, including Pacific Biosciences' (PacBio) single-molecule real-time sequencing and Oxford Nanopore Technologies' nanopore sequencing (reviewed in Amarasinghe et al., 2020). Another important aspect of the model system toolkit is the ability to develop transgenic approaches to investigate gene function. An approach that takes *Ephedra* genes and expresses them in a model plant such as *Arabidopsis* (to elicit either rescue or ectopic phenotypes) can be partially informative as a first instance. However, the heterologous approach presents difficulties in the interpretation of negative results (do we attribute the lack of performance to actual change in gene function, to phylogenetic distance, or to a different genomic environment?). The next step towards enabling functional studies may include transient transgenesis via virus induced gene silencing (Dinesh-Kumar et al., 2003), known to work in a variety of plant systems (Di Stilio, 2011) and/or gene editing techniques combined with somatic embryogenesis, as implemented in conifer trees (reviewed in Uddenberg et al., 2015). In the meantime, gene expression analyses, including *de novo* assembly of additional reference transcriptomes and RNAseq analysis, combined with validation of gene expression via in situ hybridization and real time quantitative PCR, should help jump-start the process of gene discovery. A more comprehensive genetic toolkit will further enable the

investigation of the genetic basis of developmental innovations in this fascinating gymnosperm lineage.

2 | CONCLUSIONS

In conclusion, we argue that additional gymnosperm model systems are indispensable to further explore the overarching phenomenon of repurposing of genes in the evolution of developmental pathways leading to increased biodiversity, in particular pertaining to seed plants. Diploid *Ephedra* (Gnetales) are well-positioned to fulfill this role, given their relatively smaller genomes and shorter generation times, and their vine-like habit for ease of cultivation. Moreover, *Ephedra* is an informative lineage for basic biological questions such as the effect of polyploidy on adaptation to extreme environments and the evolution of the key reproductive seed plant innovations of pollination and seed dispersal.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

Amarasinghe, S. L., Su, S., Dong, X., Dong, X., Zappia, L., Ritchie, M. E., & Gouil, Q. (2020). Opportunities and challenges in long-read sequencing data analysis. *Genome Biology*, *21*, 30.

Barker, M. (2013). Karyotype and genome evolution in pteridophytes. In I. J. Leitch, J. Greilhuber, J. Doležel & J. F. Wendel (Eds.), *Plant Genome Diversity: Physical Structure, Behaviour and Evolution of Plant Genomes* (Vol. 2, pp. 245–253). Springer-Verlag.

Bolinder, K., Humphreys, A. M., Ehrlen, J., Alexandersson, R., Ickert-Bond, S. M., & Rydin, C. (2016). From near extinction to diversification by means of ashift in pollination mechanism in the gymnosperm relict *Ephedra* (Ephedraceae, Gnetales). *Botanical Journal of the Linnean Society*, *180*, 461–477.

Burns, R. M., & Honkala, B. H. (1965). Silvics of North America Volume 1, Conifers. Technical Coordinators Timber Management Research Agriculture Handbook 654. USDA.

Carlquist, S. (2012). Wood anatomy of Gnetales in a functional, ecological, and evolutionary context. *Aliso*, *30*, 33–47.

Christenhusz, M. J. M., Reveal, J. L., Farjon, A., Gardner, M. F., Mill, R. R., & Chase, M. W. (2011). A new classification and linear sequence of extant gymnosperms. *Phytotaxa*, *19*, 55–70.

Dallimore, W., & Jackson, A. B. (1967). *A Handbook of Coniferae and Ginkgoaceae*: Ed. 4 St. Martin's Press.

Dinesh-Kumar, S., Anandalakshmi, R., Marathe, R., Schiff, M., & Liu, Y. (2003). Virus induced gene silencing. *Methods in Molecular Biology*, *236*, 287–294.

De La Torre, A. R., Birol, I., Bousquet, J., Ingvarsson, P. K., Jansson, S., Jones, S. J. M., Keeling, C. I., MacKay, J., Nilsson, O., Ritland, K., Street, N., Yanchuk, A., Zerbe, P., & Bohlmann, J. (2014). Insights into conifer giga-genomes. *Plant Physiology*, *166*, 1724–1732.

De La Torre, A. R., Li, Z., Van de Peer, Y., & Ingvarsson, P. K. (2017). Contrasting rates of molecular evolution and patterns of selection among gymnosperms and flowering plants. *Molecular Biology and Evolution*, *34*, 1363–1377.

Di Salvatore, M., Carafa, A. M., & Carratù, G. (2013). Growth and reproductive phenology of Welwitschia mirabilis Hook.f. *The Open Plant Science Journal*, *7*, 39–46.

Di Stilio, V. S. (2011). Empowering plant evo-devo: Virus induced gene silencing validates new and emerging model systems. *BioEssays*, *33*, 711–718.

Di Stilio, V. S., Martin, C., Schulfer, A. F., & Connelly, C. F. (2009). An ortholog of *MIXTA-like2* controls epidermal cell shape in flowers of *Thalictrum*. *New Phytologist*, *183*, 718–728.

Di Stilio, V. S., Melzer, R., & Hall, J. C. (2017). A broader view for plant EvoDevo: Novel approaches for diverse model systems. *Frontiers in Plant Science*, *8*, 61. https://doi.org/10.3389/fpls.2017.00061

Dörken, V. M., Nimsch, H., & Rudall, P. J. (2019). Origin of the Taxaceae aril: Evolutionary implications of seed-cone teratologies in *Pseudotaxus chienii*. *Annals of Botany*, *123*, 133–143.

Fawcett, J. A., & Van de Peer, Y. (2010). Angiosperm polyploids and their road to evolutionary success. *Trends in Evolutionary Biology*, *2*, 3.

Fuster, F., & Traveset, A. (2019). Evidence for a double mutualistic interaction between a lizard and a Mediterranean gymnosperm, *Ephedra fragilis*. *AoB Plants*, *11*(1), plz001.

Guan, R., Zhao, Y., Zhang, H., Fan, G., Liu, X., Zhou, W., Shi, C., Wang, J., Liu, W., Liang, X., Fu, Y., Ma, K., Zhao, L., Zhang, F., Lu, Z., Lee, S. M. Y., Xu, X., Wang, J., Yang, H., ... Chen, W. (2016). Draft genome of the living fossil *Ginkgo biloba*. *GigaScience*, *5*, 49.

Hilton, J., & Bateman, R. M. (2006). Pteridosperms are the backbone of seed-plant phylogeny. *The Journal of the Torrey Botanical Society*, *133*(1), 119–168.

Hizume, M., & Tominaga, K. (2016). Fluorescent band pattern of chromosomes in *Ephedra americana* var. *andina*, Ephedraceae. *Chromosome Botany*, *11*, 27–30.

Hollander, J. L., Vander Wall, S. B., & Baguley, J. G. (2010). Evolution of seed dispersal in North American *Ephedra*. *Evolutionary Ecology*, *24*, 333–345.

Ickert-Bond, S. M., & Renner, S. S. (2016). The Gnetales: Recent insights on their morphology, reproductive biology, chromosome numbers, biogeography, and divergence times. *Journal of Systematics and Evolution*, *54*, 1–16.

Ickert-Bond, S. M., Skvarla, J. J., & Chissoe, W. F. (2003). Pollen dimorphism in *Ephedra* L. (Ephedraceae). *Review of Palaeobotany and Palynology*, *124*, 325–334.

Ickert-Bond, S. M., Sousa, A., Min, Y., Loera, I., Metzgar, J., Pellicer, J., Hidalgo, O., & Leitch, I. J. (2020). Polyploidy in gymnosperms—Insights into the genomic and evolutionary consequences of polyploidy in *Ephedra*. *Molecular Phylogenetics and Evolution*, *147*, 106786

Ickert-Bond, S. M., & Wojciechowski, M. F. (2004). Phylogenetic relationships in Ephedra (Gnetales): Evidence from nuclear and chloroplast DNA sequence data. Systematic Botany 29(4): 834–849.

Jenner, R. A. (2006). Unburdening evo-devo: Ancestral attractions, model organisms, and basal baloney. *Development Genes and Evolution*, *216*, 385–394.

Knight, C. A. (2005). The large genome constraint hypothesis: Evolution, ecology and phenotype. *Annals of Botany*, *95*, 177–190.

Kubitzki, K. (1990). Gnetatae. In K. U. Kramer & P. S. Green (Eds.), *The families and genera of vascular plants* (Vol. 1, pp. 378–391). Springer Verlag.

Leebens-Mack, J. H., Barker, M. S., Carpenter, E. J., Deyholos, M. K., Gitzendanner, M. A., Graham, S. W., Grosse, I., Li, Z., Melkonian, M., Mirarab, S., Porsch, M., Quint, M., Rensing, S. A., Soltis, D. E., Soltis, P. S., Stevenson, D. W., Ullrich, K. K., Wickett, N. J., DeGironimo, L., ... Wong, G. K.-S. (2019). One thousand plant transcriptomes and the phylogenomics of green plants. *Nature*, *574*, 679–685.

Leitch, A. R., & Leitch, I. J. (2012). Ecological and genetic factors linked to contrasting genome dynamics in seed plants: Tansley review. *New Phytologist*, *194*, 629–646.

Leslie, A. B., Beaulieu, J. M., and Mathews, S. 2017. Variation in seed size is structured by dispersal syndrome and cone morphology in conifers and other nonflowering seed plants. *New Phytologist*, 216: 429–437

Little, S., Groot, E., Christianson, M., Jernstedt, J: *Ephedra monosperma*, a potential model organism for studies of development and evolution in Gnetales and gymnosperms. Paper presented at the Annual Meeting of the Botanical Society of America (Botany 2012), Columbus, OH, 7-11 June 2012. http://2012.botanyconference.org/engine/search/index.php?func=detail%26aid=959

Lodha, D., Rathore, N., Kataria, V., & Shekhawat, N. S. (2014). In vitro propagation of female *Ephedra foliata* Boiss. & Kotschy ex Boiss.: An endemic and threatened gymnosperm of the Thar Desert. *Physiology and Molecular Biology of Plants*, *20*, 375–383.

Loera, I., Ickert-Bond, S. M., & Sosa, V. (2015). Ecological consequences of contrasting dispersal syndromes in New World Ephedra: Higher rates of niche evolution related to dispersal ability. *Ecography*, *38*, 1187–1199.

Loera, I., Sosa, V., & Ickert-Bond, S. M. (2012). Diversification in North American arid lands: Niche conservatism, divergence and expansion of habitat explain speciation in the genus *Ephedra*. *Molecular Phylogenetics and Evolution*, *65*, 437–450.

Lovisetto, A., Baldan, B., Pavanello, A., & Casadoro, G. (2015). Characterization of an AGAMOUS gene expressed throughout development of the fleshy fruit-like structure produced by *Ginkgo biloba* around its seeds. *BMC Evolutionary Biology*, *15*, 139.

Lovisetto, A., Guzzo, F., Busatto, N., & Casadoro, G. (2013). Gymnosperm B-sister genes may be involved in ovule/seed development and, in some species, in the growth of fleshy fruit-like structures. *Annals of Botany*, *112*, 535–544.

Lovisetto, A., Guzzo, F., Tadiello, A., Toffali, K., Favretto, A., & Casadoro, G. (2012). Molecular analyses of MADS-box genes trace back to gymnosperms the invention of fleshy fruits. *Molecular Biology and Evolution*, *29*, 409–419.

Mathews, S. (2009). Phylogenetics relationships among seed plants: Persistent questions and the limits of molecular data. *American Journal of Botany*, *96*, 228–236.

Motomura, H., Noshiro, S., & Mikage, M. (2007). Variable wood formation and adaptation to the alpine environment of *Ephedra pachyclada* (Gnetales: Ephedraceae) in the Mustang District, western Nepal. *Annals of Botany*, *100*, 315–324.

Neale, D. B., McGuire, P. E., Wheeler, N. C., Stevens, K. A., Crepeau, M. W., Cardeno, C., Zimin, A. V., Puiu, D., Pertea, G. M., Sezen, U. U., Casola, C., Koralewski, T. E., Paul, R., Gonzalez-Ibeas, D., Zaman, S., Cronn, R., Yandell, M., Holt, C., Langley, C. H., ... Wegrzyn, J. L. (2017). The Douglas-Fir genome sequence reveals specialization of the photosynthetic apparatus in Pinaceae. *G3: Genes, Genomes, Genetics*, *7*, 3157–3167.

Nepi, M., Little, S., Guarnieri, M., Nocentini, D., Prior, N., Gill, J., Barry Tomlinson, P., Ickert-Bond, S. M., Pirone, C., Pacini, E., & von Aderkas, P. (2017). Phylogenetic and functional signals in gymnosperm ovular secretions. *Annals of Botany*, *120*, 923–936.

Noda, K., Glover, B. J., Linstead, P., & Martin, C. (1994). Flower color intensity depends on specialized cell-shape controlled by a Myb-related transcription factor. *Nature*, *369*, 661–664.

Pfannebecker, K. C., Lange, M., Rupp, O., & Becker, A. (2017). Seed plant-specific gene lineages involved in carpel development. *Molecular Biology and Evolution*, *34*, 925–942.

Piatkowski, B. T., Imwattana, K., Tripp, E. A., Weston, D. J., Healey, A., Schmutz, J., & Shaw, A. J. (2020). Phylogenomics reveals convergent evolution of red-violet coloration in land plants and the origins of the anthocyanin biosynthetic pathway. *Molecular Phylogenetics and Evolution*, *151*, 106904.

Podocarpus elatus-Useful tropical plants. http://tropical.theferns.info/viewtropical.php?id=Podocarpus+elatus. Accessed December 27, 2020.

Ran, J.-H., Shen, T.-T., Wang, M.-M., & Wang, X.-Q. (2018). Phylogenomics resolves the deep phylogeny of seed plants and indicates partial convergent or homoplastic evolution between

Gnetales and angiosperms. *Proceedings of the Royal Society B*, *285*, 20181012.

Renner, S., & Sousa dos Santos, A. (2019). The organization of nuclear ribosomal DNA in gnetophytes—Physically separate and physically linked arrangements of 35S and 5S genes. A commentary on: 'Remarkable variation of ribosomal DNA organization and copy number in gnetophytes, a distinct lineage of gymnosperms. *Annals Botany*, *123*, vi–vii.

Roddy, A. B., Théroux-Rancourt, G., Abbo, T., Benedetti, J. W., Brodersen, C. R., Castro, M., Castro, S., Gilbride, A. B., Jensen, B., Jiang, G.-F., Perkins, J. A., Perkins, S. D., Loureiro, J., Syed, Z., Thompson, R. A., Kuebbing, S. E., & Simonin, K. A. (2020). The scaling of genome size and cell size limits maximum rates of photosynthesis with implications for ecological strategies. *International Journal of Plant Sciences*, *181*, 75–87.

Rudall, P. J. (2020). Colourful cones: How did flower colour first evolve? *Journal of Experimental Botany*, *71*, 759–767.

Ruelens, P., Zhang, Z., Mourik, H., van, Maere, S., Kaufmann, K., & Geuten, K. (2017). The origin of floral organ identity quartets. *The Plant Cell*, *29*, 229–242.

Rydin, C., Khodabandeh, A., & Endress, P. K. (2010). The female reproductive unit of *Ephedra* (Gnetales): Comparative morphology and evolutionary perspectives. *Botanical Journal of the Linnean Society*, *163*, 387–430.

Stapf, O. (1889). Die Arten der Gattung *Ephedra*. *Denkschrift der Kaiserlichen Akademie der Wissenschaften, Mathematisch-Naturwissenschaftliche Klasse*, *56*, 1–122.

Stevens, K. A., Wegrzyn, J. L., Zimin, A., Puiu, D., Crepeau, M., Cardeno, C., Paul, R., Gonzalez-Ibeas, D., Koriabine, M., Holtz-Morris, A. E., Martínez-García, P. J., Sezen, U. U., Marçais, G., Jermstad, K., McGuire, P. E., Loopstra, C. A., Davis, J. M., Eckert, A., de Jong, P., ... Langley, C. H. (2016). Sequence of the sugar pine megagenome. *Genetics*, *204*, 1613–1626.

The Tomato Genome Consortium, Sato, S., Tabata, S., Hirakawa, H., Asamizu, E., Shirasawa, K., Isobe, S., Kaneko, T., Nakamura, Y., Shibata, D., Aoki, K., Egholm, M., Knight, J., Bogden, R., Li, C., Shuang, Y., Xu, X., Pan, S., Cheng, S., ... York, T. (2012). The tomato genome sequence provides insights into fleshy fruit evolution. *Nature*, *485*, 635–641.

Theißen, G., Melzer, R., & Rümpler, F. (2016). MADS-domain transcription factors and the floral quartet model of flower development: Linking plant development and evolution. *Development*, *143*, 3259–3271.

Theißen, G., & Saedler, H. (2001). Floral quartets. *Nature*, *409*, 469–471.

Uddenberg, D., Akhter, S., Ramachandran, P., Sundström, J. F., & Carlsbecker, A. (2015). Sequenced genomes and rapidly emerging technologies pave the way for conifer evolutionary developmental biology. *Frontiers in Plant Science*, *6*, 970.

Van de Peer, Y., Mizrachi, E., & Marchal, K. (2017). The evolutionary significance of polyploidy. *Nature Reviews Genetics*, *18*, 411–424.

Wan, T., Liu, Z.-M., Li, L.-F., Leitch, A. R., Leitch, I. J., Lohaus, R., Liu, Z.-J., Xin, H.-P., Gong, Y.-B., Liu, Y., Wang, W. C., Chen, L. Y., Yang, Y., Kelly, L. J., Yang, J., Huang, J. L., Li, Z., Liu, P., Zhang, L., ... Wang, X. M. (2018). A genome for gnetophytes and early evolution of seed plants. *Nature Plants*, *4*, 82–89.

Wang, B., Jiang, B., Zhou, Y., Su, Y., & Wang, T. (2015). Higher substitution rates and lower dN/dS for the plastid genes in Gnetales than other gymnosperms. *Biochemical Systematics and Ecology*, *59*, 278–287.

Wang, Y. Q., Melzer, R., & Theißen, G. (2010). Molecular interactions of orthologues of floral homeotic proteins from the gymnosperm Gnetum gnemon provide a clue to the evolutionary origin of 'floral quartets'. *Plant Journal*, *64*, 177–190.

Wellmer, F., Bowman, J. L., Davies, B., Ferrándiz, C., Fletcher, J. C., Franks, R. G., Graciet, E., Gregis, V., Ito, T., Jack, T. P., Jiao, Y., Kater, M. M., Ma, H., Meyerowitz, E. M., Prunet, N., & Riechmann, J. L. (2014). Flower Development: Open Questions and Future Directions. In J. L. Riechmann & F. Wellmer (Eds.), *Flower Development: Methods and Protocols* (pp. 103–124). Springer.

Whitmore, T. (1977). *A first look at* Agathis. Unit of Tropical Silviculture Commonwealth Forestry Institute, University of Oxford.

Wickett, N. J., Mirarab, S., Nguyen, N., Warnow, T., Carpenter, E., Matasci, N., Ayyampalayam, S., Barker, M. S., Burleigh, J. G., Gitzendanner, M. A., Ruhfel, B. R., Wafula, E., Der, J. P., Graham, S. W., Mathews, S., Melkonian, M., Soltis, D. E., Soltis, P. S., Miles, N. W., ... Leebens-Mack, J. (2014). Phylotranscriptomic analysis of the origin and early diversification of land plants. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, E4859–E4868.

Wu, H., Ma, Z., Wang, M.-M., Qin, A.-L., Ran, J.-H., & Wang, X.-Q. (2016). A high frequency of allopolyploid speciation in the gymnospermous genus *Ephedra* and its possible association with some biological and ecological features. *Molecular Ecology*, *25*, 1192–1210.

Wu, H., Yu, Q., Ran, J.-H., & Wang, X.-Q. (2020). Unbiased subgenome evolution in allotetraploid species of Ephedra and its implications for the evolution of large genomes in gymnosperms [published online ahead of print November 16, 2020]. *Genome Biology and Evolution*, evaa236. https://doi.org/10.1093/gbe/evaa236

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