

# Did selection for seed traits across the Cretaceous/Paleogene boundary sort plants based on ploidy?

KEITH BERRY<sup>1\*</sup> and GANESH K. JAGANATHAN<sup>2</sup>

<sup>1</sup>Science Department, Hoehne Re-3 School District, Hoehne, Colorado, 81046, USA;  
e-mail: keith.berry@hoehnesd.org

<sup>2</sup>University of Shanghai for Science and Technology, Shanghai 200093, China; e-mail: jganeshcbe@gmail.com

Received 22 April 2022; accepted for publication 21 December 2022

**ABSTRACT.** Paleobotanists debate whether the Cretaceous/Paleogene boundary (KPB) event was selective. As the hypothesis that the KPB event selected for plants with fast-return leaf economic traits (e.g. deciduousness) has lost empirical support in recent investigations, researchers have turned to alternative hypotheses to explain an abrupt decline in primary productivity across the KPB. Two contemporary hypotheses designed to explain selectivity among plants across the KPB are that (1) polyploids exhibited greater survivorship than their diploid progenitors or counterparts (i.e. the KPB-whole genome duplication or WGD hypothesis) and that (2) plants with desiccation-tolerant (DT), i.e. orthodox, seeds exhibited greater survivorship than plants with desiccation-sensitive (DS), also known as recalcitrant, seeds. Late embryogenesis abundant (LEA) protein gene families are perceived to confer DT and seed longevity among vascular plants. Non-parametric Wilcoxon signed-rank test for matched pairs and a Mann-Whitney U test reveal that plant lineages perceived to have undergone WGD across the KPB exhibit significantly greater numbers of LEA genes than those that did not. On the basis of these data, this investigation elicits a merger between the KPB-WGD and KPB-seed traits concepts. However, emphasis is shifted from the concept of WGD as an immediate adaptation to climatic stress at the KPB (the KPB-WGD hypothesis) to the concept that WGD was an exaptation, which, by definition, fortuitously enhanced the survival of vascular plants across the KPB but that probably evolved initially in other climatic contexts.

**KEYWORDS:** K/Pg boundary, mass extinction, whole genome duplication (WGD), macroevolution, plants, seeds

## INTRODUCTION

Contemporary hypotheses on the cause of the Cretaceous/Paleogene boundary (KPB) mass extinction event emphasize an abrupt loss of primary productivity at the KPB associated with a severe, long-lasting impact winter caused by sulfate and other stratospheric aerosols produced by the Chicxulub asteroid impact in the Yucatán Peninsula (Junium et al., 2022; Morgan et al., 2022). Although previous paleobotanical investigations emphasized a rather mild KPB impact winter associated with selection for deciduousness or

other fast-return leaf economic strategies (Wolfe and Upchurch, 1987; Blonder et al., 2014), this hypothesis lacks critical empirical support in recent investigations (Berry, 2020; Butrim et al., 2022). Long-term recovery of plants is perceived to have been from soil propagule banks, although plants would have initially faced global deforestation coupled with wildfires caused by reentering impact ejecta and a transient period of global darkness associated with stratospheric aerosols (Nichols and Johnson, 2008; Vajda and Berovic, 2014). Accordingly, paleobotanists have shifted their attention to other hypotheses,

\* Corresponding author

such as selection for desiccation-tolerant (DT), i.e. orthodox (*sensu* Roberts, 1973), seeds that are long-lived and form seed banks capable of tolerating frost, drought and other environmental factors or selection for stress-tolerant polyploids across the KPB (Berry, 2020; Van de Peer et al., 2021). Desiccation tolerance is generally viewed as a prerequisite for long-term seed viability, especially in the context of forming seed banks in freezing terrestrial environments (Jaganathan et al., 2015; Long et al., 2015). In turn, DT and seed longevity is conferred through late embryogenesis abundant (LEA) genes (Smolikova et al., 2021; Sharma, 2022). Changes in ploidy level may have resulted in divergent evolutionary trajectories and may have influenced the diversification of surviving clades.

Given that there is considerable taxonomic evidence that plants with DT seeds probably exhibited greater survivorship across the KPB (Berry, 2020), a natural extension of this concept is that plants with a greater number of LEA genes exhibited greater survivorship across the KPB. This hypothesis may explain the apparent correlation between whole genome duplication (WGD) frequency and the KPB, which was first recognized by Fawcett et al. (2009), but which has received relatively little attention (Van de Peer et al., 2021). Up to this point, connections between these concepts have largely been anecdotal, such as recognizing that two of the plant taxa with the longest-lived seeds known (i.e. seed longevity greater than a millennium), *Phoenix dactylifera* L. and *Nelumbo nucifera* Gaertn. (Sano et al., 2016), represent lineages that have undergone WGD at or near the KPB (Vanneste et al., 2014; Lohaus and Van de Peer, 2016). Despite several mentions (e.g. Berry, 2020; Shi et al., 2020), the hypothesis that DT or seed longevity was selected across the KPB in correlation with WGD has not yet been rigorously tested (Van de Peer et al., 2021).

Initially regarded as an evolutionary dead-end during the formulation of the neo-Darwinian Evolutionary or Modern Synthesis (Stebbins, 1950), polyploidy is now known to be a major feature of plant macroevolution (Soltis and Burleigh, 2009; Soltis et al., 2009, 2014). WGD constitutes a relatively high proportion of speciation events within vascular plant lineages (~15–31% of all speciation events; Wood et al., 2009), although the factors contributing to the

long-term success or establishment of polyploid lineages are less well understood (Clark and Donoghue, 2018; Clark et al., 2019). WGD has been viewed as providing either the raw genetic material for evolutionary diversification (Barrett et al., 2019) or the means of surviving major climatic upheavals (Sessa, 2019; Van de Peer et al., 2021). In either of these ways, WGD could be important for surviving or proliferating in the aftermath of global mass extinction events. The adaptive benefits of WGD are threefold: the origination of evolutionary innovation via neofunctionalization, the reduction of pleiotropy via subfunctionalization and the generation of genetic redundancy (Chain et al., 2011). In instances where these genes are maladaptive, duplicate genes are typically lost within a few million years after the WGD event (Lynch and Conery, 2000; Chain et al., 2011). Given the very short-term nature of the impact winter, which lasted decades at most (Chiarenza et al., 2020; Tabor et al., 2020; Morgan et al., 2022), it is conceivable that duplicate genes favoring plant survival and proliferation during this extreme, but short-lived event ultimately would have been lost in the Danian unless they conferred some longer-term advantage, which may extend, perhaps, even to the present day. Therefore, it seems reasonable to infer that such traits may have been exaptations rather than adaptations – i.e. features that had initially evolved in other climatic contexts, but which fortuitously enhanced survival or proliferation of plants across the KPB. In this way, the KPB-seed traits hypothesis can be viewed as conceptually distinct from the KPB-WGD hypothesis, as the latter emphasizes WGD as a means of “instant speciation” (cf. Coyne and Orr, 2004) that facilitated rapid or immediate adaptation to severe climatic upheaval at the KPB (Sessa, 2019; Van de Peer et al., 2021). Alternatively, the KPB-seed traits hypothesis emphasizes that seed dormancy was not an immediate adaptation to the climatic upheaval at the KPB but instead had initially evolved outside of this context (Berry, 2020).

In order to evaluate the above hypotheses, this investigation merges two datasets: (1) one that emphasizes WGD events in relatively close proximity to the KPB (Vanneste et al., 2014; Lee et al., 2016; Lohaus and Van de Peer et al., 2016; Olsen et al., 2016; Van de Peer et al., 2017; Wu et al., 2019) and (2) one that compiles numbers of genes for late embryogenesis

abundant (LEA) proteins (Artur et al., 2019a). LEA genes are known to confer desiccation tolerance to plants and are generally perceived to be associated with seed longevity (Smolikova et al., 2021; Sharma, 2022). For example, extreme seed longevity in both *Phoenix dactylifera* and *Nelumbo nucifera* is conferred by LEA genes (Shen-Miller et al., 2013; Aziz et al., 2021; Matilla, 2022). This study investigates whether the average number of LEA genes differs significantly between lineages that either have or have not undergone WGD. A net increase in gene diversification associated with WGD suggests LEA genes were adaptive across the KPB. Whether or not WGD represents an immediate adaptation to the KPB event is contingent upon accurate dating of WGD events. Therefore, it is also important to consider contemporary evidence on the precise timing of WGD events in order to evaluate the alternative exaptation hypothesis, which suggests that WGD may have occurred in climatic contexts other than the KPB event, but which proved fortuitous for survival across the KPB. Finally, a third hypothesis is that LEA genes are almost always retained following WGD events because they are non-adaptive spandrels that arise as byproducts of selection for other adaptive features (*sensu* Gould and Lewontin, 1979), such as occasional asexuality (Freeling, 2017). Accordingly, it is important to specifically consider whether a reduction in the number of LEA genes following WGD has been associated with the evolution of seed recalcitrance, a prediction of adaptive theory.

## MATERIALS AND METHODS

According to contemporary perspectives, most genes duplicated during WGD are eventually lost unless they confer a selective advantage (Lynch and Conery, 2000; Chain et al., 2011; Gout and Lynch, 2015; Wu et al., 2020). Previous investigations have sought to identify duplicated genes retained across the KPB in order to determine if these were selected across this interval; however, these have not previously addressed the question of whether LEA genes or gene families were among those retained across the KPB (Wu et al., 2020). For this investigation, a non-parametric Wilcoxon (1945) signed-rank test for matched pairs is used to determine whether the number of LEA genes is significantly higher among plants perceived to have undergone WGD at or near the KPB than their closest relatives that did not undergo WGD at or near the KPB (Figs 1, 2). The Wilcoxon signed-rank test for matched pairs can be used to determine whether

terminal taxa sharing a node but differing in key traits – in this case, the presence/absence of paleopolyploidy at or near the KPB – exhibit significant diversification differences (Coyné and Orr, 2004). If the number of LEA genes is significantly higher among those plant lineages perceived to have undergone WGD at or near the KPB, then it can be inferred that these duplicate genes probably had a selective advantage across this interval and were, therefore, retained (e.g. Wu et al., 2020). Considering that WGD may have occurred outside of the context of the KPB event, it is conceivable that a higher number of LEA genes could either be an exaptation (i.e. initially evolved for some other purpose, but which fortuitously contributed to survival and proliferation across the KPB) or a spandrel (i.e. a byproduct of the evolution of one or more traits, rather than as a direct product of adaptive evolution). For the purposes of this investigation, pairwise comparisons were made using the phylogenies of Vanneste et al. (2014) and Artur et al. (2019a), where sister lineages perceived either to have or have not undergone WGD at or near the KPB, respectively, were compared. The average number of LEA genes for sister lineages that either did or did not exhibit WGD at or near the KPB was calculated, and these were subjected to the Wilcoxon signed-rank test. Lineages known to exhibit additional instances of WGD following the KPB, such as *Glycine max* (L.) Merr., *Brassica rapa* L. and *Zea mays* L. (Fig. 1), were excluded from the analysis to reduce the confounding effects of additional rounds of post-KPB paleopolyploidization on the analysis. For the same reason, *Xerophyta* Juss. was not used as a control for comparison because this taxon is now perceived to have undergone WGD near the Paleocene/Eocene thermal maximum (PETM), which affected the number of LEA genes in this desiccation-tolerant, resurrection plant lineage via “rewiring” or cooptation of the seed dormancy regulatory gene network (Costa et al., 2017; Artur et al., 2019a, b; Lyall et al., 2020).

In addition to the Wilcoxon signed-rank test for matched pairs, a Mann-Whitney (1947) U test is performed to determine whether spermatophyte lineages perceived to have experienced WGD at or near the KPB exhibit a significantly higher number of LEA genes than plants that did not. These data were compiled from Artur et al. (2019a: Supplementary Table 1) and are illustrated in Figure 2. For this test, all spermatophytes sharing a most recent common ancestor (MRCA) with *Amborella trichopoda* Baill. were used. Taxa excluded from this analysis included those perceived to have undergone additional rounds of WGD following the KPB, as discussed above.

Certain genes may be preferentially retained following WGD events, such as transcription factors, signal transducers and genes encoding multiprotein complexes (Blanc and Wolfe, 2004; Maere et al., 2005; Li et al., 2016; Tasdighian et al., 2017). For this reason, the Mann-Whitney U test and Wilcoxon signed rank test for matched pairs were performed again, including taxa previously excluded from the analyses (i.e. taxa experiencing post-KPB WGD). The results of these two rounds of analyses were compared in order to determine whether duplicate LEA genes are preferentially retained following WGD events, regardless of whether they are associated with the KPB.

## DATA AND RESULTS

Plant lineages perceived to have undergone WGD at or near the KPBP exhibit a significantly higher number of LEA genes than their closest relatives that did not ( $p < 0.05$ ), as determined by both a Wilcoxon signed-rank test for matched pairs (i.e. pairwise, sister group comparisons of average numbers of LEA genes for taxa either exhibiting WGD at or near the KPBP or not exhibiting this trait) and a Mann-Whitney U test (i.e. comparison of number of LEA genes for spermatophyte taxa either exhibiting WGD at or near the KPBP or not exhibiting this trait, for all taxa sharing an MRCA with *Amborella trichopoda*). This is signified by a “+” sign in Figures 1 and 2. Therefore, it can be inferred that the number of LEA genes in these lineages was positively selected across the KPBP because they are retained in these lineages, although this inference is contingent on accurate dating of WGD events (discussed below). As the number of genes or diversification of gene families are perceived to have been affected by paleopolyploidy in these lineages (Artur et al., 2019a; Chen et al., 2019), then it can be inferred that these were selectively retained across the KPBP. Two lineages perceived to have experienced WGD at or near the KPBP, the sea grasses and Solanaceae Juss., experienced a reduction in the number of LEA genes associated with WGD (Fig. 2).

When the tests were repeated with taxa previously excluded from the analyses (e.g. *Glycine max*, *Brassica* L., *Zea mays* and *Xerophyta*), there was no change to the result of the Wilcoxon signed rank test for matched pairs. This is because there was no change to the net effect or positive shift in LEA gene diversification, as taxa exhibiting post-KPBP WGD still exhibited a greater average number of LEA genes than their respective sister taxa. Including these taxa increased the significance of the result of the Mann-Whitney U test, which lowered the p-value from  $p \approx 0.02$  to  $p \approx 0.004$ .

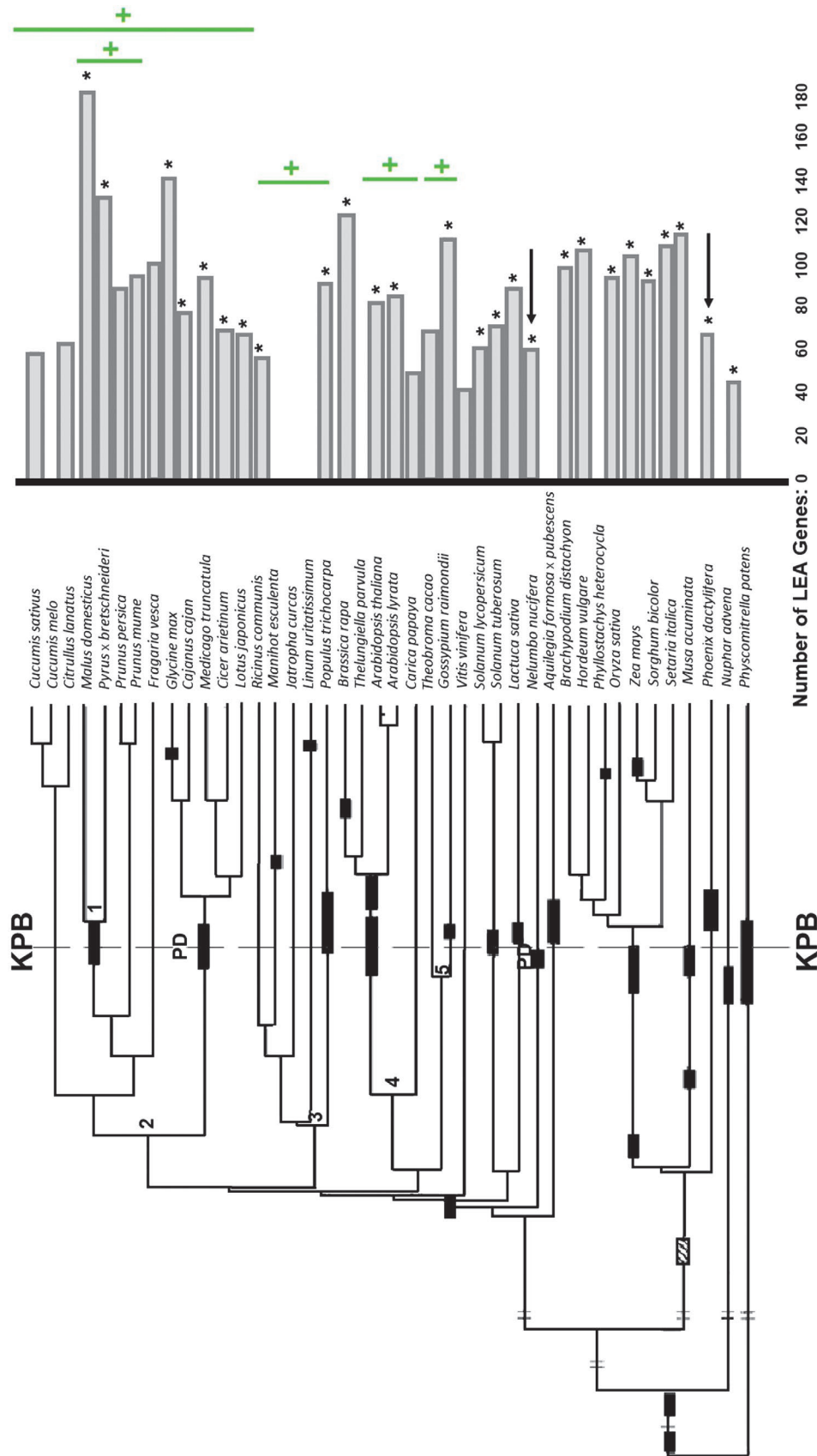
## DISCUSSION

The role of WGD in plant macroevolution, particularly in relation to global mass extinction boundaries, remains uncertain, primarily due to a lack of temporal control on the WGD events (Clark and Donoghue, 2017, 2018;

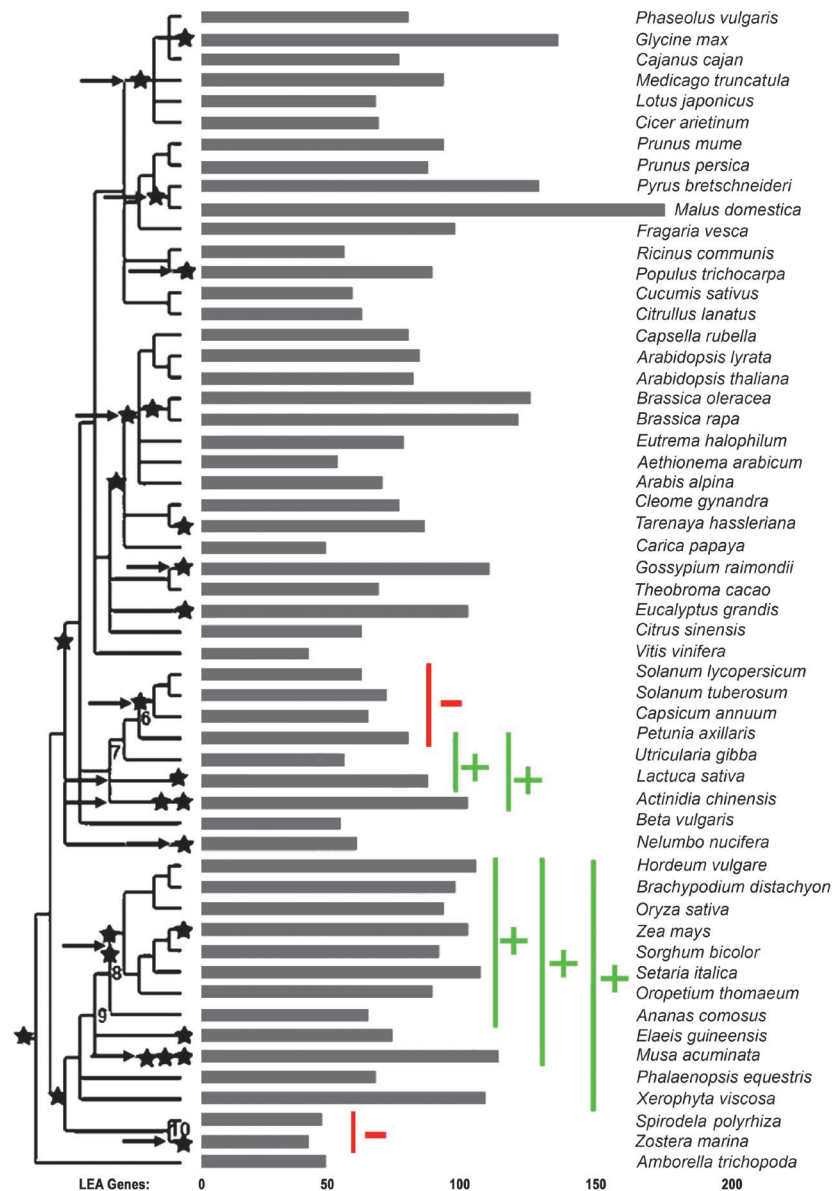
Clark et al., 2019). It was initially thought that guard cell size directly correlates with ploidy level in angiosperms and other vascular plants (Lomax et al., 2014). However, based on this guard cell size proxy evidence, paleobotanists have been critical of the concept that polyploids exhibited greater survivorship than their diploid progenitors or contemporaries at the KPBP (Lomax et al., 2014). This view is complicated by the understanding that some polyploids exhibit smaller guard cells and spores/pollen than their closest diploid relatives (Da Silva et al., 2019; Berry, 2022a).

Among those plant lineages with orthodox seeds that crossed the KPBP include *Nelumbo* Adans. and Fabaceae Lindl. The former is known to have crossed the KPBP in the Western Interior of North America (Johnson, 2002; Berry, 2020), whereas the latter is perceived to have first appeared either slightly below or just above the KPBP in this region (Lyson et al., 2019; Koenen et al., 2021; Zhao et al., 2021; Herendeen et al., 2022). *Nelumbo* is perceived to have evolved DT in association with WGD at or near the KPBP (Shi et al., 2020). Parsimony suggests that orthodox seeds evolved in Fabaceae only once; as a water-impermeable seed coat, i.e. physical dormancy is present in all of the subfamilies of this family, is comparatively rare among other angiosperm families and is lacking in its closest relatives (e.g. Polygalaceae Hoffmanns. et Link) and gymnosperms (Baskin and Baskin, 2014). DT seeds might have offered numerous benefits to survive the changing climate, such as the ability to establish a long-term seed bank. Zanne et al. (2014) postulated that DT seeds establish soil seed banks as a critical survival strategy for angiosperm radiation in freezing environments.

Accordingly, this trait may have contributed to the proliferation of Fabaceae in the Paleogene (De Casas et al., 2017), although other factors (e.g. nitrogen fixation and the ability to adapt to dry conditions) may also have contributed to this radiation (Zhao et al., 2021). Furthermore, physical dormancy or DT is explained through an LEA gene regulatory network, which is now known to have evolved through WGD (Verdier et al., 2013; Marques, 2018; Artur et al., 2019a). Although older, Fabaceae-like fruits are reported from the late Campanian in Mexico (Centeno-González et al., 2021), these fruits lack diagnostic characteristics associated with this family (Herendeen



**Figure 1.** Relative timing of whole genome duplication events (redrawn from Vanneste et al., 2014: Fig. 1) plotted against numbers of LEA genes (adapted from Artur et al., 2019a). Numbered nodes indicate clades subjected to the Wilcoxon (1945) signed-rank test for matched pairs to test the differential effects on LEA gene diversification by WGD at or near the KPB. Positive signs “+” indicate a significant, positive shift in the average number of LEA genes in clades with WGD at or near the KPB as determined by the Wilcoxon signed-rank test for matched pairs and the Mann-Whitney (1947) U tests. Clades demonstrating physical dormancy are labeled “PD”. Arrows indicate the two taxa with the longest-lived seeds known



**Figure 2.** Numbers of LEA genes in relation to phylogenetic relationships, with WGD events depicted as stars (redrawn and adapted from Artur et al., 2019a: Fig. 1). Arrows indicate WGD events reconstructed as occurring close to the KPB according to Vanneste et al. (2014), Lohaus and Van de Peer (2016), Lee et al. (2016), Olsen et al. (2016), Van de Peer et al. (2017) and Wu et al. (2019). Numbered nodes indicate clades subjected to the Wilcoxon (1945) signed-rank test for matched pairs to test the differential effects on LEA gene diversification by WGD at or near the KPB (numbering continued from Fig. 1). Positive signs “+” indicate a significant, positive shift in the number of LEA genes in clades with WGD at or near the KPB as determined by the Wilcoxon signed-rank test for matched pairs and Mann-Whitney (1947) U tests. Negative signs “-” indicate a reduction in the number of LEA genes associated with WGD near the KPB (Lee et al., 2016; Olsen et al., 2016), presumably as a result of adaptation to a warm, shallow water coastal habitat in the case of sea grasses (Artur et al., 2019a) or a loss of duplicates following WGD in the case of Solanaceae (Chen et al., 2019)

et al., 2022). The oldest confirmed Fabaceae fruits first appear in seasonal tropical forests in the Denver Basin, Colorado, slightly above the KPB (Lyson et al., 2019; Herendeen et al., 2022). Thus, it is probable that tropical wet forests favored the DS seeds, and when moved to other ecosystems with seasonal climates resulted in drying pressures, eventually mechanisms to avoid overdrying, plausibly explaining the absence of physical dormancy, in wet ecosystems. This is because the related

family Polygalaceae and these more ancient, Fabaceae-like fruits are both found in tropical wet forests (Greenwood and Christophel, 2005; Baskin and Baskin, 2014; Martínez-Cabrera and Estrada-Ruiz, 2014). This places the origin of Fabaceae near the KPB event (Herendeen et al., 2022), when multiple rounds of WGD may have occurred, including an ancestral, pan-legume WGD event first posited by Koenen et al. (2021) and later corroborated by Zhao et al. (2021). Finally, Fabaceae observed

to be DS (i.e. species where DT has been secondarily lost) display an underrepresentation of LEA genes when compared to their close relatives with DT (Delahaie et al., 2013; Marques, 2018).

Hypotheses focused on selection of leaf economic traits, such as deciduousness or other fast-return growth strategies, struggle to explain differential survival of conifers across the KPB (Royer et al., 2003; Berry, 2020). More recently, this has been explained through differences in seed traits (Berry, 2022b, c), clarifying why low-latitude (i.e. tropical evergreen) araucarian conifers (Fig. 3), for example, suffered greater extinction than other archaic conifer groups (e.g. *Classopollis* Pflug pollen-producing Cheirolepidiaceae Turutanovaketova) – a well-documented pattern (Carvalho et al., 2021; Jacobs and Currano, 2021). Carvalho et al. (2021) proposed that the disappearance of these conifers at the KPB may be due to the lesser propensity of conifers for WGD than angiosperms, which may agree with the results of this investigation (Fig. 3).

The hypothesis that plants experienced WGD at the KPB due to enhanced stress tolerance of polyploids (Fawcett et al., 2009) has been critiqued because of inaccurate dating of WGD events (Clark and Donoghue, 2017, 2018; Clark et al., 2019). For example, Vanneste et al. (2014), Lohaus and Van de Peer (2016), and Van de Peer et al. (2017) describe a WGD event in the lineage leading to *Phoenix*

*dactylifera*, which they presume occurred in close proximity to the KPB on the basis of synonymous substitution rate (Ks) peak-based estimates; however, Barrett et al. (2019) recognize that this ancient WGD event is shared by all palms and, therefore, must have significantly pre-dated the KPB. Similarly, Huang et al. (2020) identified a series of WGD events in Cyatheales A.B. Frank (tree ferns) closely bracketing the KPB; however, scrutiny of these events in the context of highly resolved, fossil-calibrated phylogenies clearly indicate that WGD occurred closer in proximity to the Triassic/Jurassic than the KPB (Berry, 2022a). Other WGD events formerly thought to have occurred close to the KPB have also been shown to either significantly predate (e.g. Vanneste et al., 2015 vs. Clark et al., 2019) or significantly post-date the KPB (Berry, 2022d).

The hypothesis that seed traits were selected across the KPB emphasizes that seed longevity is an exaptation (*sensu* Gould and Vrba, 1982) rather than an adaptation to the severe climatic conditions at the KPB, particularly if the KPB climatic event was too severe for plants to reproduce and plant recovery was contingent on preexisting soil propagule banks (Berry, 2020). Essentially the same, exaptation-focused hypothesis has already been proposed to explain differential survival of diatoms across the KPB (Gould, 2002). Therefore, WGD conferring seed longevity (e.g., duplication of LEA genes) need not have occurred at



**Figure 3.** *Araucarites longifolia* (Lesquereux) Dorf (New Mexico Museum of Natural History and Science NMMNH P-85987, L-12997) from the syndepositional late Maastrichtian Vermejo-Raton Formation, southeastern Colorado, an example of a conifer that disappeared at the KPB

the KPB, but could have occurred any time prior to the KPB, so long as differential WGD events provided the raw material (i.e., variation in LEA gene number) upon which selection could later act at the KPB.

It is well established that transcription factors, signal transducers and genes encoding multiprotein complexes have a greater chance of surviving WGD events (Blanc and Wolfe, 2004; Maere et al., 2005; Li et al., 2016; Tasdighian et al., 2017). Accordingly, it is probable that retention of duplicate LEA genes following WGD may be a general phenomenon not exclusively associated with selection for seed longevity at the KPB or during long-term climatic cooling during the Cenozoic. Furthermore, it is also conceivable that WGD may be a spandrel (*sensu* Gould and Lewontin, 1979) associated with selection for other traits, such as asexuality, across the KPB (Freeling, 2017). In either case, it may be that duplicate LEA genes are frequently retained after WGD events, but not because they are climatic adaptations.

Among the strongest evidence contradicting this spandrel hypothesis is the view that sea grasses experienced WGD at or near the KPB (~67 Ma; Lee et al., 2016; Olsen et al., 2016) coupled with a net reduction in the number of LEA genes (Fig. 2), which has been proposed as a result of adaptation to the warm, shallow marine habitat rather than adaptation to the severe climatic stress of the KPB event (Lee et al., 2016; Olsen et al., 2016; Artur et al., 2019a). This is consistent with the observation that sea grasses exhibit strongly recalcitrant (i.e. desiccation-intolerant or DS) seeds, despite retaining the potential for seed dormancy (Xu et al., 2020). Sea grasses first appeared in the Maastrichtian and are known to have crossed the KPB in its type area and vicinity (Van der Ham et al., 2007; Hart et al., 2016). Despite being desiccation-intolerant (DS), recalcitrant sea grass seeds are known to remain viable in warm, shallow sea water habitats for at least three years (Leck et al., 1989; Belzunce et al., 2008), thus forming what is referred to as a “persistent” seed bank in shallow marine sediments, with seed viability greater than a year (Orth et al., 2006: 113–114). Therefore, these plants could have recovered from a period of transient darkness lasting 1–2 years, provided that coastal sea temperatures remained warm enough for seed bank survival, as predicted in some asteroid impact climate models (e.g.

Chiarenza et al., 2020; Morgan et al., 2022). Indeed, coastal habitats may have experienced a less severe asteroid impact winter than continental landmasses due to the “thermal inertia” of the ocean (Tabor et al., 2020: p. 5), a factor already discussed at length in the context of the KPB-seed traits hypothesis (Berry, 2020). This underscores the adaptive context of LEA gene diversification. In the case of sea grasses, loss of duplicate LEA genes is presumed to have been rapid following WGD – that is, prior to the radiation of sea grasses in the Paleogene (Lee et al., 2016; Olsen et al., 2016). Therefore, these data seem to corroborate the existing theory on the fate of duplicate genes in an adaptive context (Lynch and Conery, 2000; Gout and Lynch, 2015; Wu et al., 2020). For this reason, retention or loss of LEA genes can be viewed as an adaptive phenomenon linked to climatic factors (Artur et al., 2019a).

Another group that may have experienced WGD near the KPB is the lineage leading to the Solanaceae (Wu et al., 2019), although this was also accompanied by a net reduction in the number of LEA genes (Fig. 2). *Solanum tuberosum* L. has been reported as having 72–74 LEA genes (Artur et al., 2019a; Chen et al., 2019), although the diploid or non-polyploid sister group to the Solanaceae is reconstructed as having 81 LEA genes (Artur et al., 2019a). Although LEA genes are presumed to have evolved through whole genome duplication in the Solanaceae, functional similarity may have resulted in the loss or consolidation of gene duplicates with functional overlap (Chen et al., 2019). In any case, these genes appear to have retained their function in desiccation and other stress tolerance (Chen et al., 2019). This is consistent with the view that *Solanum tuberosum* seeds are DT and can remain viable for at least two decades (Barker and Johnson, 1980).

Viviparous seeds, or those with effectively no dormancy, are characteristic of tropical mangrove palms (e.g. *Nypa fruticans* Wurmb) (Baskin and Baskin, 2014), including those known from the fossil record (Moreno-Dominguez et al., 2016). These mangrove palms are presumed to have been the principal competitors or ecological replacements of *Classopollis* pollen-producing Cheiralepidiaceae (archaic conifers) throughout the late Cretaceous and early Paleogene (Jacobs, 2004; Quattrocchio et al., 2013; Berry 2022e). In the early Paleocene (Danian), anomalous



abundance patterns or “spikes” in *Classopollis* pollen appear to have been associated with a temporary reprieve from ecological competition with these mangrove palms in brackish-water, coastal or intertidal habitats due to the non-deposition of this palm pollen at peak palynological abundances of *Classopollis* pollen (Muller, 1968; Wang et al., 1990; Morley, 1998; Barreda et al., 2012; Berry, 2022e). *Classopollis* pollen spikes have been recorded from several areas in the early Paleocene, including depositional basins along the equator (western Sarawak in Borneo) and in both the Northern (Tarim Basin, China, and vicinity) and Southern Hemispheres (Colorado and Golfo San Jorge Basins in Patagonia, Argentina) (Muller, 1968; Morley, 1998; Wang et al., 1990; Quattrocchio, 2006; Barreda et al., 2012; Berry, 2022b, e). These data are consistent with the predictions of the KPB-seed traits hypothesis (Berry, 2022b, e), as Cheirolepidiaceae is known to have exhibited seed dormancy (Baskin and Baskin, 2014). These data are also consistent with palynological evidence for a near mass extinction event among palms, where palm pollen diversity decreased by ~47% in some equatorial regions at the KPB (Pan et al., 2006). With the exception of only a few palms (e.g. coryphoid palms that include *Phoenix dactylifera*), most palms produce recalcitrant or DS seeds (Baskin and Baskin, 2014; Jaganathan, 2021). Considering that palynological records are typically too poorly resolved taxonomically to record the species-level mass extinction event among plants at the KPB (Nichols and Johnson, 2008), a near mass extinction event recorded in palm pollen diversity in equatorial habitats strongly supports predictions based on the KPB-seed traits hypothesis.

In view of all of these patterns, the results of this investigation provide key insight into the role of WGD in plant macroevolution across the KPB. Rather than representing an immediate adaptation to the severe climatic stress of the KPB event, WGD across the KPB appears to have been associated with adaptation to varying climatic conditions. Fortuitous survival and proliferation of plants across the KPB appears to have depended on traits that initially evolved in other climatic contexts – that is, exaptation. This is consistent with the view that climatic conditions at the KPB were probably too severe for plant reproduction, thus facilitating recovery from below-ground

reserves (Nichols and Johnson, 2008; Vajda and Bercovici, 2014). This perspective highlights that retention of LEA gene duplicates is clearly adaptive in climatic contexts outside of the KPB event, as seen by the inclusion of taxa, such as *Glycine max*, *Zea mays*, *Brassica* and *Xerophyta*, in the analyses. This perspective also accommodates uncertainty in the timing of WGD events, as it allows WGD to be removed from the precise temporal context of the KPB event. Although some of the WGD events may be dated precisely to the KPB interval, this is not a prerequisite for an exaptation-focused hypothesis as to why plant lineages experiencing WGD in the general vicinity of the KPB appear to exhibit a greater number of LEA genes than their closest relatives.

## CONCLUSION

The critical role of seed traits aiding the successful recovery of spermatophytes across KPB is only recently gaining renewed interest. This study reinforces the potential importance of WGD across the KPB by showing that terrestrial vascular plant lineages perceived to have undergone WGD at or near the KPB exhibit significantly greater number of LEA genes and shows a clear link between WGD and seed traits, particularly DT. Accordingly, this investigation shifts emphasis from WGD as a rapid, immediate adaptation to climatic stress at the KPB to WGD as an exaptation that is generally adaptive in other climatic contexts, but which fortuitously enhanced plant survival across the KPB.

## ACKNOWLEDGMENTS

We thank the editors and anonymous reviewers for their thoughtful and helpful critiques of this manuscript.

## REFERENCES

- Artur, M.A.S., Zhao, T., Ligterink, W., Schranz, E., Hilhorst, H.W.M., 2019a. Dissecting the genomic diversification of late embryogenesis abundant (LEA) protein gene families in plants. *Genome Biology and Evolution* 11, 459–471. <https://doi.org/10.1093/gbe/evy248>
- Artur, M.A.S., Rienstra, J., Dennis, T.J., Farrant, J.M., Ligterink, W., Hilhorst, H., 2019b. Structural plasticity of intrinsically disordered LEA proteins from *Xerophyta schlechteri* provides protection *in vitro*

- and *in vivo*. *Frontiers in Plant Science* 10, 1–15. <https://doi.org/10.3389/fpls.2019.01272>
- Aziz, M.A., Sabeem, M., Mullath, S.K., Brini, F., Mas-moudi, K., 2021. Plant group II LEA proteins: intrinsically disordered structure for multiple functions in response to environmental stresses. *Biomolecules* 11, 1–27. <https://doi.org/10.3390/biom11111662>
- Barker, W.G., Johnston, G.R., 1980. The longevity of seeds of the common potato, *Solanum tuberosum*. *American Potato Journal* 57, 601–607. <https://doi.org/10.1007/BF02854130>
- Barreda, V.D., Cúneo, N.R., Wilf, P., Currano, E.D., Scasso, R.A., Brinkhuis, H., 2012. Cretaceous/Paleogene floral turnover in Patagonia: drop in diversity, low extinction, and a *Classopollis* spike. *PLoS ONE* 7, 1–8. <https://doi.org/10.1371/journal.pone.0052455>
- Barrett, C.F., McKain, M.R., Sinn, B.T., Ge, X.-J., Zhang, Y., Antonelli, A., Bacon, C.D., 2019. Ancient polyploidy and genome evolution in palms. *Genome Biology and Evolution* 11, 1501–1511. <https://doi.org/10.1093/gbe/evz092>
- Baskin C.C., Baskin J.M., 2014. *Seeds: Ecology, Biogeography, and Evolution: Second Edition*. Academic Press, San Diego.
- Belzunce, M., Navarro, R.M., Rapoport, H.F., 2008. *Posidonia oceanica* seeds from drift origin: viability, germination and early plantlet development. *Botanica Marina* 51, 1–9. <https://doi.org/10.1515/BOT.2008.005>
- Berry, K., 2020. Seed traits linked to differential survival of plants during the Cretaceous/Paleogene impact winter. *Acta Palaeobotanica* 60, 307–322. <https://doi.org/10.35535/acpa-2020-0016>
- Berry, K., 2022a. Which is more appropriate for deciphering recursive patterns in plant recovery across the Triassic/Jurassic and Cretaceous/Paleogene boundaries, nomothetic or idiographic approaches? *New Mexico Museum of Natural History and Science Bulletin* 90: Fossil Record 8, 1–8.
- Berry K., 2022b. Was the K/Pg boundary *Classopollis* spike a singular event? Review of global palynological records suggests otherwise, with broad implications. *Rocky Mountain Geology* 57, 17–29.
- Berry, K., 2022c. Paleobiogeography of the stenochlaenoid ferns: Using fossils and molecules to investigate macroevolutionary patterns and processes. *International Journal of Plant Sciences* 183, 268–278. <https://doi.org/10.1086/718576>
- Berry, K., 2022d. Conifer turnover across the K/Pg boundary in Colorado, U.S.A., parallels South American patterns: New and emerging perspectives. *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen* 303, 11–28. <https://doi.org/10.1127/njgpa/2022/1035>
- Berry, K., 2022e. A *Classopollis* “spike” in the *Rugbivesiculites* Zone of the Kayan Sandstone, western Sarawak, Borneo, suggests a Danian age for these deposits. Review of Palaeobotany and Palynology 304, 104728. <https://doi.org/10.1016/j.revpalbo.2022.104728>
- Blanc, G., Wolfe, K.H., 2004. Functional divergence of duplicated genes formed by polyploidy during *Arabidopsis* evolution. *The Plant Cell* 16, 1679–1691. <https://doi.org/10.1105/tpc.021410>
- Blonder, B., Royer, D.L., Johnson, K.R., Miller, I., Enquist, B.J., 2014. Plant ecological strategies shift across the Cretaceous-Paleogene boundary. *PLoS Biology* 12, 1–7. <https://doi.org/10.1371/journal.pbio.1001949>
- Butrim, M.J., Royer, D.L., Miller, I.M., Dechesne, M., Neu-Yagle, N., Lyson, T.R., Johnson, K.R., Barclay, R.S., 2022. No consistent shift in leaf dry mass per area across the Cretaceous-Paleogene boundary. *Frontiers in Plant Science* 13, 1–11. <https://doi.org/10.3389/fpls.2022.894690>
- Carvalho, M.R., Jaramillo, C., de la Parra, F., Caballero-Rodríguez, F.H., Wing, S., Turner, B.L., D'Apollito, C., Romero-Báez, M., Narváez, P., Martínez, C., Gutierrez, M., Labandeira, C., Bayona, G., Rueda, M., Paez-Reyes, M., Cárdenas, D., Duque, A., Crowley, J.L., Santos, C., Silvestro, D., 2021. Extinction at the end-Cretaceous and the origin of modern neotropical rainforests. *Science* 372, 63–68. <https://doi.org/10.1126/science.abf1969>
- Centeno-González, N.K., Martínez-Cabrera, H.I., Porras-Múzquiz, H., Estrada-Ruiz, E., 2021. Late Campanian fossil of a legume fruit supports Mexico as a center of Fabaceae radiation. *Communications Biology* 4, 1–8. <https://doi.org/10.1038/s42003-020-01533-9>
- Chain, F.J.J., Dushoff, J., Evans, B.J., 2011. The odds of duplicate gene persistence after polyploidization. *BMC Genomics* 12, 1–7. <https://doi.org/10.1186/1471-2164-12-599>
- Chen, Y., Li, C., Zhang, B., Yi, J., Yang, Y., Kong, C., Lei, C., Gong, M., 2019. The role of the Late Embryogenesis-Abundant (LEA) protein family in development and the abiotic stress response: a comprehensive expression analysis of potato (*Solanum tuberosum*). *Genes* 10, 1–16. <https://doi.org/10.3390/genes10020148>
- Chiarenza, A.A., Farnsworth, A., Mannion, P.D., Lunt, D.J., Valdes, P.J., Morgan, J.V., Allison, P.A., 2020. Asteroid impact, not volcanism, caused the end-Cretaceous dinosaur extinction. *Proceedings of the National Academy of Sciences USA* 117, 17084–17093. <https://doi.org/10.1073/pnas.2006087117>
- Clark, J.W., Donoghue, P.C.J., 2017. Constraining the timing of whole genome duplication in plant evolutionary history. *Proceedings of the Royal Society of London, Series B* 284, 1–8. <https://doi.org/10.1098/rspb.2017.0912>
- Clark, J.W., Donoghue, P.C.J., 2018. Whole-genome duplication and plant macroevolution. *Trends in Plant Science* 23, 933–945. <https://doi.org/10.1016/j.tplants.2018.07.006>
- Clark, J.W., Puttick, M.N., Donoghue, P.C.J., 2019. Origin of horsetails and the role of whole-genome duplication in plant macroevolution. *Proceedings of*

- the Royal Society of London, Series B 286, 1–10. <https://doi.org/10.1098/rspb.2019.1662>
- Costa, M.-C. D., Artur, M.A.S., Maia, J., Jonkheer, E., Derks, M.F.L., Nijveen, H., Williams, B., Mundry, S.G., Jiménez-Gómez, J.M., Hesselink, T., Schijlen, E.G.W.M., Ligterink, W., Oliver, M.J., Farrant, J.M., Hilhorst, H.W.M., 2017. A footprint of desiccation tolerance in the genome of *Xerophyta viscosa*. *Nature Plants* 3, 1–11. <https://doi.org/10.1038/nplants.2017.38>
- Coyne, J.A., Orr, H.A., 2004. *Speciation*. Sinauer Associates, Oxford, 480 p.
- Da Silva, D.M., Da Silva Sylvestre, L., Ferreira Mendonça, C.B., Gonçalves-Esteves, V., 2019. Spore diversity among species of Blechnaceae in the Atlantic forest. *Acta Palaeobotanica Brasiliica* 33, 1–13. <https://doi.org/10.1590/0102-33062018abb0321>
- De Casas, R.B., Willis, C.G., Pearse, W.D., Baskin, C.C., Baskin, J.M., Cavender-Bares, J., 2017. Global biogeography of seed dormancy is determined by seasonality and seed size: a case study in the legumes. *New Phytologist* 214, 1527–1536. <https://doi.org/10.1111/nph.14498>
- Delahaie, J., Hundertmark, M., Bove, J., Leprince, O., Rogniaux, H., Buitink, J., 2013. LEA polypeptide profiling of recalcitrant and orthodox legume seeds reveals ABI3-regulated LEA protein abundance linked to desiccation tolerance. *Journal of Experimental Botany* 64, 4559–4573. <https://doi.org/10.1093/jxb/ert274>
- Fawcett, J.A., Maere, S., Van de Peer, Y., 2009. Plants with double genomes might have had a better chance to survive the Cretaceous-Tertiary extinction event. *Proceedings of the National Academy of Sciences USA* 106, 5737–5742. <https://doi.org/10.1073/pnas.0900906106>
- Freeling, M., 2017. Picking up the ball at the K/Pg boundary: The distribution of ancient polyploidies in the plant phylogenetic tree as a spandrel of asexuality with occasional sex. *The Plant Cell* 29, 202–206. <https://doi.org/10.1105/tpc.16.00836>
- Gould, S.J., 2002. *The Structure of Evolutionary Theory*. The Belknap Press of Harvard University, Cambridge, pp. 1296–1332.
- Gould, S.J., Lewontin, R.C., 1979. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. *Proceedings of the Royal Society of London, Series B* 205, 581–598. <https://doi.org/10.1098/rspb.1979.0086>
- Gould, S.J., Vrba, E.S., 1982. Exaptation – a missing term in the science of form. *Paleobiology* 8, 4–15. <https://doi.org/10.1017/S0094837300004310>
- Gout, J.-F., Lynch, M., 2015. Maintenance and loss of duplicated genes by dosage subfunctionalization. *Molecular Biology and Evolution* 32, 2141–2148. <https://doi.org/10.1093/molbev/msv095>
- Greenwood, D., Christophel, D., 2005. The origins and Tertiary history of Australian “tropical” rainforests. In: Bermingham, E., Dick, C.W., Moritz, C. (eds), *Tropical Rainforests: Past, Present, and Future*. University of Chicago Press, Chicago, 322–335.
- Hart, M.B., FitzPatrick, M.E.J., Smart, C.W., 2016. The Cretaceous/Paleogene boundary: Foraminifera, sea grasses, sea level change and sequence stratigraphy. *Palaeogeography, Palaeoclimatology, Palaeoecology* 441, 420–429. <https://doi.org/10.1016/j.palaeo.2015.06.046>
- Herendeen, P.S., Cardoso, D.B.O.S., Herrera, F., Wing, S.L., 2022. Fossil papilionoids of the Bowdichia clade (Leguminosae) from the Paleogene of North America. *American Journal of Botany* 109, 130–150. <https://doi.org/10.1002/ajb2.1808>
- Huang, C.-H., Qi, X., Chen, D., Qui, J., Ma, H., 2020. Recurrent genome duplication events likely contributed to the ancient and recent rise of ferns. *Journal of Integrative Plant Biology* 62, 433–455. <https://doi.org/10.1111/jipb.12877>
- Jaganathan, G.K., 2021. Ecological insights into the coexistence of dormancy and desiccation-sensitivity in *Arecaceae* species. *Annals of Forest Science* 78: 1–14. <https://doi.org/10.1007/s13595-021-01032-9>
- Jaganathan, G.K., Dalrymple, S., Liu, B., 2015. Towards an understanding of factors controlling seed bank composition and longevity in the alpine environment. *The Botanical Review* 81, 70–103. <https://doi.org/10.1007/s12229-014-9150-2>
- Jacobs, B.F., 2004. Palaeobotanical studies from tropical Africa: relevance to the evolution of forest, woodland and savannah biomes. *Philosophical Transactions: Biological Sciences* 359, 1573–1583. <https://doi.org/10.1098/rstb.2004.1533>
- Jacobs, B.F., Currano, E.D., 2021. The impactful origin of neotropical rainforests: A mass extinction event led to vast diversity and structural complexity of neotropical rainforests. *Science* 372, 28–29. <https://doi.org/10.1126/science.abb2086>
- Johnson, K.R., 2002. The megafloora of the Hell Creek and lower part of the Fort Union formations in the western Dakotas: vegetational response to climate change, the Cretaceous-Tertiary boundary event, and rapid marine transgression. *Geological Society of America Special Papers* 361, 329–391. <https://doi.org/10.1130/0-8137-2361-2.329>
- Junium, C.K., Zerkle, A.L., Witts, J.D., Ivany, L.C., Yancey, T.E., Liu, C., Claire, M.W., 2022. Massive perturbations to atmospheric sulfur in the aftermath of the Chicxulub impact. *Proceedings of the National Academy of Sciences USA* 119, 1–7. <https://doi.org/10.1073/pnas.2119194119>
- Koenen, E.J.M., Ojeda, D.I., Bakker, F.T., Wieringa, J.J., Kidner, C., Hardy, O.J., Pennington, R.T., Herendeen, P.S., Bruneau, A., Hughes, C.E., 2021. The origin of legumes is a complex paleopolyploid phylogenomic tangle closely associated with the Cretaceous-Paleogene (K/Pg) mass extinction event. *Systematic Biology* 70, 508–526. <https://doi.org/10.1093/sysbio/syaa041>
- Leck, M.A., Parker, V.T., Simpson, R.L., 1989. *Ecology of soil seed banks*. Academic Press, Inc., San Diego, 462 p.
- Lee, H.T., Golicz, A.A., Bayer, P.E., Jiao, Y., Tang, H., Paterson, A.H., Sablok, G., Krishnaraj, R.R., Chan,

- C.-K.K., Batley, J., Kendrick, G.A., Larkhum, A.W.D., Ralph, P.J., Edwards, D., 2016. The genome of a Southern Hemisphere seagrass species (*Zostera muelleri*). *Plant Physiology* 172, 272–283. <https://doi.org/10.1104/pp.16.00868>
- Li, Z., Defoort, J., Tasdighian, S., Maere, S., Van de Peer, Y., De Smet, R., 2016. Gene duplicability is highly consistent across all angiosperms. *The Plant Cell* 28, 326–344. <https://doi.org/10.1105/tpc.15.00877>
- Lohaus, R., Van de Peer, Y., 2016. Of dups and dinos: evolution at the K/Pg boundary. *Current Opinion in Plant Biology* 30, 62–69. <https://doi.org/10.1016/j.pbi.2016.01.006>
- Lomax, B.H., Hilton, J., Bateman, R.M., Upchurch, G.R., Jr., Lake, J.A., Leitch, I.J., Cromwell, A., Knight, C.A., 2014. Reconstructing relative genome size of vascular plants through geological time. *New Phytologist* 201, 636–644. <https://doi.org/10.1111/nph.12523>
- Long, R.L., Gorecki, M.J., Renton, M., Scott, J.K., Colville, L., Goggin, D.E., Commander, L.E., Westcott, D.A., Cherry, H., Finch-Savage, W.E., 2015. The ecophysiology of seed persistence: a mechanistic view of the journey to germination or demise. *Biological Reviews* 90, 31–59. <https://doi.org/10.1111/brv.12095>
- Lyll, R., Schlebusch, S.A., Proctor, J., Prag, M., Hussey, S.G., Ingle, R.A., Illing, N., 2020. Vegetative desiccation tolerance in the resurrection plant *Xerophyta humilis* has not evolved through reactivation of the seed canonical LAFL regulatory network. *The Plant Journal* 101, 1349–1367. <https://doi.org/10.1111/tpj.14596>
- Lynch, M., Conery, J.S., 2000. The evolutionary fate and consequences of duplicate genes. *Science* 290, 1151–1155. <https://doi.org/10.1126/science.290.5494.1151>
- Lyson, T.R., Miller, I.M., Bercovici, A.D., Weissenburger, K., Fuentes, A.J., Clyde, W.C., Hagadorn, J.W., Butrim, M.J., Johnson, K.R., Fleming, R.F., Barclay, R.S., MacCracken, S.A., Lloyd, B., Wilson, G.P., Krause, D.W., Chester, S.G.B., 2019. Exceptional continental record of biotic recovery after the Cretaceous-Paleogene mass extinction. *Science* 366, 977–983. <https://doi.org/10.1126/science.aay2268>
- Maere, S., De Bodt, S., Raes, J., Casneuf, T., Montagu, M.V., Kuiper, M., Van de Peer, Y., 2005. Modeling gene and genome duplications in eukaryotes. *Proceedings of the National Academy of Sciences, USA* 102, 5454–5459. <https://doi.org/10.1073/pnas.0501102102>
- Mann, H.B., Whitney, D.R., 1947. On a test of whether one or two random variables is stochastically larger than the other. *Annals of Mathematical Statistics* 18, 50–60.
- Marques, A., 2018. Desiccation sensitive seeds: Understanding their evolution, genetics and physiology. Ph.D. Thesis, Wageningen University, Wageningen, the Netherlands, 189 p.
- Martínez-Cabrera, H.I., Estrada-Ruiz, E., 2014. Wood anatomy reveals high theoretical hydraulic conductivity and low resistance to vessel implosion in a Cretaceous fossil forest from northern Mexico. *PLoS ONE* 9, 1–11. <https://doi.org/10.1371/journal.pone.0108866>
- Matilla, A.J., 2022. The orthodox dry seeds are alive: a clear example of desiccation tolerance. *Plants* 11, 1–20. <https://doi.org/10.3390/plants11010020>
- Moreno-Dominguez, R., Cascales-Miñana, B., Ferrer, J., Diez, J.B., 2016. First record of the mangrove palm *Nypa* from the northeastern Ebro Basin, Spain: with taphonomic criteria to evaluate the drifting duration. *Geologica Acta* 14, 101–111.
- Morgan, J.V., Bralower, T.J., Brugger, J., Wünnenman, K., 2022. The Chicxulub impact and its environmental consequences. *Nature Reviews Earth and Environment* 3, 338–354. <https://doi.org/10.1038/s43017-022-00283-y>
- Morley, R.J., 1998. Palynological evidence for Tertiary plant dispersals in the SE Asian region in relation to plate tectonics and climate. In: Hall, R., Holloway, J.D. (eds), *Biogeography and Geological Evolution of SE Asia*. Backbuys Publishers, Leiden, The Netherlands, pp. 211–234.
- Muller, J., 1968. Palynology of the Pedawan and Plateau Sandstone Formations (Cretaceous – Eocene) in Sarawak, Malaysia. *Micropaleontology* 14, 1–37.
- Nichols, D.J., Johnson, K.R., 2008. *Plants and the K-T boundary*. Cambridge University Press, Cambridge.
- Olsen, J.L., Rouzé, P., Verhelst, B., Lin, Y.-C., Bayer, T., Collen, J., Dattolo, E., De Paoli, E., Dittami, S., Maumus, F., Michel, G., Kersting, A., Lauritano, C., Lohaus, R., Töpel, M., Tonon, T., Vanneste, K., Amirebrahimi, M., Brakel, J., Boström, C., Chovatia, M., Grimwood, J., Jenkins, J.W., Jueterbock, A., Mraz, A., Stam, W.T., Tice, H., Bornberg-Bauer, E., Green, P.J., Pearson, G.A., Procaccini, G., Duarte, C.M., Schmutz, J., Reusch, T.B.H., Van de Peer, Y., 2016. The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea. *Nature* 530, 331–335. <https://doi.org/10.1038/nature16548>
- Orth, R.J., Harwell, M.C., Inglis, G.J., 2006. Ecology of seagrass seeds and dispersal strategies. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (eds), *Seagrasses: Biology, Ecology and Conservation*. Springer, Dordrecht, The Netherlands, pp. 111–133.
- Pan, A.D., Jacobs, B.F., Dransfield, J., Baker, W.J., 2006. The fossil record of palms (Arecaceae) in Africa and new records from the Late Oligocene (28–27 Mya) of north-western Ethiopia. *Botanical Journal of the Linnean Society* 151, 69–81. <https://doi.org/10.1111/j.1095-8339.2006.00523.x>
- Quattrocchio, M.E., 2006. Palynology and paleocommunities of the Paleogene of Argentina. *Revista Brasileira de Paleontologia* 9, 101–108.
- Quattrocchio, M.E., Martínez, M.A., Hinojosa, L.F., Jaramillo, C., 2013. Quantitative analysis of Cenozoic palynofloras from Patagonia, southern South

- America. *Palynology* 37, 246–258. <https://doi.org/10.1080/01916122.2013.787126>
- Roberts, E. H., 1973. Predicting the Storage Life of Seeds. *Seed Science and Technology* 1, 499–514.
- Royer, D.L., Osborne, C.P., Beerling, D.J., 2003. Carbon loss by deciduous trees in a CO<sub>2</sub>-rich ancient polar environment. *Nature* 424, 60–62. <https://doi.org/10.1038/nature01737>
- Sano, N., Rajjou, L., North, H.M., Debeaujon, I., Marion-Poll, A., Seo, M., 2016. Staying alive: molecular aspects of seed longevity. *Plant and Cell Physiology* 57, 660–674. <https://doi.org/10.1093/pcp/pcv186>
- Sessa, E.B., 2019. Polyploidy as a mechanism for surviving global change. *New Phytologist* 221, 5–6. <https://doi.org/10.1111/nph.15513>
- Sharma, G.S., 2022. Structural and functional role of plant dehydrins in enhancing stress tolerance. In: Roy, S., Mathur, P., Chakraborty, A.P., Saha, S.P. (eds), *Plant Stress: Challenges and Management in the New Decade*. Cham, Switzerland, Springer Nature, pp. 111–122
- Shen-Miller, J., Lindner, P., Xie, Y., Villa, S., Wooding, K., Clarke, S.G., Loo, R.R.O., Loo, J.A., 2013. Thermal-stable proteins of fruit of long-living Sacred Lotus *Nelumbo nucifera* Gaertn. var. China Antique. *Tropical Plant Biology* 6, 1–29. <https://doi.org/10.1007/s12042-013-9124-2>
- Shi, T., Rahmani, R.S., Gugger, P.F., Wang, M., Li, H., Zhang, Y., Li, Z., Wang, Q., Van de Peer, Y., Marchal, K., Chen, J., 2020. Distinct expression and methylation patterns for genes with different fates following a single whole-genome duplication in flowering plants. *Molecular Biology and Evolution* 37, 2394–2413. <https://doi.org/10.1093/molbev/msaa105>
- Smolikova, G., Leonova, T., Vashurina, N., Frolov, A., Medvedev, S., 2021. Desiccation tolerance as the basis of long-term seed viability. *International Journal of Molecular Sciences* 22, 1–24. <https://doi.org/10.3390/ijms22010101>
- Soltis, D.E., Burleigh, J.G., 2009. Surviving the K-T mass extinction: New perspectives on polyploidization in angiosperms. *Proceedings of the National Academy of Sciences, USA* 106, 5455–5456. <https://doi.org/10.1073/pnas.0901994106>
- Soltis, D.E., Albert, V.A., Leebens-Mack, J., Bell, C.D., Paterson, A.H., Zheng, C., Sankoff, D., DePamphilis, C.W., Wall, P.K., Soltis, P.S., 2009. Polyploidy and angiosperm diversification. *American Journal of Botany* 96, 336–348. <https://doi.org/10.3732/ajb.0800079>
- Soltis, D.E., Segovia-Salcedo, M.C., Jordan-Thaden, I., Majure, L., Miles, N.M., Mavrodiev, E.V., Mei, W., Cortez, M.B., Soltis, P.S., Gitzendammer, M.A., 2014. Are polyploids really evolutionary dead-ends (again)? A critical reappraisal of Mayrose et al. (2011). *New Phytologist*, 1105–1117.
- Stebbins, G.L., 1950. *Variation and Evolution in Plants*. Columbia University Press, New York.
- Tabor, C.R., Bardeen, C.G., Otto-Bliesner, B.L., Garcia, R.R., Toon, O.B., 2020. Causes and climatic consequences of the impact winter at the Cretaceous-Paleogene boundary. *Geophysical Research Letters* 47, 1–10. <https://doi.org/10.1029/2019GL085572>
- Tasdighian, S., Van Bel, M., Li, Z., Van de Peer, Y., Carreto-Paulet, L., Maere, S., 2017. Reciprocally retained genes in the angiosperm lineage show the hallmarks of dosage balance sensitivity. *The Plant Cell* 29, 2766–2785. <https://doi.org/10.1105/tpc.17.00313>
- Vajda, V., Bercovici, A., 2014. The global vegetation pattern across the Cretaceous-Paleogene mass extinction interval: A template for other extinction events. *Global and Planetary Change* 122, 29–49. <https://doi.org/10.1016/j.gloplacha.2014.07.014>
- Van der Ham, R.W.J.M., Van Konijnenburg-van Cittert, J.H.A., Indeherde, L., 2007. Seagrass foliage from the Maastrichtian type area (Maastrichtian, Danian, NE Belgium, SE Netherlands). *Review of Palaeobotany and Palynology* 144, 301–321. <https://doi.org/10.1016/j.revpalbo.2006.07.008>
- Van de Peer, Y., Ashman, T.L., Soltis, P.S., Soltis, D.E., 2021. Polyploidy: an evolutionary and ecological force in stressful times. *The Plant Cell* 33, 1–16. <https://doi.org/10.1093/plcell/koab149>
- Van de Peer, Y., Mizrachi, E., Marchal, K., 2017. The evolutionary significance of polyploidy. *Nature Review Genetics* 18, 411–424. <https://doi.org/10.1038/nrg.2017.26>
- Vanneste, K., Maere, S., Van de Peer, Y., 2014. Tangled up in two: a burst of genome duplications at the end of the Cretaceous and the consequences for plant evolution. *Philosophical Transactions of the Royal Society of London, Series B* 369, 1–13. <https://doi.org/10.1098/rstb.2013.0353>
- Vanneste, K., Sterck, L., Myburg, A.A., Van de Peer, Y., Mizrachi, E., 2015. Horsetails are ancient polyploids: evidence from *Equisetum giganteum*. *Plant Cell* 27, 1567–1578. <https://doi.org/10.1105/tpc.15.00157>
- Verdier, J., Lalanne, D., Pelletier, S., Torres-Jerez, I., Righetti, K., Bandyopadhyay, K., Leprince, O., Chatelain, E., Ly Vu, B., Gouzy, J., Gamas, P., Udvardi, M.K., Buitink, J., 2013. A regulatory network-based approach dissects late maturation processes related to the acquisition of desiccation tolerance and longevity of *Medicago truncatula* seeds. *Plant Physiology* 163, 757–774. <https://doi.org/10.1104/pp.113.222380>
- Wang, D., Sun, X.Y., Zhao, Y.N., 1990. Late Cretaceous to Tertiary palynofloras in Xinjiang and Qinghai, China. *Review of Palaeobotany and Palynology* 65, 95–104. [https://doi.org/10.1016/0034-6667\(90\)90060-V](https://doi.org/10.1016/0034-6667(90)90060-V)
- Wilcoxon, F., 1945. Individual comparisons by ranking methods. *Biometrics Bulletin* 1, 80–83.
- Wolfe, J.A., Upchurch, G.R., Jr., 1987. Leaf assemblages across the Cretaceous-Tertiary boundary in the Raton Basin, New Mexico and Colorado. *Proceedings of the National Academy of Sciences, USA* 84, 5096–5100.

- Wood, T.E., Takebayashi, N., Barker, M.S., Mayrose, I., Greenspoon, P.B., Rieseberg, L.H., 2009. The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences, USA* 106, 13875–13879. <https://doi.org/10.1073/pnas.0811575106>
- Wu, H., Ma, T., Kang, M., Ai, F., Dong, G., Liu, J., 2019. A high-quality *Actinidia chinensis* (kiwifruit) genome. *Horticulture Research* 6, 1–9. <https://doi.org/10.1038/s41438-019-0202-y>
- Wu, S., Han, B., Jiao, Y., 2020. Genetic contribution of paleopolyploidy to adaptive evolution in angiosperms. *Molecular Plant* 13, 59–71. <https://doi.org/10.1016/j.molp.2019.10.012>
- Xu, S., Xu, S., Zhou, Y., Gu, R., Zhang, X., Yue, S., 2020. Long-term seed storage for desiccation sensitive seeds in the marine foundation species *Zostera marina* L. (eelgrass). *Global Ecology and Conservation* 24, 1–11. <https://doi.org/10.1016/j.gecco.2020.e01401>
- Zanne, A.E., Tank, D.C., Cornwell, W.K., Eastman, J.M., Smith, S.A., FitzJohn, R.G., McGlenn, D.J., O'Meara, B.C., Moles, A.T., Reich, P.B., Royer, D.L., Soltis, D.E., Stevens, P.F., Westoby, M., Wright, I.J., Aarssen, L., Bertin, R.I., Calaminus, A., Govaerts, R., Hemmings, F., Leishman, M.R., Oleksyn, J., Soltis, P.S., Swenson, N.G., Warman, L., Beaulieu, J.M., 2014. Three keys to the radiation of angiosperms into freezing environments. *Nature* 506, 89–92. <https://doi.org/10.1038/nature12872>
- Zhao, Y., Zhang, R., Jiang, K.-W., Qi, J., Hu, Y., Guo, J., Zhu, R., Zhang, T., Egan, A.N., Yi, T.-S., Huang, C.-H., Ma, H., 2021. Nuclear phylotranscriptomics and phylogenomics support numerous polyploidization events and hypotheses for the evolution of rhizobial nitrogen-fixing symbiosis in Fabaceae. *Molecular Plant* 14, 748–773. <https://doi.org/10.1016/j.molp.2021.02.006>