Is lack of cycad (Cycadales) diversity a result of a lack of polyploidy?

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Many hypotheses exist for the relative diversity of seed plant taxa. We discuss one of them: how the relative dearth of cycad diversity throughout their 300 million year history may be a result of a lack of duplication of their entire nuclear genome, often termed polyploidy. We show theoretically how polyploidy causes speciation via cryptic reproductive isolation. Polyploidy can also cause radiations via epigenetically induced heterochrony and plasticity. Most cycads have only a few large chromosomes because of a lack of whole genome duplication or, except possibly in the genus *Zamia*, chromosomal fission. Large chromosomes and extremely small effective population sizes result in substantial linkage disequilibrium, genetic hitchhiking and genetic drift in cycads. By contrast, other seed plants have higher incidences of polyploidy and may therefore have been more prone to radiations.   © 2011 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2011, **165**, 156–167.

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INTRODUCTION

Why are there so few cycads? Why are there so many flowering plants? There are only two or three extant cycad families containing approximately 100–150 species (Stevenson, 1992; Rai *et al*., 2003). Following Scotland & Wortley (2003), we accept this conservative estimate, and not the more liberal estimates of around 300 species (Hill, Stevenson & Osborne, 2004, 2007). As we are only discussing the relative number of taxa, these comparisons will be fair if examining an equally conservative number of other seed plant taxa, such as the estimates of approximately 413 families (APG III, 2009) of extant flowering plants containing 200 000 species (Scotland & Wortley, 2003), instead of more liberal estimates of 350 000 angiosperm species (see references in Scotland & Wortley, 2003). The huge disparity in biodiversity between cycads and flowering plants cannot be the result of one group having millions of years more to evolve than the other. Fossil evidence shows that cycads originated approximately 275–300 million years ago (Zhifeng & Thomas, 1989; Axsmith *et al*., 2003), whereas flowering plants originated approximately 125 million years ago (Friis, Pedersen & Crane, 1999; Sun *et al*., 2002). Molecular evidence also supports a much earlier origin of cycads than flowering plants (Samigullen *et al*., 1999; Soltis, Soltis & Zanis, 2002). Flowering plants have simply radiated much more than cycads and other seed plants.

From the Palaeozoic to the present, cycads have never been diverse. Unlike famous cases of the coelocanth and *Welwitschia* Hook.f., which are relictual members of once diverse lineages, cycads have never shown much taxonomic or morphological diversity (Harris, 1961, 1964; Pant, 1987; Anderson & Anderson, 1989; Taylor, Taylor & Krings, 2009). Cycads have always had the same basic shape as they do today, although somewhat more slender forms are

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known from the Triassic (Delevoryas & Hope, 1971, 1976). Angiosperms, in contrast, have dominated the terrestrial landscape since their radiation in the mid to late Cretaceous or early Tertiary, and are unparalleled in morphological, ecological and taxonomic diversity (Magallón, Crane & Herendeen, 1999; Soltis & Soltis, 2004; De Bodt, Maere & Van de Peer, 2005; Soltis et al., 2008; Fawcett, Maere & Van de Peera, 2009).

Conventional wisdom holds that flowering plant diversity is high because they co-evolved with insects. At least three hypotheses have been proposed to explain this co-evolution of plants and insects, and all three find little to no support in cycad natural history. First, high diversity has been hypothesized to arise when plants are pollinated by insects (Grant, 1949, but see Smith et al., 2008). Cycad diversity has always been low despite all cycads probably being insect-pollinated (Norstog, Stevenson & Niklas, 1986; Tang, 1987; Norstog & Nicholls, 1997; Terry, 2001; Terry et al., 2004), as were their Mesozoic ancestors (Gorelick, 2001b; Klavins et al., 2005). Cycads probably evolved from medullosan seed ferns (Crane, 1985a; Serbet & Rothwell, 1995), which were all probably pollinated by arthropods (Gorelick, 2001b; Labandeira, Kvacek & Mostovski, 2007). Second, high diversity has been hypothesized to arise when seeds are distributed by animals (McKey, 1975; Herrera, 1989). Many cycads have seeds dispersed by animals (Jones, 1993; Norstog & Nicholls, 1997). How else could the large seeds of Zamia pseudoparasitica Yates (averaging 2 cm diameter) establish themselves as epiphytes or pseudo-epiphytes (Taylor et al., 2008)? Third, high diversity has been hypothesized to arise when plants, through the production of secondary chemical compounds, undergo an evolutionary arms race with insects that eat them (Ehrlich & Raven, 1964). Cycads have evolved novel chemical defensive compounds to thwart herbivores (Norstog & Nicholls, 1997). The volatiles that cycads produce to attract pollinators may have co-evolved with anti-herbivory compounds (Pellmyr et al., 1991), and hence this is probably an ancient mechanism conserved in seed plant lineages. Thus, co-evolutionary arguments for explaining seed plant diversity or a relative lack of diversity seem to be false on the basis of data from cycads.

It is thus time to look at hypotheses other than those involving co-evolution with animals to explain the extraordinary lack of cycad diversity over the past 300 million years (e.g. Gorelick, 2001b; Williams, 2008). Previously, we enumerated 20 hypotheses for the radiations of angiosperms and lack of radiations in gymnosperms (Gorelick, 2001b), including: self-incompatibility reduces extinction rates; obligate dioecy reduces speciation rates; pollen tube competi-

tion causes higher speciation rates; speciation rates are inversely proportional to duration between pollination and fertilization; speciation rates are inversely proportional to gametophyte size, especially for megagametophytes; there is a selective advantage to double fertilization and vessels; there is a selective advantage to larger seeds and deciduous leaves; there is a selective advantage to shorter generation times (especially annuals) and a lack of lignin; and polyploidy causes higher speciation rates and lower extinction rates. Although a plurality of mechanisms is simultaneously at play, and not all listed hypotheses are mutually exclusive, here we focus solely on the hypothesis involving polyploidy.

High diversity in various seed plant lineages has been hypothesized to be coupled with a high frequency of polyploidy (Soltis & Soltis, 1993). Polyploidy is common in flowering plants, especially in herbaceous plants (Otto & Whitton, 2000; Goldblatt & Johnson, 2003, 2008; Crepet & Niklas, 2009), whereas polyploidy has never been documented in cycads (Levin & Wilson, 1976; Crepet & Niklas, 2009). Sporophytic (i.e. diploid) chromosome numbers of cycads range from 16 to 28 (Moretti et al., 1993; Goldblatt & Johnson, 2003, 2008). The base number of sporophytic chromosomes seems to be 16 or 18 in Cycadales, with the number only increasing in Zamia L. (16–28, excluding 20) and Microcycas A.DC (26). Extant conifers and gnetophytes (which are probably closely related to one another) have incidences of polyploidy intermediate between those of angiosperms and cycads (Table 1). We argue that a lack of polyploidy in cycads was a major cause of their low diversity, with minor radiations only existing as a result of chromosomal fission.

This is largely a theoretical paper, albeit relying heavily on the comparative method for support. It is impossible to say in a vacuum whether cycad diversity is low or high. This is something that can only be done by comparing cycad diversity with that of other seed plants, hoping to arrive at a mechanism that can explain the differences across these disparate taxa.

LACK OF SYMPATRIC SPECIATION CAUSED BY NO ALLOPOLYPLOIDY OR AUTOPOLYPLOIDY

Lack of polyploidy translates into a lack of radiation, which is, in turn, a result of a lack of sympatric speciation. Autopolyploids (autoploids) are individuals with twice the number of copies of each chromosome, where the parents (if there is more than one) are both members of the same species. Allopolyploids (alloploids) are individuals produced by a hybridization event between two different species, where off-

Table 1. Diversity, origin and polyploidy in seed plants

	Number of extant families	Number of extant species	First appearance (millions of years)	Prevalence of polyploidy (%)
Angiosperms	~500	200 000–400 000	125	40–95
Conifers*	7	500–800	330	5–15
Gnetales†	3	100–150	200	5–15
Cycads	2	150–300	300	0
Ginkgos	1	1	280	?

*Conifers were much more diverse in the Mesozoic.
†Gnetales are probably highly derived conifers.

spring have the chromosome complements of both parents. Both autopolyploidy and allopolyploidy result in a doubling or near doubling of total genome size.

Both autopolyploidy and allopolyploidy cause quantum speciation via instantaneous reproductive isolation (Grant, 1971; Stebbins, 1971). During gametophyte formation, cells undergo meiosis and thereby halve the number of chromosomes per cell. Individuals in the ancestral diploid lineage form haploid gametes, whereas polyploid individuals form diploid gametes. When a gamete from a diploid parent fuses with a gamete from a tetraploid parent, their offspring is most often a sexually sterile triploid with an odd number of chromosomes (but see Ramsey & Schemske, 1998). Even if triploid offspring can form viable unreduced gametes, the resulting sporophytic progeny would be tetraploid if backcrossed with the original population or hexaploid if self-fertilization occurs. In either instance, the progeny would still not be diploid. Polyploidy creates new biological species, although they need not be morphologically distinct from the diploid species. Although polyploid individuals cannot backcross with diploid individuals that gave rise to them, what evidence exists that the diploids and polyploids will not resemble one another?

At least in flowering plants, where autoploids are now believed to be relatively common (Soltis *et al*., 2007; contra Stebbins, 1950; Grant, 1971), autoploid populations are believed to not only be reproductively isolated, but also possess distinct ecological and geographical niches (Soltis *et al*., 2007; Thompson & Merg, 2008). No one has yet tested such ideas on nonflowering plants. However, we assume that this argument is quite general: that autopolyploidy fosters sympatric speciation and radiations. Because cycads lack autopolyploidy, they have probably not been able to speciate sympatrically (other than possibly via co-evolution with distinct pollinating insects) and have probably been unable to radiate into as many ecological niches as have autoploid angiosperms.

Having established the respective roles of autopolyploidy in flowering plants and cycads, we shall refer to both autoploids and alloploids simply as polyploids for the remainder of this article, especially as species concepts are always in such flux (Rowley, 2007; Soltis *et al*., 2007) and the biological species' concept may be untenable in plants (Cracraft, 1989).

LACK OF ADAPTIVE RADIATIONS OF CYCADS PARTLY AS A RESULT OF GENOME ARCHITECTURE

In the previous section, we argued that a lack of polyploidy resulted in a lack of cryptic speciation and minimal diversification in cycads because of a lack of sympatric speciation. Here, we argue that this same lack of polyploidy severely suppresses any noncryptic adaptive speciation. In fact, cycad genome architecture implies that natural selection in general should play almost no role in cycad evolution. These conclusions readily flow from established population genetic theory. Cycads have a few large chromosomes, copious genetic hitchhiking and relatively high mutation rates (Barton, 1995; Lynch, 2007), and hence have almost no role for selection. Cycads also have small effective population sizes because of long generation times, small numbers of individuals and obligate dioecy (or at least sequential hermaphroditism; Osborne & Gorelick, 2007), generating genetic drift (Gorelick, 2009). Drift should therefore play a much larger role than selection in cycad evolution, rendering adaptation unlikely. In the next two paragraphs, we outline evidence for diminished selection and increased drift for cycads.

Any organism possessing a few large chromosomes will have high linkage disequilibrium and, consequently, much genetic hitchhiking. Two genes on separate chromosomes are usually assorted randomly each generation during meiosis (independent segregation), thereby resetting their linkage disequilibrium to zero. Two genes on the same chromosome

(which is much more likely to happen in cycads and any taxa with only a few large chromosomes) can only have their linkage disequilibrium reduced by recombination. With typically only one or two crossing-over recombination events per pair of chromosomes per generation (Otto & Lenormand, 2002), this is unlikely to happen for many pairs of genes. Large nonzero linkage disequilibrium is further maintained in triplets, quadruplets or larger complexes of genes (Gorelick & Laubichler, 2004). Large linkage disequilibrium means that, if you know the allele at one locus, you can predict the allele at one or more other loci (Liu & Lin, 2005; Bertram & Gorelick, 2009). If selection acts to fix an allele at one locus, linked genes will follow along for the ride, not being subject to selection on their own (hence genetic hitchhiking; Levin, 2000). A few large chromosomes result in a dramatically smaller pool of genes subject to direct selection.

By contrast with organisms with a few large chromosomes, consider an organism with the same genome split up into many smaller chromosomes (chromosomal fission). Gene linkages on either side of the centromere are broken during chromosomal fission, thereby decreasing genetic hitchhiking and allowing adaptive radiations through selection on newly unlinked genes (Todd, 1970). The aneuploid series in the genus *Zamia* is indicative of chromosomal fission (Moretti & Sabato, 1984; Moretti, 1990; Moretti *et al.*, 1991; Caputo *et al.*, 1996; Napolitano, Caputo & Moretti, 2004), supported by the combined analysis of phylogenetic and karyotypic data (Caputo *et al.*, 2004; Olson & Gorelick, 2011). Chromosomal fission may therefore account for the small-scale adaptive radiations, although the effective population size is so small in *Zamia* (and Cycadales as a whole) that genetic drift should still be much more important than selection (Gorelick, 2009).

Long-term effective population size is the harmonic mean of the time series of effective population sizes over many generations (Hartl & Clark, 1989), which disproportionally weights bottlenecks. Lynch (2007) provided empirical order-of-magnitude estimates of long-term effective population size for prokaryotes, nonparasitic protists, invertebrates, vertebrates, annual plants and perennial plants. His perennial plant data, composed of trees with generation times of roughly 20 years, had the lowest average long-term effective population sizes, on the order of 10 000 individuals. Cycad long-term effective population sizes must be substantially lower than this. Uneven sex ratios, which appear to exist in some cycads (Ornduff, 1985, 1990; Tang, 1990), reduce effective population sizes. However, the most draconian reduction in long-term effective population size is that most census values for cycad species reveal few individuals. For example, Donaldson reported less than 300 mature individuals of both *Encephalartos dolomiticus* Lavranos & D.L.Goode and *E. inopinus* R.A.Dyer in the entire world, setting an upper limit of effective population size at less than 150 (IUCN, 2008). Genetic drift is inversely proportional to long-term effective population size, which is small in cycads, and hence cycad evolution is dominated by drift.

HYPOTHESIS: POLYPLOIDY EPIGENETICALLY TRIGGERS RADIATIONS

Could polyploidy cause large-scale, rapid phenotypic changes and major macroevolutionary radiations (Stebbins, 1966; Levinton, 2001; Adams & Wendel, 2005)? Three of the great evolutionary biologists of the 20th century gave a resounding answer that polyploidy was utterly insignificant when it came to large-scale diversification over evolutionary time. Haldane (1929) asserted that, 'Often the visible effect [of polyploidy] is very small indeed, far less than that of many single factors or [aneuploidy].' Goldschmidt (1940) added that, 'in plants [polyploidy] cannot lead very far in macroevolution, as the possibilities of such differentiation are quickly exhausted.' Finally, the one botanical architect of the modern synthesis, Stebbins (1971), warned that, 'Clearly chromosome doubling by itself is not a help but a hindrance to the evolutionary success of higher plants.' We argue with three famous dead evolutionary biologists by asserting that polyploidy causes developmental changes, such as slower growth rate and shorter times to reach sexual maturity (Dobzhansky, 1937), which can appear as large morphological changes.

Polyploidy triggers epigenetic changes, such as changes in cytosine methylation or chromatin formation (Ohno, 1970; Comai *et al.*, 2000; Zhou, Cheng & Tiersch, 2002; Osborn *et al.*, 2003; Wendel & Doyle, 2005; Lukens *et al.*, 2006). Any newly inserted portion of the genome acquires disproportionate levels of cytosine methylation (except for those few aberrant animal and fungal lineages that have completely lost methylation) and chromatin formation (Holliday, 1984). Polyploidy is the most extreme form of newly inserted portions of the genome, with the largest proportion of epigenetic changes (Matzke & Matzke, 1998; Liu *et al.*, 2000; Rapp & Wendel, 2005; Lukens *et al.*, 2006). The resulting extra cytosine methylation and chromatin formation down-regulate regulatory genes, causing subfunctionalization and potentially shutting off later stages of development (Force *et al.*, 1999; Liu & Wendel, 2003; Gorelick, 2004; Wendel & Doyle, 2005; Adams, 2007; Paun *et al.*, 2007). These epigenetic changes can trigger paedomorphism and

other changes in developmental timing, i.e. heterochrony (Roth *et al.*, 1993; Roth, Nishikawa & Wake, 1997; Barrier, Robichaux & Purugganan, 2001; Lee, Mummenhoff & Bowman, 2002; Gorelick, 2004). Paedomorphism is reproduction in a state that appears morphologically juvenile, a terminology that was developed by zoologists, who were accustomed to well-defined germ and somatic lines. Although it may be impossible to distinguish various types of paedomorphism in plants (Li & Johnston, 2000; Olson, 2007), we can probably infer when paedomorphism exists. The determination of what constitutes juvenile and adult morphologies can only be made with reference to sister taxa, which regrettably for cycads may mean Palaeozoic medullosan seed ferns (Crane, 1985b). The duplication of the entire genome also has a more direct effect on heterochrony because the duration of the mitotic cell cycle is inversely proportional to the genome size (Gregory, 2002). Polyploidy is therefore one of the most likely causes of paedomorphism (in particular) and heterochrony (in general). Via epigenetic changes, whole genome duplication may induce neo-Goldschmidtian changes (Crepet & Niklas, 2009), which are not necessarily adaptive.

Evolutionary developmental biologists have convincingly argued that phenotypic novelties only arise via changes in developmental pathways (Müller & Newmann, 2003), implying that epigenetic mechanisms underlie radiations. Palaeontologists and biologists have shown that changes in developmental timing may have been the cause of major evolutionary radiations, such as of trilobites, stony corals, terebratuloid brachiopods and flowering plants (Cloud, 1948; Gorelick, 2001b; Liu & Wendel, 2003). When examined with the epigenetic consequences detailed above, palaeontological and biological viewpoints indicate that polyploidy could have been the major cause of evolutionary radiations. However, thus far, there is only a single palaeontological data point in support of (and none refuting) this chain of inferences connecting polyploidy with macroevolutionary radiations in plants (Masterson, 1994).

We hypothesize that polyploidy triggers epigenetic changes which, in turn, trigger large morphological changes and radiations [please note that both Liu & Wendel (2003) and Chong & Whitelaw (2004) also discussed this hypothesis, but only in the context of flowering plants and animals, respectively, and not in the context of gymnosperms]. Flowering plants have high incidences of polyploidy, with between 40 and 80% of taxa clearly showing signs of polyploidy (Levin, 2002). Polyploidy even appears to be common in early diverging angiosperms (Cui *et al.*, 2006).

Hypotheses regarding radiations are necessarily comparative. Radiation is a relative term used to describe a higher than average rate of speciation minus extinction. Predicted radiations in polyploid lineages are relative to sister lineages that are not polyploid. However, it should be noted that polyploidy inducing epigenetic changes is a hypothesis about speciation and diversification rates, not extinction rates. Thus, polyploidy can induce radiations without selective or adaptive advantages.

The definition of radiation as a higher than average speciation minus extinction rate is operational because we can simply count taxa (Klak, Reeves & Hedderson, 2004), but it is blind to degrees of morphological novelty. For example, there are relatively many species in the cycad genera *Cycas* L. and *Macrozamia* Miq. and the conifer genus *Pinus* L., although seemingly not huge amounts of morphological novelty in each genus. By contrast, the cycad genera *Stangeria* T.Moore (one species) and *Bowenia* Hook. (two species) and the gnetophyte genus *Welwitschia* (one species) are considered to be morphologically unusual, yet we do not count these as radiations; a rich fossil record for each of these odd monotypic and bitypic genera may eventually provide evidence for radiations of these taxa, too, at least in a previous era. Although we have a general feeling for what should be considered as morphological novelty, such notions are difficult or impossible to operationalize. What is extremely novel to one person may be a mere perturbation to another. In order to test hypotheses rigorously, we therefore stick to a more objective accounting definition of radiations, which could instead be called high diversification (Klak *et al.*, 2004).

POTENTIAL TESTS OF THE POLYPLOIDY HYPOTHESIS

Can we directly test the hypothesis that cycad diversity is low because of a lack of polyploidy? Just because extant cycads show less than a two-fold range in chromosome numbers (from 16 to 28), this does not necessarily imply that polyploidy never existed in cycads. Extinct cycads conceivably could have undergone polyploidy, followed by either extinction or diploidization.

Masterson (1994) provided the key to testing directly whether a lack of polyploidy was the cause of a lack of cycad diversity. It should be possible to take her methods and apply them to fossil cycad lineages to ascertain that no polyploidy occurred. Polyploidy causes cells to be larger; the extra chromosomes take up a significant amount of space in the cell, something which appears to be true in all eukaryotes. By establishing that the guard cell size of stomata increases with DNA content (and therefore ploidy), Masterson looked along a phylogenetic branch for

increases in guard cell size in fossil leaf cuticles, and showed that polyploidy was indeed associated with radiations of fossil flowering plants.

In modern floras, cycad leaves are particularly distinct and easy to identify. However, when looking at the fossil record, one does not see cryptic species or reproductive isolation. One only sees morphospecies. In the Mesozoic (250 to 65 million years ago), cycad leaf and stem fossils superficially resembled those of the cycadeoids (Bennettitales). In fact, most Mesozoic fossil leaves that were identified as cycads in the 1800s are now known to be bennettitalean. (This is why the Mesozoic was mistakenly called the 'age of cycads'. Similarly, the Palaeozoic was mistakenly known as the 'age of ferns', even though most of the putative Palaeozoic fern fossil leaflets have turned out to be seed ferns or even insect wings!) Bennettitales and cycad leaves can be readily distinguished by Bennettitales having paracytic (syndetocheilic) stomata and cycads having anomacytic (haplocheilic) stomata (Thomas & Bancroft, 1913). Bennettitales are believed to be much more closely related to flowering plants than to cycads (Doyle & Donoghue, 1993; Nixon *et al.*, 1994; Rothwell, Crepet & Stockey, 2009).

In order to infer polyploidy or a lack thereof in fossil cycads, we would need a substantial number of cycad fossils and a phylogeny for them. Then, along a phylogenetic branch where there is phyletic evolution, we could see whether there is an increase in size of stomatal guard cells. Sequence analyses provided direct evidence of palaeopolyploidization events in many flowering plants [most notably *Arabidopsis thaliana* (L.) Heynh.; Simillion *et al.* 2002 and references therein]. These techniques could possibly be applied to Cycadales to test directly for evidence of polyploidy in their evolutionary history.

EXTENDING THE HYPOTHESIS TO OTHER SEED PLANTS

One of the most robust tests of whether a lack of polyploidy is partly responsible for a lack of cycad diversity is to see whether the hypothesis of polyploidy epigenetically triggering radiations is corroborated for all other seed plants. Radiations make no sense in isolation, but only relative to sister taxa. For a lack of polyploidy to adequately explain evolutionary stasis and a lack of diversity in cycads, this hypothesis must also take into account the extravagantly high species richness of angiosperms and the more modest species richness of most gymnosperms (Friedman, 2009; Soltis *et al.*, 2009).

Although they have been divided into four subclasses to reflect their long evolutionary history (Chase & Reveal, 2009), extant gymnosperm taxa all seem depauperate. There is only a single extant species of *Ginkgo*. There are approximately 500 species of conifers in seven families. There are approximately 100 species in the three genera of Gnetales, contained in either one or three families (it is still debatable whether or not Gnetales belong within Coniferophyta; Friis *et al.*, 2007; Frohlich & Chase, 2007). However, modern diversity of gymnosperm taxa does not reflect their historic patterns, especially in the Cretaceous (Yang, Geng, Dilcher, Chen & Lott, 2005). There were substantial radiations, albeit not as substantial as Tertiary angiosperm radiations, among Bennettitales, Gnetales and the conifer family Cheirolepidiaceae (Watson, 1977, 1988; Gorelick, 2001b; Dilcher *et al.*, 2005; Rydin *et al.*, 2006). Subsequently, Bennettitales and Cheirolepidiaceae went extinct at the Cretaceous–Tertiary (K–T) boundary (Taylor *et al.*, 2009). The diversity of Gnetales was decimated at the K–T extinction, with only three lineages surviving into the earliest Tertiary and until today (Taylor *et al.*, 2009). Fawcett, Maere & Van de Peera (2009) proposed that genome duplication conferred greater survivability to flowering plants in the K–T extinction. Perhaps this extends to Gnetales as well: those with duplicated genomes more readily made it through the K–T mass extinction. By contrast, Cycadales have never radiated (Gorelick, 2001b and references therein).

The hypothesis of polyploidy epigenetically triggering radiations is solely about speciation and origination, not about extinction. Thus, we should examine Mesozoic gymnosperm diversity in testing this hypothesis for those taxa that suffered huge extinctions at the end of the Cretaceous.

Nobody has ever conducted a study akin to Masterson (1994) to infer ploidy levels of extinct gymnosperms. The best we can do with existing data is to ask 'How common is polyploidy in extant gymnosperms?' Polyploidy is rare or absent in extant conifers, with the one notable exception of the hexaploid coastal redwood, *Sequoia sempervirens* Endl. (Khoshoo, 1959; Saylor & Simons, 1970). *Ginkgo biloba* L. is diploid, with a sporophytic chromosome count of $2n = 24$ (Zhang, Sodmergen & Li, 1997). In contrast with conifers and ginkgos, polyploidy seems to occur in each of the three extant genera of Gnetales. *Welwitschia mirabilis* Hook.f and *Gnetum montanum* Markgr. are tetraploids (Khoshoo, 1959; Hizume *et al.*, 1993; Cui *et al.*, 2006). Some hybrid populations of *Ephedra* L. may be allotetraploid, an inference based on interspecific hybrid populations being monoecious (Wendt, 1993), whereas most extant Gnetales are strictly dioecious. The simplest way to evolve from strict dioecy to monoecy is via the formation of alloploids (Gorelick, 2003, 2005). Polyploidy in Gnetales is consistent with their radiations in the

Mesozoic, followed by a bottleneck caused by the mass extinction at the end of the Cretaceous.

Ploidy data on gymnosperms are consistent with the hypothesis that polyploidy triggers radiations. The only putatively diverse conifer lineages in the Mesozoic were Cheirolepidiaceae and Gnetales, and Gnetales are the only extant gymnosperms for which there are substantial hints of polyploidy. Many fossils of Cheirolepidiaceae are being found and identified (Axsmith, Krings & Waselkov, 2004; van Konijnenburg-van Cittert, 2008), and so it may eventually be possible to infer polyploidy in them using Masterson's methodology. Her methods could also be used to infer polyploidy in Bennettitales, for which many leaf fossils are known. Masterson's methodology should also be used to verify that no polyploidy occurred in the many never diverse gymnosperm lineages, such as cycads, Pentoxylales, Vojnovskyales and Hermanophytales (Taylor *et al.*, 2009).

CONCLUDING REMARKS

To understand why there are so many flowering plants, we must also understand the flip side of this coin: why there are so few cycads. Of all extant seed plants, cycads and ginkgos clearly have the least contemporary morphological diversity and probably the smallest number of species during the past 250 million years. Cycads and ginkgo also have shown no signs of polyploidy (whole genome duplication). Here, we have proposed a mechanism by which polyploidy triggers macroevolutionary radiation *sensu* Goldschmidt. Cycads and ginkgos are also unique amongst extant seed plants in being zoidogamous (Norstog, 1993; Friedman & Gillford, 1997; Gorelick, 2001a), but nobody has proposed a mechanism by which siphonogamy induces radiations (Poort, Visscher & Dilcher, 1996).

Polyploidy, as classically envisioned, causes reproductive isolation, which, in turn, causes nonadaptive sympatric speciation. There exists compelling evidence that polyploidy can also cause phenotypic evolution via the collateral epigenetic changes of extra cytosine methylation and chromatin formation associated with gene duplication. In particular, polyploid-induced epigenetic changes can cause paedomorphism and other forms of heterochrony. There also exists evidence, albeit largely from the zoological literature, that heterochrony is associated with radiations. In principle, polyploidy thereby provides a mechanism by which radiations can occur via epigenetically inducing heterochrony. Such radiations via polyploidy appear to be neither adaptive nor maladaptive (*sensu* Schluter, 2000), i.e. they are merely nonadaptive. When adaptive sympatric speciation and/or radiation occurs by polyploidy, it is probably through the generation of novel gene functions (although adaptive mutations do rarely occur; Force *et al.*, 1999).

We applied these theoretical arguments to explain why cycad diversity is low compared with that of flowering plants and other gymnosperms. Cycads appear to have never been polyploid, whereas flowering plants have shown a predilection for polyploidy throughout much of their evolutionary history. Other gymnosperms groups, such as gnetophytes and conifers *s.l.*, appear to have levels of diversity and levels of polyploidy that are intermediate between those of cycads and angiosperms. However, the true test of this polyploidy hypothesis remains to be conducted: Can we infer from fossil evidence, especially the size of stomatal guard cells, that cycads have never been polyploid? Could we also correlate fossil inferences from other Mesozoic gymnosperm lineages regarding polyploidy with data on their radiations or lack thereof? Can sequence analysis provide a clear answer to questions of past ploidy levels? Much work needs to be done to further bolster the hypothesis that polyploidy triggers radiations in plants, but the initial evidence seems to support it.

We also encourage others to look for chromosomal fission, and not just whole genome duplication, in plants. Simply counting chromosomes will not allow us to distinguish between the different evolutionary processes of chromosomal duplication and fission. Karyotyping, or a combination of chromsome numbers and *C*-values, will allow these processes to be readily distinguished. Unfortunately, there are only six cycad records in the *C*-value database (Ohri & Khoshoo, 1986; Leitch *et al.*, 2001; Murray, Leitch & Bennett, 2004), providing insufficient information for inferring chromosomal fission at this time. Chromosomal fission should be able to induce small adaptive radiations, possibly as seen in the clade containing *Zamia* and *Microcycas*, especially by alleviating the effects of genetic hitchhiking.

The hypothesis that polyploidy via whole genome duplication induces nonadaptive, noncryptic radiations is only one of at least 20 hypotheses for the diversity of angiosperms and dearth of diversity of cycads. Several mechanisms are undoubtedly operating simultaneously to create macroevolutionary patterns of diversity (Crepet & Niklas, 2009). Our purpose is to highlight polyploidy as a potentially large contributor to these patterns, to describe the underlying evolutionary theory and to suggest further tests.

Although most researchers have attempted to address the abominable mystery of why most seed plants are angiosperms, we take the complimentary tack of addressing why so few seed plants are gymnosperms. Being that most (all?) extant cycads are insect pollinated, as were many extinct gymnosperms,

we look to one of the other hypotheses to explain such disparities (Gorelick, 2001a, b; Williams, 2008). An increase in the number of chromosomes can be a result of whole genome duplication (polyploidy or polysomy) or chromosomal fission. Whole genome duplication results in quantum speciation via reproductive isolation, as well as large morphological/developmental changes caused by methylation of duplicated genomes. Polyploid-induced epigenetic changes probably account for angiosperm diversity, especially in herbaceous plants. By contrast, cycads have no polyploidy in their evolutionary history, making them a taxonomically and morphologically depauperate group; hence, their evolutionary stasis since the Palaeozoic. Cycads have large chromosomes, resulting in much linkage disequilibrium and genetic hitchhiking. The only escape from a small number of large chromosomes is in the genus *Zamia*, where chromosomal fission appears to be occurring, possibly explaining the small radiation in this group. However, the combination of a lack of whole genome duplication and small effective population sizes in cycads makes genetic drift a much more influential force of their evolution than selection, thereby precluding adaptive radiations.

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REFERENCES

Adams KL. 2007. Evolution of duplicate gene expression in polyploid and hybrid plants. *Journal of Heredity* **98:** 136–141.

Adams KL, Wendel JF. 2005. Novel patterns of gene expression in polyploid plants. *Trends in Genetics* **21:** 539–543.

Anderson JM, Anderson HM. 1989. *Palaeoflora of southern Africa, Molteno Formation (Triassic), Volume 2: Gymnosperms (excluding Dicroidium)*. Rotterdam: A.A. Balkema.

APG III. 2009. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161:** 105–121.

Axsmith BJ, Serbet R, Krings M, Taylor TN, Taylor EL, Mamay SH. 2003. The enigmatic Paleozoic plants *Spermopteris* and *Phasmatocycas* reconsidered. *American Journal of Botany* **90:** 1585–1595.

Axsmith BJ, Krings M, Waselkov K. 2004. Conifer pollen cones from the Cretaceous of Arkansas: implications for diversity and reproduction in the Cheirolepidiaceae. *Journal of Paleontology* **78:** 402–409.

Barrier M, Robichaux RH, Purugganan MD. 2001. Accelerated regulatory gene evolution in an adaptive radiation. *Proceedings of the National Academy of Sciences of the United States of America* **98:** 10 208–10 213.

Barton N. 1995. Linkage and the limits to natural selection. *Genetics* **140:** 821–841.

Bertram SM, Gorelick R. 2009. Quantifying and comparing mating systems using normalized mutual entropy. *Animal Behaviour* **77:** 201–206.

Caputo P, Cozzolino S, Gaudio L, Moretti A, Stevenson DW. 1996. Karyology and phylogeny of some Mesoamerican species of *Zamia* (Zamiaceae). *American Journal of Botany* **83:** 1513–1520.

Caputo P, Cozzolino S, de Luca P, Moretti A, Stevenson DW. 2004. Molecular phylogeny of *Zamia* (Zamiaceae). In: Walters T, Osborne R, eds. *Cycad classification: concepts and recommendations*. Wallingford, Oxfordshire: CABI, 149–159.

Chase MW, Reveal JL. 2009. A phylogenetic classification of the land plants to accompany APG III. *Botanical Journal of the Linnean Society* **161:** 122–127.

Chong SY, Whitelaw E. 2004. Epigenetic germline inheritance. *Current Opinion in Genetics and Development* **14:** 692–696.

Cloud PE. 1948. Some problems and patterns of evolution exemplified by fossil invertebrates. *Evolution* **2:** 322–350.

Comai L, Tyagi AP, Winter K, Holmes-Davis R, Reynolds SH, Stevens Y, Byers B. 2000. Phenotypic instability and rapid gene silencing in newly formed *Arabidopsis* allotetraploids. *Plant Cell* **12:** 1551–1567.

Cracraft J. 1989. Speciation and its ontology: the empirical consequences of alternative species concepts for understanding patterns and processes of differentiation. In: Otte D, Endler JA, eds. *Speciation and its consequences*. Sunderland, MA: Sinauer Associates, 28–59.

Crane PR. 1985a. Phylogenetic analysis of seed plants and the origin of angiosperms. *Annals of the Missouri Botanical Garden* **72:** 716–793.

Crane PR. 1985b. Phylogenetic relationships in seed plants. *Cladistics* **1:** 329–348.

Crepet WL, Niklas KJ. 2009. Darwin's second 'abominable mystery': why are there so many angiosperm species? *American Journal of Botany* **96:** 366–381.

Cui LY, Wall PK, Leebens-Mack JH, Lindsay BG, Soltis DE, Doyle JJ, Soltis PS, Carlson JE, Arumuganathan K, Barakat A, Albert VA, Ma H, dePamphilis CW. 2006. Widespread genome duplications throughout the history of flowering plants. *Genome Research* **16:** 738–749.

De Bodt S, Maere S, Van de Peer Y. 2005. Genome duplication and the origin of angiosperms. *Trends in Ecology and Evolution* **20:** 591–597.

Delevoryas T, Hope RC. 1971. A new Triassic cycad and its phyletic implication. *Postilla* **150:** 1–21.

Delevoryas T, Hope RC. 1976. More evidence for a slender growth habit in Mesozoic Cycadaphyta. *Review of Palaeobotany and Palynology* **21:** 93–100.

Dilcher DL, Bernardes-De-Oliveira ME, Pons D, Lott TA. 2005. Welwitschiaceae from the lower Cretaceous of

northeastern Brazil. *American Journal of Botany* **92:** 1294–1310.

Dobzhansky TG. 1937. *Genetics and the origin of species*. New York: Columbia University Press.

Doyle JA, Donoghue MJ. 1993. Phylogenies and angiosperm diversification. *Paleobiology* **19:** 141–167.

Ehrlich PR, Raven PH. 1964. Butterflies and plants: a study in coevolution. *Evolution* **18:** 586–608.

Fawcett JA, Maere S, Van de Peera 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 of the United States of America* **106:** 5737–5742.

Force A, Lynch M, Pickett FB, Amores A, Yan YL, Postlethwait J. 1999. Preservation of duplicate genes by complementary, degenerate mutations. *Genetics* **151:** 1531–1545.

Friedman WE. 2009. The meaning of Darwin's 'abominable mystery'. *American Journal of Botany* **96:** 5–21.

Friedman WE, Gillford EM. 1997. Development of the male gametophyte of *Ginkgo biloba*: a window into the reproductive biology of early seed plants. In: Hori T, Ridge RW, Tulecke W, Del Tredici P, Trémouillaux-Guillen J, Tobe H, eds. *Ginkgo biloba, a global treasure: from biology to medicine*. Tokyo: Springer-Verlag, 29–49.

Friis EM, Pedersen KR, Crane PR. 1999. Early angiosperm diversification: the diversity of pollen associated with angiosperm reproductive structures in early Cretaceous floras from Portugal. *Annals of the Missouri Botanical Garden* **86:** 259–296.

Friis EM, Crane PR, Pedersen KR, Bengtson S, Donoghue PCJ, Grimm GW, Stampanoni M. 2007. Phase-contrast X-ray microtomography links Cretaceous seeds with Gnetales and Bennettitales. *Nature* **450:** 549–552.

Frohlich MW, Chase MW. 2007. After a dozen years of progress the origin of angiosperms is still a great mystery. *Nature* **450:** 1184–1189.

Goldblatt P, Johnson DE, eds. 2003. *Index to plant chromosome numbers*. St. Louis, MO: Missouri Botanical Garden. Available at http://mobot.mobot.org/W3T/Search/ipcn.html

Goldblatt P, Johnson DE, eds. 2008. *Index to plant chromosome numbers, 2004–2006*. St. Louis, MO: Missouri Botanical Garden. Available at http://mobot.mobot.org/W3T/Search/ipcn.html

Goldschmidt RB. 1940. *The material basis of evolution*. New Haven, CT: Yale University Press.

Gorelick R. 2001a. Cycad prepollen: description and possible evolutionary consequences of zoidogamy. *Cycad Newsletter* **24:** 12–24.

Gorelick R. 2001b. Did insect pollination cause increased seed plant diversity? *Biological Journal of the Linnean Society* **74:** 407–427.

Gorelick R. 2003. Evolution of dioecy and sex chromosomes via methylation driving Muller's ratchet. *Biological Journal of the Linnean Society* **80:** 353–368.

Gorelick R. 2004. Resolving the phylogenetic placement of *Blossfeldia liliputana* (Cactaceae): reticulate evolution, chloroplast inheritance, and graft-chimeras. *Bradleya* **22:** 9–14.

Gorelick R. 2005. Theory for why dioecious plants have equal length sex chromosomes. *American Journal of Botany* **92:** 979–984.

Gorelick R. 2009. Evolution of cacti is largely driven by genetic drift, not selection. *Bradleya* **27:** 41–52.

Gorelick R, Laubichler MD. 2004. Decomposing multilocus linkage disequilibrium. *Genetics* **166:** 1581–1583.

Grant V. 1949. Pollination systems as isolating mechanisms in angiosperms. *Evolution* **3:** 82–97.

Grant V. 1971. *Plant speciation*. New York: Columbia University Press.

Gregory TR. 2002. Genome size and developmental complexity. *Genetica* **115:** 131–146.

Haldane JBS. 1929. The species problem in the light of genetics. *Nature* **124:** 514–516.

Harris TM. 1961. The fossil cycads. *Palaeontology* **4:** 313–323.

Harris TM. 1964. *The Yorkshire Jurassic flora. II. Caytoniales, cycadales and pteridosperms*. London: British Museum (Natural History).

Hartl DL, Clark AG. 1989. *Principles of population genetics*. Sunderland, MA: Sinauer Associates.

Herrera CM. 1989. Seed dispersal by animals: a role in angiosperm diversification? *American Naturalist* **133:** 309–322.

Hill KD, Stevenson DW, Osborne R. 2004. The world list of cycads (Appendix 1). In: Walters T, Osborne R, eds. *Cycad classification: concepts and recommendations*. Wallingford, Oxfordshire: CABI, 219–236.

Hill KD, Stevenson DW, Osborne R. 2007. The world list of cycads. *Memoirs of the New York Botanical Garden* **97:** 454–483.

Hizume M, Gu Z, Yue Z, Kondo K. 1993. Karyomorphological study on *Gnetum montanum* collected in Yunnan, China. *Chromosome Information Service* **54:** 23–25.

Holliday R. 1984. The biological significance of meiosis. In: Evans CE, Dickinson HG, eds. *Controlling events in meiosis*. Cambridge: Cambridge University Press, 381–394.

IUCN. 2008. IUCN red list of threatened species. Available at http://www.iucnredlist.org [accessed 22 January 2009].

Jones DL. 1993. *Cycads of the world*. Frenchs Forest Reed Books.

Khoshoo TN. 1959. Polyploidy in gymnosperms. *Evolution* **13:** 24–39.

Klak C, Reeves G, Hedderson T. 2004. Unmatched tempo of evolution in southern African semi-desert ice plants. *Nature* **427:** 63–65.

Klavins SD, Kellogg DW, Krings M, Taylor EL, Taylor TN. 2005. Coprolites in a Middle Triassic cycad pollen cone: evidence for insect pollination in early cycads? *Evolutionary Ecology Research* **7:** 479–488.

van Konijnenburg-van Cittert JHA. 2008. The Jurassic fossil plant record of the UK area. *Proceedings of the Geologists Association* **119:** 59–72.

Labandeira CC, Kvacek J, Mostovski MB. 2007.

Pollination drops, pollen, and insect pollination of Mesozoic gymnosperms. *Taxon* **56:** 663–695.

Lee J-Y, Mummenhoff K, Bowman JL. 2002. Allopolyploidization and evolution of species with reduced floral structures in *Lepidium* L. (Brassicaceae). *Proceedings of the National Academy of Sciences of the United States of America* **99:** 16 835–16 840.

Leitch IJ, Hanson L, Winfield M, Parker J, Bennett MD. 2001. Nuclear DNA C-values complete familial representation in gymnosperms. *Annals of Botany* **88:** 843–849.

Levin DA. 2000. *The origin, expansion, and demise of plant species*. New York: Oxford University Press.

Levin DA. 2002. *The role of chromosomal change in plant evolution*. Oxford: Oxford University Press.

Levin DA, Wilson AC. 1976. Rates of evolution in seed plants: net increase in diversity of chromosome numbers and species numbers through time. *Proceedings of the National Academy of Sciences of the United States of America* **73:** 2086–2090.

Levinton JS. 2001. *Genetics, paleontology, and macroevolution*, 2nd edn. Cambridge: Cambridge University Press.

Li P, Johnston MO. 2000. Heterochrony in plant evolutionary studies through the twentieth century. *Botanical Review* **66:** 57–88.

Liu B, Wendel JF. 2003. Epigenetic phenomena and the evolution of plant allopolyploids. *Molecular Phylogenetics and Evolution* **29:** 365–379.

Liu B, Hu B, Dong YZ, Liu ZL, He MY. 2000. Speciation-induced heritable cytosine methylation changes in polyploid wheat. *Progress in Natural Science* **10:** 601–606.

Liu ZQ, Lin SL. 2005. Multilocus LD measure and tagging SNP selection with generalized mutual information. *Genetic Epidemiology* **29:** 353–364.

Lukens LN, Pires JC, Leon E, Vogelzang R, Oslach L, Osborn T. 2006. Patterns of sequence loss and cytosine methylation within a population of newly resynthesized *Brassica napus* allopolyploids. *Plant Physiology* **140:** 336–348.

Lynch M. 2007. *The origins of genome architecture*. Sunderland, MA: Sinauer Associates.

McKey D. 1975. The ecology of coevolved seed dispersal systems. In: Gilbert LE, Raven PH, eds. *Coevolution of animals and plants*. Austin, TX: University of Texas Press, 159–191.

Magallón S, Crane PR, Herendeen PS. 1999. Phylogenetic pattern, diversity, and diversification of eudicots. *Annals of the Missouri Botanical Garden* **86:** 297–372.

Masterson J. 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science* **264:** 421–424.

Matzke MA, Matzke AJM. 1998. Polyploidy and transposons. *Trends in Ecology and Evolution* **13:** 241.

Moretti A. 1990. Karyotypic data on North and Central American Zamiaceae (Cycadales) and their phylogenetic implications. *American Journal of Botany* **77:** 1016–1029.

Moretti A, Sabato S. 1984. Karyotype evolution by centromeric fission in *Zamia* (Cycadales). *Plant Systematics and Evolution* **146:** 215–223.

Moretti A, Caputo P, Gaudio L, Stevenson DW. 1991. Intraspecific chromosome variation in *Zamia* (Zamia, Cycadales). *Caryologia* **44:** 1–10.

Moretti A, Caputo P, Cozzolino S, Gaudio L. 1993. Karyotypes of new world cycads. In: Stevenson DW, Norstog KJ, eds. *The biology, structure, and systematics of the Cycadales*. Milton, Qld: Palm & Cycad Societies of Australia, 263–269.

Müller GB, Newmann SA. 2003. Origination of organismal form: the forgotten cause in evolutionary theory. In: Müller GB, Newmann SA, eds. *Origination of organismal form: beyond the gene in developmental and evolutionary biology*. Cambridge, MA: MIT Press, 3–10.

Murray BG, Leitch IJ, Bennett MD. 2004. Gymnosperm DNA C-values database (release 3.0, Dec 2004). Available at http://www.kew.org/cvalues/homepage.html December 2004 ed.

Napolitano A, Caputo P, Moretti A. 2004. Karyology, phytogeography, and the origin of intraspecific karyotype variation in *Zamia paucijuga* and *Zamia polymorpha* (Zamiaceae). *Delpinoa* **46:** 71–83.

Nixon KC, Crepet WL, Stevenson DW, Friis EM. 1994. A reevaluation of seed plant phylogeny. *Annals of the Missouri Botanical Garden* **81:** 484–533.

Norstog KJ. 1993. Spermatogenesis in *Microcycas*: evolutionary significance of male gametes of seed plants. In: Stevenson DW, Norstog KJ, eds. *The biology, structure, and systematics of the Cycadales*. Milton, Qld: Palm & Cycad Societies of Australia, 270–278.

Norstog KJ, Nicholls TJ. 1997. *The biology of cycads*. Ithaca, NY: Cornell University Press.

Norstog KJ, Stevenson DW, Niklas KJ. 1986. The role of beetles in pollination of *Zamia furfuracea* L. fil. (Zamiaceae). *Biotropica* **18:** 300–306.

Ohno S. 1970. *Evolution by gene duplication*. Berlin: Springer-Verlag.

Ohri D, Khoshoo TN. 1986. Genome size in gymnosperms. *Plant Systematics and Evolution* **153:** 119–132.

Olson K, Gorelick R. 2011. Chromosomal fission accounts for small-scale radiations in *Zamia* (Zamiaceae; Cycadales). *Botanical Journal of the Linnean Society* **102:** 168–185.

Olson ME. 2007. Wood ontogeny as a model for studying heterochrony, with an example of paedomorphosis in *Moringa* (Moringaceae). *Systematics and Biodiversity* **5:** 145–158.

Ornduff R. 1985. Male biased sex ratios in the cycad *Macrozamia reidlei* (Zamiaceae). *Bulletin of the Torrey Botanical Club* **112:** 393–397.

Ornduff R. 1990. Geographic variation in reproductive behavior and size structure of the Australian cycad *Macrozamia communis* (Zamiaceae). *American Journal of Botany* **77:** 92–99.

Osborn TC, Pires JC, Birchler JA, Auger DL, Chen ZJ, Lee H-S, Comai L, Madlung A, Doerge RW, Colot V, Martienssen RA. 2003. Understanding mechanisms of novel gene expression in polyploids. *Trends in Genetics* **19:** 141–147.

Osborne R, Gorelick R. 2007. Sex change in cycads: cases,

causes, and chemistry. *Memoirs of the New York Botanical Garden* **97:** 335–345.

Otto SP, Lenormand T. 2002. Resolving the paradox of sex and recombination. *Nature Reviews Genetics* **3:** 252–261.

Otto SP, Whitton J. 2000. Polyploid incidence and evolution. *Annual Review of Genetics* **34:** 401–437.

Pant DD. 1987. The fossil history of the Cycadales. *Geophytology* **17:** 125–162.

Paun O, Fay MF, Soltis DE, Chase MW. 2007. Genetic and epigenetic alterations after hybridization and genome doubling. *Taxon* **56:** 649–656.

Pellmyr O, Tang W, Groth I, Bergström G, Thien LB. 1991. Cycad cone and angiosperm floral volatiles: inferences for the evolution of insect pollination. *Biochemical Systematics* **19:** 623–627.

Poort RJ, Visscher H, Dilcher DL. 1996. Zoidogamy in fossil gymnosperms: the centenary of a concept, with special reference to prepollen of late Paleozoic conifers. *Proceedings of the National Academy of Sciences of the United States of America* **93:** 11 713–11 717.

Rai HS, O'Brien HE, Reeves PA, Olmstead RG, Graham SW. 2003. Inference of higher-order relationships in the cycads from a large chloroplast data set. *Molecular Phylogenetics and Evolution* **29:** 350–359.

Ramsey J, Schemske DW. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* **29:** 467–501.

Rapp RA, Wendel JF. 2005. Epigenetics and plant evolution. *New Phytologist* **168:** 81–91.

Roth G, Nishikawa KC, Naujoksmanteuffel C, Schmidt A, Wake DB. 1993. Paedomorphosis and simplification in the nervous system of salamanders. *Brain Behavior and Evolution* **42:** 137–170.

Roth G, Nishikawa KC, Wake DB. 1997. Genome size, secondary simplification, and the evolution of the brain in salamanders. *Brain Behavior and Evolution* **50:** 50–59.

Rothwell GW, Crepet WL, Stockey RA. 2009. Is the anthophyte hypothesis alive and well? New evidence from the reproductive structures of Bennettitales. *American Journal of Botany* **96:** 296–322.

Rowley GD. 2007. Cytotypes: a case for infraspecific names. *Taxon* **56:** 983.

Rydin C, Pedersen KR, Crane PR, Friis EM. 2006. Former diversity of *Ephedra* (Gnetales): evidence from Early Cretaceous seeds from Portugal and North America. *Annals of Botany* **98:** 123–140.

Samigullen TK, Martin WF, Troitsky AV, Antonov AS. 1999. Molecular data from the chloroplast *rpoC1* gene suggest deep and distinct dichotomy of contemporary spermatophytes into two monophyla: gymnosperms (including Gnetales) and angiosperms. *Journal of Molecular Evolution* **49:** 310–315.

Saylor LC, Simons HA. 1970. Karyology of *Sequoia sempervirens*: karyotype and accessory chromosomes. *Cytologia* **35:** 294–303.

Schluter D. 2000. *The ecology of adaptive radiation*. Oxford: Oxford University Press.

Scotland RW, Wortley AH. 2003. How many species of seed plants are there? *Taxon* **52:** 101–104.

Serbet R, Rothwell GW. 1995. Functional morphology and homologies of gymnospermous ovules: evidence from a new species of *Stephanospermum* (Medullosales). *Canadian Journal of Botany-Revue Canadienne de Botanique* **73:** 650–661.

Simillion C, Vandepoele K, Van Montagu MCE, Zabeau M, Van de Peer Y. 2002. The hidden duplication past of *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America* **99:** 13 627–13 632.

Smith CI, Pellmyr O, Althoff DM, Balcázar-Lara M, Leebens-Mack J, Segraves KA. 2008. Pattern and timing of diversification in *Yucca* (Agavaceae): specialized pollination does not escalate rates of diversification. *Proceedings of the Royal Society B: Biological Sciences* **275:** 249–258.

Soltis DE, Soltis PS. 1993. Molecular data and the dynamic nature of polyploidy. *Critical Reviews in Plant Sciences* **12:** 243–273.

Soltis DE, Soltis PS, Zanis MJ. 2002. Phylogeny of seed plants based on evidence from eight genes. *American Journal of Botany* **89:** 1670–1681.

Soltis DE, Soltis PS, Schemske DW, Hancock JF, Thompson JN, Husband BC, Judd WS. 2007. Autopolyploidy in angiosperms: have we grossly underestimated the number of species? *Taxon* **56:** 13–30.

Soltis DE, Bell CD, Kim S, Soltis PS. 2008. Origin and early evolution of angiosperms. *Annals of the New York Academy of Sciences* **1133:** 3–25.

Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng CF, Sankoff D, dePamphilis CW, Wall PK, Soltis PS. 2009. Polyploidy and angiosperm diversification. *American Journal of Botany* **96:** 336–348.

Soltis PS, Soltis DE. 2004. The origin and diversification of angiosperms. *American Journal of Botany* **91:** 1614–1626.

Stebbins GL. 1950. *Variations and evolution in plants*. New York: Columbia University Press.

Stebbins GL. 1966. *Processes of organic evolution*. Englewood Cliffs, NJ: Prentice-Hall.

Stebbins GL. 1971. *Chromosomal evolution in higher plants*. London: Edward Arnold.

Stevenson DW. 1992. A formal classification of the extant cycads. *Brittonia* **44:** 220–223.

Sun G, Ji Q, Dilcher DL, Zheng SL, Nixon KC, Wang XF. 2002. Archaefructaceae, a new basal angiosperm family. *Science* **296:** 899–904.

Tang W. 1987. Insect pollination in the cycad *Zamia pumila* (Zamiaceae). *American Journal of Botany* **74:** 90–99.

Tang W. 1990. Reproduction in the cycad *Zamia pumila* in a fire-climax habitat: an eight-year study. *Bulletin of the Torrey Botanical Club* **117:** 368–374.

Taylor AS, Mendieta J, Bernal R, Silvera G. 2008. A never-before-reported characteristic of *Zamia pseudoparasitica*. *Cycad Newsletter* **31:** 8–9.

Taylor TN, Taylor EL, Krings M. 2009. *Paleobotany: the biology and evolution of fossil plants*. Amsterdam: Elsevier.

Terry I. 2001. Thrips and weevils as dual, specialist pollinators of the Australian cycad *Macrozamia communis* (Zamiaceae). *International Journal of Plant Sciences* **162:** 1293–1305.

Terry I, Moore CJ, Walter GH, Forster PI, Roemer RB, Donaldson JD, Machin PJ. 2004. Association of cone thermogenesis and volatiles with pollinator specificity in *Macrozamia* cycads. *Plant Systematics and Evolution* **243:** 233–247.

Thomas HH, Bancroft N. 1913. On the cuticles of some recent and fossil cycadean fronds. *Transactions of the Linnean Society, Botany* **8:** 155–204.

Thompson JN, Merg KF. 2008. Evolution of polyploidy and the diversification of plant–pollinator interactions. *Ecology* **89:** 2197–2206.

Todd NB. 1970. Karyotypic fissioning and canid phylogeny. *Journal of Theoretical Biology* **26:** 445–480.

Watson J. 1977. Some lower Cretaceous conifers of the cheirolepidiaceae from the U.S.A. and England. *Palaeontology* **20:** 715–749.

Watson J. 1988. The cheirolepidiaceae. In: Beck CB, ed. *Origin and evolution of gymnosperms*. New York: Columbia University Press, 382–447.

Wendel JF, Doyle J. 2005. Polyploidy and evolution in plants. In: Henry RJ, ed. *Plant diversity and evolution: genotypic and phenotypic variation in higher plants*. Wallingford, Oxfordshire: CABI Publishing, 97–117.

Wendt T. 1993. A new variety of *Ephedra torreyana* (Ephedraceae) from west Texas and Chihuahua, with notes on hybridization in the *E. torreyana* complex. *Phytologia* **74:** 141–150.

Williams JH. 2008. Novelties of the flowering plant pollen tube underlie diversification of a key life history stage. *Proceedings of the National Academy of Sciences of the United States of America* **105:** 11 259–11 263.

Yang Y, Geng BY, Dilcher DL, Chen ZD, Lott TA. 2005. Morphology and affinities of an early Cretaceous *Ephedra* (Ephedraceae) from China. *American Journal of Botany* **92:** 231–241.

Zhang Z-M, Sodmergen, Li Z-L. 1997. Micronucleus formation in microspores of *Ginkgo biloba* and its significance in evolution. *Acta Botanica Sinica* **39:** 97–101.

Zhifeng G, Thomas BA. 1989. A review of fossil cycad megasporophylls, with new evidence of *Crossozamia* Pomel and its associated leaves from the Lower Permian of Taiyuan, China. *Review of Palaeobotany and Palynology* **60:** 205–223.

Zhou RJ, Cheng HH, Tiersch TR. 2002. Differential genome duplication and fish diversity. *Reviews in Fish Biology and Fisheries* **11:** 331–337.