Evolutionary rates of nuclear and organellar genomes are linked in land plants

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Abstract

Plants carry genetic material in three separate compartments, the nuclear, mitochondrial, and chloroplast genomes. These genomes interact with each other to various degrees and are subject to shared evolutionary drivers exerted by their host organisms. Although animal mitochondrial and nuclear genomes display linked evolutionary rates, a well-documented phenomenon termed “mito-nuclear covariation”, it is not clear whether the three plant genomes display covarying evolutionary signals on a broad scale. We tested for correlated evolutionary rates between nuclear and organellar genomes using extensive data sets from the major clades of land plants (Embryophyta), including mosses, ferns, gymnosperms, and angiosperms. To examine the evolutionary dynamics in parasitic angiosperms, which are under distinctive selective pressures, we also analysed data sets from mistletoes, broomrapes, sandalwoods, and rafflesias. Evolutionary rates of nuclear and organellar genomes were positively linked in each group of land plants tested, except in the parasitic angiosperms. We also found positive correlations between rates of nonsynonymous and synonymous change. Our results reveal extensive evolutionary rate variation across land plant taxa, particularly in mitochondrial genomes in angiosperms. Overall, we find that nuclear, mitochondrial, and chloroplast genomes in land plants share similar drivers of mutation rates, despite considerable variation in life history, morphology, and genome sizes among clades. Our findings lay the foundation for further exploration of the impact of co-evolutionary interactions on shared evolutionary rates between genomes.

Introduction

Land plants (embryophytes) colonized the terrestrial environment between 583 and 459 Myr ago (Morris et al. 2018; Nie et al. 2020) and have diversified into an estimated 435,000 living species (Enquist et al. 2019), filling almost every ecological niche on Earth (Fig. 1). Whilst land plants display an immense range of morphological, ecological, and cellular differences, almost all have their genetic content carried in three separate genomes, found in their nuclei, mitochondria, and chloroplasts. Mitochondria are responsible for responsible for critical cell functions such as cellular respiration, immune function, and oxidative phosphorylation (Sloan et al. 2014; Zervas et al. 2019; Wang et al. 2022). Chloroplasts are primarily responsible for photosynthesis, transforming light and water into glucose (Martin and Herrmann 1998). Unlike the nuclear genome, chloroplasts and mitochondrial genomes are much smaller, responsible for far fewer cell functions, effectively haploid, unable to sexually recombine, and are uniparentally transmitted, usually maternally (but see Greiner et al. 2015 for exceptions).

Considerable evolutionary and structural variation can be seen across the nuclear genomes of land plants, with extensive degrees of genome duplication and polyploidy. For instance, the largest known genome (1*C* = 160.45 Gb) occurs in an octoploid New Caledonian fork fern, *Tmesipteris oblanceolata* (Fernández et al. 2024). Polyploidy is likely to have provided an important foundation for the phenotypic novelties that allowed the flowering plants to radiate and speciate (Soltis et al. 2019; Benton et al. 2022). Plant mitochondrial genomes share the same endosymbiotic origin, cellular function, and uniparental transmission as those of animals, yet they exhibit a range of structures, from circular to branched, are much larger (0.2–20 Gb), and typically have very low rates of nucleotide substitution (Wolfe et al. 1987; Drouin et al. 2008; Chen et al. 2017; Morley and Nielsen 2017). In contrast, chloroplast genomes are remarkably conserved in size, structure, and content across land plants. They have much lower substitution rates than nuclear genomes in land plants (Wolfe et al. 1987; Drouin et al. 2008; Smith 2015) Chloroplast genomes are circular, 110–200 kb in length, and include 80–90 protein-coding genes, four ribosomal RNA genes, and 30–31 transfer RNA genes (Kaur et al. 2022). However, there are massive reductions in plastome content in parasitic angiosperms (Graham et al. 2017; Zervas et al. 2019). This reduction reaches its most extreme in the family Rafflesiaceae, where two species have lost their entire chloroplast genome (Molina et al. 2014; Cai et al. 2021). Parasitic flowering plants are ‘outliers’ to the remaining land plants in other aspects; for instance, there are vastly accelerated rates of mitochondrial evolution in *Viscum* (mistletoes) along with gene loss. (Havird et al. 2019; Zervas et al. 2019).

Although there are great differences in size, structure, and content between nuclear and organellar genomes, there are well-documented interactions between these genomic compartments. Coordinated change between the nucleus and mitochondria may be integral for survival; this manifests as mito-nuclear covariation in animals, where the accumulation of deleterious mutations in mitochondrial genomes is compensated for by mutations in the nucleus (Martin and Herrmann 1998; Weaver et al. 2022). The mitochondria have protein complexes that consist of subunits variously encoded by the mitochondrial and nuclear proteins, and these subunits need to interact properly to

ensure full function (Martin and Herrmann 1998; Weaver et al. 2022). This is particularly evident in males, where mutations that impede fertility in males are non-functional in females, and are retained in the population despite their deleterious effects (David et al. 2022). Furthermore, hybrids in various animals show reduced fitness because of incompatibilities between maternally inherited mitochondrial genes and paternally inherited nuclear-encoded mitochondrial proteins (Burton et al. 2013; Sloan et al. 2017). Thus, mito-nuclear covariation can act as a post-zygotic barrier on the path to speciation (Greiner et al. 2015; Hill 2016). This is also demonstrated in hybrid plants, where expression of chimeric open reading frames in the mitochondria causes cytoplasmic male sterility, which results in a failure to produce functional pollen (Bentolila et al. 2002; Delph et al. 2007; Fujii et al. 2011). Only when there are compensatory changes in nuclear-encoded proteins can there be restoration of male fertility.

Given the co-evolutionary interactions between the nuclear and organellar genomes, we would expect their evolutionary rates to show some level of association. Such rate correlations have been observed in the nuclear and mitochondrial genomes of crustaceans, bivalves, mammals, yeast, and insects (Barreto et al. 2018; Yan et al. 2019; Piccinini et al. 2021; Formaggioni et al. 2022; Weaver et al. 2022; Biot-Pelletier et al. 2023; Tao et al. 2024). However, there has been mixed evidence of evolutionary rate correlations across nuclear and organellar genomes in plants, with some researchers discounting the existence of coevolutionary interactions between nuclear and chloroplast genomes (Hill 2020). Recent studies have found strong signals for rate correlations in data sets comprising hundreds of nuclear and chloroplast genes from 20–25 angiosperm taxa (Williams et al. 2019; Forsythe et al. 2021), as well as evidence for linkage disequilibrium in nuclear and mitochondrial genes across 3439 genomes of seven angiosperm species (Lian et al. 2024). Similar correlations have been detected in studies of individual genera and families of angiosperms (Zhang et al. 2015; Rockenbach et al. 2016; Yang et al. 2023; Khachaturyan et al. 2024). Neverthless, the amount of co-ordination between nuclear, mitochondrial, and chloroplast genomes has not been fully explored across the diversity of land plants. This leaves uncertainty about whether the interactions between the three genomes have left broad-scale signatures of covariaton in their evolutionary rates.

In this study, we characterize evolutionary rate variation and test for correlations in rates of nuclear, mitochondrial, and chloroplast genome evolution using extensive data sets from major lineages of land plants. We first conduct a simulation study to validate the performance of three phylogenetic methods used to test for evolutionary rate correlations. We then use these methods to analyse data sets of mosses, ferns, gymnosperms, and angiosperms. Our analyses reveal strong evolutionary rate correlations between nuclear, mitochondrial, and chloroplast genes across major lineages of land plants. In contrast, these patterns are not detected in parasitic angiosperms, perhaps a consequence of their unique habit and reduced genomes.

Results

Evolutionary rates across land plants

Our analyses revealed considerable evolutionary rate variation in nuclear and chloroplast genomes across the major lineages of land plants (Fig. 2A): mosses, ferns, gymnosperms, and angiosperms. We estimated evolutionary rates by dividing the mean root-to-tip distance of each phylogenetic tree by the estimated age of its root node (*SI Appendix*, Table S1). Given that the age of the angiosperms continues to be debated, with molecular estimates ranging from the Late Jurassic to the Permian (Sauquet et al. 2022), we used multiple estimates for the crown-age of this group (Fig. 2A, *SI Appendix*, Table S2). Estimated evolutionary rates of nuclear genomes ranged from 4.46×10^{-4} substitutions/site/Myr in gymnosperms to 2.16×10^{-3} substitutions/site/Myr in angiosperms. In contrast, the estimated evolutionary rate of chloroplast genomes varied from 4.56×10^{-4} substitutions/site/Myr in gymnosperms to 9.67×10^{-4} substitutions/site/Myr in ferns. Rates of mitochondrial genome evolution were substantially lower than those of nuclear and chloroplast genomes, ranging from 2.33×10^{-4} substitutions/site/Myr in mosses to 4.69×10^{-4} substitutions/site/Myr in angiosperms.

Across four major lineages of land plants, we identified disparate levels of among-lineage rate variation as measured by the coefficient of variation of rates (σ) (Fig. 2B). This metric was calculated in a Bayesian phylogenetic analysis by dividing the standard deviation of the branch rates by the mean of the branch rates, with values close to zero indicating clocklike evolution (Drummond et al. 2006). In the major lineages of land plants, the nuclear and chloroplast data sets had similar levels of moderate rate heterogeneity, with mean σ ranging from 0.694 to 1.11 across all groups (Fig. 2B, *SI Appendix*, Table S3). In angiosperms, the mitochondrial mean σ was 1.52 (95% CI 1.35–1.71), reflecting an extreme degree of rate variation among lineages. Among parasitic angiosperms, σ was especially pronounced in nuclear genes of Rafflesiaceae (σ = 1.60, 95% CI 0.702–2.59), mitochondrial genes in *Loranthus* (σ = 1.40, 95% CI 1.09–1.76), and chloroplast genes in Orobanchaceae (σ = 1.41, 95% CI 0.961–1.92). Concordant results were obtained when we estimated the degree of among-lineage rate heterogeneity using the coefficient of variation of root-to-tip distances in maximum-likelihood phylogenetic trees (*SI Appendix*, Fig. S1, Table S3).

Evolutionary rate correlations between genomes

We tested for evolutionary rate correlations across genomic compartments of the major land plant lineages using available genomic data sets (*SI Appendix*, Table S1). We began by conducting a comprehensive simulation study to validate three methods of analysis (described fully in *SI Appendix*): (i) correlations of root-to-tip distances, based on maximum-likelihood phylograms; (ii) independent sister-pair contrasts, based on maximum-likelihood phylograms; and (iii) correlations of branch rates inferred using Bayesian relaxed clocks. We found that all three methods were accurate, with false positives detected across an average of 3.6% of replicates, and that the methods were moderately powerful, with the average detection of true correlations at 88.4% (*SI Appendix*, Fig. S2–S7). The

independent sister-pair contrasts method was the most conservative, being unable to detect any rate correlations when there were only 18 ingroup taxa in the simulations. In these cases, there were only a small number of sister-pair contrasts (n=3) (*SI Appendix*, Fig. S2, S6). When the 18-taxon simulation setting was excluded for independent sister-pair contrasts, the average detection of true correlations overall increased to 99.5% across the three methods. Accordingly, we focused on sampling data sets that had more than 20 ingroup taxa (*SI Appendix*, Table S1). We found that the third method, correlations of branch rates inferred using Bayesian relaxed clocks, had the greatest accuracy and power (*SI Appendix,* Fig. S3, S7). Thus, we focus on the results obtained using this method, although the two other methods yielded concordant results (*SI Appendix*, Fig. S8–S10).

We found consistently strong, significant correlations between evolutionary rates of nuclear and chloroplast genomes (Fig. 3; $r_s \geq 0.48$ and $p \leq 10^{-8}$ for all comparisons). This result was found across all of the major land plant lineages that we assessed. Similarly, there was a significant association between evolutionary rates of nuclear and mitochondrial genomes (*SI Appendix*, Fig. S8; $r_s \geq 0.25$ and $p \leq 10^{-4}$) and between almost all mitochondrial and chloroplast genomes (*SI Appendix*, Fig. S8; $r_s \geq 0.28$ and $p < 0.05$). All tested associations were significant, with the exception of angiosperm mitochondrial and chloroplast genomes, which was weakly positive but statistically insignificant ($r_s = 0.063$, $p = 0.11$). We were also unable to detect an evolutionary rate correlation between mitochondrial and chloroplast genomes in a clade of marine algae, closely related to land plants, Chlorophyta (*SI Appendix*, Fig. S11, $r_s = 0.18$ and $p = 0.13$).

To gain additional insight into the nature of the rate correlations, we performed further analyses of protein-coding sequences by separating the 1st+2nd and 3rd codon sites. We treated these as proxies for nonsynonymous and synonymous evolutionary changes, respectively. These analyses were possible for three data sets: mosses, ferns, and angiosperms. For both nonsynonymous and synonymous rates, we detected correlations between nuclear and mitochondrial genomes (Table 1; $r_s \geq 0.12$ and $p \leq 0.024$) and between nuclear and chloroplast genomes (Table 1; $r_s \geq 0.30$ and $p \leq 0.01$). Similarly, almost all comparisons of evolutionary rates between mitochondrial and chloroplast genomes detected significant associations (Table 1). However, we were unable to detect a correlation in nonsynonymous rates ($r_s = 0.033$ and $p = 0.26$) and synonymous rates ($r_s = 0.037$ and $p = 0.24$) between the mitochondrial and chloroplast genomes of angiosperms, despite the large sample size ($n = 185$). The results were concordant with those obtained using the two other methods that we employed (*SI Appendix*, Table S4–S5). The ability to detect significant associations was reduced when we used sister-pair contrasts, as expected from the results of our simulations. However, all tested comparisons were significant when using root-to-tip distances, which was not unexpected, given the slightly inflated detection of rate correlations when using this method shown in our simulations (*SI Appendix*).

Uncoupled evolutionary rates in parasitic flowering plants

We tested for evolutionary rate correlations across the nuclear, mitochondrial, and chloroplast genomes of four groups of parasitic angiosperms. For these analyses, we targeted the most recent

genomic or multilocus studies with large taxon sampling (full description in *SI Appendix*). Our analyses failed to detect any associations in evolutionary rates between nuclear and chloroplast genomes in Orobanchaceae, *Thesium*, and *Loranthus*, and between nuclear and mitochondrial genomes in Rafflesiaceae (Fig. 4; $p > 0.90$). The results were largely concordant with those obtained using the two other methods that we employed (*SI Appendix*, Fig. S12–S14). The only discrepancy was in *Loranthus*, for which there was a correlation between the root-to-tip distances ($p = 0.04$); however, this comparison was based on few taxa ($n = 13$), and the root-to-tip method yielded 35% false positives in our simulation study when there were only 18 taxa in the data set.

Discussion

Evolutionary rate correlations between genomes in land plants

Our study has shown that evolutionary rates are positively correlated between the nuclear, mitochondrial, and chloroplast genomes in the major lineages of land plants. These correlations were detected using various methods of analysis that we validated through an extensive simulation study. The pervasive signal of rate correlations across such diverse lineages is remarkable, given their ancient age (e.g., bryophyte mosses arose 424–416 Ma; Bechteler et al. 2023) and their disparate life histories and ecologies. Contrastingly, we could not detect any evolutionary rate correlations in parasitic angiosperms. Indeed, parasitic angiosperms feature a variety of distinctive morphological and cellular traits that demarcate them from the rest of the flowering plants. For instance, the 'parasite reduction syndrome' phenomenon describes the convergent loss of vegetative tissue and photosynthetic organs and genes in parasitic flowering plants (Sanchez-Puerta et al. 2023).

Evolutionary rate correlations are consistent with the broad effects of cytonuclear covariation and interaction in land plants. These results are in line with the presence of mito-nuclear covariation in animals (Weaver et al. 2022). There is evidence that coordination between the nuclear and organellar genomes in plants is vital to fundamental processes in plant cells, including ATP synthesis, oxidative phosphorylation, and photosynthesis (Sloan et al. 2014). For instance, in flowering plants, 2000 proteins are contained in the mitochondria, but fewer than 41 of these are encoded by the mitochondrial genome (Chen et al. 2017). The vast majority of proteins are encoded in the nucleus and transferred into the mitochondria by transit peptides. The nuclear genome can thus generate regulatory factors required for effective mitochondrial function (Chen et al. 2017; Hill 2020; Wang et al. 2022). This is particularly important in the mitochondrial response to pathogen infection, activating programmed cell death, and immune function (Wang et al. 2022).

Gene exchange between genomic compartments might be partly driving the evolutionary rate correlation that we have uncovered. There are clear instances of bidirectional movement between the nuclear and mitochondrial genomes; for example, up to half of the maize mitochondrial genome is nuclear in origin (Chen et al. 2017). The movement of mitochondrial genes into the chloroplast genome appears to be much less profuse. The conserved chloroplast genome is more likely to transfer its genes to the nuclear and mitochondrial genomes, but unable to take up foreign organellar

DNA (Chen et al. 2017; Hill 2020). This is consistent with the uniformity in size and structure of the chloroplast genome in land plants. Furthermore, the sexually recombining nuclear genome has taken up hundreds of genes from the organellar compartments, as well as controlling and regulating many of the functions of the mitochondria and chloroplasts (Hill 2003; Sloan et al. 2013). These features of genomic interaction suggest a weaker association between the evolutionary rates of mitochondrial and chloroplast genomes. However, if evolutionary rates in the mitochondrial and chloroplast genomes are both strongly linked to that of the nuclear genome, an indirect association can emerge between the rates of the two organellar genomes. This is consistent with the strength of the rate correlation being somewhat reduced between the two organellar compartments in ferns, angiosperms, gymnosperms.

Our findings may also explain why higher rates of chloroplast substitution are linked to diversification in the angiosperm family Proteaceae (Duchene and Bromham 2013). A higher rate of change in the chloroplast genome predicts rapid changes in the nuclear and mitochondrial genomes, potentially driving adaptation, reproductive isolation, and eventually speciation. This is similar to findings in vertebrate and invertebrate animals, where mito-nuclear covariation is a key factor in bolstering speciation (Hill 2016; Wang et al. 2021; Weaver et al. 2022). Furthermore, a similar link between substitution rate and diversification has been found across many angiosperm clades (Lancaster 2010; Bromham et al. 2015).

Shared drivers of evolutionary rates

Although cytonuclear interactions are expected to produce some degree of evolutionary covariation between the nuclear and organellar genomes, the observed correlations in evolutionary rates might be also due to a shared response by all three genomic compartments to some other factor. For example, the genomes might be responding to the same selection pressures or might have a shared system of DNA repair (Williams et al. 2019). We looked for signatures of these factors by performing separate tests of correlations for nonsynonymous and synonymous sites. We found correlations between the nonsynonymous rates of nuclear and organellar genomes, consistent with positive selection and genomic interactions driving the shared variation in evolutionary rates. However, we also found correlations between the synonymous rates of nuclear and organellar genomes, suggesting that there is a shared driver of mutation rates across the three genomic compartments. This is supported by the results of a previous study that found correlated rates at synonymous sites, but not at nonsynonymous sites (Bromham et al. 2015). However, our results suggest that the patterns of mutation rate variation are also being propagated to nonsynonymous rates, where a weaker correlation is apparent across the three genomes. The results of our analyses, conducted at a broad scale on hundreds of nuclear genes and tens of organellar genes, contradict those of previous studies that only found correlations between mitochondrial- and nuclear-encoded mitochondrial genes known to interact (Yan et al. 2019; Piccinini et al. 2021; Weaver et al. 2022).

Previous studies have identified a number of potential drivers of mutation rates in plant genomes. For instance, generation time is closely linked to long-term rates of meiosis in plants, since each elapsed generation has undergone a meiotic cycle. Thus, generation time might be driving the mutation rates of all three genomes (Petit and Hampe 2006). Also, plant height is closely linked to generation time, and height has been found to be strongly associated with the rate of molecular evolution (Lanfear et al. 2013). This may be because taller plants have slower growth in their apical meristem and so undergo fewer cycles of mitosis (Li et al. 1996; Lanfear et al. 2013). Therefore, fewer mutations are inherited by offspring, effectively reducing the evolutionary rates of taller plants.

Environmental factors such as temperature can potentially act as mutagens in plants (Davies et al. 2004; Quiroz et al. 2023). This has been shown by experiments in *Arabidopsis thaliana*, where multi-generational exposure to higher temperature led to accumulation of mutations in genes involved in DNA repair, defense repair, and signalling, as well as increasing the mutation rate (Belfield et al. 2021; Lu et al. 2021). UV irradiation is another well-documented mutagen in plants (Davies et al. 2004; Quiroz et al. 2023), leading to oxidative DNA damage as demonstrated in Cucurbitaceae (Watanabe et al. 2006). These environmental drivers of mutation would potentially act on all three genomes in land plants.

Population size is also likely to have a broad impact on the evolutionary dynamics of nuclear, mitochondrial, and chloroplast genomes. There is less efficient selection against mutations in small populations, leading to a reduction in the efficiency of DNA repair machinery and ultimately to elevated mutation rates across the three genomes (Bergeron et al. 2023). There is evidence of unique systems of DNA repair in the nuclear, mitochondrial, and chloroplast genomes. For instance, mitochondria lack nucleotide excision repair, whereas chloroplasts are unable to fix double-strand breaks using non-homologous end joining (Boesch et al. 2011). Genome copy number and mutation rate are negatively linked in mitochondria, possibly so that unmutated copies can perform repairs on double-strand breaks, but this pattern is not seen in chloroplasts (Zwonitzer et al. 2024). Alternatively, there is mounting evidence for strong links between the epigenome and mutation rates in plants (Martincorena and Luscombe 2013; Quiroz et al. 2023). Further work on DNA repair systems in plants is needed to clarify their role in mutation rate variation across nuclear and organellar genomes.

Evolutionary rates in land plants

Across land plants, we found that nuclear genomes have evolved the most rapidly, followed by chloroplast genomes and mitochondrial genomes. These results are consistent with those of previous studies (Wolfe et al. 1987; Drouin et al. 2008; Smith 2015). Overall, land plant nuclear genomes exhibit highly dynamic evolution, with the largest genomes 2400 times the size of the smallest; nuclear genomes also undergo rampant polyploidization in land plants (Pellicer et al. 2018). Furthermore, nuclear genomes are responsible for most cell functions, encoding between 25,000–40,000 genes (Heslop-Harrison and Schmidt 2012). Thus, their fast evolutionary rate, when compared to the reduced chloroplast and mitochondria, is not unexpected. The low evolutionary rates of nuclear and organellar genomes in gymnosperms are consistent with the long generations of these taxa

(Leitch and Leitch 2012; De La Torre et al. 2017). Whilst many angiosperms can complete their life cycle in mere weeks, most gymnosperms take years to finish a single cycle.

There was a moderate amount of among-lineage rate heterogeneity overall in nuclear and chloroplast genes in the major land plant clades. Our results are consistent with previous findings of high rate heterogeneity among lineages in angiosperm mitochondrial genomes. Whilst plants have drastically slowed rates of mitochondrial evolution, the angiosperm mitochondrial genome is dynamic and exhibits highly variable evolutionary rates (Galtier 2011; Havird et al. 2017; Petersen et al. 2020). The inflated rates of mitochondrial evolution in lineages such as *Plantago*, *Silene*, and *Viscum* have been attributed to genetic transfer from bacteria, viruses, and the nuclear and chloroplast genomes (Zervas et al. 2019). Indeed, the phenomenon of mitochondrial fusion is exhibited by *Amborella trichopoda*, the sister taxon to all other angiosperms (Rice et al. 2013). The mitochondrial genome of this species has fused with the whole genomes of three green algae and a moss species, and has received 82 copies of protein and rRNA via horizontal transfer from other angiosperm species . Further insights will be gained from analysing larger amounts of data, particularly additional mitochondrial data; mitochondrial genomes are publicly available for only 142 species of plants, compared with 4445 chloroplast genomes (Petersen et al. 2020).

Evolutionary rates in parasitic angiosperms

The parasitic flowering plants included in our study exhibited high levels of among-lineage rate heterogeneity in their nuclear and organellar genomes. Rate heterogeneity was especially pronounced in nuclear genes of Rafflesiaceae, in mitochondrial genes of *Loranthus*, and in chloroplast genes of Orobanchaceae. Given the extreme levels of rate variation, it is perhaps unsurprising that we could not detect any evolutionary rate correlations in the parasitic angiosperms that we tested: *Loranthus*, Orobanchaceae, Rafflesiaceae, and *Thesium*.

Parasitism has evolved at least 12 times across the angiosperms (Westwood et al. 2010; Nickrent 2020), with an estimated 4750 species attacking the stems and roots of host plants for nutrition. Our data sets included Rafflesiaceae, a group of the most reduced holoparasites (Molina et al. 2014). Holoparasites do not perform any photosynthetic function and rely on their hosts for their nutritional requirements, with the most extreme form being endoparasitism. Indeed, Rafflesiaceae include endoparasites such as *Sapria*, which lack an independent body and grow directly on their host (Cai et al. 2021). Other parasite species that we analysed occur as hemiparasites, which have not lost photosynthetic function completely, and only partly rely on their host for heterotrophic nutrient demands. Hemiparasites can be either obligate, depending on their hosts for nutrients, or facultative, where they are able to live independently of their hosts. We analysed data from obligate parasites *Loranthus*, which reside on the stems of their hosts, and *Thesium*, which includes both obligate and facultative herbaceous parasites that attach to the roots of their hosts. Orobanchaceae includes members that span the range of parasitic modes, from free-living hemiparasites to holoparasites that

live on their hosts and leach water and nutrients directly from the hosts' roots, e.g., in desert hyacinths.

The degree of parasitism seems to be linked to the amount of chloroplast reduction. For example, the holoparasitic members of Orobanchaceae have experienced widespread chloroplast gene losses, with members of Rafflesiaceae being the most extreme iteration of this gene loss, as they appear to have lost their chloroplast genomes entirely (Molina et al. 2014; Zervas et al. 2019). The hemiparasitic *Viscum*, a genus of mistletoe, have lost 10–22% of their chloroplast genes compared with their autotrophic relatives (Petersen et al. 2015; Zervas et al. 2019). These gene losses are likely to be associated also with the loss of signalling and regulatory genes in the nuclear and mitochondrial genomes. This may have caused the lack of evolutionary rate correlations between nuclear and organellar genomes in parasitic angiosperms; given the loss of nuclear-encoded genes. Moreover, due to the intimate nature of the host–parasite association, horizontal gene transfer is rife, especially in the mitochondrial genomes of parasite species. In family Rafflesiaceae it was found that 24–41% of mitochondrial genes were donated from the host plant (Xi et al. 2013). This provides another potential explanation for the lack of evolutionary rate correlations; the foreign genes have not undergone coordinated evolution with the native nuclear genes over millions of years.

Conclusions

We have identified correlations in evolutionary rates across the nuclear, mitochondrial, and chloroplast genomes in land plants, but not in parasitic angiosperms. These associations suggest that the evolution of the three genomes is at least partly governed by a common factor that acts upon mutation rates. Further insight into these evolutionary dynamics can be gained through detailed studies of specific genes, including nuclear and mitochondrial genes that encode products that are used in the mitochondria. Our framework can be extended to other major lineages of plants, including the green algae but also red algae. We found a weakly positive (but insignificant) correlation in a small data set of chlorophytes. If there is evidence for evolutionary rate correlations in algae, this indicates that the dynamic is ancient and consistent across all Viridiplantae. Indeed, whether rate correlations exist at an even larger scale across between the genomic compartments across the Tree of Life is unknown. This is particularly pertinent as new organelles are being discovered, such as the nitrogen-fixing organelle, the 'nitroplast' (Coale et al. 2024). Another open question is whether evolutionary rate correlations would persist in other photosynthetic eukaryote lineages with plastids derived from secondary or tertiary symbioses, such as dinoflagellates (Maruyama and Kim 2020). Uncovering the drivers of evolutionary rate correlations will allow better understanding of the causes of genomic change.

Materials and Methods

Data Collection and Curation. We collected nucleotide sequence data for nuclear, mitochondrial, and chloroplast markers from a diverse range of plant taxa, including mosses, ferns, gymnosperms, angiosperms, as well as a number of genera and families of parasitic angiosperms. We use the formal terminology, as per Cantino et al. (2007) and Bechteler et al. (2023). Thus, 'mosses' refers to Bryophyta, 'ferns' refers to Monilophyta, 'gymnosperms' refers to the extant Acrogymnospermae, and 'angiosperms' to Angiospermae. Full details of the data sets and their sources are provided in the Supplementary Material (*SI Appendix*, Table S1). We targeted the most recent genomic studies, which included all described protein-coding genes for the organellar data sets, with taxon sampling to the greatest extent achievable under our computational limits. Overall, for the four major land plant clades (mosses, ferns, gymnosperms, and angiosperms), we assembled data with mean alignment lengths of 167,688 bp for nuclear DNA, 27,341 bp for mitochondrial DNA, and 60,574 bp for chloroplast DNA. The average number of taxa that were used for these comparisons was n = 86. Gene alignments were smaller for data sets of parasitic angiosperms, with mean lengths of 3,298 bp, 5,585 bp, and 51,230 bp, for nuclear, mitochondrial, and chloroplast data sets (see *SI Appendix* and Table S1). The average number of taxa for these comparisons was n = 70.

We pruned the nuclear and organellar data sets to ensure that taxa matched between the data sets. We then inferred phylograms using maximum likelihood in IQ-TREE2 (version 2.2.2.6; Bui et al. 2020) and Bayesian inference in BEAST2 (version 2.6.6; Bouckaert et al. 2019). For all analyses, tree topologies were fixed to match those from the source studies (*SI Appendix*, Table S1). However, it is evident that separate genomic compartments can produce highly divergent phylogenies. The fact that we did not account for this but still found strong correlations (despite the presence of noise) is an encouraging result, and suggests that evolutionary rate correlations are significant.

Maximum-Likelihood Phylogenetic Analysis. We inferred phylograms using maximum-likelihood analysis. When alignments of individual genes were available, we selected the partitioning scheme and nucleotide substitution models in IQ-TREE2 (-m TESTMERGEONLY). For gene data sets with sequences that were well aligned and in frame, we also split the sequences into 1st+2nd and 3rd codon sites. We used these as proxies for nonsynonymous and synonymous sites, respectively, allowing us to test for correlations at these sites separately. Branch lengths were proportionally linked between partitions. We constrained the tree topology (-g) and inferred branch lengths for each data set. Files used in all of these analyses are included in Supplementary Data Set S1.

Bayesian Phylogenetic Analysis. We then inferred trees using Bayesian analysis in BEAST2, using the same partitioning schemes as in the maximum-likelihood analyses. For each partition, the substitution model was averaged using bModeltest (Bouckaert and Drummond 2017). Uncorrelated lognormal relaxed-clock models were unlinked between genomes (nuclear, chloroplast, and mitochondrial), with one clock model having a mean rate of 1.0 and with the other clock model having

a mean rate that was inferred in the analysis (Drummond et al. 2006). The mean rate of the clock model was assigned a uniform prior with bounds of 0 and 10^6. To allow rate variation across partitions, each was assigned its own relative rate parameter. The tree topology was fixed, as in the maximum-likelihood analyses, with a birth-death tree prior on the relative node times.

Each analysis was run for at least 10^8 MCMC steps, with sampling every 10^4 steps. The sampling frequency was varied according to the chain length, with at least 10^4 trees sampled from the posterior for each run. At least three independent runs were completed for each analysis and combined in LogCombiner, with at least 10% of each run discarded as burn-in. Effective sample sizes were greater than 200 for most parameters, including the likelihood and posterior. The combined tree samples were summarized using TreeAnnotator. The MCMC log files are included in the Supplementary Files.

Method Validation. We validated the methods for detecting correlated evolutionary rates by ensuring that they were both accurate (with low rate of false-positive detection) and powerful (able to detect linked rates of evolution across a variety of settings). We performed a comprehensive simulation study, described in the Supplementary Material, to evaluate methods based on correlations of (i) root-to-tip distances, (ii) independent sister-pair contrasts, and (iii) branch rates inferred using Bayesian relaxed clocks. All three methods were found to be accurate, powerful, and fit for use when testing for evolutionary rate correlations. These results are consistent with those from our previous evaluation of these methods in detecting evolutionary rate correlations between molecular and morphological data sets (Asar et al. 2023).

Root-to-Tip Distances. Root-to-tip distances were calculated using the package *adephylo* in R. The outgroup taxa were removed from the nuclear, mitochondrial, and chloroplast phylograms inferred in our maximum-likelihood analyses above, then patristic distances between the root of the tree to each tip were calculated. We then tested for pairwise correlations between the root-to-tip distances of the three phylograms. We used a permutation correlation test (perm.cor.test function in *jmuOutlier*) (Higgins 2004) using 200,000 simulations, implemented in R (Garren 2019), to avoid violations caused by use of non-independent data points.

Independent Sister-Pair Contrasts. The second method of testing for correlated rates involved taking phylogenetically independent pairs of taxa (sister-pairs) and comparing their relative branch lengths between nuclear, mitochondrial, and chloroplast phylograms. Using the R package *diverge* (Anderson and Weir 2022), we extracted sister species that shared a most recent common ancestor to the exclusion of other sister pairs, which avoids the problem of phylogenetic non-independence but reduces the amount of data (Felsenstein 1985; Bromham et al. 2002). We computed the difference between the branch lengths of sister taxa for each of the plant organellar phylograms. We tested for correlations between these contrasts using the non-parametric Spearman's rank correlation test, to allow for violations of bivariate normality and homoscedasticity. We log-transformed and standardized all contrasts by dividing them by the square root of the sum of branch lengths (Garland et al. 1992; Freckleton 2000; Welch and Waxman 2008; Lanfear et al. 2010). After investigating whether the data met the assumptions of the independent sister-pairs contrasts method, we used a two-sample sign

test where the assumptions were violated. See Supplementary Material for full description of assumption tests and their results for all comparisons (Table S8–S16).

Branch Rates Inferred Using Bayesian Relaxed Clocks. The third method of testing for correlated rates was based on the inferred posterior mean branch rates from Bayesian phylogenetic analyses. We tested for correlations between the branch rates inferred using the uncorrelated lognormal relaxed clock for the plant nuclear, mitochondrial, and chloroplast data sets. To compute the significance of the correlation, we used Spearman's rank-order correlation test.

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Figures and Tables

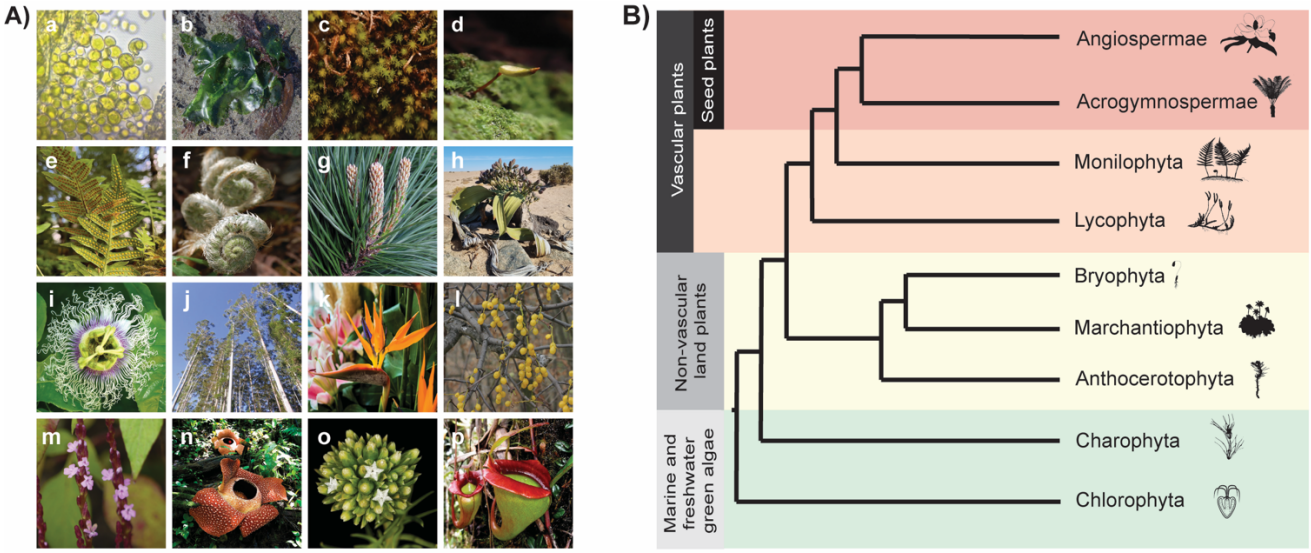

Fig. 1. Morphological diversity and phylogeny of the green plants (Viridiplantae). (A) Representatives of the major clades of land plants that were analysed in this study (c–j) and close relatives of land plants, the chlorophyte green algae (a-b). a) *Chlorella* sp., b) *Ulva* sp., c) *Takakia* sp., d) *Buxbaumia aphylla*, e) *Polypodium glycyrrhiza*, f) *Polystichium acrostichoides*, g) *Pinus radiata*, h) *Welwitschia mirabilis*, i) *Passiflora edulis*, j) *Eucalyptus grandis*, k) *Strelitzia reginae*, l) *Loranthus europaeus*, m) *Striga gesneroides*, n) *Rafflesia arnoldii*, o) *Thesium scabrum*, and p) *Nepenthes jacquelinae*. All photographs were sourced from OpenVerse (https://openverse.org). Authors and Creative Commons licenses are supplied, unless image is in the public domain: a) Owen Strickland under CC BY 4.0, b) Ryan Hodnett under CC BY-SA 4.0, f) Derek Ramsey under CC BY-SA 2.5, g) Tony Wills under CC BY 2.5, h) Marc Fradera-Soler under CC BY 4.0, i) Sam Fraser-Smith under CC BY 2.0, j) Peter Woodard under CC BY-SA 3.0, k) fotopamas under CC BY 2.0, m) Dinesh Valke under CC BY-SA 2.0, n) SofianRafflesia under CC BY-SA 4.0, o) SAplants under CC BY-SA 4.0, and p) Shawn Mayes under CC BY-SA 3.0. (B) Simplified phylogeny of Viridiplantae (the green plants). Relationships are summarized from those described in ONE KP (2019). Plant silhouettes are in the public domain and available at http://www.phylopic.org.

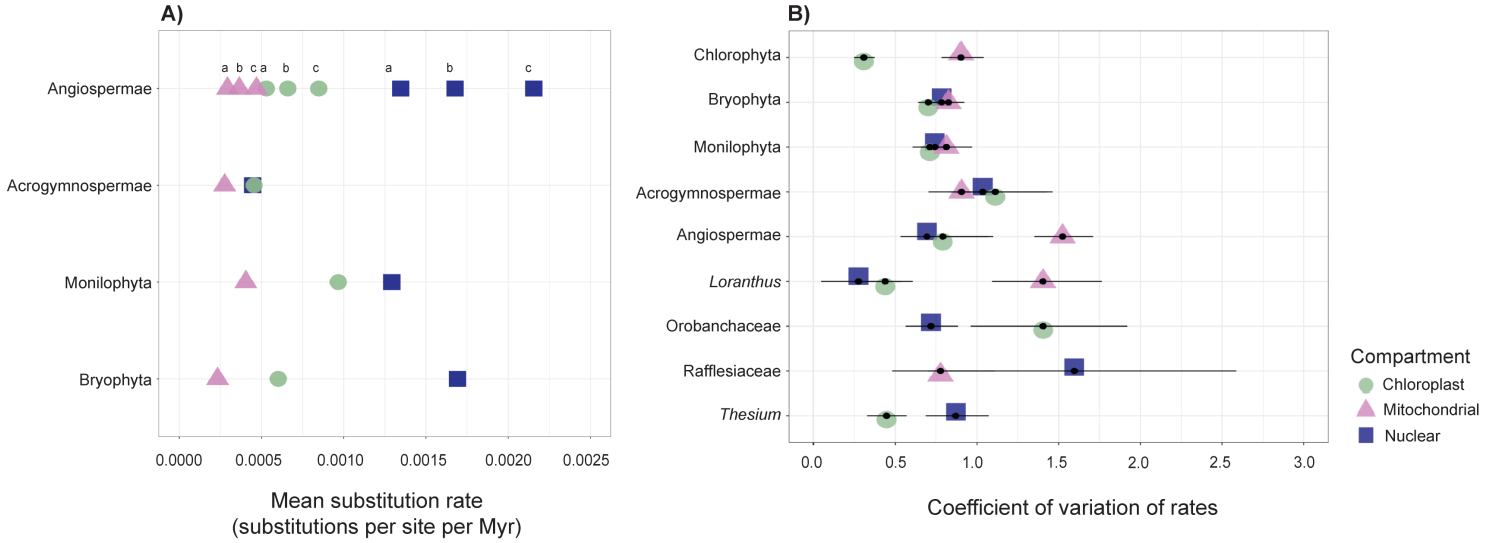

Fig. 2. There is pronounced diversity in evolutionary rates and among-lineage rate heterogeneity amongst the major lineages of land plants. (A) Estimates of evolutionary rates for nuclear markers (dark blue squares), mitochondrial markers (violet triangles), and chloroplast markers (green circles) of the major embryophyte lineages. Three estimates of evolutionary rates are provided for Angiospermae, depending on whether a Middle Triassic ~246 Ma (a), Early Jurassic ~198 Ma (b), or Late Jurassic ~ 154 Ma (c) age of origin is assumed. These age estimates are from the analyses using constrained, relaxed, and unconstrained complete calibrations reported by Ramirez-Barahona et al. (2020). Plant silhouettes are in the public domain and available at http://www.phylopic.org. (B) Coefficient of variation of rates, a measure of among-lineage rate heterogeneity, estimated for each of the plant clades in this study. The nuclear, mitochondrial, and chloroplast genomic compartments are represented by dark blue squares, violet triangles, and green circles, respectively. Estimates were obtained by Bayesian relaxed-clock analysis and are shown as posterior means accompanied by 95% credibility intervals.

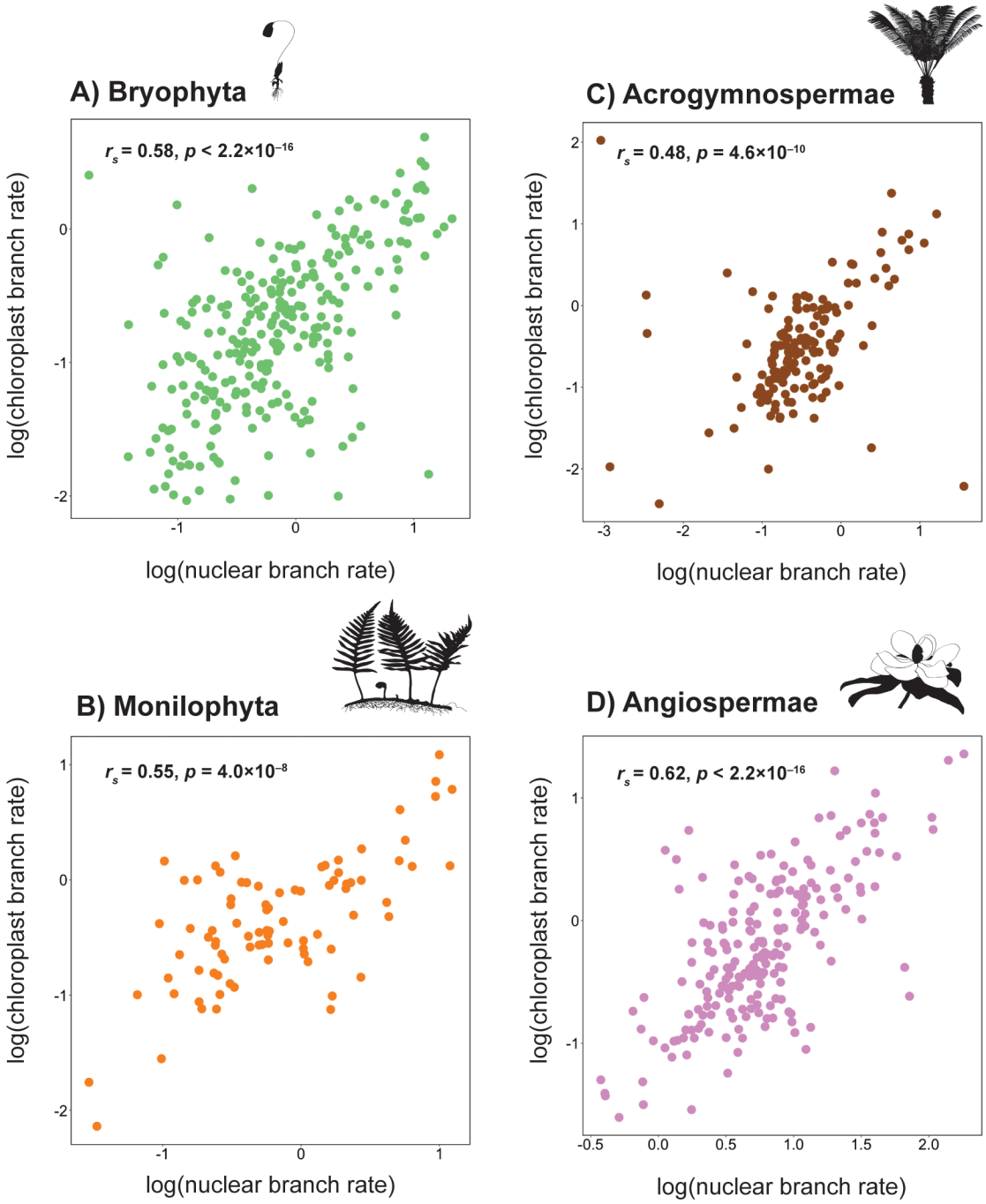

Fig. 3. Rates of nuclear and chloroplast evolution are strongly linked in the major embryophyte clades. Log-transformed relative branch rates plotted for nuclear genome against the chloroplast genome for the major land plant clades. Branch rates were estimated using Bayesian relaxed-clock analysis. The correlation coefficient (r_s) and p-value (calculated using Spearman's rank correlation test) are shown for each plot. Bold font indicates p-values below 0.05. Plant silhouettes are in the public domain and available at http://www.phylopic.org.

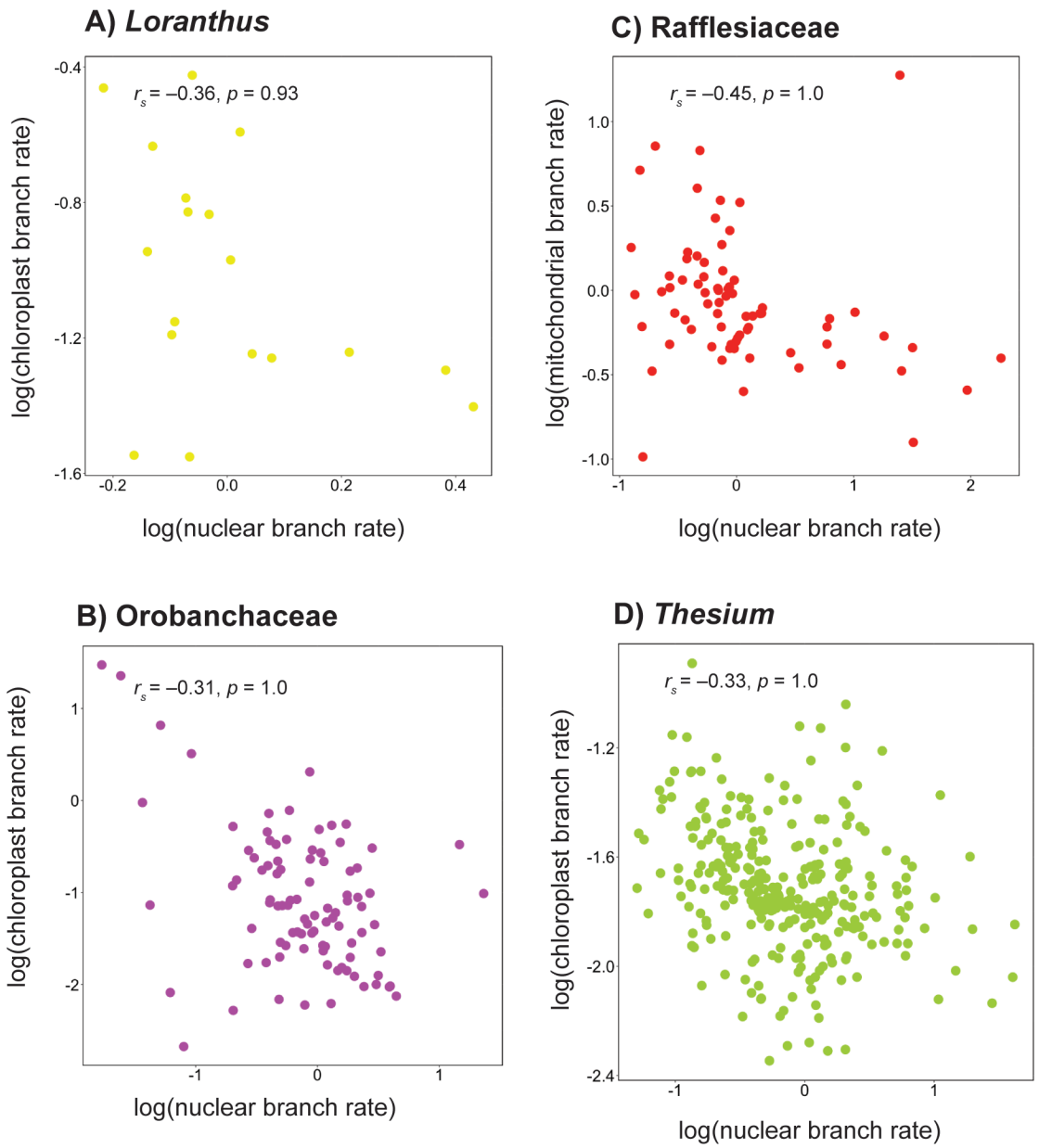

Fig. 4. Rates of organellar evolution are decoupled in parasitic angiosperms. Comparisons of evolutionary branch rates of nuclear, mitochondrial, and chloroplast genes are shown for four parasitic lineages of angiosperms. Relative branch rates were estimated using Bayesian relaxed-clock analysis. The correlation coefficient (r_s) and p-value (calculated using Spearman's rank correlation test) are shown for each plot. Bold font indicates p-values below 0.05.

Table 1. Correlations between rates at 1st + 2nd sites and 3rd sites for each of the organellar compartments in the major embryophyte clades. For each organellar comparison, the correlation coefficient (r_s) and p-values (p) for a Spearman rank correlation test are recorded. Bold font indicates p-values below 0.05.

Taxon[a]		**Nuclear–Chloroplast**		**Nuclear–Mitochondrial**		**Chloroplast–Mitochondrial**	
		1st + 2nd	**3rd**	**1st + 2nd**	**3rd**	**1st + 2nd**	**3rd**
Bryophyta	r_s	0.51	0.61	0.12	0.23	0.31	0.26
	p	**$<2.2\times10^{-16}$**	**$<2.2\times10^{-16}$**	**2.4×10^{-2}**	**9.4×10^{-5}**	**8.3×10^{-8}**	**7.4×10^{-6}**
Monilophyta	r_s	0.57	0.52	0.29	0.39	0.27	0.40
	p	**9.9×10^{-9}**	**2.3×10^{-7}**	**3.2×10^{-3}**	**1.2×10^{-4}**	**3.6×10^{-3}**	**2.2×10^{-5}**
Angiospermae[b]	r_s	0.40	0.30	-	-	0.033	0.037
	p	**1.1×10^{-3}**	**1.0×10^{-2}**	-	-	2.6×10^{-1}	2.4×10^{-1}

[a]Number of taxa (n) included for the comparisons was: n = 135 (Bryophyta), n = 44 (Monilophyta), n = 51 (Monilophyta, chloroplast–mitochondrial), n = 30 (Angiospermae, nuclear–chloroplast), and n = 185 (Angiospermae, chloroplast–mitochondrial).

[b]These comparisons refer to the data sets sourced from Zeng et al. (2014) and Ruhfels et al. (2014), and Tyzka et al. (2023). This differs from the nuclear and chloroplast data sets presented in the main figure since these did not have site partitioning data available.