How does genome size affect the evolution of pollen tube growth rate, a haploid performance trait?

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ABSTRACT

Premise of the Study – Male gametophytes of most seed plants deliver sperm to eggs via a pollen tube. Pollen tube growth rates (*PTGRs*) of angiosperms are exceptionally rapid, a pattern attributed to more effective haploid selection under stronger pollen competition. Paradoxically, whole genome duplication (WGD) has been common in angiosperms but rare in gymnosperms. Pollen tube polyploidy should initially accelerate *PTGR* because increased heterozygosity and gene dosage should increase metabolic rates, however polyploidy should also independently increase tube cell size, causing more work which should decelerate growth. We asked how genome size changes have affected the evolution of seed plant *PTGRs*.

Methods - We assembled a phylogenetic tree of 451 species with known *PTGRs*. We then used comparative phylogenetic methods to detect effects of neo-polyploidy (within-genus origins), DNA content, and WGD history on *PTGR*, and correlated evolution of *PTGR* and DNA content.

Key Results - Gymnosperms had significantly higher DNA content and slower *PTGR* optima than angiosperms, and their *PTGR* and DNA content were negatively correlated. For angiosperms, 89% of model weight favored Ornstein-Uhlenbeck models with a faster *PTGR* optimum for neo-polyploids, but *PTGR* and DNA content were not correlated. In comparisons of within-genus and intraspecific-cytotype pairs, *PTGRs* of neo-polyploids ≤ paleo-polyploids.

Conclusions – Genome size increases should negatively affect *PTGR* when genetic consequences of WGDs are minimized, as found in intra-specific autopolyploids (low heterosis) and gymnosperms (few WGDs). But in angiosperms, the higher *PTGR* optimum of neo-polyploids and non-negative *PTGR*-DNA content correlation suggest that recurrent WGDs have caused substantial *PTGR* evolution in a non-haploid state.

Keywords: DNA content, evolution of development, gametophyte, macroevolution, pollen competition, pollen tube growth rate, polyploidy, whole genome duplication.

49 **INTRODUCTION**

50 In seed plants, the male gametophyte is a highly-reduced, haploid organism that develops
51 within the pollen grain and completes its life cycle after pollination by growing a pollen tube that
52 invades female reproductive tissues. The pollen tube functions to attach the male gametophyte
53 and to absorb nutrients from female tissues, and in most seed plants (conifers, Gnetales, and
54 angiosperms), it has the novel function of transporting the sperm cells to the egg-bearing female
55 gametophyte (siphonogamy) (Friedman, 1993). Pollen tube growth rate (*PTGR*) is a central
56 aspect of male gametophyte performance that can evolve due to changes in the time between
57 pollination and fertilization, and due to changes in the intensity of pollen tube competition.
58 Strikingly, angiosperms are known to have much shorter reproductive cycles (Williams and
59 Reese, 2019), much higher potential for pollen competition (Mulcahy, 1979), and orders of
60 magnitude faster *PTGRs* (Williams, 2012) relative to gymnosperms. The pattern of exceptionally
61 fast angiosperm *PTGRs* is thought to have evolved rapidly via haploid selection on pollen-
62 expressed genes (Mulcahy, 1979; Arunkumar et al., 2013; Otto et al., 2015), which constitute a
63 large portion of the genome (Tanksley et al., 1981; Rutley and Twell, 2015; Hafidh et al., 2016).

64 If the dramatic and rapid acceleration of *PTGR*s in angiosperms has been driven by
65 haploid selection on pollen performance genes, then one might expect polyploidy to be rare in
66 angiosperms. Evolution above the haploid level is expected to reduce the efficiency of selection
67 on pollen (Otto et al., 2015). Yet, the opposite is true – ancient whole genome duplications
68 (WGDs), recent polyploids, and speciation by polyploidy have been especially common in
69 angiosperms, whereas in gymnosperms genome size has evolved largely by other processes
70 (Wood et al., 2009; Mayrose et al., 2011; Leitch & Leitch, 2012, 2013; Landis et al., 2018). In
71 fact, large changes genome size can have a number of immediate effects on *PTGR*. First, *PTGR*

72 might be faster in a neo-diploid pollen tube since increases in gene number cause: 1) heterosis,
73 due to sheltering of deleterious pollen-expressed alleles and/or new allelic interactions upon loss
74 of haploidy (Lande and Schemske, 1985; Husband and Schemske, 1997; Comai, 2005; Birchler
75 et al. 2010; Husband, 2016), and 2) gene dosage effects, due to increased capacity for protein
76 synthesis and hence the possibility for higher metabolic rates (Stebbins, 1974; Comai, 2005;
77 Conant and Wolfe, 2008). On the other hand, substantial increases in DNA content (whether by
78 WGD or other processes) are known to increase nuclear size, cell size, and the duration of the
79 cell cycle, independent of the effects of genes (Bennett, 1971, 1972; Cavalier-Smith, 1978; Price
80 1988; Cavalier-Smith, 2005). The phenotypic effects of increased bulk DNA, hereafter referred
81 to as "nucleotypic" effects (Bennett, 1971; Snodgrass et al, 2016; Doyle & Coate, 2019), cause
82 more work for the growing pollen tube cell and should therefore negatively affect *PTGR*,
83 counteracting the positive "genotypic" effects of heterozygosity and gene dosage.
84 As shown in Figure 1, if genome size expansion occurs without increasing the number of
85 genes, then nucleotypic effects will predominate and slower *PTGRs* should evolve. But if
86 genome size increase occurs by WGD, then altered gene expression patterns (due to dosage and
87 heterozygosity effects) should counteract nucleotypic effects in the stabilized neo-polyploid (Fig.
88 1). In the latter case, the balance of nucleotypic and genotypic effects varies depending on the
89 magnitude of potential heterosis, which depends directly on the amount of standing genetic
90 variation (Birchler et al. 2010). In general, at inception tetraploid sporophytes are expected to
91 have higher heterozygosity than their diploid progenitors, irrespective of mode of
92 polyploidization (auto- to allo-polyploidy) or mating system (Lande and Schemske 1985; Soltis
93 and Soltis 2000). Thus, at inception, autopolyploids that arise from outcrossing progenitors and

94 allopolyploids will have a higher potential for heterosis, relative to autopolyploids that arose
95 from selfing ancestors (Fig. 1).
96 After the initial effects of WGD, genotypic effects continue to evolve under both
97 stabilizing and directional selection on *PTGR*, mediated by shifts in mating system and
98 phenomena such as genome downsizing, biased gene retentions, recombination, and ultimately
99 the return to disomic inheritance (Conant and Wolfe, 2008; De Smet et al. 2013; Conant et al.,
100 2014; Freeling et al., 2015; Dodsworth et al., 2016; Panchy et al. 2016; Wendel et al., 2018).
101 Nucleotypic effects by definition can only evolve by changes in genome size, which after WGD
102 tend to be biased to small losses relative to the size of the WGD (Dodsworth et al. 2016). Hence,
103 with time, genotypic effects are predicted to overwhelm nucleotypic effects, irrespective of
104 initial effects and the direction of *PTGR* evolution.
105 In this study, we used model-based comparative phylogenetic analyses to determine if
106 polyploidy, DNA C-value, and WGD history have affected the evolution of *PTGRs* in seed
107 plants, and whether genome size effects have occurred predominantly during polyploid periods
108 of history or during subsequent periods of more or less diploid evolution. Because all seed plants
109 have at least one WGD in their history, we defined neo-polyploids as having a higher
110 chromosome multiple than the base chromosome number of their genus, and paleo-polyploids
111 (hereafter, "diploids") as having similar chromosome number as the genus base number (as in
112 Wood et al. 2009; Mayrose et al. 2011). This allowed us to determine if, 1) neo-polyploids have
113 faster *PTGRs* than diploids, as predicted if WGDs generally produce strong initial genotypic
114 effects that persist in the polyploid condition, or 2) neo-polyploids have slower *PTGRs* than
115 diploids, as predicted if nucleotypic effects initially outweigh genotypic effects and if fast
116 *PTGRs* generally evolve after diploidization (eg. in paleopolyploids). We also predict an

117 underlying negative correlation between *PTGR* and genome size due to nucleotypic effects,
118 which should be most apparent in intraspecific neo-polyploids and in lineages with little history
119 of WGD.
120

121 **MATERIALS AND METHODS**

122 ***Tree Construction and Dating*** – GenBank accessions for 16 gene regions (*rbcL, matK, trnL-F,*
123 *18s_rDNA, atpB, ndhF, adh, trnL, rpl32, trnT-L, psbA-trnH, rpl32-trnL, ITS, 5.8s_rRNA, rps16,*
124 and *26s_rDNA*) for 451 seed plant species with pollen tube growth rate data were retrieved,
125 cleaned, and assembled into a multiple gene alignment (length – 9263 base pairs, 16 partitions,
126 69.6% missing data) using PHLAWD and phyutility (Smith and Donoghue, 2008; Smith and
127 Dunn, 2008). Tree inference was performed using maximum likelihood in RAxML version 8
128 (Stamatakis, 2014) on CIPRES. A pruned version of the seed plant tree from Magallón et al.
129 (2015) was used as a guide tree to enforce topology of major clades. The resulting maximum
130 likelihood estimate of the tree was rooted and ultrametricized using the *ape* (Paradis et al., 2004)
131 and *geiger* packages in R (Harmon et al., 2008). Time-calibration was performed with the
132 Congruification method (Eastman et al., 2013), using the Magallón et al. (2015) phylogeny as the
133 reference tree.

134 ***Data collection and character scoring*** – Data on *PTGRs* were taken from Williams
135 (2012) and more recent literature (cited in Appendix S1; see the Supplementary Data with this
136 article). The *PTGR* value used for each species represents an estimate of maximum sustained
137 growth rate, which is consistent with other comparative analyses of physiological traits, and with
138 the fact that researchers almost always measure *PTGRs* from the longest pollen tube(s). Thus,
139 *PTGR* values for each species represent an average of maximum in vivo growth rates, or if there

140 was more than one report for a species the average of those values (as in Williams, 2012).
141 *PTGRs* were taken exclusively from within-ploidy level crosses (i.e., never from inter-ploid
142 crosses), in keeping with our overall goal of finding mechanisms underlying the pattern of *PTGR*
143 evolution within stabilized polyploids.

144 DNA content was analyzed using 1C-value: the amount of nuclear DNA in the
145 unreplicated gametic nucleus, irrespective of ploidy level (Swift, 1950; Bennett and Leitch,
146 2012). As we were primarily interested in the nucleotypic effects of bulk DNA amount, we use
147 the terms C-value, DNA content, and genome size interchangeably throughout. C-value data was
148 collected from the Kew Royal Botanic Gardens Plant C-value Database (Bennett and Leitch,
149 2012). Chromosome counts were obtained from the Index to Plant Chromosome Numbers
150 (IPCN). To examine the effect of recent polyploidy (defined as occurring at or within the genus
151 level; Wood et al., 2009; Mayrose et al., 2011) on *PTGR*, we scored taxa as "neo-polyploid" if
152 their chromosome counts were $\geq$ 1.5 times that of their generic 1x base count (from Wood et al.,
153 2009) and "diploid" (paleo-polyploid) if < 1.5 times that value (N = 206 angiosperms, 23
154 gymnosperms). To examine the effect of ancient (deeper than genus-level) duplication events on
155 *PTGR*, the number of WGDs in each genus-to-root lineage was counted for each angiosperm
156 (found in Appendix S1 of Landis et al. 2018) and gymnosperm (Li et al. 2015).

157 ***Phylogenetic Comparative Analyses*** - To visualize changes in DNA content and *PTGR*
158 along tree branches and to generate estimates of node states, ancestral state reconstructions were
159 performed and plotted using the contMap function in *phytools* (Felsenstein, 1985; Revell,
160 2012)(Appendix S2). Given many known biological differences between gymnosperms and
161 angiosperms in pollen tube growth (Friedman, 1993; Williams, 2008) and in mechanisms of
162 genome size change (see Discussion) (Ohri and Khoshoo, 1986; Leitch et al., 1998), all

163 comparative analyses were performed on gymnosperms only, angiosperms only, and the full
164 dataset (all spermatophytes). C-value and *PTGR* were $\log_{10}$-transformed for all analyses.
165 Model-based analyses were used to examine patterns of *PTGR* and C-value evolution
166 separately. The *OUwie* function was implemented in R (Beaulieu and O'Meara, 2014), and the
167 following models were tested: single- and multi-rate Brownian motion (BM1, BMS,
168 respectively), single-regime Ornstein-Uhlenbeck (OU1), and multi-regime OU models with
169 either one global $\boldsymbol{\alpha}$ and $\boldsymbol{\sigma^2}$ estimate (OUM), one $\boldsymbol{\alpha}$ and multiple $\boldsymbol{\sigma^2}$ (OUMV), or multiple $\boldsymbol{\alpha}$ and
170 one $\boldsymbol{\sigma^2}$ (OUMA). In all models, $\boldsymbol{\sigma^2}$ represents the rate of random evolution and $\boldsymbol{\alpha}$**,** the strength of
171 attraction to an optimum, θ. The single- and multiple-regime models were compared to test
172 whether or not, 1) angiosperms and gymnosperms evolve around different *PTGR* or C-value
173 optima, and 2) diploids and neo-polyploids (within all three groups) evolve around different
174 *PTGR* or C-value optima. For all analyses, AICc values were used to calculate model weights
175 and the weighted average of parameter values was then calculated using all models that
176 contributed > 1% of the model weight (Burnham & Anderson, 2002). Unless otherwise noted, all
177 measures of uncertainty around parameter estimates are standard errors.
178 Since each *PTGR* value represents a species mean obtained from multiple measurements,
179 we attempted to incorporate error into phylogenetic comparative analyses. Since species means
180 were $\log_{10}$-transformed for analysis, $\log_{10}$-transformed *SE*s are also required. As there is no
181 reliable way to calculate the $\log_{10}$-transformed *SE* from the literature without the original data for
182 each species, we took the following approach. First, we assumed all species had similar *SE*s in
183 *PTGR*, and we applied an empirically-determined *SE* from an exemplar species to all. *Magnolia*
184 *grandiflora* has an average *PTGR* of 828 ± 141 μm h^{-1} (N = 25 outcrosses), close to the
185 angiosperm median of 587 μm h^{-1} (Williams, 2012 and this study) (Appendix S3). The standard

186 deviation (*SD*) of $\log_{10}$-transformed data was calculated and divided by the mean of the $\log_{10}$-
187 transformed data to acquire a coefficient of variation (*CV*) of 0.0237. We then multiplied the
188 $\log_{10}$-transformed mean *PTGR* of each species by 0.0237 to provide an estimate of the $\log_{10}$
189 taxon-specific standard deviation. The standard deviation (*SD*) was used as a conservative
190 estimate of error because sample sizes were generally not available for calculating *SE*. Secondly,
191 we performed a sensitivity analysis by evaluating each evolutionary model in *OUwie* with *SDs*
192 calculated from hypothetical global *CV*s of 0.00, 0.05, 0.10, 0.25, and 0.50 (Appendix S4).
193 The association between recent polyploidy and *PTGR* was also assessed among 10
194 diploid-polyploid near-relative pairs (appearing as sister taxa on the tree at the within-genus
195 level). Only polyploid taxa with a single diploid sister on the tree were used. The *PTGRs* of 11
196 intraspecific diploid-autopolyploid pairs from the literature were also compared. A two-tailed
197 binomial (sign) test was used to test significance in both.
198 The cumulative effect of ancient polyploid events was explored with phylogenetic
199 generalized least squares (*PGLS*) regression using the *phylolm* package in R (Ho & Ane, 2014).
200 The number of ancient duplication events in the history of each tip taxon (inferred from Landis et
201 al., 2018) was used as the predictor variable with *PTGR* as the response variable.
202 The relationship between pollen tube growth rate and gametophytic DNA content was
203 also assessed with *PGLS* regression. Gametophytic DNA content was used as the predictor
204 variable and *PTGR* the response variable. BM (Grafen, 1989) and OU (Martins and Hansen,
205 1997) models were both used, in addition to Pagel's lambda, kappa, and delta models (Pagel,
206 1997, 1999). To examine the effect of ploidy and the interaction between ploidy and C-value on
207 *PTGR*, a phylogenetic ANCOVA was implemented with C-value as the covariate in *phylolm*.

208 Shifts among convergent *PTGR* and C-value optima were determined with a maximum
209 likelihood approach to detect multiple optima within seed plants, using *SURFACE* in R (Ingram
210 and Mahler, 2013). Using an OU model with a global α and σ^2, a single-optimum model was
211 subdivided into multiple-optima models in a stepwise fashion until adding another optimum
212 decreased the model likelihood by ΔAIC > -2. Separate optima were then collapsed (i.e. two
213 regimes were assigned the same optimum) in a pairwise fashion until further collapses decreased
214 model likelihood. Shifts in *PTGR* and C-value optima that occurred at the same node, or within
215 two nodes of each other, were identified manually. Nodes with *PTGR* or C-value regime shifts
216 were also manually compared to the Landis et al. (2018) WGD map to see if a WGD had
217 occurred at that node or up to two nodes *prior* to the regime shift.
218

219 **RESULTS**

220 ***PTGR evolution and C-value evolution in angiosperms versus gymnosperms –*** The *PTGR* tree
221 comprised 451 seed plants, with 28 species from 7 of 8 gymnosperm orders (Christenhusz et al.
222 2011) including Cycads, *Ginkgo,* conifers and Gnetales; and 423 species from 38 of 64 (59%) of
223 angiosperm orders (APG IV 2016), including representatives from all three ANA grade lineages,
224 Chloranthaceae, eumagnoliids, and a broad distribution of both monocots and eudicots (full tree
225 in Appendix S2). Gymnosperm *PTGR*s ranged from < 1 to 19 μm h^{-1} (mean ± *SD* = 3.29 ± 4.34,
226 median = 1.49 μm h^{-1}), whereas angiosperm *PTGR*s ranged from < 5 to > 30,000 μm h^{-1} (mean ±
227 *SD* = 1744 ± 3576 μm h^{-1}, median = 587 μm h^{-1}). The maximum likelihood (ML) reconstruction
228 indicated that ancestral $\log_{10}$ *PTGR* of extant angiosperms was 2.44 μm h^{-1} (95% CI: 1.09-3.69)
229 versus 0.215 μm h^{-1} (95% CI: -1.48-1.92) for extant gymnosperms (Appendix S2).

230 Model-based analyses of seed plant *PTGRs* and C-values favored OU models with
231 separate optima for angiosperms and gymnosperms, accounting for > 99.9 % of the model
232 weight in both analyses (Appendix S5, S6). Log_{10} *PTGR* optima were more than an order of
233 magnitude faster in angiosperms (2.69 ± 0.048 μm h^{-1}) than in gymnosperms (0.187 ± 0.123 μm
234 h^{-1}).

235 The C-value tree included 183 species from the *PTGR* tree for which DNA content data
236 could be obtained. The resulting log_{10} C-value optimum for angiosperms (0.184 ± 0.051 pg) was
237 more than a magnitude of order smaller than that of gymnosperms (1.231 ± 0.041 pg). Ancestral
238 Log_{10} DNA content was also smaller for angiosperms than for gymnosperms, 0.29 pg (95% CI: -
239 0.45-1.04) versus 1.10 pg (95% CI: -0.28-2.47), consistent with larger comparative analyses of
240 DNA content (see Leitch and Leitch, 2013).

241 ***Joint evolution of PTGR and ploidy*** – In model-based analyses of angiosperms using the
242 empirical error rate, 89% of the model weight favored a separate and higher optimum for neo-
243 polyploids (N = 68) than for diploids (N = 138) (model averaged log_{10} *PTGR* = 3.2 ± 0.23 vs. 2.8
244 ± 0.08 μm h^{-1}; Table 2). In the sensitivity analysis, OU models with separate and faster *PTGR*
245 optima for neo-polyploids than diploids received > 50% of model weight when the error
246 calculated from *CV*s ranged from 0 to 25 %, but above 25% single-regime and BM models had
247 the majority of the weight (Appendix S4). These are conservative results, since *SDs,* not *SEs,*
248 were used to model error on the tree. The gymnosperm-only analysis was not performed due to
249 low sample size (2 of 23 species were polyploid).

250 A survey of intraspecific cytotypes found autopolyploids had slower *PTGR* than diploids
251 in 9 of 11 pairs and no difference in the remaining two (Binomial test, P = 0.002; Appendix
252 S7b). In the nearest-relative comparisons, within-genus polyploids had slower *PTGR* than

253 diploids in four pairs, faster *PTGR* in five, and no difference in one (Two-tailed binomial test, *P*
254 = 0.623)(Appendix S7a).
255 The historical effect of number of ancient genome duplications on *PTGR* was non-
256 significant, whether or not recent (within-genus) WGDs were included (kappa model weight >
257 99.9%, $N = 451$; $P > 0.3$ in both analyses).
258 ***Joint evolution of PTGR, DNA content and ploidy -*** For seed plants, ordinary least
259 squares (*OLS*) regression showed a significant negative correlation between DNA content and
260 *PTGR* ($N = 183$, $P<0.0001$), but that result was clearly driven by the large DNA contents and
261 slow *PTGRs* of gymnosperms relative to angiosperms (Fig. 2), because the *PGLS* regression was
262 non-significant (Table 1). Taking these two clades separately, DNA content was negatively
263 correlated with *PTGR* in gymnosperms in the *PGLS* regression ($N = 23$; model-averaged slope: -
264 1.09 ± 0.49 $\log_{10}$ *PTGR*; Table 1). In angiosperms, a positive correlation using *OLS* ($N = 161$;
265 $P=0.0005$), was non-significant using *PGLS* (Table 1). The patterns of *PTGR* and C-value
266 evolution in seed plants can be visualized in Figure 3. In a smaller phylogenetic ANCOVA
267 analysis, after controlling for C-value, the effect of ploidy on *PTGR* was non-significant in
268 angiosperms ($N = 100$) and seed plants ($N = 118$) (non-significant ploidy x C-value interaction
269 removed; Appendix S8).
270 ***Coincident regime shifts in PTGR and DNA content*** – Maximum likelihood analysis of
271 convergent evolution of *PTGRs* detected 13 distinct optima ($N = 451$ taxon tree), with 51 shifts
272 (22 to faster and 29 to slower optima). For C-value, there were 9 distinct optima ($N = 184$ taxon
273 tree), with 4 shifts to larger and 7 shifts to smaller optima. Regime shifts in both traits were
274 coincident at only two nodes: a *PTGR* acceleration (from $\theta = 0.147$ to $\theta = 2.47$ $\log_{10}$ μm h^{-1}) and
275 genome downsizing ($\theta = 2.71$ to $\theta = 0.702$ $\log_{10}$ pg) in the CA of extant angiosperms; and a

276 *PTGR* slowdown ($\theta = 2.78$ to $\theta = 2.47$ $\log_{10}$ μm h^{-1}) and genome size decrease ($\theta = 0.209$ to $\theta =$
277 -0.386 $\log_{10}$ pg) in the CA of rosids and Saxifragales (i.e. superrosids; Fig. 4). When the search
278 was relaxed to include adjacent nodes, an additional coincidence occurred, with shift to higher
279 *PTGR* followed by a shift to higher C-value near the base of monocots. Ancient WGDs
280 coincided with the shifts in *PTGR* and C-value at the CA of angiosperms (above) and with a
281 decrease in C-value in the CA of eudicots.
282

283 **DISCUSSION**

284 The impact of genome size on *PTGR* is determined by the magnitudes of conflicting nucleotypic
285 and genotypic effects. Such effects depend on the mechanism of genome size change.
286 Nucleotypic effects decelerate *PTGR* and are always present irrespective of mode of genome size
287 change, whereas large-scale genetic effects are only possible after WGD. We predicted that
288 angiosperms and gymnosperms should have different patterns of *PTGR* evolution based on their
289 contrasting patterns of genome size change. Gymnosperm *PTGRs* should be most susceptible to
290 nucleotypic effects because they have evolved large genomes sizes and WGDs have been rare. In
291 contrast, angiosperms have evolved smaller genome sizes despite recurrent WGDs and
292 widespread present-day polyploidy. Thus, gene duplication and sorting have played a much
293 greater role in the evolution of angiosperm *PTGRs*, allowing genotypic effects to counterbalance
294 or overwhelm nucleotypic effects. Below we discuss our findings in light of these expected
295 patterns.
296

297 ***The evolution of* PTGR *in angiosperms versus gymnosperms -*** We found that seed plant
298 *PTGRs* best fit an OU model, indicating less *PTGR* variation among lineages than expected

299 under a Brownian motion evolutionary model, with a faster optimum for angiosperms than for
300 gymnosperms. Phylogenetic half-lives were similar (5.6 and 5.7 MY, respectively) and very
301 short (only 3.9% and 2.3 % of their respective crown ages), indicating a strong attraction to their
302 optimum values. Such a pattern is consistent with stabilizing selection on *PTGR* imposed by
303 slower evolution of linked sporophytic traits, such as the timing of stigma receptivity relative to
304 egg receptivity, pollen tube pathway length, or maternal provisioning. Gymnosperm *PTGRs* may
305 have been constrained by a hard boundary such as by biophysical or physiological limitations, or
306 a soft boundary, such as by lack of selection for fast rates. Angiosperms have clearly not been
307 bound by those same limitations, given their much higher *PTGR* optimum, the convergent
308 evolution of extremely fast *PTGRs* in many unrelated derived lineages of monocots and eudicots,
309 and occasionally large within-genus differences in *PTGR*.

310 Our results suggest that most of the accelerations of angiosperm *PTGR*, and their higher
311 *PTGR* variance relative to gymnosperms, have largely evolved *after* the origin of angiosperms
312 and their novel pollen tube cell biology. First, estimates of angiosperm ancestral *PTGR* and
313 ancestral optimum under OU (275 and 295 μm h^{-1}, respectively) are slower than the angiosperm-
314 wide OU optimum of 490 μm h^{-1} and the angiosperm median of 587 μm h^{-1}. Secondly, the higher
315 among-lineage variance is due to many transitions to both faster and slower *PTGR* optima within
316 extant angiosperms. Transitions to slower rates within angiosperms are concentrated on lineages
317 that have evolved delayed fertilization, such Fagales, orchids and others, or high selfing rates,
318 which suggests relaxation of directional selection on *PTGR* (Williams and Reese, 2019). In
319 contrast, gymnosperm *PTGRs* were likely ancestrally slow (Figure 4).

320 There are several non-mutually exclusive hypotheses for what triggered the evolution of
321 fast *PTGRs* in angiosperms. First, Mulcahy (1979) invoked a shift to much higher intensity of

322 pollen competition in angiosperms as a driver of the origin and continued evolution of faster
323 growth rates. Notably, no other type of tip-growing cell in land plants (whether gametophytic or
324 sporophytic) has evolved comparably fast tip-growth rates and none of those cell types, including
325 gymnosperm pollen tubes, experience intense competition for resources (Williams et al., 2016).
326 Secondly, gymnosperm *PTGRs* may be slow because they lack novel biophysical or
327 physiological attributes of pollen tubes and/or those attributes enabled faster *PTGRs* to evolve in
328 angiosperms (Hoekstra, 1983; Derksen et al., 1999; Fernando et al., 2005; Williams, 2008,
329 2009). Thirdly, with or without pollen competition, rapid *PTGRs* may have been necessary as
330 angiosperm sporophytes transitioned to a much faster reproductive cycle (Stebbins, 1974;
331 Williams, 2012; Williams and Reese, 2019). Finally, our results suggest a new possibility, that
332 strong differences in genome-level processes have impacted the evolution of angiosperm *PTGRs*
333 relative to their living and extinct seed plant relatives.
334
335 ***Mechanisms of genome size change and PTGR evolution within seed plants*** – A major finding
336 of this study is that angiosperm neo-polyploids evolved around a much faster *PTGR* optimum
337 (1648 μm h^{-1}) than diploids (595 μm h^{-1}), despite several sources of variation in the data. First,
338 neo-polyploids were by definition derived within genera, and their smaller sample size and
339 shorter branch lengths reduced the power to estimate parameters relative to diploids, as reflected
340 by the larger standard error around the neo-polyploid optimum. Nevertheless, the proportion of
341 neo-polyploids in our data set (33% of angiosperms) is almost exactly that found in the full
342 Wood et al. (2009) data set and similar to that in other studies (Mayrose et al., 2011; Barker et
343 al., 2016; Landis et al., 2018).

344 There was also biological variability in our dataset. In our taxon sampling, we were
345 agnostic to variation in mating systems and modes of polyploid origins, since our interest was in
346 how *PTGR* has evolved in natural stabilized polyploids. In retrospect, our sample does seem
347 representative. Of 14 angiosperm polyploids whose mode of origin has been studied, seven were
348 autopolyploid and seven allopolyploid, similar to the nearly-equal proportions found by Barker
349 et al. (2016). Furthermore, among 16 polyploids for which mating system has been studied, eight
350 were fully outcrossing, seven were self-compatible (two autogamous, two mixed mating, and
351 three unknown), and one was apomictic – a not unusual distribution (Goodwillie et al., 2005;
352 Gibbs, 2014; Ashman et al., 2014). Thus, our taxon sampling seems not be have been greatly
353 biased. Even with such information, predicting the magnitude of genetic variation in polyploids
354 is not so simple. For example, autotetraploids originate with a subset of the genetic variation in
355 the diploid progenitor population but they often outcross and hybridize, whereas allopolyploids
356 can be highly heterozygous when they originate, but often are highly selfing (Stebbins, 1974;
357 Soltis and Soltis, 1999; Barringer 2007; Whitney et al., 2010). Hence, despite several sources of
358 heterogeneity, the faster *PTGR* optimum of neo-polyploids indicates that *PTGR* acceleration
359 evolves either at the time of WGDs or during the time period in which the descendant species
360 retain a polyploid chromosome number.
361 The closest approximation of the initial effect of polyploidy on *PTGR* is the comparison
362 of diploids with their intraspecific, autopolyploid cytotypes. In all 11 pairs, *PTGRs* of
363 autopolyploid cytotypes were slower than or equal to those of their intraspecific diploid
364 progenitors. We should re-emphasize that all studies involved in vivo crosses among diploid
365 sporophytes (1x pollen on 2x pistils) compared to crosses among tetraploid sporophytes (2x
366 pollen on 4x pistils), in keeping with our goal of generalizing effects on *PTGR* in stabilized

367 polyploids. Nucleotypic effects acting to slow *PTGR* should be most apparent in autopolyploids
368 at inception, because there is lower potential for heterosis. Thus, the lack of any examples of
369 faster *PTGR* in neo-autotetraploid cytotypes than in their diploid progenitors suggests that
370 increased gene dosage by itself generally does not initially fully offset nucleotypic effects.

371 Nucleotypic effects on *PTGR* could be substantial. Tube size affects *PTGR* in a linear
372 fashion, because larger tubes must make more tube wall per unit time, and since tube diameter is
373 constant during growth, the rate of wall production is directly proportional to tip extension rate
374 (Williams et al., 2016). Kostoff & Prokofieva (1935) reported in vivo pollen tubes to be 39%
375 larger in diameter in an allotetraploid *Nicotiana* relative to the mean of its presumed diploid
376 progenitors, and Iyengar (1938) found 8-53% larger tube diameters in tetraploid versus diploid
377 species of *Gossypium*.

378 Taken together our results suggest that nucleotypic effects are strong and act as a brake
379 on *PTGR* at inception (intraspecific polyploid analysis), but as neo-polyploids become stabilized
380 and persist over time, nucleotypic effects are more than offset by genotypic effects (within-genus
381 pairs and model-based analyses) which often produce faster *PTGRs* in angiosperms.

382 We found that DNA content has evolved around a significantly lower optimum in
383 angiosperms than in gymnosperms, even though angiosperms have a broad range of DNA C-
384 values that encompass the entire range of seed plant genome sizes (Fig. 3; see Leitch and Leitch,
385 2013 for a larger survey). Angiosperms also have great variation in ploidy level, a history of
386 speciation by polyploidy, and much evidence of past genome duplication (Ahuja, 2005; Wood et
387 al., 2009; Husband et al., 2013; Van de Peer et al., 2017; Landis et al., 2018). There were at least
388 1-7 WGDs in the lineages leading from the seed plant root to each of the tips in our *PTGR* tree,
389 and 33% of taxa (68/206 angiosperms versus 2/23 gymnosperms) were identified as neo-

390 polyploids. The often low DNA content and high ploidy levels of angiosperms are not surprising
391 given that genome duplication is commonly followed by rapid loss of DNA sequences, gene
392 fractionation by large-scale deletions, biased retention of genes with beneficial dosage effects,
393 and ultimately a return to an apparent diploid state in sporophytes (Conant and Wolfe, 2008;
394 Conant et al., 2014; Freeling et al., 2015; Dodsworth et al., 2016; Wendel et al., 2018). Thus, one
395 explanation for the much faster *PTGRs* of angiosperms relative to gymnosperms is that
396 widespread gene duplication by WGDs have often enabled transgressive evolution of faster
397 *PTGRs* leading to the observed pattern of convergent evolution of extremely fast *PTGRs* in many
398 unrelated lineages of monocots and eudicots.

399 WGDs have been rare in gymnosperms (Ahuja, 2005; Leitch et al., 2005; Wood et al.,
400 2009; Soltis et al., 2009; Husband et al., 2013; Leitch and Leitch, 2013; Lee and Kim, 2014) and
401 their high DNA contents are thought to be due mainly to high transposon activity without
402 repeated rounds of genome duplication (Leitch & Leitch, 2013; Lee and Kim, 2014). Hence,
403 gymnosperms may have experienced the nucleotypic effects of higher DNA content on pollen
404 tube dimensions, which is predicted to reduce *PTGR,* without the potential for counter-balancing
405 effects, such as initially higher gene dosage and heterozygosity followed by gene sorting during
406 the diploidization process. Our finding of a negative correlation between *PTGR* and DNA
407 content in gymnosperms, but not in angiosperms supports that hypothesis.

408 Though gymnosperm *PTGRs* are likely affected by tube sizes, nucleotypic effects do not
409 account for the magnitude of the difference in their slow *PTGRs* relative to those of angiosperms.
410 Gymnosperm pollen tubes can range up to 300 μm in diameter (Coulter and Chamberlain, 1928;
411 Gifford and Foster, 1989), but many species of siphonogamous conifers and Gnetales have
412 angiosperm-like pollen tube diameters in the 10 to 20 μm range. Yet no gymnosperm has

413 evolved a *PTGR* faster than 20 μm h^{-1}. It has been argued that their pecto-cellulosic wall
414 structure is a limitation relative to angiosperm pollen tube walls, which use the plasma
415 membrane-bound enzymes callose synthase and pectin-methylesterase in a novel way to more
416 rapidly synthesize a strong and durable tube cell wall and callose plugs (Derksen, 1999,
417 Abercrombie et al., 2012; Wallace and Williams, 2017). However, other types of pecto-cellosic
418 tip-growing cells, such as root hairs, grow faster than gymnosperm pollen tubes (Williams et al.,
419 2016). Thus, it seems likely that the extremely slow growth rates of gymnosperm pollen tubes
420 reflect an ancestrally antagonistic relationship between maternal tissues and pollen tubes that
421 functioned as invasively growing rhizoids, coupled with a lack of selection for faster growth rate
422 due to the absence of pollen competition and a long period between pollination and fertilization.
423 Our results also suggest a lack of opportunity for genotypic effects to evolve due to the rarity of
424 WGDs.
425
426 ***Conclusions*** **-** Studies across the tree of life have consistently shown that ploidy level and DNA
427 content are correlated with cell size and metabolic rate (Cavalier-Smith, 1978; Gregory, 2001;
428 Cavalier-Smith, 2005). Pollen tube dimensions and energetics affect the amount of cell wall
429 material produced per unit of growth and the rate at which cell wall is produced, which together
430 determine *PTGR*. In gymnosperms, *PTGR* was negatively correlated with genome size, but in
431 angiosperms, where the effects of WGDs are much more prevalent, there was no such
432 correlation, and neo-polyploids evolved around a higher *PTGR* optimum than diploids. These
433 results support the expectation that genome size increases incur nucleotypic effects that act as a
434 brake on growth rate. The degree to which genotypic effects counterbalance nucleotypic effects
435 depends on the historical nature and time since genome size increase in any particular lineage.

436 Understanding causal relationships between genome size, ploidy and *PTGR* will involve
437 mechanistic studies of tube cell dimensions and wall synthesis rates in haploid and polyploid
438 gametophytes. On the other hand, there appears to be great variation in the tug of war between
439 genotypic and nucleotypic effects, and there are likely to be deeper evolutionary patterns
440 underlying that variation.
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442
443
444 **ACKNOWLEDGEMENTS**

445 We thank B. O'Meara and J. Beaulieu for advice on phylogenetic analyses, I. Leitch for data on
446 DNA content, and J. Edwards and M. Rankin for assistance in the lab. We are tremendously
447 grateful to several anonymous reviewers for their perceptive and useful comments. Partial
448 support to J.B.R. was provided by National Science Foundation award IOS 1052291 to J.H.W.
449
450
451 **Authors Contributions:** J.B.R. and J.H.W. jointly conceived of the study and wrote the paper;
452 J.B.R. collected data on genome sizes and ploidy levels, constructed the phylogenetic tree and
453 performed all comparative analyses; J.H.W. collected data on *PTGRs* and diploid-autopolyploid
454 *PTGRs*.
455
456 **Data Accessibility Statement:** Scripts written during the creation of this manuscript are
457 available on GitHub: https://github.com/jbr1848/PTGR.genome.evolution. The phylogenetic tree
458 created during this study can be found on TreeBase:
459 http://purl.org/phylo/treebase/phylows/study/TB2:S24291.

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463 **LITERATURE CITED**

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677 **Table 1:** Phylogenetic generalized least squares regression of $\log_{10}$ *PTGR* as a function of $\log_{10}$
678 C-value. Only models contributing more than 1% of total weight are included. *P* values are for
679 the slope of the regression. Gymnosperm averaged model: Log_{10} *PTGR* = 1.46 (± 0.62) - 1.09 (±
680 0.49)*($\log_{10}$ C-value).
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Seed plants (*N* = 183)			**Angiosperms (*N* = 161)**			**Gymnosperms (*N* = 23)**		
Model	**Weight**	***P***	**Model**	**Weight**	***P***	**Model**	**Weight**	***P***
kappa	0.999	0.463	kappa	0.975	0.284	OU	0.265	0.020
			lambda	0.024	0.221	delta	0.257	0.006
						kappa	0.193	0.445
						BM	0.121	0.001
						lambda	0.119	0.327
						EB	0.044	0.001

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689 **Table 2*:* Parameter estimates for angiosperm *PTGR* analyses under different evolutionary**
690 **models.** Note that OU1 is a single optimum model, and the rest specify separate "diploid"
691 (paleo-polyploid) and neo-polyploid optima. BM1 and BMS models contributed <1% model
692 weight and were excluded.
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Model	ΔAICc	Model weight	Diploid σ^2	Polyploid σ^2	Diploid α	Polyploid α	Diploid optimum	Polyploid optimum
OUMA	[332.76]	0.373	0.099		0.077	0.074	2.776	3.285
OUMV	0.23	0.333	0.099	0.057	0.077		2.768	3.262
OUM	1.53	0.174	0.090		0.080		2.760	3.263
OU1	2.26	0.120	0.089		0.077		2.812	
AVERAGED MODEL		~1.0	0.096 ± 0.177	0.082 ± 0.220	0.078 ± 0.213	0.077 ± 0.217	2.775 ± 0.079	3.217 ± 0.231

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697 **FIGURE LEGENDS**

698 **Figure 1. Predicted initial effects of large increases in genome size on pollen tube growth**
699 **rate (*PTGR*).** The dashed line indicates an ancestral haploid (1x) *PTGR*. Upon transition to a
700 larger (> 1x) genome size, nucleotypic effects should act to decrease *PTGR* regardless of
701 mechanism of change. Genotypic effects are only present after WGD or large-scale gene
702 duplications and are predicted to increase *PTGR* via increased gene dosage and heterozygosity.
703 The magnitude of heterosis due to initial increase in heterozygosity is expected to scale with
704 genetic variation in the descendent taxon. The ancestral haploid *PTGR* can only be conserved
705 when genotypic and nucleotypic effects perfectly offset each other.

706

707 **Figure 2: Relationship between pollen tube growth rate (*PTGR*) and DNA content (1C-**
708 **value) in seed plants.** The model- averaged slope of the PGLS regression is shown for
709 gymnosperms (green points, $N = 161$), whereas slopes for seed plants (all points, $N = 183$) and
710 angiosperms (purple points, $N = 23$) were non-significant. Optima (with standard error bars) for
711 each group (from model-based analyses in Tables S3, S4) are included for illustrative purposes.

712

713 **Figure 3: Inferred pattern of pollen tube growth rate (*PTGR*) and genome size changes in**
714 **seed plants.** Contour plot comparing *PTGR* evolution (left, $\mu m\ h^{-1}$) and C-value evolution (right,
715 picograms) ($N = 183$). Scale bar at the bottom of each phylogeny indicates 100 million years.
716 GYM = gymnosperms; ANA = Amborellales, Nymphaeales, Austrobaileyales, Chloranthales,
717 eumagnoliids; MONO = monocots.

718

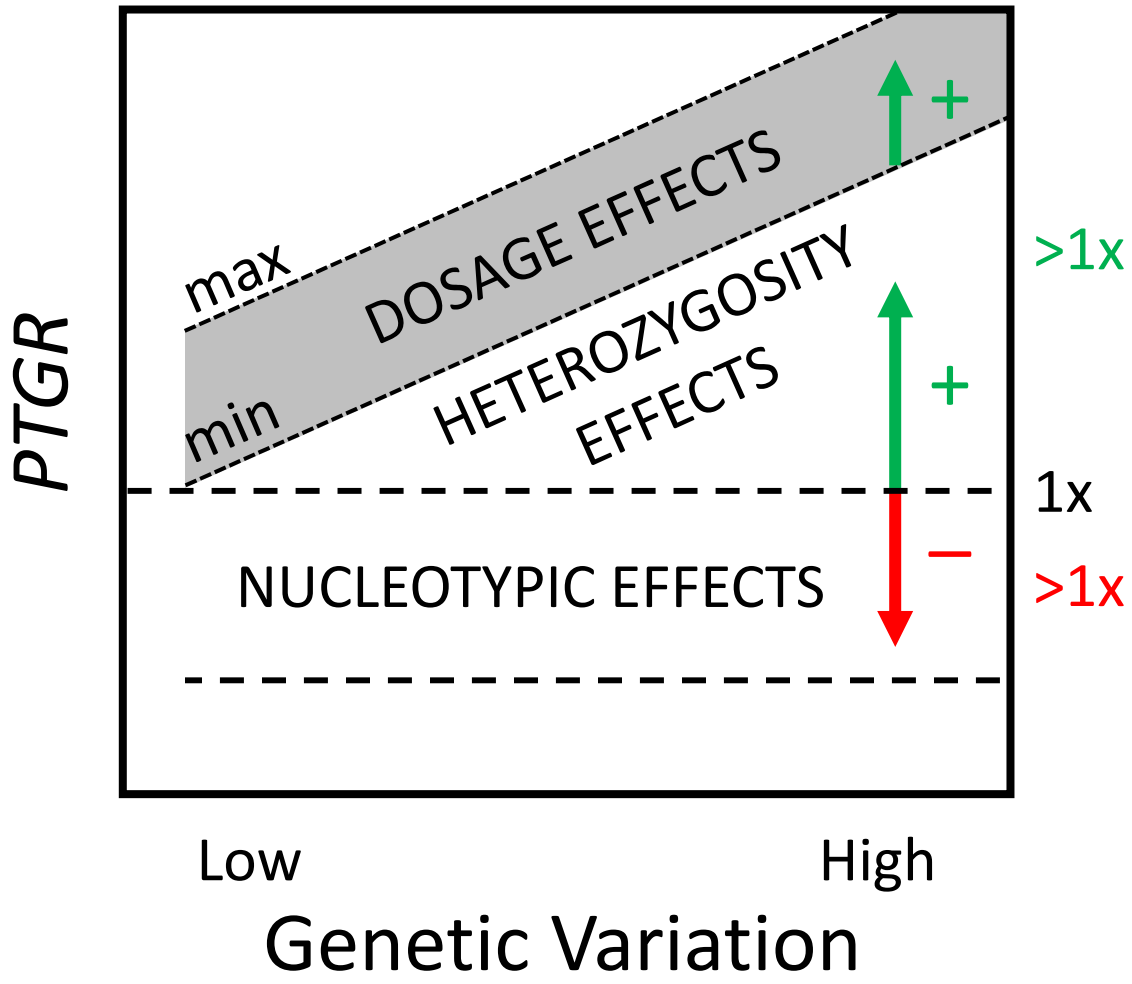
719 **Figure 4: Coincident evolution of pollen tube growth rate (*PTGR*) and DNA content (C-**
720 **value).** Paired *SURFACE* plot showing regime shifts in *PTGR* (left) versus DNA content (right)
721 (N = 183). Nodes which have experienced a regime shift along the stem leading to it are marked
722 with magenta diamonds (not all *PTGR* shifts are shown, since *PTGR* tree has been pruned to
723 match C-value tree). Branch colors: *gray* = seed plant ancestral optimum (*PTGR* $\theta = 0.147$; C-
724 value $\theta = 2.71$); *green* = ancestral optimum for angiosperms (*PTGR* $\theta = 2.47$; C-value $\theta =$
725 0.702); *red* = derived lineages following a shift to a higher optimum than previously; *blue* =
726 derived lineages following a shift to a lower optimum than previously. Black arrows indicate
727 instances where shifts in *PTGR* and C-value coincide. Scale bar at the bottom of each phylogeny
728 indicates 100 million years. GYM = gymnosperms; A = Amborellales, Nymphaeales,
729 Autrobaileyales, Chloranthales, eumagnoliids; MONO = monocots.
730
731 **Additional Supporting Information may be found online in the supporting information**
732 **section at the end of the article:**
733
734 **Appendix S1**: **119 additional *PTGR* values and references not reported in Williams 2012.**
735
736 **Appendix S2: Pollen tube growth rate (*PTGR*) evolution across Spermatophytes.**
737
738 **Appendix S3: Summary statistics for pollen tube growth rate (*PTGR*) of *Magnolia***
739 ***grandiflora*.**
740
741 **Appendix S4: Sensitivity analysis for the magnitude of $\log_{10}$ *PTGR* error estimates.**
742
743 **Appendix S5: *PTGR* evolution in gymnosperms vs. angiosperms.**
744
745 **Appendix S6: C-value evolution in gymnosperms vs. angiosperms.**

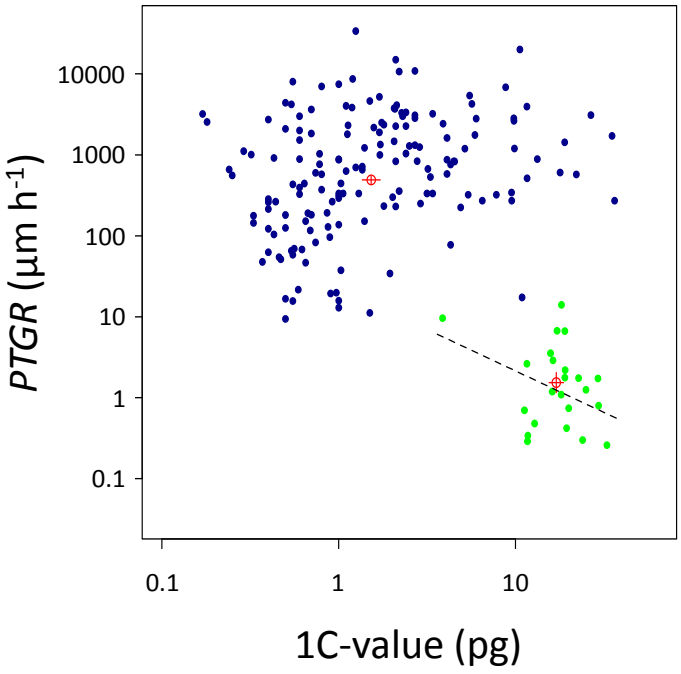
746

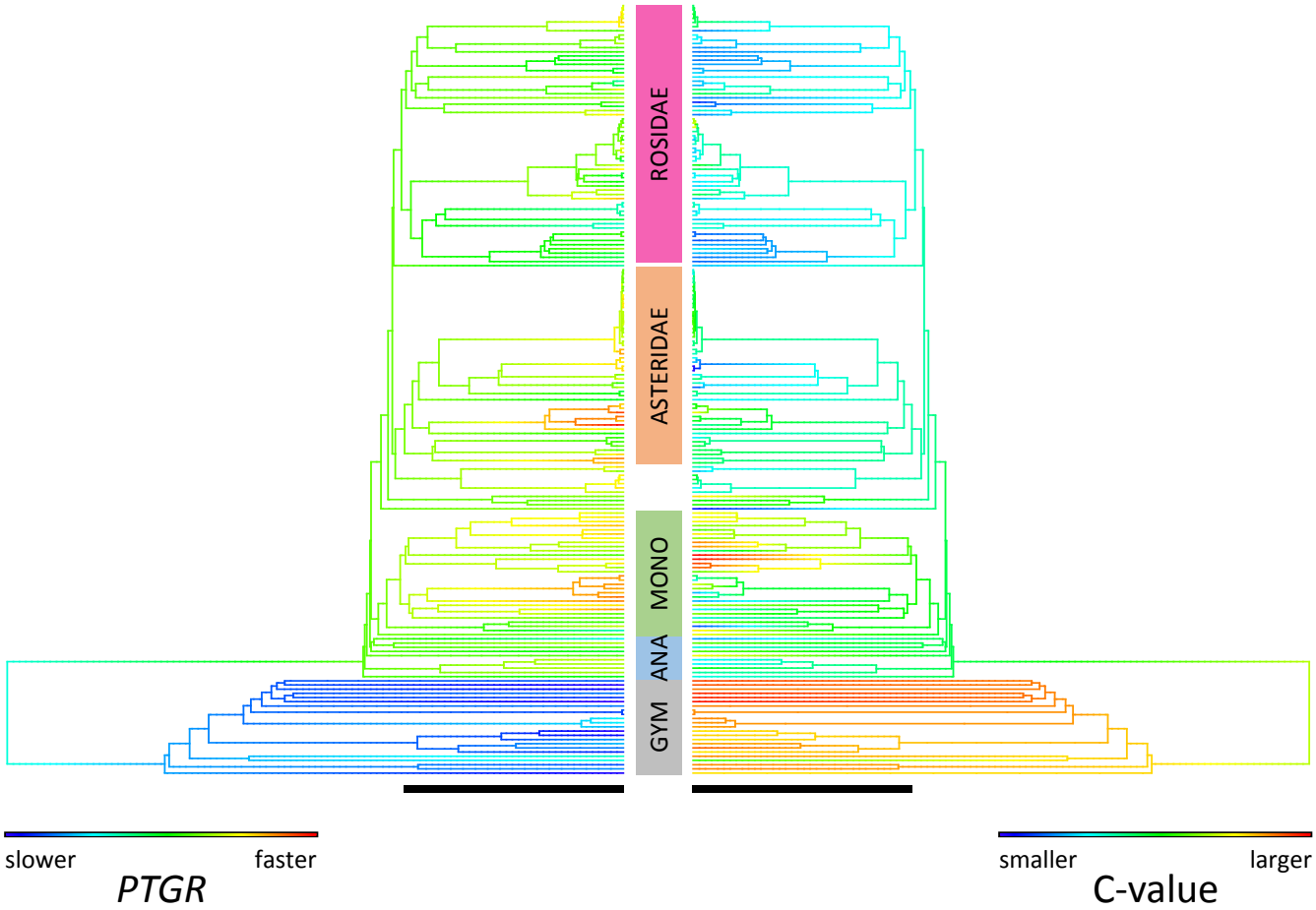
747 **Appendix S7: Closely-related taxon analyses**.

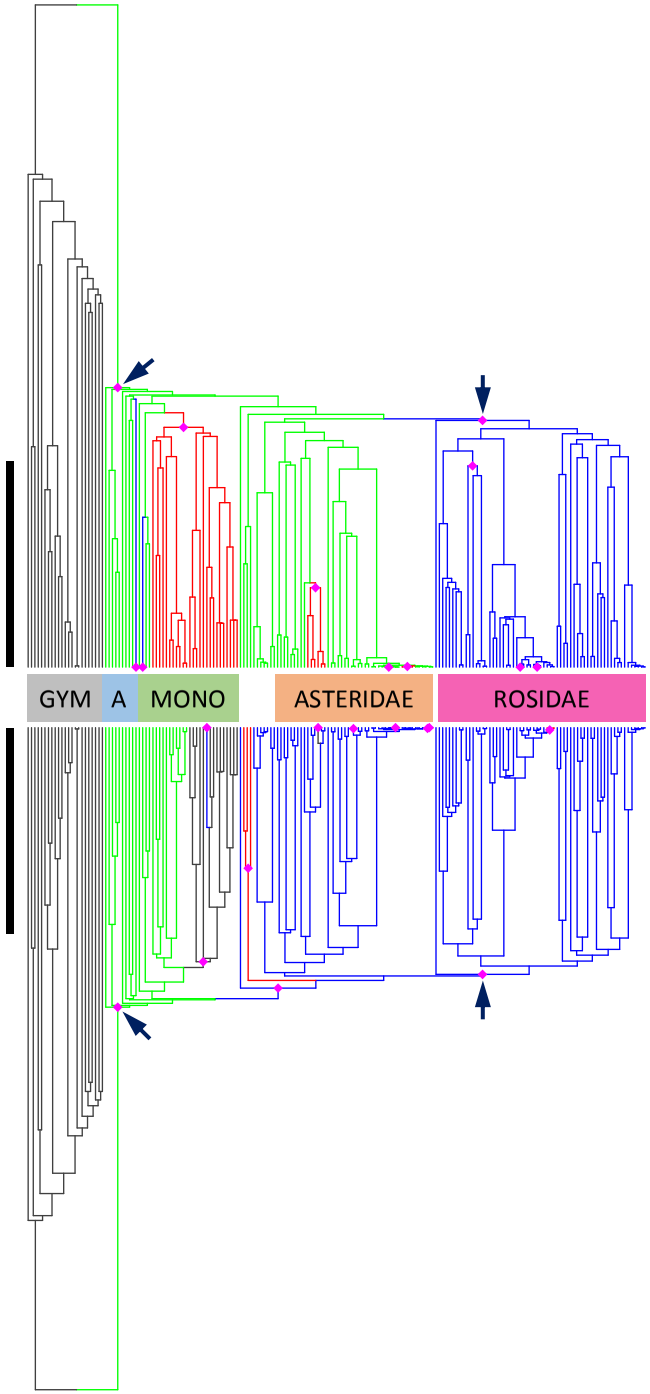
748

749 **Appendix S8: Phylogenetic ANCOVA results.**


PTGR
max
min
DOSAGE EFFECTS
HETEROZYGOSITY EFFECTS
+
+
>1x
1x
NUCLEOTYPIC EFFECTS
−
>1x
Low
High
Genetic Variation


10000
1000
100
10
1
0.1
PTGR (µm h-1)
0.1
1
10
1C-value (pg)


ROSIDAE
ASTERIDAE
MONO
ANA
GYM
slower
faster
PTGR
smaller
larger
C-value


GYM
A
MONO
ASTERIDAE
ROSIDAE

Appendix S1: 119 additional *PTGR* values and references not reported in Williams 2012.

Taxon	***PTGR*** **(μm h^{-1})**	**Reference**
Abelmoschus esculentus	5217	Patil et al 2013
Abutilon x hybridum	1292	Cited in Sears 1937
Acacia mangium	46.5	Ngheim et al 2013
Acacia_auriculiformis	116	Ngheim et al 2013
Acer rubrum	182	van Ryn et al 1988, Radford et al 1968
Aegle marmelos	181	Bhardwaj and Tandon 2013
Albuca canadensis	1000	Johnson et al 2012
Albuca setosa	2477	Johnson et al 2012
Alstroemeria aurea	3091	Aizen and Raffaele 1998, De Jeu et al. 1996
Alstroemeria pelegrina	574	De Jeu et al. 1996
Anathallis	4320	Gontijo et al 2010
Antirrhinum controversum	131	Cario and Guemes 2014
Antirrhinum valentinum	160	Cario and Guemes 2014
Aureolaria pedicularia	15.2	Ramstetter and Mulcahy 1986
Bertholletia excels	800	Moritz and Ludders 1993
Betula papyrifera	11.1	Williams unpbl.
Boswellia serrata	182	Sunnichan et al 2005
Brassica rapa	371	Hiroi et al 2013
Calluna vulgaris	387	Behrend et al 2012, Mahy and Jacquemart 1999
Cambessedesia	587	dos Santos et al 2012
Camellia oleifera	338	Gao et al 2015
Carica papaya	214	Traub and O'Rork 1939
Ceiba pentandra	2500	Gribel et al 1999
Ceratonia siliqua	152	Von Haselberg et al. 2004
Chamaecrista fasciculata	637	Tucker 1996, Fenster and Sork 1988
Citrullus lanatus	1151	Sedgley and Buttrose 1978
Citrus maxima	58.3	Distephano et al 2012
Citrus medica	122	Distefano et al 2012
Citrus reticulata	51.3	Distefano et al 2012
Clarkia xantiana	630	Hove and Mazer 2013
Commiphora wrightii	42.4	Geetha et al 2013
Cornus florida	70.8	Reed 2004
Corylus heterophylla	62.5	Liu et al 2014
Cucumis anguria	774	Matsumoto et al. 2012
Cucumis melo	870	Matsumoto et al. 2012
Cucumis metulifer	1009	Matsumoto et al. 2012
Cybistax antisyphilitica	583	Bittencourt et al 2010

Cyrtandra kauaiensis	261	Johnson et al 2015
Cyrtandra longifolia	221	Johnson et al 2015
Cyrtandra platyphylla	305	Johnson et al 2015
Cytisus multiflorus	4.48	Valtueña et al 2010
Cytisus striatus	19.8	Rodriguez-Riaño et al 1999
Dalzellia zeylanica	1159	Sehgal et al 2011
Dianthus caryophyllus	3002	Larsen et al 1995
Downingia bacigalupii	853	Kaplan 1969
Echium vulgare	590	Melser et al 1997
Eruca vesicaria	275	Cited in Sears 1937
Eucalyptus globulus	58.3	Gore et al 1990
Faramea occidentalis	2183	Travers 1999
Ficus carica	47.6	Beck and Lord 1988
Fumana	273	Carrio and Guemes 2013
Guihaiothamnus acaulis	188	Xie et al 2013
Haberlea rhodopensis	152	Bogacheva-Milkoteva 2013
Handroanthus ochraceus	1617	Oliveira, pers. comm.
Handroanthus serratifolius	1617	Oliveira, pers. comm.
Hedyosmum brasiliense	97.2	Williams and Edwards, unpbl.
Hedyotis acutangula	991	Wu et al 2010
Helleborus foetidus	514	Vesprini and Pacini 2000
Heuchera micrantha	181	Rabe and Soltis 1999
Hippophae rhamnoides	20.9	Mangla et al 2013
Hymenaea	1667	Gibbs et al 1999
Ipomoea purpurea	7450	Shu-Mei Chang, pers. comm. 2014
Ipomopsis aggregata	2409	Sage et al 2006, Wolf et al 2001
Jathropa curcas	915	Abdelgadir et al 2012
Lactoris fernandeziana	40	Bernardello et al 1999
Lactuca sativa	3085	Einset 1944
Lagerstroemia indica	1175	Pounders et al 2006
Lathyrus chloranthus	271	Herrick et al 1993
Lathyrus odoratus	321	Herrick et al 1993
Limnocharis	467	Hall 1902
Linaria	392	Cited in Sears 1937
Lupinus arizonicus	442	Wainwright 1978
Magnolia grandiflora	828	Edwards, Rankin, and Williams, unpbl. 2014
Medicago rigidula	82.2	Sangduen et al 1983
Medicago sativa	192	Barnes and Cleveland 1963
Morinda parvifolia	957	Liu et al 2012
Mussaenda kwangtungensis	963	Luo et al 2015
Mussaenda shikokiana	816	Chen et al 2014
Nemesia strumosa	333	Sears 1937

Nivenia corymbosa	873	Goldblatt and Bernhardt 1990
Nivenia stokeii	1217	Goldblatt and Bernhardt 1990
Nuphar advena	835	Taylor and Williams, unpbl.
Nyctanthes arbor tristis	526	Bhatnagar and Uma 1969
Orchis anthropophora	357	Luca et al 2015
Orchis italica	357	Luca et al 2015
Oreocharis acaulis	1318	Guo et al 2013
Oroxylum indicum	3000	Gautam et al 2009
Paeonia brownii	20.8	Bernhardt et al 2013
Parthenium	1333	Gerstel and Riner 1950
Passiflora edulis	2174	Rego et al 2000
Phalaenopsis	208	Zhang and O-Neill 1993
Phoenix dactylifera	315	Reuveni et al 1986
Platanthera	462	Stickler et al 2013 (poster)
Plumbago zeylanica	12741	Russell 1985
Polypleurum stylosum	153	Khosla et al 2000
Potamogeton intortusifolius	267	Zhang et al 2010
Potamogeton perfoliatus	1585	Zhang et al 2010
Potamogeton wrightii	1483	Zhang et al 2010
Pseudopiptadenia	39	Pires and Freitas 2008
Restrepia	99	Millner et al 2015
Schisandra sphenanthera	88.9	Du et al 2012
Silene vulgaris	2323	Glaeti 2006
Solanum chacoense	396	Liu et al 2012
Solanum laxum	333	Lewis and Crowe 1958
Sorghum bicolor	3638	Heslop-Harrison et al 1984, Hodnett et al 2005
Spathodea campanulata	4028	Bittencourt et al 2003
Sporobolus anglicus	8943	Li et al 2008
Thryptomene calycina	320	Beardsell et al 1993
Ticodendron incognitum	382	Sogo and Tobe 2008
Torenia baillonii	3200	Kikuchi et al 2007
Torenia concolor	1900	Kikuchi et al 2007
Trimezia	2000	Bystedt and Vennigerholz 1991
Vaccinium corybosum	191	Knight and Scott 1964
Vaccinium myrtillus	67.1	Jacquemart and Thompson 1996
Vaccinium uliginosum	54.3	Jacquemart and Thompson 1996
Vaccinium vitis idaea	98.6	Jacquemart and Thompson 1996
Zeyheria montana	2554	Bittencourt and Semir 2004
Zeylanidium lichenoides	354	Chaudhary et al 2014, Sehgal et al 2014

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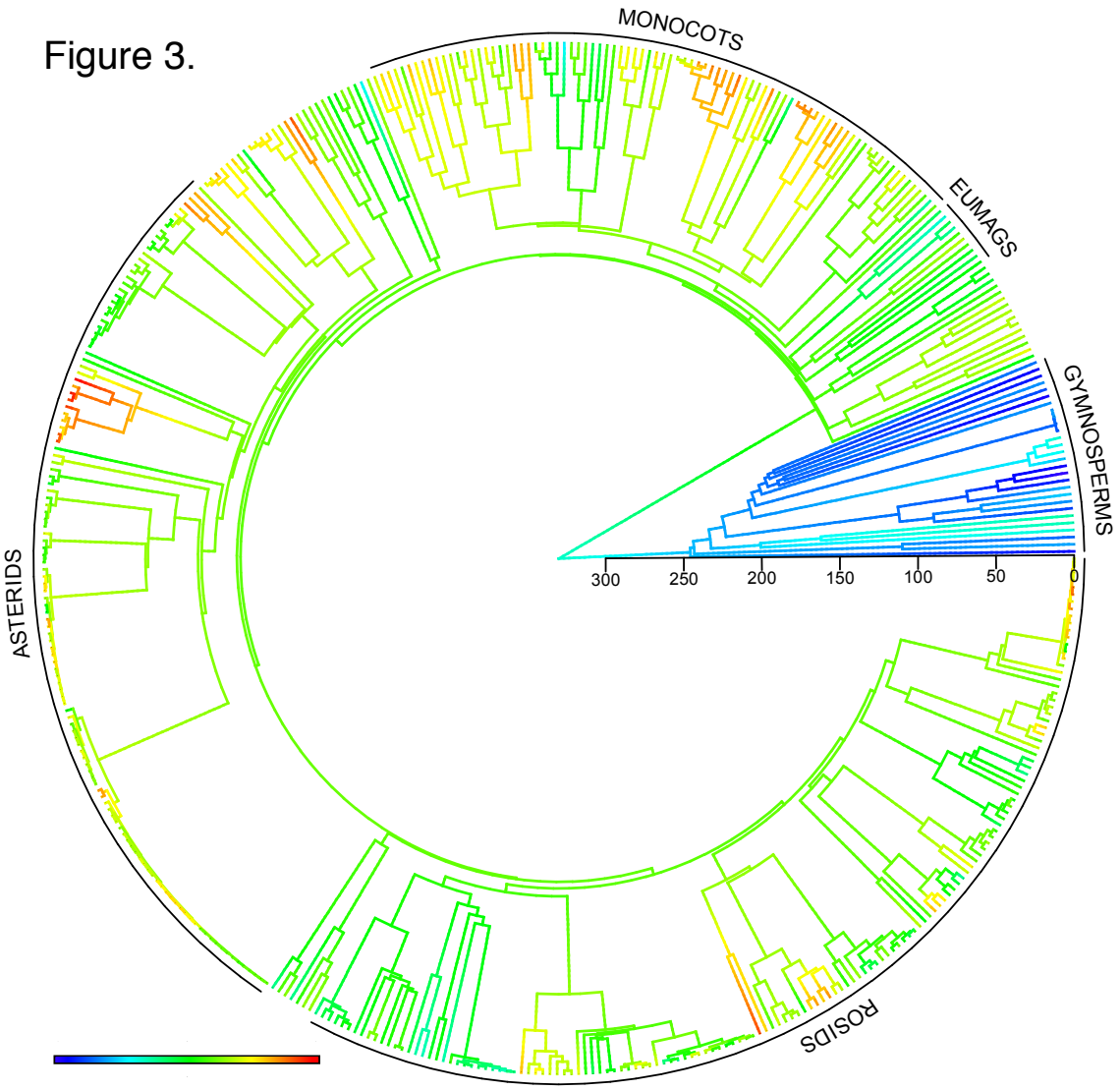
Appendix S2: Pollen tube growth rate (*PTGR*) evolution across Spermatophytes. Contour plot showing reconstructed history of *PTGR*. Cool colors indicate *PTGR*s closer to the minimum value in seed plants while warm colors indicate *PTGR*s closer to the maximum value in seed plants. Scale bar indicates millions of years before present.



Appendix S3: Summary statistics for pollen tube growth rate (*PTGR*) of *Magnolia grandiflora*.

Statistic	*PTGR*
raw mean (*N = 25* crosses)	827.6 μm h^{-1}
raw SD (*N = 25*)	141.3 μm h^{-1}
raw CV	0.1708
Log(10) mean	2.912 μm h^{-1}
transformed SD	0.0689 μm h^{-1}
transformed CV	0.0237

Appendix S4: Sensitivity analysis for the magnitude of $\log_{10}$ *PTGR* error estimates. Values in each column represent model weights from separate analyses of angiosperm diploids (*N* = 138) vs. polyploids (*N* = 68). Column headings indicate the coefficient of variation (CV), ranging from zero to 0.50, used to calculate estimated species-specific standard deviations around *PTGRs* in each analysis. The best-fitting model at each CV is indicated in bold. [a], Empirically-determined CV of *Magnolia grandiflora*.

	Coefficient of Variation					
Model	**0.00**	**0.0237[a]**	**0.05**	**0.10**	**0.25**	**0.50**
OUMV	**0.376**	**0.373**	**0.459**	**0.461**	0.119	0.045
OUMA	0.363	0.333	0.307	N/A	0.172	0.070
OUM	0.157	0.174	0.137	0.309	0.212	0.080
OU1	0.104	0.120	0.097	0.230	**0.390**	0.188
BM1	1.74E-31	1.77E-25	3.20E-20	4.58E-14	0.089	**0.528**
BMS	5.45E-32	5.52E-26	4.81E-24	1.30E-12	0.017	0.089

Appendix S5: *PTGR* evolution in gymnosperms vs. angiosperms. Selective regime 1 represents gymnosperms (N = 28) and selective regime 2 represents angiosperms (N = 423). Models representing <1% of the model weight are excluded.

model	ΔAICc	model weight	sigma sq1	alpha1	sigma sq2	alpha2	optimum1	se1	optimum2	se2
OUMV	(822.1)	0.338	0.091	0.122	0.139	0.122	0.187	0.123	2.690	0.048
OUM	0.040	0.332	0.137	0.124	0.137	0.124	0.188	0.150	2.690	0.047
OUMA	0.051	0.330	0.091	0.120	0.091	0.123	0.187	0.123	2.690	0.048
AVERAGED MODEL		~1	0.106	0.122	0.122	0.123	0.187	0.132	2.690	0.048

Appendix S6: C-value evolution in gymnosperms vs. angiosperms. Selective regime 1 represents gymnosperms (*N* = 23) and selective regime 2 represents angiosperms (*N* = 161). Models representing <1% of the model weight are excluded.

model	ΔAICc	model weight	sigma sq1	alpha1	sigma sq2	alpha2	optimum1	se1	optimum2	se2
OUMV	(172.4)	0.502	0.007	0.095	0.005	0.095	1.231	0.041	0.184	0.051
OUMA	0.02	0.498	0.006	0.085	0.006	0.096	1.231	0.042	0.184	0.051
AVERAGED MODEL		~1	0.007	0.09	0.005	0.095	1.231	0.041	0.184	0.051

Appendix S7: Closely-related taxon analyses.

Appendix S7a. Closely-related species pairs extracted from ploidy dataset. *PTGRs* in μm h^{-1}. Binomial test ($P = 0.623$; $N = 10$).

DIPLOID TAXON	POLYPLOID TAXON	DIPLOID *PTGR*	POLYPLOID *PTGR*	FASTER TAXON
Anagallis arvensis	*Anagallis monelli*	233.33	105.56	diploid
Hemerocallis thunbergii	*Hemerocallis fulva*	4166.67	6266.67	polyploid
Ipomoea purpurea	*Ipomoea batatas*	7450	4625	diploid
Iris mandshurica	*Iris pseudacorus*	278.65	4255.50	polyploid
Lythrum junceum	*Lythrum salicaria*	722.22	493.60	diploid
Medicago rigidula	*Medicago sativa*	82.23	192.17	polyploid
Prunus avium	*Prunus domestica*	260.88	177.5	diploid
Tabebuia rosea	*Tabebuia chrysotricha*	1111.11	1342.45	polyploid
Trifolium pratense	*Trifolium polymorphum*	103.89	444.44	polyploid
Ulmus pumila	*Ulmus americana*	56.25	56.25	equivocal

Appendix S7b. Intraspecific diploid-polyploid cytotypes taken from the literature. All are autopolyploids. Binomial test, $N = 11$, $P = 0.0020$. Percent difference is calculated relative to the diploid.

REF.	TAXON	DIPLOID *PTGR*	POLYPLOID *PTGR*	POLYPLOID +/- (% DIFF)
1	*Beta vulgaris* 2x,4x	241.2 µm/h	142.7 µm/h	slower (-69%)
2	*Cucumis melo* 2x,4x	"no difference"		equivocal
3	*Datura stramonium* 2x,4x	2953.7 µm/h	2812.5 µm/h	slower (-4.8%)
4	*Lactuca sativa* 2x,4x	"faster"	"slower"	slower
5	*Malus domestica* 2x,4x	3.8 units/96 h	3.1 units/96 h	slower (-18.4%)
6	*Malus domestica* 2x,3x	682 µm/h	465 µm/h	slower (-31.8%)
7	*Secale cereale* 2x,4x	12.24 units/h	12.08 units/h	slower (-1.3%)
8	*Solanum sp*. 2x,4x	"faster"	"slower"	slower
9	*Trifolium pratense* 2x,4x	2322 µm/h	1950 µm/h	slower (-16%)
10,11	*Zea mays* 2x,4x	Slower pollen germination and pollen tube growth rate in 4x		slower

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Appendix S8: Phylogenetic ANCOVA results. Models comprising <1% of the model weight are excluded.

Appendix S8a: Angiosperms only (*N* = 100).

Full model

Model	Weight	$P_{C\text{-}value}$	P_{ploidy}	$P_{interaction}$
kappa	0.688	0.028	0.565	0.334
OU	0.287	0.005	0.679	0.414
lambda	0.025	0.076	0.867	0.626

Model averaged slope for C-value: 0.399 ± 0.167

No interaction

Model	Weight	$P_{C\text{-}value}$	P_{ploidy}
kappa	0.652	0.046	0.748
OU	0.313	0.006	0.895
lambda	0.035	0.083	0.979

Model averaged slope for C-value: 0.344 ± 0.153

C-value only

Model	Weight	$P_{C\text{-}value}$
kappa	0.642	0.045
OU	0.322	0.005
lambda	0.036	0.079

Model averaged slope for C-value: 0.344 ± 0.153

Appendix S8b: All seed plants (*N* = 118).

Full model

Model	Weight	$P_{C\text{-}value}$	P_{ploidy}	$P_{interaction}$
kappa	0.998	0.080	0.486	0.624

Slope for C-value: 0.288 ± 0.163

No interaction

Model	Weight	$P_{C\text{-}value}$	P_{ploidy}
kappa	0.997	0.092	0.562

Slope for C-value: 0.263 ± 0.155

C-value only

Model	Weight	$P_{C\text{-}value}$
kappa	0.997	0.090

Slope for C-value: 0.264 ± 0.154