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 seed plants deliver sperm to eggs via a pollen tube. Pollen tube growth rate (*PTGR*) may evolve rapidly due to pollen competition and haploid selection, but many angiosperms are currently polyploid and all have polyploid histories. Polyploidy should initially accelerate *PTGR* via "genotypic effects" of increased gene dosage and heterozygosity on metabolic rates, but "nucleotypic effects" of genome size on cell size should reduce *PTGR.* How are such cell-level consequences of genome size change related to the evolution of orders-of-magnitude faster *PTGRs* in angiosperms versus gymnosperms?

Methods - We assembled a phylogenetic tree of 451 species with known *PTGRs* and added ploidy and DNA content values from the literature. We then used comparative phylogenetic methods to detect phylogenetic signal, differences in selective optima, and correlated evolution of *PTGR* and genome size.

Key Results - Gymnosperms had significantly higher C-value and slower *PTGR* optima than angiosperms. DNA content was negatively correlated with *PTGR* in gymnosperms, but non-significant in angiosperms. Among angiosperms, model-based analyses indicated a single *PTGR* selective optimum for diploids and polyploids. Sister-taxon and intraspecific-cytotype comparisons indicated either no difference or slower *PTGRs* in polyploids than diploids.

Conclusions – Results from closely-related taxa indicate nucleotypic effects are initially equal to or outweigh genotypic effects, and none of the analyses found that genome duplication accelerated *PTGR.* Thus, our results suggest that gradual and sustained expansion of *PTGRs* in angiosperms has mostly occurred in diploid or diploidized species, where pollen tubes are most subject to haploid selection.

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

51 **INTRODUCTION**

52 In seed plants, the male gametophyte is a highly-reduced haploid organism, developing
53 within the pollen grain until pollination, when it germinates and a pollen tube emerges to invade
54 maternal tissues. The pollen tube has been recruited for the novel function of siphonogamy, the
55 transport of non-motile sperm cells to the egg-bearing female gametophyte, in conifers and
56 Gnetales (two related gymnosperm groups) and in angiosperms (Friedman, 1993). In
57 siphonogamous groups, pollen germination speed and pollen tube growth rate (*PTGR*) together
58 determine fertilization timing. Much of the haploid genome of the male gametophyte is
59 expressed during pollen tube development and also after fertilization, in the diploid sporophyte
60 generation (Tanksley et al., 1981; Twell et al., 2006; Otto et al. 2015). Pollen competition is
61 thought to be much more intense in angiosperms, causing greater exposure to haploid selection
62 and faster evolution of genes for pollen germination speed and tube growth rate (Mulcahy, 1979;
63 Snow, 1990; Arunkumar et al. 2013; Otto et al. 2015). Indeed, angiosperms have evolved orders-
64 of-magnitude faster pollen tube growth rates than gymnosperms (Williams 2009, 2012).

65 One complication with the idea that pollen performance genes have evolved rapidly
66 under haploid selection is that whole genome duplications (WGDs) have been especially
67 common in angiosperms relative to gymnosperms (Leitch & Leitch, 2012, 2013). WGDs elevate
68 pollen tube gene expression above the haploid level, which has consequences for growth rates.
69 First, WGDs mask haploid selection on pollen-expressed genes via the effects of heterosis
70 (sheltering of deleterious alleles and/or new allelic interactions) (Lande and Schemske, 1985;
71 Husband and Schemske, 1997; Comai, 2005; Birchler et al. 2010; Husband, 2016). Secondly,
72 WGDs enable gene dosage effects that could increase the capacity for protein synthesis and
73 hence increase metabolic rates (Stebbins, 1974; Comai, 2005; Conant and Wolfe, 2008). Thirdly,

74 large changes in DNA content (whether by WGD or other processes) are known to have
75 "nucleotypic" effects on nuclear size, cell size and the duration of the cell cycle, independent of
76 "genotypic" effects at individual loci (Bennett, 1971, 1972; Cavalier-Smith, 1978; Price 1988;
77 Cavalier-Smith, 2005).

78 We know surprisingly little about the mechanistic effects of WGDs on gametophyte
79 performance in general, and how they might balance each other out in any one species, or if there
80 are any universal effects of WGDs on pollen performance specifically. Almost all studies of the
81 effects of increased DNA content on cell size and growth rate have been on relatively isotropic
82 cells with isotropic growth (Commoner, 1964; Holm-Hansen, 1969; Price et al., 1973; Grime,
83 1983; Shuter et al., 1983; Beaulieu et al., 2008). In these, increases in DNA content, whether by
84 WGDs or other mechanisms are known to have the following effects: 1) a longer S-phase is
85 required to replicate more DNA (Van't Hof, 1965; Bennett, 1972), and 2) a longer period of cell
86 growth is needed to grow a larger cell, because more DNA requires a larger nucleus and a
87 proportionally larger cytoplasm (Gregory, 2001; Cavalier-Smith, 2005). As a result, in cell
88 populations, including multicellular tissues, the *population (tissue) growth rate* is slower because
89 the cell cycles of cell lineages are longer. Importantly, the *volumetric growth rate* of individual
90 cells is rarely of interest, and may in fact stay the same after WGD, if the longer duration of the
91 cell cycle is proportional to the increase in cell size.

92 If increases in cell cycle duration and cell size are disproportionate after an increase in
93 DNA content, then volumetric growth rate of an individual isotropic cell must also evolve. Most
94 roughly isotropic plant cells grow by diffuse growth, in which cell wall construction occurs
95 simultaneously across the entire expanding plasma membrane surface (Albersheim et al., 2011).
96 In such cells, surface area increases as the 2/3 power of volume, and consequently ever more cell

97 wall material must be made per unit time during growth. This means that wall and plasma
98 membrane production rates must either keep increasing just to maintain a constant rate of
99 volumetric growth or that volumetric growth rate slows down as surface area increases. Diffuse-
100 growing plant cells can partially reduce this problem by delaying some of their cell wall
101 production until after they reach mature size (Cosgrove, 2005; Albersheim et al., 2011). But a
102 large increase in cell size, such as after WGD, is expected to cause a disproportionate amount of
103 extra construction costs, making it likely that a slower cell growth (expansion) rate will be the
104 consequence.

105 The problem is quite different for anisometric, tip-growing cells, such root hairs and
106 pollen tubes. First, pollen tubes are terminally-differentiated cells, so their cell growth rate is
107 entirely restricted to G1 of the cell cycle. Secondly, as shown in Fig. 1, new cell wall production
108 in a pollen tube occurs only in a very small region near the elongating tube apex (Chebli et al.,
109 2012), and growth rate is directly determined by the rate of wall production (Rounds et al.,
110 2011). Thus, in a pollen tube, cell surface area and cell volume are directly proportional to each
111 other throughout growth, because the area of active wall synthesis remains constant during
112 growth (Fig. 1). Given a constant nutrient supply and temperature, *PTGR* is also constant
113 (Brewbaker and Majumder, 1961). Tube diameter is determined as the tube tip is formed and
114 after germination a tube of constant diameter is maintained by self-similar growth (Fayant et al.,
115 2010; Geitmann, 2011; Nezhad et al., 2013). This means the same amount of work is done at all
116 stages of elongation, and consequently wall production rate does not become limiting at later
117 stages and larger sizes as it does in isotropic, diffuse-growing cells. Furthermore, since a pollen
118 tube only functions during growth, rather than as a mature cell, wall production is not delayed
119 until after mature size is attained. In sum, the volumetric growth rate of a pollen tube directly

120 reflects the amount of wall material being produced per unit of time (Williams *et al.*, 2016). For
121 a neo-diploid pollen tube relative to its haploid ancestor, the nucleotypic effect on *PTGR* will not
122 depend on cell cycle duration or mature cell size per se, but only on the degree to which the
123 amount of wall materials has been modified to accommodate its new DNA content (Fig. 1).

124 How should the nucleotypic effect play out in a cell that only functions during growth? In
125 isotropic, diffuse-growing cells, genome size scales with cell size because the nucleo-
126 cytoplasmic ratio is thought to be conserved for optimal function of the mature cell (Bennett,
127 1972; Cavalier-Smith, 1978; Price, 1988; Cavalier-Smith, 2005; Barow, 2006). In a pollen tube
128 (Fig. 1), most of the functional cytoplasm, including the sperm cells, tube nucleus, and
129 organelles, are confined to a space of constant size between the leading edge of the enlarging
130 tube vacuole and the elongating tip (Hepler and Winship, 2015). Hence, a reasonable expectation
131 is that the volume *per unit of tube length* of a growing diploid pollen tube would be up to double
132 that of its progenitor haploid tube. Doubling volume per unit length results in a 41% increase in
133 tube diameter and circumference (Fig. 1). That value is consistent with in vivo observations of a
134 39% larger diameter tube in an allotetraploid *Nicotiana* relative to the mean of its presumed
135 diploid progenitors (Kostoff & Prokofieva, 1935), or of 8-53% larger tube diameters in tetraploid
136 versus diploid species of *Gossypium* (Iyengar, 1938).

137 Tube shank wall thickness is generally seen as being near its minimal value to function in
138 resisting turgor pressure and compression stress (Parre & Geitmann, 2005). Wall thickness did
139 not scale with tube diameter in several angiosperms (Williams et al. 2016), and turgor did not
140 scale with either tube diameter in fungal hyphae (Harold *et al.*, 1996) or with *PTGR* lily (Benkert
141 *et al.*, 1997). Therefore, all else being equal, nucleotypic effects of genome doubling are

142 expected to cause up to a 41% increase in the amount of wall material needed to extend a pollen
143 tube tip a unit of length, which in turn is predicted to slow *PTGR* by up to 41%,.
144 Genome doubling can also affect *PTGR* by its genotypic effects on pollen tube
145 energetics, which ultimately affect the rate of wall synthesis. Pollen tube energetics are still not
146 well understood, and there are redundant aerobic and anaerobic respiratory systems (Rounds et
147 al. 2010, 2011; Colaço et al., 2012; Obermeyer et al., 2013; Selinski and Scheibe, 2014). Yet it is
148 well accepted that most of the metabolic energy expended by a growing pollen tube is allocated
149 in one way or another to the production of new wall material (Bove et al., 2008). Genome
150 duplication might be expected to increase wall production rates because of the effect of increased
151 gene dosage on metabolic processes. Although gene expression levels can become rapidly
152 modified during and after polyploidization due to gene loss, gene silencing, and dosage
153 compensation (Freeling et al., 2015; Schoenfelder and Fox, 2015; Dong et al., 2016; Panchy et
154 al., 2016), there is evidence that dosage effects have played an important role in the evolution of
155 metabolic rates. For example, in yeast cells, alcohol dehydrogenase activity per cell increased
156 linearly with ploidy across four ploidy levels (Dilorio *et al.*, 1987). In plant tissues,
157 endopolyploidy is common in cells that have secretory or transfer functions and endopolyploid
158 cells have higher metabolic rates (d'Amato, 1984; Galbraith *et al.*, 1991; Scholes & Paige,
159 2015). Finally, WGD-generated paralogs involved in metabolic pathways, such as glycolysis,
160 can be preferentially retained as genomes undergo gene losses after WGD (Conant and Wolfe
161 2007).
162 Another genetic consequence of WGD on *PTGR* occurs via the fitness effects of diploidy
163 over haploidy at the gene level. Diploidy is expected to initiate or maintain faster *PTGRs* for two
164 reasons. First, via the sheltering of deleterious performance alleles that were present in one or

165 both haploid ancestors or that arose after WGD (Husband and Schemske, 1997; Husband, 2016).
166 Secondly, via the effect of heterozygosity (overdominance), which is known to cause heterosis
167 for growth rates of sporophytes in intra- and inter-specific hybrids (Lippman and Zamir, 2007;
168 Feys et al., 2018). These potential effects of diploidy on *PTGR* are expected in all neo-polyploids
169 (Lande and Schemske, 1985), but the heterotic effects of allelic interactions and sheltering
170 become more evident as genetic variation in the polyploid increases. Still, in all but the special
171 case where there is no genetic variation, diploidization of an ancestrally haploid pollen tube
172 predicts genetic effects on performance speed to result in greater than or equal to ancestral
173 haploid speed.

174 The predicted effects of WGDs on pollen performance speed are summarized in Fig. 2.
175 Increases in DNA content, by WGD or any other process, are expected to *reduce PTGR* by
176 nucleotypic effects, primarily that of increased tube cell size on wall volume. In contrast,
177 increases in DNA content by WGD involves genome-wide gene duplication, and are expected to
178 *increase PTGR* through the genotypic effects of gene dosage and heterosis. Given that haploid
179 selection on pollen tubes has been seen as an important arbiter of the evolution of flowering plant
180 sporophytes, how robust has pollen tube growth and development been to WGDs? Is there
181 evidence that WGDs been involved in generating the pattern of accelerated *PTGRs* within
182 angiosperms?

183 In this study, we test the hypothesis that changes in DNA content have affected *PTGR* by
184 asking if there are consistent macroevolutionary patterns that might distinguish among the
185 alternative predictions described above. Specifically, we tested for differences in *PTGR* between
186 diploids and polyploids at the intraspecific, sister-taxon, and macroevolutionary scale; and for
187 correlations between *PTGR* and DNA content at the macroevolutionary scale. We examined the

188 macroevolutionary pattern at the level of all plants that have pollen tubes (seed plants), and
189 within the two major lineages that have different pollen tube cell biology and vastly different
190 levels of species diversity, gymnosperms and angiosperms.

191 **MATERIALS AND METHODS**

192 ***Data collection*** – Data on *PTGRs* were taken from Williams (2012) and more recent literature
193 (cited in Supplemental Information). Consistent with other comparative analyses of
194 physiological traits, and with the way researchers measure *PTGR* from the longest pollen tubes,
195 *PTGR* values for each species represent an average of maximum in vivo growth rates, or if there
196 was more than one report for a species the average of those values (see Williams 2012 for
197 details). *PTGRs* were taken from within-diploid or within-polyploid (i.e., never interploidy)
198 crosses, in keeping with our overall goal of finding mechanisms underlying the pattern of *PTGR*
199 evolution in stabilized polyploids. DNA content was analyzed using C-value: the amount of
200 nuclear DNA in the unreplicated gametic nucleus, irrespective of ploidy level (Swift, 1950;
201 Bennett and Leitch, 2012). While DNA content is equivalent to genome size in diploid
202 organisms, it is a multiple of genome size in polyploids, thus we use the term DNA content
203 throughout. C-value data was collected from the Kew Royal Botanic Gardens Plant C-Value
204 Database (Bennett and Leitch, 2012). Ploidy level data was extracted from the same source as
205 *PTGR* data where possible, and otherwise from the Kew Plant C-Value Database, TRY
206 databases, or the Index to Plant Chromosome Numbers. As ploidy level is determined either by
207 chromosome count or segregation pattern in these data, paleopolyploids and ancient duplication
208 events are not included.

209 ***Tree Construction and Dating*** – GenBank accessions for 16 gene regions (rbcL, matK,
210 trnL-F, 18s_rDNA, atpB, ndhF, adh, trnL, rpl32, trnT-L, psbA-trnH, rpl32-trnL, ITS,

211 5.8s_rRNA, rps16, and 26s_rDNA) for the 451 seed plant species with pollen tube growth rate
212 data were retrieved, cleaned, and assembled into multiple gene alignments using PHLAWD and
213 phyutility (Smith and Donoghue, 2008; Smith and Dunn, 2008). Tree inference was performed
214 using maximum likelihood in RAxML version 8 (Stamatakis, 2014) on CIPRES. A pruned
215 version of the seed plant tree from Magallón et al. (2015) was used as a guide tree to enforce
216 topology of major clades. The resulting maximum likelihood estimate of the tree was rooted and
217 ultrametricized using the *ape* (Paradis et al., 2004) and *geiger* packages in R (Harmon et al.,
218 2008). Time-calibration was performed using the Congruification method (Eastman et al., 2013).
219 ***Character scoring***- The *PTGR* value used for each species represents an estimate of
220 maximum sustained growth rate. Since each *PTGR* value represents a species mean obtained
221 from multiple measurements, we attempted to incorporate error into phylogenetic comparative
222 analyses. *OUwie* allows for incorporating estimates of standard error (*SE*), and since species
223 means were log-transformed for analysis, log-transformed *SE*s are required. As there is no
224 reliable way to calculate the log-transformed *SE* from the literature without the original data for
225 each species, we used the following conservative approximation. First, we assumed all species
226 had similar SEs in *PTGR*, and we applied an empirically-determined *SE* from an exemplar
227 species to all. *Magnolia grandiflora* has an average *PTGR* of 828 (±141) μm h^{-1} (N = 25
228 outcrosses), close to the angiosperm median of 587 μm h^{-1} (Williams, 2012 and this study)
229 (Table S1). The standard deviation (*SD*) of log-transformed data was calculated and divided by
230 the mean of the log-transformed data to acquire a coefficient of variation (*CV*) of 0.0237. We
231 then multiplied the log-transformed mean *PTGR* of each species by 0.0237 to provide an
232 estimate of taxon-specific standard deviation. The standard deviation was used as a conservative
233 estimate of error because sample sizes were generally not available for calculating *SE*. We also

234 performed a sensitivity analysis by running the *OUwie* analyses with hypothetical error values
235 obtained set as 0, 0.05, 0.1, 0.25, and 0.5 (Table S2). Unless otherwise noted, all measures of
236 uncertainty around parameter estimates are in standard error.

237 We analyzed two different sets of ploidy data. In the first, 'restricted dataset' (N = 273
238 taxa), we recognized only ploidy levels as stated in the literature. In the second 'expanded
239 dataset' (N = 335 taxa), we inferred ploidy levels of 62 additional species by comparing their
240 chromosome count to their generic base count as reported in Wood et al. (2009). Taxa with a
241 chromosome number equal to or greater than twice the generic base count were considered
242 polyploid.

243 ***Phylogenetic Comparative Analyses*** - Phylogenetic signal was assessed for both *PTGR*
244 and gametophytic DNA content using Blomberg's K in the *picante* package in R (Blomberg et
245 al., 2003; Kembel et al., 2010). C-values and *PTGRs* were $\log_{10}$ transformed in this and all other
246 phylogentic comparative analyses below. To visualize changes in DNA content and *PTGR* along
247 tree branches and to generate estimates of node states, ancestral state reconstructions were
248 performed and plotted using the contMap function in *phytools* (Felsenstein, 1985; Revell, 2012).
249 Given many known biological differences between gymnosperms and angiosperms for both
250 pollen tube growth (Friedman, 1993; Williams, 2008) and DNA content /polyploidy (see
251 Discussion) (Ohri and Khoshoo, 1986; Leitch et al., 1998), all analyses were performed on
252 gymnosperms only, angiosperms only, and the full dataset (all spermatophytes). Whether *PTGR*
253 evolves under different selective regimes between the two seed plant groups was also evaluated
254 using the *OUwie* function (*OUwie* package) in R (Beaulieu and O'Meara, 2014) to compare
255 different models of evolution (detailed below). In multiple-regime models, the regimes were

256 either gymnosperms (1) or angiosperms (2). The same analysis was used to test for differences in
257 DNA content evolution between gymnosperms and angiosperms as well.
258 To detect whether pollen tube growth rates of polyploid species evolve under a different
259 selective regime than diploid species, *OUwie* was used to compare single-regime and two-regime
260 models. The following models were tested: single-rate Brownian motion (BM1), multi-rate
261 Brownian Motion (BMS), single-regime Ornstein-Uhlenbeck (OU1), multi-regime OU with
262 single *alpha* and *sigma^2* estimates (OUM), multi-regime OU with single *alpha* and multiple
263 *sigma^2* (OUMV), and multi-regime OU with multiple *alpha* and single *sigma^2* (OUMA).
264 The association between polyploidy and *PTGR* was also assessed among 20 diploid-
265 polyploid sister taxa on the tree (within-genus or within-family level). Only polyploid taxa with a
266 single diploid sister were used. The *PTGRs* of 11 intraspecific diploid-polyploid pairs from the
267 literature were also compared. A two-tailed binomial (sign) test was used to test significance.
268 The relationship between the continuous traits, pollen tube growth rate and gametophytic
269 DNA content, was assessed with phylogenetic generalized least squares (*PGLS*) regression using
270 the *phylolm* package in R. Gametophytic DNA content was used as the predictor variable and
271 *PTGR* the response variable. BM (Grafen, 1989) and OU (Martins and Hansen, 1997) models
272 were both used, in addition to Pagel's lambda, kappa, and delta models (Pagel, 1997, 1999).
273 To explore the level of convergent evolution within each trait and determine coincidence
274 of selective regime shifts in *PTGR* and DNA content, the *SURFACE* package in R was used
275 (Ingram and Mahler, 2013). *SURFACE* adds selective regime shifts to a single regime model in a
276 stepwise fashion until adding another regime shift decreases the model likelihood. Regimes are
277 then collapsed in a pairwise fashion until further collapses decrease the likelihood. Nodes that
278 included a regime shift in both traits were identified.

279

280 **RESULTS**

281 ***PTGR evolution and C-value evolution in angiosperms versus gymnosperms -*** Blomberg's K
282 indicated significant phylogenetic signal across seed plants and within angiosperms, for both the
283 *PTGR* and C-value datasets (Table 1). *OUwie* analyses of seed plants rejected both the Brownian
284 Motion (BM) and Ornstein-Uhlenbeck (OU) single-optimum models for the evolution of *PTGR*
285 and C-value. OU models that allowed separate selective regimes for angiosperms and
286 gymnosperms accounted for > 99.9 % of the model weight in both *PTGR* and C-value (Tables
287 S3, S4). Log_{10} *PTGR* selective optima were more than a magnitude of order higher in
288 angiosperms (2.69 ± 0.048 μm h^{-1}) than in gymnosperms (0.187 ± 0.123 μm h^{-1}). Log_{10} DNA
289 content selective optima were more than a magnitude of order smaller in angiosperms (0.184 ±
290 0.051 pg) than in gymnosperms (1.231 ± 0.041 pg). The maximum likelihood (ML)
291 reconstructions of angiosperm and gymnosperm common ancestors (CAs) showed a similar
292 pattern. Ancestral Log_{10} *PTGR* for angiosperms was higher than that of gymnosperms, 2.44 μm
293 h^{-1} (95% CI: 1.09-3.69) versus 0.215 μm h^{-1} (95% CI: -1.48-1.92), respectively (Fig. 3), and
294 ancestral Log_{10} DNA content was 0.29 pg (95% CI: -0.45-1.04) for angiosperms and 1.10 pg
295 (95% CI: -0.28-2.47) for gymnosperms.

296 ***Joint evolution of PTGR and ploidy*** - In the restricted ploidy dataset, the best fitting
297 *OUwie* model for angiosperms using the *Magnolia* error estimate of 0.024 was the single-rate
298 OU model which had 50.0% of the model weight (Table 2a); whereas the three two-regime OU
299 models accounted for the remaining 50.0% of the model weight (both BM models were rejected).
300 This pattern held up for a wide range of error values in the sensitivity analysis (Table S2), except
301 that when error reached 0.5, a value > 20 times that seen in *Magnolia*. In the expanded ploidy

302 dataset (Table 2b), the weight of the single-regime OU model was 57.2% compared to a
303 combined 42.8% for the three two-regime models. However, the estimates for the polyploid
304 selective optima are different from those of the restricted dataset; diploid optima in both datasets
305 were 2.7 ± 0.07 $\log_{10}$ µm h^{-1}, whereas polyploid optima were 1.4 ± 1.5 $\log_{10}$ µm h^{-1} in the
306 restricted dataset versus 2.8 ± 1.00 l og_{10} µm h^{-1} in the expanded dataset.

307 A survey of intraspecific cytotypes found autopolyploids had slower *PTGR* than diploids
308 in 9 of 11 pairs and no difference in the remaining two (Binomial test, $P = 0.002$; Table S4b). In
309 the within-genus sister-taxon comparison, polyploids had slower *PTGR* than diploids in 9 pairs,
310 faster *PTGR* in 2, and no difference in two (Two-tailed binomial test, $P = 0.065$). If expanded to
311 within-family sisters, there were twelve, six, and two pairs, respectively ($P = 0.238$; Table S4a).

312 ***Joint evolution of PTGR and DNA content -*** For seed plants, simple linear regression
313 (*SLR*) showed a significant negative correlation between DNA content and *PTGR* ($P<0.0001$),
314 but that result was clearly driven by the large DNA contents and slow *PTGRs* of gymnosperms
315 relative to angiosperms (Fig. 5), because the *PGLS* regression was non-significant ($P=0.463$;
316 Table 3). Taking these two clades separately, DNA content was negatively correlated with *PTGR*
317 in gymnosperms in the *PGLS* regression (*SLR*: $P = 0.200$; *PGLS*: $P \leq 0.02$, OU model; Table 3),
318 but not *SLR* (phylogenetic signal was not significant for either trait in gymnosperms; Table 1).
319 For angiosperms, it was positively correlated using *SLR* ($P=0.0005$), but non-significant using
320 *PGLS* ($P = 0.284$; Table 3). In angiosperms, the *PGLS* regression was non-significant when
321 polyploids were excluded ($P = 0.457$, kappa model).

322 ***Detecting coincident regime shifts in PTGR and DNA content within seed plants*** - The
323 *SURFACE* analysis of *PTGRs* found 13 distinct selective regimes ($N = 451$ taxon tree), with 51
324 selective regime shifts (22 to faster and 29 to slower regimes). For C-value, there were 9 distinct

325 selective regimes (*N* = 184 taxon tree), with 4 shifts to larger and 7 shifts to smaller regimes.
326 Regime shifts in *PTGR* and DNA content were coincident at only two nodes, a *PTGR*
327 acceleration and genome downsizing in the CA of extant angiosperms and a *PTGR* slowdown
328 and genome size decrease in the CA of rosids and (Fig. 6). Excluding terminal, branches, all
329 other regime shifts were at least 3 nodes away from each other. *PTGR* shifts on the smaller tree
330 (Fig. 6) were in the same locations as on the larger *PTGR* tree (not shown).
331

332 **DISCUSSION**

333 ***DNA content variation and the evolution of PTGR in angiosperms versus gymnosperms -*** We
334 found that *PTGR* has evolved around a significantly faster selective optimum in angiosperms
335 than in gymnosperms. Though it has long been known that most angiosperm *PTGRs* far exceed
336 those of gymnosperms, this result provides quantitative support to the near-universal assumption
337 that shifts to faster angiosperm-like *PTGRs* began *prior* to the origin of extant angiosperms.
338 However, the slow angiosperm-wide selective optimum and reconstructed ancestral *PTGRs* (both
339 < 490 µm/h), are consistent with gradual evolution of faster *PTGRs* in angiosperms.
340 There are several hypotheses for how and why angiosperms evolved such fast *PTGRs*.
341 First, Mulcahy (1979) invoked a shift to much higher intensity of pollen competition in
342 angiosperms as a driver of the origin and continued evolution of faster growth rates. Notably, no
343 other type of tip-growing cell (haploid or diploid) in land plants has evolved comparably fast tip-
344 growth rates and none of those cell types, including gymnosperm pollen tubes, experience
345 intense competition for resources (Williams et al., 2016). Secondly, rapid *PTGR* may have been
346 advantageous as angiosperms transitioned to a much faster reproductive cycle (Stebbins, 1974;
347 Williams, 2012; Williams and Reese, *in review*). Thirdly, biophysical or physiological attributes

348 of pollen tubes may have either constrained *PTGRs* of gymnosperms to be slow or enabled faster
349 *PTGRs* in angiosperms, or both (Hoekstra, 1983; Derksen et al., 1999; Fernando et al., 2005;
350 Williams, 2008, 2009). A fourth possibility, tested here, is that strong differences in genome-
351 level processes between angiosperms and gymnosperms have impacted the evolution of
352 angiosperm *PTGRs* relative to their living and extinct seed plant relatives.

353 We found that DNA content has evolved around a significantly lower selective optimum
354 in angiosperms than in gymnosperms, even though angiosperms have a broad range of DNA C-
355 values that encompass the entire range of seed plant genome sizes (Fig. 5; see Leitch and Leitch,
356 2013 for a larger survey). Paradoxically, angiosperms have great variation in ploidy level, a
357 history of speciation by polyploidy, and much evidence of past genome duplication in both
358 diploids and polyploids, including a WGD in a common ancestor of extant angiosperms (Wood
359 et al., 2009; Husband et al., 2013; Van de Peer et al., 2017; Landis et al., 2018). Evidence for
360 more recent WGDs is found in most modern diploids, which is consistent with studies of
361 experimental WGDs that show that genome duplication is commonly accompanied by rapid loss
362 of DNA sequences, gene fractionation by large-scale deletions, biased retention of genes with
363 beneficial dosage effects, and ultimately a return to the diploid state (Conant and Wolfe, 2008;
364 Conant et al., 2014; Freeling et al., 2015; Dodsworth et al., 2016; Wendel et al., 2018).

365 In contrast, WGDs have been very rare in gymnosperms (Leitch et al., 2005; Wood et al.,
366 2009; Soltis et al., 2009; Husband et al., 2013; Leitch and Leitch, 2013), and their high DNA
367 contents are thought to be due mainly to high transposon activity without repeated rounds of
368 genome duplication (Leitch & Leitch, 2013; Lee and Kim, 2014). Hence, gymnosperms have
369 experienced the effects of higher DNA content on pollen tube dimensions, which is predicted to

370 reduce *PTGR,* without the potential counter-balancing effects of gene dosage and heterosis
371 incurred by repeated rounds of genome duplications (Fig. 2), gene sorting, and diploidization.
372 Angiosperm pollen (Hoekstra, 1983) and sporophytes (Bond, 1989; Feild and Arens,
373 2005; Beaulieu et al., 2007) are known to have evolved much higher respiration rates than the
374 pollen and sporophytes of gymnosperms. Respiration rates of angiosperm pollen are ~10 times
375 higher than those of sporophytic tissues (Tadege and Kuhlemeier, 1997), and other energetic
376 pathways also function (Rounds et al., 2011; Obermeyer et al., 2013). A major consequence of
377 WGDs over single-gene duplication events is that WGDs can increase gene dosage of whole
378 metabolic pathways at once (Conant and Wolfe, 2007). When particular metabolic pathways are
379 limiting to growth rate, duplication can upregulate the pathway via gene dosage effects, and
380 positive selection on dosage can shelter gene duplicates, allowing time for sub- or neo-
381 functionalization, and hence much faster evolution of those genes (Kondrashov and Kondrashov,
382 2006; Conant and Wolfe, 2007). Pollen tube growth rate genes are widespread in the genome, for
383 example, occurring on every chromosome arm of corn (Sari-Gorla et al., 1992). Thus, *PTGR* is a
384 trait governed by many interacting genes that are widespread in the genome, which is exactly the
385 kind of trait in which WGDs are favored over individual gene duplications or segmental
386 duplications of chromosomes, due to the need to conserve gene dosage balance (Birchler et al.,
387 2012; Conant et al., 2014; Freeling et al., 2015).
388 Growth rate evolution in sporophytic systems is consequent on growth of tissues,
389 populations of cells, and rates and durations of many individual cell cycles. In contrast, *PTGR*
390 genes with gametophytic expression govern a very restricted aspect of individual cell growth –
391 mostly the intracellular transport system and cell wall synthesis machinery operating within a
392 single, terminal cell cycle stage (G_1, or G_0) (Russell et al., 2012; Geitmann and Nebenführ,

393 2015). This means that the evolution of faster *PTGR* in angiosperms is likely to have imposed
394 selection on a restricted set of gametophytically-expressed genes in metabolic and intracellular
395 transport pathways (Arunkumar et al., 2015; Gossmann et al., 2016). Genes within such
396 biochemical pathways might be preferentially retained after WGD due to the need to maintain
397 balanced gene dosage effects (Birchler and Veita, 2012; Conant et al., 2014). Starting with the
398 origin of flowering plants, WGDs have provided repeated opportunities for genome-wide, gene-
399 level effects (gene sorting via positive and purifying selection, sheltering of deleterious alleles,
400 and heterosis) to affect *PTGR* evolution. At the same time, given the small genome sizes of so
401 many plants with a history of WGDs, the nucleotypic effect of doubled DNA content on cell size
402 must often have been tempered by rapid genome downsizing following WGD (Freeling et al.,
403 2015; Dodsworth et al., 2016; Wendel et al., 2018).

404 In summary, increases in DNA content by any mechanism are expected to initially
405 decrease *PTGR* via the nucleotypic effects of larger genome size on cell size and growth,
406 whereas increases in DNA content by WGD are expected to initially increase *PTGR* via the
407 genotypic effects of increased gene/allele numbers (Fig. 2). *PTGR* evolution of gymnosperms
408 may have been relatively stagnant for many reasons, such as lack of pollen competition, relaxed
409 selection on *PTGR* due to slow reproductive cycles, and developmental constraints on their
410 pollen tube structure and physiology. However, it may also be that their high degree of
411 heterochromatin and rarity of WGDs have given them all the limitations of high DNA content
412 but none of the evolutionary opportunities provided by large-scale redundancy due to WGD and
413 fractionation cycles. Angiosperm *PTGRs* have evolved via repeated cycles WGDs, followed by
414 fractionation, gene retentions due to advantageous dosage effects, sub- or neo-functionalization
415 of paralogs, and eventually diploidization. Consequently, growth rate genes have alternately

416 experienced periods of relaxed selection due to WGD and haploid selection on performance. Our
417 findings that angiosperms have evolved around a higher optimum *PTGR* but a lower optimum C-
418 value than gymnosperms, no coincident regime shifts to higher *PTGR* and higher C-value, and
419 the lack of evidence for faster *PTGRs* in polyploids than diploids, suggests that the rapid *PTGRs*
420 of angiosperms have largely evolved at the haploid level, in diploid or diploidized species.

421 ***Polyploidy and PTGR evolution within angiosperms -*** Diploid and polyploid
422 angiosperms evolved under a single selective regime, irrespective of how ploidy level was
423 scored. However, in both analyses the three two-regime OU models also accounted for
424 substantial model weight (43% and 50%), and in these, the polyploid *PTGR* optimum was
425 similar or slightly slower than the diploid optimum. Thus, we find no evidence that WGDs result
426 in higher *PTGRs.* Two caveats are first, only 13-14% of the taxa in our two analyses were scored
427 as polyploids versus 25-35% estimated for angiosperms (Wood et al*.,* 2009; Landis et al., 2018);
428 and secondly, on our tree polyploid taxa were generally recently-derived with relatively short
429 branches, comprising a very small proportion of the total branch length of the tree. Hence, we
430 may have had lower power to estimate parameters for polyploids relative to diploids. Still, the
431 sister-taxon comparisons avoided these weaknesses and were consistent with the model-based
432 results – polyploids had similar or slightly slower *PTGR* than diploids in most sister taxa on our
433 tree.

434 Neo-polyploid *PTGR* is affected by the balance between genotypic and nucleotypic
435 effects (Fig. 2). Since polyploid genetic variation can vary but affects only the potential for
436 genotypic effects, our species might not have been a random sample of polyploid variation.
437 Mating systems, modes of polyploid origins, and patterns of chromosomal segregation have
438 effects on the degree of genetic variation and the nature of recombination in neo-polyploids. Yet,

439 among 16 species scored as polyploid in both our analyses, eight were fully outcrossing, seven
440 were self-compatible (two autogamous, two mixed mating, and four unknown), and 1 was
441 apomictic – a not unusual distribution (Goodwillie et al., 2005; Gibbs, 2014; Ashman et al.,
442 2014). Whitney et al. (2010) found only a weak correlation between DNA content and mating
443 system, and this may reflect the fact that genetic variation in polyploids cannot be predicted
444 easily. For example, autotetraploids originate with a subset of the genetic variation in the diploid
445 progenitor population but they often outcross and hybridize, whereas allopolyploids can be
446 highly heterozygous when they originate, but often are highly selfing (Stebbins, 1974; Soltis and
447 Soltis, 1999; Whitney et al., 2010). In the special case in which there is no genetic variation in a
448 diploid progenitor and its neo-autopolyploid descendant, *PTGR* would be solely determined by
449 the balance between nucleotypic effects and gene dosage effects (which also depend on the
450 degree of dosage compensation; Guo et al., 1996) (Fig. 2).

451 The closest approximation of the initial effect of genome duplication on *PTGR,*
452 independent of levels of genetic variation, is the comparison of diploids with their intraspecific,
453 autopolyploid cytotypes. In all 11 pairs, *PTGRs* of autopolyploid cytotypes were slower than or
454 equal to those of their intraspecific diploid progenitors. We should re-emphasize that all studies
455 involved in vivo diploid crosses (1x pollen on 2x pistils) compared to tetraploid crosses (2x
456 pollen on 4x pistils). The lack of any examples of faster *PTGR* in neo-autotetraploid cytotypes
457 than in their diploid progenitors (in which the effects of heterosis are minimized) seems to
458 suggest that increased gene dosage generally cannot fully offset nucleotypic effects, causing
459 slower or at best similar *PTGR* upon autotetraploid formation.

460 ***Disentangling nucleotypic from genotypic effects on* PTGR *evolution*** – Genome size is
461 positively correlated with cell size and often negatively correlated with organismal growth rate

462 across all kingdoms of life (Cavalier-Smith, 1978; Gregory, 2001). One major proposed
463 explanation for the correlation between DNA content (which strongly influences nuclear size)
464 and cell size in isotropic cells is that there is an optimal ratio of nuclear to cytoplasmic volume
465 for metabolic efficiency (nucleoskeletal theory) (Cavalier-Smith, 1978, 2005). "Growth rate" in
466 most studies refers to the doubling time of lineages of cells, and hence to the average duration of
467 cell cycles, not to individual cell growth rates per se. Cell cycles are longer after genome
468 duplication due to a longer DNA replication phase and also to the longer growth period needed
469 to reach a larger size. Thus, the speed of cell enlargement in such studies is conflated with the
470 duration of S-phase. This is not the case for a pollen tube, which is a terminally-differentiated
471 cell that only grows and functions in the G_1-phase of its cell cycle. Therefore, in pollen tubes,
472 unlike in diffuse-growing cells of multicellular tissues, volumetric cell growth rate is the primary
473 target of natural or sexual selection.

474 Large-scale genome size increases, whether by WGD or any other method, are expected
475 to increase cell size to maintain the nucleo-cytoplasmic ratio. *PTGR* is directly determined by the
476 amount and rate of cell wall production, which depends on tube size (Winship et al. 2010;
477 Williams et al. 2016). A neo-diploid pollen tube with doubled tube volume must produce 41%
478 more cell wall material per unit time to maintain its ancestral haploid *PTGR* (Fig. 1). Size
479 increases of that order have been observed in diploid over haploid pollen tubes (Kostoff &
480 Prokofieva, 1935; Iyengar, 1938). Hence, without increases in metabolic rates that could
481 compensate for that extra work, *PTGR* and genome size are expected to be negatively correlated.

482 Importantly, we found such a negative correlation in gymnosperms. Nucleotypic effects
483 should outweigh genotypic effects in gymnosperms, since the group has experienced large
484 genome expansions without widespread gene duplication by WGD after their origin (Leitch &

485 Leitch, 2013; Lee and Kim, 2014). Similar support for the existence of nucleotypic effects is that
486 *PTGR* was generally slower in the youngest angiosperm polyploids (eg. intraspecific cytotypes
487 or within-genus sisters). Over time, nucleotypic effects remain more or less constant in
488 polyploids, but positive genotypic effects can accumulate via all the usual population genetic
489 processes, counterbalancing or outweighing nucleotypic effects. Hence, the lack of correlation
490 between DNA content and *PTGR* in angiosperms as a whole should be interpreted as a
491 consequence of variation in the degree of compensating genotypic effects on *PTGR,* due to time
492 since origin and genetic variation.

493 A weakness of comparative analyses of the question of DNA content and pollen
494 performance is that all angiosperms, including "diploids" as determined by diploid segregation
495 of chromosomes or by diploid base chromosome number, have at least one WGD in their
496 ancestry (Landis, 2018, and reference therein). Thus, the most relevant measures of ploidy level
497 are probably cryptic. For example, is pollen tube gene expression in a particular taxon haploid or
498 polyploid? Perhaps a more relevant measure for comparisons is the number of WGDs among
499 different lineages, traced back from each tip. Such data are already becoming available as more
500 genomic data is accumulated.

501 ***Conclusions -*** Studies across the tree of life have consistently shown that ploidy level and
502 DNA content are correlated with cell size and metabolic rate. Pollen tube dimensions and
503 energetics affect the amount of cell wall material produced per unit of growth and the rate at
504 which cell wall is produced, which together determine *PTGR.* In gymnosperms, *PTGR* was
505 negatively correlated with genome size, but in angiosperms, where the effects of WGDs are more
506 prevalent, *PTGR* seems to be somewhat robust to genome duplication. Neo-autopolyploids
507 inherited similar or slower *PTGRs* than their diploid ancestors, and polyploids compared to their

508 diploid near-relatives seem to follow the same pattern. With changes in genome size, nucleotypic
509 effects act as a brake on growth rate and are always present, but the degree to which genotypic
510 effects counterbalance these depends on the historical nature and time since genome size
511 increase in any particular lineage. Understanding causal relationships between genome size,
512 ploidy and *PTGR* will involve mechanistic studies of tube cell dimensions and wall synthesis
513 rates in haploid and polyploid gametophytes. On the other hand, there appears to be great
514 variation in the tug of war between genotypic and nucleotypic effects, and there are likely to be
515 deeper evolutionary patterns underlying that variation.
516
517
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527 performed all comparative analyses; J.H.W. collected data on *PTGRs* and diploid-autopolyploid
528 *PTGRs.*
529

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827 **FIGURE LEGENDS**

828 **Figure 1: Pollen tube wall construction.** A primary cell wall is generated at the tip (red/blue
829 areas), whereas a secondary wall (angiosperms only) is synthesized in a short region behind the
830 tip (yellow area). Genome duplication is predicted to double the volume of a mature cell, but
831 since the tube cell is constantly growing and its cytoplasm is largely confined to the tip region, a
832 pollen tube can only change its effective volume by changing its fixed diameter (circumference)
833 or its length per time (pollen tube growth rate, *PTGR*). If wall thickness (*W*) and *PTGR* (*L/t*) are
834 held constant, a diploid tube cell with doubled volume per unit time will have a 41% larger
835 circumference. As a result, wall production rate would increase 1.41-fold, not two-fold, over that
836 of its haploid ancestor: WPR_{1x} ($\mu m^3\ h^{-1}$) = C_0 x W x L/t; WPR_{2x} ($\mu m^3\ h^{-1}$) = 1.41C_0 x W x L/t
837

838 **Figure 2. Predicted direction of effects of WGD on pollen tube growth rate (*PTGR*).**
839 Genotypic effects (gene dosage and heterozygosity) are predicted to increase *PTGR,* whereas
840 nucleotypic effects should decrease *PTGR,* after transition from a haploid (1x) to a diploid (2x)
841 pollen tube. The magnitude of genotypic effects is expected to scale with genetic variation in the
842 polyploid. The ancestral haploid *PTGR* will be conserved in the derived diploid male
843 gametophyte when genotypic and nucleotypic effects perfectly offset each other.
844

845 **Figure 3: Pollen tube growth rate (*PTGR*) evolution across Spermatophytes.** Contour plot
846 showing reconstructed history of *PTGR*. Cool colors indicate *PTGR*s closer to the minimum
847 value in seed plants while warm colors indicate *PTGR*s closer to the maximum value in seed
848 plants. Scale bar indicates millions of years before present.
849

850 **Figure 4: Pollen tube growth rate (*PTGR*) and genome size evolution across seed plants.**
851 Contour plot comparing *PTGR* evolution (left, μm h^{-1}) and c-value evolution (right, picograms).
852 Scale bars at the bottom of each phylogeny indicate 100 million years. Center bars indicate the
853 following major groups: gray = gymnosperms, blue = early-divergent angiosperms and
854 eumagnoliids, green = monocots, orange = asterids, pink = rosids.
855
856 **Figure 5: Relationship between pollen tube growth rate (*PTGR*) and genome size (C-value)**
857 **in seed plants.** Separate analyses of gymnosperms (green) and angiosperms (blue) were non-
858 significant. Selective optima (with standard errors) for each group (from *OUwie* analyses; Tables
859 S3-S4) are included for illustrative purposes.
860
861 **Figure 6: Coincident evolution of pollen tube growth rate (*PTGR*) and DNA content (C-**
862 **value).** Paired *SURFACE* plot showing selective regime shifts in *PTGR* (left) versus genome size
863 (right). Nodes which have experienced a regime shift along the stem leading to it are marked
864 with magenta x's. Branch colors – *gray* = seed plant ancestral selective regime; *green* = ancestral
865 selective regime for angiosperms; *red* = derived selective regimes faster/larger than angiosperm
866 ancestral regime; and *blue* = derived selective regimes slower/smaller than angiosperm ancestral
867 regime. Yellow arrows indicate instances where shifts in *PTGR* and genome size coincide. Scale
868 bars at the bottom of each phylogeny indicate 100 million years. Center bars indicate the
869 following major groups: gray = gymnosperms, blue = early-divergent angiosperms and
870 eumagnoliids, green = monocots, orange = asterids, pink = rosids.
871
872 **Figure S1: Pollen tube growth rate and ploidy level evolution across Spermatophytes.**
873

874 **Table S1: Summary statistics for pollen tube growth rate (*PTGR*) of *Magnolia grandiflora.***

875

876 **Table S2: Sensitivity analysis for pollen tube growth rate (*PTGR*) error estimates**.

877

878 **Table S3: Pollen tube growth rate (*PTGR*) evolution in gymnosperms vs. angiosperms.**

879

880 **Table S4: C-value evolution in gymnosperms vs. angiosperms.**

881

882 **Table S5: Sister taxon analyses**.

883

884

885 **Table 1: Phylogenetic signal (Blomberg's *K*) for *PTGR* and C-value**.

	K	***P*-value**
PTGR seed plants	0.213	<0.0001
PTGR angiosperms	0.075	<0.0001
PTGR gymnosperms	0.060	0.0553
C-value seed plants	0.146	<0.0001
C-value angiosperms	0.099	<0.0001
C-value gymnosperms	0.371	0.169

886

887

Table 2*: OUwie* model comparison and parameter estimates.

a. Results using "restricted" dataset.

model	ΔAICc	model weight	Diploid σ^2	Diploid α	Polyploid σ^2	Polyploid α	Optimum (± *SE*)	Diploid optimum (± *SE*)	Polyploid optimum (± *SE*)
OU1	[418.4]	0.500	0.078	0.067	0.078	0.067	2.728 ± 0.068		
OUM	1.315	0.259	0.078	0.067	0.078	0.067		2.742 ± 0.070	1.421 ± 1.509
OUMA	2.765	0.125	0.082	0.070	0.082	0.068		2.744 ± 0.069	1.246 ± 1.480
OUMV	2.921	0.116	0.079	0.066	0.059	0.066		2.742 ± 0.070	1.375 ± 1.343
BM1	130.164	2.72E-29	0.033	NA	0.033	NA	2.489 ± 0.533		
BMS	131.651	1.29E-29	0.034	NA	0.000	NA		2.503 ± 0.556	1.297 ± 7.097

b. Results using "expanded" dataset.

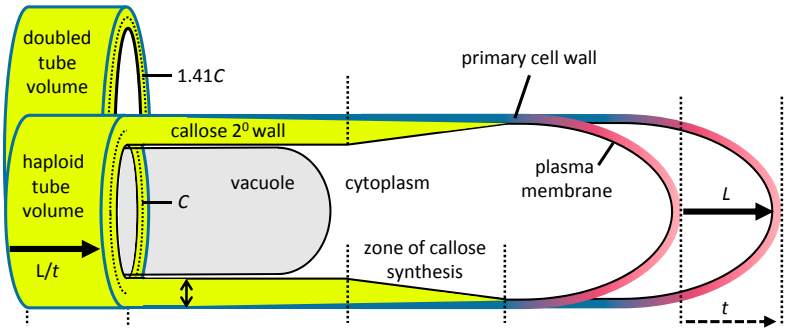
model	ΔAICc	model weight	Diploid σ^2	Diploid α	Polyploid σ^2	Polyploid α	Optimum (± *SE*)	Diploid optimum (± *SE*)	Polyploid optimum (± *SE*)
OU1	[530.8]	0.572	0.076	0.061	0.076	0.061	2.714 ± 0.064		
OUM	2.045	0.206	0.076	0.061	0.076	0.061		2.713 ± 0.066	2.805 ± 1.060
OUMA	3.155	0.118	0.078	0.061	0.078	0.060		2.713 ± 0.066	2.797 ± 1.085
OUMV	3.393	0.105	0.077	0.060	0.058	0.060		2.712 ± 0.067	2.760 ± 0.973
BM1	153.391	2.81E-34	0.036	NA	0.036	NA	2.469 ± 0.552		
BMS	156.317	6.51E-35	0.037	NA	0.021	NA		2.514 ± 0.572	-0.589 ± 8.214

895 **Table 3:** Phylogenetic generalized least squares regression model comparisons of $\log_{10}$ DNA
896 content versus $\log_{10}$ *PTGR*.
897

Seed plants			Angiosperms only			Gymnosperms only		
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	0.001	0.368	lambda	0.024	0.221	delta	0.257	0.006
OU	6.26E-28	0.644	OU	2.90E-04	0.005	kappa	0.193	0.445
delta	1.22E-35	0.471	delta	3.08E-21	0.276	BM	0.121	0.001
BM	1.48E-42	0.373	BM	2.18E-33	0.365	lambda	0.119	0.327
EB	5.45E-43	0.373	EB	8.03E-34	0.365	EB	0.044	0.001

898
899

900 **Figure 1**



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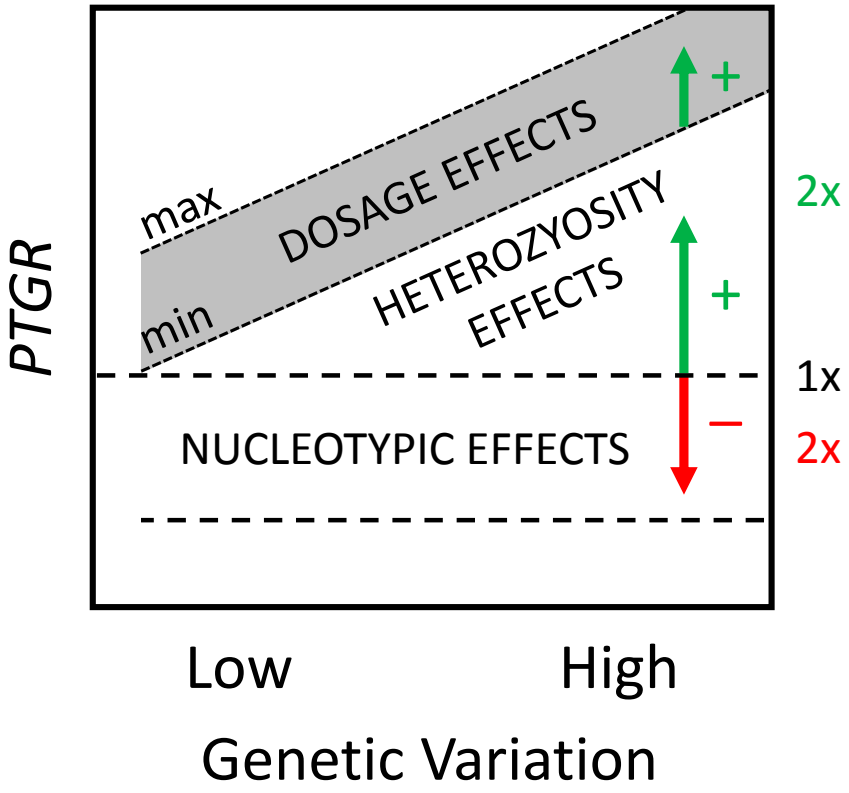
903 **Figure 2**



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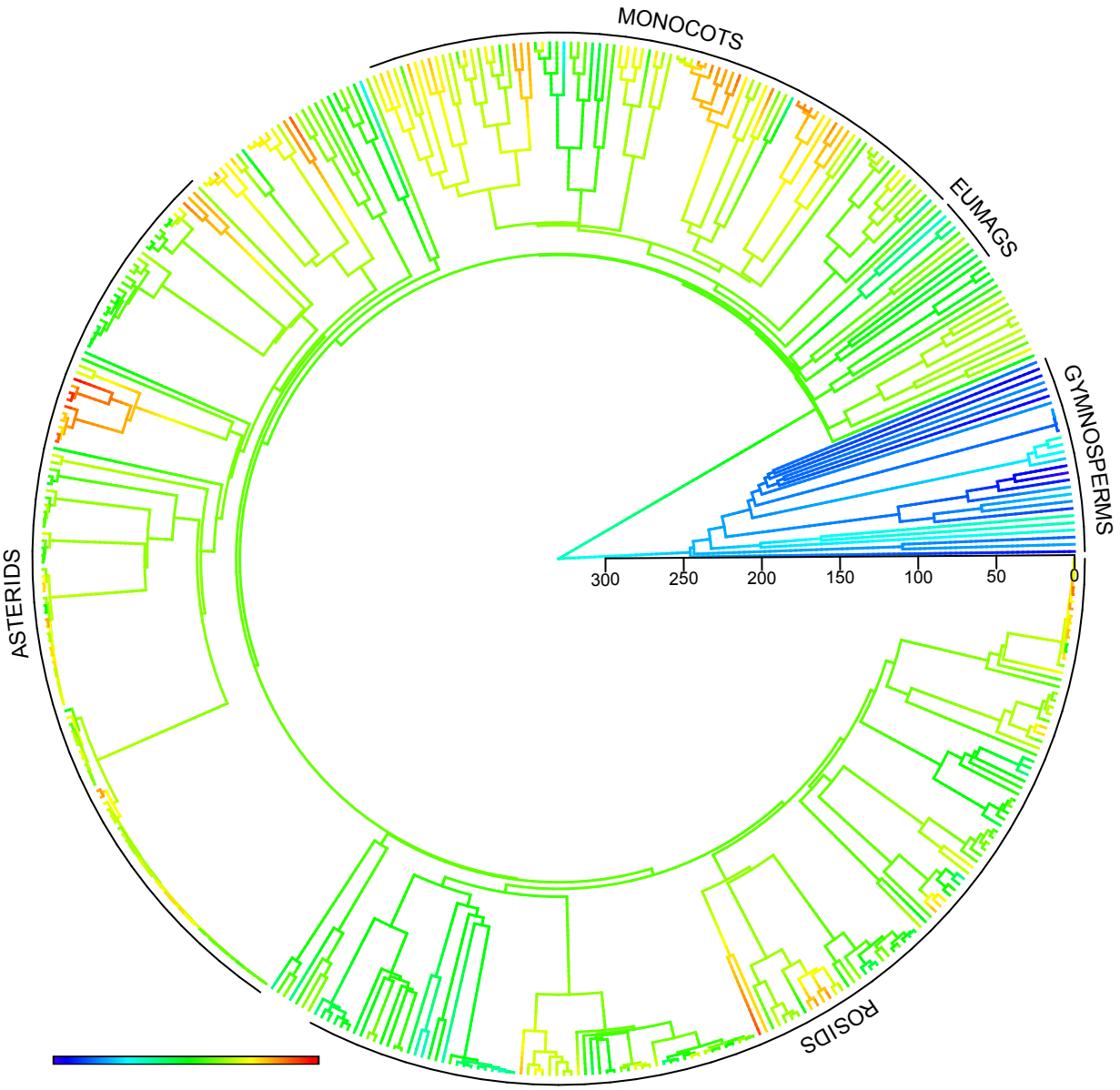

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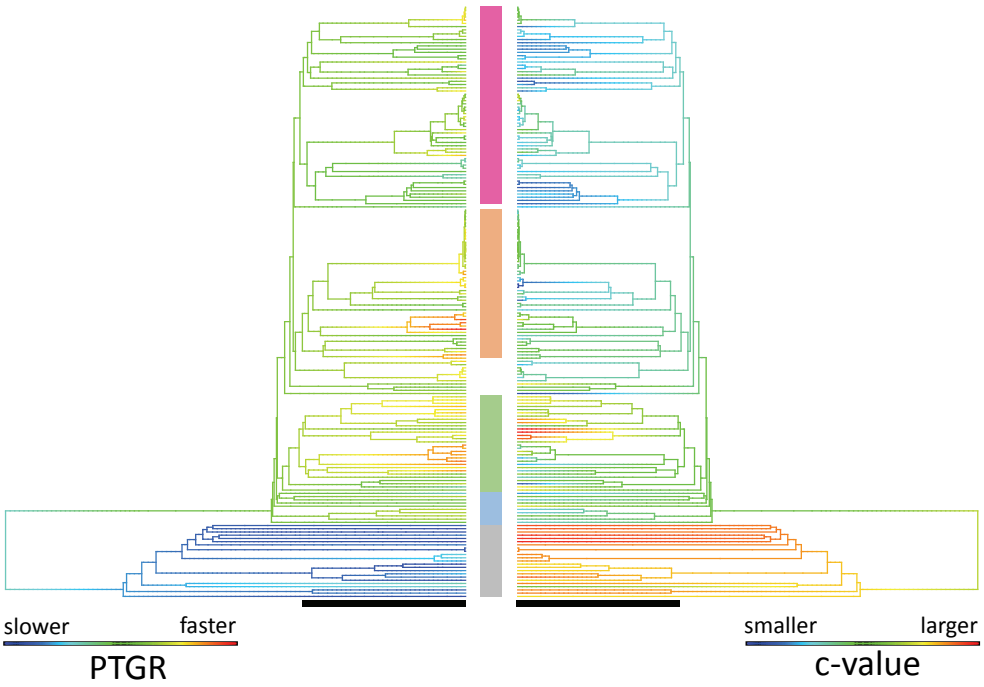
Figure 4



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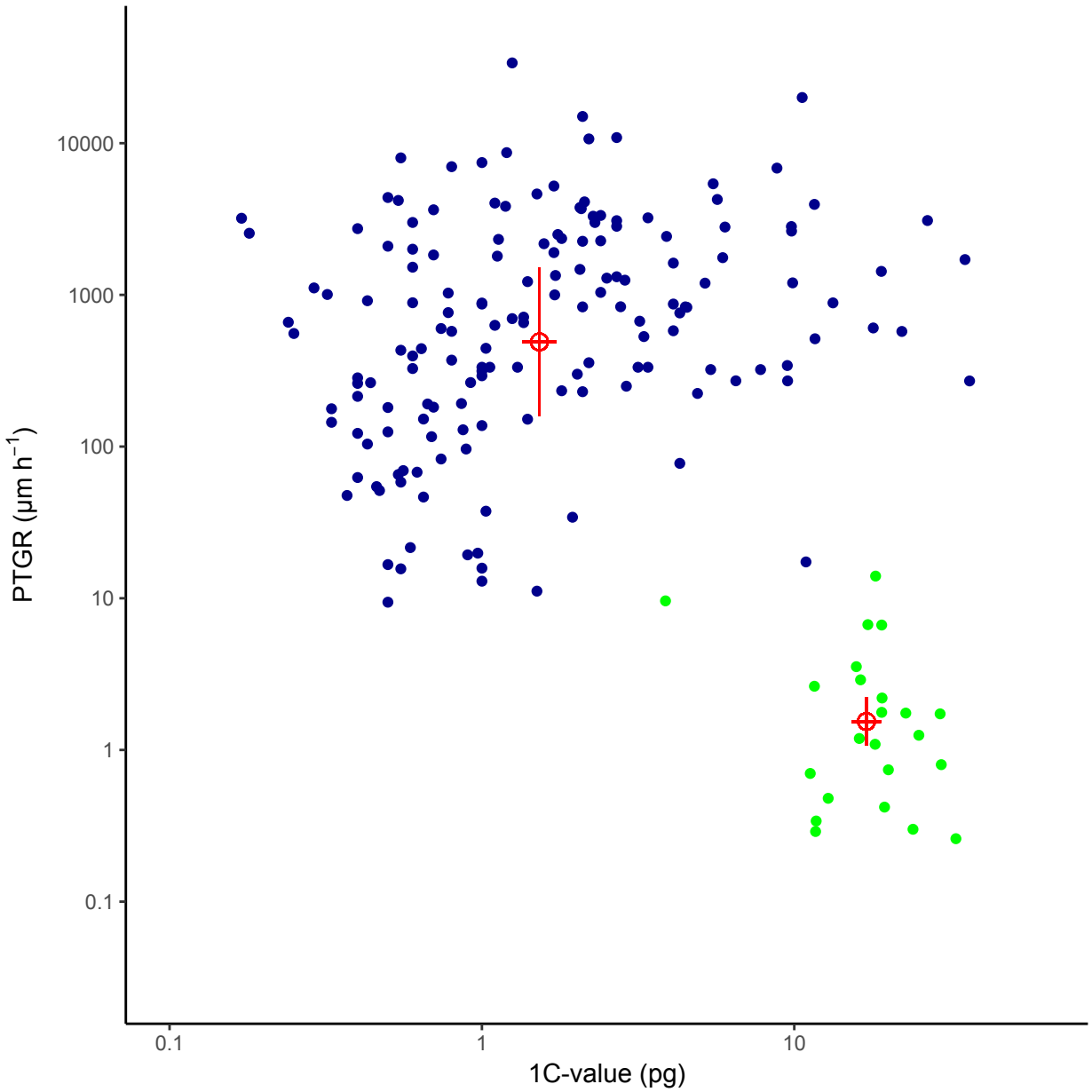

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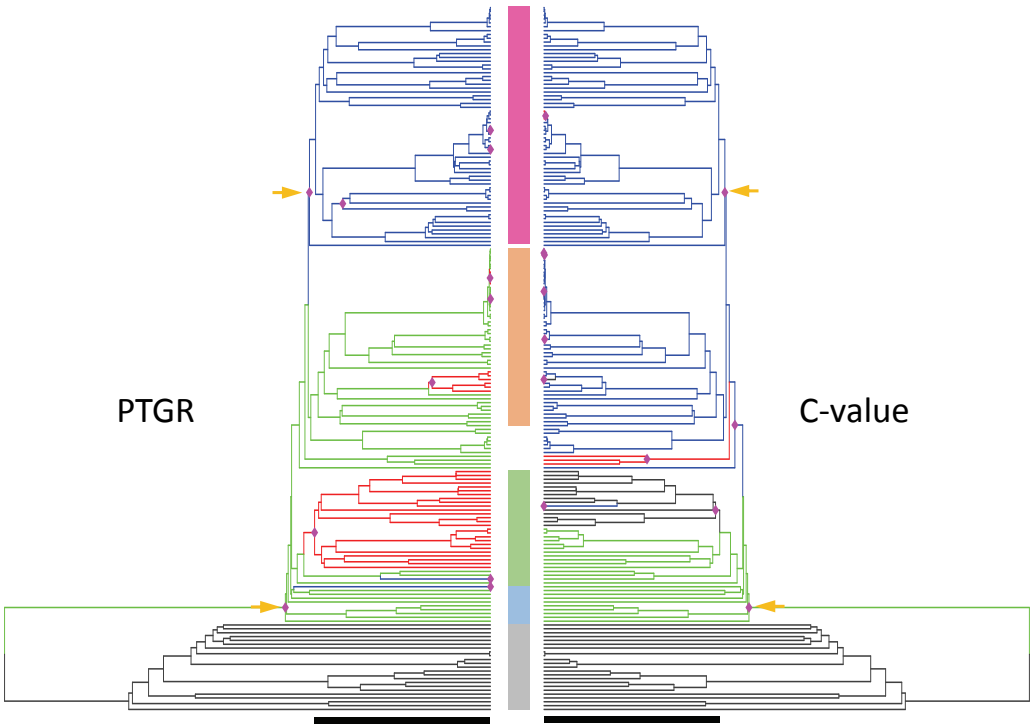
915

916 **Figure 6**



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