

1 The scaling of genome size and cell size limits maximum rates 2 of photosynthesis with implications for ecological strategies

3

4 Adam B. Roddy¹, Guillaume Thérroux-Rancourt², Tito Abbo³, Joseph W. Benedetti⁴, Craig R.
5 Brodersen¹, Mariana Castro⁵, Silvia Castro⁵, Austin B. Gilbride⁴, Brook Jensen⁶, Guo-Feng
6 Jiang⁷, John A. Perkins⁸, Sally D. Perkins⁹, João Loureiro⁵, Zuhah Syed¹⁰, R. Alexander
7 Thompson³, Sara E. Kuebbing¹¹, Kevin A. Simonin³

8

9 1 School of Forestry & Environmental Studies, Yale University, New Haven, CT 06511 USA

10 2 Institute of Botany, Universität für Bodenkultur, Vienna, Austria

11 3 Department of Biology, San Francisco State University, San Francisco, CA, 94132 USA

12 4 Amity Regional High School, Woodbridge, CT 06525 USA

13 5 Centre for Functional Ecology, Department of Biology, University of Coimbra, Calçada
14 Martim de Freitas, 3000-456, Coimbra, Portugal

15 6 Department of Biological Sciences, California State University-Stanislaus, Turlock, CA
16 95382 USA

17 7 State Key Laboratory of Conservation and Utilization of Subtropical Agrobioresources and
18 Guangxi Key Laboratory of Forest Ecology and Conservation, College of Forestry, Guangxi
19 University, Nanning, Guangxi 530004, China

20 8 Azalea Society of America, Washington, D.C., USA

21 9 American Rhododendron Society, Great River, NY 11739 USA

22 10 High School in the Community, New Haven, CT 06511, USA

23 11 Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260 USA

24

25 Author for contact:

26 Email: adam.rodny@yale.edu

27 Phone: +1.510.224.4432

28 ORCID: 0000-0002-4423-8729

29

30 **Abstract**

31 A central challenge in plant ecology is to define the major axes of plant functional variation
32 with direct consequences for fitness. Central to the three main components of plant fitness
33 (growth, survival, and reproduction) is the rate of metabolic conversion of CO₂ into carbon
34 that can be allocated to various structures and functions. Here we (1) argue that a primary
35 constraint on the maximum rate of photosynthesis per unit leaf area is the size and packing
36 density of cells and (2) show that variation in genome size is a strong predictor of cell sizes,
37 packing densities, and the maximum rate of photosynthesis across terrestrial vascular
38 plants. Regardless of the genic content associated with variation in genome size, the simple
39 biophysical constraints of encapsulating the genome define the lower limit of cell size and
40 the upper limit of cell packing densities, as well as the range of possible cell sizes and
41 densities. Genome size, therefore, acts as a first-order constraint on carbon gain and is
42 predicted to define the upper limits of allocation to growth, reproduction, and defense. The
43 strong effects of genome size on metabolism, therefore, have broad implications for plant
44 biogeography and for other theories of plant ecology, and suggest that selection on
45 metabolism may have a role in genome size evolution.

46

47

48

49 Introduction

50 Quantifying major axes of plant functional variation has given rise to an ever-growing list of
51 traits that impact growth, reproduction, and survival, the three components of individual
52 fitness (Violle et al. 2007). These traits have traditionally been viewed from a reductionist
53 perspective that scales form-function relationships of individual plant organs (e.g. leaves,
54 stems, and roots) to whole organism ecological strategies. As the ultimate source of energy
55 and matter for growth and reproduction, photosynthetic capacity represents a first-order
56 constraint on the emergent properties between whole plant form and function and
57 individual fitness. Here we provide evidence that genome-cellular allometry directly
58 influences interspecific variation in photosynthetic metabolism and provide a mechanistic
59 framework that links genome size and metabolism to other aspects of plant ecology and
60 evolution.

61 One of the three components of fitness is growth, which is ultimately limited by
62 photosynthetic metabolism. Relative growth rate (RGR) varies considerably across species
63 and is driven by photosynthetic rate and the resource investment to support
64 photosynthesis as:

$$65 \quad RGR = A_{mass} \cdot LMR$$

66 where A_{mass} is the photosynthetic rate per unit leaf biomass and LMR is the leaf mass ratio
67 (the proportion of leaf dry mass to total plant dry mass). A_{mass} is, therefore, frequently
68 considered a major plant strategy axis (Poorter and Remkes 1990; Poorter et al. 1990;
69 Reich et al. 1992). However, A_{mass} can be decomposed as:

$$70 \quad A_{mass} = SLA \cdot A_{area}$$

71 where SLA is the specific leaf area (leaf area per leaf dry mass) and A_{area} is the net carbon
72 assimilation rate per unit canopy leaf area. Because of its direct effect on A_{mass} , SLA is often
73 considered a major predictor of interspecific variation in RGR. A_{area} , on the other hand,

74 varies orthogonally to SLA (Wright et al. 2004), and, therefore, determines the upper limit
75 of the relationship between A_{mass} , SLA, and RGR. Maximum potential A_{area} represents, then,
76 a fundamental limitation on the maximum amount of carbon available for allocation to
77 growth, reproduction, and survival relative to species ecological strategies.

78 The centrality of A_{area} to plant ecological strategy suggests two questions:

- 79 • First, what are the fundamental features of plant structure that determine maximum
80 potential A_{area} ?
- 81 • Second, to what extent do these relationships scale up to affect plant ecological
82 strategies and evolutionary dynamics?

83 Here we present a mechanistic framework to address both of these questions, that is based
84 on the positive scaling between genome size and cell size. Although the relationship
85 between genome size (i.e. nuclear volume) and cell size has long been of interest (von
86 Sachs 1893), the mechanisms are still not fully understood (Doyle and Coate 2019), and its
87 implications for organismal metabolism have not been fully articulated. We show that the
88 allometry between genome size and cell size influences rates of photosynthetic metabolism
89 and argue that the scaling of genome size and metabolism affects ecological distributions
90 and evolutionary dynamics. In this way, any factor affecting rates of metabolism is a
91 potential agent of selection on genome size and, potentially, on genome structure as well.

92 It is now widely recognized that variation in genome size can have significant
93 consequences for organismal structure and function, independent of the genes that define
94 the genotype (Bennett 1971). Positive scaling between genome size and cell size across
95 terrestrial plants has given rise to numerous studies characterizing the many other
96 phenotypic correlates of genome size independent of variation in genome structure,
97 commonly referred to as “nucleotype” effects, although some of these correlations are
98 disputable after accounting for shared phylogenetic history (Bennett 1971; Cavalier-Smith
99 1978; 1982; Bennett and Leitch 2005). Correlates of genome size encompass an incredible
100 diversity of plant phenotypes, including, for example, the sizes of plant structures, rates of
101 cell division, rates of physiological processes, and tolerances and responses to abiotic
102 conditions (Table 1).

103 Our goal is not to recapitulate the many reviews about the nucleotype-phenotype
104 relationship but, instead, to align these studies more systematically with the field of plant
105 functional biology. We believe that the diverse impacts of genome-cellular allometry on the
106 body plan of terrestrial vascular plants strongly influences the coordination between plant
107 functional traits and, ultimately, whole organism form-function relationships. Here we
108 summarize previous research, perform new analyses of existing data, and present new data
109 to show how genome size may, through its impacts on cell size and tissue structure,
110 determine the biophysical limits of plant metabolic rates, and, therefore, influence other
111 aspects of ecology and evolution. That genome size may be a key functional trait is not a
112 new idea (Grime 1998). Yet, despite numerous reports of the phenotypic and ecological
113 correlates of genome size (Table 1), it has not been fully integrated into the functional trait
114 literature. Our goal, therefore, is to more directly show how genome size influences plant
115 traits that impact maximum rates of photosynthetic metabolism. Metabolism is central to
116 all three aspects of plant fitness, providing the carbon necessary for allocation to growth,
117 reproduction, and survival. As such, genome size may not itself be a functional trait but
118 instead may define the limits of variation in numerous other functional traits.

119 **Genome-cellular allometry limits rates of resource transport and** 120 **metabolism**

121 **Allometry of genome size and cell size**

122 The role of the genome in limiting cell size has been postulated since at least the late 1800s
123 (von Sachs 1893) and was critical in shaping early modern views of the evolution of plant
124 vascular systems (Bailey and Tupper 1918). At a minimum, a cell must contain its genome,
125 and there is a strong relationship between the volumes of meristematic cells and genome
126 size (Šímová and Herben 2012). Cellular expansion from this meristematic minimum size is
127 cell type-specific (Doyle and Coate 2019). Within a cell type, size can be influenced by
128 various environmental and developmental factors (Melaragno et al. 1993). Despite this
129 substantial growth in cell volume during development, there remains a significant effect of
130 genome size on cell size, particularly for stomatal guard cells (Beaulieu et al. 2008; Knight

131 and Beaulieu 2008; Lomax et al. 2013; Simonin and Roddy 2018). For example, stomatal
132 guard cell size and density, which regulate the fluxes of water and CO₂ between the
133 biosphere and atmosphere, vary within species depending on light, water availability, and
134 atmospheric CO₂ concentration (Hetherington and Woodward 2003; Franks and Beerling
135 2009). Furthermore, in the vascular transport network, the sizes of xylem conduits and
136 their density in the leaf are also affected by variation in genome size (Maherali et al. 2009;
137 Hao et al. 2013; De Baerdemaeker et al. 2018; Simonin and Roddy 2018). Yet why genome
138 size and final cell size are correlated within a cell type remains unclear (Doyle and Coate
139 2019).

140 We tested whether smaller genomes allow not only for smaller initial and final cell sizes
141 but also for a greater range in final cell size using published data for terrestrial C₃ plants.
142 We used data for stomatal guard cells because they are the most commonly measured cell
143 sizes in plants and because their sizes and abundance determine the leaf surface
144 conductance to CO₂ and water vapor and, therefore, directly control rates of resource
145 transport for use in photosynthetic metabolism. Sizes of guard cells for angiosperms
146 (Beaulieu et al. 2008), gymnosperms, and ferns were compiled previously by Simonin and
147 Roddy (2018), and here we include data for mosses and hornworts from Field et al. (2015)
148 and Renzaglia et al. (2017). We assumed that stomatal guard cells are shaped as capsules,
149 which are composed of a central cylinder with hemispherical ends, such that cell volume
150 could be estimated from cell length as:

151
$$V = \pi \cdot r^2 \cdot \left(\frac{4}{3}r + a\right)$$

152 where r is the radius of the cylinder and of the hemispherical ends and a is the height of the
153 cylinder. We assumed that a is equal to $2r$, such that the guard cell length is equal to $4r$.
154 Simplifying this equation allowed cell volume to be calculated from guard cell length as:

155
$$V = \frac{5}{96} \pi \cdot (\text{guard cell length})^3.$$

156 The dumbbell-shaped guard cells present among monocots would likely violate these
157 assumptions about cell shape and so we excluded from this analysis data for the Poaceae,

158 which are known to have dumbbell-shaped guard cells. Data for meristematic cell volume
159 and genome size were taken from Šímová and Herben (2012). We used linear regression (R
160 package *stats*) to fit the mean response and quantile regression (R package *rq*) to test
161 whether there was greater variation in cell volume among taxa with smaller genomes (i.e.
162 heteroskedasticity), based on differences between quantile regression slopes, using the
163 functions 'rq' and 'anova.rq'.

164 Across over two orders of magnitude in genome size, meristematic cell volume defined the
165 lower limit of guard cell volume (Figure 1); the smallest guard cells were only slightly
166 larger than meristematic cells of the same genome size. Genome size was a strong and
167 significant predictor of meristematic cell volume ($\log(\text{volume}) = 0.69 \log(\text{genome size}) +$
168 2.68 ; $R^2 = 0.98$, $P < 0.001$; Šímová and Herben 2012). Though it explained less of the
169 variation, genome size was a significant predictor of final guard cell volume among
170 terrestrial vascular plants ($\log(\text{cell volume}) = 0.55 \log(\text{genome size}) + 3.44$; $R^2 = 0.48$, $P <$
171 0.001). Including mosses and hornworts, however, substantially reduced the explanatory
172 power of genome size on cell volume to under 10%. Quantile regression revealed that for
173 vascular plants the slope through the 10th quantile was steeper (slope = 0.66 ± 0.07 ,
174 intercept = 2.98 ± 0.07) than the slope through the 90th quantile (0.47 ± 0.09), although
175 this difference was not significant ($P = 0.07$). While there was no significant difference
176 between the 10% and 90% quantile slopes, lower quantiles had consistently steeper slopes
177 when considering all species and also angiosperms alone (Figure S1), suggesting that the
178 smaller minimum cell size allowed by smaller genomes enables greater variation in final
179 cell size. In fact, for a given genome size, interspecific variation in mature guard cell volume
180 could vary by as much as two orders of magnitude among vascular plants. Theoretically,
181 maximum cell size is not as tightly constrained by genome size, such that other cell types
182 can be much larger than guard cells. The greater variation among species with smaller
183 genomes implies that smaller genomes allow for greater plasticity in cell sizes and cell
184 packing densities which directly influence maximum rates of leaf surface conductance to
185 CO_2 and water and ultimately photosynthetic metabolism per unit leaf surface area
186 (Simonin and Roddy 2018). Further, the greater diversity of cell sizes observed in plants
187 with small genomes suggests that the correlation between genome size and cell size is

188 simply the result of occupying available space within the cell. A small genome can be
189 housed in either a small or a large cell, but a large genome cannot be housed in a cell
190 smaller than its nucleus.

191 The greater variation in cell volume allowed by smaller genomes (Figure 1) further
192 suggests that smaller genomes allow for greater variation in cell packing densities. For
193 guard cell lengths, stomatal densities, and vein densities, smaller genomes allowed for
194 greater variation in traits across ferns, gymnosperms and angiosperms (Simonin and
195 Roddy 2018). Species with smaller genomes in these datasets are predominantly
196 angiosperms, and these analyses compared distantly related species. We further tested for
197 greater variation in cell sizes and packing densities with smaller genomes among closely
198 related species using taxa in *Rhododendron* (Ericaceae) sect. *Schistanthe* Schltr. (= sect.
199 *Vireya* Blume) and a collection of deciduous *Rhododendron* cultivars that vary in ploidy
200 from diploids to hexaploids. The monophyletic *Schistanthe* clade has a stepwise
201 phylogeographic history, having radiated eastward from the Malay Peninsula and reached
202 New Guinea within the last 15 Ma (Goetsch et al. 2011). We sampled leaves from 19 taxa
203 growing under common garden conditions at the Rhododendron Species Foundation
204 Botanical Garden in Federal Way, WA, USA. Genome sizes were measured following
205 standard protocols (Dolezel et al. 2007) at the Benaroya Research Institute in Seattle, WA,
206 USA. For measurements of stomatal size and density, epidermal impressions were made on
207 fresh leaves using dental putty (Coltene Whaledent President Light Body), transferred
208 using clear nail polish, mounted in water, and imaged using a light microscope.
209 Measurements of leaf vein density were made on leaf sections cleared by soaking in 4%
210 NaOH, 3% sodium hypochlorite, stained with 1% Safranin O, counterstained with 1% Fast
211 Green, mounted in ethanol, and imaged with a light microscope. Stomatal traits were
212 averaged across ten images per taxon, and leaf vein density was averaged across five
213 images per taxon. Genome sizes for the *Rhododendron* cultivars were measured at the
214 University of Coimbra, Portugal, and all anatomical measurements were made on leaf
215 sections cleared in 4% NaOH, stained in 1% Safranin and mounted in ethanol and Cytoseal
216 (Fisher Scientific). The two datasets of congeners were pooled in statistical analyses.
217 Quantile regression through the 10th and 90th percentile of the species means were used

218 to quantify the variation in traits associated with variation in genome size. Consistent with
219 previous results across terrestrial vascular plants (Simonin and Roddy 2018), among
220 *Rhododendron* taxa, there was greater variation in the sizes and packing densities of veins
221 and stomata among species with smaller genomes (Figure 2). This was apparent due to
222 significant differences between the 10th and 90th quantiles for guard cell length (10th: $2.40 \pm$
223 1.14 , 90th: -0.72 ± 1.06 ; $F = 7.11$, $P < 0.01$) and for stomatal density (10th: 2.99 ± 10.63 , 90th:
224 -24.51 ± 12.41 ; $F = 5.90$, $P = 0.02$), but not for vein density (10th: 0.14 ± 0.20 , 90th: $-0.36 \pm$
225 0.19 ; $F = 3.22$, $P = 0.07$). Further corroborating the significant differences between the 10th
226 and 90th quantile slopes were the more negative slopes among higher quantiles of the data
227 for all traits (Supplementary Figure S2), consistent with the results for guard cell volume
228 among both angiosperms and vascular plants (Figures 1, S1). Thus, across phylogenetic
229 scales, smaller genomes allow for greater variation in the sizes and packing densities of
230 cells.

231 **Genome size limits maximum photosynthetic metabolism**

232 A major limitation on photosynthetic capacity is the ability to deliver resources to, and
233 export products from, the sites of metabolic processing (Enquist et al. 1998; West et al.
234 1999a; Brown et al. 2004). At the level of an individual cell—the fundamental unit of living
235 organisms—rates of resource transport are strongly influenced by cell size because the ratio
236 of cell surface area to cell volume increases exponentially with decreasing cell size. Because
237 genome size constrains minimum cell size and the maximum packing densities of cells
238 (Figures 1-2), genome size is predicted to limit the maximum rate of photosynthetic
239 metabolism across vascular plants.

240 Previous work has hypothesized that genome size would be linked to maximum
241 photosynthetic rate but found little support (Knight et al. 2005; Beaulieu et al. 2007). One
242 major reason for not finding support is that these previous studies attempted to predict
243 variation in A_{mass} , which accounts for the construction costs of leaves, rather than A_{area} ,
244 which is the maximum metabolic rate regardless of the construction costs. As described
245 above, A_{area} would define the maximum amount of carbon assimilated, but how the plant
246 allocates the total assimilated carbon—to growth, reproduction, defense, more durable

247 leaves, etc.–would reflect the numerous factors that influence plant form and other aspects
248 of plant function (Bazzaz et al. 1987). Thus, A_{area} , which is orthogonal to SLA and A_{mass}
249 (Wright et al. 2004), is predicted to be constrained by cell and genome sizes. Consistent
250 with this prediction, genome size is a strong predictor of the sizes and densities of stomatal
251 guard cells and leaf veins across vascular plants (Simonin and Roddy 2018), and we
252 predicted, therefore, that genome size would, via its effects on the sizes and packing
253 densities of cells, limit A_{area} . It is important to clarify that many factors can influence A_{area}
254 of a given leaf. For example, nutrient deficiency and water stress can reduce A_{area} below its
255 theoretical maximum–independent of the effects of cell and genome size–by limiting either
256 the biochemical or stomatal contributions to carbon assimilation. When these other factors
257 are not limiting, then cell size is predicted to limit A_{area} , and, as a result, we predicted that
258 genome size would define the upper limit (estimated using quantile regression) of A_{area} .

259 Data for area-based maximum photosynthetic rate were compiled from the primary
260 literature (Supplemental Table 1) and merged with the Kew Plant DNA C-Values Database
261 (Bennett and Leitch 2012). This dataset included 210 species, of which 138 were
262 angiosperms, 46 were gymnosperms, and 26 were ferns. We tested whether genome size
263 limits A_{area} using quantile regression. Like above, we estimated the upper limit of A_{area} as
264 the 90th quantile, but include slope estimates across quantiles (Figure S3). Standard errors
265 around these quantile slopes were estimated by bootstrapping 300 replicates. There is no
266 phylogenetically corrected method for estimating quantile slopes, so we tested whether the
267 pattern observed across all species was also apparent only among the angiosperms, which
268 exhibit the largest range in genome size of the three main groups of vascular plants. This
269 analysis helped to determine whether the effects of genome size on A_{area} were driven solely
270 by the divergences between the three major clades.

271 Smaller genomes enabled higher maximum photosynthetic rates across and within major
272 plant clades (Figure 3). Across all terrestrial vascular plants, the upper limit (the 90th
273 quantile) of A_{area} was defined by genome size (slope = -0.18 ± 0.03). A nearly identical
274 slope of the 90th quantile was apparent only among the angiosperms (-0.19 ± 0.05),
275 suggesting that the effect of genome size on maximum A_{area} was not due solely to the
276 divergences between the three major clades. Across all quantiles there was little difference

277 between the quantile slopes estimated for all species versus the angiosperms alone, and
278 these quantile slopes were mostly within the confidence interval of the regression slope
279 through the entire dataset (Figure S3).

280

281 The scaling relationship between A_{area} and genome size follows naturally from the
282 relationships between genome size and the sizes and densities of veins and stomata.
283 However, veins and stomata are not the only cells responsible for driving variation in
284 photosynthetic rates. While the maximum rate of CO₂ diffusion into the leaf is defined by
285 the sizes and densities of stomata (Franks and Beerling 2009), once inside the leaf, CO₂
286 must diffuse through the leaf intercellular airspace and into the chloroplasts lining the
287 interior surfaces of mesophyll cells. Thus, the three-dimensional structure and organization
288 of the mesophyll is predicted to be a prime target for selection on photosynthetic
289 metabolism (Tholen et al. 2012; Ren et al. 2019) and to be critical to leaf photosynthetic
290 function (Earles et al. 2019). The limited evidence on *Arabidopsis thaliana* mutants
291 suggests that cell size is critical to this mesophyll architecture (Lehmeier et al. 2017). Based
292 on the results presented here (Figure 3) and elsewhere (Simonin and Roddy 2018), we
293 predict that the scaling relationships between genome size and cell size that coordinate
294 veins and stomata extend also to the sizes of cells and their organization within the leaf
295 mesophyll.

296 **Genome size may limit the rate of metabolic up- or down-regulation**

297 Although maximum potential rate of leaf gas exchange is an important parameter
298 determining a species' physiological capacity, the actual rate of leaf gas exchange at any
299 given moment is often substantially lower, depending on a variety of physiological and
300 environmental factors (e.g. light level, atmospheric humidity, leaf temperature, plant water
301 status). Changes in sun angle, shading by passing clouds, and self-shading by fluttering
302 leaves all drive changes in incoming solar radiation, and these rapid dynamics have
303 influenced the evolution of photosynthetic biochemistry (Pearcy 1990). Under naturally
304 varying conditions, leaf gas exchange fluctuates dramatically and rarely reaches its
305 maximum rate, with greater variation occurring at the top of the plant canopy. How

306 frequently a leaf can reach its maximum gas exchange rate and how well it can optimize its
307 physiological processes to environmental conditions depend on how rapidly the leaf can
308 respond to dynamic, fluctuating conditions.

309 There is an emerging consensus that smaller stomata respond more rapidly to fluctuating
310 conditions than larger stomata, allowing leaves with smaller stomata to more closely tune
311 their physiological rates with environmental conditions (Drake et al. 2013; Lawson and
312 Blatt 2014; Lawson and Vialet-Chabrand 2019). Leaf physiological processes change at
313 different rates, with changes in stomatal conductance occurring an order of magnitude
314 more slowly than changes in photosynthesis (McAusland et al. 2016). This difference in
315 response times between physiological processes (e.g. photosynthetic assimilation rate and
316 stomatal conductance) can reduce water use efficiency when stomata are closing and
317 reduce photosynthetic efficiency when stomata are opening (Lawson and Vialet-Chabrand
318 2019), limiting total photosynthesis by up to 20% (Lawson and Blatt 2014). If stomatal
319 response times are directly limited by the size of stomata then genome-cellular allometry
320 may limit not only the maximum rate of metabolism but also how quickly metabolism can
321 respond to fluctuating environmental conditions. Of the species for which stomatal
322 response times were measured by McAusland et al. (2016) and Drake et al. (2013), twelve
323 were included in the Kew Plant DNA C-Values database. Consistent with previous reports,
324 there was a positive correlation between genome size and guard cell length ($R^2 = 0.36$, $P <$
325 0.05 ; Figure 4a), and stomatal response rate exhibited a triangular relationship with
326 genome size such that smaller genomes exhibited both higher maximum stomatal response
327 rates but also a greater variation in stomatal response rate. While the available data on
328 stomatal response rates measured using standard protocols are limited, these preliminary
329 results suggest that genome size indirectly limits the maximum rate of stomatal opening
330 and closing via its effects on the sizes and densities of stomata.

331 **How genome size-metabolism scaling may impact plant biogeography**

332 **Polyploidy thought to increase niche breadth**

333 Variation in genome size and structure associated with polyploidization has long been
334 considered to be an important driver of plant evolution and to be associated with shifts in
335 environmental tolerances, habitat breadth, trait variation, and interspecific interactions
336 (Stebbins 1940; Otto and Whitton 2000; Soltis et al. 2003; Soltis et al. 2014; Barker et al.
337 2016a,b), and niche differentiation between polyploids and their diploid parentals has
338 been considered a prerequisite for the successful establishment of newly arisen polyploids
339 (Levin 1975; Fowler & Levin, 1984). Describing the types of polyploids and how they are
340 has been thoroughly reviewed elsewhere (e.g. Stebbins 1947; Soltis et al. 2015), and we
341 focus our discussion here on how and why ploidy—via its relationship with genome size—
342 may or may not correlate with species distributions and habitat breadth. Until they can be
343 more rigorously tested, these ideas will remain speculative.

344 Polyploids have been hypothesized to be better adapted to extreme habitats, to have
345 greater hardiness, and to have greater ecological adaptability (reviewed by Stebbins 1985;
346 Brochmann et al. 2004). The possible mechanisms for these effects can be roughly grouped
347 into two categories: one involving the genetic and genic content of the polyploid genome
348 and one involving the nucleotypic effects of ploidy and genome size. Because polyploid
349 genomes commonly have additional genome copies, they have higher absolute genic
350 contents, would enable neofunctionalization of duplicated genes, and typically have higher
351 heterozygosity, all of which can promote higher tolerances of environmental conditions.
352 The nucleotypic effects of ploidal variation, though long recognized (Stebbins 1940), are
353 often confounded with nucleotypic effects of genome size variation.

354 While ploidy and genome size are commonly assumed to be synonymous, at broad
355 phylogenetic scales there is generally no relationship between genome size and ploidy
356 (Leitch and Bennett 2004), reflecting the complex history of both ancient and
357 contemporary whole genome duplications, particularly among the angiosperms (Jiao et al.
358 2011; Clark and Donoghue 2018; Landis et al. 2018; Ren et al. 2018). In contrast to

359 pteridophytes, which also frequently undergo whole genome duplications (Clark et al.
360 2016), angiosperm genomes readily rediploidize after polyploidization such that genome
361 size and ploidy are positively correlated only for narrowly defined phylogenetic groups (i.e.
362 within genera and families, Figure 5; Leitch and Bennett 2004; Dodsworth et al. 2016). If
363 leaf and plant structure and function influence ecological tolerances and habitat breadth
364 (i.e. if plant structure-function is adaptive), then the nucleotypic effects of genome size are
365 predicted to influence environmental tolerances.

366 **Smaller genomes enable greater phenotypic plasticity**

367 One long-standing hypothesis is that higher ploidy is related to wider habitat breadth
368 because polyploids can tolerate greater ecological stress. Higher ploidy is associated with
369 greater heterozygosity (i.e. greater genetic diversity) and, frequently, higher genic content
370 due to multiple genome copies, both of which are thought to promote plasticity and enable
371 polyploids to withstand a greater range of environmental conditions than diploids.
372 However, several studies testing this hypothesis have not observed polyploids to have
373 greater habitat breadth (e.g. Stebbins 1985; Martin and Husband 2009; Glennon et al. 2014;
374 Johnson et al. 2014). Furthermore, these tests frequently find that diploids exhibit greater
375 habitat breadth than polyploids (Petit and Thompson 1999; Hijmans et al. 2007;
376 Brittingham et al. 2018; Castro et al. 2019). One reason is that traits are not necessarily
377 more variable in polyploids than in diploids (Stebbins 1985; Wei et al. 2018).

378 We predict that one reason ploidy is not commonly found to correlate with ecological
379 breadth is because genome size—rather than ploidy per se—drives variation in the absolute
380 range of potential cell sizes and, by extension, phenotypic plasticity in rates of resource
381 transport and metabolism. Thus, the phylogenetic scale-dependence of the relationship
382 between genome size and ploidy (Figure 5), particularly among the angiosperms, could
383 lead to confounding patterns depending on the phylogenetic scale at which comparisons
384 are made. For example, in the analysis of Rice et al. (2019), ploidy was determined relative
385 to other closely related species, such that within genera or families ploidy and genome size
386 are positively correlated, suggesting that the bias towards higher abundances of polyploids
387 at higher latitudes may reflect nucleotypic effects of genome size on cell size and

388 metabolism. The complex, fluctuating process of polyploidization and rediploidization,
389 which can winnow the genome nonrandomly (Wendel 2015), would promote the
390 proliferation of beneficial elements associated with genome duplications (e.g. more gene
391 copies that can neofunctionalize) while reducing the size of the genome needed to maintain
392 high rates of development and metabolism (Table 1).

393 We posit here that the nucleotypic effects of genome size, regardless of ploidy, may
394 influence environmental tolerances. Because smaller genomes allow for greater variation
395 in cell size and metabolism (Figures 1-3), species with smaller genomes may be better able
396 to fine tune their tissue structure to environmental conditions. This flexibility would allow
397 species with smaller genomes to better optimize their metabolic rates in order to occupy a
398 wider range of environmental conditions. Combined with the effects of genome size on
399 rates of cell division (Van't Hof and Sparrow 1963; Van't Hof 1965; Šímová and Herben
400 2012), the greater plasticity in cell size and higher metabolic rates attainable by species
401 with small genomes may enable them to better colonize new habitats.

402 **Community-scale patterns in genome size across gradients in productivity**

403 If habitats filter species based on rates of metabolism and if there are nucleotypic effects of
404 genome size on metabolism, then community-scale distributions of genome size may vary
405 across gradients of productivity. In habitats that can support high rates of productivity and
406 primary metabolism, species with small genomes are expected to predominate because
407 they can maintain higher rates of metabolism and more rapidly adjust their physiology to
408 match environmental conditions. This strategy would be one of maintaining steady state
409 physiological processes. At a broad scale, this prediction holds because angiosperms,
410 which have, on average, smaller genomes than other vascular plants are dominant in most
411 ecosystems, particularly those characterized by high productivity. However, high rates of
412 metabolism and maintaining steady-state physiology, even among the angiosperms, are not
413 always favorable. Two such habitats are those characterized by extreme water and nutrient
414 limitation, such as deserts and epiphytic habitats, and by extreme cold, such as high
415 latitudes. Higher incidences of polyploids have been commonly reported in higher latitudes

416 and among arctic floras (Brochmann et al. 2004; Rice et al. 2019), but arid habitats have
417 received less attention.

418 Arid and epiphytic habitats are characterized by low productivity and may support species
419 with large genomes. In these habitats, high rates of metabolism are not always favored,
420 which may relax selection for small genomes. One strategy common in arid and epiphytic
421 habitats is succulence, which is often associated with Crassulacean acid metabolism (CAM)
422 photosynthesis. The CAM syndrome limits water loss by restricting CO₂ uptake and water
423 loss to nighttime when humidity is high and the atmospheric demand for evaporation
424 relatively low. As a result, CAM species typically rely more heavily on resource storage (e.g.
425 CO₂, H₂O) or non-steady-state physiology to maintain photosynthetic metabolism and limit
426 water loss. If metabolism is one agent of selection on genome size, then we would predict
427 that in arid, resource poor environments, selection for small genomes (associated with
428 small cells and high metabolic rates) may be weak among CAM species, allowing genomes
429 of CAM species to expand in size. We tested this hypothesis using the taxonomic
430 distributions of CAM photosynthesis from Smith and Winter (1996) and genome size data
431 from the Kew Plant DNA C-Values Database (Bennett and Leitch 2012). For C3, we used the
432 broad distribution of angiosperms reported in Simonin and Roddy (2018), which are
433 representative of extant angiosperm diversity. We scored as CAM the narrowest taxonomic
434 level in the Kew DNA C-Values Database that was listed as containing CAM by Smith and
435 Winter (1996). For example, if a genus were listed as containing any CAM species, all
436 species in the genus were assumed to exhibit CAM photosynthesis. This approach was
437 biased against observing differences in genome size between C3 and CAM species because
438 it necessarily grouped some C3 species as CAM. To account for phylogenetic history, we
439 constructed a dated, family-level supertree using the methods described in Simonin and
440 Roddy (2018), and compared C3 and CAM genome sizes using the *phylANOVA* function in
441 'phytools' (Revell 2012). Log-normalized genome sizes were significantly larger among
442 CAM species than among C3 species ($t = 8.11$, $df = 284.03$, $P < 0.001$) even after accounting
443 for shared phylogenetic history ($t = 7.51$, $P < 0.05$; Figure 6), consistent with the prediction
444 that large genomes may evolve when selection for high rates of metabolism is weak.
445 However, future analyses that incorporate better determination of the phylogenetic

446 distributions of photosynthetic pathways is needed to more rigorously test whether the
447 evolution of CAM photosynthesis and its associated switch towards non-steady-state
448 physiological processes is indeed associated with increases in genome size.

449 Arid, resource poor habitats are not exclusively composed of species with large genomes.
450 Rather, they may harbor a diversity of strategies associated with divergent niches. In
451 deserts, physiological strategies can be arrayed along a spectrum from strict non-steady-
452 state physiology characterized by low rates of metabolism (e.g. obligate CAM) to quasi-
453 steady-state physiology (e.g. C3 species) characterized by high rates of metabolism (Nobel
454 and Jordan 1983; Hunt and Nobel 1987). While CAM species can rely on resource storage
455 during periods of limited water availability, C3 species in deserts tend to function during a
456 relatively narrow period of time when water is available. Thus, because their carbon gain is
457 limited to such a short time period, C3 desert plants may have small genomes and cells that
458 enable high rates of metabolism. In fact, desert shrubs have the highest rates of stem
459 hydraulic conductance measured in C3 plants (Mencuccini 2003), and even among species
460 from humid tropical forests, dry forest species have higher hydraulic conductance than wet
461 forest species (Brenes-Arguedas et al. 2013). Thus, less productive habitats may select not
462 simply for larger genomes but instead allow for multiple strategies that encompass a
463 broader range of metabolic rates and, by extension, greater variation in genome size at the
464 community level.

465 **Smaller genomes increase the probability of invasiveness**

466 The multifaceted effects of genome size on plant structure, function, and ecology (Table 1)
467 is particularly relevant to the study of invasive species. Identifying the traits that allow an
468 introduced species to establish, naturalize, and invade into a new environment is a central
469 aim of invasion biology (Simberloff 2011), with broader implications for plant
470 biogeographic patterns. Here we distinguish between nonnative species—those that survive
471 and reproduce in their introduced range—and nonnative invasive species—those that can
472 disperse, establish, and spread far from their original source of introduction (Richardson et
473 al. 2011). This distinction is important because prior studies on the traits of ‘invaders’
474 focus on these different subsets of species, which have slightly different, but overlapping,

475 sets of traits that determine whether they can survive and reproduce versus invade non-
476 native regions (Kleunen et al. 2015).

477 Early theory on the distinguishing traits of invasive plants postulated that “ideal weeds”
478 should grow rapidly, produce seed continuously and in high number throughout the
479 growing season, be tolerant to a wide range of environmental conditions, exhibit high trait
480 plasticity, and be able to reproduce vegetatively from fragments (Baker 1974). On average,
481 these predictions have been upheld, with nonnative invasive plants tending to exhibit traits
482 consistent with high fitness (e.g. number of flowers, fruits, or seed or germination rates),
483 high relative growth rates, high dispersal abilities (e.g. smaller seeds), and more efficient
484 carbon-capture strategies (e.g. high specific leaf area), relative to co-occurring native
485 species (Leishman et al. 2007; Kleunen et al. 2010; Ordonez et al. 2010; Kuester et al. 2014)
486 or naturalized but not invasive nonnative species (Rejmánek and Richardson 1996;
487 Gallagher et al. 2014). Combined, these traits confer a growth advantage, such that plants
488 with small seeds can disperse further distances, have shorter generation times, and higher
489 relative growth rates, owing to the greater rates of cell division and higher metabolic rates
490 provided by smaller genomes (Pandit et al. 2014; Suda et al. 2015). Indeed, even within
491 species, populations with smaller genomes are more likely to successfully invade new
492 habitats (Pysek et al. 2018).

493 Because many of the traits linked with invasiveness can be influenced by both ploidy and
494 genome size, both have been implicated as underlying features driving invasion (Pandit et
495 al. 2014; Suda et al. 2015). Because polyploids are thought to be better able to tolerate
496 environmental fluctuations and to be better able to adapt to new environments, polyploids
497 tend to be overrepresented among nonnative invasives compared to native angiosperms
498 (Rejmánek and Richardson 1996; Prentis et al. 2008; Beest et al. 2011; Pandit et al. 2014).

499 Similarly, nonnative invasive species tend to have smaller genomes than non-invasive
500 plants (both native and non-native), which is thought to be due to the diverse effects of
501 genome size on metabolism, rates of development and growth, and seed size (Rejmánek
502 and Richardson 1996; Bennett et al. 1998; Kubešová et al. 2010; Pandit et al. 2014).

503 However, the complex, scale-dependent relationship between ploidy and genome size
504 (Figure 5) complicates a clear understanding of the effects of ploidy versus genome size on

505 invasiveness (Rejmánek and Richardson 1996; Pandit et al. 2014). Because angiosperms,
506 which predominate among nonnative invasives, readily rediploidize and downsize their
507 genomes subsequent to whole genome duplications (Leitch and Bennett 2004), assessing
508 the relative effects of ploidy versus genome size on invasiveness can be difficult. For
509 example, the likelihood of being invasive increases with chromosome number and ploidy
510 but decreases with genome size (Rejmánek and Richardson 1996; Pandit et al. 2014). The
511 multiple paths to polyploidization and the selective retention of only certain parts of the
512 genome during subsequent genome downsizing (Wendel 2015) could explain how both
513 higher ploidy and smaller genomes are correlated with invasiveness.

514 **A possible role for metabolism in genome size evolution**

515 As the major source of energy and matter for the biosphere, photosynthetic metabolism
516 represents a first-order control over ecological processes globally. This fundamental link
517 between metabolic and ecological processes has driven the development of the Metabolic
518 Theory of Ecology (MTE) that provides a mechanistic framework for predicting variation in
519 organismal life history attributes, population dynamics, and larger scale ecosystem
520 processes from organismal-level traits related to resource supply for metabolism (West et
521 al. 1997; Enquist et al. 1998; West et al. 1999a; West et al. 1999b; West et al. 2002; Price et
522 al. 2010). While appealing and seemingly endowed with incredible explanatory power, a
523 number of criticisms of the theory and its assumptions have been consistently raised
524 (Kozłowski and Konarzewski 2004; Kozłowski and Konarzewski 2005; Price et al. 2012).
525 One primary assumption is that the sizes of terminal units in vascular networks (e.g.
526 capillaries in circulatory systems or terminal veins in plant leaves) are invariant. The
527 problems with this assumption have been thoroughly detailed for animal circulatory
528 systems with the allometry of genome size and cell size emerging as a critical factor
529 influencing how body size scales with metabolism (Kozłowski et al. 2003). Furthermore,
530 the allometry of genome size and cell size (Figure 1) and the effects of genome size on
531 maximum metabolic rate (Figure 3) presented here suggest that this assumption is violated
532 in plants, as well. Modifications to the original model that relax some of its assumptions
533 have improved model predictions for plants, particularly by allowing for variation in the
534 packing of xylem conduits (Savage et al. 2010). However, the nucleotypic effects of genome

535 size have yet to be incorporated, although they may further improve models and help to
536 clarify the constraints and major innovations driving botanical form, function, and
537 diversity.

538
539 The effects of genome size on cell sizes and packing densities across vascular plants
540 (Figures 1,2; Beaulieu et al. 2008; Simonin and Roddy 2018) and the importance of cell size
541 in metabolism (Savage et al. 2010) together suggest that there may be a role for
542 metabolism in the evolution of genome size. While it is appealing to expect that genome
543 size may predict metabolic rate, the effects of genome size are likely more nuanced.
544 Because genome size defines only the lower limit of cell size, genome size may limit only
545 the maximum possible rate of energy and matter exchange (Figure 3), rather than being a
546 clear predictor of metabolism more generally. This suggests that evolutionary increases in
547 metabolic capacity may be tied to the evolution of genome size, such as has been described
548 in birds (Wright et al. 2014). How selection on genome size *per se* may be translated into
549 alterations of genome sequence structure is unclear but would be an important step
550 towards understanding the drivers of genome size variation. Independent evidence for the
551 role of metabolism in shaping genome-cellular allometry can be evaluated by comparing
552 structures with similar developmental origins such as flowers and leaves (Olson and
553 Pittermann 2019). Flowers, unlike leaves, need not support high rates of energy and
554 matter exchange for use in photosynthetic metabolism and generally have larger cells and
555 lower cell packing densities than their conspecific leaf counterparts (Roddy et al. 2013,
556 2019; Zhang et al. 2018; Roddy *in press*). Thus, under different selection regimes due to
557 differences in metabolism, traits can diverge even within the same organism (Olson and
558 Arroyo-Santos 2015). Furthermore, defining the biophysical limits of phenotypic variation
559 is central to understanding the diversity of plant form and function, and our analyses
560 suggest that genome size defines one bound to the range of possible cell sizes.

561

562 Table 1. Brief summary of traits shown previously to correlate with genome size.

563

Sizes	Reference
Pollen volume	Bennett 1972; Knight et al. 2010
Cell mass	Martin 1966
Epidermal cell size	Beaulieu et al. 2008; Knight and Beaulieu 2008
Nuclear volume	Van't Hof and Sparrow 1963; Baetcke et al. 1967
Nuclear dry mass	Bennett et al. 1983; White and Rees 1987
Seed mass	Grotkopp et al. 2004; Beaulieu et al. 2007
Xylem vessel diameter	Maherali et al. 2009; Hao et al. 2013; De Baerdemaeker et al. 2018
Rates	
Cell division rate, meiosis, mitosis	Van't Hof and Sparrow 1963; Van't Hof 1965; Bennett 1971
Minimum generation time	Bennett 1972
Leaf expansion rate	Grime et al. 1985
Phenology	Grime and Mowforth 1982
Frost tolerance	MacGillivray and Grime 1995

564

565

566

567

568 **Figure legends**

569 Figure 1. Genome size determines the minimum size of cells, and smaller genomes enable
570 greater variation in final cell size. Data for meristematic cells (blue triangles) were taken
571 from Šímová and Herben (2012), and the solid black line is the regression through these
572 points. Data for mature stomatal guard cells of extant plants (circles and squares) for ferns
573 (dark green), gymnosperms (pink), and angiosperms (light blue) were taken from Simonin
574 and Roddy (2018), and data for mosses and hornworts (light green) were taken from Field
575 et al. (2015) and Renzaglia et al. (2017). The two dashed lines represent the 10th (lower)
576 and 90th (upper) quantile regressions through mature guard cell data for vascular plants
577 with their respective confidence intervals shaded. The dotted line represents the 90th
578 quantile through all guard cell data (vascular and non-vascular plants).

579 Figure 2. Variation in the sizes and packing densities of stomatal guard cells and leaf veins
580 with variation in genome size among *Rhododendron* sect. *Schistanthe* species (circles) and
581 polyploid *Rhododendron* cultivars (triangles). Lines represent regressions through the 90th
582 (upper) and 10th (lower) quantiles. These quantile regression were significantly different
583 for guard cell length and stomatal density (dashed) but not for vein density (dotted).
584 Genome size limits the lower limit of cell size and the upper limit of cell packing densities,
585 and there is greater variation in anatomical traits among species with smaller genomes.

586 Figure 3. Genome size limits the maximum rate of photosynthesis (A_{area}) across C3
587 terrestrial plants. (a) Untransformed relationship and (b) log-transformed relationship.
588 Dashed black lines are regressions through the upper 90th quantile of all data with grey
589 shading representing the 95% confidence interval. Blue dashed lines and blue shading
590 represent the 90th quantile regression and its 95% confidence interval for angiosperms
591 alone, showing that the same slope defines the upper limit among only the angiosperms as
592 across all three major clades of vascular plants.

593 Figure 4. Genome size may limit the maximum rate of stomatal response (i.e. how fast
594 stomata can open or close). Data taken from McAusland et al. (2016) and Kew Plant DNA C-
595 values Database.

596 Figure 5. Relationship between genome size and ploidy for angiosperms. Each line
597 represents the linear regression within a genus. At narrow taxonomic scales, ploidy and
598 genome size are correlated, but at broad taxonomic scales (i.e. among all angiosperms),
599 there is no relationship between genome size and ploidy due to rediploidization.

600 Figure 6. Distributions of genome size for C3 and CAM species show CAM lineages have
601 significantly larger genomes than C3 lineages. Lineages identified as CAM likely include
602 many C3 species; see text for details on identification of photosynthetic pathways. There
603 was a significant difference in log-normalized genome size for the two photosynthetic
604 pathways, even after accounting for shared phylogenetic history.

605

606 Figure S1. Quantile regression slopes and bootstrapped standard errors for of cell volume
607 and genome size data plotted in Figure 1 for vascular plants. Quantiles were calculated for
608 every 5% of the data (5% to 95%) for all vascular plants (ferns, gymnosperms,
609 angiosperms; black points) and for angiosperms only (blue points). Points are jittered
610 horizontally so they do not plot on top of each other. The OLS slope through the entire
611 dataset (solid red line) and its confidence interval (dotted red lines) are included for
612 comparison. Lower quantiles of the data have consistently steeper slopes.

613

614 Figure S2. Quantile regression slopes and bootstrapped confidence intervals for (a) guard
615 cell length, (b) stomatal density, and (c) vein density of *Rhododendron* subsect. *Schistanthe*
616 species and *Rhododendron* cultivars. Original data plotted in Figure 2. Quantiles were
617 calculated for every 5% of the data (5% to 95%), with standard errors estimated by
618 bootstrapping 300 replicated. The OLS slope (solid red line) and its confidence interval
619 (dotted red lines) are included for comparison. For all three traits, lower quantiles of the
620 data have consistently steeper slopes.

621

622 Figure S3. Quantile regression slopes and bootstrapped standard errors for A_{area} and
623 genome size data plotted in Figure 3. Quantiles were calculated for every 5% of the data
624 (5% to 95%) for all vascular plants (ferns, gymnosperms, angiosperms; black points) and
625 for angiosperms only (blue points). Points are jittered horizontally so they do not plot on
626 top of each other. The OLS slope through the entire dataset (solid red line) and its
627 confidence interval (dotted red lines) are included for comparison. Lower quantiles of the
628 data have consistently steeper slopes.

629

630 **References**

- 631 Baetcke K, A Sparrow, C Nauman, SS Schwemmer 1967 The relationship of DNA content to
632 nuclear and chromosome volumes and to radiosensitivity (LD50). Proceedings of
633 the National Academy of Sciences of the United States of America 58:533.
- 634 Bailey IW, WW Tupper 1918 Size variation in tracheary cells: I. a comparison between the
635 secondary xylems of vascular cryptogams, gymnosperms and angiosperms.
636 Proceedings of the American Academy of Arts and Sciences 54:149–204.
- 637 Baker HG 1974 The evolution of weeds. Annual Review of Ecology and Systematics 5:1–24.
- 638 Barker MS, N Arrigo, AE Baniaga, Z Li, DA Levin 2016a On the relative abundance of
639 autopolyploids and allopolyploids. New Phytologist 210:391–398.
- 640 Barker MS, BC Husband, JC Pires 2016b Spreading Winge and flying high: The evolutionary
641 importance of polyploidy after a century of study. American Journal of Botany
642 103:1139–1145.
- 643 Bazzaz FA, NR Chiariello, P Coley, LF Pitelka 1987 Allocating resources to reproduction and
644 defense. BioScience 37:58–67.
- 645 Beaulieu JM, IJ Leitch, CA Knight 2007 Genome size evolution in relation to leaf strategy
646 and metabolic rates revisited. Annals of Botany 99:495–505.
- 647 Beaulieu JM, AT Moles, IJ Leitch, MD Bennett, JB Dickie, CA Knight. 2007. Correlated
648 evolution of genome size and seed mass. New Phytologist 173:422-437.
- 649 Beaulieu JM, IJ Leitch, S Patel, A Pendharkar, CA Knight 2008 Genome size is a strong
650 predictor of cell size and stomatal density in angiosperms. New Phytologist
651 179:975–986.
- 652 Beest M te, JJ Le Roux, DM Richardson, AK Brysting, J Suda, M Kubešová, P Pyšek 2011 The
653 more the better? The role of polyploidy in facilitating plant invasions. Annals of
654 Botany 109:19–45.

- 655 Bennett M, J Heslop-Harrison, J Smith, J Ward 1983 DNA density in mitotic and meiotic
656 metaphase chromosomes of plants and animals. *Journal of Cell Science* 63:173–179.
- 657 Bennett MD 1971 The duration of meiosis. *Proceedings of the Royal Society B* 178:277–
658 299.
- 659 Bennett MD 1972 Nuclear DNA content and minimum generation time in herbaceous
660 plants. *Proceedings of the Royal Society of London Series B Biological Sciences*
661 181:109–135.
- 662 Bennett MD, IJ Leitch 2005 The evolution of the genome. In: Gregory TR, editor. Elsevier.
663 pp. 89–162.
- 664 ——— 2012 Plant DNA C-values database (release 6.0). <http://data.kew.org/cvalues/>
- 665 Bennett MD, IJ Leitch, L Hanson 1998 DNA amounts in two samples of angiosperm weeds.
666 *Annals of Botany* 82:121–134.
- 667 Brenes-Arguedas T, AB Roddy, TA Kursar 2013 Plant traits in relation to the performance
668 and distribution of woody species in wet and dry tropical forest types in Panama.
669 *Functional Ecology* 27:392–402.
- 670 Brittingham HA, MH Koski, T-L Ashman 2018 Higher ploidy is associated with reduced
671 range breadth in the Potentilleae tribe. *American Journal of Botany* 105:700–710.
- 672 Brochmann C, A Brysting, I Alsos, L Borgen, H Grundt, A-C Scheen, R Elven 2004 Polyploidy
673 in arctic plants. *Biological Journal of the Linnean Society* 82:521–536.
- 674 Brown JH, JF Gillooly, AP Allen, VM Savage, GB West 2004 Toward a metabolic theory of
675 ecology. *Ecology* 85:1771–1789.
- 676 Castro M, J Loureiro, M Serran, D Tavares, BC Husband, C Siopa, S Castro 2019 Mosaic
677 distribution of cytotypes in a mixed-ploidy plant species, *Jasione montana*: nested
678 environmental niches but low geographical overlap. *Botanical Journal of the*
679 *Linnean Society* 190:51–66.

- 680 Cavalier-Smith T 1978 Nuclear volume control by nucleoskeletal DNA, selection for cell
681 volume and cell growth rate, and the solution of the DNA c-value paradox. *Journal of*
682 *Cell Science* 34:247–278.
- 683 ——— 1982 Skeletal DNA and the evolution of genome size. *Annual Review of Biophysics*
684 *and Bioengineering* 11:273–302.
- 685 Chamberlain S 2016 brranching: Fetch 'phylogenies' from many sources. <[https://CRAN.R-](https://CRAN.R-project.org/package=brranching)
686 [project.org/package=brranching](https://CRAN.R-project.org/package=brranching)>
- 687 Clark J, O Hidalgo, J Pellicer, H Liu, J Marquardt, Y Robert, M Christenhusz, S Zhang, M Gibby,
688 IJ Leitch, et al. 2016 Genome evolution of ferns: Evidence for relative stasis of
689 genome size across the fern phylogeny. *New Phytologist* 210:1072–1082.
- 690 Clark JW, PC Donoghue 2018 Whole-genome duplication and plant macroevolution. *Trends*
691 *in Plant Science*.
- 692 de Baerdemaeker NJF, N Hias, J van den Bulcke, W Keulemans, K Steppe 2018 The effect of
693 polyploidization on tree hydraulic functioning. *American Journal of Botany*
694 105:161–171.
- 695 Dodsworth S, M Chase, AR Leitch 2016 Is post-polyploidization diploidization the key to the
696 evolutionary success of the angiosperms? *Botanical Journal of the Linnean Society*
697 180:1–5.
- 698 Dolezel J, J Greilhuber, J Suda 2007 Estimation of nuclear DNA content in plants using flow
699 cytometry. *Nature Protocols* 2:2233–44.
- 700 Doyle JJ, JE Coate 2019 Polyploidy, the nucleotype, and novelty: The impact of genome
701 doubling on the biology of the cell. *International Journal of Plant Sciences* 180:1–52.
- 702 Drake PL, RH Froend, PJ Franks 2013 Smaller, faster stomata: Scaling of stomatal size, rate
703 of response, and stomatal conductance. *Journal of Experimental Botany* 64:495–505.

- 704 Earles JM, TN Buckley, CR Brodersen, FA Busch, FJ Cano, B Choat, JR Evans, GD Farquhar, R
705 Harwood, M Huynh, et al. 2019 Embracing 3D complexity in leaf carbon–water
706 exchange. *Trends in Plant Science* 24:15–24.
- 707 Enquist BJ, JH Brown, GB West 1998 Allometric scaling of plant energetics and population
708 density. *Nature* 395:163–165.
- 709 Field KJ, JG Duckett, DD Cameron, S Pressel 2015 Stomatal density and aperture in non-
710 vascular land plants are non-responsive to above-ambient atmospheric CO₂
711 concentrations. *Annals of Botany* 115:915–922.
- 712 Fowler NL, Levin DA 1984 Ecological constraints on the establishment of a novel polyploid
713 in competition with its diploid progenitor. *American Naturalist* 124:703-711.
- 714 Franks PJ, DJ Beerling 2009 Maximum leaf conductance driven by CO₂ effects on stomatal
715 size and density over geologic time. *Proceedings of the National Academy of
716 Sciences* 106:10343–10347.
- 717 Gallagher RV, RP Randall, MR Leishman 2014 Trait differences between naturalized and
718 invasive plant species independent of residence time and phylogeny. *Conservation
719 Biology* 29:360–369.
- 720 Glennon KL, Ritchie ME, Segraves KA 2014 Evidence for shared broad-scale climatic niches
721 of diploid and polyploid plants. *Ecology Letters* 17: 574-582.
- 722 Goetsch LA, LA Craven, BD Hall 2011 Major speciation accompanied the dispersal of *Vireya*
723 *Rhododendrons* (Ericaceae, *Rhododendron* sect. *Schistanthe*) through the Malayan
724 archipelago: Evidence from nuclear gene sequences. *Taxon* 60:1015–1028.
- 725 Grime J 1998 Plant classification for ecological purposes: Is there a role for genome size?
726 *Annals of Botany* 82:117–120.
- 727 Grime J, M Mowforth 1982 Variation in genome size—an ecological interpretation. *Nature*
728 299:151.

- 729 Grime J, J Shacklock, S Band 1985 Nuclear DNA contents, shoot phenology and species co-
730 existence in a limestone grassland community. *New Phytologist* 100:435–445.
- 731 Grotkopp E, M Rejmánek, M Sanderson, TL Rost 2004 Evolution of genome size in pines
732 (*Pinus*) and its life-history correlates: supertree analyses. *Evolution* 58:1705–1729.
- 733 Hao G-Y, ME Lucero, SC Sanderson, EH Zacharias, NM Holbrook 2013 Polyploidy enhances
734 the occupation of heterogeneous environments through hydraulic related trade-offs
735 in *Atriplex canescens* (Chenopodiaceae). *New Phytologist* 197:970–978.
- 736 Hetherington A, F Woodward 2003 The role of stomata in sensing and driving
737 environmental change. *Nature* 424:901–908.
- 738 Hijmans RJ, T Gavrilenko, S Stephenson, J Bamberg, A Salas, DM Spooner 2007 Geographical
739 and environmental range expansion through polyploidy in wild potatoes (*Solanum*
740 section *petota*). *Global Ecology and Biogeography* 16:485–495.
- 741 Hunt ER, PS Nobel 1987 Non-steady state water flow for three desert perennials with
742 different capacitances. *Australian Journal of Plant Physiology* 14:363-375.
- 743 Hof JV 1965 Relationships between mitotic cycle duration, S period duration and the
744 average rate of DNA synthesis in the root meristem cells of several plants.
745 *Experimental Cell Research* 39:48–58.
- 746 Jiao Y, NJ Wickett, S Ayyampalayam, AS Chanderbali, L Landherr, PE Ralph, LP Tomsho, Y
747 Hu, H Liang, PS Soltis, et al. 2011 Ancestral polyploidy in seed plants and
748 angiosperms. *Nature* 473:97–100.
- 749 Johnson AL, R Govindarajulu, T-L Ashman 2014 Bioclimatic evaluation of geographical
750 range in *Fragaria* (Rosaceae): Consequences of variation in breeding system, ploidy
751 and species age. *Botanical journal of the Linnean Society* 176:99–114.
- 752 Kleunen M van, W Dawson, N Maurel 2015 Characteristics of successful alien plants.
753 *Molecular Ecology* 24:1954–1968.

- 754 Kleunen M van, E Weber, M Fischer 2010 A meta-analysis of trait differences between
755 invasive and non-invasive plant species. *Ecology Letters* 13:235–245.
- 756 Knight CA, JM Beaulieu 2008 Genome size scaling through phenotype space. *Annals of*
757 *Botany* 101:759–766.
- 758 Knight CA, NA Molinari, DA Petrov 2005 The large genome constraint hypothesis:
759 evolution, ecology and phenotype. *Annals of Botany* 95:177–190.
- 760 Knight CA, RB Clancy, L Götzenberger, L Dann, JM Beaulieu. 2010. On the relationship
761 between pollen size and genome size. *Journal of Botany* 2010:612017..
- 762 Kozłowski J, M Konarzewski 2004 Is West, Brown and Enquist’s model of allometric scaling
763 mathematically correct and biologically relevant? *Functional Ecology* 18:283–289.
- 764 Kozłowski J, M Konarzewski 2005 West, Brown and Enquist’s model of allometric scaling
765 again: the same questions remain. *Functional Ecology* 19:739–743.
- 766 Kozłowski J, M Konarzewski, AT Gawelczyk 2003 Cell size as a link between noncoding
767 DNA and metabolic rate scaling. *Proceedings of the National Academy of Sciences*
768 100:14080–14085.
- 769 Kubešová M, L Moravcova, J Suda, V Jarošík, P Pyšek, others 2010 Naturalized plants have
770 smaller genomes than their non-invading relatives: A flow cytometric analysis of the
771 Czech alien flora. *Preslia* 82:81–96.
- 772 Kuester A, JK Conner, T Culley, RS Baucom 2014 How weeds emerge: A taxonomic and
773 trait-based examination using united states data. *New Phytologist* 202:1055–1068.
- 774 Landis JB, DE Soltis, Z Li, HE Marx, MS Barker, DC Tank, PS Soltis 2018 Impact of whole-
775 genome duplication events on diversification rates in angiosperms. *American*
776 *Journal of Botany* 105:348–363.
- 777 Lawson T, MR Blatt 2014 Stomatal size, speed, and responsiveness impact on
778 photosynthesis and water use efficiency. *Plant Physiology* 164:1556–1570.

- 779 Lawson T, S Violet-Chabrand 2019 Speedy stomata, photosynthesis and plant water use
780 efficiency. *New Phytologist* 221:93–98.
- 781 Lehmeier C, R Pajor, MR Lundgren, A Mathers, J Sloan, M Bauch, A Mitchell, C Bellasio, A
782 Green, D Bouyer, et al. 2017 Cell density and airspace patterning in the leaf can be
783 manipulated to increase leaf photosynthetic capacity. *The Plant Journal* 92:981–994.
- 784 Leishman MR, T Haslehurst, A Ares, Z Baruch 2007 Leaf trait relationships of native and
785 invasive plants: community- and global-scale comparisons. *New Phytologist*
786 176:635–643.
- 787 Leitch I, M Bennett 2004 Genome downsizing in polyploid plants. *Biological journal of the*
788 *Linnean Society* 82:651–663.
- 789 Levin DA 1975 Minority cytotype exclusion in local plant populations. *Taxon* 24:35-43.
- 790 Lomax BH, J Hilton, RM Bateman, GR Upchurch, JA Lake, IJ Leitch, A Cromwell, CA Knight
791 2013 Reconstructing relative genome size of vascular plants through geological
792 time. *New Phytologist* 201:636–644.
- 793 MacGillivray CW, J Grime 1995 Genome size predicts frost resistance in British herbaceous
794 plants: Implications for rates of vegetation response to global warming. *Functional*
795 *Ecology* 9:320–325.
- 796 Magallón S, S Gómez-Acevedo, LL Sánchez-Reyes, T Hernández-Hernández 2015 A
797 metacalibrated time-tree documents the early rise of flowering plant phylogenetic
798 diversity. *New Phytologist* 207:437–453.
- 799 Maherali H, AE Walden, BC Husband 2009 Genome duplication and the evolution of
800 physiological responses to water stress. *New Phytologist* 184:721–731.
- 801 Martin PG 1966 Variation in the amounts of nucleic acids in the cells of different species of
802 higher plants. *Exp Cell Res* 44:84–94.
- 803 Martin SL, BC Husband 2009 Influence of phylogeny and ploidy on species ranges of north
804 american angiosperms. *Journal of Ecology* 97:913–922.

- 805 McAusland L, S Violet-Chabrand, P Davey, NR Baker, O Brendel, T Lawson 2016 Effects of
806 kinetics of light-induced stomatal responses on photosynthesis and water-use
807 efficiency. *New Phytologist* 211:1209–1220.
- 808 Melaragno JE, B Mehrotra, AW Coleman 1993 Relationship between endopolyploidy and
809 cell size in epidermal tissue of arabidopsis. *The Plant Cell* 5:1661–1668.
- 810 Mencuccini M 2003 The ecological significance of long-distance water transport: Short-
811 term regulation, long-term acclimation and the hydraulic costs of stature across
812 plant life forms. *Plant, Cell & Environment* 26:163–182.
- 813 Nobel PS, PW Jordan 1983 Transpiration stream of desert species: resistances and
814 capacitances for a C3, a C4, and a CAM plant. *Journal of Experimental Botany*
815 34:1379-1391.
- 816 Olson ME and A Arroyo-Santos 2015 How to study adaptation (and why to do it that way).
817 *The Quarterly Review of Biology* 90:167-191.
- 818 Olson ME, J Pittermann 2019 Cheap and attractive: water relations and floral adaptation.
819 *New Phytologist* 223:8-10.
- 820 Ordonez A, IJ Wright, H Olf 2010 Functional differences between native and alien species:
821 a global-scale comparison. *Functional Ecology* 24:1353–1361.
- 822 Otto SP, J Whitton 2000 Polyploid incidence and evolution. *Annual Review of Genetics*
823 34:401–437.
- 824 Pandit MK, SM White, MJO Pocock 2014 The contrasting effects of genome size,
825 chromosome number and ploidy level on plant invasiveness: a global analysis. *New*
826 *Phytologist* 203:697–703.
- 827 Pearcy RW 1990 Sunflecks and photosynthesis in plant canopies. *Annual Review of Plant*
828 *Biology* 41:421–453.
- 829 Petit C, JD Thompson 1999 Species diversity and ecological range in relation to ploidy level
830 in the flora of the Pyrenees. *Evolutionary Ecology* 13:45–65.

- 831 Poorter H, C Remkes 1990 Leaf area ratio and net assimilation rate of 24 wild species
832 differing in relative growth rate. *Oecologia* 83:553–559.
- 833 Poorter H, C Remkes, H Lambers 1990 Carbon and nitrogen economy of 24 wild species
834 differing in relative growth rate. *Plant physiology* 94:621–627.
- 835 Prentis PJ, JR Wilson, EE Dormontt, DM Richardson, AJ Lowe 2008 Adaptive evolution in
836 invasive species. *Trends in Plant Science* 13:288–294.
- 837 Price CA, JF Gilooly, AP Allen, JS Weitz, KJ Niklas 2010 The metabolic theory of ecology:
838 Prospects and challenges for plant biology. *New Phytologist* 188:696–710.
- 839 Price CA, JS Weitz, VM Savage, J Stegen, A Clarke, DA Coomes, PS Dodds, RS Etienne, AJ
840 Kerkhoff, K McCulloh, et al. 2012 Testing the metabolic theory of ecology. Chave J,
841 editor. *Ecology Letters* 15:1465–1474.
- 842 Pysek P, H Skalova, J Cuda, W-Y Guo, J Suda, J Dolezal, O Kauzal, C Lambertini, M Lucanova,
843 T Mandakova, et al. 2018 Small genome separates native and invasive populations in
844 an ecologically important cosmopolitan grass. *Ecology* 99:79–90.
- 845 Reich P, M Walters, D Ellsworth 1992 Leaf life-span in relation to leaf, plant, and stand
846 characteristics among diverse ecosystems. *Ecological monographs* 62:365–392.
- 847 Rejmánek M, DM Richardson 1996 What attributes make some plant species more
848 invasive? *Ecology* 77:1655–1661.
- 849 Ren R, H Wang, C Guo, N Zhang, L Zeng, Y Chen, H Ma, J Qi 2018 Wide-spread whole genome
850 duplications contribute to genome complexity and species diversity in angiosperms.
851 *Molecular Plant* 11:414–428.
- 852 Ren T, SM Weraduwage, TD Sharkey 2019 Prospects for enhancing leaf photosynthetic
853 capacity by manipulating mesophyll cell morphology. *Journal of Experimental*
854 *Botany* 70:1153–1165.

- 855 Renzaglia KS, JC Villarreal, BT Piatkowski, JR Lucas, A Merced 2017 Hornwort stomata:
856 Architecture and fate shared with 400-million-year-old fossil plants without leaves.
857 Plant Physiology 174:788–797.
- 858 Revell LJ 2012 phytools: an R package for phylogenetic comparative biology (and other
859 things). Methods in Ecology and Evolution 3:217-223.
- 860 Rice A, P Šmarda, M Novosolov, M Drori, L Glick, N Sabath, S Meiri, J Belmaker, I Mayrose
861 2019 The global biogeography of polyploid plants. Nature Ecology & Evolution
862 3:265–273.
- 863 Richardson DM, P Pyšek, JT Carlton 2011 A compendium of essential concepts and
864 terminology in invasion ecology. In: Fifty years of invasion ecology: The legacy of
865 Charles Elton. Wiley-Blackwell Oxford, UK. pp. 409–420.
- 866 Roddy AB, CM Guilliams, T Lilittham, J Farmer, V Wormser, T Pham, PVA Fine, TS Field, TE
867 Dawson 2013 Uncorrelated evolution of leaf and petal venation patterns across the
868 angiosperm phylogeny. Journal of Experimental Botany 64:4081-4088.
- 869 Roddy AB, G-F Jiang, K-F Cao, KA Simonin, CR Brodersen 2019 Hydraulic traits are more
870 diverse in flowers than in leaves. New Phytologist 223:193-203.
- 871 Roddy AB (*in press*) Energy balance implications of floral trait involved in pollinator
872 attraction and water balance. International Journal of Plant Sciences.
- 873 von Sachs J 1893 Physiologische Notizen. vi. Über einige Beziehungen der spezifischen
874 Grösse der Pflanzen zu ihren Organisation. Flora 77:49–81.
- 875 Savage VM, LP Bentley, BJ Enquist, JS Sperry, DD Smith, PB Reich, EI von Allmen 2010
876 Hydraulic trade-offs and space filling enable better predictions of vascular structure
877 and function in plants. Proceedings of the National Academy of Sciences 107:22722–
878 22727.
- 879 Simonin KA, AB Roddy 2018 Genome downsizing, physiological novelty, and the global
880 dominance of flowering plants. PLoS Biology 16:e2003706.

- 881 Smith J, K Winter 1996 Crassulacean acid metabolism. In: Winter K; Smith J, editors.
882 Springer Verlag: Berlin. pp. 427–436.
- 883 Soltis DE, PS Soltis, JA Tate 2003 Advances in the study of polyploidy since plant speciation.
884 New Phytologist 161:173–191.
- 885 Soltis DE, CJ Visger, PS Soltis 2014 The polyploidy revolution then...and now: Stebbins
886 revisited. American Journal of Botany 101:1057–1078.
- 887 Soltis PS, DB Marchant, Y Van de Peer, DE Soltis 2015 Polyploidy and genome evolution in
888 plants. Current Opinion in Genetics & Development 35:119–125.
- 889 Stebbins GL 1940 The significance of polyploidy in plant evolution. The American
890 Naturalist 74:54–66.
- 891 Stebbins GL 1985 Polyploidy, hybridization, and the invasion of new habitats. Annals of the
892 Missouri Botanical Garden:824–832.
- 893 Stebbins Jr GL 1947 Types of polyploids: Their classification and significance. In: Advances
894 in genetics. Vol. 1. Elsevier. pp. 403–429.
- 895 Suda J, LA Meyerson, IJ Leitch, P Pysek 2015 The hidden side of plant invasions: The role of
896 genome size. New Phytologist 205:994–1007.
- 897 Šímová I, T Herben 2012 Geometrical constraints in the scaling relationships between
898 genome size, cell size and cell cycle length in herbaceous plants. Proceedings of the
899 Royal Society B: Biological Sciences 279:867–875.
- 900 Tholen D, C Boom, X-G Zhu 2012 Prospects for improving photosynthesis by altering leaf
901 anatomy. Plant Science 197:92–101.
- 902 Van't Hof J, AH Sparrow 1963 A relationship between dna content, nuclear volume, and
903 minimum mitotic cycle time. Proceedings of the National Academy of Sciences of the
904 United States of America 49:897.

- 905 Violle C, M-L Navas, D Vile, E Kazakou, C Fortunel, I Hummel, E Garnier 2007 Let the
906 concept of trait be functional! *Oikos* 116:882–892.
- 907 Webb CO, DD Ackerly, SW Kembel 2008 Phylocom: Software for the analysis of
908 phylogenetic community structure and trait evolution. *Bioinformatics* 24:2098–
909 2100.
- 910 Wei N, R Cronn, A Liston, T Ashman 2018 Functional trait divergence and trait plasticity
911 confer polyploid advantage in heterogeneous environments. *New Phytologist*
912 221:2286–2297.
- 913 Wendel JF 2015 The wondrous cycles of polyploidy in plants. *American Journal of Botany*
914 102:1753–1756.
- 915 West GB, JH Brown, BJ Enquist 1997 A general model for the origin of allometric scaling
916 laws in biology. *Science* 276:122–126.
- 917 ——— 1999a A general model for the structure and allometry of plant vascular systems.
918 *Nature* 400:664–667.
- 919 ——— 1999b The fourth dimension of life: Fractal geometry and allometric scaling of
920 organisms. *Science* 284:1677–1679.
- 921 West GB, WH Woodruff, JH Brown 2002 Allometric scaling of metabolic rate from
922 molecules and mitochondria to cells and mammals. *Proceedings of the National*
923 *Academy of Sciences* 99:2473–2478.
- 924 White J, H Rees 1987 Chromosome weights and measures in *Petunia*. *Heredity* 58:139.
- 925 Wright IJ, PB Reich, M Westoby, DD Ackerly, Z Baruch, F Bongers, J Cavender-Bares, T
926 Chapin, JH Cornelissen, M Diemer, et al. 2004 The worldwide leaf economics
927 spectrum. *Nature* 428:821.
- 928 Wright NA, TR Gregory, CC Witt 2014 Metabolic 'engines' of flight drive genome size
929 reduction in birds. *Proceedings of the Royal Society B* 281: 20132780.

930 Zhang F-P, MR Carins Murphy, AA Cardoso, GJ Jordan, TJ Brodribb 2018 Similar geometric
931 rules govern the distribution of veins and stomata in petals, sepals and leaves. New
932 Phytologist 219:1224-1234.











