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1 How environment and genetic
2 architecture of unreduced gametes
3 shape the establishment of
4 autopolyploids

5
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20
21 **Running title:** Unreduced gametes and polyploidy establishment

22
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25 **Abstract:** It is broadly assumed that polyploidy success is due to an increase in fitness
26 associated with whole genome duplication due to higher tolerance to stressful conditions. In
27 agreement, several theoretical models found that, among other factors, a better tolerance to
28 new environmental conditions can promote polyploidy establishment. These models,
29 however, often made strong hypotheses, for example considering that diploids cannot adapt
30 to new conditions, or that unreduced gametes production is not a limiting factor and that it is
31 of a fixed quantity. In this paper, we challenged some of these hypotheses. We developed a
32 theoretical model in which we modeled the joint evolution of a quantitative trait under
33 selection and the production of unreduced gametes, this trait also being a quantitative trait;
34 both traits were pleiotropically linked. We followed the adaptation of initially diploid
35 populations to a new environment to which neo-tetraploid individuals were directly adapted.
36 The generation of these autotetraploid individuals was enabled by the genetic production of
37 unreduced gametes and by the environmental change modifying the average production of
38 these gametes. We found that for realistic values of unreduced gametes production,
39 adaptation to new environmental conditions was mainly achieved through adaptation of
40 diploids to the new optimum rather than the fixation of newly adapted tetraploid individuals.
41 In broader parameter sets, we found that the adaptation process led to mixed-ploidy
42 populations, except when the populations were swamped with unreduced gametes, and that
43 pleiotropy and environmental effects favored the co-existence of both cytotypes.

44

45 **Keywords:** unreduced gametes, pleiotropy, autopolyploidy, neo-polyploidy, quantitative
46 genetics, environment.

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53 **Introduction:**

54

55 Polyploids are organisms known for having more than two chromosome sets compared with
56 their diploid congener. Polyploidy resulting from whole genome duplication (WGD) is
57 frequently accompanied by meiotic abnormalities and alters gene dosage, consequently
58 affecting the phenotypes and long-term evolution of populations (Otto 2007). There is
59 evidence that polyploidization is an important mechanism in speciation and diversification
60 (Soltis *et al.* 2015), with blooms of diversity associated with ancient WGD events, both in
61 plants, particularly the angiosperms, animals and fungi (Jiao *et al.* 2011; Van De Peer *et al.*
62 2017). For example, it has been suggested that all angiosperms descended from an ancient
63 WGD event (Jiao *et al.*, 2011; *Amborella* Genome Project, 2013), and 25 to 35% of
64 angiosperm species have been estimated to be recently formed polyploid (i.e. neo-polyploid)
65 species (Wood *et al.*, 2009; Mayrose *et al.*, 2011; Barker *et al.*, 2016a). The geographic
66 distribution of neo-polyploids in the angiosperms shows a latitudinal trend, with a higher
67 frequency of polyploids at higher latitudes (Rice *et al.* 2019). It is an ongoing debate if
68 polyploidy fosters adaptability to stressful environments (Madlung 2013), and if in particular
69 neo-polyploidy is associated with stressful conditions, such as cold and dry conditions, as
70 reviewed by (Van De Peer *et al.* 2021). This environmental association becomes particularly
71 intriguing when considering the mechanisms leading to the formation of neo-polyploids.

72 Although somatic doubling could potentially play a role in the formation of neo-
73 polyploids, it is currently assumed that it usually involves unreduced gametes (UG), which
74 are gametes with the somatic ploidy level, such as diploid gametes in a diploid organism; for
75 the cytological mechanisms and molecular regulatory networks underlying UG formation see
76 (Brownfield & Kohler 2011; De Storme & Geelen 2013). Pathways to polyploidy can be
77 ‘one-step’ (fusion of two unreduced gametes; rather rare because of the rarity of unreduced

78 gamete formation), ‘triploid bridge’ (fusion of unreduced and reduced gamete within a
79 diploid population, with triploid offspring producing unreduced gametes that backcross with
80 the reduced gamete parent), and ‘hybrid bridge’ (same as triploid bridge, only that it refers to
81 interspecific crosses; rather common, as unreduced gamete formation is higher in hybrids)
82 (Mason & Pires 2015). Unreduced gamete formation has been shown to depend on abiotic
83 and biotic factors, such as temperature, moisture, nutrition and herbivory (Kreiner *et al.*
84 2017a; Mason & Pires 2015; Ramsey & Schemske 1998a).

85 Once polyploids are formed, they face numerous challenges to finally establish in a
86 population, and to form a distinct polyploid lineage that may eventually speciate from its
87 diploid progenitor. Upon emergence, polyploids tend to experience a lack of mating
88 opportunities (minority cytotype exclusion; Levin 1975). The lack of mating opportunities in
89 short-lived neo-polyploid organisms can be theoretically overcome by a strong fitness
90 advantage of neo-polyploids (Husband, 2004), polyploidy-associated mating system
91 transitions (Griswold 2021; Oswald & Nuismer 2011), higher clonal reproduction of
92 polyploids (Van Drunen & Friedman 2022), but also certain spatial components of neo-
93 polyploid establishment (Kauai *et al.* 2023). Unreduced gametes production per se is assumed
94 to come with an immediate fitness disadvantage, due to meiotic mishap and low triploid
95 fitness (Kreiner *et al.* 2017), but also as a result of the genetic architecture of UG formation.
96 In the formation of unreduced gametes, several genes have been found to be involved
97 (Bretagnolle & Thompson 1995; Brownfield & Kohler 2011; De Storme & Geelen 2013), for
98 which pleiotropic effects (i.e. the fact that several traits are affected by a single locus) can be
99 assumed. Under the assumption of a correlated production of male and female unreduced
100 gametes, polyploid fixation is more likely in combination with mating system transitions, as
101 selfing of an individual that produces unreduced gametes of both sexes will likely result in
102 polyploid progeny. For flowering plants, self-fertilization is indeed assumed to be more

103 common in polyploids compared to diploids (Barringer 2007), although this study did not
104 discriminate between autopolyploids (i.e. multiplied chromosome set within a species) and
105 allopolyploids (i.e. multiplied chromosome set from two species), which does not allow to
106 distinguish between the effect of WGD and hybridization. Later, Husband et al. (2008) found
107 that autopolyploids are predominantly outcrossing, while allopolyploids are largely selfing.
108 However, all above mentioned theoretical considerations strongly depended on key
109 assumptions. The first one is that newly formed polyploid individuals are at least as fit as
110 their diploid progenitors (Husband 2004; Kauai *et al.* 2023; Oswald & Nuismer 2011; Van
111 Drunen & Friedman 2022). However, neo-polyploid lineages are generally less fit than their
112 diploid progenitors (at least in controlled conditions, see Porturas et al., 2019; Clo and Kolář,
113 2021 for synthetic neo-auto/allopolyploids). Considering such an initial fitness cost strongly
114 affects the theoretical outcome, such as the evolution toward self-fertilization being
115 disfavored in these conditions (Clo *et al.* 2022; Gaynor *et al.* 2023). In the longer term,
116 however, autopolyploids may exhibit a fitness advantage over their diploid progenitors (Clo,
117 2022a), especially in scenarios of environmental change (Van De Peer *et al.* 2021b).

118

119 The second strong assumption is that UG production is high enough for the
120 production of neo-polyploid lineages not to be a limiting factor in polyploid evolution (Baack
121 2005; Husband 2004; Li *et al.* 2004; Van Drunen & Friedman 2021). However, the
122 production of unreduced gametes is generally much lower in natural populations of non-
123 hybrid species (between 0.1 to 2%; Kreiner et al., 2017) compared to what is found in
124 theoretical models (Felber 1991; Husband 2004; Oswald & Nuismer 2011; Van Drunen &
125 Friedman 2022). Few models attempted to model the effect and evolution of UG production
126 on the probability of polyploid evolution (Clo *et al.* 2022; Gerstner *et al.* 2024). Clo *et al.*
127 (2022) treated the evolution of unreduced gametes as a quantitative trait and found that under

128 realistic conditions high enough levels of UG production for autopolyploidy to invade are
129 only reached when genetic drift is strong. Gerstner *et al.* (2024) modeled the inter-generation
130 variation of unreduced gametes following the empirical data by Kreiner *et al.* (2017b) and
131 found that such variation can lead to the evolution of autopolyploidy in initially diploid
132 populations. While both models are informative, they are also built on strong assumptions.
133 Clo *et al.* (2022) made the genetic architecture of UG production too simplistic, omitting, for
134 example, the fact that mutations increasing UG production are pleiotropically linked to
135 fitness and strongly affect fitness components such as pollen and/or ovule production
136 (d'Erfurth *et al.* 2010; Erilova *et al.* 2009; Ravi *et al.* 2008; Wang *et al.* 2010; see also
137 Brownfield & Kohler 2011 for a review). They also neglected the effects of the environment,
138 while such factors, like temperature, are known to have major effects on UG production rates
139 (De Storme *et al.* 2012; Mason *et al.* 2011; Pecrix *et al.* 2011; Schindfessel *et al.* 2023; Wang
140 *et al.* 2024). On the opposite, Gerstner *et al.* (2024) made the hypothesis that the variation in
141 UG production is only environmental, while it is known that this trait is determined by both
142 environmental and genetic factors (Parrott & Smith 1986; Tavoletti *et al.* 1991a). Considering
143 a more realistic genetic architecture of UG production could strongly impact the conclusions
144 of the above-mentioned models, and could make us understand the relative contribution of
145 both genetic and environmental effects in UG production and polyploidy evolution.

146

147 In this paper, we investigate the effect of the genetic architecture of UG production in
148 the origin and establishment of autopolyploids in both stable and disturbed environments, by
149 considering both environmental stochasticity and the pleiotropic effect of mutations affecting
150 UG production on fitness. We build an individual-based simulation model extended from Clo
151 *et al.* (2022), in which we test the following parameters: environmental factor and pleiotropy
152 as effects of studying how unreduced gametes as a quantitative trait evolve and affect the

153 origin and establishment of polyploids. In conclusion, we found that for realistic values of
154 UG production, adaptation to new environmental conditions was mainly realized by
155 adaptation of diploids to the new optimum rather than the fixation of newly adapted tetraploid
156 individuals. In broader parameter sets, we found that the adaptation process led to mixed-
157 ploidy populations, except when the populations were swamped with unreduced gametes, and
158 that pleiotropy and environmental effects favored the co-existence of both cytotypes.

159

160

161 **Material and methods:**

162

163 *General assumptions*

164 We considered the joint evolution of a quantitative trait Z and the production of
165 unreduced gametes (UG) in a population of constant size N , initially made of diploid
166 individuals reproducing through obligate random mating. The phenotypic value z of an
167 individual was determined by the additive action of L_Z loci each with an infinite possible
168 number of alleles and was given by

$$169 \quad z = g_z + e_z, (1)$$

170 where g_z was the genetic component of the individual's phenotype and was given by $g_{z-2x} =$
171 $\sum_j^L (g_j^M + g_j^P)$, where g_j^M (respectively g_j^P) was the additive allelic effect at locus j inherited
172 from the maternal (respectively paternal) gamete in the diploid population. After
173 polyploidization and with tetrasomic inheritance, the genetic component became $g_{4x} =$
174 $\sum_j^L d \cdot (g_j^{M1} + g_j^{M2} + g_j^{P1} + g_j^{P2})$, where g_j^{M1} and g_j^{M2} (respectively g_j^{P1} and g_j^{P2}) were the
175 additive allelic effects at locus j inherited from the maternal (respectively paternal) gametes.
176 The parameter d controlled for the dosage effect and determined the effect of

177 polyploidization on the tetraploid genotypic values compared to diploid ones. The additive
178 value of a new mutant allele was drawn from a Gaussian distribution of mean 0 and variance
179 α^2 . The random environmental effect on the phenotypic trait e_z was drawn from a Gaussian
180 distribution of mean 0 and variance V_E and was considered to be independent of the genetic
181 components of fitness.

182 The trait underwent stabilizing selection around an arbitrary optimal phenotypic value,
183 denoted Z_{opt} and being equal to zero in this model. The fitness value W_Z of an individual with
184 phenotype z was thus described by the Gaussian function:

$$185 \quad W_Z = e^{-\delta^2/2\omega^2}, (2)$$

186 where δ was the distance between the individual's phenotype z and the optimum trait value
187 and ω^2 was the width of the fitness function, representing the strength of selection. There was
188 no dominance at the phenotypic scale in this model, but recessivity of mutations arose
189 naturally at the fitness scale due to the non-linearity of the phenotype-fitness function (see
190 Manna *et al.* 2011 for diploids and Clo, 2022 for tetraploids).

191 The trait “unreduced gametes production” was coded for both male and female
192 reproductive functions by $L_{G-M} = L_{G-F}$ freely recombining bi-allelic loci. The average UG
193 production for male and female functions p_{UG-M} and p_{UG-F} of an individual was determined by
194 the additive action of alleles at different loci each with an infinite possible number of alleles
195 and was given by

$$196 \quad p_{UG-M} = g_{UG-M} + e_{UG} (3.1)$$

197 and

$$198 \quad p_{UG-F} = g_{UG-F} + e_{UG} (3.2)$$

199 where g_{UG-M} (respectively g_{UG-F}) was the genetic component of the individual's male
200 (respectively female) UG production and was given by $g_{UG-M-2x} = \sum_j^L (g_j^{UG-M-M} +$
201 $g_j^{UG-M-P})$, (respectively $g_{UG-F-2x} = \sum_j^L (g_j^{UG-F-M} + g_j^{UG-F-P})$), where g^{UG-M-M}_j
202 (respectively g^{UG-M-P}_j) was the additive allelic effect at locus j inherited from the maternal
203 (respectively paternal) gamete in the diploid population. After polyploidization and with
204 tetrasomic inheritance, the genetic component became (for male) $g_{UG-M-4x} =$
205 $\sum_j^L (g_j^{UG-M-M1} + g_j^{UG-M-M2} + g_j^{UG-M-P1} + g_j^{UG-M-P2})$, where $g^{UG-M-M1}_j$ and $g^{UG-M-M2}_j$
206 (respectively $g^{UG-M-P1}_j$ and $g^{UG-M-P2}_j$) were the additive allelic effects at locus j inherited from
207 the maternal (respectively paternal) gametes. The same applied for female (un)reduced
208 gametes production. The additive value of a new mutant allele was drawn from a Gaussian
209 distribution of mean 0 and variance a^2 . The environmental effect e_{UG} was fixed during
210 simulations but could vary between the two steps of the simulation process. If by chance p_{UG-}
211 M and/or p_{UG-F} were < 0 (respectively > 1), then p_{UG-M} and/or p_{UG-F} were forced to be equal to
212 0 (respectively 1). In this model, we simulated three levels of pleiotropy: 1) all the different
213 loci of the different traits can be independent, or 2) pleiotropy between loci involved in UG
214 production for male and female functions can happen, or 3) pleiotropy between all kind of
215 loci (phenotypic trait and unreduced gametes for male and female functions) can occur.

216

217

218 ***Simulation process:***

219

220 The simulations took place in two steps: a first one of stabilizing selection, and a
221 second one of directional selection (adaptation process). For this step, we initiated each

222 simulation from a population of genetically identical diploid individuals that are homozygous
223 for the ancestral allele at all loci ($z = 0$ & $p_{UG-M} = p_{UG-F} = 0$ at $t = 0$). The life cycle can be
224 summarized in five successive events. First, there is a fitness-dependent choice of the first
225 parent (selection), followed by the choice of the second parent, which is also fitness-
226 dependent. Selection takes place as follows: an individual is sampled randomly among the N
227 individuals, but its sampling probability is weighted by its fitness. Once the two parents are
228 chosen, the type of gametes (reduced or unreduced) they will produce is chosen. For each
229 reproducer, one number is sampled in a uniform distribution between 0 and 1. If p_{UG} (male or
230 female) is higher than the sampled value, the reproducer generates an unreduced gamete. For
231 reduced (n) gametes ($n = x$ and $n = 2x$ gametes for diploid and tetraploid individuals,
232 respectively), one allele per locus of each sister chromatid is sampled randomly for each trait.
233 This phase is then followed by the introduction of mutations for each trait, the number of
234 which is sampled from a Poisson distribution with parameter U , the haploid genomic
235 mutation rate (with $U = \mu L$, where μ is the per-locus mutation rate and L is the number of loci
236 underlying the trait under study). If the resulting offspring is not diploid or tetraploid, it will
237 not contribute to the next generation (i.e. triploid individuals are considered inviable or
238 sterile). The reproduction phase stops when N viable offspring are formed. The first step
239 stops when the population is at Mutation-Selection-Drift (M-S-D) equilibrium. The
240 population is considered to be at M-S-D equilibrium when the average population fitness
241 value calculated over the last thousand generations does not differ by more than one percent
242 from the mean fitness calculated over the previous thousand generations.

243 The second step of the simulations began once the population reached M-S-D
244 equilibrium, by introducing an environmental change (done by changing the phenotypic
245 optimum Z_{opt} from 0 to 2.5). After the environmental change, we assumed the polyploids are
246 adapted to their new conditions (while before the change the fitness of each cytotype is

247 approximately the same) and have a fitness advantage over diploids. In addition, the
248 environmental change can induce or not a change in UG production (i.e. there is a positive
249 non-zero effect of the environment on UG production). The population adapted either by (i)
250 making diploid individuals to adapt to the new conditions thanks to standing genetic diversity
251 and/or *de novo* mutations, (ii) increasing the number of newly adapted tetraploid individuals
252 thanks to genetic diversity for unreduced gametes and the potential effect of the environment,
253 or (iii) a mix of the two solutions (mixed-ploidy populations). The population evolved during
254 500 generations after the change, and the frequency and variance of unreduced gametes, the
255 average fitness of the population, and the frequency of tetraploids in the population were
256 inferred at each generation.

257

258 ***Parameter sets:***

259

260 Simulations were run for several parameter sets. The values chosen for the mutation rate U
261 range from 0.005 to 0.1, reflecting the per-trait haploid genomic mutation rate found in the
262 literature (Halligan & Keightley 2009). We used parameter set values similar to those
263 explored in similar theoretical models (Bürger *et al.* 1989; Ronce *et al.* 2009; Oswald &
264 Nuismer 2011), with the number of freely recombining loci under selection $L = 100$, and $a^2 =$
265 0.05, $\text{var}(e_Z) = \text{var}(e_{UG}) = 1$. The strength of stabilizing selection is set to $\omega^2 = 9$ to fit
266 empirical observations (Clo & Opedal 2021). We considered a population size of $N = 200$
267 individuals. The dosage parameter d is equal to 0.65 ($g_{Z-4x} = 1.3 * g_{Z-2x}$, which is found in neo-
268 polyploid and established polyploid populations on average; see Porturas *et al.*, 2019 and Clo
269 & Kolář, 2021 for meta-analyses). When pleiotropy is considered, the number of pleiotropic
270 loci can be equal to 0 (no pleiotropy), 40 (moderate pleiotropy), or 80 (high pleiotropy). As

271 mentioned earlier, before the environmental change, the fitness of neo-polyploids was close
272 to the one of diploids (slightly lower because of the gigas phenotypic effect) but was initially
273 higher at the beginning of the environmental change. Before the change, e_{UG} equals 0 but can
274 be equal to 0, 0.1, or 0.3 after the change when considering that the environmental stress
275 (such as change in temperature) also affects UG production. Finally, Z_{opt} switched from 0 to
276 2.5 after the environmental change. A summary of the parameters used and their values can
277 be found in Table 1.

278

279 **Table 1.** Description of the model parameters, their abbreviations, and tested values in
280 simulations.

Parameter	Abbreviation	Value(s)
Population size	N	200
Number of loci	L	100
Haploid genomic mutation rate	U	0.005, 0.05 or 0.1
Variance of mutational effects	a^2	0.05
Dosage effect	d	0.65
V_E of environmental effects	V_E	1
Strength of stabilizing selection	ω^2	9

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282

283

284 **Results:**

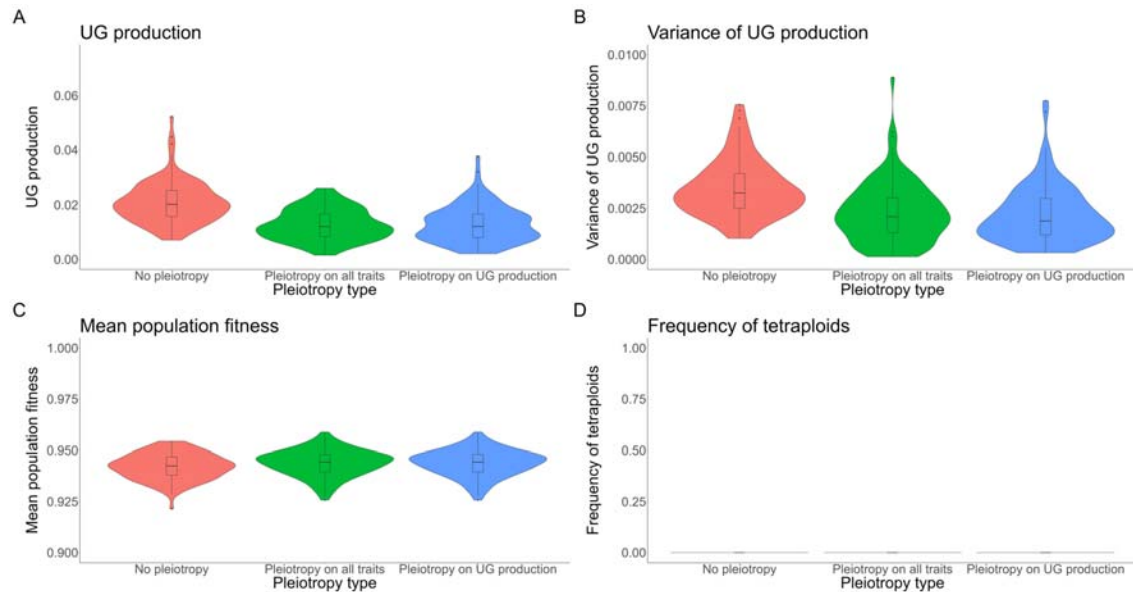
285

286 **Description of populations at Mutation-Selection-Drift equilibrium:**

287

288 Under stable environmental conditions (i.e. $Z_{\text{opt}} = 0$), the main factors affecting population
289 metrics were pleiotropy and the mutation rate (Figure 1; Supplementary Figures 1 and 2).
290 Under a fixed mutation rate ($U = 0.005$; see below for details), pleiotropy on the quantitative
291 trait and on UG production generally led to more efficient purging of deleterious mutations
292 (both on the quantitative trait under stabilizing selection and on mutations increasing UG
293 production), resulting in lower UG production (median 1% with pleiotropy vs. median 2%
294 without pleiotropy; Figure 1A), lower genetic diversity for UG production (median 0.18%
295 with pleiotropy vs. median 0.30% without pleiotropy; Figure 1B) and slightly increased
296 population fitness (median 94.4% with pleiotropy vs. median 94.2% without pleiotropy;
297 Figure 1C). Higher mutation rates ($U = 0.05$ and 0.1) did not change the patterns
298 qualitatively, but generally led to higher average UG production (median 18% without
299 pleiotropy for $U = 0.05$ vs. median 35% for $U = 0.1$; Supplementary Figures 1 and 2), higher
300 genetic variance of unreduced gametes (median 18% without pleiotropy for $U = 0.05$ vs.
301 median 36% for $U = 0.1$; Supplementary Figures 1 and 2) and lower population fitness
302 (median 91% without pleiotropy for $U = 0.05$ vs. median 85% for $U = 0.1$; Supplementary
303 Figures 1 and 2). Under low mutation rates, we did not find mixed-ploidy populations at the
304 M-S-D equilibrium (0% of tetraploids, the populations were purely diploid; Figure 1D), while
305 intermediate and high mutation rates can lead to stable mixed-ploidy populations (between 1
306 to 10% of tetraploids, the higher when no pleiotropic effects were modeled; Supplementary
307 Figures 1 and 2). Because the simulations with low mutation rates gave the most realistic
308 pattern of UG production for empirical rates of UG production (1–2%), we described
309 separately the evolution of polyploidy with this biologically realistic scenario in the first time
310 and other scenarios in the second time.

311



312

313

314 *Figure 1: Effect of pleiotropy type (no, all traits, unreduced gametes; number of pleiotropic loci = 80) on*
315 *unreduced gametes production and its variance, population fitness and tetraploid fixation, simulated from a*
316 *population of genetically identical diploid individuals under the mutation rate $U = 0.005$. Pleiotropy on all traits*
317 *means that pleiotropy affected both the quantitative trait under directional selection and unreduced gametes*
318 *production. (A) Proportion of unreduced gametes production in diploid individuals; (B) variance of unreduced*
319 *gametes production in diploid individuals; (C) population fitness; (D) frequency of tetraploids in the population.*

320

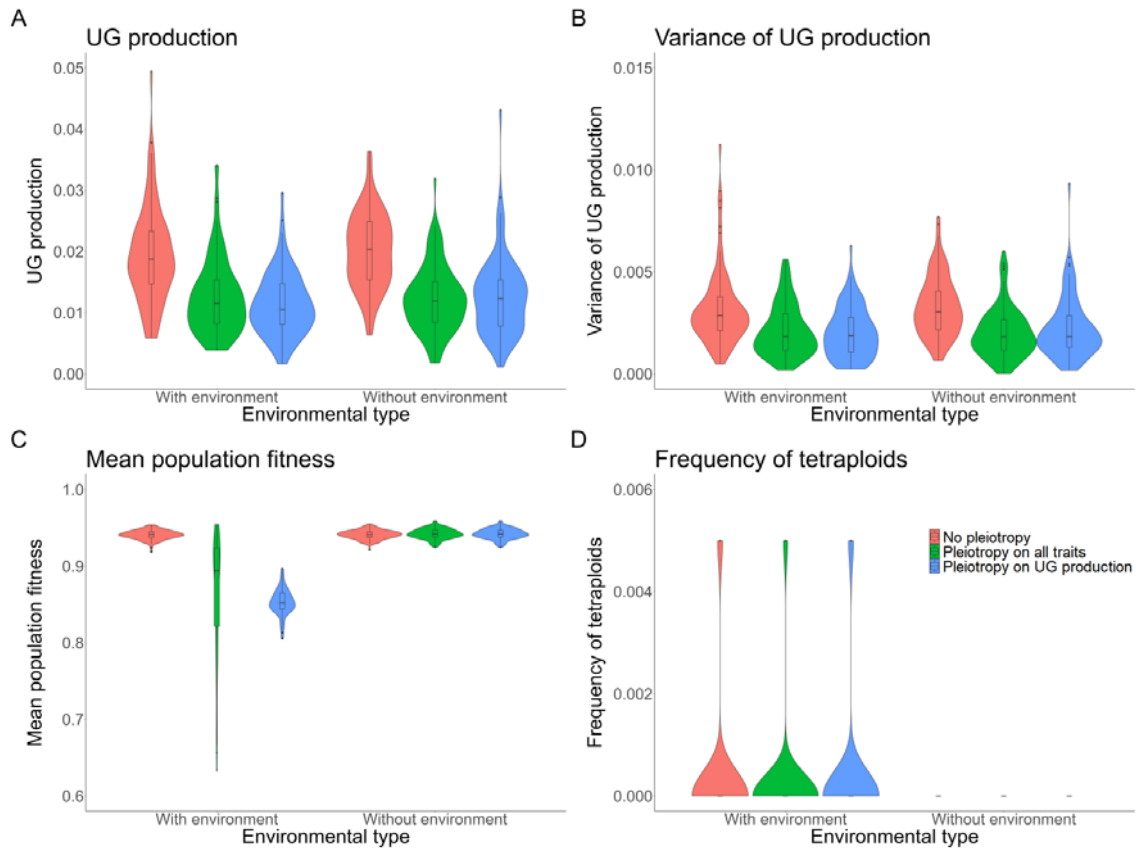
321

322 **The evolution of polyploidy under environmental change for realistic parameter sets**

323

324 Here we focused on simulations with low mutation rates ($U = 0.005$) and realistic
325 environmental conditions (directional selection, Z_{opt} shifting from 0 to 2.5, and an
326 environmental effect on UG production of 10%). It was observed that environmental
327 conditions did not significantly alter the population cytotype structure and fitness after the
328 directional selection episode (Figure 2). Both UG production and variance remained similar
329 (UG production: median 2% with(out) environmental effect, no pleiotropy; Figure 2A; UG

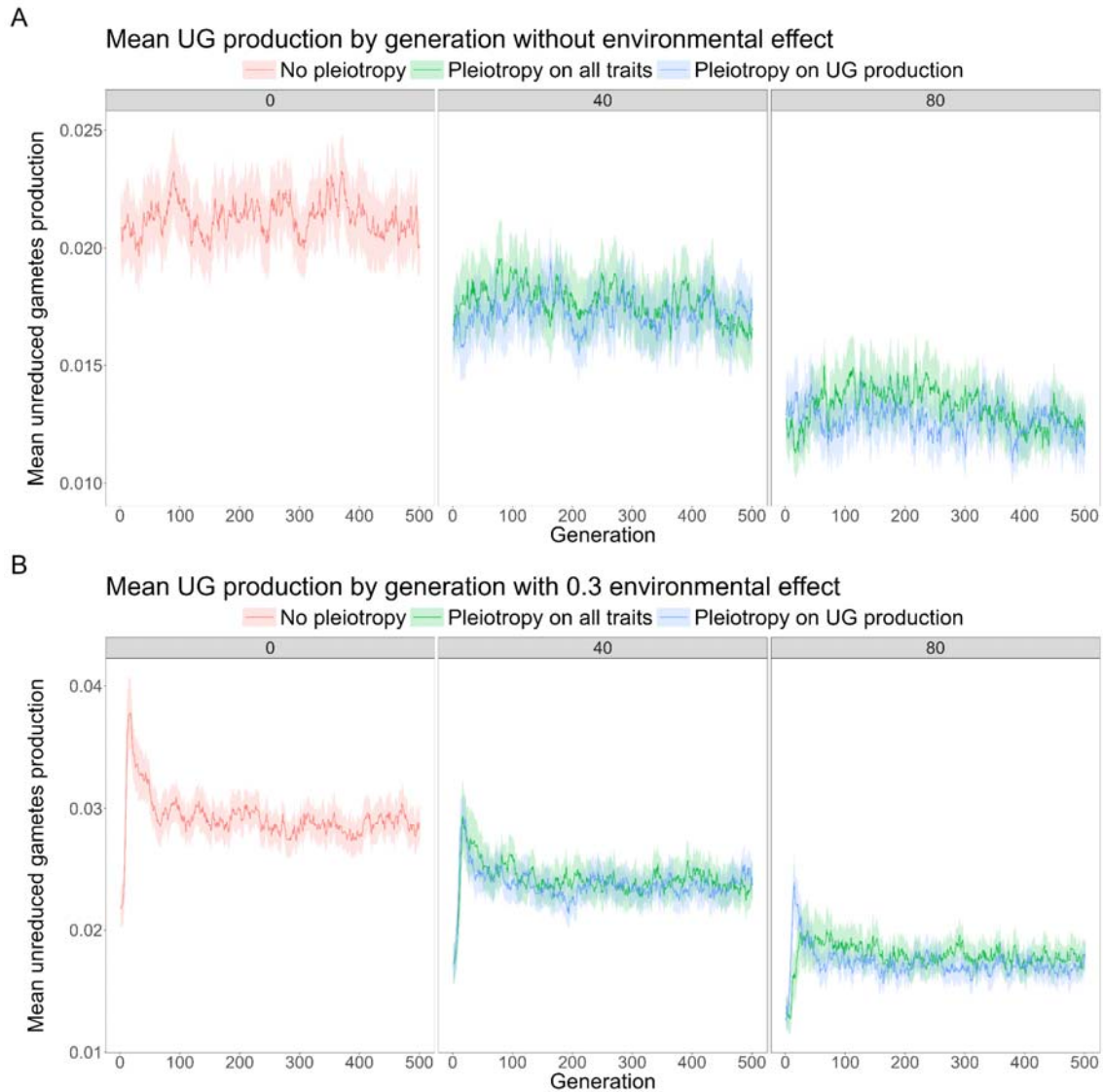
330 variance: median 0,3% with(out) environmental effect, no pleiotropy; Figure 2B). Mean
331 population fitness also remained comparable after 500 generations when pleiotropy is absent
332 (median 94%; Figure 2C), but with both environmental effect and pleiotropy population
333 fitness decreased substantially, the highest being when pleiotropy only affected UG
334 production (median 85%; Figure 2C). At the end of the simulations, the populations were
335 only made of diploid individuals (Figure 2D), suggesting that the recovery of fitness is made
336 by diploid individuals adapting to new conditions rather than the invasion of newly adapted
337 tetraploids. Environmental effects on UG production rarely led to mixed-ploidy populations
338 (Figure 2D). We attributed this to a conflict between environmental effect and new mutations
339 that could have favored the selection of newly adapted tetraploids (but that had a negative
340 pleiotropic effect on the trait under directional selection) to the detriment of the evolution of
341 diploid individuals to the new optimum (Figure 3B; see Figure 3A for a comparison without
342 environmental effect). Indeed, one can see that the production of unreduced gametes slightly
343 increased at the beginning of the environmental change (generally doubling in the first 20
344 generations, shifting for example from 2% to 4% when no pleiotropy is modeled; Figure 3B).
345 The production then decreased during the 500 generations of adaptation (Figure 3B).
346
347



348

349 *Figure 2: Effect of pleiotropy type (no, all traits, unreduced gametes; number of pleiotropic loci = 80) and*
350 *environmental factor (0.1) on the evolution of polyploidy after 500 generations of directional selection,*
351 *simulated from a population of genetically identical diploid individuals under the mutation rate $U = 0.005$.*
352 *Pleiotropy on all traits means that pleiotropy affected both the quantitative trait under directional selection*
353 *and unreduced gametes production. (A) Proportion of unreduced gametes production in diploid individuals; (B)*
354 *variance of unreduced gametes production in diploid individuals; (C) population fitness; (D) frequency of*
355 *tetraploids in the population.*

356



357

358 *Figure 3: Effect of pleiotropy type (no, all traits, unreduced gametes; number of pleiotropic loci = 0, 40, 80) on*
359 *unreduced gametes production (A) without effect of environmental factor; (B) with effect of environmental*
360 *factor (0.3), simulated from a population of genetically identical diploid individuals under the mutation rate $U =$*
361 *0.005 for 500 generations. Pleiotropy on all traits means that pleiotropy affected both the quantitative trait*
362 *under directional selection and unreduced gametes production.*

363

364

365

366

367 ***The general effects of environment and genetic architecture on the evolution of***
368 ***polyploidy:***

369

370 At the beginning of the directional selection episode, the frequency of unreduced gametes,
371 due to genetic effects, increased in the first generations and then decreased back to an
372 equilibrium value (Figures 3B and 4). This steep initial increase was due to the fact that at the
373 beginning of the adaptation process, selection favored both the formation and the increase in
374 frequency of newly adapted tetraploid individuals. Once diploid individuals were closer to
375 the new phenotypic optimum, formation of tetraploids was either selected against and
376 tetraploids remained in the minority within the population due to minority cytotype exclusion
377 (between 0 to 10% of tetraploids when no environmental effect was modeled; Figure 5A;
378 between 0 to 55% of tetraploids with a realistic environmental effect of 0.1 on UG
379 production; Figure 5B), or tetraploid formation was continuously favored until fixation of
380 tetraploidy (100% of tetraploids when the effect of the environment on UG production is
381 strong, independent of the mutation rate; Figure 5C). As previously described, the overall
382 effect of increased mutation rates was to increase both the average production of unreduced
383 gametes and of tetraploids within populations (Figures 4 and 5). It was, however, notable that
384 the mutation rate has a synergistic effect with the environmental effect on unreduced
385 gametes: the higher the environmental effect, the higher the genetic response to increase UG
386 production (Figure 4). This is because the production of unreduced gametes is under
387 frequency dependence: the higher UG production is in the population, the less it is counter-
388 selected. Thus, the stronger the effect of the environment, the easier it is for a mutation
389 increasing UG production to be maintained in the population. Pleiotropy had a generally
390 negative effect by decreasing the frequency of unreduced gametes and tetraploids (Figures 4
391 and 5). Interestingly, the effect of pleiotropy on limiting both UG production and the
392 frequency of tetraploids was stronger when pleiotropy was limited to UG production of both

393 male and female functions, rather than affecting both UG production and the quantitative trait
394 under directional selection (Figures 4B and 5B). This was due to the fact that if the mutation
395 affecting both UG production and the phenotypic trait had a positive effect on fitness (i.e. the
396 mutation brought the phenotype closer to the new optimum), the mutation was selected
397 despite its costly effect on UG production.

398 Overall, the fixation of polyploidy remained rare (Figures 5 and 6). With low
399 mutation rates, populations adapted to new conditions either by remaining diploid, with the
400 individuals adapting to the new conditions, or by forming mixed-ploidy populations of
401 adapted diploids and tetraploids, notably when the effect of the environment on UG
402 formation is high (Figures 5 and 6). With higher mutation rates, the formation of a mixed-
403 ploidy population was much more frequent, with tetraploids reaching 10 to 55% in the
404 populations (Figure 5B). The fixation of polyploidy was frequent when unreduced gametes
405 swamped the population, i.e. with a high mutation rate and a strong effect of the environment
406 (Figures 5C and 6).

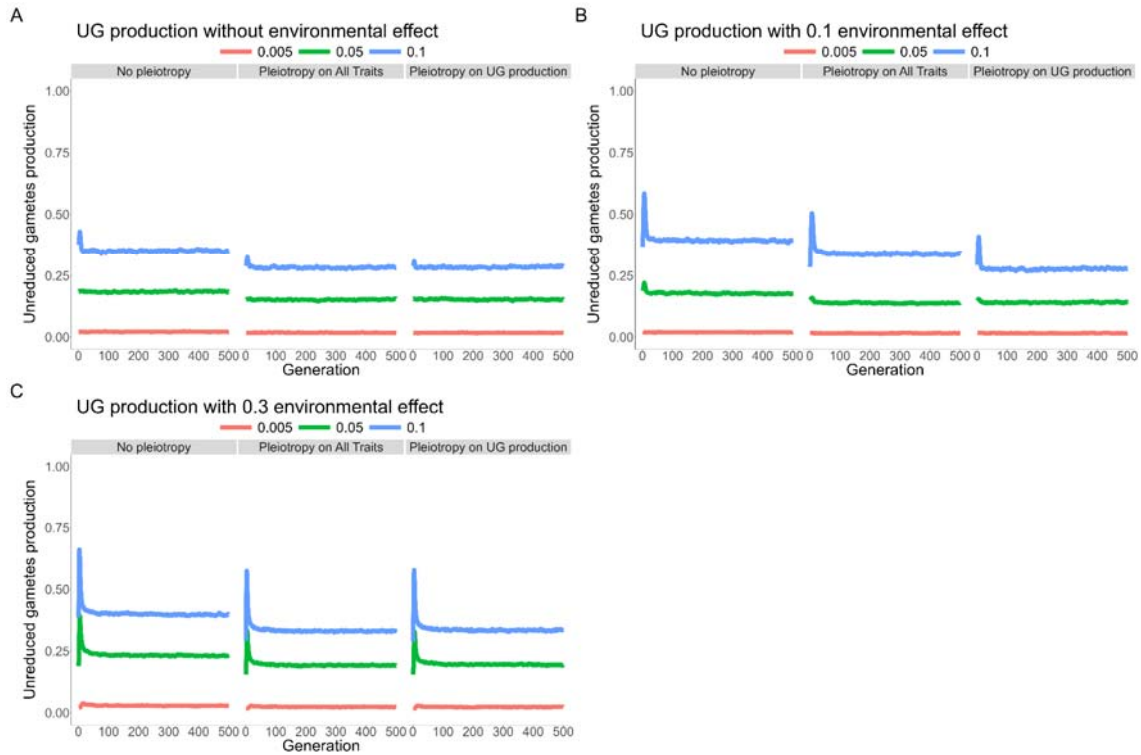
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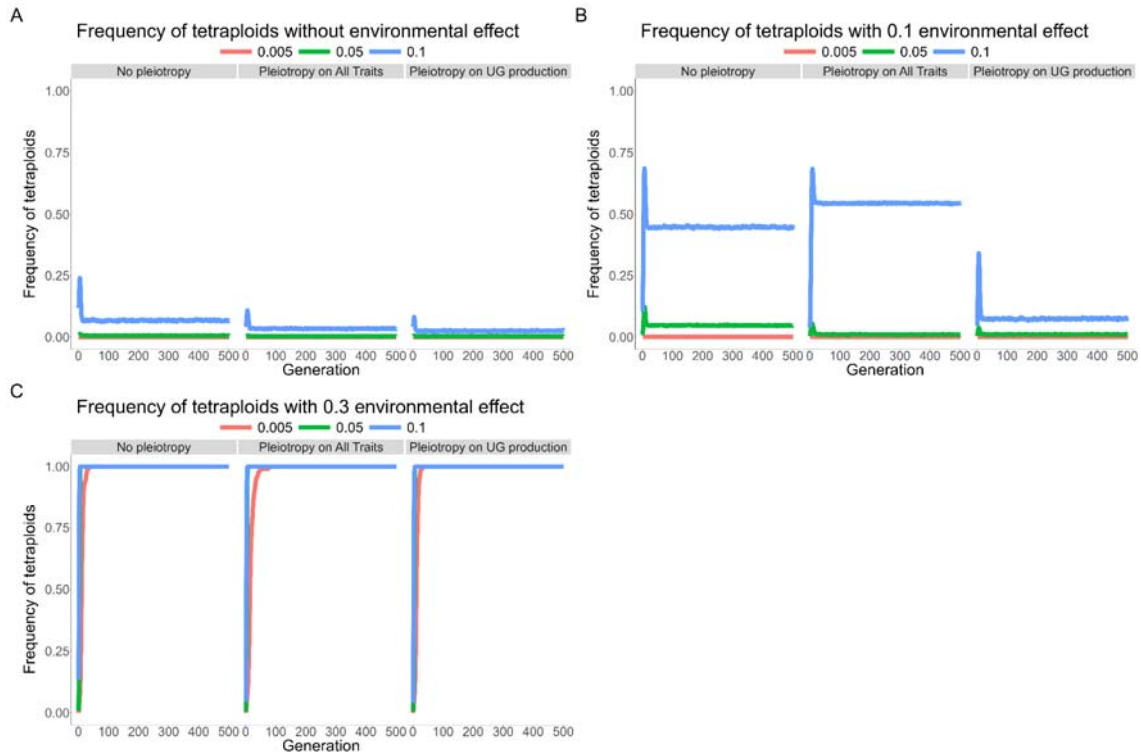
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413 *Figure 4: Effect of pleiotropy type (no, all traits, unreduced gametes; number of pleiotropic loci = 40) and the*
414 *environmental factor (0, 0.1, 0.3) on unreduced gametes production under different mutation rates ($U = 0.005,$*
415 *0.05, 0.1; differently colored lines), simulated from a population of genetically identical diploid individuals for*
416 *500 generations. Pleiotropy on all traits means that pleiotropy affected both the quantitative trait under*
417 *directional selection and unreduced gametes production. (A) No environmental factor; (B) environmental factor*
418 *0.1; (C) environmental factor 0.3.*

419



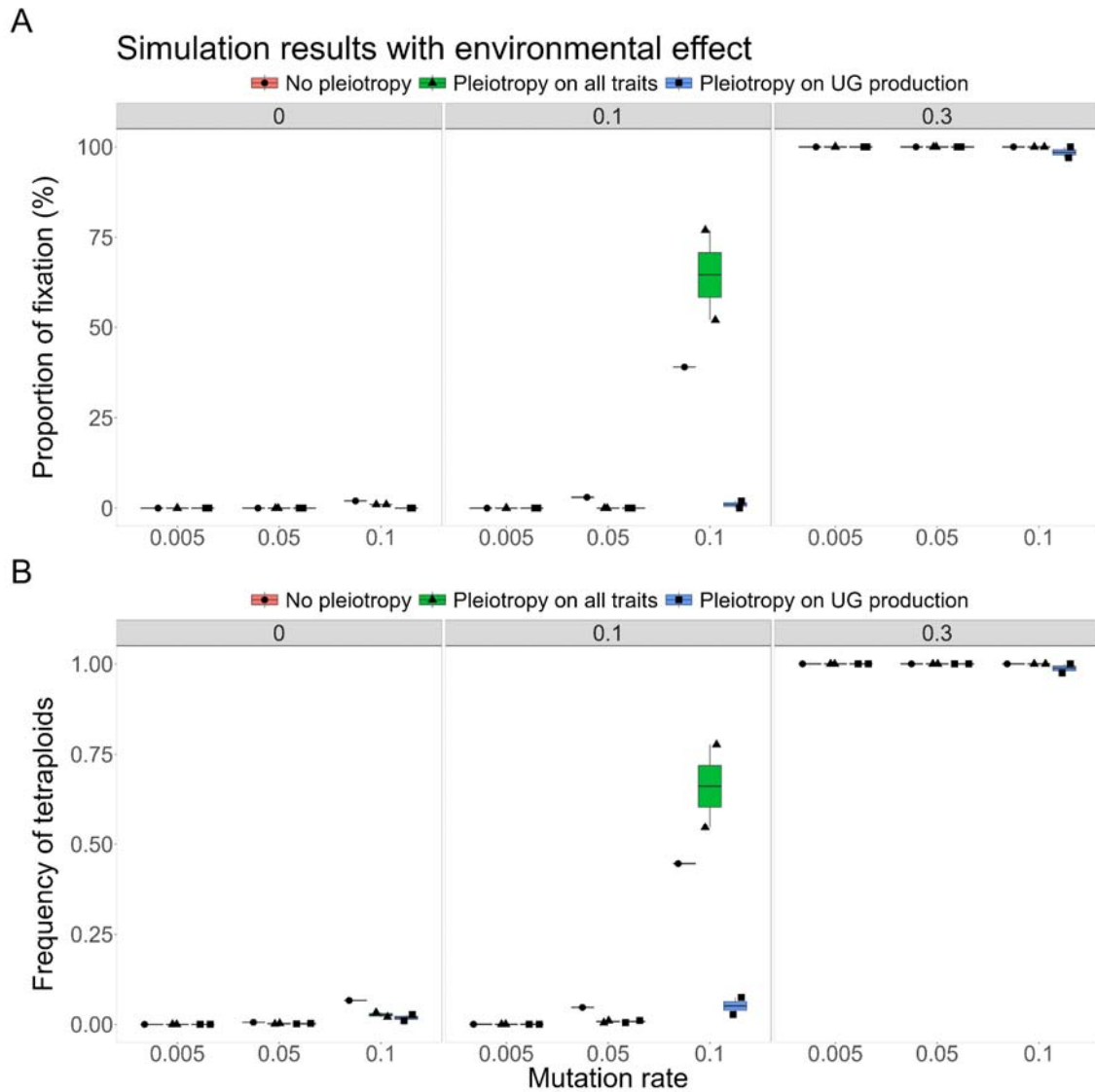
420

421 *Figure 5: Effect of pleiotropy type (no, all traits, unreduced gametes; number of pleiotropic loci = 40) and the*
422 *environmental factor (0, 0.1, 0.3) on the frequency of tetraploids in the population under different mutation*
423 *rates ($U = 0.005, 0.05, 0.1$; differently colored lines). simulated from a population of genetically identical diploid*
424 *individuals for 500 generations. Pleiotropy on all traits means that pleiotropy affected both the quantitative*
425 *trait under directional selection and unreduced gametes production. (A) No environmental factor; (B)*
426 *environmental factor 0.1; (C) environmental factor 0.3.*

427

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429



430

431 *Figure 6: Effect of pleiotropy type (no, all traits, unreduced gametes; number of pleiotropic loci = 40) and the*
432 *environmental factor (0, 0.1, 0.3) on the (A) fixation and (B) frequency of tetraploids in the population under*
433 *different mutation rates ($U = 0.005, 0.05, 0.1$; differently colored lines). simulated from a population of*
434 *genetically identical diploid individuals for 500 generations. Pleiotropy on all traits means that pleiotropy*
435 *affected both the quantitative trait under directional selection and unreduced gametes production.*

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441 **Discussion & Conclusion:**

442 We simulated the interplay between stabilizing selection and directional selection in response
443 to an environmental factor during the origin and fixation of autopolyploids. In this process,
444 the pleiotropic effect of mutations on UG production and the quantitative trait affecting
445 population fitness were tested. Under stabilizing selection, pleiotropy had a negative effect on
446 the production and genetic variation of unreduced gametes. Under a realistic low mutation
447 rate in the focal genes, we found no fixation of tetraploidy. Higher mutation rates led to
448 higher heritable rates of UG production and to the formation of stable mixed-ploidy
449 populations, however, with tetraploids remaining rare (< 10% in frequency). Under
450 directional selection, the environmental factor had a complex effect on UG production and
451 population cytotype structure. Taken as an isolated parameter, the higher the effect of the
452 environment on UG production, the higher the frequency of polyploidy at the end of the 500
453 simulated generations following the environmental change. In addition, environment and
454 genetic architecture had a synergistic effect: the stronger the effect of the environment, the
455 higher the genetic component of UG production. This is mainly because the production of
456 unreduced gametes is frequency-dependent, i.e. the higher it is, the less costly it is.

457

458 *Evolution of tetraploidy during stressful conditions*

459 Environmental stress is considered to be important to the origin of polyploids, as it affects
460 UG production (Kreiner *et al.* 2017a; Mason & Pires 2015; Ramsey & Schemske 1998a). In
461 particular, high and low temperatures have been shown to impact male UG formation (De
462 Storme *et al.* 2012; Mason *et al.* 2011; Pecrix *et al.* 2011; Schindfessel *et al.* 2023; Wang *et*
463 *al.* 2017), although the plants used therein were mainly of hybrid origin, which makes them
464 generally more prone to form unreduced gametes (Ramsey & Schemske 1998a). Treatment

465 with high temperatures is commonly used to generate unreduced male gametes for polyploid
466 breeding (Mai *et al.* 2019). In our simulations, tetraploids got fixed in a population either
467 under extreme environmental effects or through higher genetic mutation rates that increase
468 UG production to ca. 20 to 40% at equilibrium ($U = 0.05, 0.1$; Figure 5C; Supplementary
469 Figure 2). These results are in line with the recent Gerstner *et al.* (2024) model which found
470 that environmental stochasticity for UG production can lead to the evolution of polyploidy.
471 However, as UG production in natural populations of non-hybrid species is typically much
472 lower, ranging from 0.1 to 2% (Kreiner *et al.* 2017), our high mutation rates seem very
473 unrealistic. If we assumed a realistic mutation rate for an average of 2% of UG production,
474 tetraploids only got fixed in a population under a strong environmental effect in our
475 simulations, while a moderate environmental effect resulted in a mixed-ploidy population
476 with rare tetraploids ($< 10\%$; Figure 5C). However, we found that the higher the effect of the
477 environment on UG production, the higher the genetic increase of UG formation. If the
478 environmental stress also affects the mutation rate (like U.V. exposure that could affect both
479 temperature and alter the mutation rate; Gengyo-Ando & Mitani 2000), it is possible that
480 higher rates of polyploids could be found.

481 In the first simulated generations after the onset of the directional selection episode, which
482 represents the environmental change, we observed increased UG production and an increased
483 frequency of newly adapted tetraploid individuals in the population compared to later
484 generations (Figure 4). Under directional selection, mutations leading to UG formation might
485 be beneficial for tetraploid establishment, while counteracting diploid adaptation. Polyploids
486 may initially benefit from phenotypic shifts associated with WGD (Clo & Kolář 2021;
487 Porturas *et al.* 2019). However, both of these meta-analyses are based on studies of
488 established natural populations, which prevents filtering out the effects of selection and
489 genetic drift that are known to have shaped population dynamics of established polyploids,

490 but have less affected the first generations after a WGD event. The immediate, short-term
491 effect of a WGD can only be assessed using experimentally induced, synthetic
492 autopolyploids. For example, Westermann *et al.* (2024) showed that pollen tube tip growth is
493 drastically challenged in immediate response to a WGD event: in synthetic autotetraploids of
494 *Arabidopsis arenosa* and *Arabidopsis thaliana* pollen tubes grew slowly and abnormally,
495 while in established tetraploids of *A. arenosa* pollen tube physiology and morphology
496 returned to normal. This suggests initially reduced polyploid formation and fitness shortly
497 after a WGD event - a scenario not modeled here. Empirical frequencies of neo-polyploids in
498 natural populations might thus be lower. Decreased polyploid origin and fitness may also
499 stem from the formation of triploid offspring, which have low viability due to the triploid
500 block (Burton & Husband 2000a; Ramsey & Schemske 1998b). However, as we did not
501 account for triploid formation in our model, this does not affect the simulations.

502 Our findings are in contrast to that of (Clo 2022b) in that the author found neo-tetraploidy to
503 be associated with an initial decrease in their adaptive potential compared to their diploid
504 progenitors, while within less than 100 generations of the simulation they reached or
505 exceeded the additive variance of the diploids. In our simulations, tetraploids were directly
506 adapted and selected for an initial around 50 generations, but then were selected against once
507 diploid individuals were closer to the new phenotypic optimum, and they remained in the
508 minority within populations, resulting in mixed-ploidy populations under moderate
509 environmental effects. Even if the link between polyploidy and adaptive potential is probably
510 highly multifactorial (Clo 2022a), modeling non-adapted tetraploids with smaller genetic
511 diversity might have favored the diploid cytotype in mixed-ploidy populations.

512 Especially for plants, mixed-ploidy populations have been frequently reported in
513 multiple diploid-autotetraploid species, with varying proportions of diploids and their

514 polyploid derivatives (Kolář *et al.* 2017). The strength of selection against polyploid
515 cytotypes in these populations further depends on the mating system and clonal propagation,
516 although their interactions are not fully understood, based on both empirical data (Barringer
517 2007; Hojsgaard 2018; Van Drunen & Husband 2019) and theoretical modeling (Griswold
518 2021; Oswald & Nuismer 2011; Van Drunen & Friedman 2022). Eventually, gene flow
519 between ploidy levels may transfer novel adaptive alleles (e.g., in *A. arenosa*, Baduel *et al.*
520 2018; Australian burrowing frogs of the genus *Neobatrachus*, Novikova *et al.* 2020) and may
521 contribute to tetraploid fixation in the long-term, but we did not model this scenario. In our
522 simulations, only under a strong environmental effect tetraploids came to fixation. This is in
523 line with the theoretical framework of Oswald & Nuismer (2011) who showed that neo-
524 tetraploid populations can adapt more efficiently than their diploid progenitors if the
525 populations undergo an environmental change, and that this change is aligned with the
526 phenotypic modifications found in tetraploid individuals. The duplication of entire genomes
527 immediately creates redundant informational ‘modules’ in the genome, offering possibilities
528 for a wider exploration of genotypic and phenotypic space (Yao *et al.* 2019). It also supports
529 the hypothesis that polyploids are expected to face higher extinction rates than their diploid
530 progenitors (Levin 2019) in the absence of environmental change, due to their smaller
531 geographical ranges upon emergence. In contrast, polyploids may thrive in stressful
532 environments and broaden their ranges, leading to their prevalence in such environments and
533 during historical periods of climate change (Bomblies 2020; Rice *et al.* 2019; Van De Peer *et*
534 *al.* 2021b). In sum, while our model did not include post-WGD environmental adaptation, it
535 shows that environmental change may support polyploid establishment by directly affecting
536 the rate of UG formation, moreover, with a further positive feedback effect on its underlying
537 genes.

538

539 *The consequences of UG genetic architecture on polyploidy evolution*

540 The production of unreduced gametes is genetically determined (Parrott & Smith 1986;
541 Tavoletti *et al.* 1991b). It varies among individuals within and between populations, such that
542 most individuals in natural populations of non-hybrid species produce male unreduced
543 gametes at zero or low frequency and only a small number of individuals produce
544 substantially more (Kreiner *et al.* 2017a). Unreduced gamete mutations affect various
545 cytological stages (Bretagnolle & Thompson 1995; Brownfield & Kohler 2011; De Storme &
546 Geelen 2013), and they involve pre-meiotic, meiotic and post-meiotic pathways. Key proteins
547 comprise DYAD, AtPS1, JAS, TES, MPK4 (meiotic) and INCENP, RBR (post-meiotic).
548 Some of these genes are polygenic (Bretagnolle & Thompson 1995; Brownfield & Kohler
549 2011; De Storme & Geelen 2013), which may account for the variation in UG frequencies in
550 natural populations and the variance of UG production found in our simulations. A clear
551 theoretical and empirical framework for the effect of genetic architecture of UG production,
552 in particular pleiotropic effects, on the fixation of polyploidy is lacking. It is unclear if a few
553 major effect mutations or numerous small effect mutations are involved, and how they affect
554 the further trajectory of polyploid adaptive evolution.

555 In our simulations, one of the main findings is that pleiotropy always has a negative effect on
556 UG production and its variance. For example, a higher number of pleiotropic loci
557 significantly reduces UG production (Figure 3). This indicates that pleiotropy constrains UG
558 production by increasing the cost of complexity, a general effect of pleiotropy (Bombliès &
559 Peichel 2022; Orr 2000). While UG production and variance under pleiotropic effects is
560 lowered during stabilizing selection in our simulations, the overall fitness of the population is
561 often increased and thus seems to benefit from pleiotropic effects (Figure 1). However, we
562 found that during directional selection, if there is pleiotropy between UG formation and

563 fitness-related loci, a mutation having both a positive effect for diploid adaptation and
564 increasing UG production can be temporarily selected. It is known that mutations affecting
565 UG formation in plants are often linked to male and/or female fitness (Brownfield & Kohler
566 2011). If this kind of pleiotropic mutations can increase in frequency during an environmental
567 change, as explored by us, polyploidy could evolve by chance at least at low frequency,
568 highlighting once again the role of stochasticity in polyploid evolution (Clo *et al.* 2022;
569 Gerstner *et al.* 2024; Kauai *et al.* 2023).

570 According to empirical data, UG production is a deleterious phenotype, maintained at a low
571 frequency in the population (Kreiner *et al.* 2017b). In general, pleiotropy on quantitative traits
572 can reduce the selection efficacy in natural populations (Fraisse *et al.* 2019; Zhang 2023).
573 Consequently, in our simulations individuals with UG production faced strong purifying
574 selection but were still maintained in the population. Pleiotropy is discussed to have an
575 antagonistic effect between fitness and reproduction, in accordance with the theory of
576 pleiotropy constraints on adaptive traits (Hughes & Leips 2017). Antagonistic pleiotropy or
577 the trade-off between traits has also been found for reproduction versus aging/longevity
578 (Austad & Hoffman 2018; Wu *et al.* 2022). Empirical work has shown that intermediate
579 pleiotropy is a driver of adaptation in *A. thaliana* (Frachon *et al.* 2017). Besides, through
580 evolution experiments of yeast (*Saccharomyces cerevisiae*), pleiotropy resulting from a
581 changing environment contributed to molecular adaptation (Chen & Zhang 2020). In our
582 simulations, environment and genetic architecture have a synergistic effect: with both an
583 environmental effect and pleiotropy, population fitness decreased. Similarly, under
584 environmental change, higher pleiotropic effects on single cell morphology in yeast were
585 observed compared to a stable environment (Geiler-Samerotte *et al.* 2020). Reduction of
586 population fitness resulting from pleiotropy could eventually lead to a reduction of the
587 population size, and the population could then be more affected by genetic drift. This

588 scenario would be in accordance with previous models showing that the fixation of
589 polyploids relies on strong genetic drift more than on natural selection (Clo *et al.* 2022),
590 favoring polyploids under environmental change.

591

592 *Limitations of the model*

593

594 If our model is informative, we had to avoid some genetic and ecological aspects
595 related to ploidy for the model to remain understandable. A first simplification is that we
596 hypothesized triploid and higher polyploid levels to be non-viable. Triploids may play a
597 crucial role in mixed-ploidy population dynamics (Husband 2004; Kauai *et al.* 2024).
598 However, triploids are typically very rare in natural populations of autopolyploids, possibly
599 being abundant only during the earliest stage of polyploid formation in a population (Burton
600 & Husband 2000b; Husband & Sabara 2004; Kolář *et al.* 2017; Ramsey & Schemske 1998a).
601 Also, we decided to model the fact that polyploidy is adaptive in the short-term, which can be
602 true in some conditions (Jiang *et al.* 2022; Maherali *et al.* 2009; Wang *et al.* 2019; Wu *et al.*
603 2019), but not in general (Clo & Kolář 2021; Porturas *et al.* 2019). Considering equivalent
604 fitness for diploids and tetraploids, or a decrease in fitness in polyploids, would not have
605 drastically changed the results. If the population is swamped with unreduced gametes (high
606 mutation rate and strong environmental effect), tetraploids would fix in a population
607 independently of their fitness (see Clo *et al.* 2022 for an example). However, for most
608 simulations for which we found mixed-ploidy populations, it is likely that with no fitness
609 advantage the populations would have remained diploid most of the time.

610 We modeled obligately outcrossing populations, while any form of assortative mating
611 or spatial structure favoring mating within cytotypes could increase the likelihood of fixation
612 of polyploidy (Otto & Whitton 2000). The first reason is that the aim of our model was to
613 simulate a more realistic architecture of UG production, and that this novelty should not
614 modify the outcomes of previous models (Husband 2004; Van Drunen & Friedman 2022).
615 Second, association between ploidy and different forms of assortative mating remains
616 controversial (Clo 2022b). If the association between clonality and polyploidy is widespread
617 (Van Drunen & Husband 2019), no clear patterns emerge for the effect of ploidy on the
618 evolution of selfing (Husband *et al.* 2008) or plant-pollinator interactions (Rezende *et al.*
619 2020). Finally, we did not model demographical events following the environmental change.
620 One of our major results is that, for realistic simulations (with UG production fitting
621 empirical data), the adaptation of populations to new conditions was more likely by diploid
622 individuals reaching the new phenotypic optimum, rather than by adapted tetraploids
623 reaching fixation. We could have simulated fluctuating population size, with low-fitness
624 individuals (diploids in our case) going extinct before reaching the reproductive stage for
625 example. With a lower population size, any increase in tetraploid frequency would have
626 lowered the strength of frequency-dependent selection more efficiently than in our
627 simulations, possibly leading to mixed-ploidy or tetraploid populations more often.

628

629

630 **Data availability:** Simulation code is available at

631 https://github.com/JosselinCLO/Model_UG_pleiotropy_environment.

632

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636

637

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642

643

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