

1 **Neopolyploidy-induced changes in the giant duckweed (*Spirodela polyrhiza*) alter herbivore**
2 **preference, performance, and plant population performance**

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12 **ABSTRACT**

13 **Premise:** Polyploidy is a widespread mutational process in angiosperms that may alter
14 population performance of not only plants but also their animal associates. Yet, knowledge of
15 whether ploidy affects plant-herbivore dynamics is scarce. Here, we test whether aphid
16 herbivores exhibit preference for diploid or neopolyploid plants, whether ploidy impacts plant
17 and herbivore performance, and whether these interactions depend on plant genetic background.

18 **Methods:** Using multiple pairs of independently synthesized neotetraploid greater
19 duckweed (*Spirodela polyrhiza*) and their diploid progenitors, we evaluated the effect of
20 neopolyploidy on duckweed's interaction with the water-lily aphid (*Rhopalosiphum nymphaeae*).
21 Using two-way choice experiments, we first evaluated feeding preference by the herbivore. We
22 then evaluated the consequences of ploidy on aphid and plant performance by measuring
23 population growth over multiple generations.

24 **Key Results:** Aphids preferred neopolyploids over diploids when the plants were
25 provided at equal abundances but not when they were provided at equal surface area, indicating
26 the role of plant size in driving this preference. Additionally, neopolyploidy increased aphid
27 population performance, but this result was highly dependent on the genetic lineage of the plant.
28 Lastly, the impact of herbivory on neopolyploids vs. diploid duckweed varied greatly with
29 genetic lineage, but overall, neopolyploids appeared to be generally less tolerant than diploids.

30 **Conclusions:** We conclude that polyploidization can impact the preference and
31 performance of herbivores on their plant hosts, whereas plant performance depends on complex
32 interactions between herbivory, ploidy, and genetic lineage. These results have significant
33 implications for the establishment and persistence of plants and herbivores in nature.

34 **Key words:** Araceae; cytotype; duckweed; freshwater herbivory; host preference; macrophyte;
35 neopolyploidy; plant-herbivore interactions; water-lily aphid; whole-genome duplication

36 INTRODUCTION

37 Plant polyploidy, or whole genome duplication, is a dramatic and prevalent mechanism of
38 differentiation in plants (Nuismer and Thompson, 2001; Thompson et al., 2004; Arvanitis et al.,
39 2010; Ramsey and Ramsey, 2014; Segraves and Anneberg, 2016). Thirty-five percent of extant
40 angiosperms are of recent polyploid origin, and all angiosperms have at least one whole-genome
41 duplication event in their evolutionary past (Soltis et al., 2009; Wood et al., 2009; Jiao et al.,
42 2011). Polyploidy is extremely widespread; polyploids are found across the globe, with some
43 terrestrial biomes containing as high as 51% polyploid frequency and mixed-ploidy species
44 (exhibiting both diploid and polyploid cytotypes) are also common (Kolář et al., 2017; Rice et
45 al., 2019). Similarly, 52% of aquatic plant species are thought to be either polyploid or mixed-
46 ploidy species (Magalhães et al., 2021). Researchers have long been trying to explain this
47 apparent enhanced ecological and evolutionary success of polyploids, particularly by
48 investigating the genotypic and phenotypic differences of polyploids from their diploid
49 progenitors (Comai, 2005; Madlung, 2013; Levin and Soltis, 2018; Fox et al., 2020; Van-de-Peer
50 et al., 2020). However, while studies of the mechanisms behind polyploid success in terrestrial
51 species have been accumulating over the last few decades, comparatively less is known for
52 aquatic species.

53 Polyploid plants often differ from their diploid ancestors in a variety of ways that can
54 impact their interaction with abiotic and biotic factors (Gross and Schiestl, 2015; Wei et al.,
55 2019; Clo and Kolář, 2021). It is argued polyploidy enhances tolerance to abiotic stressors, such
56 as heat, cold, salt, and nutritional stress (Yang et al., 2014; Godfree et al., 2017; Song et al.,
57 2020; Tossi et al., 2022; Anneberg et al., 2023a). Yet, tolerance to biotic stressors has received
58 much less attention, despite the likelihood that phenotypic differences also impact species

59 interactions (Segraves and Anneberg, 2016; Forrester and Ashman, 2017; Rezende et al., 2020;
60 Anneberg et al., 2023b). Specifically, polyploidy-induced phenotypic and genotypic changes can
61 lead to novel interactions with other species, such as herbivores, pollinators, and microbes
62 (Arvanitis et al., 2010; McCarthy et al., 2016; Porturas et al., 2019; Walczyk and Hersch-Green,
63 2019; Forrester et al., 2020; Rezende et al., 2020; Curé et al., 2022; Anneberg et al., 2023b).

64 Numerous changes due to polyploidy including plant body size, trichome size/number,
65 leaf thickness, secondary metabolite production, and cellulose content may alter herbivore
66 performance and preference as well as plant tolerance and resistance to herbivores (Bagheri and
67 Mansouri, 2015; Corneillie et al., 2018; Wei et al., 2019; Bomblies, 2020; Hamarashid et al.,
68 2022; Malacrinò et al., 2022). For example, the gigas effect, or the enlargement of plant cells due
69 to the increased amounts of DNA, often leads to polyploid plants being larger in size than their
70 diploid progenitors (Doyle and Coate, 2019; Bomblies, 2020; Clo and Kolář, 2021). This
71 phenotypic change may increase their apparency to herbivores leading to a greater number of
72 herbivores being found on polyploids. This change in size often comes at the cost of slower
73 growth rate, which may in turn impact the plant's ability to tolerate and recover from herbivore
74 damage (Züst and Agrawal, 2016; Corneillie et al., 2018; DeRose et al., 2022; Anneberg et al.,
75 2023a). Similarly, ploidy-driven changes in the photosynthetic rate of the plant could allow for
76 faster or slower recovery depending on the direction (Warner and Edwards, 1993; Cao et al.,
77 2018). Further, polyploidization may impact secondary-metabolite production, and polyploids
78 may acquire higher levels of defenses against herbivores (Lavana et al., 2012; Bagheri and
79 Mansouri, 2015; Edger et al., 2015; Gaynor et al., 2020).

80 While much effort has been focused on capturing the variation in the responses to
81 polyploidy across different species, is also important to also recognize the intraspecific variation

82 in response to polyploidy due to genetic differences in progenitor diploids (Soltis et al., 2016;
83 Castro et al., 2020; Anneberg et al., 2023b; Bafort et al., 2023). Polyploidy can arise multiple
84 times independently within a single taxon across genetically divergent individuals, leading to
85 differences in whole-genome duplication's affect within a single species (Soltis et al., 1993;
86 Segraves et al., 1999). For example, in the context of species interactions, Anneberg et al.
87 (2023a) recently found that the effect of polyploidy on duckweed microbiomes was different
88 across multiple, independently synthesized neopolyploid lineages of duckweed. Yet, our
89 knowledge of how other species interactions may be impacted by the interactions of
90 polyploidization and genetic background, such as plant-herbivore interactions, is still limited.

91 Compared to other biotic interactions, evidence for polyploidy's effect on plant-herbivore
92 interactions are heavily weighted toward herbivore attraction, attack, and resultant plant
93 performance, with none experimentally addressing the impact on both herbivore and plant
94 populations (Arvanitis et al., 2010; Gross and Schiestl, 2015; Münzbergová et al., 2015;
95 O'Connor et al., 2019). Additionally, results from these studies are mixed. For example,
96 Halverson et. al (2008) found that neither diploid nor polyploid *Solidago altissima* were
97 consistently attacked more frequently by five species of herbivores. Similarly, Thompson et al.
98 (1997) found that the moth *Greya potentilla* was sometimes more likely to attack tetraploids over
99 diploid *Heuchera grossularifolia*, but they were still able to colonize both diploids and
100 polyploids of separate origin. Indeed, while insightful, general conclusions about the overall
101 impact on the plant-herbivore relationship are hard to synthesize, as these studies are often
102 conducted in a wide variety of habitat types, using a mixture of established and newly
103 synthesized polyploids of different ages, and do not quantify herbivore or plant performance
104 (Thompson et al., 1997; Walczyk and Hersch-Green, 2019; Harkin and Stewart, 2021; Harms

105 and Walter, 2021). Notably, however, a recent study by Curé et al. (2022) did test the preference
106 and performance of a specialist pea aphid, *Acyrtosiphon pisum*, on two different host plants.
107 They found no ploidal-dependent preference in two species, red clover and alfalfa, but they did
108 find that aphids that originated from populations specialized on diploid red clover had higher
109 fecundity on that host than on synthesized neotetraploid red clover. To our knowledge, this is the
110 only study to quantify herbivore performance on diploids and neopolyploids, and now opens the
111 door to build on these results by also quantifying plant performance and expanding to new plant-
112 herbivore systems.

113 While quantifying herbivory on natural established populations has provided key insights
114 into the effects of polyploidy on plant-herbivore interactions, most prior studies suffer from three
115 limitations. First, these can confound the impact of polyploidy, interspecific hybridization, and
116 evolution following whole-genome duplication (Parisod et al., 2010; Drunen and Husband, 2018;
117 Bomblies, 2020). By using newly established or synthetic polyploids, or neo-polyploids, one can
118 isolate the immediate consequences of whole-genome duplication alone. Second, the outcome of
119 whole-genome duplication may vary on the genotypic background of the individual, so multiple
120 genotypes should be used, and genetic variance must be accounted for (Drunen and Husband,
121 2018; Wei et al., 2020; Bafort et al., 2023). Third, most studies of polyploidy-herbivory
122 interactions are conducted over only a small portion of the life-history of the plant or herbivore
123 and usually on single individuals. This may limit our ability to draw conclusions on the fitness
124 impacts of polyploidy under herbivory. To our knowledge, there are no population-level
125 experimental studies investigating both plant and herbivore performance and herbivore
126 preference in the context of neopolyploidy. Curé et al. (2022) were the first and only to
127 investigate this relationship using neopolyploids but focused on individual-level plant response.

128 Consequently, to further our understanding of the outcomes of whole-genome duplication on
129 species interactions, there is a need for experiments examining the immediate effects of whole-
130 genome duplication at the population level, using multiple independently created polyploid
131 genotypes (Parisod et al., 2010; Kolář et al., 2017; Spoelhof et al., 2017; Drunen and Husband,
132 2018; Anneberg et al., 2023b).

133 Duckweed is well suited to fill this knowledge gap in how neopolyploidy affects plant-
134 herbivore interactions (Laird and Barks, 2018). Duckweeds are globally distributed, small,
135 aquatic floating plants that primarily reproduce asexually via budding. An individual consists of
136 a single frond, a small leaf-like structure making up the entire shoot, and multiple roots (Ziegler
137 et al., 2015; Acosta et al., 2021). Duckweeds reproduce rapidly (within four days in optimal
138 conditions), and thus multiple generations can be studied in the span of several weeks (Ziegler et
139 al., 2015; Hart et al., 2019). While there is natural variation in many duckweed species, Greater
140 duckweed (*S. polyrhiza*) is gaining traction as model system for polyploidy and herbivory owing
141 to the amenability of population-level studies using synthesized neotetraploid plants, affording
142 direct comparison of neopolyploid populations to those of their diploid progenitors (Anneberg et
143 al., 2023a). Combined with experimental studies of herbivores, population-level impacts of
144 herbivory can be precisely studied from both the plant and herbivore perspective (Mariani et al.,
145 2020; Subramanian and Turcotte, 2020, 2023).

146 The water-lily aphid, *Rhopalosiphum nymphaeae*, is a globally-distributed generalist
147 herbivore of duckweeds (Halder et al., 2020; Subramanian and Turcotte, 2020, 2023). Aphids are
148 phloem-feeding herbivores that reproduce facultatively parthenogenetically via live birth with a
149 population doubling time of around two days (Hance et al., 1994). Because both aphids and
150 duckweeds are fast-reproducing, asexual organisms, together they provide a unique opportunity

151 to evaluate the effect of neopolyploidy on population growth rates of both host and herbivore
152 over multiple generations.

153 Here, we addressed four questions. 1) Do aphids exhibit a preference for diploid or
154 neopolyploid duckweed, and if so, is this a function of the effect of ploidy on plant body size? 2)
155 Does duckweed ploidy alter aphid population performance? 3 Do aphids differentially affect the
156 performance of neopolyploid and diploid duckweed populations? 4) Are the results of the
157 previous questions dependent upon the genetic lineage of duckweed?

158

159 **MATERIALS AND METHODS**

160 *Cultivation of duckweed and aphids-*

161 We used colchicine-induced autotetraploid and colchicine-exposed but unconverted
162 diploids as described in Anneberg et al. (2023a) to answer our questions. To obtain these, we had
163 previously applied the mitotic inhibitor colchicine to induce whole-genome duplication in six
164 genetically distinct diploid *S. polyrhiza* collected from eastern Pennsylvania and western Ohio,
165 U.S.A. (See Table S1 for collection site info) (Xu et al., 2018; Anneberg et al., 2023a; Kerstetter
166 et al., 2023). Then, in 2019 and 2020, ploidy was confirmed using flow cytometry following Wei
167 et al. (2020). Although we did not observe any residual effects of colchicine treatment, to be
168 conservative, we used the colchicine-treated but unconverted diploid individuals in the
169 experiment (Anneberg et al., 2023a). Prior to the experiment, we grew individual lineages of
170 duckweed in the growth chamber at 23.5°C, 50% humidity, 50 $\mu\text{mol}/\text{m}^2$ /s light, and 16:8
171 light/dark cycle.

172 Water-lily aphids were collected from a duckweed community composed of a mixture of
173 several species of duckweed (*Spirodela polyrhiza*, *Lemna minor*, and *Wolffia brasiliensis*) at

174 Twin Lakes Park in Westmoreland County, Pennsylvania, USA (40.323383333, -79.472383333)
175 in September of 2017 (Subramanian and Turcotte, 2020). Stock aphid populations were then
176 maintained on monocultures of diploid *S. polyrhiza* populations in the growth chamber.

177 ***Preference Experiments-***

178 To determine whether aphids exhibit feeding preference for diploid or neotetraploid
179 duckweed, we conducted several two-way aphid choice trials following the basic set-up
180 established in Subramanian and Turcotte (2020) in January and February of 2023. Each trial
181 consisted of a diploid and its corresponding derived neopolyploid in preference arenas. On
182 average, our neopolyploid duckweed were 46% larger in surface area than their diploid
183 progenitors (See Supplemental Table 2). Considering this size difference, we conducted two
184 separate sets of trials in preference arenas. The first was the ‘Abundance-controlled’ trial – we
185 added exactly six fronds of each ploidy of a given lineage to the arenas. The second was the
186 ‘Area-controlled’ trial – we added approximately equal population surface area of each ploidy of
187 a given lineage to the preference arenas. We equalized ploidal surface area by first placing
188 duckweed in 3.5 cm² cells of a culture plate, such that there was a single, non-overlapping layer
189 of duckweed floating on the surface before moving pairs of them to the preference arenas. The
190 preference arenas consisted of 60 mL jars with 19.6 cm² openings that were filled with 50 mL of
191 0.1x strength diluted, sterile plant growth media (Appenroth et al., 1996). In the center of the 60
192 mL jar, we floated a 0.6 cm diameter circle of white plastic as a platform for the aphid in the
193 middle of the duckweed population. The diploid and neopolyploid plants were intermixed around
194 the platform and then we placed a single 3rd instar aphid on the platform. We tested preference
195 on five of the six genetic lineage pairs of diploid-neopolyploid duckweeds. One genetic lineage
196 (SP.07) was omitted due to contamination with algae that was later cleaned, allowing for use in

197 the performance trials (see below). Both the Area- and Abundance-controlled trials were
198 replicated 20 times per genetic lineage, for a total of 200 paired trials (5 genetic lineages x 2 trial
199 types x 20 replicates). All aphids and duckweeds were only used once. We determined
200 preference by observing which ploidy the aphid chose to insert its stylet (Subramanian and
201 Turcotte, 2020). We recorded aphid preference after 1, 5, 30, 60 and 90 minutes and 24 hours. If
202 the aphid died or crawled out of the jar (which only occurred 4 times out of 200 trials), no choice
203 was recorded, and it was removed from the data analysis. For 93% of the trials, the aphid stayed
204 on the same individual originally chosen (usually in the first 1 or 5 minutes), so only final choice
205 at 24 hours was used in the analysis.

206 *Performance Experiment-*

207 We assessed aphid and duckweed performance in a separate, full factorial experiment
208 where we crossed ploidy, genetic lineage and aphid presence in a growth chamber. We added
209 220 mL of 0.5x strength growth media to 240 mL glass jars. Into each, we added six individuals
210 of a single ploidy from a single genetic lineage of duckweed. We then randomly chose jars to
211 add either five 3rd instar aphids or no aphids. Each combination was replicated 10 times for a
212 total of 240 jars (6 genetic lineages x 2 ploidies x 2 aphid treatments x 10 replicates). The 10
213 replicates were split into two time-blocks of five, run consecutively. Each experiment lasted 15
214 days, allowing for three-four generations of both duckweeds and aphids. We quantified
215 population growth of the aphids by counting their abundances two-three times per week. We
216 quantified duckweed performance in two ways, both of which represent multigenerational
217 fitness. First, we quantified duckweed population abundance over time by counting their
218 abundances two-three times per week. Because aphids and duckweed reproduce asexually under
219 these conditions, abundance serves as a direct measure of population performance. Second, at the

220 end of the 15 days, we measured final biomass by harvesting all duckweeds at the end of the
221 experiment, drying them at 55°C for one week, and weighing them on Cahn C-31 microbalance
222 scale to the nearest 0.0001 g.

223 *Statistical Analyses-*

224 We tested for an effect of neopolyploidy on aphid preference, aphid performance and
225 plant performance (in terms of both abundances and final biomass). All analyses were performed
226 in R version 4.1.2 (R Core Team, 2021). For the preference trials, we conducted *G*-tests of
227 goodness-of-fit for each lineage in each trial type. For each trial type (Area- or Abundance-
228 controlled), we computed the total *G* (summed across lineages), pooled *G* and calculated the
229 heterogeneity *G* to assess whether there was significant variation among genetic lineages using
230 the *RVAideMemoire* package (Herve, 2023).

231 For the performance experiments, we constructed separate generalized linear models with
232 aphid population growth, plant population growth and final plant biomass as response variables.
233 For aphid population growth, we used a generalized linear mixed model (GLMM) with a
234 negative binomial probability distribution, with aphid abundances as the response variable, and
235 ploidy (diploid or neopolyploid) and genetic lineage (as a categorical factor) and their interaction
236 as main effects. We also included experimental time-block as a fixed effect. Lastly, to account
237 for repeated measures over the course of the experiment, ‘Day’, or day of sampling, and ‘Jar ID’,
238 which was the individual experimental unit, were included as crossed random effects. We
239 removed the abundance on day one from this analysis because all experimental units started with
240 the same exact number of aphids (five). Linear mixed effects models were run using the
241 *glmmTMB* package (Brooks et al., 2017). We used a negative binomial distribution to account

242 for overdispersion in the aphid population data. All model residuals were assessed using the
243 DHARMA package (Hartig, 2022).

244 For duckweed population abundances, we used a GLMM with a Poisson probability
245 distribution with duckweed abundances as the response variable and similar explanatory
246 variables and random effects as above, but we also included a main effect for herbivory
247 (presence or absence) as well as a three-way interaction term (Ploidy:Lineage:Herbivory). We
248 also removed the first day's data point from this analysis because all experimental units started
249 with the same exact number of duckweed fronds (six).

250 Finally, we analyzed final duckweed dry biomass using a linear model with a normal,
251 Gaussian probability distribution with ploidy, genetic lineage and herbivory as main effects, with
252 all interactions and time-block as a fixed effect.

253 We also ran lineage-specific GLMMs for all models (aphid abundance, duckweed
254 abundance and duckweed biomass) post-hoc to see which, if any, genetic lineages were driving
255 significant effects. Lineage-specific models had the same structure as the overall models but
256 without the 'genetic lineage' response variable.

257

258 **RESULTS**

259 *Herbivore preference and performance-*

260 When duckweed ploidies were matched by frond abundance in the Abundance-controlled
261 trial, we found a significant aphid preference for neopolyploid plants over diploids (Fig. 1A,
262 Table 1). Pooled across all lineages, aphids chose polyploids 66 out of 100 trials, and this
263 preference was consistent across all genetic lineage pairs. Given that diploids were only chosen
264 31 times implies that neopolyploids were 213% as likely to be attacked by aphids than diploids.

265 However, when duckweed ploidies were matched by total surface area in the Area-controlled
266 trial, aphids did not exhibit significant preference (Fig. 1B, Table 1). Specifically, in 54 of the
267 trials, the aphid chose the neopolyploid, and in 46 of the trials, the aphid chose the diploid. This
268 was consistent across lineages (Table 2).

269 Plant polyploidy alone increased aphid performance but genetic lineage and its
270 interaction with ploidy also significantly affected aphid performance ($P < 0.001$, Fig. 2, Table
271 S3). Neopolyploids, on average, hosted 14% more aphids than diploids at the end of the
272 experiment. The significant ploidy-lineage interaction indicates that the effect of ploidy on
273 performance varied by lineage; for example, neopolyploid SP.11 hosted an average of 33% *more*
274 aphids than diploid SP.11 by the end of the experiment, whereas aphids on SP.07 performed 4%
275 worse on polyploids than the diploid (Ploidy:Lineage interaction, $df = 5$, $P < 0.001$, Fig. 2, Table
276 S3). Only one lineage, SP.11, showed significant effects of neopolyploidy on aphid abundance in
277 the lineage-specific models (Table S4).

278 ***Duckweed Performance-***

279 *Abundance-*

280 Overall, neopolyploid duckweed reached lower abundances in the face of herbivory than
281 diploid duckweed in a lineage-dependent manner (three-way Ploidy:Herbivory:Lineage
282 interaction, $P = 0.031$, Fig. 3, Table S5). For most lineages, neopolyploid duckweed abundance
283 was more impacted by herbivory than diploid abundance, but the result was highly dependent on
284 the genetic lineage. Independent duckweed lineages also responded to neopolyploidy differently
285 (Ploidy:Lineage interaction, $df = 5$, $P < 0.001$, Fig. 3, Table S5). And while there was no
286 Ploidy:Herbivory interaction (neopolyploids *overall* were not significantly more or less tolerant
287 than diploids in terms of abundance), there were strong lineage-dependent responses to herbivory

288 (Lineage:Herbivory interaction, $df = 5$, $P < 0.001$, Fig 3, Table S5). For example, SP.01 was very
289 tolerant, with its average final abundance only decreased by 11% (across diploids and
290 neopolyploids) in the face of herbivory, whereas SP.43 declined by 38% suggesting lower
291 tolerance. It is worth noting, however, that lineage-specific models reveal that SP.05 was the
292 only lineage to exhibit a significant Ploidy:Herbivory interaction, wherein the neopolyploid's
293 abundance was more significantly impacted and less tolerant to aphids than the diploid (Table
294 S6). Diploid SP.05 final abundance declined by an average of 14% in the face of herbivory,
295 whereas neopolyploid's final abundance declined by an average 35%.

296 *Biomass-*

297 Aphid presence significantly reduced duckweed final biomass across all treatments but
298 did so unevenly among ploidies (Fig. 4, Table S7, Table S8). Aphids impacted neopolyploid
299 biomass similarly to diploid biomass (2% difference in their tolerance) at a marginally
300 significant level (Fig. 4, Table S7, Ploidy:Herbivory interaction, $df = 1$, $P = 0.063$), but the
301 biological effect size was very small (Table S7). This result, however, varied among genetic
302 lineages at a marginally significant level (Ploidy:Herbivory:Lineage interaction, $df = 5$, $P =$
303 0.065); neopolyploids of SP.05, SP.11 and SP.41 were all less tolerant than their diploid
304 progenitors in terms of biomass, whereas diploids of SP.01, SP.07 and SP.43 were all less
305 tolerant than neopolyploids.

306

307 **DISCUSSION**

308 Our results demonstrated significant differences in the effect of polyploidy and genetic
309 lineage on herbivore preference and performance, and plant performance in response to
310 herbivory. Preference experiments indicated that aphids preferred neopolyploid duckweed across

311 all genetic lineages, but that this result was largely driven by differences between ploidies in
312 frond size, as the preference disappeared when we controlled for size. In addition to aphids
313 preferring the neopolyploid duckweed, aphids also often performed better -- reaching higher
314 abundances-- on neopolyploid duckweed. However, this result was highly dependent on plant
315 genetic lineage. Lastly, neopolyploids appeared to be slightly less tolerant than diploids in the
316 face of herbivory, but the effects were small and highly dependent on genetic lineage.

317 The relationships between herbivory, polyploidy, and plant genetic background on plant
318 performance were complex. The absence of a significant two-way interaction between
319 polyploidy and herbivory on plant performance, but the presence of three-way interactions,
320 suggests that the effects of polyploidy and herbivory do not depend on each other in a
321 straightforward manner, and that the genetic background of the plant plays a large role. For
322 example, in the lineage SP.05, aphids reached similar abundances on neopolyploids and diploids,
323 but abundance and biomass of neopolyploid SP.05 were more negatively impacted by aphid
324 herbivory than its diploid progenitor (Fig. 3, Fig. 4, Table S6, Table S8.). This would imply that
325 neopolyploid duckweed are, in fact, less tolerant per herbivore. However, neopolyploid SP.11
326 hosted more aphids than diploid SP.11, but there were no significant differences in how they
327 responded to herbivory. This may actually imply that neopolyploid SP.11 is *more* tolerant per
328 aphid than diploid SP.11. Such findings emphasize the need for a nuanced understanding of how
329 multiple factors interact to shape ecological outcomes. Had we only quantified herbivore
330 performance or plant performance, these complex relationships could have been overlooked.
331 However, to truly make comparisons and broad conclusions across multiple plant-herbivore
332 systems, more population-level data on the plant and the herbivore in the context of polyploidy
333 are needed (Curé et al., 2022; Anneberg et al., 2023b).

334 Overall, aphids preferred neopolyploids and performed better on them. Indeed, this trend
335 has been found in other plant herbivore systems with mixed ploidies, such as *Greya politella*
336 moths and plants in the *Lithophragma* genus, and Alligator weed (*Alternanthera philoxeroides*)
337 and the flea beetle (*Agasicles hygrophila*) (Janz and Thompson, 2002; Krug and Sosa, 2019).
338 However, many studies reporting this pattern also confirm that their results depended on other
339 factors, such as plant origin, year sampled, and environmental context, and the opposite trend has
340 also been found in some plant-herbivore systems as well (Janz and Thompson, 2002; Hull-
341 Sanders et al., 2009; König et al., 2014, 2016; Münzbergová et al., 2015). Our results with
342 synthetic neopolyploids show that, in particular, it is important to account for the differences in
343 size between the two ploidies as a potential mechanism driving herbivore preference, yet this
344 control is not commonly conducted. Corroborating the preference study result that plant size
345 plays a large role in driving these interactions, in the performance study, the lineage with the
346 smallest size difference between diploids and neopolyploids, SP.07, exhibited very little
347 differences in aphid population sizes (See Supplemental Table 2). Similarly, SP.11
348 neopolyploids saw the largest increase in aphid abundances compared to diploids, and they are
349 approximately 56% larger than their diploid progenitors. However, this trend was not always
350 consistent across lineages; neopolyploid SP.05 are nearly double the size of their diploid
351 progenitors, but they exhibited little differences in the number of aphids hosted. Interestingly,
352 this may indicate that something other than size may be contributing to polyploidizations effect
353 on the duckweed-aphid relationship for this lineage. There is very little mechanistic work behind
354 what might drive differences between herbivore preference and performance *in the context of*
355 *polyploidy*. However, we do know that, in addition to ‘apparency’ can be driven by visual cues
356 such as size, preferences are also driven by olfactory, mechanical, and chemical cues, and that

357 variation in plant chemistry exists even at the intraspecific level (Powell et al., 2006; Jakobs and
358 Müller, 2018; Endara et al., 2023). For example, in *Centaurea phrygia*, Münzbergová et al.
359 (2015) cite differences in levels of secondary compounds gallic acid and several polyphenols in
360 diploid *Centaurea phrygia*, as a potential mechanism for diploids suffering less seed damage
361 than polyploids. Further, polyploidization may have varied effects on herbivores with alternative
362 modes of feeding, such as chewing or mining, as compared to phloem-suckers like aphids.
363 Feeding modes vary amount and type of damage they inflict on host plants, and thus plants may
364 use different strategies to tolerate and resist different herbivores (Ali and Agrawal, 2012;
365 Marmolejo et al., 2021; Xu et al., 2021). How chemical versus structural defenses change with
366 whole-genome duplication, however, remains unknown. Thus, one avenue for future research
367 could involve investigating the chemical profiles of neopolyploid and diploid duckweed and
368 whether these impact varying types of herbivory differently.

369

370 **CONCLUSIONS**

371 Our experiment, using multiple neopolyploid lineages, revealed that that polyploidy and
372 genetic lineage impact herbivore preferences for plant hosts and herbivore fitness, and that this
373 trend is, in part, driven by size differences between neopolyploids and diploids. This study
374 represents one of the initial attempts to investigate these dynamics in aquatic plants, and further
375 work should consider expanding its scope to encompass a broader range of aquatic species. The
376 extent to which neopolyploids' fitness is affected by herbivory was strongly dependent on
377 genetic lineage. By combining results on herbivore performance and plant performance across
378 multiple plant genotypes, we were able to uncover complex relationships between ploidy,
379 herbivory, plant genetic background. Our work here lays the groundwork for future experimental

380 studies to explore the longer-term and mechanistic drivers of the impact of polyploidy on plant-
381 herbivore relationships, as well as to further understand how these impacts might impact plant
382 and herbivore establishment and persistence in nature.

383 ***Future directions-***

384 Long-term, polyploidy can alter species interactions in two ways: either via the direct
385 changes caused by whole-genome duplication, or by the indirect changes incurred via evolution
386 that occurs following whole-genome duplication. Here, we were able to investigate the effects of
387 the direct changes caused by polyploidization, leaving the latter still up for investigation. A
388 recent study by Malacrinò et al. (2022), however, showed that exposure to herbivory rapidly
389 increased *S. polyrhiza* resistance in only 30 generations. Future research using long-term
390 experimental studies with both neopolyploids and diploids would be needed to address whether
391 polyploids would evolve differently than diploids in the presence/absence of herbivores.

392 Given our results, it is possible that, in addition to facilitating establishment of polyploids
393 in natural communities, whole-genome duplication in plants may also contribute to the
394 evolutionary diversification of the herbivore. Aphids not only incorporated neopolyploid
395 duckweed in their diets, but also preferred neopolyploids and performed better on them; this
396 implies that neopolyploidy may be a mechanism to advance the migration of herbivores and
397 facilitate aphid expansion outside of their current ranges (Curé et al., 2022).

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407

408 **Author Contributions**

409 HRA, T-LA and MMT conceptualized and designed the study. HRA carried out the
410 experiments. HRA, T-LA and MMT conceptualized the analysis. HRA analyzed the data and
411 wrote the first draft of the manuscript, and HRA, T-LA and MMT edited subsequent drafts.

412

413 **Data Availability Statement**

414 Data and R scripts will be archived in an online repository by the time of publication.

415

416 **Supporting Information**

417 Additional supporting information may be found online in the Supporting Information section at
418 the end of the article.

419

420 Literature Cited

- 421 Acosta, K., K. J. Appenroth, L. Borisjuk, M. Edelman, U. Heinig, M. A. K. Jansen, T. Oyama, et
422 al. 2021. Return of the Lemnaceae: duckweed as a model plant system in the genomics
423 and postgenomics era. *The Plant Cell* 33: 3207–3234.
- 424 Ali, J. G., and A. A. Agrawal. 2012. Specialist versus generalist insect herbivores and plant
425 defense. *Trends in Plant Science* 17: 293–302.
- 426 Anneberg, T. J., E. M. O’Neill, T.-L. Ashman, and M. M. Turcotte. 2023a. Polyploidy impacts
427 population growth and competition with diploids: multigenerational experiments reveal key
428 life history tradeoffs. *New Phytologist*.
- 429 Anneberg, T. J., M. M. Turcotte, and T.-L. Ashman. 2023b. Plant neopolyploidy and genetic
430 background differentiates the microbiome of duckweed across a variety of natural freshwater
431 sources. *bioRxiv*: 2023.04.29.538806.
- 432 Appenroth, K.-J., S. Teller, and M. Horn. 1996. Photophysiology of turion formation and
433 germination in *Spirodela polyrhiza*. *Biologia Plantarum* 38: 95–106.
- 434 Arvanitis, L., C. Wiklund, Z. Münzbergova, J. P. Dahlgren, and J. Ehrlén. 2010. Novel
435 antagonistic interactions associated with plant polyploidization influence trait selection and
436 habitat preference. *Ecology Letters* 13: 330–337.
- 437 Bafort, Q., T. Wu, A. Natran, O. D. Clerck, and Y. V. de Peer. 2023. The immediate effects of
438 polyploidization of *Spirodela polyrhiza* change in a strain-specific way along environmental
439 gradients. *Evolution Letters*.
- 440 Bagheri, M., and H. Mansouri. 2015. Effect of Induced Polyploidy on Some Biochemical
441 Parameters in *Cannabis sativa* L. *Applied Biochemistry and Biotechnology* 175: 2366–2375.
- 442 Bomblies, K. 2020. When everything changes at once: finding a new normal after genome
443 duplication. *Proceedings of the Royal Society B: Biological Sciences* 287: 20202154.
- 444 Brooks, M., K. Kristensen, K. van Benthem, A. Magnusson, C. Berg, A. Nielsen, H. Skaug, et al.
445 2017. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated
446 Generalized Linear Mixed Modeling. *The R Journal* 2: 378–400.
- 447 Cao, Q., X. Zhang, X. Gao, L. Wang, and G. Jia. 2018. Effects of ploidy level on the cellular,
448 photochemical and photosynthetic characteristics in *Lilium* FO hybrids. *Plant Physiology and*
449 *Biochemistry* 133: 50–56.
- 450 Castro, M., J. Loureiro, A. Figueiredo, M. Serrano, B. C. Husband, and S. Castro. 2020.
451 Different Patterns of Ecological Divergence Between Two Tetraploids and Their Diploid
452 Counterpart in a Parapatric Linear Coastal Distribution Polyloid Complex. *Frontiers in*
453 *Plant Science* 11: 315.

- 454 Clo, J., and F. Kolář. 2021. Short- and long-term consequences of genome doubling: a meta-
455 analysis. *American Journal of Botany* 108: 2315–2322.
- 456 Comai, L. 2005. The advantages and disadvantages of being polyploid. *Nature Reviews Genetics*
457 6: 836–846.
- 458 Corneillie, S., N. D. Storme, R. V. Acker, J. U. Fangel, M. D. Bruyne, R. D. Rycke, D. Geelen,
459 et al. 2018. Polyploidy Affects Plant Growth and Alters Cell Wall Composition . *Plant*
460 *Physiology* 179: 74–87.
- 461 Curé, A. E., D. M. Althoff, and K. A. Segraves. 2022. Host expansion in a specialist herbivore is
462 facilitated by whole-genome duplication in the host plant. *Ecological Entomology*.
- 463 DeRose, R. J., R. S. Gardner, R. L. Lindroth, and K. E. Mock. 2022. Polyploidy and growth—
464 defense tradeoffs in natural populations of western quaking Aspen. *Journal of Chemical*
465 *Ecology*: 1–10.
- 466 Doyle, J. J., and J. E. Coate. 2019. Polyploidy, the Nucleotype, and Novelty: The Impact of
467 Genome Doubling on the Biology of the Cell. *International Journal of Plant Sciences* 180: 1–
468 52.
- 469 Drunen, W. E. V., and B. C. Husband. 2018. Immediate vs. evolutionary consequences of
470 polyploidy on clonal reproduction in an autopolyploid plant. *Annals of Botany* 122: 195–205.
- 471 Edger, P. P., H. M. Heidel-Fischer, M. Bekaert, J. Rota, G. Glöckner, A. E. Platts, D. G. Heckel,
472 et al. 2015. The butterfly plant arms-race escalated by gene and genome duplications.
473 *Proceedings of the National Academy of Sciences* 112: 8362–8366.
- 474 Endara, M.-J., D. L. Forrister, and P. D. Coley. 2023. The Evolutionary Ecology of Plant
475 Chemical Defenses: From Molecules to Communities. *Annual Review of Ecology, Evolution,*
476 *and Systematics* 54.
- 477 Forrester, N. J., and T.-L. Ashman. 2017. The direct effects of plant polyploidy on the legume–
478 rhizobia mutualism. *Annals of Botany* 121: 209–220.
- 479 Forrester, N. J., M. Rebolleda-Gómez, J. L. Sachs, and T.-L. Ashman. 2020. Polyploid plants
480 obtain greater fitness benefits from a nutrient acquisition mutualism. *New Phytologist* 227:
481 944–954.
- 482 Fox, D. T., D. E. Soltis, P. S. Soltis, T.-L. Ashman, and Y. V. de Peer. 2020. Polyploidy: A
483 Biological Force From Cells to Ecosystems. *Trends in Cell Biology* 30: 688–694.
- 484 Gaynor, M. L., S. Lim-Hing, and C. M. Mason. 2020. Impact of genome duplication on
485 secondary metabolite composition in non-cultivated species: A systematic meta-analysis.
486 *Annals of Botany* 126: mcaa107-.

- 487 Godfree, R. C., D. J. Marshall, A. G. Young, C. H. Miller, and S. Mathews. 2017. Empirical
488 evidence of fixed and homeostatic patterns of polyploid advantage in a keystone grass
489 exposed to drought and heat stress. *Royal Society Open Science* 4: 170934.
- 490 Gross, K., and F. P. Schiestl. 2015. Are tetraploids more successful? Floral signals, reproductive
491 success and floral isolation in mixed-ploidy populations of a terrestrial orchid. *Annals of*
492 *Botany* 115: 263–273.
- 493 Halder, J., A. B. Rai, S. Chakrabarti, and D. Dey. 2020. Distribution, Host Range and Bionomics
494 of *Rhopalosiphum nymphaeae* (Linnaeus, 1761) a Polyphagous Aphid in Aquatic Vegetables.
495 *Defence Life Science Journal* 5: 49–53.
- 496 Hamarashid, S. H., Y. Khaledian, and F. Soleimani. 2022. In vitro polyploidy-mediated
497 enhancement of secondary metabolites content in *Stachys byzantina* L. *Genetic Resources*
498 *and Crop Evolution* 69: 719–728.
- 499 Hance, Th., D. Nibelle, Ph. Lebrun, G. Impe, and C. Hove. 1994. Selection of *Azolla* forms
500 resistant to the water lily aphid, *Rhopalosiphum nymphaeae* Life history of *Rhopalosiphum*
501 *nymphaeae*. *Entomologia Experimentalis et Applicata* 70: 11–17.
- 502 Harkin, C., and A. J. A. Stewart. 2021. Differential outcomes of novel plant-herbivore
503 associations between an invading planthopper and native and invasive *Spartina* cordgrass
504 species. *Oecologia* 195: 983–994.
- 505 Harms, N. E., and D. J. Walter. 2021. Influence of *Butomus umbellatus* L. lineage and age on
506 leaf chemistry and performance of a generalist caterpillar. *Aquatic Botany* 172: 103391.
- 507 Hart, S. P., M. M. Turcotte, and J. M. Levine. 2019. Effects of rapid evolution on species
508 coexistence. *Proceedings of the National Academy of Sciences* 116: 201816298.
- 509 Hartig, F. 2022. DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed)
510 Regression Models. R package version 0.4.6.
- 511 Herve, M. 2023. RVAideMemoire: Testing and Plotting Procedures for Biostatistics. R package
512 version 0.9-83.
- 513 Hull-Sanders, H. M., R. H. Johnson, H. A. Owen, and G. A. Meyer. 2009. Influence of
514 polyploidy on insect herbivores of native and invasive genotypes of *Solidago gigantea*
515 (Asteraceae). *Plant Signaling & Behavior* 4: 893–895.
- 516 Jakobs, R., and C. Müller. 2018. Effects of intraspecific and intra-individual differences in plant
517 quality on preference and performance of monophagous aphid species. *Oecologia* 186: 173–
518 184.
- 519 Janz, N., and J. N. Thompson. 2002. Plant polyploidy and host expansion in an insect herbivore.
520 *Oecologia* 130: 570–575.

- 521 Jiao, Y., N. J. Wickett, S. Ayyampalayam, A. S. Chanderbali, L. Landherr, P. E. Ralph, L. P.
522 Tomsho, et al. 2011. Ancestral polyploidy in seed plants and angiosperms. *Nature* 473: 97–
523 100.
- 524 Kerstetter, J. E., A. L. Reid, J. T. Armstrong, T. A. Zallek, T. T. Hobbie, and M. M. Turcotte.
525 2023. Characterization of microsatellite markers for the duckweed *Spirodela polyrhiza* and
526 *Lemna minor* tested on samples from Europe or the United States of America. *bioRxiv*:
527 2023.02.15.528655.
- 528 Kolář, F., M. Čertner, J. Suda, P. Schönswetter, and B. C. Husband. 2017. Mixed-Ploidy
529 Species: Progress and Opportunities in Polyploid Research. *Trends in Plant Science* 22:
530 1041–1055.
- 531 König, M. A. E., C. Wiklund, and J. Ehrlén. 2016. Butterfly oviposition preference is not related
532 to larval performance on a polyploid herb. *Ecology and Evolution* 6: 2781–2789.
- 533 König, M. A. E., C. Wiklund, and J. Ehrlén. 2014. Context-dependent resistance against butterfly
534 herbivory in a polyploid herb. *Oecologia* 174: 1265–1272.
- 535 Krug, P., and A. J. Sosa. 2019. Mother knows best: plant polyploidy affects feeding and
536 oviposition preference of the alligator weed biological control agent, *Agasicles hygrophila*.
537 *BioControl* 64: 623–632.
- 538 Laird, R. A., and P. M. Barks. 2018. Skimming the surface: duckweed as a model system in
539 ecology and evolution. *American Journal of Botany* 105: 1962–1966.
- 540 Lavania, U. C., S. Srivastava, S. Lavania, S. Basu, N. K. Misra, and Y. Mukai. 2012.
541 Autopolyploidy differentially influences body size in plants, but facilitates enhanced
542 accumulation of secondary metabolites, causing increased cytosine methylation. *The Plant*
543 *Journal* 71: 539–549.
- 544 Levin, D. A., and D. E. Soltis. 2018. Factors promoting polyploid persistence and diversification
545 and limiting diploid speciation during the K–Pg interlude. *Current Opinion in Plant Biology*
546 42: 1–7.
- 547 Madlung, A. 2013. Polyploidy and its effect on evolutionary success: old questions revisited with
548 new tools. *Heredity* 110: 99–104.
- 549 Magalhães, T. L., K. Murphy, A. Efremov, V. Chepinoga, T. A. Davidson, and E. Molina-
550 Navarro. 2021. Ploidy state of aquatic macrophytes: Global distribution and drivers. *Aquatic*
551 *Botany* 173: 103417.
- 552 Malacrino, A., L. Böttner, S. Nouere, M. Huber, M. Schäfer, and S. Xu. 2022. Induced responses
553 contribute to rapid plant adaptation to herbivory. *bioRxiv*: 2022.11.24.517793.

- 554 Mariani, F., A. D. Giulio, S. Fattorini, and S. Ceschin. 2020. Experimental evidence of the
555 consumption of the invasive alien duckweed *Lemna minuta* by herbivorous larvae of the moth
556 *Cataglyphis lemnae* in Italy. *Aquatic Botany* 161: 103172.
- 557 Marmolejo, L. O., M. N. Thompson, and A. M. Helms. 2021. Defense Suppression through
558 Interplant Communication Depends on the Attacking Herbivore Species. *Journal of Chemical*
559 *Ecology* 47: 1049–1061.
- 560 McCarthy, E. W., M. W. Chase, S. Knapp, A. Litt, A. R. Leitch, and S. C. L. Comber. 2016.
561 Transgressive phenotypes and generalist pollination in the floral evolution of *Nicotiana*
562 polyploids. *Nature Plants* 2: 16119.
- 563 Münzbergová, Z., J. Skuhrovec, and P. Maršík. 2015. Large differences in the composition of
564 herbivore communities and seed damage in diploid and autotetraploid plant species.
565 *Biological Journal of the Linnean Society* 115: 270–287.
- 566 Nuismer, S. L., and J. N. Thompson. 2001. Plant polyploidy and non-uniform effects on insect
567 herbivores. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 268:
568 1937–1940.
- 569 O'Connor, T. K., R. G. Laport, and N. K. Whiteman. 2019. Polyploidy in creosote bush (*Larrea*
570 *tridentata*) shapes the biogeography of specialist herbivores. *Journal of Biogeography* 46:
571 597–610.
- 572 Parisod, C., R. Holderegger, and C. Brochmann. 2010. Evolutionary consequences of
573 autopolyploidy. *New Phytologist* 186: 5–17.
- 574 Porturas, L. D., T. J. Anneberg, A. E. Curé, S. Wang, D. M. Althoff, and K. A. Segraves. 2019.
575 A meta-analysis of whole genome duplication and the effects on flowering traits in plants.
576 *American Journal of Botany* 106: 469–476.
- 577 Powell, G., C. R. Tosh, and J. Hardie. 2006. Host plant selection by aphids: Behavioral,
578 evolutionary, and applied perspectives. *Annual Review of Entomology* 51: 309–330.
- 579 Ramsey, J., and T. S. Ramsey. 2014. Ecological studies of polyploidy in the 100 years following
580 its discovery. *Philosophical Transactions of the Royal Society B: Biological Sciences* 369:
581 20130352.
- 582 R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for
583 Statistical Computing, Vienna, Austria.
- 584 Rezende, L., J. Suzigan, F. W. Amorim, and A. P. Moraes. 2020. Can plant hybridization and
585 polyploidy lead to pollinator shift? *Acta Botanica Brasílica* 34: 229–242.
- 586 Rice, A., P. Šmarda, M. Novosolov, M. Drori, L. Glick, N. Sabath, S. Meiri, et al. 2019. The
587 global biogeography of polyploid plants. *Nature Ecology & Evolution* 3: 265–273.

- 588 Segraves, K. A., and T. J. Anneberg. 2016. Species interactions and plant polyploidy. *American*
589 *Journal of Botany* 103: 1326–1335.
- 590 Segraves, K. A., J. N. Thompson, P. S. Soltis, and D. E. Soltis. 1999. Multiple origins of
591 polyploidy and the geographic structure of *Heuchera grossulariifolia*. *Molecular Ecology* 8:
592 253–262.
- 593 Soltis, D. E., V. A. Albert, J. Leebens-Mack, C. D. Bell, A. H. Paterson, C. Zheng, D. Sankoff, et
594 al. 2009. Polyploidy and angiosperm diversification. *American Journal of Botany* 96: 336–
595 348.
- 596 Soltis, D. E., P. S. Soltis, and L. H. Rieseberg. 1993. Molecular Data and the Dynamic Nature of
597 Polyploidy. *Critical Reviews in Plant Sciences* 12: 243–273.
- 598 Soltis, D. E., C. J. Visger, D. B. Marchant, and P. S. Soltis. 2016. Polyploidy: Pitfalls and paths
599 to a paradigm. *American Journal of Botany* 103: 1146–1166.
- 600 Song, X.-M., J.-P. Wang, P.-C. Sun, X. Ma, Q.-H. Yang, J.-J. Hu, S.-R. Sun, et al. 2020.
601 Preferential gene retention increases the robustness of cold regulation in Brassicaceae and
602 other plants after polyploidization. *Horticulture Research* 7: 20.
- 603 Spoelhof, J. P., P. S. Soltis, and D. E. Soltis. 2017. Pure polyploidy: Closing the gaps in
604 autopolyploid research. *Journal of Systematics and Evolution* 55: 340–352.
- 605 Subramanian, S. K., and M. M. Turcotte. 2023. Experimentally quantifying impact of herbivory
606 on duckweed communities in natural pond ecosystems.
- 607 Subramanian, S. K., and M. M. Turcotte. 2020. Preference, performance, and impact of the
608 water-lily aphid on multiple species of duckweed. *Ecological Entomology* 45: 1466–1475.
- 609 Thompson, J. N., B. M. Cunningham, K. A. Segraves, D. M. Althoff, and D. Wagner. 1997.
610 Plant Polyploidy and Insect/Plant Interactions. *The American Naturalist* 150: 730–743.
- 611 Thompson, J. N., S. L. Nusimer, and K. Merg. 2004. Plant polyploidy and the evolutionary
612 ecology of plant/animal interactions. *Biological Journal of the Linnean Society* 82: 511–519.
- 613 Tossi, V. E., L. J. M. Tosar, L. E. Laino, J. Iannicelli, J. J. Regalado, A. S. Escandón, I. Baroli, et
614 al. 2022. Impact of polyploidy on plant tolerance to abiotic and biotic stresses. *Frontiers in*
615 *Plant Science* 13: 869423.
- 616 Van-de-Peer, Y., T.-L. Ashman, P. S. Soltis, and D. E. Soltis. 2020. Polyploidy: an evolutionary
617 and ecological force in stressful times. *The Plant Cell* 33: 11–26.
- 618 Walczyk, A. M., and E. I. Hersch-Green. 2019. Impacts of soil nitrogen and phosphorus levels
619 on cytotype performance of the circumboreal herb *Chamerion angustifolium*: implications for
620 polyploid establishment. *American Journal of Botany* 106: 906–921.

- 621 Warner, D. A., and G. E. Edwards. 1993. Effects of polyploidy on photosynthesis.
622 *Photosynthesis Research* 35: 135–147.
- 623 Wei, N., R. Cronn, A. Liston, and T.-L. Ashman. 2019. Functional trait divergence and trait
624 plasticity confer polyploid advantage in heterogeneous environments. *The New Phytologist*
625 221: 2286–2297.
- 626 Wei, N., Z. Du, A. Liston, and T.-L. Ashman. 2020. Genome duplication effects on functional
627 traits and fitness are genetic context and species dependent: studies of synthetic polyploid
628 *Fragaria*. *American Journal of Botany* 107: 262–272.
- 629 Wood, T. E., N. Takebayashi, M. S. Barker, I. Mayrose, P. B. Greenspoon, and L. H. Rieseberg.
630 2009. The frequency of polyploid speciation in vascular plants. *Proceedings of the National*
631 *Academy of Sciences* 106: 13875–13879.
- 632 Xu, J., X. Wang, H. Zu, X. Zeng, I. T. Baldwin, Y. Lou, and R. Li. 2021. Molecular dissection of
633 rice phytohormone signaling involved in resistance to a piercing-sucking herbivore. *New*
634 *Phytologist* 230: 1639–1652.
- 635 Xu, N., F. Hu, J. Wu, W. Zhang, M. Wang, M. Zhu, and J. Ke. 2018. Characterization of 19
636 polymorphic SSR markers in *Spirodela polyrhiza* (Lemnaceae) and cross-amplification in
637 *Lemna perpusilla*. *Applications in Plant Sciences* 6: e01153.
- 638 Yang, P.-M., Q.-C. Huang, G.-Y. Qin, S.-P. Zhao, and J.-G. Zhou. 2014. Different drought-stress
639 responses in photosynthesis and reactive oxygen metabolism between autotetraploid and
640 diploid rice. *Photosynthetica* 52: 193–202.
- 641 Ziegler, P., K. Adelman, S. Zimmer, C. Schmidt, and K. -J. Appenroth. 2015. Relative in vitro
642 growth rates of duckweeds (Lemnaceae) – the most rapidly growing higher plants. *Plant*
643 *Biology* 17: 33–41.
- 644 Züst, T., and A. A. Agrawal. 2016. Trade-Offs Between Plant Growth and Defense Against
645 Insect Herbivory: An Emerging Mechanistic Synthesis. *Annual Review of Plant Biology* 68:
646 1–22.
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656 **TABLES**

657 **Table 1.** Preference trial results and G-values in the Abundance controlled trial (when *equal*
 658 *number of duckweed (Spirodela polyrhiza) fronds* were used). G-values, degrees of freedom, and
 659 p-values are given for each lineage, their sum, as well as pooled across all lineages.

Lineage	Diploid chosen	Neopolyploid chosen		G-value	d.f.	P-value
SP.01	5	14		4.43	1	0.035
SP.05	6	14		3.29	1	0.069
SP.11	7	12		1.33	1	0.25
SP.41	6	13		2.64	1	0.10
SP.43	7	13		1.83	1	0.18
			total G	13.53	5	0.019
pooled	31	66	pooled G	12.92	1	0.00033
			heterogeneity G	0.6121	4	0.96

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661 **Table 2.** Preference trial results and G-values in the Area Controlled trial (when *equal total area*
662 *of duckweeds were used*). G-values, degrees of freedom, and p-values are given for each lineage,
663 their sum, as well as pooled across all lineages.

Lineage	Diploid chosen	Neopolyploid chosen		G-value	d.f.	P-value
SP.01	10	10		0	1	1
SP.05	8	12		0.81	1	0.82
SP.11	10	10		0	1	1
SP.41	6	14		3.29	1	0.07
SP.43	10	9		0.05	1	0.82
			total G	4.15	5	0.53
pooled	44	55	pooled G	1.22	1	0.27
			heterogeneity G	2.92	4	0.57

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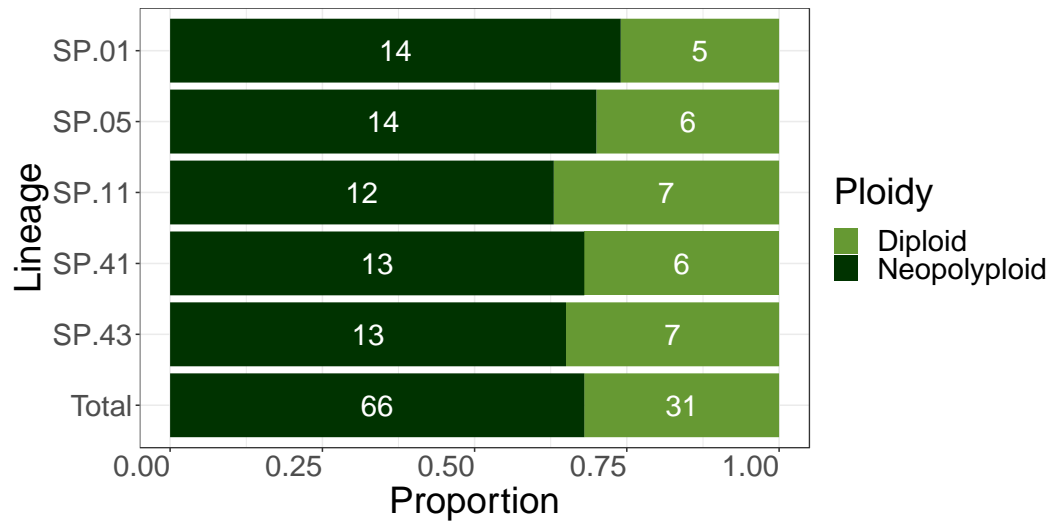
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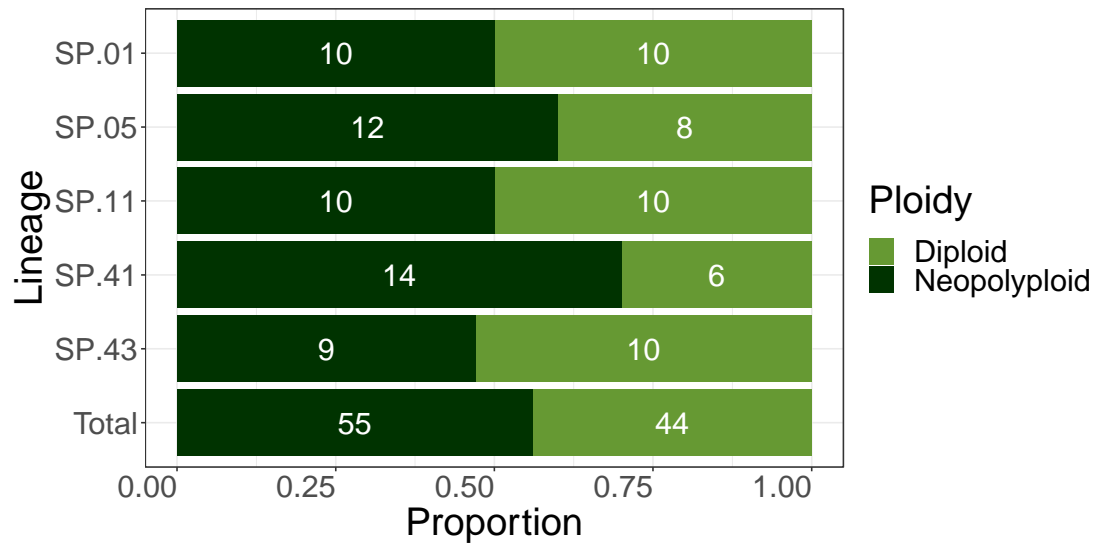
669 **Figures**

670 a)



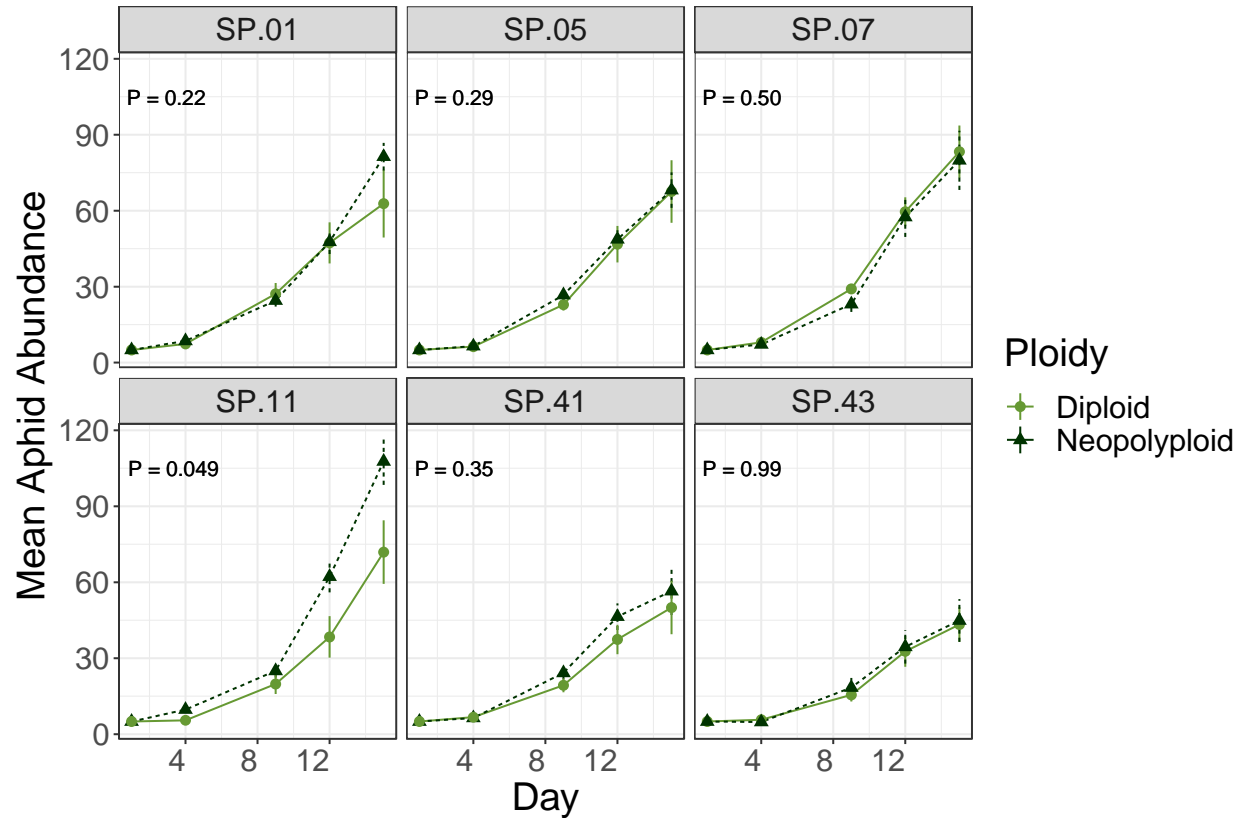
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672 b)



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674 **Figure 1** Water-lily aphid herbivore preference (number of trials aphids chose diploid or
675 neopolyploid plant) in the **a)** Abundance-controlled trial and the **b)** Area-controlled trial. Results
676 are presented by lineage (number) and summed across all lineages (Total).



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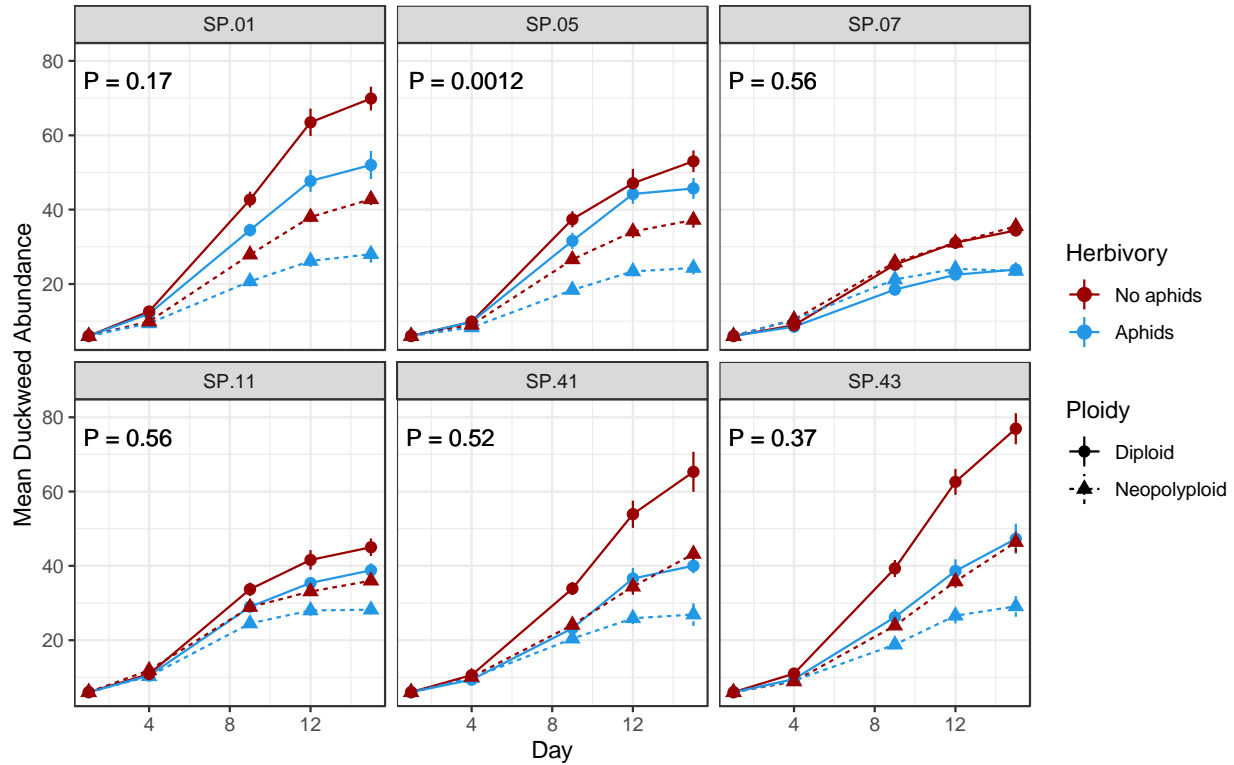
678 **Figure 2** Aphid abundances over time when feeding on diploid or neopolyloid duckweed. Each

679 panel represents a different duckweed lineage, and each point is the mean and standard error of

680 10 replicates. P-values on the graph represent the significance of ploidy in the lineage-specific

681 GLMMs (See Table S4 for full model results)

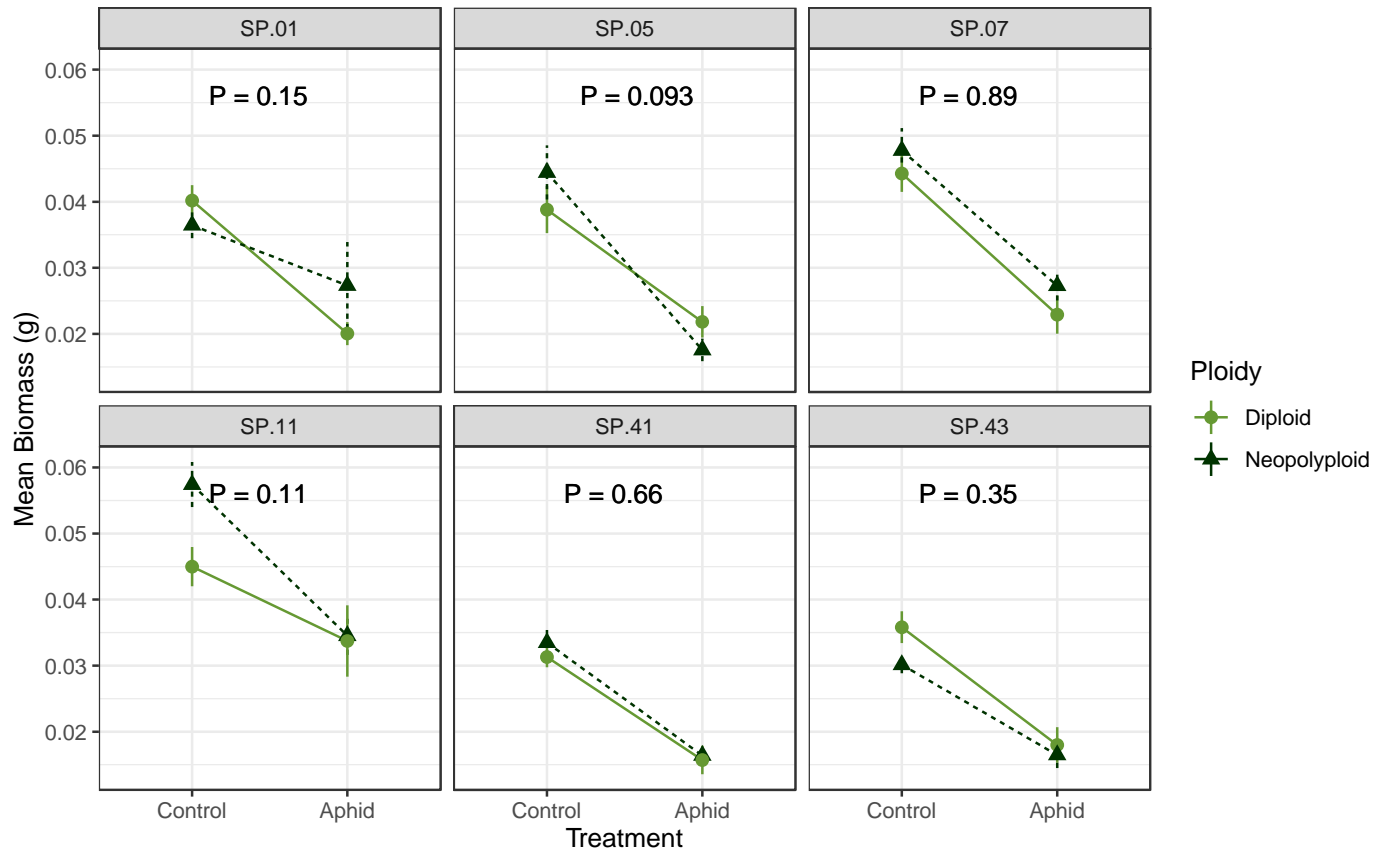
682



683

684 **Figure 3** Duckweed abundance over time for diploid and neopolyploids with (red) and without
685 aphids (blue). Each panel represents a different duckweed lineage, and each point represents the
686 mean and standard error of 10 replicates. P values represent the Ploidy:Herbivory interaction and
687 are calculated from the lineage-specific Poisson GLMMs (See Table S6 for full model results).

688



689

690 **Figure 4)** Duckweed biomass by genotype: Genotype-specific changes in biomass following the

691 addition of herbivory in both the diploid and the polyploid treatments.