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

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Abstract

Premise: Polyploidy is a widespread mutational process in angiosperms that may alter population performance of not only plants but also their interacting species. Yet, knowledge of whether polyploidy affects plant–herbivore dynamics is scarce. Here, we tested whether aphid herbivores exhibit preference for diploid or neopolyploid plants, whether polyploidy impacts plant and herbivore performance, and whether these interactions depend on the plant genetic background.

Methods: Using independently synthesized neotetraploid strains paired with their diploid progenitors of greater duckweed (*Spirodela polyrhiza*), we evaluated the effect of neopolyploidy on duckweed's interaction with the water-lily aphid (*Rhopalosiphum nymphaeae*). Using paired-choice experiments, we evaluated feeding preference of the herbivore. We then evaluated the consequences of polyploidy on aphid and plant performance by measuring population growth over multiple generations.

Results: Aphids preferred neopolyploids when plants were provided at equal abundances but not at equal surface areas, suggesting the role of plant population surface area in driving this preference. Additionally, neopolyploidy increased aphid population performance, but this result was dependent on the plant's genetic lineage. Lastly, the impact of herbivory on neopolyploid vs. diploid duckweed varied greatly with genetic lineage, where neopolyploids appeared to be variably tolerant compared to diploids, sometimes mirroring the effect on herbivore performance.

Conclusions: By experimentally testing the impacts of polyploidy on trophic species interactions, we showed that polyploidization can impact the preference and performance of herbivores on their plant hosts. These results have significant implications for the establishment and persistence of plants and herbivores in the face of plant polyploidy.

KEYWORDS

Araceae, cytotype, duckweed, freshwater herbivory, host preference, neopolyploidy, plant-herbivore interactions, performance, water lily aphid, whole-genome duplication

Whole-genome duplication is a dramatic and prevalent mechanism of differentiation in plants (Nuismer and Thompson, 2001; Thompson et al., 2004; Arvanitis et al., 2010; Ramsey and Ramsey, 2014; Segraves and Anneberg, 2016). Polyploidy is increasingly being recognized as an important driving force in the evolution of all angiosperms; 35% of extant angiosperms are of recent polyploid origin, and all have at least one whole-genome duplication event in their evolutionary past (Soltis et al., 2009; Wood et al., 2009; Jiao et al., 2011). Polyploidy is extremely widespread, with frequencies reaching as high as 51% in some terrestrial biomes (Rice et al., 2019). Additionally, mixed-ploidy species, which exhibit both diploid and polyploid cytotypes, are also commonly found

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across the globe (Kolář et al., 2017). Researchers have long sought to understand why polyploids seem to experience greater ecological and evolutionary success, particularly by investigating the genotypic and phenotypic differences of polyploids from their diploid progenitors (Roose and Gottlieb, 1976; Comai, 2005; Baldwin and Husband, 2013; Madlung, 2013; Levin and Soltis, 2018; Fox et al., 2020; Van de Peer et al., 2020)

Polyploid plants often differ from their diploid ancestors in a variety of ways that can impact their interaction with abiotic and biotic factors (Gross and Schiestl, 2015; Wei et al., 2019; Clo and Kolář, 2021). Specifically, plant size, trichome size/number, leaf thickness, secondary metabolite production, and cellulose content are just some of the traits that may be affected by whole-genome duplication (Wei et al., 2019; Bomblies, 2020; Hamarashid et al., 2022; Malacrinò et al., 2024). For example, the gigas effect, or the enlargement of plant cells due to the increased amounts of DNA, often leads to polyploid plants being larger than their diploid progenitors (Doyle and Coate, 2019; Bomblies, 2020; Clo and Kolář, 2021).

It has been argued that the changes associated with polyploidy can lead to an enhanced tolerance to abiotic stressors such as heat, cold, salt, and nutritional stress (Yang et al., 2014; Godfree et al., 2017; Song et al., 2020; Tossi et al., 2022; Anneberg et al., 2023a). Yet, tolerance to biotic stressors has received much less attention, despite the likelihood that phenotypic differences also impact species interactions (Segraves and Anneberg, 2016; Forrester and Ashman, 2017; Rezende et al., 2020; Anneberg et al., 2023a, 2023b). Specifically, polyploidy-induced phenotypic and genotypic changes can lead to novel interactions with other species, such as herbivores, pollinators, and microbes (Arvanitis et al., 2010; McCarthy et al., 2016; Porturas et al., 2019; Walczyk and Hersch-Green, 2019; Forrester et al., 2020; Rezende et al., 2020; Curé et al., 2022; Anneberg et al., 2023b).

Many of the phenotypic changes associated with polyploidy may impact the herbivores that feed on the plant (Bagheri and Mansouri, 2015; Corneillie et al., 2018; Hamarashid et al., 2022; Malacrinò et al., 2024). For example, the enlargement of plants through the gigas effect may increase their visibility and detectability, or their apparency, to herbivores, leading to a greater number of herbivores being found on polyploids (Martini, 2021). This change in size often comes at the cost of slower growth rate, which may in turn impact the plant's ability to tolerate and recover from herbivore damage (Züst and Agrawal, 2016; Corneillie et al., 2018; DeRose et al., 2022; Anneberg et al., 2023a). Similarly, ploidy-driven changes in the photosynthetic rate of the plant could allow for faster or slower recovery depending on the direction (Warner and Edwards, 1993; Cao et al., 2018). Further, polyploidization may impact secondary-metabolite production, and polyploids may acquire higher levels of defenses against herbivores (Lavania et al., 2012; Bagheri and Mansouri, 2015; Edger et al., 2015; Gaynor et al., 2020).

Compared to other biotic interactions, evidence for polyploidy's effect on plant–herbivore interactions are heavily weighted toward herbivore attraction, attack, and resultant plant performance (Arvanitis et al., 2010; Gross and Schiestl, 2015; Münzbergová et al., 2015; O'Connor et al., 2019). Additionally, results from these studies are mixed. For example, Halverson et al. (2008) found that neither diploid nor polyploid plants of *Solidago altissima* were consistently attacked more frequently by five species of herbivores. Similarly, Thompson et al. (1997) found that the moth *Greya potentilla* was sometimes more likely to attack tetraploids over diploid plants of *Heuchera grossularifolia*, but they were still able to colonize both diploids and polyploids of separate origin.

While insightful, conclusions about the impact on the plant–herbivore relationship are hard to synthesize from these studies, as most prior studies suffer from four limitations. First, these studies did not simultaneously address the impact on both herbivore and plant populations. By studying both concurrently, one can understand the reciprocal consequences of polyploidy on the entire relationship. Specifically, one can potentially gain insight into how coevolutionary dynamics might be impacted. For instance, if polyploidy enhances plant tolerance and/or resistance to herbivory, is this effect mirrored in the herbivore, resulting in decreased performance on polyploid plants compared to their diploid counterparts?

Second, prior studies typically used established polyploids in their research as opposed to newly formed, or neopolyploids. While naturally occurring polyploids provide valuable insights into the long-term evolution of polyploids, they have some drawbacks, as they confound the effects of hybridization and subsequent evolution following polyploidization (Parisod et al., 2010; Drunen and Husband, 2018; Bomblies, 2020). By using synthetic autopolyploids, or neopolyploids, one can isolate the immediate consequences of whole-genome duplication alone on the plant–herbivore interaction. Notably, however, Curé et al. (2022) did test the preference and performance of a specialist pea aphid, *Acyrthosiphon pisum*, on diploid and neotetraploid cytotypes of two different host plants. They found no ploidal-dependent preference in two species, red clover and alfalfa, but they did find that aphids that originated from populations specialized on diploid red clover had higher fecundity on that host than on synthesized neotetraploid red clover. To our knowledge, this is the only study to quantify herbivore performance on diploids and neopolyploids, and it has opened the door to build on these results by also quantifying plant performance and expanding to new plant–herbivore systems.

The third limitation of previous studies is that the outcome of whole-genome duplication may vary depending on the genotypic background of the individual, so multiple genotypes should be used in a single study (Drunen and Husband, 2018; Wei et al., 2020; Bafort et al., 2023). While much effort has been focused on capturing the variation in the responses to polyploidy across different species, it is also important to recognize the intraspecific variation in response to polyploidy due to genetic differences in progenitor diploids (Soltis et al., 2016; Castro et al., 2020; Bafort et al., 2023; Anneberg et al., 2023b). Polyploidy can arise multiple times independently within a single taxon across genetically divergent individuals, leading to differences in the effects of whole-genome duplication within a single species (Soltis et al., 1993; Segraves et al., 1999). For example, in the context of species interactions, Anneberg et al. (2023b) recently found that the effect of polyploidy on duckweed microbiomes was different across multiple, independently synthesized neopolyploid lineages of duckweed. Yet, our knowledge of how other species interactions may be impacted by the interactions of polyploidization and genetic background, such as plant–herbivore interactions, is still limited.

Lastly, most studies of polyploidy–herbivory interactions are conducted over only a small portion of the life history of the plant or herbivore and usually on single individuals. This limited scope may thus constrain our ability to draw conclusions on the fitness impacts of polyploidy under herbivory, as fitness measured on single individuals ignores density-dependence and may not correlate with population performance. To our knowledge, there are no population-level experimental studies investigating both plant and herbivore performance and herbivore preference in the context of neopolyploidy. Consequently, there is a need for manipulative plant–herbivore experiments examining the immediate effects of whole-genome duplication at the population level, using multiple independently created polyploid genotypes (Parisod et al., 2010; Kolář et al., 2017; Spoelhof et al., 2017; Drunen and Husband, 2018; Anneberg et al., 2023b).

Duckweed (Araceae) is well suited to fill this knowledge gap in how neopolyploidy affects plant–herbivore interactions (Laird and Barks, 2018). Duckweeds are globally distributed, small, aquatic, floating plants that primarily reproduce asexually via budding. An individual consists of a single frond, a small leaf-like structure making up the entire shoot, and multiple roots (Ziegler et al., 2015; Acosta et al., 2021). Duckweeds reproduce rapidly (within 4 days in optimal conditions), and thus multiple generations can be studied in the span of several weeks (Ziegler et al., 2015; Hart et al., 2019). While there is natural variation in many duckweed species, greater duckweed (*Spirodela polyrhiza*) is gaining traction as a model system for polyploidy and herbivory owing to the amenability of population-level studies using synthesized neotetraploid plants, affording direct comparison of neopolyploid populations to those of their diploid progenitors (Bafort et al., 2023; Anneberg et al., 2023a; Turcotte et al., 2024). Combined with experimental studies of herbivores, population-level impacts of herbivory can be precisely studied from both the plant and herbivore perspective (Mariani et al., 2020; Subramanian and Turcotte, 2020, 2023). Further, while studies of the mechanisms behind polyploid success in terrestrial species have been accumulating over the last few decades, comparatively less is known for aquatic species. Polyploidy may have non-uniform effects on aquatic plants when compared to their terrestrial counterparts (Lodge, 1991; Prančl et al., 2014; Magalhães et al., 2021). For example, aquatic plants have, on average, higher rates of primary production, and the nutrient dynamics in aquatic systems are different than terrestrial ones (Cyr and Pace, 1993; Nowlin et al., 2008). Furthermore, the reproductive strategies of some aquatic plants, such as the rapid reproduction in duckweed, may result in differences in their abilities to resist or tolerate herbivory (Subramanian and Turcotte, 2020).

The water-lily aphid, *Rhopalosiphum nymphaeae*, is a globally distributed generalist herbivore of duckweeds (Halder et al., 2020; Subramanian and Turcotte, 2020, 2023). Aphids are phloem-feeding herbivores that reproduce facultatively parthenogenetically via live birth with a population doubling time of around 2 days (Hance et al., 1994). Because both aphids and duckweeds are fast-reproducing, asexual organisms, together they provide a unique opportunity to evaluate the effect of neopolyploidy on population growth rates of both host and herbivore over multiple generations.

Here, we addressed four questions using independently synthesized neotetraploid strains paired with their diploid progenitors of greater duckweed in a paired-choice experiment and fully randomized, factorial growth chamber experiment: (1) Do aphids exhibit a preference for diploid or neopolyploid duckweed at equal frequencies, and does this preference change if we control for total plant surface area? (2) Does duckweed ploidy alter aphid population performance? (3) Do aphids differentially affect the performance of neopolyploid and diploid duckweed populations? (4) Are the results of the previous questions dependent upon the genetic lineage of duckweed?

MATERIALS AND METHODS

Cultivation of duckweed and aphids

We used colchicine-induced autotetraploid and colchicine-exposed but unconverted diploids as described by Anneberg et al. (2023a) to answer our questions. To obtain these, we had previously applied the mitotic inhibitor colchicine to induce whole-genome duplication in six genetically distinct diploid *S. polyrhiza* collected from eastern Pennsylvania and western Ohio, United States (see Appendix S1, Table S1 for collection site info; Xu et al., 2018; Kerstetter et al., 2023; Anneberg et al., 2023a). Then, in 2019 and 2020, ploidy was confirmed using flow cytometry following the methods of Wei et al. (2020). Although we did not observe any residual effects of colchicine treatment, to be conservative, we used the colchicine-treated but unconverted diploid individuals in the experiment (Anneberg et al., 2023a). Before the experiment, we grew individual lineages of duckweed in the growth chamber at 23.5°C, 50% humidity, 50 µmol m^{-2} s^{-1} light, and 16 h light/8 h dark.

We established laboratory stock populations of water-lily aphids from a single individual collected from a duckweed community that comprised several species of duckweed (*Spirodela polyrhiza*, *Lemna minor*, and *Wolffia brasiliensis*) at Twin Lakes Park in Westmoreland County, Pennsylvania (40.323383333, −79.472383333) in September 2017 (Subramanian and Turcotte, 2020). Populations formed from a single isofemale lineage were then maintained on monocultures of diploid *S. polyrhiza* populations in the growth chamber.

Preference experiments

To determine whether aphids exhibit feeding preference for diploid or neotetraploid duckweed, we conducted several paired-choice trials following the basic set-up established in Subramanian and Turcotte (2020) in January and February 2023 (see Figure S1 in Appendix S1 for photos of the experimental set-ups). Each trial consisted of a diploid and its corresponding derived neopolyploid in preference arenas. On average, our neopolyploid duckweed were 46% larger in surface area than their diploid progenitors (Appendix S1, Table S2). Considering this size difference, we wanted to investigate whether total plant population surface area affected aphid choice. To do so, we conducted two separate sets of trials in preference arenas. The first was the abundance-controlled trial; we added exactly six fronds of each ploidy of a given lineage to the arenas. The second was the area-controlled trial; we added approximately equal population surface area of each ploidy of a given lineage to the preference arenas. We equalized ploidal surface area by first placing duckweed in 3.5-cm^2 cells of a culture plate, such that there was a single, non-overlapping layer of duckweed floating on the surface before moving pairs of them to the preference arenas. The preference arenas consisted of 60-mL jars with 19.6-cm^2 openings that were filled with 50 mL of 0.1× strength, sterile plant growth medium (Appenroth et al., 1996). This strength is relatively high in nutrients compared to natural conditions and was used to ensure nutrients were not limited during the 24-h experiment. In the center of the 60-mL jar, we floated a 0.6-cm-diameter circle of white plastic as a platform for the aphid in the middle of the duckweed population. The diploid and neopolyploid plants were intermixed around the platform, and then we placed a single third instar aphid on the platform. We tested preference on five of the six genetic lineage pairs of diploid–neopolyploid duckweeds. One genetic lineage (SP.07) was omitted due to contamination with algae that was later cleaned, allowing its use in the performance trials (see below). Both the area- and abundance-controlled trials were replicated 20 times per genetic lineage, for a total of 200 paired trials (5 genetic lineages × 2 trial types × 20 replicates). All aphids and duckweeds were only used once. We determined preference by observing which ploidy the aphid chose to insert its stylet (Subramanian and Turcotte, 2020). We used feeding over 24 h here as a proxy for preference, but we recognize our results may be a conservative estimate. We recorded aphid preference after 1, 5, 30, 60, and 90 min and 24 h. If the aphid died or crawled out of the jar (which only occurred four times out of 200 trials), no choice was recorded, and it was removed from the data analysis. For 93% of the trials, the aphid stayed on the same individual originally chosen (usually in the first 1 or 5 min), so only the final choice at 24 h was used in the analysis.

Performance experiment

We assessed aphid and duckweed performance in a separate, full factorial experiment where we crossed ploidy, genetic lineage, and aphid presence in a growth chamber. We added 220 mL of 0.5 × strength growth medium to 240 mL glass jars. Into each, we added six individuals of a single ploidy from a single genetic lineage of duckweed. Stronger media was used for the performance experiment because this experiment ran for 15 days as opposed to 24 h, and we wanted to ensure nutrients were not limiting at the start of the experiment. We then randomly chose jars to add either five third instar aphids or no aphids. Each combination was replicated 10 times for a total of 240 jars (6 genetic lineages × 2 ploidies × 2 aphid treatments × 10 replicates). The 10 replicates were split into two time-blocks of five, run consecutively. Each experiment lasted 15 days, allowing for three to four generations of both duckweeds and aphids. We quantified population growth of the aphids by counting their abundances five times over 15 days. We quantified duckweed performance in two ways, both of which represent multigenerational fitness. First, we quantified duckweed population abundance over time by counting their abundances two to three times per week. Because aphids and duckweed reproduce asexually under these conditions, abundance serves as a direct measure of population performance. Second, at the end of the 15 days, we measured final biomass by harvesting all duckweeds at the end of the experiment, drying them at 55°C for 1 week, and weighing them using a microbalance scale to the nearest 0.0001 g.

Statistical analyses

We tested for an effect of neopolyploidy on aphid preference, aphid performance, and plant performance (in terms of both abundances and final biomass). All analyses were performed in R version 4.1.2 (R Core Team, 2021). For the preference trials, we conducted *G*-tests of goodness of fit for each lineage in each trial type to test whether there was an overall deviation from the expected proportion of no preference or of half choosing diploids and half choosing neopolyploids. For each trial type (area- or abundance-controlled), we computed the total *G* (summed across lineages) and pooled *G* and calculated the heterogeneity *G* to assess whether there was significant variation among genetic lineages using the RVAideMemoire package (Herve, 2023). For the abundance-controlled trials, we also performed supplemental *G*-tests, where the null hypothesis for preference used in the calculation was derived from the ratio of the surface-area differences between each individual lineage. We did this as a secondary way (in addition to the area-controlled trials) to test whether the observed preferences could be attributed to variations in surface area among the different genetic lineages.

For the performance experiments, we constructed separate generalized linear models (GLMMs) with aphid population abundance, plant population abundance, and final plant biomass as response variables. For aphid population abundance, we used a GLMM with a negative binomial probability distribution, with aphid abundances as the response variable and ploidy (diploid or neopolyploid) and genetic lineage (as a categorical factor) and their interaction as main effects. We also included experimental time-block as a fixed effect. Lastly, to account for repeated measures during the experiment, we included day (day of sampling) and jar ID (individual experimental unit) as crossed random effects. We initially attempted to include day with interactions with the main effects, but the model was overfit, so we instead included it as a crossed random effect. We removed the abundance on day 1 from this analysis because all experimental units started with the same number of aphids (five). Linear mixed effects models were run using the glmmTMB package (Brooks et al., 2017). We used a negative binomial distribution to account for overdispersion in the aphid population data. All model residuals were assessed using the DHARMa package (Hartig, 2022).

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

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

We ran Type III ANOVAs on all preference model outputs using the car package (Fox and Weisberg, 2019). We also ran lineage-specific GLMMs for all performance models (aphid abundance, duckweed abundance, and duckweed biomass) post hoc to see which, if any, genetic lineages were driving significant effects. Lineage-specific models had the same structure as the overall models but without the genetic lineage response variable. We also calculated estimated group means using the emmeans package (Lenth, 2021) for the performance models to effectively compare effects between the different treatments.

RESULTS

Herbivore preference and performance

When duckweed ploidies were matched by frond abundance in the abundance-controlled trial, we found a significant aphid preference for neopolyploid plants over diploids (Figure 1A, Table 1). Pooled across all lineages, aphids chose polyploids in 66 of 100 trials, and this preference was consistent across all genetic lineage pairs. Given that diploids were only chosen 31 times implies that neopolyploids were 213% as likely to be attacked by aphids than diploids. Our aphid results, however, did not differ significantly from a null hypothesis based on the measured surface-area ratios (Appendix S1, Table S2) between ploidies for any lineage (*G*-test; Appendix S1, Table S3). This finding was confirmed in the area-controlled trial; when duckweed ploidies were matched by total surface area, aphids did not exhibit significant preference at the individual lineage level or across all lineages (Figure 1B, Table 2). Specifically, in 54 of the trials, the aphid chose the neopolyploid, and in 46 of the trials, the aphid chose the diploid.

Plant polyploidy alone increased aphid performance ($P = 0.0031$; Figure 2; Appendix S1, Table S4), but genetic lineage and its interaction with ploidy also significantly affected aphid performance ($P < 0.001$; Figure 2; Appendix S1, Table S4). Across all lineages, neopolyploids hosted more aphids than diploids at by the end of the experiment (Appendix S1, Table S6). Yet, the significant ploidy × lineage interaction indicates that the effect of ploidy on performance varied by lineage; for example, aphids on neopolyploid SP.11 reached higher abundances than aphids on diploid SP.11, whereas aphids on neopolyploid SP.07 reached lower abundances than aphids on diploid SP.07 (Appendix S1,

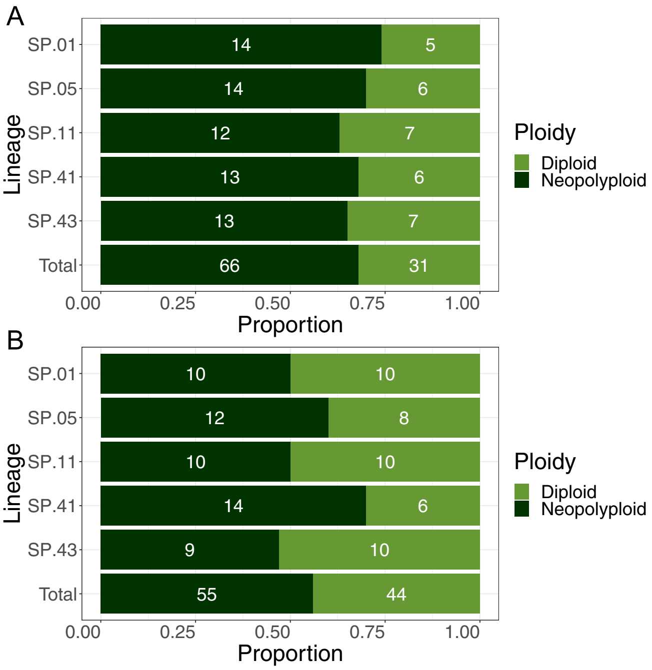

FIGURE 1 Water-lily aphid herbivore preference (number of trials that aphids chose diploid or neopolyploid plants) in the (A) abundance-controlled trial and the (B) area-controlled trial. Results are presented by lineage (number) and summed across all lineages (total).

TABLE 1 Preference trial results and *G*-values in the abundance-controlled trial when an equal number of duckweed (*Spirodela polyrhiza*) fronds were used.

Lineage	Diploid chosen	Neopolyploid chosen		*G*	df	*P*
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*	0.61	4	0.96

Notes: G-values, degrees of freedom, and P-values are given for each lineage, their sum, and pooled across all lineages.

TABLE 2 Preference trial results and *G*-values in the area-controlled trial when an equal total area of duckweeds was used.

Lineage	Diploid chosen	Neopolyploid chosen		*G*	df	*P*
SP.01	10	10		0	1	1
SP.05	8	12		0.81	1	0.37
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

Notes: G-values, degrees of freedom, and *P*-values are given for each lineage, their sum, and pooled across all lineages.

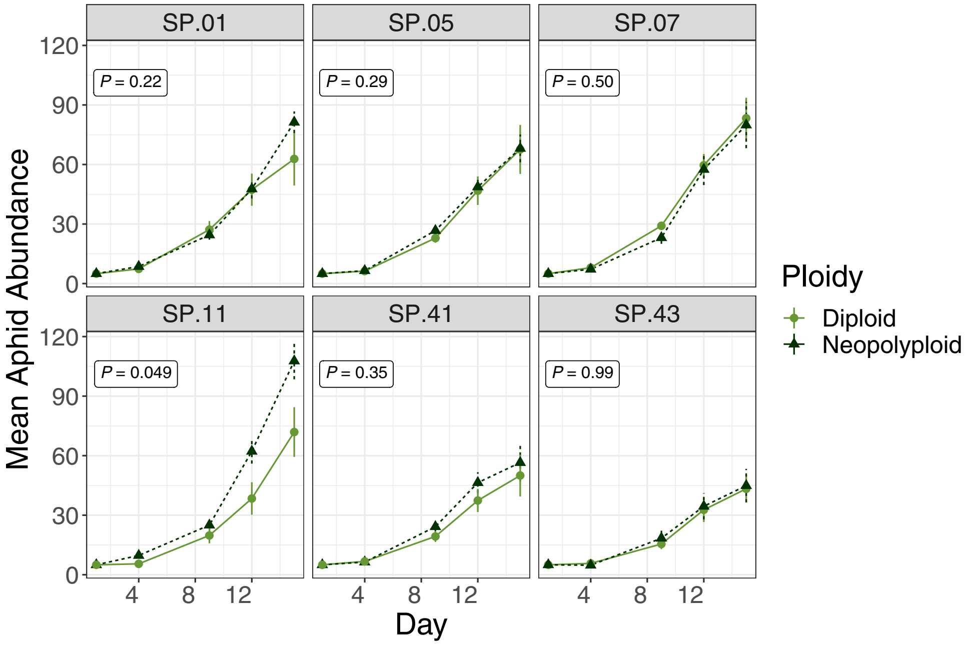

FIGURE 2 Aphid abundances over time after feeding on diploid or neopolyploid duckweed. Each panel represents a different duckweed lineage (given at top of each graph), and each point is the mean and standard error of 10 replicates. P-values represent the significance of ploidy in the lineage-specific GLMMs (see Appendix S1, Tables S4 and S5 for full model results).

Tables S4, S6). Only one lineage, SP.11, showed significant effects of neopolyploidy on aphid abundance in the lineage-specific models ($P = 0.049$; Appendix S1, Table S5).

Duckweed performance

Abundance

Overall, the difference between neopolyploid and diploid duckweed abundance in the face of herbivory is significantly dependent on both ploidy and lineage (three-way ploidy × herbivory × lineage interaction, $P = 0.061$; Figure 3; Appendix S1, Table S7). For some lineages (SP.05 and SP.11), neopolyploid duckweed abundance was more impacted by herbivory than diploid abundance was impacted, but overall, the result was highly dependent on the genetic lineage (Figure 3; Appendix S1, Tables S7, S9). Independent duckweed lineages also responded to neopolyploidy differently (ploidy × lineage interaction, $P < 0.001$; Figure 3; Appendix S1, Table S7). And while there was no significant ploidy × herbivory interaction ($P = 0.077$; Appendix S1, Table S7), indicating neopolyploids overall were not significantly more or less tolerant to herbivory than diploids in terms of abundance, there were strong lineage-dependent responses to herbivory (lineage × herbivory interaction, $P < 0.001$; Figure 3; Appendix S1, Tables S7, S9). It is worth noting, however, that lineage-specific models reveal that SP.05 was the only lineage to exhibit a significant ploidy × herbivory interaction, wherein the neopolyploid's abundance was more significantly impacted and less tolerant to aphids than the diploid (Appendix S1, Table S8).

Biomass

Aphid presence significantly reduced duckweed final biomass across all treatments but did so unevenly among ploidies (Figure 4; Appendix S1, Tables S10, S11). Aphids impacted neopolyploid biomass slightly more diploid biomass at a marginally significant level (ploidy × herbivory interaction, $P = 0.063$; Figure 4; Appendix S1, Table S10), but the differences were small (Appendix S1, Table S12). This result, however, also varied among genetic lineages at a marginally significant level (ploidy × herbivory × lineage interaction, $P = 0.065$; Appendix S1, Table S10); neopolyploids of SP.05, SP.11, and SP.41 were all less tolerant than their diploid progenitors in terms of biomass, whereas neopolyploids of SP.01, SP.07, and SP.43 were all more tolerant than diploids.

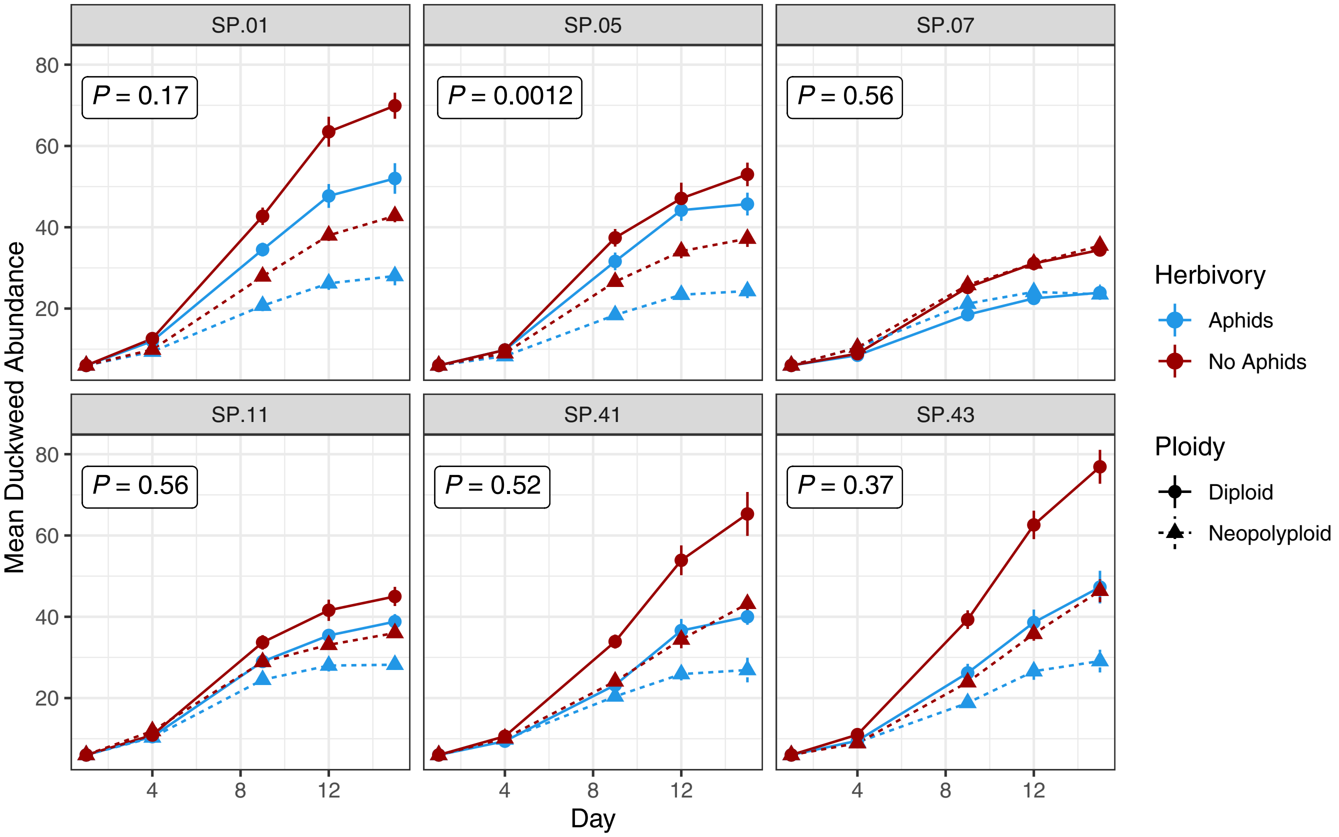

FIGURE 3 Duckweed abundance over time for diploid and neopolyploids with (red) and without aphids (blue). Each panel represents a different duckweed lineage (given at the top of each graph), and each point represents the mean and standard error of 10 replicates. *P*-values represent the ploidy × herbivory interaction and are calculated from the lineage-specific Poisson GLMMs (see Appendix S1 [Tables S7, S8] for full model results).

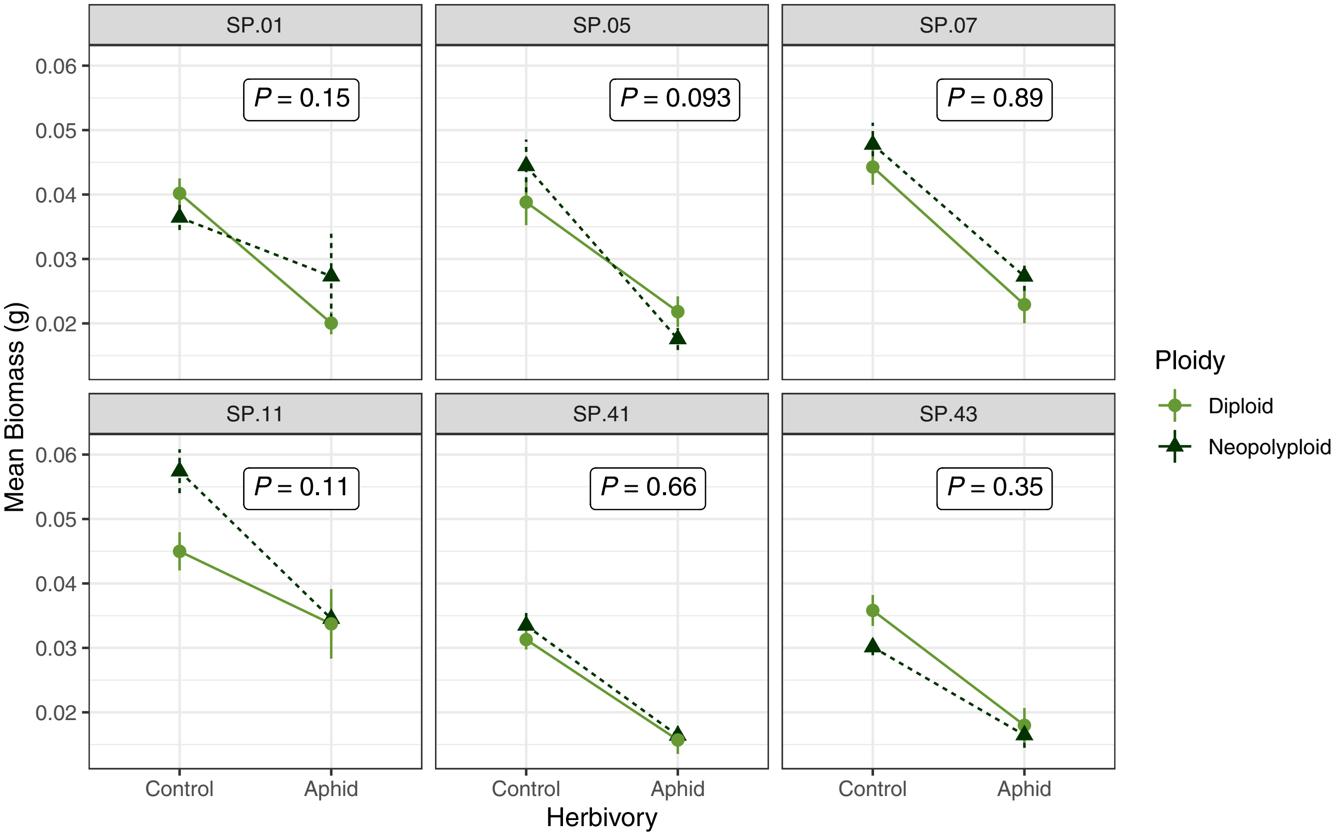

FIGURE 4 Duckweed biomass by genotype. Genotype-specific changes in biomass after the addition of herbivory in the diploid and the neopolyploid treatments. Each panel represents a different duckweed lineage (given at the top of each graph), and each point represents the mean and standard error of 10 replicates. *P*-values represent the ploidy × herbivory interaction and are calculated from the lineage-specific Poisson GLMMs (see Appendix S1 [Tables S10, S11] for full model results).

DISCUSSION

Our results demonstrated significant differences in the effect of polyploidy and genetic lineage on herbivore preference and performance, and plant performance in response to herbivory. Preference experiments indicated that aphids preferred neopolyploid duckweed across all genetic lineages, but that this result was largely driven by differences between ploidies in population surface area, as the preference disappeared when we controlled for surface area statistically and experimentally. In addition to aphids preferring the neopolyploid duckweed, aphids also often performed better—reaching higher abundances—on neopolyploid duckweed. However, this result was highly dependent on plant genetic lineage. Lastly, neopolyploids appeared to be slightly less tolerant than diploids in the face of herbivory, but the effects were small and highly dependent on genetic lineage.

The relationships between herbivory, polyploidy, and plant genetic background on plant performance were complex. The absence of a significant two-way interaction between polyploidy and herbivory on plant performance, but the presence of three-way interactions, suggests that the effects of polyploidy and herbivory do not depend on each other in a straightforward manner, and that the genetic background of the plant plays a large role. For example, in the lineage SP.05, aphids reached similar abundances on neopolyploids and diploids, but abundance and biomass of neopolyploid SP.05 were more negatively impacted by aphid herbivory than its diploid progenitor (Figures 3 and 4; Appendix S1, Tables S7, S10). This observation, where a similar number of aphids had a more negative impact on the abundance and biomass of neopolyploids compared to diploids, implies that neopolyploid duckweed are, in fact, less tolerant per herbivore. In contrast, neopolyploid SP.11 hosted more aphids than diploid SP.11, but there were no significant differences in how they responded to herbivory. This opposing result, where a higher number of aphids did not lead to any differences between neopolyploid and diploid abundance and biomass, may actually imply that neopolyploid SP.11 is more tolerant per aphid herbivore than diploid SP.11. Had we only quantified herbivore performance or plant performance, these complex relationships could have been overlooked. However, our experiment only lasted 15 days, and ecological feedbacks between the two could become apparent in longer experiments. For example, if herbivores reach higher abundances on neopolyploids, then cumulative negative effects on the plant could eventually arise, even if the tolerance per aphid is higher.

Overall, aphids preferred neopolyploids and performed better on them. Indeed, this trend has been found in other plant–herbivore systems with mixed ploidies, such as *Greya politella* moths and plants in the *Lithophragma* genus, and alligator weed (*Alternanthera philoxeroides*) and flea beetle (*Agasicles hygrophila*) (Janz and Thompson, 2002; Krug and Sosa, 2019). However, many studies reporting this pattern also confirmed that their results depended on other factors, such as plant origin, year sampled, and environmental context, and the opposite trend has also been found in some plant–herbivore systems as well (Janz and Thompson, 2002; Hull-Sanders et al., 2009; Münzbergová et al., 2015; König et al., 2014, 2016). Our results with synthetic neopolyploids show that, in particular, it is important to account for the differences in population surface area between the two ploidies when the plants are given at similar frequencies as a potential mechanism driving herbivore preference, yet this control is not commonly included.

Corroborating the preference study result that population surface area contributes to aphid choice in driving these interactions, in the performance study, duckweed with the largest surface areas often supported the most aphids (Figure 2; Appendix S1, Table S2). For example, SP.011 neopolyploids hosted the most aphids, and they have one of the largest mean surface areas of all our lineages. Further, the lineage with the smallest size difference between diploids and neopolyploids, SP.07, exhibited very little differences in aphid population sizes (Figure 2; Appendix S1, Table S2). Similarly, SP.11 neopolyploids supported the largest increase in aphid abundances compared to diploids, and they are also approximately 56% larger than their diploid progenitors (Figure 2; Appendix S1, Table S2). However, this trend was not always consistent across lineages; neopolyploid SP.05 are nearly double the size of their diploid progenitors, but they exhibited little differences in the number of aphids hosted. The absence of an effect of size seen here may indicate that something other than size may be contributing to polyploidizations effect on the duckweed–aphid relationship for this lineage. There is very little mechanistic work behind what might drive differences between herbivore preference and performance in the context of polyploidy. However, we do know that, in addition to apparency, preferences are also driven by olfactory, mechanical, and chemical cues and that plant chemistry varies even at the intraspecific level (Powell et al., 2006; Jakobs and Müller, 2018; Endara et al., 2023). For example, in *Centaurea phyrgia*, Münzbergovà et al. (2015) cited differences in levels of secondary compounds gallic acid and several polyphenols in diploid *C. phyrgia* as a potential mechanism for diploids suffering less seed damage than polyploids. Further, polyploidization may have varied effects on herbivores with alternative modes of feeding, such as chewing or mining, as compared to phloem suckers like aphids. For example, phloem feeders generally induce the salicylic acid defense pathways, whereas chewers often cause much more tissue damage and may induce the jasmonic acid and ethylene defense pathways (Ali and Agrawal, 2012; Xu et al., 2021). How chemical versus structural defenses to herbivory change with whole-genome duplication, however, remains largely unknown.

CONCLUSIONS

Our experiment, using multiple neopolyploid lineages, revealed that polyploidy and genetic lineage impact herbivore preferences for plant hosts and herbivore fitness, and that this trend is, in part, driven by population surface area differences between neopolyploids and diploids when they are given at similar abundances. The extent to which neopolyploids' fitness is affected by herbivory was strongly dependent on genetic lineage and sometimes mirrored the effect on the herbivores. By combining results on herbivore performance and plant performance across multiple plant genotypes, we were able to uncover complex relationships among ploidy, herbivory, and plant genetic background. Our work here lays the groundwork for future experimental studies to explore the longer-term and mechanistic drivers of the impact of polyploidy on plant–herbivore relationships, and to further understand how these changes might impact plant and herbivore establishment and persistence in nature.

Future directions

This study represents one of the initial attempts to investigate these dynamics in aquatic plants, and further work should consider expanding its scope to encompass a broader range of aquatic consumers and producers. For example, in our system, the plant and the herbivore exhibited similar generation times. Systems in which the plant and herbivore have very different generation times may have altered reciprocal interactions and thus differential responses to whole-genome duplication. Additionally, there are likely other changes associated with whole-genome duplication other than size worth investigating that may affect herbivore preference and performance, such as differences in chemical or morphological defenses discussed above or in host quality.

Long term, polyploidy can alter species interactions in two ways: by direct changes caused by whole-genome duplication or by the indirect changes incurred via evolution after whole-genome duplication. Here, we were able to investigate the effects of the direct changes caused by polyploidization, leaving the latter still up for investigation. A recent study by Malacrinò et al. (2024), however, showed that exposure to herbivory rapidly increased *S. polyrhiza* resistance in only 30 generations. Future research using long-term experimental studies with both neopolyploids and diploids are needed to address whether polyploids evolve differently than diploids in the presence/absence of herbivores.

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

AUTHOR CONTRIBUTIONS

H.R.A., T.-L.A., and M.M.T. conceptualized and designed the study. H.R.A. carried out the experiments and acquired the data. H.R.A., T.-L.A., and M.M.T. conceptualized the analysis. H.R.A. analyzed the data and wrote the first draft of the manuscript, and H.R.A., T.-L.A., and M.M.T. edited subsequent drafts.

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DATA AVAILABILITY STATEMENT

All data that are not already available have been archived through Figshare: doi:10.6084/m9.figshare.25000562

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Sampling locations and surface areas of genetic lineages, supplemental statistical tests and results, and photos of the experimental setups.

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