New Phytologist

Research

Polyploidy impacts population growth and competition with diploids: multigenerational experiments reveal key life-history trade-offs

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Received: *31 October 2022*
Accepted: *24 January 2023*

New Phytologist (2023)
doi: 10.1111/nph.18794

Key words: Araceae, duckweed, nutrient limitation, stoichiometry, synthetic polyploid, whole-genome duplication.

Summary

- Ecological theory predicts that early generation polyploids ('neopolyploids') should quickly go extinct owing to the disadvantages of rarity and competition with their diploid progenitors. However, polyploids persist in natural habitats globally. This paradox has been addressed theoretically by recognizing that reproductive assurance of neopolyploids and niche differentiation can promote establishment. Despite this, the direct effects of polyploidy at the population level remain largely untested despite establishment being an intrinsically population-level process.
- We conducted population-level experiments where life-history investment in current and future growth was tracked in four lineage pairs of diploids and synthetic autotetraploids of the aquatic plant *Spirodela polyrhiza*. Population growth was evaluated with and without competition between diploids and neopolyploids across a range of nutrient treatments.
- Although neopolyploid populations produce more biomass, they reach lower population sizes and have reduced carrying capacities when growing alone or in competition across all nutrient treatments. Thus, contrary to individual-level studies, our population-level data suggest that neopolyploids are competitively inferior to diploids. Conversely, neopolyploid populations have greater investment in dormant propagule production than diploids.
- Our results show that neopolyploid populations should not persist based on current growth dynamics, but high potential future growth may allow polyploids to establish in subsequent seasons.

Introduction

Whole-genome duplication is a common macromutational process across the tree of life (Doyle & Coate, 2019; Fox *et al.*, 2020), resulting in the formation of an incipient 'neopolyploid' within an otherwise diploid population. At the local scale, neopolyploid individuals are expected to almost always go extinct due to competitive exclusion by their established diploid progenitors in their shared niche through mechanisms such as minority cytotype exclusion (Levin, 1975; Husband, 2000; Arrigo & Barker, 2012). However, the expectation of rapid extinction is discordant with the observation that established populations of polyploid plants persist globally in natural environments, with polyploidy acting as a common speciation mechanism in plants (Spoelhof *et al.*, 2017; Rice *et al.*, 2019). This establishment paradox has been addresssed via theoretical models that focus on population-level dynamics, which emphasize the importance of asexuality and niche differentiation in promoting establishment success (Rodriguez, 1996; Rausch & Morgan, 2005; Oswald & Nuismer, 2011; Fowler & Levin, 2016; Spoelhof *et al.*, 2020b). However, nearly all empirical tests of the ecological effects of polyploidy have been conducted at the individual level. This creates a significant gap in our understanding of polyploid establishment because the performance of an individual does not necessarily equate to the performance of the population. In particular, neopolyploids may differ from diploids in population intrinsic growth rate and in their sensitivity to intraspecific competition with themselves or interspecific competition with their diploid ancestors (Hart *et al.*, 2018). Moreover, neopolyploidy may alter life-history strategies, such as investment in actively growing progeny vs storage that affects current and future population growth, respectively (Van Noordwijk & Dejong, 1986; Stearns, 1989). The lack of experiments on the immediate ecological and evolutionary effects of neopolyploidy on population-level processes is a crucial missing link to understanding the persistence and establishment of polyploid lineages.

At the individual-level, neopolyploidy can lead to instantaneous phenotypic differentiation from their diploid progenitors (Ramsey & Schemske, 2002; Clo & Kolar, 2021). When strictly considering autopolyploidy, which does not include the

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confounding effects of interspecific hybridization, the direct effect of increased genomic content ('nucleotypic' effects) and gene dosage effects (Bomblies, 2020) can lead to immediate phenotypic novelty, such as slower cell division, larger cells (e.g. stomates), or increased body size (Doyle & Coate, 2019; Bomblies, 2020). Neopolyploidy can also lead to phenotypic shifts in the reproductive traits of individuals, such as altered reproductive phenology, increased seed production or clonality (Ramsey & Ramsey, 2014; Van Drunen & Husband, 2018a; Anneberg & Segraves, 2020), which could dramatically affect future population recruitment rates. Additionally, these novel phenotypes can vary with the genotype of the progenitor diploid (Van Drunen & Husband, 2018b; Doyle & Coate, 2019; Wei *et al.*, 2020). Thus, the genetic origin of a neopolyploid may be as important as selective tuning and later adaptation in determining the success of neopolyploid populations. It is therefore critical to study the effect of neopolyploidy on population performance using multiple genome duplication events from unique diploid genotypes.

The increased body size associated with neopolyploidy at the individual level (Otto, 2007) is predicted to be adaptive when neopolyploids directly compete with their smaller-bodied diploid ancestors, but there may be population-level costs associated with this individual-level benefit that are rarely considered. For instance, larger-bodied neopolyploids may have a stronger *per capita* competitive effect on their diploid progenitors (Levin, 1983; Hin & de Roos, 2019), but larger-sized individuals could also imply stronger intraspecific competition and hence a lower carrying capacity. This would be consistent with individual-level studies showing neopolyploid growth and fitness requires greater nutrient supplies than their diploid progenitors (Guignard *et al.*, 2017; Walczyk & Hersch-Green, 2019; Anneberg & Segraves, 2020). Given that neopolyploids often have higher nutrient requirements, we expect them to have slower population growth and should be more sensitive to diploid competition when resources are limited as a result (Guignard *et al.*, 2017; Hart *et al.*, 2018). These patterns could lead to a trade-off between increased biomass production and population abundance. For instance, metabolic scaling theory predicts that while larger individuals have relatively lower metabolisms and grow more slowly than small individuals, they have one demographic advantage – their metabolic demands per unit mass are lower so populations can achieve higher biomass (Marshall, 2022). Additionally, the ratio of nutrient supply (e.g. nitrogen (N) : phosphorus (P)) and the relative differences in requirements for these nutrients by diploids and neopolyploids may alter competitive outcomes (MacArthur, 1972; Tilman, 1982). Thus, by manipulating not only the concentration but also the ratio of nutrients, we can test whether nutrient stoichiometry can mediate the competitive dynamics between neopolyploids and their diploid ancestors. This may be especially important if whole-genome duplication causes a niche shift, reducing competition among the ploidal types and the likelihood that neopolyploids go extinct in all resource environments (Rodriguez, 1996; Oswald & Nuismer, 2011; Fowler & Levin, 2016).

While previous individual-level studies focus on current productivity (e.g. biomass) (Collins *et al.*, 2011; Thompson *et al.*, 2015), none to our knowledge have explored how alternative life-history strategies are affected by competition. Beyond growth in the current season, many species can facultatively engage in storage by banking dormant individuals for future growth, especially when resources become scarce (Venable & Brown, 1988). Examples include egg or seed banking, investment into rhizomes, and recalcitrant spore production (Baskin & Baskin, 2014; Martinez-Garcia & Tarnita, 2017). At the individual level, neopolyploidy can lead to an immediate increase in storage investment (e.g. root buds or seed mass) (Van Drunen & Husband, 2018a; Anneberg & Segraves, 2020). As such, neopolyploid persistence may be mediated by both the timing and the total production of stored propagules, given that they could circumvent times of low nutrient supply or competitively stressful growing conditions through dormancy (Caceres, 1997; Angert *et al.*, 2009). Furthermore, whether the competitive interactions between diploid and neopolyploid populations in current (active) growth mirror those on future growth potential (dormant propagules) remains untested.

To fill these gaps in knowledge, we ask: (1) Is there an advantage to neopolyploidy at the population level, and does it depend on resource availability or the genetic background of the progenitor diploid? (2) When growing together, do neopolyploid or progenitor diploid populations have a competitive advantage over the other in growth and abundance? (3) Do the determinants of ploidal-specific investment into future population growth mirror current population growth? We addressed these questions by synthesizing neopolyploids from four genetically distinct diploid genotypes of a floating aquatic plant, the 'greater duckweed' *Spirodela polyrhiza* (Araceae; Supporting Information Methods S1) that reproduces asexually through either clonal division of actively growing fronds or dormant propagules (turions) (Appenroth & Nickel, 2010) (Fig. 1).

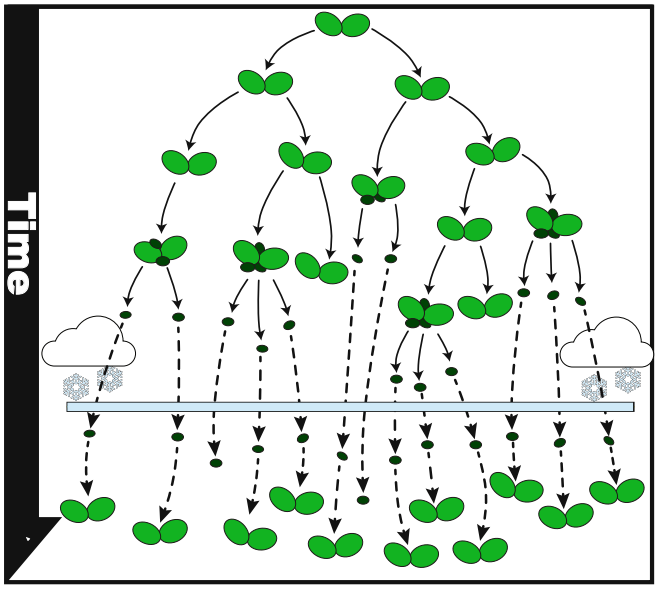

Fig. 1 Greater duckweed (*Spirodela polyrhiza*) populations propagate via multiple generations (black arrows) of actively growing fronds (green plants) over a growing season and produce turions (black circles) that are dormant propagules that can overwinter (dotted arrows that pass through the blue bar) and regrow to produce future populations of fronds.

We chose to study an asexually reproducing species since current theory predicts that reproductive assurance, such as via clonal reproduction, can alleviate minority cytotype challenges and contribute to neopolyploid establishment (Fowler & Levin, 2016; Van Drunen & Husband, 2019; Spoelhof *et al.*, 2020a). The potential importance of asexual reproduction in polyploid establishment is also supported in empirical reviews (Van Drunen & Husband, 2019). As a result, *S. polyrhiza* is a biologically relevant and tractable system to address the factors governing polyploid establishment especially at the population level. Additionally, we used *S. polyrhiza* since it is one of the fastest reproducing macroscopic plants (Ziegler *et al.*, 2015), which allows us to conduct large replicated multigenerational population dynamic experiments. In this study, we evaluated the population-level responses of neopolyploids and their progenitor diploids when grown both alone or in competition across a range of N and P that varied in concentration and stoichiometry. Synthetic neopolyploids are recognized as a powerful tool for studying the direct effects of genome duplication because they avoid the confounding effects of evolution after duplication that exist in wild polyploid–diploid comparisons (Bomblies, 2020). Furthermore, including multiple genotypes of synthetic neopolyploids allows us to discern how repeatable the immediate effects of genome doubling are when considering the standing intraspecific genetic diversity of progenitor diploids.

Materials and Methods

See Methods S1 for an extended description of all methods.

Study system and synthesis of neopolyploids

Spirodela polyrhiza (Araceae) is a model system for testing population responses to different ecological settings (Lam *et al.*, 2014; Wang *et al.*, 2014; Laird & Barks, 2018; Hart *et al.*, 2019). This freshwater aquatic plant has a relatively small genome size (158 Mbp) with rapid generation times (Ziegler *et al.*, 2015), whose populations propagate mostly through clonal growth via budding in as little as 3–4 d, rather than sexually reproducing via rare flowering events (Jacobs, 1947). Specifically, *S. polyrhiza* populations are clonally propagated via: the production of actively growing fronds that separate from their maternal plant and contribute to current-season populations; and production of dormant propagules (turions) that overwinter and contribute to future populations (Jacobs, 1947; Appenroth & Nickel, 2010) (Fig. 1). Since turions are produced in response to stress (e.g. nutrient scarcity or onset of winter) and remain dormant until growing conditions become favorable (i.e. transition from winter to spring), their production can be considered investment in future growth (Appenroth & Nickel, 2010).

Four genetically distinct ancestral diploid lineages of *S. polyrhiza* were collected in western Pennsylvania, USA (Table S1 for collection site info). These diploid lineages were genotyped using several microsatellite markers (Hart *et al.*, 2019; Kerestetter *et al.*, 2023). In 2019 and 2020, we generated a synthetic neotetraploid (neopolyploid) lineage from each of the four diploid *S. polyrhiza* lineages via application of the mitotic inhibitor colchicine (Sigma Aldrich, CAS: 64-86-8; see Methods S1 for details). We then confirmed ploidy level using flow cytometry as outlined by Wei *et al.* (2020). We compared population growth rates of colchicine-treated diploids that did not convert to tetraploids with diploids belonging to the same lineage that were not treated with colchicine ($n = 360$) and found that they were not significantly different from each other (Fig. S1). Nevertheless, to be conservative, the following comparative analyses were conducted on colchicine-treated but unconverted diploid populations vs the corresponding neotetraploids from each lineage.

Experimental setup

Experimental populations were seeded with six fronds in 950-ml plastic containers filled with 500 ml of modified Appenroth media (Appenroth *et al.*, 1996), according to their prescribed nutrient treatment (Table S2 for recipes). Populations grew in a glasshouse in the summer of 2021 at the University of Pittsburgh for 17 d. Treatments in each experiment were applied in a randomized block design. Since duckweeds have very rapid generation time (3–4 d from bud to reproductive maturity), up to 5–6 generations can occur in 17 d, making this duration on par with other multigenerational population dynamic studies with duckweeds (Armitage & Jones, 2019; Hart *et al.*, 2019).

We conducted two experiments. The first experiment tested how monocultures of each genetic lineage and ploidy respond to a factorial manipulation of N and P while holding all other nutrients constant. We either increased or decreased the concentration of N and/or P by one order of magnitude, respectively. Thus, we had nine nutrient treatment levels, which varied in both the concentration and ratio of N and P (Table S2). We grew five replicates of each ploidy (autotetraploids and colchicine-treated diploids) by lineage (four levels), by nutrient treatment (nine levels) ($n = 360$).

The second experiment tested whether variation in nutrient supply can mediate the outcomes of neopolyploid competition with their diploid progenitors by growing diploids and neopolyploids either alone or together in an additive competition design. Since we did not detect an interaction between ploidy and nutrient stoichiometry in our first experiment, we used the three nutrient treatments from the first experiment with a fixed N : P stoichiometry of 14 (corresponding to low N and low P – low; medium N and medium P – medium; and high N and high P – high; Table S2). Thus, in experiment two, we factorially manipulated competition (diploid alone, neopolyploids alone, or both together), genetic lineage (four levels), and nutrients (three levels), and included 10 replicates for a total of 360 experimental units.

In each experiment, we quantified duckweed population performance by measuring three population level responses: the total number of individual fronds every 2 d from which we also estimated carrying capacity (see below), the total number of turions every 2 d, and total dry biomass from the final day of the experiments as an estimate of productivity. Turions from mixed cytotype treatments were assigned using flow cytometry (Methods

S1). We measured pretreatment size as the surface area covered by plants at the beginning of the experiment. For the first experiment, we also quantified how polyploidy affects individual turion biomass by separately collecting fronds and turions for three of the five replicates during harvest. The harvested fronds and turions were dried in a 55°C drying oven for 1 wk before weighing them for their biomass.

Statistical analyses

For the first experiment, we analyzed each of the three population level traits (frond abundance, biomass, and turion) with either linear or generalized linear mixed effect models. In these models, to account for any pretreatment differences, we included a covariate of initial surface area covered by the six starting individual fronds in each sample. To test the population-level performance in the first experiment, we fit linear mixed models to the frond and turion count data separately since the dormant turions do not contribute to population growth in the current season. In these models, we specified ploidy, lineage, N concentration, P concentration, day of experiment, and their full factorial of interactions, as fixed effects and we added pretreatment size a covariate without interactions. We accounted for repeated measures in these models by including a random nested effect of 'sublineage' (which was the lineage-specific ploidy level) within day of experiment.

We further estimated the carrying capacity for each replicate population by fitting a logistic growth model to the frond data using the three-parameter logistic growth model of Pinheiro & Bates (2000):

$$\text{Population size} = \frac{\text{Asymptote}}{1 + \exp(x_{\text{mid}} - x)/scal}$$

where the asymptote is the estimated population size at equilibrium (carrying capacity), x_{mid} represents the inflection point (in days) where population growth was fastest, x is the distance from the origin along an x-axis, and $scal$ is the inverse of population growth rate at the time of fastest population growth. We used the nls_table function from the FORESTMANGR package (Braga *et al.*, 2020) and solved for the asymptote in the self-starting logistic function 'SSlogis' in the BASE STATS package in R (R Core Team, 2021). After $\log_{10}$-transformation to improve normality, we fit a linear model with the estimated carrying capacity data as the response variable to be explained by ploidy, genetic lineage, N concentration, P concentration, and their full factorial of interactions as factors, we did not include initial area as covariate.

Moreover, we tested for differences in productivity between diploid and neopolyploid populations with their respective final dry biomass from day of harvest. Since final dry biomass data measurements had uneven residual errors, we analyzed these data with a generalized mixed effect model with a gamma distribution (Bates *et al.*, 2015) that had the same predictors as our population size analyses. To analyze how polyploidy affects the size of individual turions, we regressed total dry mass of turions with the total count of turions for each ploidy level separately (Fig. S2). Using the best-fit line from these regression equations, we estimated the mass per turion for all samples and filtered out samples that were reared in high nutrient treatments since they did not produce turions. We used a linear model to test how ploidy, lineage, nutrient treatment, and their interactions affected the estimated mass per turion. In all statistical models, we calculated a *post hoc* least square mean estimate for each treatment level using the LSMEANS package (Lenth, 2016) in R (R Core Team, 2021). From the least square means estimates, we derived the relative percent increase or decrease in neopolyploid population performance in relation to diploids.

For the second experiment, we analyzed how competition between diploids and neopolyploids affects their population performance by fitting the same models as described in the first experiment except we included competition and the interactions with the other factors as main effects. When estimating carrying capacities from the competition experiment, we observed that populations in high nutrient treatments were still growing too quickly, causing spurious carrying capacity estimates. Thus, we omitted the high nutrient treatment from our main analysis of carrying capacity (all estimates are reported in Data availability section). Last, we tested for niche differentiation in resource use between diploids and neopolyploids by comparing their carrying capacities when grown in competition to a null expectation of half their carrying capacities in monoculture. This null expectation is based on the principal that if two competing taxa perfectly overlap in niche space and are competitively equivalent, they should perform half and in monoculture (de Wit, 1960; Firbank & Watkinson, 1985). We built two generalized linear models with a quasi-poisson distribution, one for each ploidy level that included low and medium nutrient treatments and all four genetic lineages. The generalized linear models compared the carrying capacity of either diploids or neopolyploids when grown in competition to 50% of their respective carrying capacity when grown in monoculture, with competition treatment, lineage, and their interaction fixed effects.

Results

Effect of polyploidy on current population growth

The current growth of neopolyploid populations was slower than their diploid progenitors, ultimately reaching smaller population sizes by the end of the experiment across all nutrient treatment levels (ploidy by day interaction; $F_{1,49} = 135.32$, $P < 0.001$; Fig. 2; Table S3). For example, averaging over all genetic lineages, neopolyploid population sizes were 42% and 28% lower than their diploids progenitors in the lowest and highest nutrient treatments, respectively. Our estimation of carrying capacity corroborated the current growth data, with neopolyploids having significantly lower estimated carrying capacities than their diploid progenitors across all nutrient treatments (Fig. 3; Table S4). In addition to polyploidy depressing carrying capacity, the significant ploidy-by-day interaction indicates that polyploids are also slower growing in general (Table S3).

Despite neopolyploids never reaching larger populations sizes than diploids, neopolyploids responded more to N enrichment, regardless of phosphorus supply: averaging across all levels of

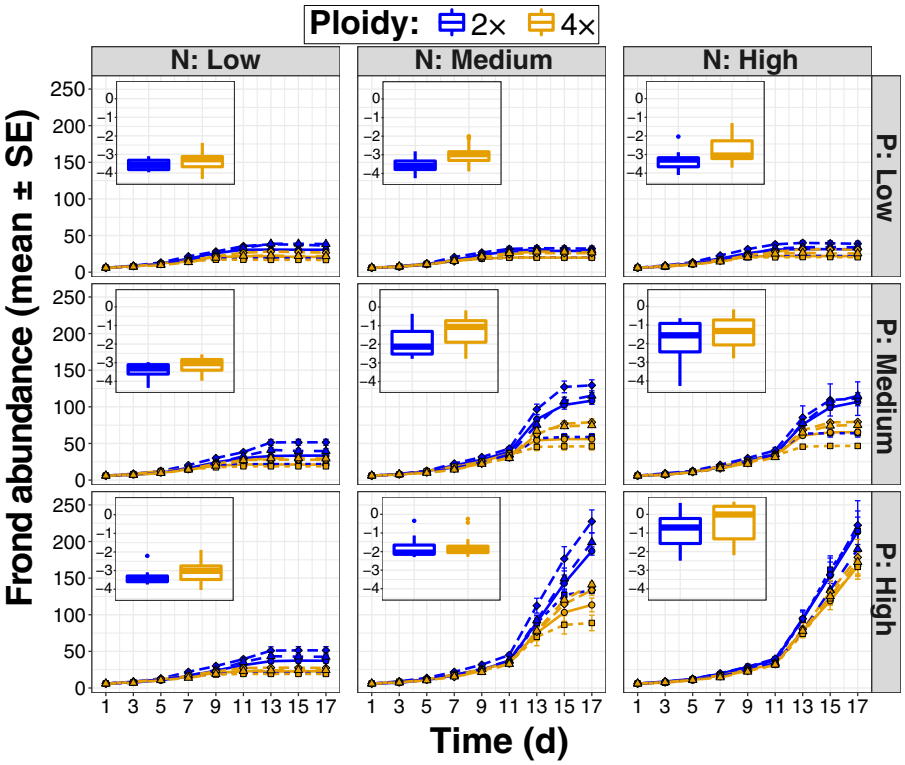

Fig. 2 Population sizes ±SE (based on counts of number of fronds) from the first experiment of diploids (2x) and neopolyploids (4x) of *Spirodela polyrhiza* growing separately, across a gradient of nitrogen 'N' and phosphorus 'P' concentrations for each of the four genetic lineages separately (different shapes and line types). Neopolyploid populations grow slower and reach smaller population sizes across all nutrient levels and genetic lineages. Boxplot insets show the median and interquartile range of neopolyploid population productivity ($\log_e$ dry g of frond biomass) is greater than diploids by Day 17 across all nutrient treatments.

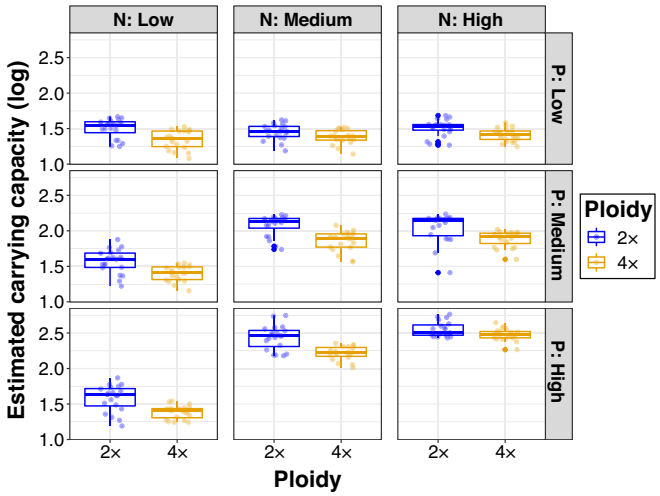

Fig. 3 Estimated carrying capacity (boxplots show median depicted as horizontal lines within each box, interquartile range depicted as whiskers, and any outliers are represented as bolded dots flanking the whiskers) of current growth fronds ($\log_{10}$) for diploid (2x) and neopolyploid (4x) *Spirodela polyrhiza* populations grown alone across the nine nutrient treatments (Nitrogen and Phosphorus) in the first experiment. Replicate populations used to estimate carrying capacity are denoted by the translucent dots overlaid on boxplots.

phosphorus, neopolyploid population sizes increased 43% more than diploids in response to nitrogen enrichment, but this pattern strongly depended on genetic background over the course of the experiment (i.e., four-way interaction between ploidy, nitrogen, genetic lineage, and day; $F_{6,3035} = 3.00$, $P = 0.006$; Table S3). Conversely, diploid population sizes increased by 15% more than neopolyploids in response to P enrichment over the course of the experiment (i.e., ploidy, phosphorus, and day interaction; $F_{2,3035} = 3.27$, $P = 0.038$), a pattern that did not vary with nitrogen supply or among the four genetic lineages (Table S3). Yet, diploids and neopolyploids did not differ in their response to variation in the N : P stoichiometric ratio (Table S5). The negative effect of neopolyploidy on population size when grown alone, regardless of nutrient supply or genetic lineage, indicates that the disadvantage of slower population growth is a universal cost of genome duplication for this aquatic plant.

In contrast to population size, and in agreement with predictions from metabolic scaling theory (Marshall, 2022) and many individual-level studies (Clo & Kolar, 2021), neopolyploid populations were more productive (final dry biomass) across all nutrient conditions. A least square mean comparison showed that neopolyploid populations accumulated 85% more biomass via current growth than diploids (Fig. 2; $\chi^2 = 6.23$, df = 1, $P = 0.013$; Table S6). Although N and P enrichment significantly increased biomass productivity, polyploidy did not interact with nutrient treatments or genetic lineage on productivity (Figs 2, S3; Table S6). Thus, the main effect of polyploidy was a substantial increase in population biomass productivity.

Competitive dynamics of diploids and neopolyploids

When we grew diploids and neopolyploids either alone or in competition in the second experiment, we showed that diploids grew faster and produced greater population sizes than their neopolyploid descendants, and this pattern held true for each

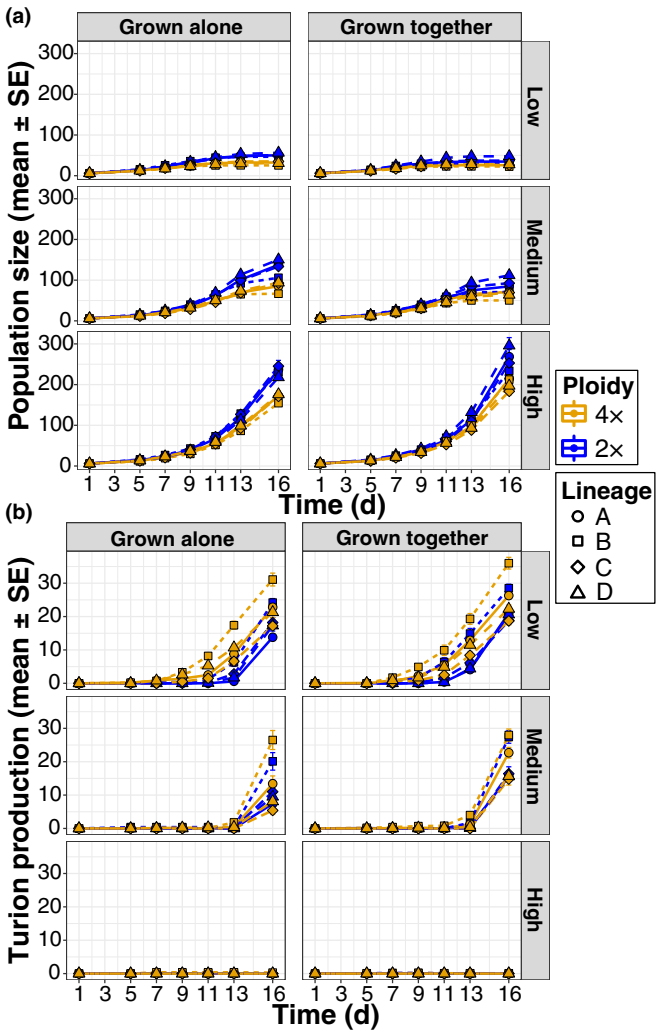

Fig. 4 Diploid and neopolyploid population dynamics (frond population size or turion production over day in experiment ±SE) of *Spirodela polyrhiza* from the second experiment, when grown alone or together in three nutrient levels for the four genetic lineages (different shapes and line types). (a) Current population size of diploids (2x) is greater than polyploids (4x) in all treatments, regardless of competition. (b) Neopolyploid populations had earlier and greater investment in turions than diploids, and competition caused both ploidy levels to increase propagule production.

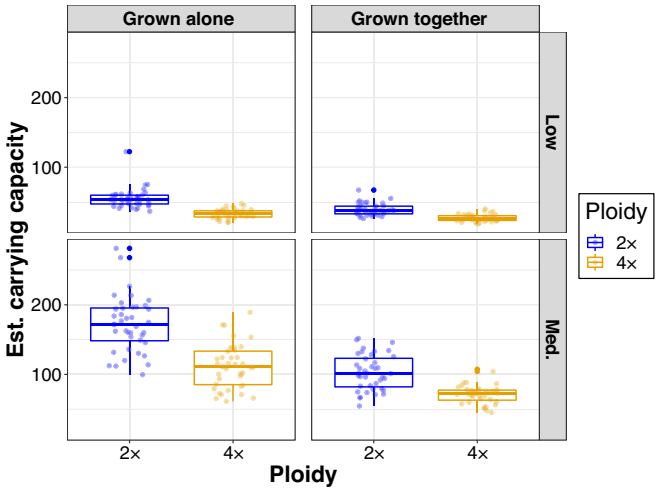

Fig. 5 Estimated carrying capacities of current growth fronds of diploid and neopolyploid *Spirodela polyrhiza* populations from the second experiment, when grown in monoculture or competition and either in low or medium nutrient treatment. Individual populations from which we estimated carrying capacity are denoted by the translucent dots overlaid on boxplots. Each boxplot shows the median as the middle horizontal line, whiskers indicate the interquartile range, and bolded points are outliers. High nutrient treatment samples not shown due to poor fit to a logistic growth model.

nutrient treatment level and for all four genetic lineages (Fig. 4; Table S7). Surprisingly, competition between diploids and neopolyploids did not interact with nutrient treatment on population sizes over the course of the experiment (Table S7). However, the effect of competition between diploids and neopolyploids did strongly interact with genetic background (Fig. 4; $F_{3,3224} = 2.877$, $P = 0.035$; Table S7), suggesting that the effect of polyploidy on competition is more complex than simple genome size doubling alone.

Our analysis of the estimated carrying capacities corroborated the analysis of population sizes. Neopolyploidy reduced carrying capacity across all nutrient treatments and regardless of competition by 34% on average (Fig. 5; Table S8, $P = 0.0047$). Both diploids and neopolyploids displayed evidence of niche differentiation in resource use. Neopolyploid populations had significantly higher carrying capacities in competition than expected based on half their carrying capacity in monoculture (+63%, $\chi^2 = 20.53$, df = 1, $P < 0.001$; Table S9). Diploid populations also had higher carrying capacities in competition than expected, but nutrient treatment significantly influenced this pattern (nutrient treatment by competition interaction: $\chi^2 = 4.30$, df = 1, $P = 0.038$; Table S9). This interaction was driven by diploid populations having carrying capacities that were 41% higher than expected in low nutrients than 19% higher than expected in medium nutrients.

While neopolyploid populations again produced more biomass than diploids in monoculture (37% greater final dry biomass than diploids; Fig. S4), competition caused the biomass productivity of neopolyploids to decline more than diploids. However, neopolyploids still produced more biomass in competition than diploids overall, especially with high nutrient supply (neopolyploid biomass productivity decline of 65% in high nutrients vs 47% in low nutrients; Fig. S4; Table S10; $\chi^2 = 13.42$, df = 2, $P = 0.001$; Table S11), and this pattern was consistent among genetic lineages ($\chi^2 = 7.11$, df = 6, $P = 0.31$; Table S11).

Ploidal-specific investment into future population growth

Unlike current (frond) growth, neopolyploids outperformed diploids in future (turion) growth potential when grown as monocultures (Fig. 1). Neopolyploid populations produced larger turions, accumulated more of these dormant propagules, and began producing them earlier than diploids, especially in lower nitrogen environments (Figs 6, S5, S6; Tables S12, S13). For instance, when grown alone across the nine nutrient levels, neopolyploid

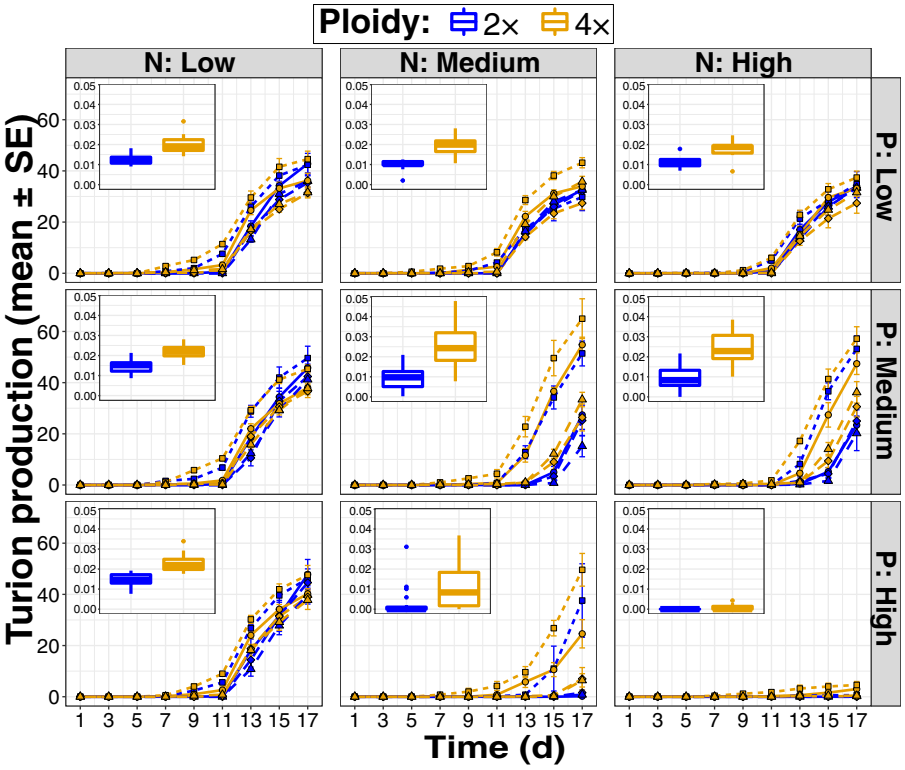

Fig. 6 Future growth investment (in number of turions produced ±SE) of diploid (2*x*) and neopolyploid (4*x*) populations of *Spirodela polyrhiza* in the first experiment when grown alone. Insets show boxplots of the $\log_e$ grams of dry biomass of turions across various N and P concentrations with medians as the bolded horizontal line, whiskers as the interquartile range, and bolded points as outliers. Neopolyploid populations produced more turions than diploids, and this pattern depends on genetic lineage (represented by the four different line-types).

populations started producing turions on average 6 d earlier in response to low nitrogen supply ($\chi^2 = 6.01$, df = 2, $P = 0.05$; Table S12). A separate generalized model of the total turion production over time found that the effect of neopolyploidy was highly dependent on N and P concentrations, in which neopolyploids produced significantly more turions in lower nutrient environments ($\chi^2 = 21.59$, df = 4, $P \leq 0.001$; Table S13). Regardless of nutrient treatment or genetic lineage, neopolyploids produced significantly heavier (78%) turions than diploids ($F_{1,247} = 7712.48$, $P < 0.001$; Fig. S5). Furthermore, there was no interaction between ploidy level and genetic lineage on turion production over the course of the experiment ($F_{3,49} = 1.457$, $P = 0.239$).

When grown with or without competition, neopolyploid populations again produced more turions and began production earlier (Fig. 4). Interestingly, competition elicited a disproportionately greater investment in storage by neopolyploid populations under medium nutrient supply than diploids, whereas diploid populations increased turion production disproportionately more with low nutrients ($\chi^2 = 49.33$, df = 2, $P < 0.001$; Fig. 4; Table S14). Nutrient supplies strongly dictated which ploidy level produced more turions in response to competition. A least square means comparison of turions produced from populations under competition relative to monoculture showed that diploid populations increased turion production more than neopolyploids under low resources (diploid increase 25% vs 12% for neopolyploids), but neopolyploids increased turion production more than diploids in medium nutrient environments (neopolyploid increase of 75% vs 67% for diploids).

Discussion

Our population-level experiments on growth and competitive dynamics revealed not only a hidden cost to whole-genome duplication – larger bodied neopolyploids grow more slowly and reach lower carrying capacities overall (Figs 2–5), but also a hidden advantage – higher population productivity. We also revealed an important change in life-history strategy as neopolyploids shift toward an increased investment in future population growth potential (Figs 4, 6). This allocation shift was evident not only at the population level but also at the individual level, since the average neopolyploid frond produced more turions that were heavier than their diploid ancestors (Figs S5, S6). Although we tracked populations over only a single growing season, the life-history strategy shift to increased future growth potential may greatly improve the odds of neopolyploid establishment. Recent theoretical work has predicted that establishment success of neopolyploid populations is strongly dependent on the number of propagules they can produce when growing in competition with their diploid progenitors (Fowler & Levin, 2016; Levin, 2021). We provide evidence to support this expectation since neopolyploids still produced more turions than diploids even when directly competing with diploids. Importantly, we also show that the population-level patterns we detected vary with their genetic background and thus are not simply a product of genome doubling *per se*, but may also be the result of a higher level genetic effect of neopolyploidy (Osborn *et al.*, 2003).

If establishment and persistence of neopolyploids depends only on current population growth and carrying capacities, then our

results indicate they would likely be eliminated over time across the broad range of resource environments tested. This was indicated by not only reduced population growth rates of neopolyploids (Fig. 2), but also lower carrying capacities (Fig. 3) over the duration of five to six generations. In fact, future modeling of the conditions for polyploid establishment should incorporate unequal carrying capacities rather than the often-assumed equality. Surprisingly, although we expected competition to favor populations of neopolyploids over their diploid progenitors, we found that the current growth of neopolyploid populations was inferior to diploids, regardless of competition (Fig. 4). However, even though population sizes of diploids and neopolyploids decreased in response to competition, since they both had significantly higher carrying capacities when grown in competition than null expectations (de Wit, 1960), we found evidence of at least partial niche segregation. Furthermore, since neopolyploids invested more in future growth than diploids, the current population growth patterns of diploids and neopolyploids may not be the best predictor of establishment.

Counter to our current growth data on population sizes, neopolyploids were more productive in terms of accumulated biomass than their diploid ancestors (Fig. 2 insets). Since neopolyploid populations consisted of larger-bodied fronds that were more productive regardless of nutrient treatment (Fig. 2), our results are consistent with metabolic size scaling rules for larger cells (Marshall, 2022) in which larger cells are metabolically more active but also more efficient. Although we expected that by providing higher nutrient supplies to neopolyploid populations, it would alleviate any growth disadvantage compared with their diploid progenitors, and we found that was not the case (Fig. 2). Therefore, although increasing nutrients may benefit neopolyploid individuals (Walczyk & Hersch-Green, 2019; Anneberg & Segraves, 2020), this does not necessarily manifest at the population level. The pattern of neopolyploid populations comprising larger-bodied fronds that achieve lower population sizes overall highlights a limitation in the individual-based paradigm, which often focuses exclusively on biomass productivity when assessing neopolyploid performance. If establishment was based on biomass productivity patterns alone, we would conclude neopolyploids are unambiguously the winner against their diploid progenitors; however, the larger population sizes of diploids reveal that neopolyploids are poorer off and may not persist.

Compared with the current growth investment of neopolyploids, our results on future growth results reveal an unexpected mechanism that could provide an early numerical advantage to neopolyploid populations by shifting their life-history strategy (Stearns, 1989), which could allow establishment. Had we only considered the total number of individuals produced by summing the fronds and turions together over the course of the growing season, we only would have found that the neopolyploids never produced more individuals than their diploids ancestors. However, when we consider only the production of dormant turions that carry forward to the next growing season, we found that neopolyploid populations invested more heavily into their future population growth than their diploid progenitors. This is an underappreciated mechanism that could help explain how polyploids establish and why mixed-ploidy populations of species are common in nature (Duchoslav *et al.*, 2010; Kolar *et al.*, 2017) as storage of dormant individuals can be a mechanism of coexistence among competing taxa (Warner & Chesson, 1985; Caceres, 1997; Angert *et al.*, 2009; Armitage & Jones, 2019). Specifically, environments with oscillations between benign and harsh growing conditions may lead to a situation where polyploid establishment is favored since neopolyploids invested more in future growth than diploids. If neopolyploid populations overwinter with more dormant propagules than diploids, they could have a numerical advantage in the spring which could allow either coexistence or exclusion of their diploid progenitors. Additionally, the generally greater investment into future growth and earlier initiation of it by neopolyploids constitutes a shift towards a more conservative risk-averse strategy that would allow them to circumvent hostile growing periods such as an early onset freeze (Jacobs, 1947; Childs *et al.*, 2010) or unexpected harsh conditions. This shift could be one explanation of the global pattern of established polyploids in polar and stressful habitats and with their evolutionary resilience to cataclysmic climate events, such as the K–T extinction (Madlung, 2013; Van De Peer *et al.*, 2017) and the relative absence of established polyploids in the tropics with a low degree of seasonality (Rice *et al.*, 2019). Our future growth data showing that neopolyploids produce larger and more dormant propagules than their diploid ancestors is an important step in understanding the factors promoting the long-term establishment of neopolyploid populations. Future work that compares the overwintering ability and fate of turions between diploids and neopolyploids across multiple seasons will reveal whether neopolyploids investing more into future growth in one season is advantageous in the long term. Furthermore, while our study in an asexually reproducing species can not address the roles of mating system in the minority cytotype exclusion principle, the current results confirm that dynamics based on asexual reproduction alone can be critical to coexistence (Rausch & Morgan, 2005). To thoroughly address whether diploids and polyploids can coexist would require experiments that test whether either of them can recover from low density in the presence of the other ploidy level (reviewed in Godwin *et al.*, 2020).

The consequences of neopolyploidy on competition at the population-level transcends a simple genome doubling effect (Doyle & Coate, 2019). Competitive interaction strength between neopolyploids and their diploid progenitors strongly depended on their genotype of origin (Fig. 4), indicating that the effect of neopolyploidy on competitive dynamics carries with it a genetic or epigenetic feature that differs among our four genetic backgrounds of neopolyploids. It is worth noting, however, that our experiment used single genotype pairs of diploids and their neopolyploid progeny in competition. Because we expect genetic variation among individuals to be much greater in the wild, it is unclear how greater intraspecific variation among either diploids or neopolyploids would affect the patterns we observed (Bolnick *et al.*, 2011). Because each of the four synthetic neopolyploid genetic lineages represents an independent evolutionary experiment (Bomblies, 2020), this variation suggests that genetic

chance events can dictate the outcome of future interannual competitive dynamics and be predictive of long-term establishment of neopolyploid populations. Therefore, if genetic lineages of neopolyploid populations that produce more dormant propagules can overcome the short-term numerical costs in current growth, then establishment may be favored in environments with a high degree of seasonality where neopolyploid populations would benefit from their greater future growth investment.

Acknowledgements

We thank Jae Kerstetter for assistance in maintaining the *S. polyrhiza* founding genotypes; Kayla Downs and Jason Simmons for polyploid–diploid stock creation and maintenance; Laurie Follweiler for greenhouse assistance; Ashman and Turcotte labs for feedback, discussion, and helping with experiments. This project was supported by the Dietrich School of Arts & Sciences and grants from the National Science Foundation (no. 2109452 to TJA, nos. 2027604 and 1912180 to T-LA, and no. 1935410 to MMT).

Competing interests

None declared.

Author contributions

TJA, T-LA and MMT conceptualized and designed the study. EMO synthesized the neopolyploids, confirmed ploidy by flow cytometry and maintained all materials. TJA carried out the experiments. TJA analyzed the data and wrote the first draft of the manuscript, and TJA, T-LA and MMT edited subsequent drafts. TJA, T-LA and MMT contributed equally to this work.

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Data availability

All data associated with this work will be freely available on the data repository Zenodo at https://zenodo.org/record/7644292#.Y-2mfi-B2gQ (doi: 10.5281/zenodo.7644292).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Population growth rates (number of fronds) for each lineage and colchicine treatment for diploids.

Fig. S2 Scatterplot showing regression lines of total turion biomass by the total count of turions for both ploidy levels.

Fig. S3 Dry frond biomass productivity ($\log_{10}$) responses of the two ploidy levels for each of the four genetic lineages across the nine nutrient treatments.

Fig. S4 Population productivity of $\log_{10}$ frond dry biomass from day of harvest for diploids and neopolyploids in response to competition and nutrient enrichment.

Fig. S5 Estimated dry biomass produced per turion of diploids and neopolyploids.

Fig. S6 Turions produced per frond in populations of diploids or neopolyploids.

Methods S1 Extended methods section, outlining the induction of neopolyploidy, testing the effect of colchicine, and the design of the two experiments.

Table S1 Genetic lineages of *Spirodela polyrhiza* used in this study and their corresponding latitude and longitude of the collection site.

Table S2 Nitrogen and Phosphorus treatment recipe including micronutrients in Appenroth media.

Table S3 ANOVA table of population sizes over the days of the monoculture experiment. Significant factors are bolded with their *P*-value.

Table S4 ANOVA table of estimated carrying capacities in response to polyploidy, nutrient treatment, genetic lineage, and their interactions in the monoculture experiment.

Table S5 ANOVA table of population sizes over the days of the monoculture experiment in response to N : P ratio.

Table S6 Chi-square table of a generalized linear mixed model with a gamma distribution of dry biomass productivity in the monoculture experiment.

Table S7 ANOVA table of population sizes over the days of the second experiment testing competition.

Table S8 Chi-square table of a generalized linear model of estimated carrying capacities in the competition experiment.

Table S9 Chi-square tables of generalized linear models testing for niche segregation between diploids and neopolyploids.

Table S10 *Post hoc* least square means estimates of biomass productivity from the harvested tissues in the competition experiment.

Table S11 Chi-square table of a generalized linear mixed model of biomass productivity in the competition experiment.

Table S12 Chi-square table of a generalized linear mixed model with binomial distribution of the timing of turion production in the monoculture experiment.

Table S13 ANOVA table of a linear mixed model of $\log_{10}$ transformed (log + 1) total turion production over the course of the monoculture experiment.

Table S14 Chi-square table of a generalized linear mixed model with a poisson distribution of total turion production (count) over the course of the competition experiment.

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