Plant Soil (2020) 456:439–453
https://doi.org/10.1007/s11104-020-04727-6

REGULAR ARTICLE

Nutrient enrichment and neopolyploidy interact to increase lifetime fitness of *Arabidopsis thaliana*

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Received: 13 July 2020 / Accepted: 23 September 2020 / Published online: 28 September 2020

Abstract
Aims Nascent polyploids, or neopolyploids, frequently arise within diploid plant lineages and are expected to experience increased requirements for growth-limiting nutrients because of building a larger genome. Because this may have important consequences for the ecology of neopolyploids, we need studies that track the lifetime fitness effects of whole genome duplication. Here we investigated how multiple origins of neopolyploidy and nutrient supply rate affected fitness-related traits of *Arabidopsis thaliana*.
Methods We investigated the interaction between cytotype, independent neopolyploid origins, and soil fertility by conducting a greenhouse experiment with five nutrient treatments that varied nitrogen and phosphorus supply. We compared biomass, flowering phenology, fecundity, average mass per seed, and offspring germination rates of diploids and their descendent neotetraploids from four independent origins.
Results The results supported the hypothesis that neopolyploidy increases nutrient limitation. Diploids outpaced their neotetraploid descendants in growth and composite fitness in all nutrient treatments except with high supply of nitrogen and phosphorus, where neotetraploid growth and composite fitness exceeded diploids. In contrast, we did not detect an interaction between cytotype and nutrient treatment for flowering phenology, but neotetraploids flowered later, and low nutrient supply caused earlier flowering. We additionally found that the trait responses of neotetraploids were strongly contingent on their independent, maternal origin.
Conclusions Polyploidy has myriad effects on plant physiology, but few studies have tested how neopolyploid-induced physiological changes can affect plant environmental interactions. By showing that neopolyploid fitness is more constrained by nutrient supply, we conclude that neotetraploidy increases nutrient limitation in *A. thaliana*.

Keywords Brassicaceae · Ecological stoichiometry · Nutrient limitation · Phenology · Polyploidy · Nitrogen · Phosphorus

Introduction

Whole genome duplication (WGD) is one of the most pervasive forces in the plant kingdom, with first – generation polyploids, or "neopolyploids", arising recurrently within diploid lineages almost as frequently as the genetic mutation rate (Ramsey and Schemske 1998). When neopolyploids first arise they must immediately

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Responsible Editor: Jeffrey Walck.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11104-020-04727-6) contains supplementary material, which is available to authorized users.

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overcome a demographic mating disadvantage from their diploid parents in order to become established or else they are expected to quickly go extinct (Arrigo and Barker 2012; Mayrose et al. 2015). Although models indicate that neopolyploid populations should be ephemeral (Fowler and Levin 2016; Rodriguez 1996), we often observe established polyploids at rates that are higher than expected (Levin 2019). Although there may be no simple mechanism that fully explains the abundance of polyploids in nature, recent efforts have hypothesized that the high incidence of established polyploid populations may be a result of both ecological opportunity and the apparent ability of extant polyploids to tolerate harsh conditions (Levin 2019; Levin and Soltis 2018). As a result, there has been a call for work characterizing how neopolyploidy can alter plant physiology and how those alterations affect performance under environmentally challenging conditions.

One of the major assumptions about the physiological effects of polyploidy is that it increases the need for growth-limiting nutrients (Guignard et al. 2017; Leitch and Leitch 2008). This assumption is rooted in the idea that WGD causes a greater need for nitrogen and phosphorus because polyploids synthesize more nucleic acids (Lewis 1985). Furthermore, polyploids may be more nutritionally constrained by a need to produce additional proteins as compared to diploids, since they must maintain gene balance in their genetic networks (Birchler and Veitia 2010; Osborn et al. 2003). Since plant protein production may not linearly scale with changes in genome size because rRNA production requires more phosphorus to facilitate growth (Hessen et al. 2010), WGD may shift the ratio of required nitrogen to phosphorus. For example, if WGD causes a doubling in nucleic acid production but not protein production, it may result in altered stoichiometric requirements for growth limiting nutrients such as nitrogen and phosphorus. Because we expect altered nitrogen and phosphorus stoichiometry at the cellular level, we also expect an increase in the total requirements of these nutrients at the tissue or whole-organism level, since WGD is strongly associated with increased tissue size at the macroscopic scale, otherwise referred to as the 'gigas' effect (Muntzing et al. 1936; Stebbins 1971). Although the gigas effect results in lower cell densities that may compensate for elevated nutrient needs, the net effect of polyploidy is predicted to result in a more nutrient limited plant because building overall larger tissues will incur a greater need for growth limiting nutrients. We thus expect that WGD will cause neopolyploids to be intrinsically more limited in their ability to grow and reproduce under nutrient-limited environments than their diploid parents.

Although our understanding of how neopolyploidy affects plant nutrient requirements is limited, we do have some evidence suggesting that polyploidy elicits a greater need for nutrients. For instance, two long-term fertilization studies in grasslands found that productivity in established polyploid species increased more with nutrient enrichment than co-occurring diploid species (Guignard et al. 2016; Šmarda et al. 2013). These findings highlight how polyploids that have become established and evolved over many generations can outperform diploid species, but the immediate effects of neopolyploidy on plant nutritional requirements remains unclear. Thus, to investigate the direct effects of WGD on plant nutrient needs, we need studies that examine early generation polyploids that have not had substantial time for evolutionary processes to act. Furthermore, we also need direct comparisons between diploids and their neopolyploid progeny in order to control for phylogenetic relatedness between diploids and polyploids. In doing so, we can directly test the immediate effects of WGD on plant nutrient limitation.

Indeed, there has been one study thus far to consider the effect of neopolyploidy on plant performance under variable nutrient supply rates. Neotetraploid *Chamerion angustifolium* grew more than diploids in response to nutrient enrichment, but these neotetraploids always produced fewer flowers than their diploid progenitors (Walczyk and Hersch-Green 2019), suggesting a tradeoff between productivity and reproductive output in polyploids. Indeed, a recent meta-analysis showed that WGD typically reduces the reproductive output of neopolyploid plants, while also causing an increase in the size of reproductive tissues, suggesting a negative relationship between fecundity and reproductive biomass investment (Porturas et al. 2019). Although neopolyploidy can negatively affect reproductive rates, we do not know how other complex fitness-related traits such as individual seed mass or progeny germination rates are affected by WGD. Since there is a negative relationship between genome size and seed mass (Beaulieu et al. 2007), we expect a negative relationship between reproductive output and the allocation to individual seeds. There may thus be a compensatory effect of neopolyploidy on plant fitness; if plant fecundity is reduced by WGD, but neopolyploids invest more into seeds, then the lifetime fitness of neopolyploids may exceed that of their diploid parents. By taking fecundity, seed mass investment, and progeny germination rate into account, we will be able to track the complex lifetime fitness response of plants to neopolyploidy and nutrient supply rate.

Although there is evidence that neopolyploidy will cause plant reproductive traits to be more constrained by the nutrient environment, we do not know how WGD and nutrient supply rates also affect other fitness-related traits such as ontogeny. Polyploids often grow more slowly than related diploids (Levin 1983), and we expect this slower growth to result in delayed reproductive phenology of neopolyploids, which can indirectly affect plant lifetime fitness (Munguia-Rosas et al. 2011). There is some evidence that established polyploids have a delayed phenology compared to diploids (Simon-Porcar et al. 2017), yet there are other instances in which established polyploids have earlier flowering phenology than their diploid ancestors (Bretagnolle and Thompson 1996; Petit et al. 1997). These mixed results may be due to a genotype by environment interaction, that can differentially shape the flowering phenology of diploids versus polyploids. For example, a common garden study found that tetraploids flowered earlier in a mixed-ploidy population in the field, but the opposite pattern was observed in a common garden environment, suggesting that environment has a strong effect on polyploidy-derived phenology differences (Segraves and Thompson 1999). Therefore, the effect of environmental nutrient supply may strongly interact with neopolyploidy on flowering phenology. Since WGD causes cells to divide more slowly and neopolyploids are expected to be more growth limited by the soil nutrient environment, we predict that neopolyploidy and soil fertility will interact to affect flowering phenology.

Here we use *Arabidopsis thaliana* (Brassicaceae) to test how nutrient limitation differs between diploids and their neopolyploid offspring. *Arabidopsis thaliana* is a good model for testing how neopolyploidy affects plant responses to nutrient addition because it is a fast-growing annual, making it possible to assess lifetime fitness. Furthermore, multiple, independent autotetraploid lineages have recently been synthesized by Luca Comai and were made publicly available by (Solhaug et al. 2016). By inducing autopolyploidy, an intraspecific WGD event, it allows us to discern the direct effect of WGD, and thus avoid the confounding effect of interspecific hybridization. These *A. thaliana* lines also represent a broad geographic sampling, providing a unique opportunity to assess how multiple independent origins of neopolyploidy affect the ecophysiological responses of plants to nutrient supply. The fact that these independent neotetraploid origins were synthesized from geographically disparate diploid maternal lineages also allows us to avoid confounding effects of local adaptation, and thus we can draw conclusions about whether the effects of polyploidy are consistent among origins. In the present study, we addressed three questions: 1) Are neotetraploid *A. thaliana* more nutrient limited than their diploid parents? 2) Are fitness related traits and flowering phenology differentially affected by plant cytotype and nutrient supply rates? 3) Are the fitness responses of plants consistent across multiple origins of neotetraploidy?

Materials and methods

Study organism and growth conditions

We used *A. thaliana* to study how neotetraploidy affects plant responses to nitrogen and phosphorus manipulation. We acquired eight accessions, comprised of four diploid ecotypes and their corresponding neotetraploid descendants that were synthesized with colchicine. Thus, these four ecotypes represent four independent origins of neotetraploidy. Seed stocks were sourced from the Arabidopsis Biological Resource Center (ABRC, Columbus, Ohio; Table S1). Although three of the four seed stocks used in this study are from Germany, they were acquired from broadly distributed sites and represent independent ecological backgrounds.

To encourage proper germination, we stratified a subset of the seeds from each maternal line for 4 days before planting into 108 cubic cm pots filled with autoclaved quartz sand. The plants were grown in a climate-controlled greenhouse maintained at 21–24 °C daytime and 18–21 °C nighttime, under ambient light conditions. We watered plants by placing the pots into nursery trays and bottom watering with deionized water. Approximately 2 weeks after the seeds had germinated and true leaves had begun to emerge, we thinned to one plant per pot to avoid competitive effects. The plants were allowed to become established for 2 weeks before treatments were applied, and during this time, we supplied the nursery trays with a modified Hoagland's fertilizer solution that is considered optimal for growth of *A. thaliana* (Cai et al. 2017).

Experimental design

To investigate how neotetraploidy affects the growth and fitness of *A. thaliana* under different nutrient supply rates, we conducted a factorial nutrient manipulation experiment. This experiment was conducted from late May 2019 to early September 2019. During this time, the eight accessions were grown under five nutrient treatment levels. We planted 60 pots of each of the eight accessions, for a total of 480 pots. The plants were distributed evenly among 20 nursery trays, such that each tray contained three pots of each of the eight accessions. We avoided artifacts driven by microclimatic variation within the greenhouse by rotating the nursery trays twice per week. Tray rotations were carried out by systematically moving the trays on the greenhouse benches while also rotating each tray 180 degrees, so that each tray occupied every possible tray location during the course of the experiment.

We investigated how cytotype and nutrient supply affected *A. thaliana* performance by varying both the nitrogen and phosphorus of nutrient treatments (Table 1). By varying both the concentration and stoichiometry of nitrogen and phosphorus in the nutrient treatments, it allowed us to discern how these nutrients differentially affected growth and reproduction. Following (Cai et al. 2017), the control nutrient treatment was a modified Hoagland fertilizer solution with a molar N:P ratio of 16, a value considered to be optimally colimiting for growth of terrestrial plants (Koerselman and Meuleman 1996). In addition to the control, we applied four experimental nutrient treatment levels: low N & P, high N & P, low N:P, and high N:P. The concentrations of each nutrient treatment were determined by either quartering (low N & P) or quadrupling (high N & P) the concentration of the control level of nitrogen and phosphorus. We also investigated how altered stoichiometric supply ratios of nitrogen to phosphorus affected diploid versus neotetraploid growth by either supplying plants with a molar N:P ratio of 256 for the high N:P treatment level, or a molar N:P ratio of 1 for the low N:P treatment level. The molar ratio of the high N:P treatment was calculated by quadrupling the concentration of nitrogen and quartering the concentration of phosphorus relative to control, whereas the low N:P treatment molar ratio was calculated by quartering the concentration of nitrogen and quadrupling the concentration of phosphorus relative to control. The high N:P treatment is well within the theoretical zone of phosphorus limitation, and the low N:P treatment is considered a strongly nitrogen limited environment (Gusewell 2004; Koerselman and Meuleman 1996). All nutrient solutions were adjusted to a pH of 5.5–5.75 to ensure nutrient availability to plants.

Table 1 Description of nitrogen and phosphorus concentrations for the nutrient treatments as well as the concentrations of micronutrients that were added to each treatment

Treatment	Nutrient Name	Molarity
Control	NH_4NO_3	4 mM
	KH_2PO_4	0.25 mM
Low Both	NH_4NO_3	1 mM
	KH_2PO_4	0.0625 mM
High Both	NH_4NO_3	16 mM
	KH_2PO_4	1 mM
Low N:P	NH_4NO_3	1 mM
	KH_2PO_4	1 mM
High N:P	NH_4NO_3	16 mM
	KH_2PO_4	0.3402 mM
Micronutrients	K_2SO_4	0.75 mM
	$MgSO_4$	0.65 mM
	$MnSO_4$	1 μM
	$CuSO_4$	0.1 μM
	$ZnSO_4$	1 μM
	Na_2MoO_4	0.035 μM
	H_3BO_3	0.01 mM
	Fe-EDTA	0.1 mM
	$CaCl_2$	2 mM

We applied the nutrient treatments by placing the nutrient solution into bottom-watering trays and allowing it to sit for 5 days per week. To avoid differential accumulation of micronutrients in the watering trays, each week we replaced the nutrient solution with deionized water for 2 days. In this way, we supplied the same volume to each bottom-watering tray for 5 days per week during the experiment; the only variation in nutrient supply was based on the relative concentration of nitrogen and phosphorus in the nutrient treatment solutions.

Fitness – related trait measurements

We investigated how neotetraploidy and soil nutrient treatments interact by measuring a suite of fitness-related traits in *A thaliana*. First, we determined how key flowering phenology events in diploids and their neotetraploid progeny were affected by altered nitrogen and phosphorus supply rates. We recorded the days to bolting, days to first flowering, and the latency between bolting and first flowering. For a subset of the plants (~4 plants per treatment level: ecotype x cytotype x nutrient treatment), we also collected three ripe, intact siliques to assess seed traits. From the siliques, we determined average seed set per silique and seed weight. A subset of these seeds were used to assess germination rate by placing approximately 20 stratified seeds on an autoclaved filter paper moistened with deionized water. The seeds were given 2 weeks to germinate and then germination success was scored using a dissecting microscope.

Plants were harvested after a majority of the tissues had senesced in order to assess productivity and fitness responses. The harvested aboveground tissues were dried in a 60 °C drying oven for 3 days before the dry weights were determined. At harvest, we also recorded the total number of siliques per plant. From the counts of the total number of siliques per plant, we multiplied by the average number of seeds per silique to estimate the plant fecundity, or the total number of seeds produced per plant. Rather than relying on fecundity alone as our estimate of plant fitness, we created a composite fitness metric by incorporating average seed mass and germination rate (Campbell 1991). Thus, our final estimate of plant fitness was the multiplicative product of fecundity, average mass per seed, and average seed germination rate. All data in this study are included in this article and its supplementary information files.

Statistical analyses

We assessed the effects of cytotype, nutrient supply rate, and independent origin of neotetraploidy by fitting individual linear mixed effect (lme) models on phenology measurements, fitness-related traits, and biomass. We used a three-way ANOVA to analyze the interaction between the main effects of cytotype, nutrient treatment, and ecotype (independent origins of neotetraploidy). By incorporating the ecotype of the plants as a main effect in our models, we were able to determine if there was an effect of independent origins of neotetraploidy on trait responses. In all our models, we included bottom watering tray as a random effect. Since we were also interested in the direction and magnitude of the differences when comparing diploids and neotetraploids, we used Tukey's HSD post hoc tests. We dropped 55 plants from the analyses because they had lost a large fraction of their potting medium due to the flushing of nutrient solutions and water during the experiment and we wanted to avoid potential artifacts caused by this stressful disturbance. To build our statistical models, we used the lme4 package (Bates et al. 2015) for lme models in R (R Core Team 2019).

Results

Phenological effects of neotetraploidy and nutrient treatment

Although there was no interaction between cytotype and nutrient treatment for the three measures of flowering phenology (Table 2), we did observe that neotetraploids generally had delayed flowering phenology compared to their diploid parents (Fig. 1). Additionally, plants in the high N & P and high N:P treatments took longer to bolt than the low N:P treatment level, whereas the control and low N & P treatment level were not statistically different from any other group. The effect of nutrient treatment on the days to first flowering was driven by delayed flowering with high N:P and high N & P treatment levels (Fig. 1; Table 2). We also observed a three-way interaction between cytotype, nutrient treatment, and ecotype on the latency between bolting and first flowering, in which there were idiosyncratic responses by the ecotypes to variation in nutrient supply and cytotype (Table 2; Fig. 2).

Reproductive and biomass effects of neotetraploidy and nutrient treatment

We found that the number of seeds per silique and average weight per seed were not responsive to nutrient treatment; however, neotetraploids had consistently fewer and heavier seeds than diploids (Table 3; Fig. 3). Although neotetraploids produced heavier and fewer seeds than diploids, the cytotype by ecotype interaction on both average weight per seed and seeds per silique was driven by variation in the magnitude of ecotype-specific responses to neotetraploidy for average weight per seed and seeds per silique, respectively.

Table 2 Summary of linear mixed effect models for three phenology traits

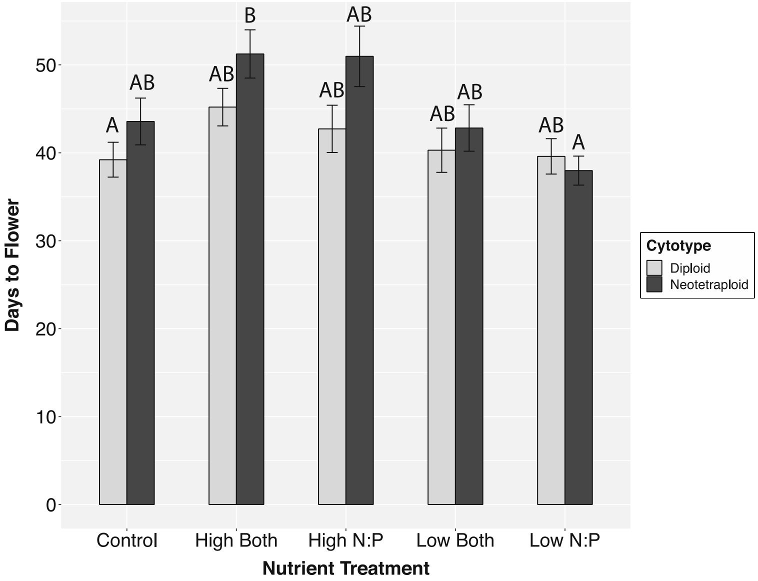
Measurement	Fixed Effect	df (treatment, error)	F	*P*
Days to Bolt	Treatment	4, 343	7.12	**<0.001**
	Cytotype	1, 343	8.13	**0.005**
	Ecotype	3, 343	122.96	**<0.001**
	Treatment x Cytotype	4, 343	1.65	0.161
	Treatment x Ecotype	12, 343	1.95	**0.028**
	Cytotype x Ecotype	3, 343	5.30	**0.001**
	Treatment x Cytotype x Ecotype	12, 343	0.94	0.512
Days to Flower	Treatment	4, 331	8.40	**<0.001**
	Cytotype	1, 331	10.72	**0.001**
	Ecotype	3, 331	131.75	**<0.001**
	Treatment x Cytotype	4, 331	1.65	0.163
	Treatment x Ecotype	12, 331	2.01	**0.023**
	Cytotype x Ecotype	3, 331	5.49	**0.001**
	Treatment x Cytotype x Ecotype	12, 331	1.12	0.341
Bolt to Flower	Treatment	4, 331	2.60	**0.036**
	Cytotype	1, 331	18.71	**<0.001**
	Ecotype	3, 331	12.68	**<0.001**
	Treatment x Cytotype	4, 331	0.86	0.491
	Treatment x Ecotype	12, 331	2.33	**0.007**
	Cytotype x Ecotype	3, 331	6.02	**<0.001**
	Treatment x Cytotype x Ecotype	12, 331	1.85	**0.040**

Signficant results are bolded

Overall, there appeared to be a negative relationship between average mass per seed and the average number of seeds per silique (Fig. 3).

There was a cytotype by ecotype interaction for fecundity (Table 3), where the C24 and Landsberg neotetraploid origins caused fecundity to be generally

Fig. 1 Days to first flowering for diploid and neotetraploid *Arabidopsis thaliana.* Grey bars represent diploids, and black bars correspond to neotetraploids (± standard error). Letters denote groupings of Tukey's post hoc tests of the nutrient supply by cytotype interaction, where groups that do not significantly differ at $p = 0.05$ are marked with the same letter



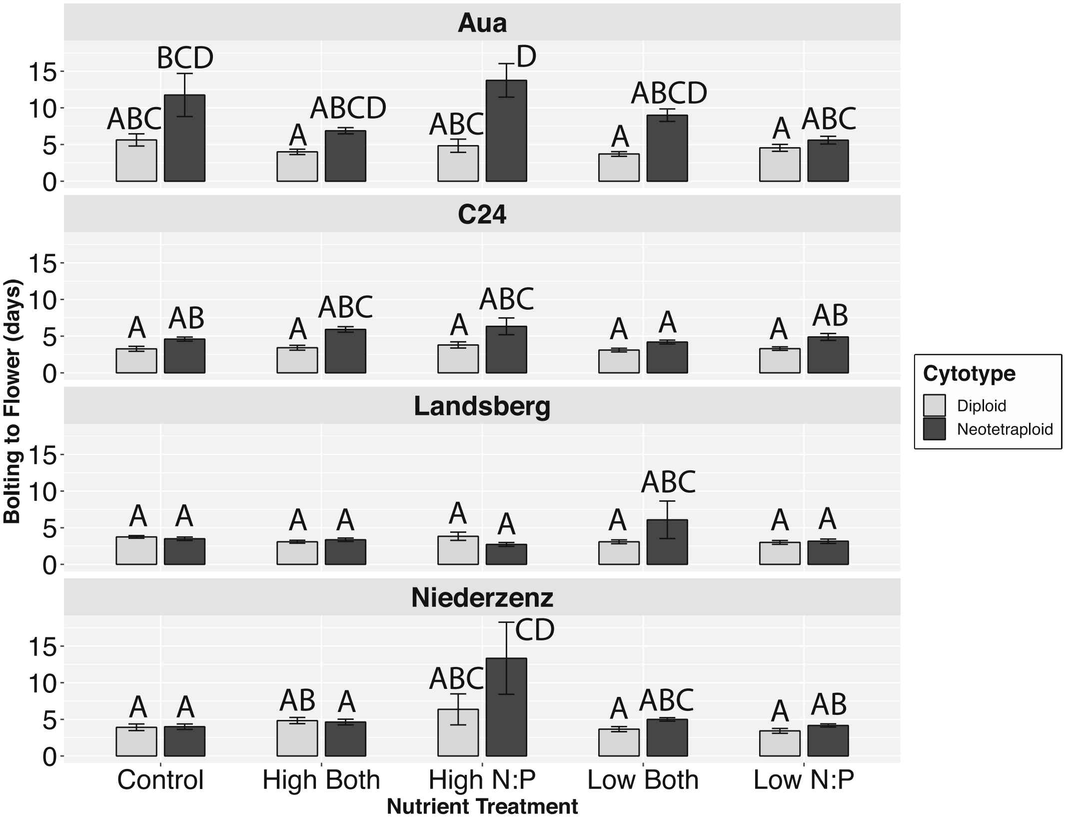


Fig. 2 The latency in days between time of first bolting and first flowering. Each subpanel represents an ecotype or "independent origin" of neotetraploidy, with grey bars representing the diploid ancestor and black bars representing the neotetraploid offspring lineage (± standard error). Letters denote groupings of Tukey's post hoc tests of the ecotype by nutrient supply by cytotype interaction, where groups that do not significantly differ at $p \geq 0.05$ are marked with the same letter

similar between diploids and neotetraploids, but neotetraploids from Aua and Niederzenz had lower fecundity than diploids. The main effect of neotetraploidy caused a significant reduction in plant fecundity, whereas high N & P and the control nutrient treatment increased fecundity compared to the other nutrient treatments. The germination success of these seeds was also increased by neotetraploidy, and there was variation among ecotypes in germination rate. We also compiled all three of the independent reproductive measures of fitness by multiplying fecundity, average mass per seed, and average germination rate of seeds as a composite measure of fitness and found a significant interaction between cytotype and nutrient treatment as well as between cytotype and ecotype (Fig. 4; Table 3). The cytotype by nutrient treatment interaction was driven by an increase in neotetraploid composite fitness with high N & P nutrient treatment, but was not different in the other nutrient treatments (Fig. 4).

Lastly, we assessed the total aboveground biomass of plants as another fitness-related measure.

There were significant interaction effects between nutrient treatment and cytotype (Fig. 5; Table 3) where neotetraploids had greater biomass production with high N & P treatment compared to no differences in biomass between diploids and neotetraploids in the other nutrient treatments. The high N & P treatment also caused greater biomass production for both diploids and neotetraploids, but there was no difference among the other nutrient treatments in biomass production.

The effect of independent origins of neotetraploidy on trait responses

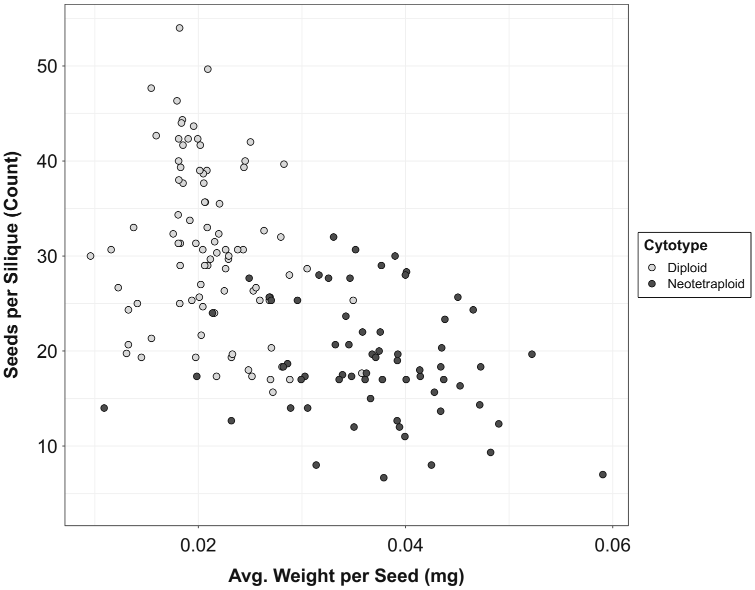
We addressed how multiple independent origins of neotetraploidy affected the cytotype-specific responses of plants to nutrient treatment. We found only one instance of a three-way interaction between ecotype or

Table 3 Summary of generalized least squares models of biomass and fitness-related traits

Measurement	Fixed Effect	df (treatment, error)	F	*P*
Avg. Weight per Seed	Treatment	4, 116	1.01	0.408
	Cytotype	1, 116	302.77	**<0.001**
	Ecotype	3, 116	12.10	**<0.001**
	Treatment x Cytotype	4, 116	1.452	0.221
	Treatment x Ecotype	12, 116	0.85	0.603
	Cytotype x Ecotype	3, 116	5.25	**0.002**
	Treatment x Cytotype x Ecotype	12, 116	0.89	0.558
Seeds per Silique	Treatment	4, 120	2.27	0.065
	Cytotype	1, 120	156.95	**<0.001**
	Ecotype	3, 120	21.79	**<0.001**
	Treatment x Cytotype	4, 120	0.91	0.460
	Treatment x Ecotype	12, 120	1.95	**0.035**
	Cytotype x Ecotype	3, 120	4.11	**0.008**
	Treatment x Cytotype x Ecotype	12, 120	1.08	0.386
Fecundity	Treatment	4, 310	22.64	**<0.001**
	Cytotype	1, 310	22.62	**<0.001**
	Ecotype	3, 310	10.63	**<0.001**
	Treatment x Cytotype	4, 310	0.65	0.631
	Treatment x Ecotype	12, 310	1.30	0.219
	Cytotype x Ecotype	3, 310	8.85	**<0.001**
	Treatment x Cytotype x Ecotype	12, 310	1.30	0.220
Progeny Germination Rate	Treatment	4, 118	0.58	0.675
	Cytotype	1, 118	6.67	**0.011**
	Ecotype	3, 118	3.25	**0.024**
	Treatment x Cytotype	4, 118	0.82	0.516
	Treatment x Ecotype	12, 118	1.46	0.149
	Cytotype x Ecotype	3, 118	0.52	0.669
	Treatment x Cytotype x Ecotype	12, 118	0.71	0.736
Composite Fitness	Treatment	4, 310	30.98	**<0.001**
	Cytotype	1, 310	0.45	0.502
	Ecotype	3, 310	10.95	**<0.001**
	Treatment x Cytotype	4, 310	3.96	**0.004**
	Treatment x Ecotype	12, 310	1.74	0.058
	Cytotype x Ecotype	3, 310	7.29	**<0.001**
	Treatment x Cytotype x Ecotype	12, 310	1.39	0.169
Biomass	Treatment	4, 325	39.59	**<0.001**
	Cytotype	1, 325	1.51	0.220
	Ecotype	3, 325	11.72	**<0.001**
	Treatment x Cytotype	4, 325	3.79	**0.005**
	Treatment x Ecotype	12, 325	2.38	**0.006**
	Cytotype x Ecotype	3, 325	4.81	**0.003**
	Treatment x Cytotype x Ecotype	12, 325	1.08	0.374

Significant results are bolded

Fig. 3 Scatterplot showing the effect of cytotype on the relationship between the average weight per seed and the number of seeds per silique. Grey dots indicate diploids and black dots neotetraploids



"multiple origins", cytotype, and nutrient treatment for the latency from date of bolting to first flowering (Table 2; Fig. 2). This three-way interaction was driven by the Landsburg and C24 neotetraploid origins not differing between diploids and neotetraploids in bolting to flowering time, the Aua origin of neotetraploidy had delayed bolting to flowering phenology, whereas the Niederzenz origin of neotetraploidy had delayed phenology only with high N:P treatment. For all other traits that were measured, the ecotype of neotetraploid origin and plant cytotype significantly interacted (Tables 2 and 3; Fig. 6), indicating that multiple origins of neotetraploidy has a strong effect on trait responses of *A. thaliana.*

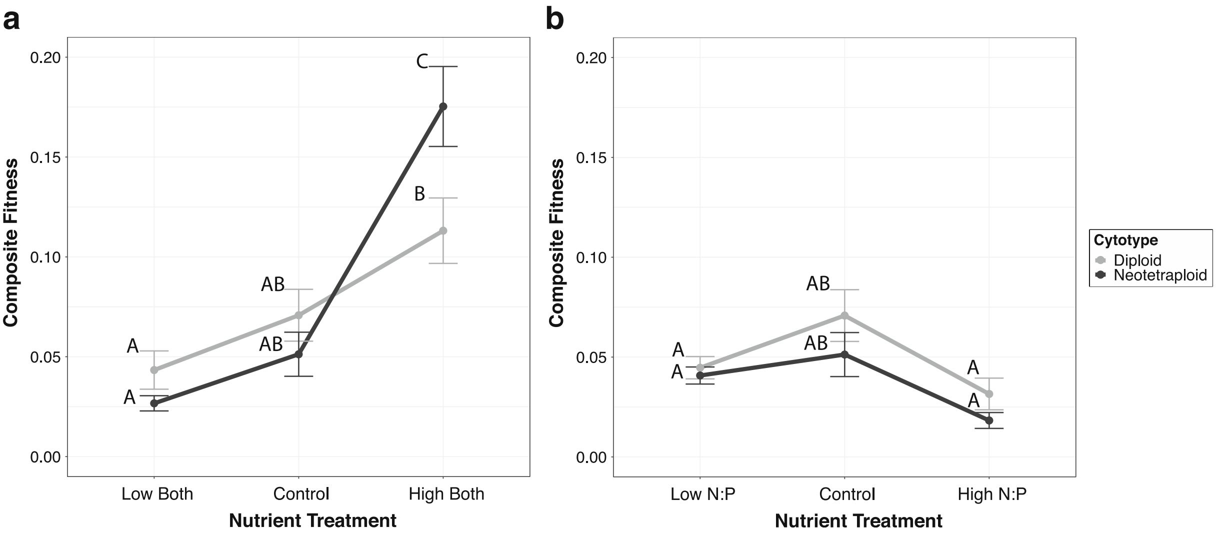


Fig. 4 Reaction norm plots of composite fitness variation in response to nutrient supply. Balanced nutrient treatments (**a**) and adjusted stoichiometry treatments (**b**). Grey lines are diploids, and black lines are neotetraploids (± standard error). Letters denote groupings of Tukey's post hoc tests of the nutrient supply by cytotype interaction, where groups that are significant have different letters

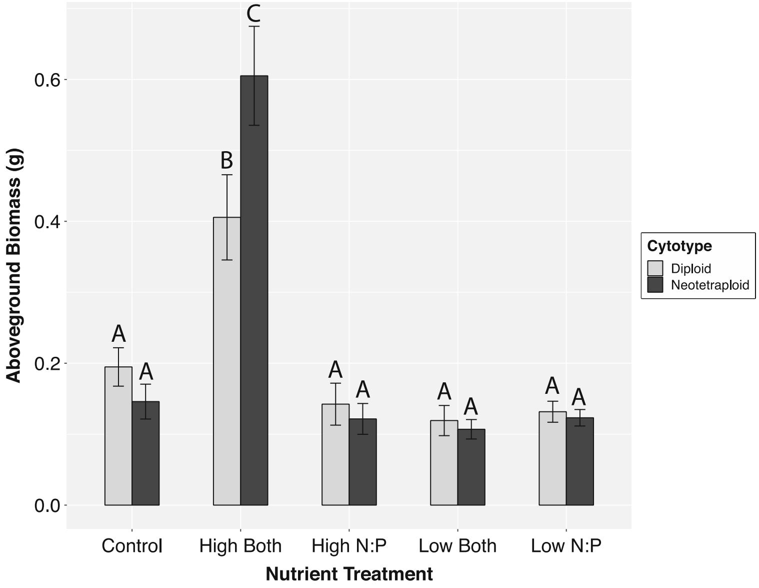


Fig. 5 Aboveground biomass production of *Arabidopsis thaliana* diploids and neotetraploids across nutrient treatments. Grey bars indicate diploids and black bars neotetraploids (± standard error). Letters denote groupings of Tukey's post hoc tests of the nutrient supply by cytotype interaction, where groups that are significant have different letters

Discussion

Polyploidy is predicted to increase the nutrient requirements of plants and thus constrain their ability to grow and reproduce in resource-limited environments. Although a few studies have shown that polyploids can be more productive with high nutrient supply rates (Guignard et al. 2016; Walczyk and Hersch-Green 2019; Šmarda et al. 2013), we do not know how other corresponding traits associated with lifetime fitness are affected. In this study, we specifically tested how neotetraploidy affects plant nutrient limitation by varying nitrogen and phosphorus supply rates and comparing the responsiveness of fitness-related traits of neotetraploids to their diploids parents. The results were consistent with the prediction that WGD causes greater nutrient requirements in plants. Nutrient limitation is often defined by the responsiveness of a plant to an increasing supply of the nutrients of interest (Vitousek et al. 2010). In the present study, we observed that growth and key fitness-related traits of neotetraploid *A. thaliana* responded more positively to a high supply of nitrogen and phosphorus than their diploid parents (Figs. 4 and 5), indicating that there is strong evidence that neopolyploidy increases nutrient limitation in *A. thaliana*. Similar to previous studies that have observed that polyploids are more responsive to nutrient environment than diploids (Guignard et al. 2016; Šmarda et al. 2013), we found that the reproductive output of neotetraploids was more responsive to the nutrient content of the soil. Interestingly, with low and control nutrient supply, diploids and neotetraploids had equivalent performance (Table 3). The comparatively stronger response of neotetraploid composite fitness to the high nutrient treatment suggests that neotetraploids were more plastic to nutrient environments (Figs. 4 and 5). This result supports the prediction that WGD causes an increase in phenotypic plasticity (Parisod et al. 2010). The enhanced performance of neotetraploids when given a high supply of nitrogen and phosphorus suggests that high resource soil environments may be more favorable for the establishment and persistence of neopolyploids.

Although we expected that neotetraploid *A. thaliana* would respond differently than their diploid parents to an altered ratio of nitrogen to phosphorus, we saw no interaction between cytotype and altered N:P stoichiometry. In fact, when we compared the growth and fitness responses of plants between all nutrient treatments, we observed that diploids and neotetraploids responded similarly to altered N:P stoichiometry as they did to low nitrogen and phosphorus. This observation was likely due to the fact that both nitrogen and phosphorus are essential nutrients for plant growth, and neither resource is substitutable for the other when a plant uses them for physiological processes (Sperfeld et al. 2016). Thus, variation in the quantity of non-substitutable nutrients supplied to plants, rather than their molar ratio of supply, is the only situation that is expected to result in substantial variation of trait responses, indicating that

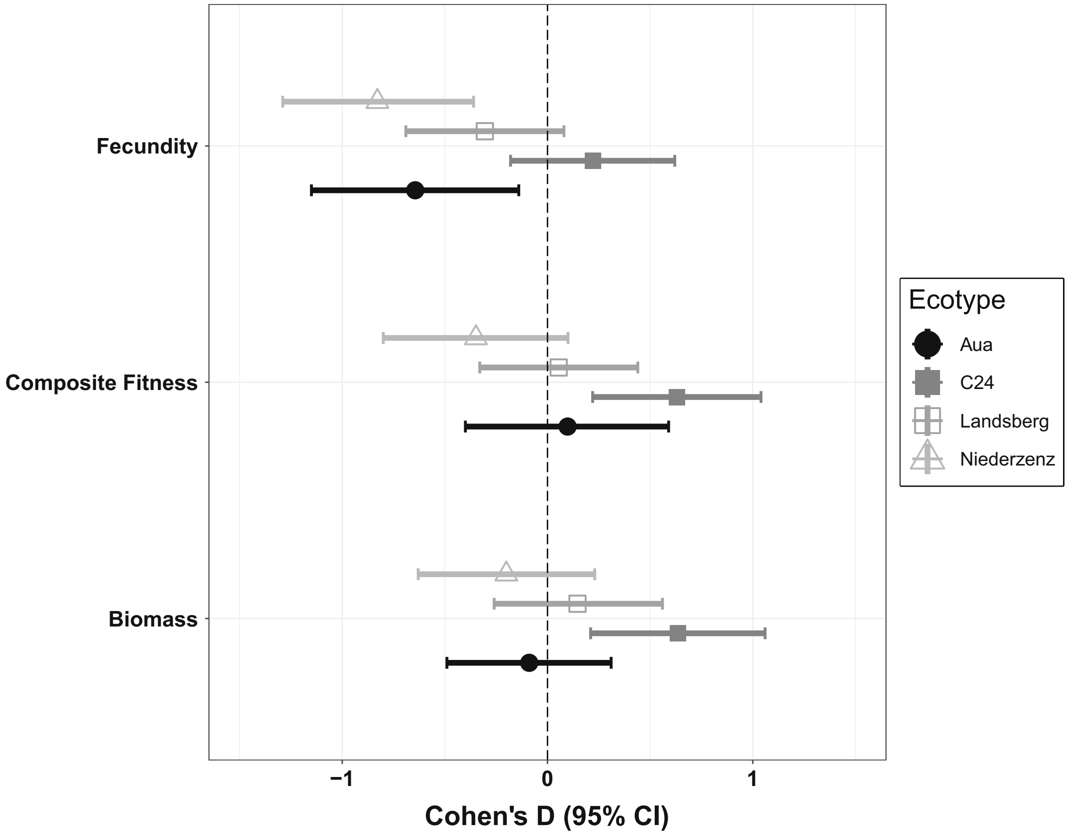


Fig. 6 Effect of multiple origins of neotetraploidy. Cohen's D was determined as the difference between diploids and neotetraploids for each ecotype (origin). Dots represent the average Cohen's D measurement and bars denote the 95% confidence interval

they are co-limiting resources (Sperfeld et al. 2016). Indeed, when we simultaneously supplied both nitrogen and phosphorus along a molar N:P ratio of 16, a value often considered to be co-limiting for many terrestrial plants, we observed an appreciable increase in growth and fitness in the plants. Consequently, we conclude that neotetraploidy does not affect the sensitivity of *A. thaliana* to nitrogen and phosphorus stoichiometry, rather, WGD affects plant sensitivity to the concentration of these co-limiting nutrients.

Similar to the measure of composite fitness, we found that neotetraploid *A. thaliana* produced significantly more aboveground biomass than their diploid progenitors, but only when supplied with a balanced and high supply of nitrogen and phosphorus. This enhancement in productivity of neotetraploids in response to nutrient enrichment corroborates long-term fertilization studies in grasslands that show that nutrient enrichment causes established polyploid plants to have a greater response in biomass productivity than co-occurring, unrelated diploid species (Guignard et al. 2016; Šmarda et al. 2013). The greater responsiveness of neotetraploids to increased nutrient supply in this study was likely driven by an increase in the nutritional cost of building inherently larger polyploid tissues, where a low nutrient supply allowed for only minimal growth of both cytotypes, but the neotetraploids achieved greater maximum growth with high nutrient supply. Although some mechanisms have been proposed to explain the greater responsiveness of polyploids to high nutrient supply, such as greater nutrient uptake efficiency, the examples we have are mostly species dependent (Cacco et al. 1976). As more studies that compare diploids and their neopolyploid progeny emerge from a broad phylogenetic sampling, we will be able to disentangle the species-specific effects from the universal effects of WGD that affect neopolyploid responses to increased nutrient supplies.

In addition to finding that neotetraploidy promoted the biomass production of *A. thaliana,* we also observed a strong interaction between cytotype and nutrient supply on the lifetime fitness of plants. The measure of lifetime fitness of plants in this study was an emergent property of multiple fitness-related traits. For instance, when we considered fecundity alone, we did not detect an interaction between plant cytotype and nutrient environment, but when we factored in the average weight per seed and the germination rates of those seeds, we saw a strong interaction between cytotype and nutrient treatment. The disparity between the results for fecundity versus composite fitness was caused by diploids outpacing their neotetraploid progeny in seed production, but neotetraploids made heavier seeds that germinated at higher rates (Fig. 3). This suggests that fecundity is the most labile reproductive effect of neopolyploidy and thus serves as a compensatory mechanism to overcome the inherently greater costs of building larger neopolyploid seeds. Similar to the negative effect of neotetraploidy on fecundity that we observed, (Walczyk and Hersch-Green 2019) also observed that neotetraploidy in *C. angustifolium* caused a reduction in the number of flowers per plant, suggesting that reduced fecundity occurs in neotetraploid *C. angustifolium*. Given that numerous studies have found a strong positive correlation between seed mass and genome size (Caceres et al. 1998; Chung et al. 1998; Richardson et al. 2015), increased nutrient supplies may commonly promote the lifetime fitness of neopolyploid plants more than their diploid parents when we take into account the greater investment into individual seeds.

Despite finding a strong interaction between cytotype and nutrient supply on growth and fitness related traits in *A. thaliana,* we did not observe a similar effect on plant flowering phenology. Instead, we observed that both the main effect of nutrient treatment and cytotype independently explained a significant amount the variation in flowering phenology. Specifically, neotetraploids had delayed flowering phenology compared to their diploid ancestors at all nutrient treatment levels, but the high N & P treatment also caused both diploids and neotetraploids to flower later. This suggests that temporal separation may segregate diploids and their neopolyploid descendants. The divergence of flowering phenology between potentially competing cytotypes has been viewed as a mechanism that can promote the odds of neopolyploid establishment (Fowler and Levin 2016; Husband and Sabara 2004; Oswald and Nuismer 2011; Rodriguez 1996; Segraves and Thompson 1999). Although the pattern of delayed flowering in neotetraploids was consistent across nutrient treatments, we do not know if our findings from the greenhouse are directly relatable to field settings. Specifically, the greenhouse growth conditions used in this study likely deviate from natural *A. thaliana* growing conditions in terms of light and temperature. However, because the greenhouse conditions were common to all plants in the experiment, we consider the differences in performance observed between plants to be the result of treatments, rather than the climate controls. Furthermore, we were unable to conclude if the differences in flowering phenology between diploids and neotetraploids are adaptive, since *A. thaliana* is primarily a selfing annual, although rare outcrossing events do occur (Abbott and Gomes 1989; Hoffmann et al. 2003; Platt et al. 2010). Future experimental work that investigates the evolutionary lability of flowering phenology in synthetic neopolyploids will help to resolve whether temporal isolation affects neopolyploid persistence.

An additional interesting finding was that the trait responses of *A. thaliana* neotetraploids often interacted with the ecotype of origin, supporting the hypothesis that variation in neopolyploid phenotypes is increased by the repeated genesis of independent neopolyploid lineages. Although the only instance in which we observed a three-way interaction between nutrient treatment, cytotype, and ecotype of neotetraploid origin was for the latency between date of bolting to first flower, we observed a significant interaction between cytotype and ecotype on individual traits in all other cases (Table 3 and Fig. 4). Numerous studies have highlighted the idea that multiple origins of neopolyploidy within a species can support the odds of polyploid establishment by integrating more of the standing genetic variation present in diploids into nascent polyploid populations (Soltis and Soltis 1999). Other recent studies have also supported this idea through comparisons of independent origins neopolyploidy, where they observed that trait responses of neopolyploids varied with maternal origin (Husband et al. 2016; Pacey et al. 2020; Wei et al. 2020). Although the four ecotypes of origin were chosen based on their availability at the time, we recognize that having only four ecotypes constrains our ability to make conclusions about the universal effects of polyploidy on *A. thaliana*. Despite this constraint, we found evidence that the trait responses of cytotypes of *A. thaliana* to nutrient supply were contingent on their independent ecotype of origin.

We expect that the three independent origins of synthetic neotetraploidy in *A. thaliana* used in this study will more closely resemble naturally derived neotetraploids since outcrossing in *A. thaliana* is rare (Abbott and Gomes 1989; Hoffmann et al. 2003; Platt et al. 2010). In contrast, colchicine-induced synthetic neopolyploids of outcrossing species may not be representative of naturally occurring neopolyploids, since colchicine is typically applied to zygotes, meaning that there are only two alleles possible per locus in the resulting neopolyploid (Dhooghe et al. 2011). Natural neopolyploids are expected to experience higher allelic diversity, since WGD primarily occurs through the union of unreduced gametes, leading to a maximum of four unique alleles per locus in neotetraploids (Otto and Whitton 2000). Additionally, we recognize that the application of mitotic inhibitors, such as colchicine, can have trans-generational effects on plant physiology, but a colchicine treatment effect is improbable in the neotetraploid maternal lines that were used here. This is because other studies have shown that the effects of colchicine effect dissipate after the first generation (Husband et al. 2016), and the neotetraploid seed stocks used here have had a few generations to remove this treatment effect.

The results show that neotetraploidy causes *A. thaliana* to be more responsive to nitrogen and phosphorus addition, supporting the hypothesis that WGD causes greater nutrient limitation in plants, and also that multiple origins of neopolyploidy can affect trait responses to nutrient supply. This study, along with other recent examples, underscores the need to investigate first-generation polyploids to capture the immediate consequences of WGD on plant ecophysiology. Studies that incorporate multiple independent genetic origins of neopolyploidy will reveal how WGD affects plant ecological interactions. In addition to highlighting the need to incorporate multiple genetic origins of neopolyploidy, this study has uniquely shown that neopolyploidy promotes the complex lifetime fitness response of *A. thaliana* to increased nutrient supply.

Acknowledgements We thank D. Althoff and S. Wang for assisting in the experimental design, and D. Althoff and A. Curé for help harvesting the plants. The authors thank D. Althoff, D. Frank, M. Ritchie, and M. Vidal for providing technical advice, and D. Althoff, S. Wang, A. Curé, P. Šmarda, M. Vidal, and an anonymous reviewer provided comments on earlier versions of the manuscript. This research was supported by a Sigma Xi Grants-In-Aid of Research to TJA, and NSF DEB 1556568 and 1655544 to KAS.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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