Autopolyploids of Arabidopsis are more phenotypically plastic than their diploid progenitors

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Summary

Polyploids have been hypothesized to have increased phenotypic plasticity compared to their diploid progenitors, but recent work has tended to suggest more complicated relationships between whole genome duplication (WGD) and plasticity. Impacts of WGD on plasticity are moderated by other evolutionary processes in nature, which has impeded generalizations regarding the effects of WGD alone. To isolate WGD effects on plasticity, we manipulated stressors in a common garden experiment comparing two diploid lineages of *Arabidopsis thaliana* to corresponding autotetraploids. For all cases in which diploids and polyploids differed in plasticity, polyploids were more plastic, consistent with previous work suggesting WGD increases plasticity. Increased plasticity was often adaptive (associated with higher total seed mass) but showed some neutral relationships to fitness under stress. Mean trait values were also affected by WGD, such as slowed phenology in polyploids. Slowed phenology was adaptive in one polyploid lineage under amenable conditions but was maladaptive in the other lineage under stress, highlighting context-dependency in the adaptive consequences of WGD. Our work shows that increased phenotypic plasticity can result from WGD alone, independent of other processes like natural selection or hybridization.

Key words: *Arabidopsis*, autopolyploidy, common garden, niche breadth, phenotypic plasticity, polyploidy, salt, whole genome duplication

Introduction

Species must rapidly adapt or go extinct. Rapid adaptation has been driven by large genomic rearrangements like whole genome duplication (WGD), evidenced in ancient speciation events (Van de Peer *et al.,* 2017; Novikova *et al.,* 2018) and in contemporary evolution (te Beest *et al.,* 2012; Pandit *et al.,* 2014). Immediately following WGD, gene redundancy instantaneously produces dosage effects that can alter expression (del Pozo & Ramirez-Parra, 2015). In later generations, further genomic and evolutionary processes will shape a new polyploid lineage (Parisod *et al.,* 2010), scaling up into traits, environmental tolerances, and geographic distributions that diverge from the lower-ploidy progenitor.

Though the ecological impacts of WGD are diverse, many have sought to reach generalizations. Polyploids have been hypothesized to occupy narrower niche space than their progenitors, due to polysomic inheritance patterns that tend to increase heterozygosity (Stebbins, 1950, 1971; Soltis *et al.,* 2014). In contrast, recent work has revealed many examples of polyploids with niche breadths similar to or broader than those of lower-ploidy relatives. Many tests of the impact of WGD on niche breadth and divergence have been ecological niche models comparing geographic distributions of related species or cytotypes of the same species (Laport *et al*., 2013; Glennon *et al.,* 2014; Marchant *et al.,* 2016; Gaynor *et al.,* 2018; Banaiga *et al.,* 2020). Common gardens and reciprocal transplants offer complementary approaches to ecological niche models (Glennon *et al.,* 2014). These experiments measure niche divergence as functional trait differentiation and often proxy niche breadth as phenotypic plasticity—the change in trait values measured across an environmental gradient.

Whole genome duplication has been hypothesized to increase phenotypic plasticity—a phenomenon invoked in part to explain how WGD facilitates niche divergence. This hypothesis derives from genomic evidence; WGD increases number of gene copies and produces regulatory, epigenetic, and epistatic changes that make polyploids more genomically plastic, or able to vary gene expression more widely, than progenitors (Jackson & Chen, 2010; Van de Peer *et al.,* 2017). However, manipulative studies show mixed results. Some find increased plasticity in higher ploidy levels (Meerts, 1992; Hahn *et al.,* 2012) while others do not (Bretagnolle & Thompson, 2001; Mraz *et al.,* 2014; Gallego-Tévar *et al.,* 2018; Wei *et al.,* 2019).

Mixed support is likely in part explained by the fact that, in many systems, the effects of WGD may be confounded with other evolutionary processes. First, for about half of all polyploids (Barker *et al.,* 2016), WGD is accompanied by interspecific hybridization (allopolyploidy; in contrast to autopolyploidy, in which WGD is not coupled with hybridization). Further complicating this issue are instances of cryptic auto- or allopolyploidy. Autopolyploids can be difficult to morphologically distinguish from diploids, taxa once thought to be autopolyploid have been revealed to be of hybrid origin, and—even for known allopolyploids—parents are often unknown or extinct (Parisod *et al.,* 2010). These problems preclude efforts to assess ecological effects of WGD because in allopolyploids, effects of WGD are coupled with the influence of two separate genomes. Studies that have estimated the separate the genomic effects of hybridization and WGD have shown that hybridization impacts allopolyploid genomes more than WGD (Jackson & Chen, 2010). Assuming genomic effects are mostly additive, one would predict hybridization—which has also been associated with increased plasticity (Cara *et al.,*

2013)—would drive most differences between allopolyploids and their parents, potentially masking the smaller impacts of WGD. Consistent with the idea that hybridization impacts traits more than polyploidy, a recent study found that plasticity values did not differ much between higher- and lower-ploidy hybrids sharing the same parental taxa, but that plasticity in the higher-ploidy hybrid shifted more from parents than did the lower-ploidy hybrid (Gallego-Tévar *et al.,* 2018). This study has been the only empirical test of this hypothesis of which we are aware, and many have recently called for studies that separate the effects of polyploidy from hybridization (Parisod *et al.,* 2010; Spoelhof *et al.,* 2017; Wei *et al.,* 2019).

Other circumstances of origin can influence the evolutionary trajectory of a polyploid lineage. New polyploid individuals are often rare within a population and are isolated from backcrossing with a progenitor (Levin 1975); even if extinction is avoided, WGD can present a genetic bottleneck. However, polyploid lineages characterized by multiple origins and subsequent admixture can overcome a bottleneck (Parisod *et al.,* 2010; Soltis *et al.,* 2014). Life history may further interact with genetic diversity. For example, selfing or clonal reproduction will tend to reduce genetic diversity (Igic *et al.,* 2008). Trajectories of lineages that suffer suppressed genetic diversity may be driven by drift, meaning that their traits reflect chance rather than WGD. Traits in admixed lineages may disproportionately reflect that additional variation and heterozygosity—similar to the issue of interspecific hybridization masking WGD effects. These processes and their effects on genetic diversity may also determine whether plasticity is adaptive. In cases of suppressed genotype diversity, phenotypic plasticity may comprise an important component of adaptive potential (Castillo *et al.,* 2018). Circumstances of origin and early demography, and their relationships to genetic diversity, are unknown for most natural polyploid lineages, limiting inferences regarding the signature of WGD in their traits.

Finally, the effects of natural selection on a polyploid lineage will be context specific (by definition) and therefore can modify WGD effects variously. Traits of more ancient polyploid lineages might have been so shaped by selection (combined with the processes mentioned above) as to impede any interpretation of their traits as having resulted from WGD. A newly generated polyploid lineage may also be strongly influenced by selection, as it competes with its progenitor and may face strong pressure for niche differentiation (Levin 1975). In both neo- and paleopolyploids, the potential influences of selection and drift make them less appropriate for measuring WGD effects on traits than synthesized polyploid lineages in which genome doubling is induced in the lab via colchicine or other mutagens. Synthesized polyploids exist outside of a natural selective context, but biologically relevant contexts can be imposed in a manipulative

experiment. This ability to impose novel selective contexts is a benefit because potentially maladaptive WGD effects can also be measured (in nature, maladaptive traits would presumably be selected out of a lineage). Phenotypic plasticity may be maladaptive (associated with fitness costs; Dechaine *et al.,* 2007), such as when one phenotype is optimal across all environments. Plasticity is likely to be adaptive (associated with fitness gains) under certain conditions, such as spatially or temporally variable environments (Berrigan & Scheiner, 2004). The adaptive values of plasticity shifts associated with WGD have rarely been quantified (but see Wei *et al.,* 2019). Such assessments are important because even if WGD does not produce large plasticity shifts, plasticity may still confer a fitness advantage for polyploid taxa over lower-ploidy relatives (Wei *et al.,* 2019).

Arabidopsis thaliana is an excellent system for studying the trait effects of WGD, in isolation from other evolutionary processes. Autotetraploidy has arisen naturally multiple times for *A. thaliana* (Vergara *et al.,* 2017). Synthetic autotetraploid *A. thaliana* genotypes have also been developed (e.g. Chen *et al.,* 1998, Pignatta *et al.,* 2010). Most previous work comparing *A. thaliana* diploids and tetraploids has focused on the genomic impacts of WGD, but some have also considered WGD effects on traits and adaptation (reviewed by del Pozo & Ramirez-Parra, 2015). Existing phenotyping and molecular work suggests abiotic stress may be an appropriate biological context in which to compare diploids and tetraploids. These cytotypes have been shown to vary in gene expression (Wang *et al.,* 2004; Pignatta *et al.,* 2010; Liu *et al.,* 2017), including for genes related to stress response (Yu *et al.,* 2010; Ng *et al.,* 2012; del Pozo & Ramirez-Parra, 2014). Shifts in expression also vary by tissue type (Wang *et al.,* 2004; Yu *et al.,* 2010; del Pozo & Ramirez-Parra, 2014), and in some cases these changes scale up into differences in functional traits and stress responses, such as under experimental salt (NaCl) stress (Chao *et al.,* 2013; del Pozo & Ramirez-Parra, 2014). Phenotypic plasticity has not been assessed for these taxa, but, in general, abiotic stressors appear to be important modulators of *A. thaliana* traits, appropriate for measuring plasticity (Pigliucci *et al.,* 1995; Pigliucci & Schlichting, 1998).

To assess WGD effects on phenotypic plasticity, we grew two lineages of synthesized autotetraploid *A. thaliana* alongside related diploids. Use of synthesized autotetraploids allowed us to examine the consequences of WGD alone, independent of the other potentially synergistic or obfuscating processes that operate on natural lineages, such as hybridization, drift, natural selection, or other demographic factors. In a common garden, we imposed treatments that would produce variation in fitness, phenological, and vegetative traits, allowing us to measure

phenotypic plasticity. We chose edaphic stress treatments relevant to the biology of *A. thaliana*. Salt (NaCl), a natural component of many soils, is a common stressor for many plant species and presents an important selective gradient for *A. thaliana* lineages growing in coastal (Busoms *et al.,* 2015) or anthropogenically disturbed habitats (Pigliucci, 2002). Salt stress could be exacerbated by interactive effects with other stressors. Examining multiple stressors is useful in common garden studies because *A. thaliana* has been observed to respond idiosyncratically to different types of abiotic stress (Pigliucci *et al.,* 1995; Pigliucci & Schlichting, 1998). Salinity impacts plant nutrient uptake ability to different degrees, depending on *A. thaliana* genotype (Busoms *et al.,* 2015), suggesting the interaction between salt and nutrients is selectively relevant for these taxa. Nutrients are heterogeneously distributed in space and time, and there is a selective advantage to being able to plastically respond to abundance of these resources. For *A. thaliana,* there may be an adaptive benefit to plasticity associated with nutrient variability because an annual mother plant's decomposition creates a nutrient pulse that can be accessed by germinating offspring (Pigliucci & Schlichting, 1998). For our experiment, investigating realistic selective contexts was crucial for allowing us to go beyond capturing WGD-induced trait and plasticity differences to also facilitating assessment of potential adaptive consequences.

Our experiment assessed: 1) shifts in phenotypic plasticity accompanying WGD, 2) shifts in mean trait values, and 3) the adaptive consequences of these shifts (association with fitness). Question 1 tested the hypothesis that polyploids are more plastic that their lower-ploidy progenitors. Question 2 has already been investigated to some extent for these taxa (Wang *et al.,* 2004; Pignatta *et al.,* 2010; Ng *et al.,* 2012; Chao *et al.,* 2013; del Pozo & Ramirez-Parra, 2014), but the adaptive values of these shifts have not been assessed (Question 3).

We predicted the genomic effects of WGD would induce shifts in phenotypes and plasticities (Jackson & Chen, 2010; Van de Peer *et al.,* 2017). We also expected WGD would slow mean values for phenology, a consequence of increased cell size (del Pozo & Ramirez-Parra, 2015). We were also interested in comparing results for Questions 1 and 2, asking whether WGD was more likely to affect mean phenotypes or phenotypic plasticity. Question 3 extended our results to the larger evolutionary consequences of WGD.

Materials and Methods

Plant Material

We obtained the following accessions of *Arabidopsis thaliana* (L.) Heynh. from the Arabidopsis Biological Resource Center (ABRC, Ohio State University). We considered one synthesized tetraploid Col-0 ("Col4x": CS3151, Chen *et al.,* 2004) as matched to two diploid accessions of Col-0 ("Col2x": CS1092, Griod *et al.,* 1993; CS69113, Pignatta *et al.,* 2010). Our Col4x was originally synthesized from a different diploid Col-0 lineage (CS3176; ABRC, 2020c), an accession for which we had low germination, but all Col-0 accessions are known to be genetically similar (ABRC, 2020a). Two synthesized tetraploid Ler-1 ("Ler4x": CS3900, created from CS20, Chen *et al.,* 1998; CS69112, created from CS1642, Solhaug *et al.,* 2016) were matched with two diploid Ler-1 ("Ler2x": CS69111, Solhaug *et al.,* 2016; CS1642, ABRC, 2020b). Col2x CS69113 and Ler2x CS69111 were both descended from plants exposed to the mutagen colchicine, which is used to generate polyploids, but that did not undergo WGD; their inclusion accounted for other variability potentially resulting from colchicine-derived mutations. Each accession had been selfed for an unknown number of generations prior to being donated to ABRC and were selfed for an additional 1-2 generations under common conditions by ABRC. Though genome changes can continue to accumulate over generations after WGD (Weiss & Maluszynska, 2001), synthetic autotetraploid *A. thaliana* are generally stable over many generations (Yu *et al.,* 2009; Liu *et al.,* 2017). For different accessions, we found that focal trait values did not differ significantly within taxa (accessions sharing ploidy and lineage [Col-0 or Ler-1]) so we grouped these for analysis.

Experimental Design

We sowed seeds into germination trays containing moist Sunshine Redi-Earth Plug and Seedling Propagation Mix (contains no fertilizer) on June 21, 2018. We cold stratified seeds by storing them in the dark at 4°C for 7 days (June 28), after which they were moved to a growth chamber kept at 22°C with 16-hour days. Fifteen days later (July 13, 22 days after sowing), we transplanted seedlings into individual pots (10 cm diameter * 9 cm height). We prepared 24 pots of each of Col2x (CS1092, n = 12; CS69113, n = 12), Col4x (CS3151, n = 24), Ler2x (CS1642, n = 12; CS69111, n = 12), and Ler4x (CS3900, n = 12; CS69112, n = 12), for a total of 96 plants in the experiment. We placed pots into 24 trays, each containing a full representation of the four taxa, to facilitate treatment applications by tray. We spread the 24 trays evenly across three growth chambers (8 trays each). We randomly assigned location of each pot within a tray. During each watering, we shuffled both the locations of trays within chambers and the locations of pots within trays. We bottom-watered pots every three days by adding 2cm of water to the tray and allowing pots to sit until the top of the soil was moist (at least one hour), then dumping

remaining water from the tray. After transplants had acclimated for six days, on July 19 (28 days after sowing), we began replacing waterings with treatment solutions. We used four different experimental treatments, replicated six times in total (twice per growth chamber).

Treatments

We manipulated two abiotic conditions: nutrients and salt (NaCl). Of salt and nutrient limitation, salt is the primary stressor. At commonly applied concentrations (e.g. 100 mM), salt decreases fitness (Chao *et al.,* 2013), delays phenology (Wang *et al.,* 2013) and affects vegetative and physiological traits (Awlia *et al.,* 2016). Nutrient limitation decreases fitness, but unamended soil is not lethal for these taxa (Pigliucci & Schlichting, 1998). We applied treatments by bottom-watering with the following solutions: 100 mM NaCl solution ("Salt"), 0.1x Hoagland's nutrient solution ("nut," Hoagland & Arnon, 1950), 100 mM NaCl in 0.1x Hoagland's ("Salt&Nut"), and tap water ("Ambient"). All solutions were made with municipal tap water, Columbus, OH. Solutions were applied every three days between July 19 (28 days after sowing) and the end of the experiment (October 27, 128 days after sowing). To measure the total load of salt delivery, we collected a soil sample from each pot at harvest. We measured electrical conductivity, a proxy for the concentration of dissolved ions in the soil, of 2 g dried soil dissolved in 12 mL distilled deionized water. Conductivity readings suggested our treatment delivery methods were effective. The highest conductivity readings were seen for Salt (97.517 ± 2.403 mS cm^{-1}, mean ± SE) and Salt&Nut (96.808 ± 1.812 mS cm^{-1}). These were higher than both nut (69.058 ± 3.399 mS cm^{-1}) and Ambient (59.721 ± 2.211 mS cm^{-1}). These readings were in line with the soil receiving and retaining inputs of fertilizer and salt required for our experiment. Conductivity also decreased over time at comparable rates across treatments, about 0.544 ± 0.071 mS cm^{-1} per day (β ± SE, linear mixed effects model with random effect of tray nested within growth chamber), as organic acids and ions leached from the (baseline unamended) growing medium over time.

Data Collection

We measured or estimated fitness-related, phenological, and vegetative traits to address our hypotheses about how whole genome duplication (WGD) affects phenotypes and plasticity (Table 1). A few traits were monitored every three days during the experiment (bolting time and rosette diameter measurements, Table 1), but we measured most traits after harvest. We harvested plants at senescence, which we defined as the point at which an individual was no longer producing flowers and had fully lengthened all fruits. Harvests took place between

August 13 (53 days after sowing) and October 27 (128 days after sowing). See Table S1 for the raw data used for analyses.

We measured some traits which showed similar patterns to the five focal traits reported here. Similar to lifespan was bolting time (days after sowing to bolting, defined as the first extension of an inflorescence from the basal rosette). Compared to bolting, lifespan also had the advantage of being more reflective of treatment effects accumulated over time. Trends for root mass ratio were similar to those for belowground biomass. Total biomass results were similar to aboveground biomass and diameters of basal leaf rosettes measured on the day treatment application began, at bolting, and at senescence.

Principal components analysis

We performed principal components analysis (PCA, stats::prcomp; R Core Team, 2020) to examine multivariate patterns in the five focal traits (Question 2), for which pairwise correlations were all $|r| < 0.65$. We standardized (centered and scaled) traits before analysis. We used permutational multivariate analysis of variance (PERMANOVA, vegan::adonis; Oksanen *et al.,* 2018) to test significance of explanatory variables in describing variation in the standardized trait matrix. We used 9999 permutations constrained within nested blocks (setBlocks argument set to growth chamber, strata set to tray number within a growth chamber). Explanatory variables tested via PERMANOVA included taxon, ploidy level (Question 2), treatment, total fitness (seed mass, Question 3), and the interaction between taxon and treatment—one way of thinking about trait plasticity across treatment environments (Question 1). We also compared plasticity (Question 1) across taxa using multivariate β-dispersion (vegan::betadisper; Anderson *et al.,* 2006; Oksanen *et al.,* 2018). Testing β-dispersion also was important to assess whether multivariate homogeneity of variance of discrete predictors, an assumption of PERMANOVA, has been violated (Anderson, 2006).

Genotype by environment models

One method for examining plasticity for each trait separately (Question 1) is via genotype by environment (GxE) models. In this framework, plasticity is captured by the GxE interaction term, or the taxon-specific change in trait values across environments (Valladares *et al.,* 2006). We analyzed raw data using linear mixed effects models (LMEs, nlme::lme, Pinheiro *et al.,* 2020) with a random effect of tray nested within growth chamber. We examined residual plots to check assumptions. Residuals showed heterogeneity of variance, which we resolved by weighting the variance on the basis of taxon (nlme::varIdent for estimating different variance for each factor

level of taxon, Zurr *et al.,* 2009). We report *P*-values based on type III sums of squares for main and interaction effects (car::Anova, Fox & Weisberg, 2019).

The main effect for taxa represents a comparison of mean phenotypes averaged across treatments (Question 2). We included all four taxa within a single GxE model for a given trait. This allowed us to quantify effects of WGD on trait means for each lineage by comparing polyploids to diploids of a given lineage (Col4x vs. Col2x and Ler4x vs. Ler2x). We were also able to measure the magnitude of trait differences based on lineage, regardless of ploidy (Col2x and Col4x vs. Ler2x and Ler4x). We coded these three comparisons in the models as planned contrasts.

The environment component of these models included the four treatments. We were not necessarily interested in the main effect of treatment, which describes how traits are expressed differently across environments, averaged across all taxa. Instead we were primarily interested in the interaction term, which provides information about taxon-specific plasticity in trait expression across our environmental contexts (Question 1) and which we coded as three planned contrasts: 1) nutrient addition ("Nut+," Nut vs. Ambient treatments), 2) salt stress ("Salt+Nut+", Salt&Nut vs. Nut), 3) salt stress under nutrient limitation ("Salt+Nut−," Salt vs. Ambient). We did not examine all treatment contrasts to maintain degrees of freedom and to focus on biologically and evolutionarily relevant environmental contexts. For instance, we did not contrast the most benign with the most stressful treatments (Nut vs. Salt), even though doing so would have estimated the highest plasticities; we expect that shifts between these two extreme conditions would be experienced much less commonly by a given taxon/population compared to the three comparisons for which we report results. We considered this realism an essential prerequisite to our consideration of the potential adaptive value of plasticity (Question 3), via structural equation models.

Structural equation models

We used a type of structural equation modeling (SEM) called confirmatory path analysis to describe the adaptive consequences (Question 3) of WGD's impacts on plasticity (Question 1) and mean trait values (Question 2). We used modified relative density plasticity index (RDPI, Valladares *et al.,* 2006), given this metric's positive performance compared to other plasticity indices (Valladares *et al.,* 2009), and its use in previous studies comparing plasticity across ploidy (Wei *et al.,* 2019). For each pair of individual trait values (x, y) that differ in treatment received, RDPI is calculated:

$$\mathrm{RDPI} = \frac{|x - y|}{(x + y)/2}$$

We modified this calculation in that we did not take the absolute value of the numerator because we were interested in the direction of trait shifts between treatments.

We calculated RDPI for each trait across the same three environmental contexts examined for GxE models: 1) Nut+ (Nut vs. Ambient treatments), 2) Salt+Nut+ (Salt&Nut vs. Nut), 3) Salt+Nut− (Salt vs. Ambient). Because RDPI is calculated across all possible pairs of same-taxon individuals for a given treatment combination, sample size is falsely inflated. So that RDPI could be analyzed in subsequent analyses, we similarly calculated pairwise means for all other variables included in these models. We defined a separate SEM for each of the three environmental contexts, five traits, and two lineages. Following the formula *i*j* to calculate all possible pairwise combinations of two groups having respective sizes of *i* and *j* yields, for example: 6 Ler2x Nut * 6 Ler2x Ambient = 36 observations. This example SEM will also include the calculations for Ler4x in the Nut+ context (6 Ler4x Nut * 6 Ler4x Ambient = 36), bringing the full number of model observations to 72.

All trait means and plasticities were standardized (centered and scaled) to allow for comparison of model coefficients across SEMs. Standardization centered the mean at zero, obscuring the sign of RDPI. Because pre-standardized RDPI signs were biologically meaningful (a positive value indicating an increase in that trait value in response to environmental context, and a negative value a decrease), to allow interpretation of RDPI, we marked positive and negative RDPI values before standardization and used this information to overlay a “true zero” line onto density plots (ggridges::geom_density_ridges, Wilke 2020) of standardized RDPI values.

We defined the following SEM configuration to examine the adaptive consequences of shifts in trait means and plasticities (RPDI) due to WGD. The SEMs first linked WGD to the mean and plasticity for a given trait (paths 1 and 2 in Fig. 1), similar to the goal of GxE analyses. Then, trait mean and plasticity were linked to fitness (seed mass, paths 3 and 4 in Fig. 1). We interpreted the adaptive consequences based on context: for a given environmental context (Nut+, Salt+Nut+, Salt+Nut−), a significant relationship of a trait to fitness could be either positive (higher values of that trait are adaptive, lower values are maladaptive) or negative (lower values are adaptive, higher are maladaptive). A non-significant relationship of a trait to fitness would indicate neutral consequences with respect to fitness. The SEMs also included a path linking WGD directly to fitness (path 5 in Fig. 1), to account for previously observed direct

impacts of WGD on fitness in these taxa (Henry *et al.,* 2005; Chao *et al.,* 2013). Therefore, in SEMs, we included these paths to account for these relationships, although they were not the primary biological motivation of SEMs. Some SEMs failed to converge due to oversaturation of variables. To yield convergence, we tried SEMs lacking either the mean or plasticity variable (and both paths linked with that variable). In all cases, one or the other of these reduced SEMs converged.

Individual models making up SEMs were generalized least squares linear models (nlme::gls, Pinheiro *et al.,* 2020). We performed typical assumption-checking procedures and examination of residual plots. We also checked assumptions that specifically may have been violated by the pairwise calculations of RDPI. We checked for autocorrelation using Mantel tests and found autocorrelation due to shared pair identity. We accounted for this autocorrelation with a Gaussian correlation structure (nlme::corGaus, Pinheiro *et al.,* 2020). We also used Levene's tests to check for unequal variance in RDPI within and among taxa (Kreyling *et al.,* 2019). We found unequal variance among taxa, which prompted us to weight model variance based on taxon (nlme::varIdent, Zurr *et al.,* 2009, Pinheiro *et al.,* 2020). Inclusion of correlation and variance structures improved performance of the individual models making up the SEMs.

We configured the individual models into SEMs using piecewiseSEM (Lefcheck 2016). We used Fisher's C and tests of d-separation to assess SEM fit and evaluate whether significant paths were missing from the analysis (Shipley 2009). Full SEMs (those including all 5 paths, Fig. 1) had only one missing path, linking mean and plasticity, while SEMs facing convergence issues (for which we removed two paths) had no missing paths. For the former, C and d-separation sometimes suggested inclusion of the missing path ($P < 0.05$), but we did not include it in final SEMs because there are often no straightforward biological explanations for such relationships and because correlations between trait means and plasticity can be artefacts of scaling issues (i.e., larger trait means can allow for higher variance and plasticity). For SEMs with no missing paths, C = 0.000 and $P = 1.000$.

Results

Principal components analysis

Based on PCA (Fig. 2), we found most of the trait variation across taxa and environments was explained by the combined effect of four of our five traits (loading scores positively correlated

with axis 1 for: lifespan, 0.499; number of inflorescences, 0.492; maximum height, 0.440; biomass, 0.553). Fitness (Question 3) also coincided with higher values of these four traits and correlated with axis 1 (PERMANOVA, $F = 42.512$, $P < 0.001$, $df_1 = 1$, $df_2 = 88$; Fig. 2c). The single other trait—root mass ratio—drove axis 2 (loading = 0.926; Fig. 2a). Notably, the β-dispersion values for comparisons of diploids to polyploids showed that polyploids were neither more or less plastic than diploids (Question 1) across these traits and across all treatments (β-dispersion all diploids vs. polyploids, $P = 0.901$, $df_1 = 1$, $df_2 = 88$; β-dispersion Col4x vs. Col2x, $p = 0.847$, $df_1 = 1$, $df_2 = 43$; β-dispersion Ler4x vs. Ler2x, $P = 0.602$, $df_1 = 1$, $df_2 = 43$, Fig. 2b), or for subsets of treatments corresponding to our three focal environmental contexts (Nut+, Salt+Nut+, Salt+Nut−; all $P > 0.2$, $df_1 = 1$, $df_2 = 19$-22). Trait centroids of diploids and polyploids diverged from each other (Question 2), a relationship that was significant for Ler4x vs. Ler2x ($F = 2.748$, $P = 0.038$, $df_1 = 1$, $df_2 = 43$; for Col4x vs. Col2x: $F = 0.741$, $P = 0.539$, $df_1 = 1$, $df_2 = 43$; for all diploids vs. polyploids: $F = 1.756$, $P = 0.131$, $df_1 = 1$, $df_2 = 88$). Another way to think about plasticity (Question 1) in a multivariate context is the interaction between taxon and environment—a significant interaction indicates that taxa respond to environmental factors differently. This interaction was nonsignificant ($F = 0.923$, $P = 0.544$, $df_{1Taxon} = 3$, $df_{1Trt} = 3$, $df_{1Int} = 9$, $df_2 = 74$, Fig. 2b,d), though we saw significant main effects for both taxon ($F = 5.772$, $P < 0.001$, $df_1 = 3$, $df_2 = 86$; β-dispersion $P = 0.439$, $df_1 = 3$, $df_2 = 86$) and treatment ($F = 12.145$, $P < 0.001$, $df_1 = 3$, $df_2 = 86$; β-dispersion $P < 0.001$, $df_1 = 3$, $df_2 = 86$).

Genotype by environment models

Next, we examined each trait separately using genotype by environment (GxE) models (see Table S2 for full summary statistics). For these models, plasticity (Question 1) was captured by planned contrasts within the interaction term and visualized in reaction norm plots (Fig. 3). All vegetative traits were significantly structured by both treatment and taxon (all $P < 0.025$, Table S2). Interaction terms were significant for two traits, root mass ratio ($\chi^2 = 26.209$, $P = 0.002$, $df_{1Taxon} = 3$, $df_{1Trt} = 3$, $df_{1Int} = 9$, $df_2 = 66$, Fig. 3c) and total biomass ($\chi^2 = 20.634$, $P = 0.014$, $df_{1Taxon} = 3$, $df_{1Trt} = 3$, $df_{1Int} = 9$, $df_2 = 66$, Fig. 3d). The interaction for biomass was driven not by WGD but by differences between Col and Ler such that Col lineages increased their biomass a mean of 47.8% more than Ler in response to Nut+ ($\beta = 0.182$, $\beta_{Std} = 0.598$, $P = 0.011$, Fig. 3d, 4c) and decreased biomass under Salt+Nut− a mean of 36.3% more than Ler, though this comparison was marginally significant ($\beta = -0.108$, $\beta_{Std} = -0.354$, $P = 0.077$, Fig. 3d, 4c). For root mass ratio, the interaction was driven by WGD, wherein we saw a marginally significant divergence of Ler4x from Ler2x ($\beta = 0.033$, $\beta_{Std} = 0.366$, $P = 0.065$, Fig. 3c, 4c). Root mass ratio

of Ler4x increased in response to Salt+Nut−, while Ler2x decreased, showing that the stress response strategies differed for polyploids and diploids. The magnitude of the shift (absolute value) was a mean of 101.0% greater in Ler4x than Ler2x, meaning that polyploids were more plastic than diploids in their ability to reallocate root mass in response to Salt+Nut−. For other traits and environmental contexts, the lack of significant interaction terms in GxE models (Fig. 4c) suggests that plasticity of polyploids across these environments did not differ from diploids.

The main effects for taxon also captured mean trait shifts as a result of WGD (Question 2, Fig. 4a). The single significant contrast showed Ler4x had a mean of 14.6% longer lifespans than Ler2x ($\beta = 6.698$, $\beta_{Std} = 0.236$, $P = 0.013$, Fig. 3a, 4a). Similarly, we saw a mean of 7.5% longer lifespan in Col4x than Col2x, though this trend was nonsignificant ($\beta = 2.196$, $\beta_{Std} = 0.091$, $P = 0.228$, Fig. 3a, 4a).

Structural equation models

Results of SEMs showed that the consequences of WGD included 10 significant paths (Table 2) for both plasticities (Question 1) and mean traits (Question 2), suggesting that effects of WGD on mean trait values may be just as prevalent as effects on plasticity (RDPI). In every case where plasticity was at least marginally significant, polyploids were more plastic (Table 2; Fig. 5c,e,f,i). Plasticity was not adaptive in every case (Question 3); some of these responses were exaggerated stress syndromes that were adaptively neutral (Table 2; Fig. 5f,i).

In the Nut+ context, WGD increased lifespan, which was adaptive in this context (Table 2, Fig. 5a); Ler4x had lifespans that were a mean of 107.2% longer than Ler2x. Whole genome duplication also increased inflorescence numbers by a mean of 79.0% in Ler4x, an adaptive shift (Table 2, Fig. 5b). Plasticity in inflorescence numbers was 334.6% higher in Col4x than in Col2x, a shift that was adaptive (Table 2, Fig. 5c). Col4x also had a mean of 57.0% less total biomass than Col2x, which was maladaptive (Table 2, Fig. 5d).

In response to Salt+Nut+, Ler4x exhibited a mean of 461.4% greater plasticity in lifespan than Ler2x, which was adaptive (Table 2, Fig. 5e). Both polyploid lineages showed increased plasticity in inflorescence number. Salt+Nut+ decreased inflorescence numbers strongly in Col4x but had little effect on this trait in Col2x, actually increasing slightly (Table 2, Fig. 5f)—Col4x plasticity magnitude was a mean of 32 times higher than Col2x (comparison of absolute values). Both Ler taxa decreased inflorescence numbers, but Ler4x was a mean of five times more plastic than Ler2x (Table 2, Fig. 5f). Shifts in inflorescence number plasticity due to WGD were adaptive for Col4x but were adaptively neutral for Ler4x (Table 2). As seen in Nut+, WGD

also decreased total biomass in Col4x for Salt+Nut+ (Table 2, Fig. 5g). Col4x had a mean of 53.0% less biomass than Col2x, a maladaptive shift (Table 2).

In the Salt+Nut− context, WGD increased mean lifespan in Col4x (Table 2, Fig. 5h). Col4x lived a mean of 342.8% longer than Col2x, a shift which was maladaptive in this context (Table 2). Root mass ratios responded to Salt+Nut− differently in Ler2x and Ler4x, plastically decreasing in Ler2x but increasing in Ler4x, a consequence of WGD that was adaptively neutral (Table 2, Fig. 5i). Comparing the absolute values of these opposite-direction shifts, the mean change in Ler4x root mass ratio was 86.4% more than for Ler2x.

Discussion

Known genomic consequences of WGD have led to predictions of increased phenotypic plasticity in polyploids (Jackson & Chen, 2010; Van de Peer *et al.,* 2017), but existing literature has not consistently found polyploids to be more plastic than lower-ploidy relatives. Using inbred synthesized taxa, we found that polyploids were more plastic than their diploid progenitors (Question 1), for all differences between diploids and polyploids that were at least marginally significant. Our support for the hypothesis of increased plasticity in polyploids represents an important advancement, potentially providing a mechanism to explain how the genomic effects of WGD scale up into ecological consequences: accelerating adaptation (Banaiga et al. 2020), producing niche differentiation (Laport *et al*., 2013), and spurring major speciation events (Van de Peer *et al.,* 2017). Many studies that have failed to find consistent effects of WGD on plasticity use natural taxa in which WGD effects may have been masked by those of hybridization, selection, or other evolutionary processes. In such cases, it is impossible to isolate the effects of WGD alone (on plasticity, or any other aspect of biology, such as niche differentiation or gene expression). Our use of synthesized polyploids allowed us to perform a controlled test of WGD effects. Our results might be in part unique *A. thaliana.* Additional studies are needed to test the hypothesis of increased phenotypic plasticity in polyploids of other species and genotypes, particularly those in which WGD is experimentally induced (Parisod *et al.,* 2010; Spoelhof *et al.,* 2017).

The largest magnitude plasticity shift we observed was for root mass ratio in Ler4x, which indicated opposite stress response strategies in Ler2x and Ler4x. In response to the most stressful environment (Salt+Nut−), Ler2x slightly decreased relative allocation to roots, but Ler4x strongly increased root mass ratio. Increasing root mass ratio in response to salt stress

can be a stress tolerance response (Morais *et al.,* 2012). This aligns with previous research in *A. thaliana* showing that WGD increases response to and tolerance of salt stress (Chao *et al.,* 2013; del Pozo & Ramirez-Parra, 2014). Roots appear to be a crucial part of the salt stress tolerance mechanism in polyploids Chao *et al.* (2013). Our work shows that increased plasticity in polyploids in other aboveground traits can add to their stress tolerance.

Previous work showed that *A. thaliana* autotetraploids' general salt stress response syndrome was adaptive (Chao *et al.,* 2013). Breaking the stress response into traits, we found that the WGD-induced shift in relative root mass was adaptively neutral (Question 3)—a response that maintained homeostasis rather than fitness (Dudley, 2004). Two other WGD-induced shifts we documented were adaptive under stress: increased lifespan plasticity in Ler4x, wherein polyploids decreased lifespan more strongly in response to stress, and increased inflorescence number plasticity in Col4x, in which polyploids decreased inflorescence number more strongly under stress. Both of these adaptive shifts were in the salt-stress only condition (Salt+Nut+), while we saw the neutral shift in root mass ratio under the more stressful condition, combined salt stress and nutrient limitation (Salt+Nut−). Likewise, neutral and maladaptive trait shifts are often seen under greater stress (Dudley, 2004). These same responses may be adaptive under different circumstances; we applied sustained over the course of individuals' lifespans, but for an individual facing a short-term stressor, temporary allocation away from fitness and towards homeostasis could produce later fitness benefits (Sih, 2004). In a short-lived annual, the duration and timing of the stressor in the life cycle might be especially important.

In considering whether stress responses are adaptive, it is also important to consider that traits differ widely in their linkage to fitness (Sih, 2004). Though roots appear to be an important part of stress tolerance in these polyploids, root mass ratio may not be as linked to fitness as other root traits. Further elucidating WGD effects on stress tolerance in this system should prioritize examining additional root traits. In our study, we could not examine further root traits because root architecture and fine roots were affected during the washing process. We grew plants in potting mix, but agar medium or hydroponic growing methods could allow root traits to be quantified.

We found that impacts of WGD on plasticity were comparable in prevalence and magnitude to impacts on mean trait values, suggesting plasticity effects are equally as important in structuring polyploid ecology as the better characterized, somewhat generalizable mean trait effects of WGD (del Pozo & Ramirez-Parra, 2015). As we have discussed, WGD effects on plasticity are difficult to generalize, due to methodological issues and the fact that plasticity (by definition)

depends on environmental context. In contrast, mean trait consequences of WGD (Question 2) are more generalizable. One common outcome is slowed growth rate due to increased cell size in polyploids (del Pozo & Ramirez-Parra, 2015). We likewise found increased mean lifespan in polyploids was the largest and most significant effect of WGD that we examined. For comparison of lineages (Col and Ler), we found greater effects of WGD on lifespan in Ler4x, the opposite of what has previously been shown (Chen, 2010; Pacey *et al.,* 2019), likely due to differences in WGD effects across growing conditions, which were not manipulated in previous studies. We saw the biggest lifespan differences in Ler4x under Nut+, but it was the most stressful condition (Salt+Nut−) that produced the biggest differences in Col4x. Large impacts of WGD on phenology likely affect many other trait, developmental, and fitness outcomes in polyploids. We found that in Ler4x, longer lifespan was associated with increased fitness under Nut+, implying that the ability to live longer had the most adaptive benefits under ameliorated conditions. For the other focal traits, none were highly correlated to each other by design (chosen to reduce redundancy and to produce an informative PCA solution). Examining linkages between phenology and other traits is likely to be particularly important for an annual like *A. thaliana,* in which circadian clock genes govern many processes (Dodd *et al.,* 2005; Chen, 2010).

We also saw that WGD decreased biomass in Col4x. This finding contrasts the general observation that polyploids often have increased biomass (Stebbins, 1971) due to heterosis (East 1936). Whole genome duplication increases heterozygosity by doubling allele number, but heterotic effects in polyploids are mostly driven by processes other than WGD. Heterosis is much greater in allopolyploids than autopolyploids (Chen, 2010), and in taxa in which heterozygosity is increased by admixture (Abel & Becker, 2007). In this light, finding the opposite of a heterotic biomass effects is less surprising. For *A. thaliana,* we are aware of no previous comparisons of total biomass between diploids and synthesized autotetraploids because most studies do not include belowground material. For aboveground biomass, early observers reported no shift in autotetraploids (Chen, 2010), but later more exhaustive comparisons revealed that several lineages showed increased aboveground biomass (measured at bolting, Pacey *et al.,* 2019). Our differing results seem to be due in part to inclusion of belowground material. Trends for aboveground biomass (data not shown) were similar to those for total biomass, but aboveground biomass was not significantly decreased in Col4x in any environmental context. Our results may also be explained in part by the environmental contexts included in our study. Interestingly, WGD decreased Col4x biomass in

all contexts except the most stressful (Salt+Nut−), suggesting that the consequences of WGD depend on the selective environment.

Though WGD affected Col4x total biomass, Ler4x did not experience the same effect. We have discussed several trait shifts that were specific to either Col or Ler. The decreases in biomass for Col4x are the most evolutionarily relevant of these differences because they were strongly maladaptive, having relationships to fitness higher in magnitude than any other WGD-induced shift. Maladaptive effects of WGD in Col have been previously documented. Col4x make fewer fruits and seeds than Col2x under non-stressful conditions (Henry *et al.,* 2005; Chao *et al.,* 2013), and seed viability is negatively affected by ploidy changes in Col but not Ler (Scott *et al.,* 2013). The transcriptome of Col4x is also considerably more affected by WGD than Ler4x (Yu *et al.,* 2010). Genome size also differs within *A. thaliana,* with Col having a particularly small genome (Schmuths *et al.,* 2004). Genome size variation can have ecological consequences (te Beest *et al.,* 2012; Pandit *et al.,* 2014), including effects on plasticity (Meyerson *et al.,* 2020). Taxa with different genome sizes could be differentially affected by WGD. Different adaptive consequences of WGD in Ler and Col help to explain why some polyploid lineages persist and others go extinct.

The polyploid taxa we considered presumably expressed maladaptive and neutral traits because selection has not yet acted on these newly generated lineages—at least for the selective factors tested here. In nature, selection will quickly remove genotypes expressing traits that are maladaptive or even adaptively neutral (Dechaine *et al.,* 2007). Selection generally acts more strongly under more stressful conditions. Selection for WGD also appears to be greater under stress, evidenced by the evolutionary signature of WGD in periods of large climactic change (Van de Peer *et al.,* 2017). Strong selection under stress would quickly remove unfit genotypes, permitting rare, high performing genotypes to establish without being swamped by less fit genotypes. The trait shifts consistent with stress tolerance that we and others (Chao *et al.,* 2013; del Pozo & Ramirez-Parra, 2014) have observed for the *A. thaliana* autotetraploids support the idea that WGD could be particularly advantageous under stress. Our results also demonstrate that WGD can be advantageous under amenable conditions. We found that adaptively neutral and maladaptive effects of WGD were expressed only under stressful conditions (Salt+Nut+ and Salt+Nut−), not under amenable conditions (Nut+). In such cases (where WGD induces shifts that are not adaptive), polyploids might have more of an advantage under amenable conditions than under stress. Weak selection in amenable conditions could also allow a minority cytotype to succeed by filtering out fewer individuals and potentially

allowing large enough effective population sizes for the minority cytotype to establish. Thus selection, demography, and chance can act together in different scenarios to produce a successful polyploid lineage.

Our findings regarding the adaptive consequences of WGD depend on how we defined fitness. We considered fitness as total seed mass rather than total seed number, the metric used by previous fitness comparisons of *A. thaliana* autotetraploids and diploids (Henry *et al.,* 2005; Chao *et al.,* 2013). Reanalysis of our data with seed number as our fitness proxy would result in entirely different results for Question 3. However, we consider total seed mass is a more equitable estimate of an individual's fitness allocation when comparing across cytotypes, given the well documented tradeoff between seed size and seed number across polyploid taxa (Bretagnolle *et al.,* 1995) and in *A. thaliana* (increased seed size: Chen *et al.,* 2010; del Pozo & Ramirez-Parra, 2014; Fort *et al.,* 2016; decreased seed number: Henry *et al.,* 2005; Chao *et al.,* 2013 [in control]). How "fitness" is best defined depends on context. We focused on abiotic conditions known to manipulate individuals' fitness allocation, so we were best served by a metric estimating total fitness allocation. In other contexts, either more seeds or larger seeds might be advantageous. Seed number might be a more appropriate fitness metric in situations where having more seeds could be advantageous, such as when intraspecific competition is an important selective factor. The alternate hypothesis, though, is that larger seeds, which contain more maternal investment, have a competitive advantage. Previous authors have framed the larger seeds of polyploids as providing just such an advantage (Bretagnolle *et al.,* 1995). *Arabidopsis thaliana* has so far provided mixed support for the hypothesis of a large-seed advantage in polyploids. Autotetraploid seeds do not have significantly higher percent germination than diploids (del Pozo & Ramirez-Parra, 2014) but do show faster initial growth rates (Fort *et al.,* 2016). For small-seeded taxa like *A. thaliana*, increased seed size might have fewer ecological effects than for other taxa, like those with large animal-dispersed seeds. Future studies should consider the potential contributions of seed number and seed size to ecological differentiation among cytotypes.

More studies are also needed that compare the ecological effects of WGD to effects of other evolutionary processes. Our study aimed to quantify WGD effects alone, but in extant taxa, WGD has clearly operated in combination with other processes and factors, including drift, genetic diversity, demography, life history, natural selection, and hybridization. It would be useful to separate each of these factors to measure their relative contributions to aspects of

polyploid ecology, such as phenotypic plasticity. Hybridization might merit particular focus. Half of all polyploids are allopolyploids (Barker *et al.,* 2016), and even in autopolyploid evolution, intraspecific admixture plays an important role (Abel & Becker, 2007). In experiments that have separated the influences of WGD and hybridization in allopolyploids, hybridization had greater genomic impacts (Chen, 2010), including for *Arabidopsis* (Jackson & Chen, 2010). The ecological effects of these two processes, like their potential contributions to shifts in phenotypic plasticity, have been less studied (but see Gallego-Tévar *et al.,* 2018; Wei *et al.,* 2019). The ecological synergy of WGD with hybridization has been implicated by observations that allopolyploids are overrepresented among invasive species (te Beest *et al.,* 2012) and crops (Renny-Byfield & Wendel, 2014). We join other recent calls for studies that separate the ecological effects of WGD and hybridization (Parisod *et al.,* 2010; Spoelhof et al. 2017; Wei *et al*., 2019). Different experimental approaches will work together to generalize the ecological effects of WGD, alone and in conjunction with other factors. A holistic picture will emerge from both macroevolutionary approaches, like ecological niche modeling, and microevolutionary approaches, like reciprocal transplants and common gardens.

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Author Contribution

KZM and SMH together designed the project. KZM performed the experiment, analyses, and data collection, supervised by SMH. KZM wrote the manuscript with contributions from SMH. KZM and SMH reviewed and approved the submitted manuscript.

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Tables

Table 1 Summary of fitness metric and the five focal traits included in our study.

Trait	Details
Fitness (total seed mass, mg)	Fitness allocation of an individual, estimated as the product of: Fruit number (count): counted from harvested aboveground material for each individual. Fruit length (mm): mean length to the nearest 0.01 mm of five fruits, measured from harvested aboveground material of each individual. We selected the five fruits to represent the full range of variation in fruit length within an individual. Seeds mm^{-1} of fruit (count): allometric for each of the 16 taxon/treatment combinations describing the number of seeds inside a fruit divided by fruit length to the nearest 0.01 μm. These values were means from one fruit of representative size taken across 4-8 individuals from each taxon/treatment. These plants were not the same individuals included in this experiment but were rather from a preliminary experiment in which plants of the same taxa were treated with the same treatment solutions used here (differing only in that plants were top-watered and grown outdoors). This metric varied among taxa but not among treatments. Mass per seed (mg): allometric describing the mass of an individual seed, for each taxon/treatment. These values were obtained by taking the mean across 5-6 sub-samples of seeds per taxon/treatment. Naturally shed seeds were sampled from 4-5 different individuals. For each sample (27-190 seeds), we measured mass to the nearest 0.01 mg, counted seeds under a dissecting microscope, and divided mass by the count. The final allometric was the mean of all samples representing a given taxon/treatment.
Lifespan (days)	Days (after sowing) to senescence (the point at which an individual was no longer producing flowers and had fully lengthened fruits).
Inflorescence number (count)	Total number of inflorescences or basal branches growing from the center of the basal rosette of leaves. Counted from the harvested aboveground material.
Root mass ratio (unitless)	Belowground biomass as a proportion of total biomass (aboveground + belowground). Aboveground harvest was measured to the nearest 0.1 mg after drying at 65°C for three days. Belowground harvest (pot with roots in soil) was stored at 4°C for no more than 10 days before washing soil from roots, drying at 65°C for three days, and massing to the nearest 0.01 mg.
Total biomass (g)	Sum of aboveground and belowground biomass.
Maximum height (cm)	Height of the tallest inflorescence growing from the center of the basal rosette of leaves, measured to the nearest cm from the harvested aboveground material.

Table 2 Significant structural equation model paths showing the effects of whole genome duplication (WGD) on standardized trait means (Fig. 1, path 1) and plasticities (relative density plasticity index, path 2), and the adaptive consequences of these shifts (paths 3 and 4).

Fig. 5	Envtl context	Trait	Lineage	WGD effect on trait ($\beta_{1/2}$)	Explanation of WGD effect on trait ($\beta_{1/2}$)	Trait effect on fitness ($\beta_{3/4}$)	Net direction WGD effect on fitness ($\beta_{1/2} * \beta_{3/4}$)	Explanation of net WGD effect on fitness	Model statistics
5a	Nut+	Lifespan (days)	Ler	1.289**	In Nut+, Ler4x had longer lifespan than Ler2x.	0.105***	+	Longer lifespan adaptive	C = 2.573, *P* = 0.276, df = 2, n = 72
5b	Nut+	Inflorescence number (count)	Ler	0.425§	In Nut+, Ler4x had more inflorescences than Ler2x.	0.165***	+	Higher inflorescence number adaptive	C = 115.947, *P* < 0.001, df = 2, n = 72
5c	Nut+	Plasticity in inflorescence number	Col	0.921*	Nut+ generally increased inflorescence number. Col4x increased more than Col2x. **Polyploids more plastic**.	0.241***	+	More plastic increases in inflorescence number adaptive	C = 0.000, *P* = 1.000, df = 0, n = 72
5d	Nut+	Total biomass (g)	Col	−0.557§	In Nut+, Col4x had less total biomass than Col2x.	0.364***	−	Lower biomass maladaptive	C = 6.266, *P* = 0.044, df = 2, n = 66
5e	Salt+Nut+	Plasticity in lifespan	Ler	−1.044*	Salt+Nut+ generally decreased lifespan. Ler4x decreased more than Ler2x. **Polyploids more plastic.**	−0.149***	+	More plastic decreases in lifespan adaptive	C = 0.000, *P* = 1.000, df = 0, n = 72
5f	Salt+Nut+	Plasticity in inflorescence number	Col	−0.768*	Salt+Nut+ did not affect Col2x inflorescence number much but decreased Col4x. **Polyploids more plastic.**	−0.139*	+	More plastic decreases in inflorescence number adaptive	C = 82.816, p < 0.001, df = 2, n = 72
5f	Salt+Nut+	Plasticity in inflorescence number	Ler	−0.625§	Salt+Nut+ generally decreased inflorescence number. Ler4x decreased more than Ler2x. **Polyploids more plastic.**	ns	ns	More plastic decreases in inflorescence number adaptively neutral	C = 35.132, *P* < 0.001, df = 2, n = 72
5g	Salt+Nut+	Total biomass (g)	Col	−0.621§	In Salt+Nut+, Col4x had less total biomass than Col2x.	0.502***	−	Lower biomass maladaptive	C = 2.157, *P* = 0.340, df = 2, n = 72
5h	Salt+Nut−	Lifespan (days)	Col	0.656*	In Salt+Nut−, Col4x had longer lifespan than Col2x.	−0.109*	−	Longer lifespan maladaptive	C = 19.336, *P* < 0.001, df = 2, n = 72
5i	Salt+Nut−	Plasticity in root mass ratio	Ler	1.218**	Salt+Nut− decreased root mass ratio in Ler2x but increased Ler4x. Greater magnitude change in Ler4x than Ler2x. **Polyploids more plastic.**	ns	ns	More plastic increases in root mass ratio adaptively neutral	C = 12.432, *P* = 0.002, df = 2, n = 72

Significance values: § 0.05 < *P* < 0.10, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns: non-significant

Environmental contexts: Nut+ (nutrient addition): Ambient vs. Nut treatments; Salt+Nut+ (salt stress): Salt&Nut vs. Nut; Salt+Nut− (salt stress under nutrient limitation): Salt vs. Ambient

Figure Legends

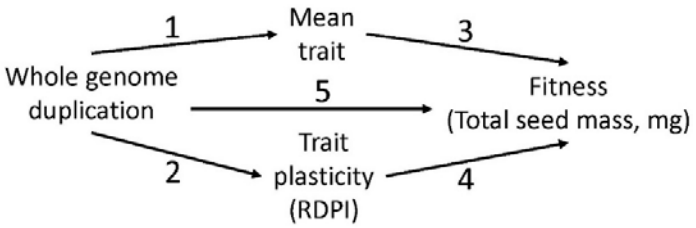

Fig. 1 Structural equation model describing the effects of whole genome duplication (WGD) on a given vegetative or phenological trait mean (path 1) and plasticity (path 2, relative density plasticity index, RDPI) and the adaptive consequences of these shifts (paths 3 and 4). Each model also included a covariate for the direct relationship between WGD and fitness (path 5).

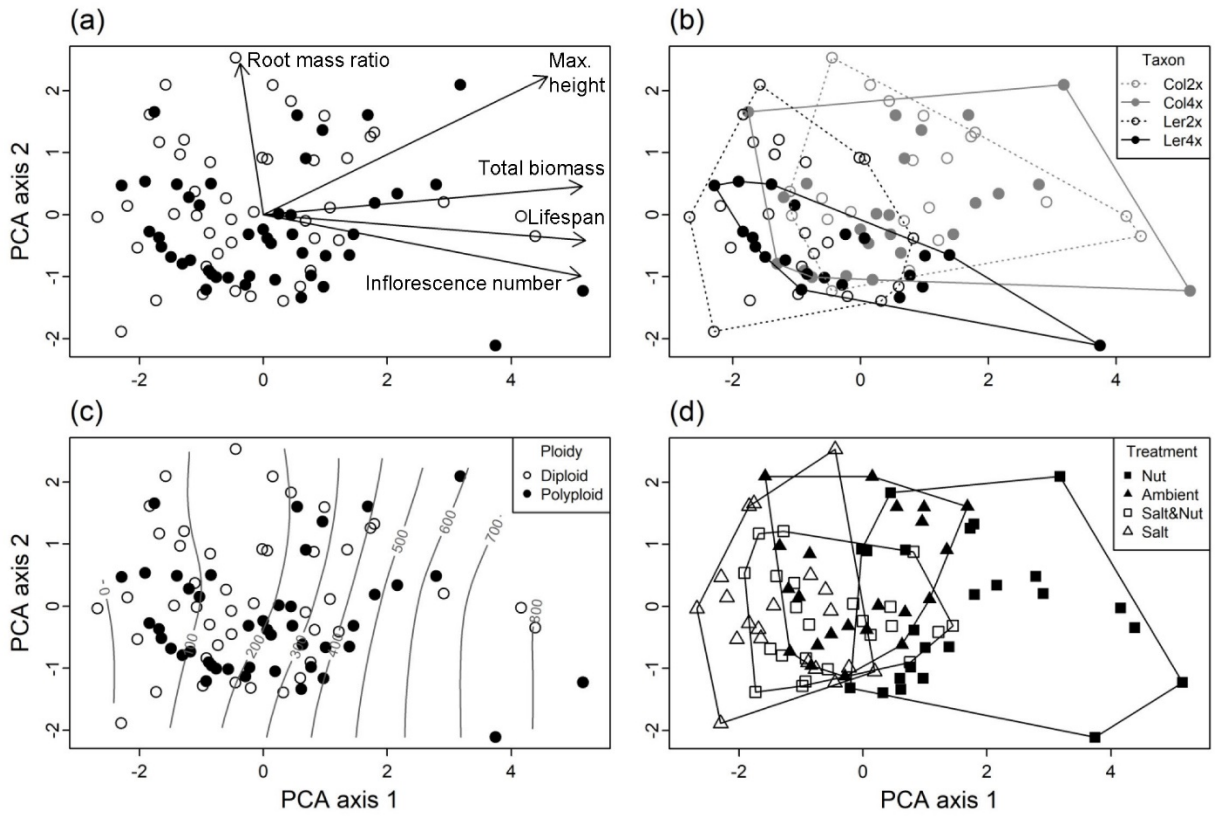

Fig. 2 Principal components analysis (PCA) of five standardized trait values across all 90 individuals having no missing data for any trait. The solution assigned 49.78% of variation to axis 1 and 20.61% to axis 2. **(a)** Biplot vectors for each trait showing the direction of the relationship between traits and PCA axes (arrow length arbitrary): root mass ratio, maximum height (cm), total biomass (g), lifespan (days), and inflorescence number (count). **(b)** Polygons grouping individuals within a taxon. **(c)** Smoothing surface for fitness (total seed mass, mg), defined with generalized additive models using thin-plate regression splines (vegan::ordisurf, Oksanen *et al.,* 2018). **(d)** Polygons grouping individuals receiving the same treatment.

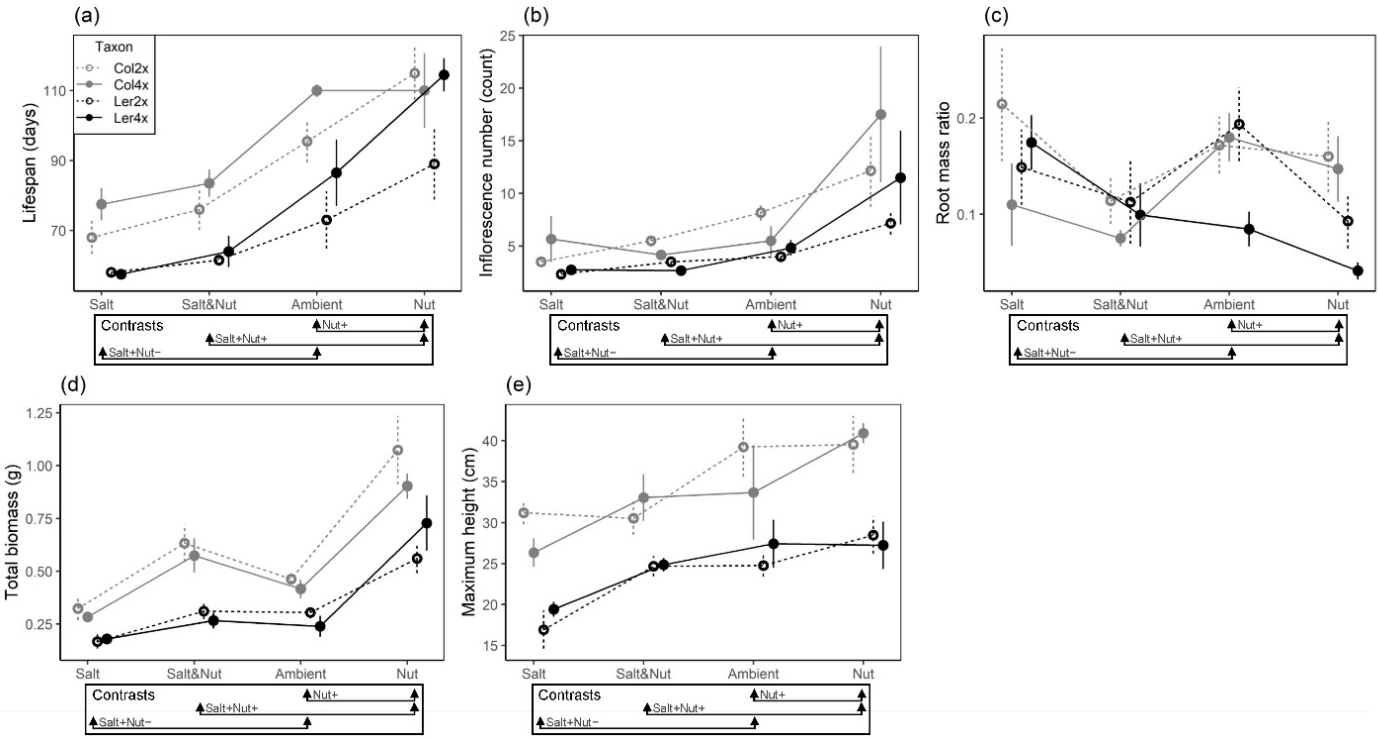

Fig. 3 Reaction norms comparing means (±SE) for five traits.

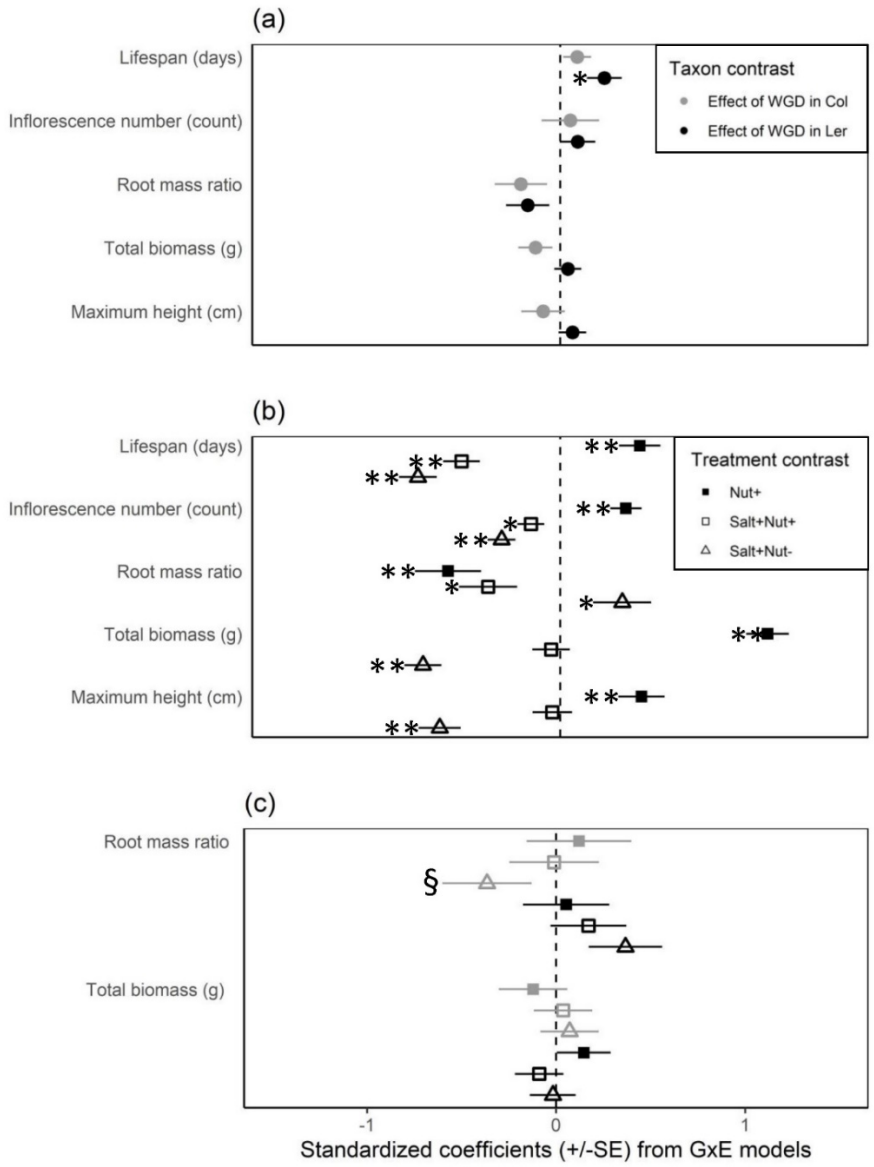

Fig. 4 Standardized model coefficients (±SE) from genotype by environment (GxE) models, including **(a)** main effects for taxon and **(b)** environment, and **(c)** the GxE interaction (when significant and thus estimated in final models). These are coefficients for planned contrasts, meaning that a significant divergence from the zero line is, for example: for **(a)** how much higher a mean trait value was for Col4x vs. Col2x ("Effect of WGD in Col"), for **(b)** how much Nut+ affected a given trait compared to Ambient condition ("Nut+"), for **(c)** how much more or less a given trait changed in response to an environmental context for each polyploid compared to their diploid progenitors. Symbols mark significant (*$P < 0.05$, **$P < 0.01$) or marginally significant (§ $0.05 < P < 0.10$) trait shifts.

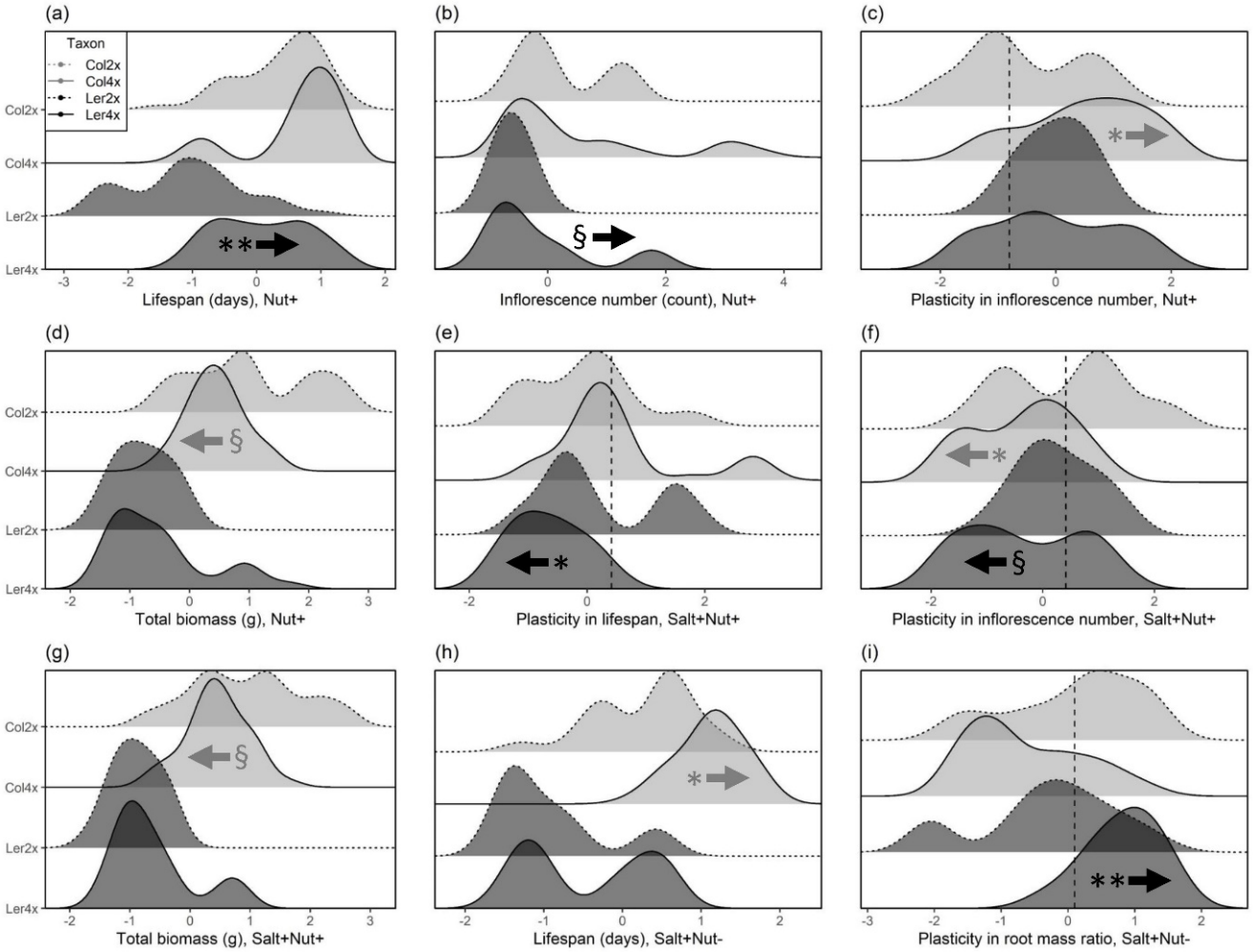

Fig. 5 For the 10 significant structural equation model paths linking whole genome duplication (WGD) to trait means and plasticities (relative density plasticity index) (Table 2), density plots showing the full distribution of standardized trait values, by taxon. Because these values are standardized, for plasticity, we added vertical dashed lines corresponding to the unstandardized "true zero" for that trait and environmental context, to allow for interpretation (e.g. for **(c)**, in response to Nut+, values falling to the left of the line indicate that inflorescence number decreased in response to Nut+, values to the right indicate an increase). The arrows mark the direction of significant (*$P < 0.05$, **$P < 0.01$) or marginally significant (§ $0.05 < P < 0.10$) trait shifts due to WGD.

Supporting Information

Table S1 Raw data used for analyses.

Table S2 Full results of genotype by environment (GxE) models.

Table S3 Full results of structural equation models (SEMs).