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Letter

The immediate effects of polyploidization of *Spirodela polyrhiza* change in a strain-specific way along environmental gradients

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Abstract

The immediate effects of plant polyploidization are well characterized and it is generally accepted that these morphological, physiological, developmental, and phenological changes contribute to polyploid establishment. Studies on the environmental dependence of the immediate effects of whole-genome duplication (WGD) are, however, scarce but suggest that these immediate effects are altered by stressful conditions. As polyploid establishment seems to be associated with environmental disturbance, the relationship between ploidy-induced phenotypical changes and environmental conditions is highly relevant. Here, we use a common garden experiment on the greater duckweed *Spirodela polyrhiza* to test whether the immediate effects of WGD can facilitate the establishment of tetraploid duckweed along gradients of two environmental stressors. Because successful polyploid establishment often depends on recurrent polyploidization events, we include four genetically diverse strains and assess whether these immediate effects are strain-specific. We find evidence that WGD can indeed confer a fitness advantage under stressful conditions and that the environment affects ploidy-induced changes in fitness and trait reaction norms in a strain-specific way.

Keywords: polyploidy, whole-genome duplication, *Spirodela polyrhiza*

Layman summary

Polyploidization, resulting from genome duplication and/or merger, is a major force in plant evolution. Despite high numbers of polyploid species and intraspecific karyotypical variation, the challenges faced by a newly formed polyploid are multifarious. Higher resource requirements, difficulties finding compatible polyploid mates, genomic instability, and competition with their well-adapted diploid ancestors all impede establishment. Nevertheless, while many polyploids are short-lived, some of them manage to survive, reproduce, and even thrive. The basis for this inequality amongst polyploids remains largely unknown. Part of the key to polyploid success must lie in the immediate effects of genome doubling. Genome size is correlated with cell size and as such, genome duplication triggers a cell size increase and a whole battery of cascading effects on the phenotype. Although these immediate effects are well characterized, variation between and even within species is considerable. Using a common garden experiment in which we exposed four genetically different diploid-tetraploid pairs of the greater duckweed *Spirodela polyrhiza* to stress gradients, we hope to improve our understanding of what determines the fate of neopolyploid plants. Here, we show that although the immediate effects of WGD can result in a decreased fitness of the tetraploid in the prevailing environment, they can confer a fitness advantage in another and that ploidy-induced changes in trait plasticity and fitness homeostasis are strain-specific. Consequently, we show that the fate of neopolyploid plants depends at least partially on the interplay of the identity of the individuals present in the polyploid population (the genotypes), and the environmental conditions they face. Our findings support the idea that stressful environments can facilitate polyploid establishment and that recurrent polyploidization can help polyploid establishment through variation in the outcomes of independent polyploidization events. Whenever possible, future studies on nucleotypic effects should include diverse ecotypes and environmental conditions in their designs.

Introduction

During much of the twentieth century, successful polyploidizations in natural settings were considered rare events. Consequently, the idea that recurrent whole genome duplication (WGD) could give rise to a new polyploid species consisting of multiple lineages, each with their own origin, i.e., the multiple origins hypothesis, was rarely verbalized. But, there are exceptions. From his early publications onward, Hagerup (1932) seemed to believe that unreduced gamete

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production in stressful environments was high. Later, he used the disjunct distribution and asexual nature of the hexaploid cranberry *Oxycoccus gigas* (currently *Vaccinium oxycoccus*, see Suda & Lysak, 2001) to deduce recurrent polyploidization (Hagerup, 1940). Based on crosses, morphological and geographical evidence, several other researchers came to similar conclusions (e.g., Rozanova (1938), see Mavrodiev & Soltis, 2001); Ownbey (1950); Ownbey & McCollum (1953); Seiler (1961); Skalinska (1947); and Sprague (1944)). Curiously, these cases did not make it into the more influential works on polyploidy from Grant (1981); Stebbins (1950); and Wagner (1970). The development of molecular markers such as allozymes brought the first conclusive molecular evidence for the presence of multiple, independently originated, lineages within the same polyploid species, i.e., Werth (1985). In subsequent years, evidence for the multiple origins hypothesis accumulated (summarized by Soltis et al. (1993) and Soltis & Soltis (1999)). Together with Ramsey & Schemske's (1998) estimates of polyploidization rates, these reviews largely helped the popularization of the multiple origins idea.

The gap between the numbers of independent polyploid origins detected using molecular methods and those predicted based on unreduced gamete formation is large. Restricted sampling most likely results in an underestimation of the number of independent origins detected (Soltis & Soltis, 1999), but can only account partially for this discrepancy. A possible explanation for the remaining difference is variation in the outcomes of different independent polyploidization events. For instance, if the immediate effects of whole genome duplication (WGD) depend on the (epi)genetic context, it is possible that only a small subset of the generated polyploids has what it takes to contribute to the establishment.

Although the immediate effects of polyploidizations were established over a century ago (Lutz, 1907; de Vries, 1901), remarkably little is known about their mechanistic underpinnings. Many of these morphological, anatomical, developmental, phenological, and physiological effects (reviewed in Bomblies, 2020; Clo & Kolar, 2021) are believed to be downstream effects of a cell size increase as a direct consequence of the doubled bulk amount of DNA, i.e., a nucleotypic effect (see Bennett, 1971, 1972; Doyle & Coate, 2019; Levin, 1983). Extensive variation in the effects between cells, tissues, individuals, and species implies that scaling rules depend on the (epi)genomic context (Katagiri et al., 2016; Robinson et al., 2018; Tsukaya, 2013), supporting the idea of variation in the outcomes of independent WGDs.

As phenotypic shifts due to polyploidy can cause a mismatch with the prevailing environmental conditions, most of these immediate changes can be expected to be disadvantageous, resulting in a reduction in overall fitness (Bomblies, 2020; Clo & Kolar, 2021). Nevertheless, if the environment changes, some of these changes might be beneficial and could facilitate autopolyploid establishment as co-opted spandrels, i.e., exaptations (Gould & Vrba, 1982). For example, the root anatomy of neotetraploid *Arabidopsis thaliana* increases salt tolerance (Chao et al., 2013) and neotetraploidy confers *Achillea borealis* an advantage in xeric dune habitats (Ramsey, 2011). The exact contribution of exaptation to polyploid establishment is unknown, but it might partly explain the association of polyploidy with stressful environments and times (Van de Peer et al., 2020).

Autopolyploid establishment seems notoriously difficult. Despite high rates of unreduced gamete formation, guaranteeing a constant production of polyploids (Ramsey & Schemske, 1998), only 16.2% of all flowering plants occur in multiple ploidies (Rice et al., 2015). Several papers indicate that the extinction rates of recent polyploids are higher than these of contemporary diploids (Arrigo & Barker, 2012; Mayrose et al., 2011, 2015 but see Soltis et al., 2014). Additionally, the long-term establishment of paleopolyploidization events seems to have been exceedingly rare (Van de Peer et al., 2017). The challenges faced by neopolyploids are many. First, they must survive while competing with their lower ploidy ancestors, which is difficult given their associated genomic instability (Comai, 2005), higher resource requirements (Guignard et al., 2016), and phenotypical changes. Second, they must manage to reproduce and become independent from new polyploidization events. Here, neopolyploids are not only hindered by reduced fertility due to meiotic hurdles (Comai, 2005; Yant et al., 2013) but also by minority cytotype exclusion (Levin, 1975). Finally, neopolyploids start with low genetic diversity, especially when they have a single origin. Although genetic diversity can increase over time, initially this lack of diversity reduces their evolutionary potential.

The challenging nature of autopolyploid establishment makes it attractive for further investigation in both natural and experimental systems. After more than a century of polyploidy research, the intrinsic and environmental factors contributing to polyploid success, and the interplay between these factors, are still intensively studied. Environmental upheaval (described above), clonality (Van Drunen & Friedman, 2022), and intraspecific variation, e.g., through independent polyploidization events (Levin, 2019; Soltis & Soltis, 1999; Stebbins, 1985), are believed to be of crucial importance for the establishment of polyploids. Combining both factors, we here study the intraspecific variation in fitness and trait plasticity of diploid and neo-autotetraploid duckweed (*Spirodela polyrhiza*) along gradients of two environmental stressors, namely, the heavy metal cadmium (Cd), and salt (NaCl). This approach allows us to address: (a) whether the relationship between diploid and tetraploid fitness changes along an environmental gradient, (b) whether these relationships are strain-specific, and (c) whether there are environments where autopolyploids have higher fitness than their diploid progenitors.

Materials and methods

Strains

Four genetically divergent strains (nr. 0013, 9316, 9242, and 9346) of the greater duckweed *Spirodela polyrhiza* (Araceae, Alismatales) were kindly provided by the Landolt collection Zurich. Each of these strains belongs to a different population genetic cluster as distinguished by Xu et al. (2019) (see Supplementary Table S1). After flow cytometric verification (Wu et al., 2022) of the diploidy of these strains, we induced WGD using a colchicine treatment (Supplementary methods 1: Colchicine treatment). Tetraploid lines were selected, and their ploidy was monitored closely for a minimum of 2 months to ensure the stability of the lines. We randomly selected a single plant from each strain-ploidy combination and used these to start our experimental lines.

Stress gradients

We studied plastic responses along three environmental gradients of two different stressors that are relevant in a global change context (Mclaughlin et al., 1999; Jamil et al., 2011), i.e., a heavy metal ($CdCl_2$) and salt (NaCl) stress. Our Cd gradient (gradient 1) was composed of regular Hoagland's E medium (Cross, 2002), and medium supplemented with 0.3 and 0.5 mg/L $CdCl_2$. For gradient 2, we used the same benign condition, and medium supplemented with 2.5 g/L, and 3 g/L NaCl. The cultures of gradients 1 and 2 were grown axenically in transparent 124 × 112 × 80.5 vitro vent boxes, covered with a 125 × 125 square petri dish, on temperature-controlled shelves at 24°C, under a 16-8 light-dark regime at 40–45 µmol m^{-2} s^{-1} PPFD. Due

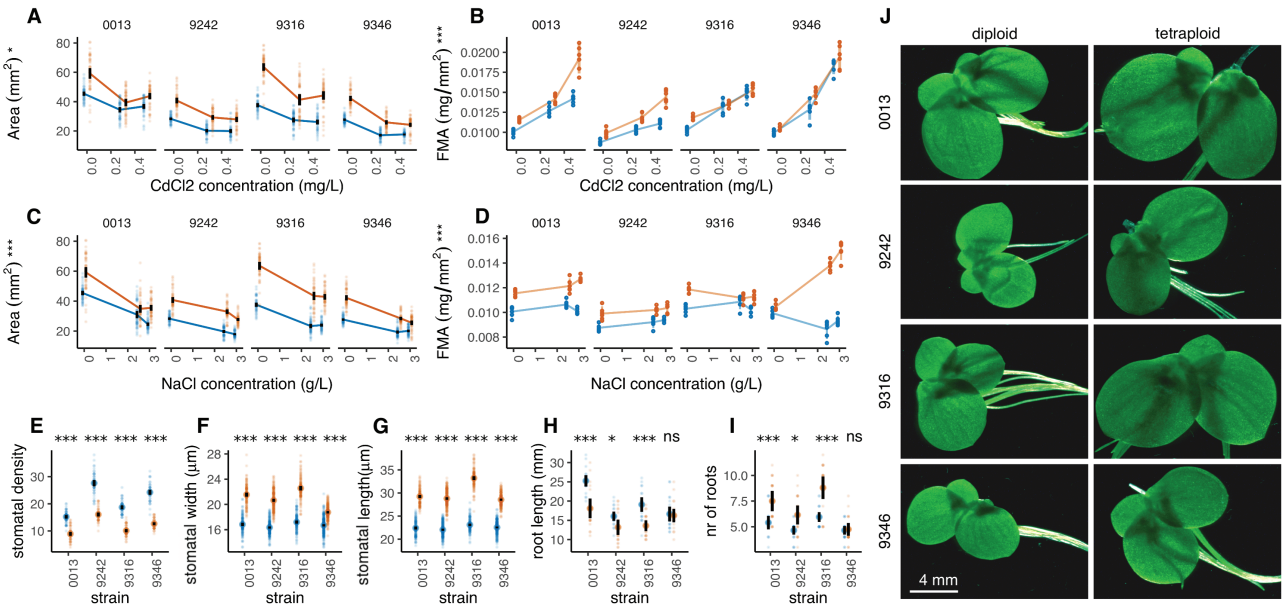

Figure 1. The immediate phenotypical effects of polyploidization (A)–(J), and their response curves along gradients 1 (A) and (B) and 2 (C) and (D) with diploids in blue and tetraploids in orange. Error bars represent 95% confidence intervals. Asterisks indicate significance levels (*ns: non-significant, *:$p \leq 0.5$, **:$p \leq 0.01$, ***:$p \leq 0.001$). The asterisks next to the dependent variables refer to the significance of the ploidy*strain*condition effect.

to space limitations, all conditions were handled separately. Because the stress response in gradient 2 was rather limited and confounding time effects could not be excluded in the first two gradients, a second salinity gradient (gradient 3) was set up in a different growth room under similar but open (non-axenic) conditions, assessing the benign and all five stress levels (i.e., concentrations of 2, 2.5, 3, 4, and 6 g/L NaCl) simultaneously.

Growth rate

All starting material was derived from a single frond, ensuring the isogeneity of the lines, and was pretreated in its respective stressful environment for a minimum of four weeks to eliminate potential carry-over effects (Landolt, 1987; Ziegler et al., 2015). During this acclimatization period, the medium was refreshed weekly. When fully acclimatized, we took 12 samples of approximately 16 fronds (4 plants) and used these for growth trials. Briefly, we measured surface area, frond number, and dry and fresh weight to calculate relative growth rate (RGR) (Supplementary methods 2: Growth rate).

Photosynthetic parameters

For the first two gradients, growth measurements on day 7 were preceded by an assessment of photosynthetic parameters (Murchie & Lawson, 2013) (Supplementary methods 3: Photosynthetic parameters).

Plant morphology and anatomy

In addition to the growth and photosynthetic parameter assessment, we selected 50 mature fronds from the acclimatized precultures of gradients 1 and 2 and measured frond size in ImageJ (Schindelin et al., 2012), by determining the surface area of each of these fronds using the same set-up as described above (Supplementary methods 2: Growth rate). Frond thickness was calculated using the common proxy of the frond mass per area (FMA), i.e., dry weight/surface area (Witkowski & Lamont, 1991). For the benign condition, this information was supplemented with root length, root number, stomatal density, stomatal width, and stomatal length (Supplementary methods 4: Microscopy).

Pigment content

Chlorophyll a (chl a), chlorophyll b (chl b), carotenoid (car), and anthocyanin (AC) content of six replicates per line were estimated following Lichtenthaler (1987) and Mancinelli (1975) (Supplementary methods 5: Pigment quantification).

Statistical analysis

For each of the parameters, i.e., RGR fn, RGR sa measured for all three gradients and frond size, thickness, RGR fw, RGR dw, NPQ, Fv/Fm, YII, car, chl a, chl b measured for gradients 1 and 2, we modeled the dependence on ploidy, strain, concentration, and all their interaction effects using a general linear model as implemented in the SAS 9.4 proc glm procedure. The significance of the modeled effects was evaluated using F-tests with type III sum of squares. Because different ploidies and strains showed response curves that could not be linearized using the same transformation, we included the condition as a discrete variable. Although requiring less elaborate parametrization, models with concentration as a continuous variable (without any transformation, and as such often violating the assumption of linearity) in general showed a lower AIC value (see Supplementary Table S2).

The variables measured uniquely in the benign condition of gradients 1 and 2, i.e., root number, stomatal width, stomatal length, and stomatal density, were modeled using strain, ploidy, and their interaction effect as dependent variables (Supplementary method 6: Statistics). Additionally, we calculated the overall and the condition-specific Pearson correlations between the average trait values of each line (Supplementary Figure S1).

Results

General phenotypes

Under all culturing conditions, tetraploid plants are on average larger than their isogenic diploids. For all pairwise comparisons, except strain 0013 in Hoagland supplemented with 2.5g/L NaCl

Table 1. General linear models of the different phenotypical and fitness measures for the three gradients, test statistics shown are the *F* values, asterisks indicate significance levels (*:$p \leq 0.5$, **:$p \leq 0.01$, ***:$p \leq 0.001$).

	df	RGR sa	RGR fn	RGR fw	RGR dw	NPQ	Fv/Fm	YII	chla	chlb	car	AC	FMA	Size
Gradient 1 ($CdCl_2$)														
cond	2	232.43***	323.1***	276.02***	191.61***	24.18***	107.55***	226.76***	620.44***	334.81***	271.02***	121.98***	679.07***	512.21***
strain	3	48.14***	59.51***	37.87***	39.79***	1.88	13.89***	37.86***	304.5***	331.18***	439.45***	540.22***	133.21***	487.94***
ploidy	1	114***	99.08***	60.47***	65.26***	0.14	18.41***	23.3***	873.41***	807.29***	845.43***	10.93**	186.54***	912.65***
cond*strain	6	4.87***	5.6***	13.47***	8.07***	2.67*	1.26	2.52*	18.28***	13.7***	4.1***	6.66***	32.32***	5.84***
ploidy*cond	2	0.89	1.99	8.66***	12.19***	1.2	3.82*	9.77***	5.64**	3.46*	3.56*	18.82***	11.02***	36.95***
ploidy*strain	3	5.71**	13.23***	10.34***	9.48***	2.06	0.15	0.81	35.27***	32.44***	27.33***	87.03***	11.97***	39.86***
ploidy*cond*strain	6	3.07**	5.19***	5.94***	8.29***	1.42	0.71	1.38	8.39***	7.79***	4.87***	11.44***	8.45***	2.31*
Gradient 2 (NaCl)														
cond	2	5.21**	17.43***	38.33***	60.66***	18.05***	35.78***	193.14***	7479.62***	3072.32***	406.48***	81.42***	38.32***	789.43***
strain	3	17.98***	35.43***	17.97***	27.78***	2.39	36.83***	10.26***	549.64***	339.04***	492.35***	606.37***	127.35***	334.95***
ploidy	1	144.48***	196.6***	165.53***	133.69***	5.57*	4.38*	6.37*	867.6***	528.16***	750.1***	199.62***	836.32***	1376.45***
cond*strain	6	3.34**	5.34***	2.51*	4.2***	3.2**	0.73	4.22***	49.75***	23.76***	13.58***	13.67***	17.44***	24.05***
ploidy*cond	2	2.67	11.51***	4.66*	3.45*	0.44	2.86	10.63***	232.88***	91.97***	18.72***	10.48***	38.27***	24.38***
ploidy*strain	3	6.28***	8.19***	12.28***	12.64***	4.02**	0.94	0.28	136.25***	69.45***	86.7***	82.18***	98.73***	65.89***
ploidy*cond*strain	6	7.54***	10.37***	5.16***	8.32***	0.98	0.76	0.96	17.56***	6.24***	14.18***	14.51***	44***	6.12***
Gradient 3 (NaCl)														
cond	5	307.1***	277.67***											
strain	3	24.28***	4.7**											
ploidy	1	40.02***	26.17***											
cond*strain	15	5.84***	3.18*											
ploidy*cond	5	11.36***	17.39***											
ploidy*strain	3	19.89***	0.01											
ploidy*cond*strain	15	2.47**	1.64											

(p adj = 0.0539), these differences are significant at the 5% significance level (see Figure 1A and C, Supplementary Tables S3 and S4). Although the magnitude of the size difference depends on the combination of the specific strain and the environment (see Figure 1A and C and the significant ploidy*strain*environment interaction in Table 1), for all strains, the frond size of both diploids and tetraploids is reduced in all saline and Cd-enriched environments compared to that in the benign environment (see Figure 1A and C). The corresponding contrasts are all highly significant (p adj < 0.0001, see Supplementary Tables S3 and S4). On average, tetraploids have thicker fronds than their corresponding diploids in the same environment, but the difference is not always significant at the 5% significance level (see Figure 1B and D, Supplementary Tables S3 and S4). The exact difference in thickness depends on the combination of strain and environment (significant ploidy*strain*environment interaction in Table 1). For all lines, the average frond thickness increases with increasing cadmium stress (Figure 1B) and this is supported by significant differences in all but one (strain 9242 dip 0.3 vs. 0.5 p adj = 0.8312) pairwise comparisons between adjacent points along both gradients (see Supplementary Table S3). The direction and magnitude of the change in frond thickness of salt-stressed strains are more variable and depend on the combination of the ploidy level and the strain (see Figure 1D, Supplementary Table S4). Both root number and the length of the longest root are dependent on the strain, their ploidy, and their interaction (see Table 2). On average, all tetraploid lines have more but shorter roots (see Figure 1H and I), but the magnitude of the difference again depends on the strain. Furthermore, the difference is not significant for strains 9346 (root number and root length) and 9242 (root number) (see Supplementary Table S6).

Stomata

Stomatal length, width, and density are all dependent on the strain and the ploidy. The magnitude of the effects induced by WGD are strain specific (Table 2). At the 5% significance level, all tetraploids have on average a lower density of stomata than their isogenic diploids (a bit less than half the density), and their stomata are wider and longer than those of the diploids (see Figure 1E and F).

Pigments

Chlorophyll a, b and carotenoid content per unit of fresh weight are highly correlated (Figure 2E and F). Within the same conditions, these three pigments are negatively correlated with frond size (see Supplementary Figure S1). The slope of the relationship between the carotenoids and chlorophyll seems to differ slightly for the highest salt concentration (more carotenoids than expected), and within that high salt environment, the correlation between frond size and chl a and chl b content is non-significant (see Supplementary Figure S1). In the benign condition, the average chlorophyll a, b, and carotenoid content per unit fresh weight of all tetraploid lines is significantly lower (p adj < 0.0001) than that of their isogenic diploids, but the magnitude of the difference depends on the strain (see Figures 2A and C, Supplementary Figures S2 and S3, and Supplementary Tables S3 and S4). The pigmentation changes with increasing $CdCl_2$ and NaCl concentrations in a ploidy-specific way that differs for the four strains (significant three-way interaction see Table 1). Nevertheless, there is a consistent pattern. Pigment contents remain constant at lower stress levels (i.e., 0.3 mg/L $CdCl_2$ and 2.5 g/L NaCl, while they drop under the benign levels if the stress is further increased (Supplementary results 1: Pigments).

Table 2. Models for root length (general linear), root number (generalized linear), stomatal length, width, and density (linear mixed model), test statistics are the F values for all models except root number where chi-square is given, asterisks indicate significance levels (*:$p \leq 0.5$, **:$p \leq 0.01$, ***:$p \leq 0.001$), the interclass correlation coefficient is given for the random effects.

	df	rootlength	rootnr (chi-square)	Stomatal width	Stomatal length	Stomatal density
ploidy	1	44.62***	33.34***	454.44***	638.54***	332.46***
strain	3	22.97***	58.17***	22.38***	19.18***	69.3***
ploidy*strain	3	5.64**	11.28*	14.02***	9.39***	6.11**
random effect ICC				0.1186894	0.21989621	0.22181221

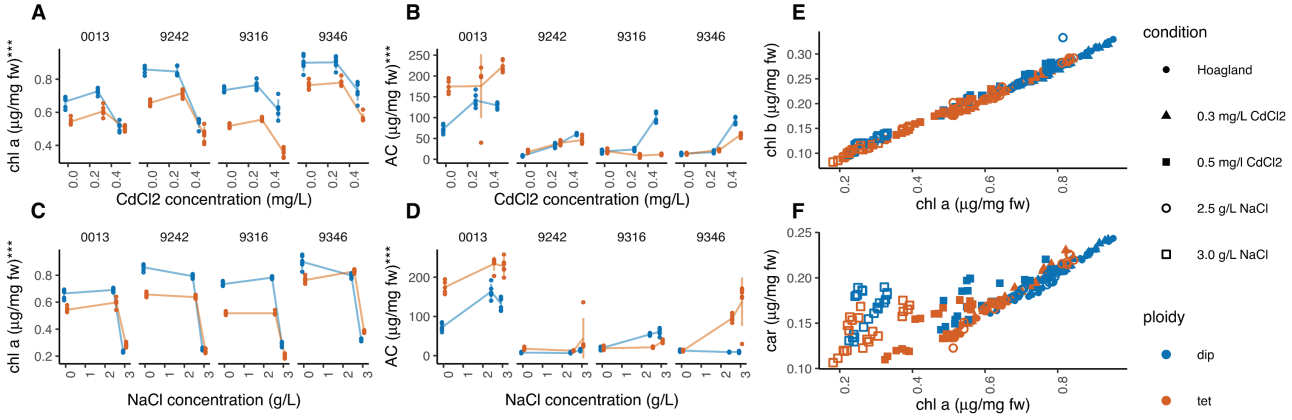

Figure 2. The effects of WGD on chlorophyll a and anthocyanin content per unit fresh weight along gradients 1 (A) and (B) and 2 (C) and (D) and the correlation of the chlorophyll a content with the chlorophyll b (E) and carotenoid content (F). Error bars represent 95% confidence intervals. The asterisks next to the dependent variables refer to the significance of the ploidy*strain*condition effect.

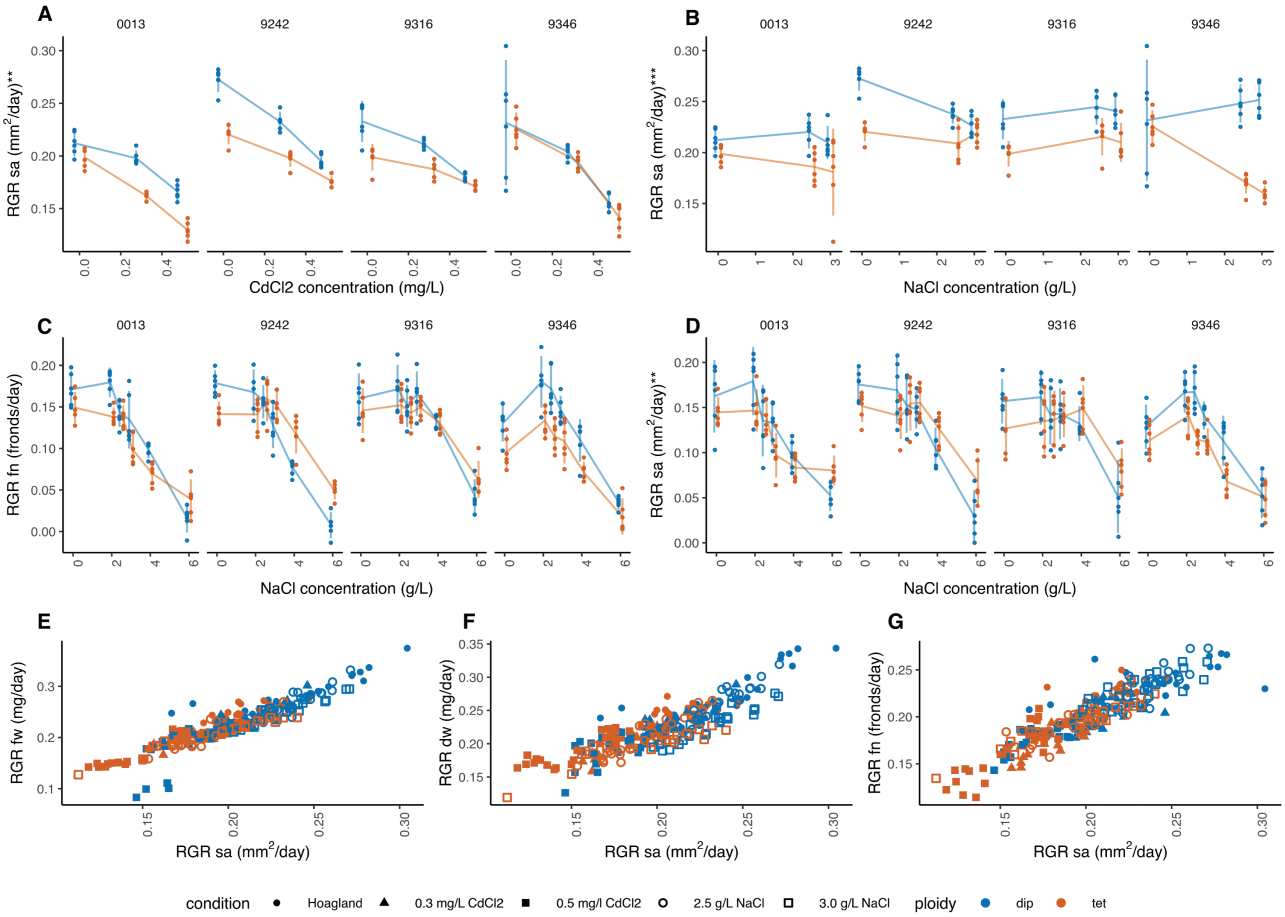

Figure 3. Fitness homeostasis, as proxied by relative growth rate along gradient 1 (A), gradient 2 (B), and gradient 3 (C) and (D). The correlation between the different fitness proxies, RGR using surface area vs. fresh weight (E), RGR using surface area vs. dry weight (F), RGR using surface area vs. frond number (G). Error bars represent 95% confidence intervals. The asterisks next to the dependent variables refer to the significance of the ploidy*strain*condition effect.

As the anthocyanin content is only poorly correlated with the other three pigments (see Supplementary Figure S1), its response is completely different. Both lines of strain 0013 contain more anthocyanin than all other lines, and the tetraploids' anthocyanin content exceeds that of the diploids significantly in all assessed conditions (see Supplementary Tables S2 and S3, Figure 2B and D). The anthocyanin content changes with increasing $CdCl_2$ and NaCl concentration in a ploidy-specific way that differs for the four strains (significant three-way interaction see Table 1) but exceeds the content under benign conditions at the highest stress levels significantly for most of the lines. This difference is, however, non-significant for diploids and tetraploids of strain 9242, tetraploids of strain 9316, and diploids of strain 9346 under salt stress, and tetraploids of strains 9316 and 9242 (but p adj = 0.0592) under Cd stress (see Supplementary Tables S2 and S3).

Growth rate

The four measurements for fitness, i.e., RGR, are highly correlated and are negatively correlated with the FMA, our proxy for leaf thickness (see Figures 3E–G and Supplementary Figure S1). For all gradients, all assessed proxies for the RGR depend on the three-way interaction term, except the RGR fn for the third gradient ($p = 0.0682$, see Table 1). Consequently, the RGR changes over the different stress conditions in a ploidy-specific way that differs between strains. In the benign condition of the first two gradients, only tetraploids of strain 9242 grow significantly slower than their isogenic diploids for all four proxies of the RGR; for strain 9316, this difference is only significant for fn and SA; for strain 9346 for fw and for strain 0013 for fn (see Figure 3A and B, Supplementary Tables S3 and S4). In the benign condition of the third gradient, the RGR of the tetraploids is on average lower than that of their isogenic diploids, but the difference is only significant for the RGR of strain 9242 as calculated by surface area (Supplementary Table S5).

$CdCl_2$ reduces the RGR (Figure 3A) and for all lines, the RGR at 0.5 mg/L $CdCl_2$ is significantly lower than in the benign condition (Supplementary Table S3). The magnitude of the decrease is strain specific, e.g., both diploid and tetraploid lines of strain 9346 have a stronger decrease than the lines of strain 9316 (Supplementary Table S3). In addition to the genetic background, the effect of stress is influenced by ploidy in a strain-specific way. Whereas tetraploids of strain 0013 seem to do increasingly worse than diploids with increasing Cd concentration (consistently significant at the 5% level, see Supplementary Table S3), the average difference seems to decrease for the other strains. For strains 9316 (fw, dw, and fn) and 9346 (fw, dw), the average RGR of the tetraploids does even exceed that of the diploids. These differences are however non-significant, but for the fw of strain 9346, it is nearly so (p = 0.055, Supplementary Table S3).

Because the third gradient encompasses the second gradient, we restrict our report of the impact of salinity on the RGRs to the results obtained using the third gradient (detail on the second

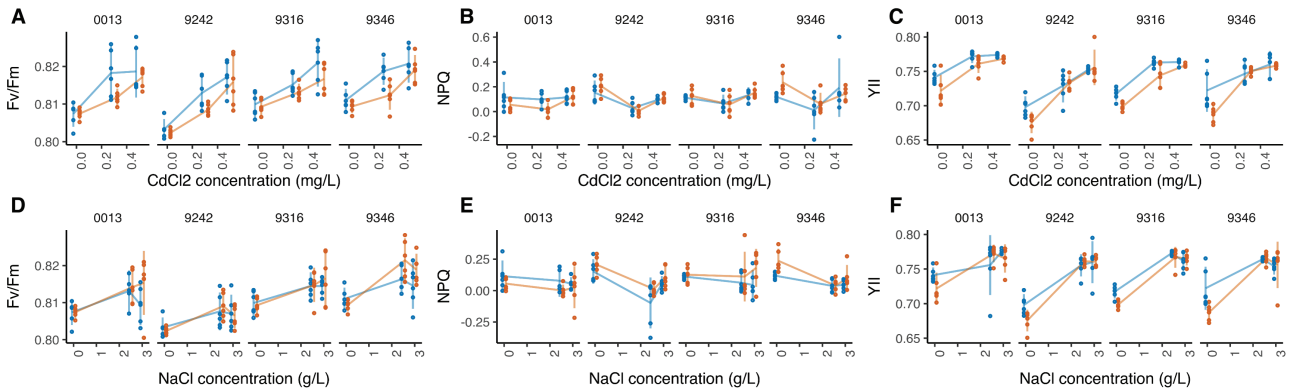

Figure 4. Reaction norm of the photosynthetic parameters of diploid (blue) and tetraploid (orange) Spirodela, along gradients 1 (A)–(C) and 2 (E)–(G). Error bars represent 95% confidence intervals. The asterisks next to the dependent variables refer to the significance of the ploidy*strain*condition effect.

gradient can be found in Supplementary results 2: Growth rate). Along the third gradient, the RGR of some lines initially increases but is consistently reduced at higher stress levels. Although the reduction is strong, we continued to observe growth even at the highest stress level. Despite this strong reduction in the RGR, the difference with the RGR in the benign environment becomes only significant at the higher salt levels of 4 or 6 g/L, depending on the specific combination of strain and ploidy (Figure 3C and D, Supplementary Supplementary Table S5). For all strains, the average difference between diploids and tetraploids changes considerably along the gradient, producing significant differences in the advantage of the diploid lines for strains 0013 and 9346 at 2 g/L (sa) for strain 9346 at 2.5 g/L (fn) and for strain 9346 at 4 g/L (sa and fn) (Supplementary Table S5). Surprisingly, for three of the four strains, i.e., 0013, 9242, and 9316, the tetraploid lines have on average a higher RGR than their isogenic diploids at 6 g/L. For strain 9242 this difference is even significant for the RGR calculated using surface area (Figure 3C and D, Supplementary Table S5).

Photosynthesis

The non-photochemical quenching (NPQ) along the Cd gradient is fairly constant, nevertheless, we did detect a small but significant interaction effect between strain and condition (see Table 1). All lines show a decrease of NPQ when going from 0 to 0.3 mg/L, but this is only significant for the tetraploids of strain 9242 (see Figure 4B and Supplementary Table S3). Additionally, all lines show an increase when going from 0.3 to 0.5 mg/L, but this difference is only significant for the diploids of strain 9346 (see Figure 4B and Supplementary Table S3). Similarly, there is little variation in NPQ along the salinity gradient. Nevertheless, there is a significant ploidy*strain interaction (see Table 1), driven by the diploids of strain 0013 having on average more NPQ than the tetraploids, while the inverse is true for the other strains (see Figure 4E and Supplementary Table S4). The significance of the condition*strain interaction terms (see Table 1) is caused by a significant reduction in NPQ of diploid and tetraploid lines of strain 9242 at 2.5 g/L NaCl, compared to the benign condition (see Figure 4E and Supplementary Table S4). Both the maximum quantum efficiency of PSII photochemistry *(Fv/Fm)* and the correlated PSII operating efficiency (YII) (see Supplementary Figure S1) increase with increasing $CdCl_2$ concentration in a ploidy-specific way. The environmental impact on the realized quantum efficiency of PSII differs significantly between the different strains, whereas, the strain-specific differences in the potential quantum efficiency remain constant over the different conditions(see Figure 4A and C and Table 1). The *Fv/Fm* of the second gradient depends on the strain, the condition, and the ploidy (Table 1). Although there is a clear trend of elevated *Fv/Fm* values under higher stress, the difference between benign and stressed is only significant for the tetraploids of line 9346, and for the benign and 3 g/L for strain 0013 (see Supplementary Table S4). For some of the lines, the potential quantum efficiency of PSII is lower than at the highest stress level, but these differences are not significant (see Supplementary Table S4). The YII values follow a similar pattern, but here the difference between the benign and the stressful condition is significant for nearly all lines; only diploids of strain 0013 grown in 2.5 g/L NaCl do not differ significantly in YII from those grown in benign conditions (Supplementary Table S4). Another striking pattern is the lower realized PSII efficiencies of the tetraploids in the benign environment (although only significant for strain 9346, Supplementary Table S4), whereas the differences are small or non-existing at higher stress levels (Figure 4C and F, Supplementary Tables S3 and S4)

Discussion

To investigate the importance of both the effects of stressful environments and the genetic background on polyploid establishment, we created neoautotetraploid lines of four genetically divergent diploid ecotypes of the greater duckweed *Spirodela polyrhiza* and phenotyped the diploids and their corresponding tetraploids along gradients of two environmental stressors. Many of the observed traits, including fitness, are determined by the interaction of ploidy, genetic background, and environment, indicating that the effect of WGD in a specific environmental context is not fixed, but depends on the genetic background of the individual. Furthermore, our results show that the immediate effects of WGD can confer a fitness advantage in a saline environment to at least one of the studied ecotypes, i.e., strain 9242. Together, our results make a strong case for the importance of both stressful environments and recurrent polyploidization events in polyploid establishment.

Determinants of the immediate effects of genome duplication

Comparing neocolchitetraploids with their diploid ancestors is by far the most informative way to study the effects of genome duplication. It is free from the confounding effects of genome merging and evolutionary changes in the aftermath of the polyploidization

that flaw comparisons between natural polyploids and the contemporary descendants of their assumed diploid ancestors. We note that the colchicine (Münzbergová, 2017) and DMSO (Iwatani et al., 2006; Li et al., 2016), used to create synthetic polyploids, can have some effect. However, we are convinced that the observed interploidy differences are almost entirely the result of genome doubling, as the four-week period, generally believed to be needed to wash away the effects of previous environments in duckweeds (Landolt, 1987; Ziegler et al., 2015), was largely exceeded at the start of our experiment. Nevertheless, future research should include colchitetraploids coming from independent polyploidization events. As mentioned in the introduction, the mechanisms behind the effects of genome duplication are poorly understood. But, they can be ascribed either to an increased dosage of genetic and or regulatory elements (dosage effects) or by the increased bulk amount of genetic material (nucleotypic effects) (see Doyle & Coate, 2019). Our results do not allow us to determine whether or to what extent the observed alterations are the results of nucleotypic or dosage change. But as 23 of our 28 statistical models of traits (see Tables 1 and 2) along the gradients include a significant interaction between ploidy and strain or ploidy, strain and environment, any nucleotypic control must be modified by the epi(genotype), much as originally proposed by Bennett (1972).

The dependence of the immediate effects of WGD on the interplay of genetic background and the environment is largely in line with previous research. Strain specificity has also been observed by Münzbergová and Haisle (2019); Van Drunen and Husband (2018); and Wei et al. (2020), while the environmental dependence, especially of the RGR, fits within observations around improved stress resistance of polyploid plants (Chao et al., 2013; Maherali et al., 2009; Ramsey, 2011). Nevertheless, a three-way interaction between ploidy, genotype, and environment, to our knowledge, has only been reported once before, in a study on the effect of WGD on asexual reproduction in *Fragaria vesca* (Van Drunen & Husband, 2018). The paucity of similar results is not surprising as most of the studies on nucleotypic effects restrict themselves to a single genotype in a benign environment. There is, however, a growing awareness of the importance of intraspecific variation and environmental variation in the immediate effects of genome duplication (see, e.g., Chao et al., 2013, Eliášová & Münzbergová, 2014, 2017; Husband et al., 2016; Meimberg et al., 2009; Münzbergová and Haisel, 2019; Oswald & Nuismer, 2011; Ramsey, 2011; Segraves & Thompson, 1999; Van Drunen & Husband, 2018; Wei et al., 2020).

Despite low genetic differentiation between the ecotypes (Ho et al., 2019; Xu et al., 2019), large ecotypical variation in phenotype and stress response is common in *Spirodela polyrhiza* (Davidson & Simon, 1981; Kuehdorf et al., 2014; Sree et al., 2015; Wozakowska-Natkaniec, 1977; Ziegler et al., 2015). We here show that the effects of genome duplication are often of the same order of magnitude as those of the genotype and that the strain-specific differences on the effect of genome duplication can be quite large in some environments, e.g., the FMA under NaCl stress, the RGR at 6 g/L NaCl and the anthocyanin content in general. We expect that the differences in the effect of WGD between different strains would be larger for other, more genetically diverse species, and even more so in the case of genome merging. Indeed, multiple origins in natural populations should yield more intraploidy variation, promoting polyploid establishment (see below).

In contrast to other traits such as pigment content and RGR, the increase in frond size and thickness proved to be rather consistent over all strains and conditions (the magnitude of the increase is variable though). Similarly, the WGD-induced increase in stomatal size and decrease in stomatal density was observed in all four strains. It seems that these traits are strongly canalized against (epi)genetic and environmental variation. All the more so, as these results are expandable to plants in general, where an increase in stomal size is one of the most consistent effects of plant polyploidization (Bomblies, 2020) and the *gigas* effect is one of the most characteristic polyploidy syndromes (Muntzing, 1936; Niazian & Nalousi, 2020; Ramsey & Schemske, 2002; Stebbins, 1971).

Several of the assessed traits follow a hormetic stress response, where the inhibitory effects of high stress are preceded by a stimulatory response at lower dosages. These seemingly beneficial effects under a low-stress regime are the results of overcompensation. Hormesis is quite common (reviewed in Agathokleous et al., 2020) and has even been observed for other duckweed species under similar concentrations of NaCl (Oláh et al., 2021). Although the stimulatory effects are rarely significant, most of our fitness response curves along the salinity gradient (gradient 3) show a hormetic response. The hormetic response of the potential and the realized quantum efficiency of PSII photochemistry as measured along our smaller salinity gradient (gradient 2), seems to corroborate this. Genome duplication seems to induce a strain-specific shift in these response curves. Previous research has revealed considerable intrapopulational variation in the hormetic response of slow and fast-growing individuals in a population (Belz et al., 2018; Belz & Sinkkonen, 2019). Similarly, it is possible that ploidy-induced dosage and/or nucleotypic changes affecting the overcompensation mechanisms are at least partially responsible for some of the observed differences in stress responses, opening an avenue for further research.

Multiple origins and polyploid success

Recurrent polyploidizations could facilitate establishment in at least three non-mutually exclusive ways. First, theoretical studies show that recurrent polyploidization per se can drive polyploid establishment, if unreduced gamete formation is very high (Felber, 1991). When drift is involved, the neutral establishment becomes even feasible under realistic rates of unreduced gamete formation (Clo et al., 2022). Second, gene flow between independently established polyploid populations can increase the genetic diversity of the polyploid metapopulation (Soltis & Soltis, 1999). Third, independent origins increase the phenotypic variation in the population and as such the chances that one line might have an immediate fitness advantage in the prevailing or in future environmental conditions (Levin, 2019). Our experiment was specifically designed to test the third hypothesis. The strain and environmental specificity of the immediate effects of genome duplication (discussed above) clearly support the idea that independent polyploidization events within the same species can have different outcomes, and that these differences are influenced by environmental conditions. Furthermore, the crossing fitness homeostasis curves of the diploid and tetraploid lines of strain 9242 along the large salt gradient (gradient 3) illustrate that polyploidy can indeed be advantageous in certain environments.

Conclusion

Here, we provide evidence that the magnitude and even the direction of the immediate effects of polyploidization change with the environmental conditions in a strain-specific way. Consequently, the immediate effects of genome duplication should not be considered as a static effect of WGD but as a plastic response to an internal change depending on both the internal (the exact genotype) and the external environment. Additionally, we show that genome duplication can create a

fitness increase for some strains in some stressful environments, and we find indications that the observed fitness differences are caused by differences in hormesis. Future studies aiming to determine whether WGD can confer an advantage ideally should include a wide range of diploid and polyploid genotypes and relevant environmental settings.

Supplementary material

Supplementary material is available online at *Evolution Letters* (https://academic.oup.com/evlett/qrac005)

Figure S1. Global (in gray) and within environment (in color) Pearson correlations between all measured phenotypical traits for gradients 1 and 2.

Figure S2. The effects of WGD on chlorophyll b (A) and carotenoid (B) concentration per unit fresh weight and on the relative growth rate measured using fresh weight (C), dry weight (D), and frond number (E) along gradient 1. Error bars represent 95% confidence intervals, diploids in blue and tetraploids in orange. The asterisks next to the dependent variables refer to the significance of the ploidy*strain*condition effect.

Figure S3. The effects of WGD on chlorophyll b (A) and carotenoid (B) concentration per unit fresh weight and on the relative growth rate measured using fresh weight (C), dry weight (D), and frond number (E) along gradient 2. Error bars represent 95% confidence intervals, diploids in blue and tetraploids in orange. The asterisks next to the dependent variables refer to the significance of the ploidy*strain*condition effect.

Figure S4. Graphical representation of the methods.

Table S1. Origin of the strains used and link to the population genetic clusters in Xu et al. (2019).

Table S2. Differences in the AIC values between models with stress as a categorical and as a continuous factor.

Table S3. Post-hoc analysis (t-test) of gradient 1 ($CdCl_2$), estimated differences, and MaxT-adjusted *p* values.

Table S4. Post-hoc analysis (t-test) of gradient 2 (NaCl), estimated differences, and MaxT-adjusted *p* values.

Table S5. Post-hoc analysis (t-test) of gradient 3 (NaCl), estimated differences, and MaxT-adjusted *p* values.

Table S6. Post-hoc analysis of traits measured uniquely in Hoagland E medium, estimated differences, and MaxT-adjusted *p* values (t-test except for rootnr z-test).

Data availability

Our raw data and SAS code are available in FigShare doi 10.6084/m9.figshare.21534192.

Author contributions

Q.B., T.W., and Y.V.d.P. conceived the study; Q.B., T.W., and A.N. designed the experiments; T.W., A.N., and Q.B. collected the data; Q.B., T.W., A.N. analyzed the data; Q.B. drafted the initial version of the manuscript and all authors contributed to later versions of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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