Parental environmental effects are common and strong, but unpredictable, in Arabidopsis thaliana

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Research

Parental environmental effects are common and strong, but unpredictable, in *Arabidopsis thaliana*

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Summary

- The phenotypes of plants can be influenced by the environmental conditions experienced by their parents. However, there is still much uncertainty about how common and how predictable such parental environmental effects really are.
- We carried out a comprehensive experimental test for parental effects, subjecting plants of multiple *Arabidopsis thaliana* genotypes to 24 different biotic or abiotic stresses, or combinations thereof, and comparing their offspring phenotypes in a common environment.
- The majority of environmental stresses caused significant parental effects, with −35% to +38% changes in offspring fitness. The expression of parental effects was strongly genotype-dependent, and multiple environmental stresses often acted nonadditively when combined. The direction and magnitude of parental effects were unrelated to the direct effects on the parents: Some environmental stresses did not affect the parents but caused substantial effects on offspring, while for others, the situation was reversed.
- Our study demonstrates that parental environmental effects are common and often strong in *A. thaliana*, but they are genotype-dependent, act nonadditively, and are difficult to predict. We should thus be cautious with generalizing from simple studies with single plant genotypes and/or only few individual environmental stresses. A thorough and general understanding of parental effects requires large multifactorial experiments.

Introduction

Phenotypic variation is at the heart of ecology and evolution. The variation in phenotype that we observe among individuals of the same species either reflects underlying genetic differences, and thus the evolutionary potential of a species, or results from plastic responses to the environment, and could thus be related to a species' environmental tolerance. A third source of phenotypic variation is parental environmental effects, where the environmental conditions of parents affect the phenotypes of their progeny (Falconer, 1965; Badyaev & Uller, 2009; Herman & Sultan, 2011; Auge *et al.*, 2017; Yin *et al.*, 2019). Parental effects are sometimes also called 'transgenerational plasticity', or – if they are transmitted only through one parent – maternal or paternal environmental effects. Parental effects are somewhat peculiar in that they can generate resemblance among relatives that would usually be considered evidence for underlying genetic variation, while in fact they represent special cases of phenotypic plasticity that extend across generations. The biological mechanisms that cause parental effects include not only simple nutritional effects such as differential seed provisioning but also physiological effects mediated by hormones, toxins or other cytosol components, or even epigenetic mechanisms where differential DNA methylation or chromatin changes are passed on to offspring (Blödner *et al.*, 2007; Jablonka & Raz, 2009; Elwell *et al.*, 2011; Herman & Sultan, 2011; Richards *et al.*, 2017).

Previous studies showed that parental effects can be ecologically important (e.g. Galloway & Etterson, 2007; Colicchio, 2017; Baker *et al.*, 2019) and also influence evolution (e.g. Falconer, 1965; Kirkpatrick & Lande, 1989; Marshall & Uller, 2007; Räsänen & Kruuk, 2007; Badyaev, 2008; Bonduriansky & Day, 2009). In particular, the demonstration that some parental effects are adaptive, with offspring thriving better in parental than in nonparental environments (e.g. Galloway & Etterson, 2007; Whittle *et al.*, 2009; Latzel *et al.*, 2014; González *et al.*, 2017; Lampei, 2019; Puy *et al.*, 2021), triggered a debate

to what extent parental effects may be evolved mechanisms and a means of rapid adaptation to environmental change (e.g. Badyaev, 2009; Dyer *et al.*, 2010; Burgess & Marshall, 2011; Herman & Sultan, 2011; Laland *et al.*, 2015). However, despite great current interest in parental effects, many important questions remain unresolved.

One of the key challenges in the study of parental effects is to understand how general and how strong they really are. An increasing number of studies showed that parental effects can be substantial and that they can both increase or decrease offspring fitness (e.g. Galloway & Etterson, 2007; Latzel *et al.*, 2009, 2010; Sultan *et al.*, 2009; Whittle *et al.*, 2009; Kochanek *et al.*, 2013; Suter & Widmer, 2013a,b; Groot *et al.*, 2017; Baker *et al.*, 2019; Puy *et al.*, 2021), but many of these studies tested a single environmental factor on a single species, sometimes using only a single genotype (but see e.g. Bossdorf *et al.*, 2009; Suter & Widmer, 2013b; He *et al.*, 2014; Vu *et al.*, 2015; González *et al.*, 2018; Lampei, 2019). As a consequence, we still do not have a good idea of how widespread parental effects are across different environmental factors, and how consistent they are across species and genotypes. Given that nonsuccessful tests for parental effects are more likely to end up in file drawers, researchers skeptical of parental effects might suspect that studies such as the ones cited above merely represent outlier cases that cannot be generalized. Ultimately, the debate can only be settled through strong experiments that test for parental effects across multiple species, genotypes, and/or environmental factors.

Another fundamental question about parental effects is how predictable they are. For instance, is the magnitude and direction of a parental effect related to (and thus predictable from) the direct effect of an environmental stress on the parental generation? Intuitively, one should expect that environmental factors with stronger effects on parents are more likely to also affect their offspring and that environmental factors with little or no effects on the parents should neither affect their offspring. But is this really true? We are not aware of any published study that has tested these simple but important assumptions.

Environmental change usually involves simultaneous changes in multiple environmental factors (Tylianakis *et al.*, 2008; Niinemets, 2010; Hof *et al.*, 2011; IPCC, 2021). Still, most previous studies on parental effects worked with single environmental factors. We know, however, that the direct effects of multifactorial environmental changes are often nonadditive (e.g. Shaw *et al.*, 2002; Shears & Ross, 2010; Hof *et al.*, 2011; Rasmussen *et al.*, 2013; Zhang & Sonnewald, 2017; Zandalinas *et al.*, 2021). It thus appears critical to also compare the transgenerational effects of single vs multiple environmental changes, to test the predictability of complex parental effects and assess the meaningfulness of previous simplified studies. However, so far only few studies (e.g. Lau *et al.*, 2008; Latzel *et al.*, 2009, 2010; Münzbergová *et al.*, 2017; Lampei, 2019) have tested for the parental effects of multiple simultaneous environmental changes.

Here, we used the model species *Arabidopsis thaliana* to thoroughly assess the generality and predictability of parental effects. We subjected multiple genotypes of *A. thaliana* to a broad range of biotic or abiotic environmental stresses, or combinations of these, altogether 24 different stress treatments, and then assessed phenotypic variation in the offspring of these plants. Our experimental setup allowed us to address the following questions: (1) How common and how consistent are parental effects across different environmental stresses and plant genotypes? (2) Can the direction and magnitude of parental effects be predicted from the direct effects of environmental stresses on the parental generation? (3) Are the parental effects of multiple simultaneous environmental stresses additive or nonadditive?

Materials and Methods

Plant material

Arabidopsis thaliana (L.) Heynh. is an annual species from open or disturbed habitats of the northern hemisphere. Because of its small genome size, predominant selfing, and rapid life cycle, the species is a popular model species in plant biology as well as ecological and evolutionary genetics and genomics (Meinke *et al.*, 1998; Pigliucci, 2002). In our study, we worked with three ecologically and geographically distinct genotypes of *A. thaliana*: the common laboratory strain *Col-0* (Versailles Center ID 168AV), the *Sha* genotype (VC ID 236AV) originating from Tajikistan, and the *Tsu-0* genotype (VC ID 91AV) from Japan. All three genotypes are frequently used in genetics and plant biology and have served as parents for populations of recombinant inbred lines. All seeds originally came from the *Arabidopsis* stock center but were bulked for one generation in a growth chamber in Bern before the main experiment. We grew 10 replicate plants per genotype for 2 wk under short-day conditions (8 h : 16 h, light : dark) at 21°C : 16°C, and then moved them to long-day conditions (16 h : 8 h, light : dark) to induce flowering and fruiting. We harvested the plants sequentially when one-third of siliques had reached maturity, and let all seeds after-ripen in paper bags for 2 wk at room temperature. We pooled all seeds of a genotype and distributed identical seed batches from these pools to all four laboratories where experiments took place.

Parental generation

We subjected the plants to 12 different individual biotic and abiotic parental stress treatments, plus 12 pairwise combinations of these stresses, resulting in a total of 24 different stress treatments. For logistic reasons, the 24 treatments were distributed across four different laboratories (henceforth referred to as 'locations') in Bern, Hohenheim, Nijmegen, and Vienna. In Bern, we tested the effects of light stress, heavy metal, pathogens, and all pairwise combinations of these. In Hohenheim, we tested the effects of cold treatment, shading and leaf removal (simulated grazing), and their combinations. In Nijmegen, we tested the effects of drought, salt stress and jasmonic acid (JA; simulation of herbivore attack), and their combinations, and in Vienna, we tested two different kinds of heat stress, as well as the effects of low nutrients, and their pairwise combinations (see next section for more details on the treatments).

At each location, we grew the plants in temperature-controlled growth chambers under the same standardized temperature and daylength conditions (16 h : 8 h, 21°C : 16°C, light : dark), and we further minimized location differences by growing plants in the same pots (7 × 7 cm) and substrate (Einheitserde ED 63T) everywhere. We stratified seeds on wet filter paper at 4°C for 3 d and transplanted similar-sized seedlings to individual pots. All plants were bottom-watered twice a week throughout the study. Sixteen days after sowing, we started the parental stress treatments, with six treatments (see above) plus a control treatment in each location, and seven replicates per treatment and genotype, that is, 147 plants per location and 588 plants overall. Within genotypes, the seedlings were randomly assigned to the experimental treatments. Where possible, treatments were terminated when the plants started to bolt.

To minimize potential influences of phenology variation among the three genotypes on seed quality, the plants were harvested sequentially, each at the same developmental stage when approximately one-third of the siliques had reached maturity. We harvested each plant aboveground and placed it in a paper bag for drying and after-ripening at room temperatures. After 14 d, we collected the seeds from the paper bags, dried the remaining biomass at 70°C for 24 h, and weighed it. We pooled the seeds of all replicate plants per genotype and parental treatment and used these to establish the offspring generation (see below).

Parental treatments

We subjected the parental plants to 12 different experimental treatments. (1) *Light stress* was imposed by increasing light levels from *c.* 250 μmol m^{-2} s^{-1} in the control environment to 450 μmol m^{-2} s^{-1} in the treated plants. (2) *Heavy metal stress* was created by adding 5 ml of a 8 mmol solution of $CuSO_4$ to each treated pot every second day, with the last addition on Day 28 after sowing. (3) For *pathogen infection*, we sprayed the plants four times (starting on Day 16 after sowing, and then every third day) with a water solution containing 8×10^8 bacteria of *Pseudomonas syringae* pv *tomato* DC3000 per ml. The *P. syringae* DC3000 strain is strongly virulent and causes disease symptoms in *A. thaliana*. (4) *Cold stress* was imposed by regularly subjecting plants to 16 h of 4°C temperature for 1 wk (16-h cold followed by 8 h at 21°C; a total of 112 h of cold). To keep plants at long-day conditions, the 16-h cold was divided into 8 h at light and 8 h at dark conditions. (5) *Shading* was created by growing plants under a shading filter foil (122 Fern Green; Lee Filters, Andover, UK) that reduced light by 50% and lowered the red : far red ratio to 0.2. The plants were kept shaded until the control plants began to flower. (6) *Leaf removal* was applied by cutting off all cotyledons, which at this time represented 50% of the leaf area, on Day 16. Twenty days later, we repeated the treatment and again cut 50% of the leaf area of each plant. (7) *Drought stress* was created by not watering the treated plants unless they showed signs of wilting, whereas all other plants were watered regularly. (8) To create *salt stress*, we added a 4 g l^{-1} NaCl solution on Day 16 and after that treated plants twice a week with a 8 g l^{-1} NaCl

solution until Day 30. (9) *Jasmonic acid* was applied by spraying treated plants with a 0.5 mM JA solution (Cipollini, 2002) and control plants with a mock treatment of 0.5% ethanol every second day starting at Day 16 after sowing. (10) *Low nutrient stress* was created by transplanting plants into a nutrient-poor substrate (Huminsubstrat N3, Neuhaus, Germany) instead of the standard substrate used for all other plants. (11) *Short intense heat* stress was created by moving plants for 24 h to a 37°C growth chamber at Day 16 and then back to control conditions, whereas for the (12) *prolonged mild heat* treatment, plants were moved to a 30°C growth chamber for 10 d, starting at Day 16. For the combination of the two heat treatments, the plants were first moved to the 37°C chamber for 24 h and then to the 30°C chamber for another 9 d.

Offspring phenotyping

To test for the effects of parental stress treatments, or their combinations, on offspring phenotypes, we used the seeds collected from the parental generation to grow offspring of all genotypes and parental treatments in a common glasshouse environment. Using the same protocols for germination and growth and the same pots and substrate as for the parental generation, we grew 10 replicate plants per genotype and treatment (= a total of 25 × 3 × 10 = 750 plants, 24 stress treatments and their combinations plus controls) in a glasshouse with a 16 h : 8 h, 27°C : 16°C, light : dark cycle. The germination rates of the seed lots were generally high (all 75–90%), and there were also little differences in germination speed, indicating that parental effects, if they occurred, were not mainly driven by changes in seed dormancy (potentially removing a 'hidden fraction' with particular characteristics) or other aspects of seed quality.

The plants were arranged in a fully randomized order and watered regularly. To estimate the growth rates of plants originating from different parental environments, we measured the rosette diameter of each plant at 16, 20, 24, 28, and 32 d after sowing, fitted a power function $y = ab^x$ to each plant's data, and used the parameter b as a measure of growth rate. Throughout the experiment, we continuously monitored phenology and recorded the date of first flowering (= first petals visible) of each plant. As in the parental generation, we harvested the plants sequentially, when approximately one-third of the siliques had reached maturity. We harvested each plant aboveground, counted its fruit number, then dried the biomass at 70°C for 24 h, and weighed it.

Statistical analyses

To get an idea of the direct, within-generation effects of the stress treatments, we analyzed the biomass data from the parental generation with a generalized linear model in R (R Core Team, 2021) that included stress treatments, plant genotypes, and their interactions. We did this separately for each of the four locations as each had a unique set of treatments. We used models with a Gaussian error distribution, and to achieve homoscedasticity, we log-transformed the biomass data before the analysis. Afterward,

we adjusted the *P*-values associated with different model effects for false discovery rates, using Storey's *q*-values (QVALUE package; Storey *et al.*, 2022).

For the offspring generation data, we first examined how large the differences between the four parental locations were, in spite of our efforts to standardize conditions. A simple analysis of variance (ANOVA) testing for location and genotype effects only among the control plants showed that there were still significant differences among locations ($P < 0.001$ for all traits), and we therefore decided to also analyze the offspring data separately for each location. We analyzed the offspring data with similar generalized linear models as above, with Gaussian error distributions and log-transformed data for plant biomass and growth rate, and quasi-Poisson distributions for flowering time and fruit number. We also FDR-adjusted *P*-values of these analyses table-wide, that is, across all four tested offspring traits.

To understand the direction and magnitudes of the parental effects of different environmental stresses, we calculated the % changes observed in biomass, fruit number, growth rate, and flowering time for each stress, or combination of stresses, relative to the control treatment in the respective experimental location, and plotted these effect sizes comparatively for each of the four measured traits. To understand how consistent these effect sizes were across the three studied genotypes, we then additionally ran contrasts for each stress and variable that tested for differences between the mean values of stress (combination) vs control of individual genotypes. We used the directions of effects and significance levels of these contrasts to visualize these genotype-specific effects next to the cross-genotype results.

To test for a relationship between the magnitude and direction of parental and offspring stress responses, we calculated the cross-genotype % change caused by each treatment when compared to the respective control plants. We did this for the parental and offspring data and then used linear regression to test for a relationship between the two.

Results

As expected, many of the studied environmental stresses had significant direct effects on the growth of *A. thaliana* (Supporting Information Table S1). In most cases, these effects were negative (Fig. 2; *x*-axis). There were also significant genotype effects in all four experimental locations, as well as several significant genotype by stress interactions, confirming the genetic variation in mean phenotypes and phenotypic plasticity that has already been demonstrated by many previous studies on *A. thaliana*.

Generality and consistency of parental effects

Several of the studied abiotic or biotic environmental stresses, or their combinations, caused significant parental effects in our experiment. The strongest parental effects were on plant biomass and fruit production, where some stresses experienced by mother plants increased or decreased the performance of their offspring by 30–40% (Fig. 1). For instance, exposure of mother plants to cold, mild heat or shading transgenerationally increased biomass

and reproduction by 20–35%, whereas intense heat, or salt in combination with drought, had the opposite effect and decreased both biomass and fruit production by similar amounts (Fig. 1). After correcting for false discovery rates, 5 of the 12 individual stresses had significant transgenerational effects on plant biomass and plant reproduction (Table 1). Compared with plant biomass and reproduction, the growth rate and flowering time of plants were much less subject to parental effects, with only few percent changes across generations (Fig. 1), and few individual stresses with significant transgenerational effects (Table 1).

The three *Arabidopsis* genotypes included in our study often differed in the degree and magnitude of transgenerational effects (Fig. 1; significant genotype interactions in Table 1). Sometimes, the effects were even in opposite directions, resulting in non- or hardly significant main effects of an environmental stress across genotypes. For instance, drought and salt stress had negative transgenerational effects (i.e. lower performance of offspring than the offspring of control plants) on the *Col* genotype, but positive effects on *Tsu*, and none at all on *Sha* (Fig. 1).

Effects on parental vs offspring generation

We found no consistent relationship between the biomass responses of mothers and offspring to the different stresses or combinations of stresses in our experiment ($R^2 = 0.038$; $P = 0.358$). For some stresses, the direct (within-generation) effects on mother plants were similar to the transgenerational effects on the offspring. For instance, the combination of short intense heat with continuing mild heat significantly decreased the biomass of both mother plants and their offspring (Fig. 2). However, there were also cases where within- and across-generation effects were in opposite directions. For instance, high light intensity increased the growth of mother plants, but it decreased offspring biomass, and for mild heat, it was vice versa (Fig. 2). There were also cases where stress treatments affected mother plants but not the offspring, for example, for salt addition or intense heat, which strongly decreased the biomass of parents but had no effects across generations (Fig. 2). Finally, we also observed cases where the direct, within-generation effects of stresses were almost zero, but there were significant transgenerational effects. Examples are cold and drought, which did not at all affect the mother plants in our experiment, but they both strongly increased offspring biomass (Fig. 2).

Parental effects of multiple simultaneous environmental stresses

We found that for five of the 12 pairwise combinations of environmental stresses, there were significant interactions in their effects on plant biomass and/or fruit number (plus three marginally significant interactions; Table 1), indicating nonadditivity of stresses when occurring in combination. For instance, high light intensity and pathogen infection caused negative parental effects on plant biomass when tested individually, but in combination, they increased the biomass of offspring plants (Fig. 1c). Positive parental effects of cold and shading turned into a negative effect
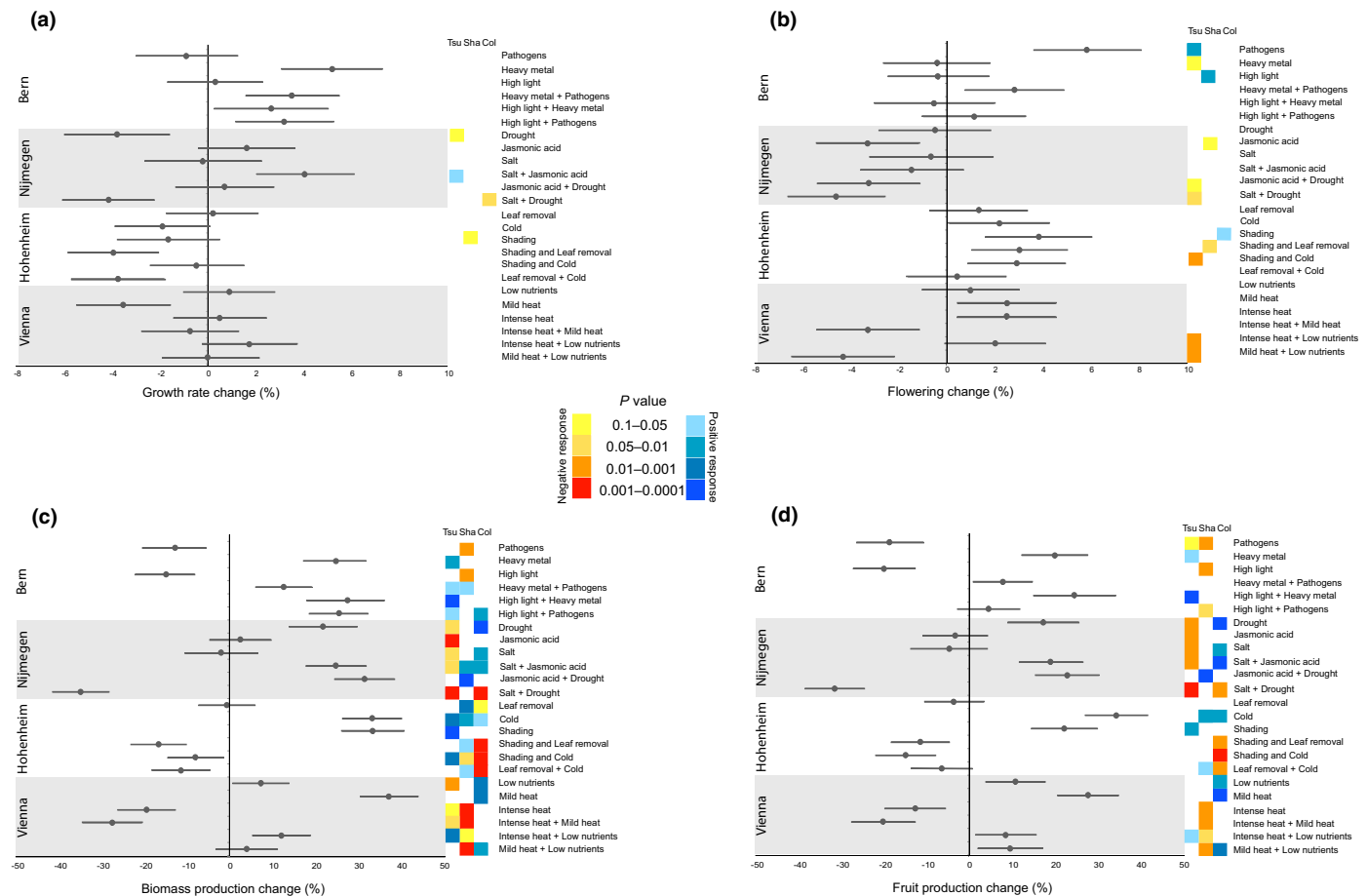
Fig. 1 Effect sizes of parental effects of different environmental stresses, or their combinations, on (a) growth rate, (b) flowering time, (c) biomass production and (d) fruit production of *Arabidopsis thaliana* plants. The values are % differences (mean ± SE) in performance between the offspring of treated parents and the offspring of control parents. Note that the parental generation was grown in four different experimental locations. The coloured squares indicate the significance levels (from contrasts) of parental effects for individual genotypes (red spectrum = negative effects; blue spectrum = positive effects).

when the two stresses were combined, and while drought and salt caused positive or neutral parental effects, their combination caused the strongest negative parental effect on plant biomass observed in our experiment (Fig. 1c). In addition to the general interactions between environmental stresses, we also found several significant three-way interactions between two stresses and plant genotype (Table 1), that is, the nonadditivity of multiple stresses depended to some degree on the plant genotype.

Discussion

Parental environmental effects are an intriguing but not well-understood source of phenotypic variation in plants. In spite of much debate about their eco-evolutionary significance (Kirkpatrick & Lande, 1989; Marshall & Uller, 2007; Räsänen & Kruuk, 2007; Badyaev, 2008; Bonduriansky & Day, 2009), we still do not know how frequent, consistent, and predictable parental effects really are. We tested the effects of 24 different environmental stresses, or their combinations, on the offspring of *A. thaliana* plants, and we found that parental effects are indeed very common, but that they are strongly genotype-dependent, act nonadditively, and are difficult to predict.

Generality and consistency of parental effects

The majority of the tested environmental stresses (16 of 24 stress treatments) caused significant parental effects, in particular on plant biomass and reproduction. The observed effect sizes, from around 35% decrease to almost 40% increase in biomass or reproduction (Fig. 1), are well within the range of that reported for parental effects in *A. thaliana* and other species (e.g. Andalo *et al.*, 1998; Blödner *et al.*, 2007; Boyd *et al.*, 2007; Galloway & Etterson, 2007; Bossdorf *et al.*, 2009; Kochanek *et al.*, 2013; Latzel *et al.*, 2014; González *et al.*, 2017; Puy *et al.*, 2021). Thus, parental effects appear to be common in *A. thaliana*, and they can be elicited through a broad range of biotic and abiotic environmental stresses – with likely consequences for ecological interactions and evolutionary trajectories (Kirkpatrick & Lande, 1989; Marshall & Uller, 2007; Badyaev, 2008). Interestingly, while parental effects were frequent and strong for biomass and fruit number, they were much less frequent and weaker for flowering time and growth rate. Clearly, some plant traits seem to be much less prone to parental effects than others, possibly because they are under tighter developmental control. A good example is flowering time, which is strongly differentiated among

Table 1 Results of analysis of variance testing for parental effects of individual stresses, or their combinations, on the growth and fitness of three genotypes of *Arabidopsis thaliana*.

			Biomass		Growth rate		No. of fruits		Flowering time	
		df	F	P (> F)	F	P (> F)	Dev.	P (> Chi)	Dev.	P (> Chi)
Bern	High light (L)	1	0.1	0.304	0.3	0.301	17	0.294	0.322	0.278
	Heavy metal (M)	1	11.3	0.007	4.5	0.094	453	0.003	0.028	0.304
	Pathogens (P)	1	1.0	0.269	0.0	0.304	2	0.304	3.47	0.018
	Genotype	2	1.6	0.234	6.8	0.009	424	0.013	31.25	<0.001
	L : M	1	0.4	0.298	0.7	0.282	1	0.304	0.61	0.236
	L : P	1	24.3	<0.001	1.5	0.240	507	0.001	0.377	0.270
	M : P	1	0.4	0.299	0.1	0.304	26	0.281	0.064	0.304
	L : Genotype	2	1.1	0.269	1.0	0.279	201	0.125	5.736	0.006
	M : Genotype	2	12.7	<0.001	0.5	0.303	628	0.001	0.638	0.291
	P : Genotype	2	2.7	0.145	0.5	0.303	255	0.078	0.666	0.289
	L : M : Genotype	2	1.0	0.277	0.4	0.304	115	0.225	3.293	0.058
	L : P : Genotype	2	2.2	0.183	0.2	0.304	103	0.239	0.396	0.303
	M : P : Genotype	2	1.0	0.281	1.4	0.246	34	0.303	0.129	0.304
	Residuals	176					8846		108.5	
Hohenheim	Leaf removal (LR)	1	28.4	<0.001	1.5	0.237	641	0.002	0.693	0.242
	Cold (C)	1	0.6	0.288	0.2	0.303	2	0.304	0.032	0.304
	Shading (S)	1	7.7	0.029	0.7	0.286	355	0.031	0.534	0.262
	Genotype	2	8.5	0.003	2.6	0.152	269	0.132	50.6	<0.001
	LR : C	1	0.1	0.304	0.6	0.289	24	0.294	0.048	0.304
	LR : S	1	0.4	0.298	1.5	0.240	12	0.303	0.973	0.209
	C : S	1	37.5	<0.001	1.1	0.259	1281	<0.001	0.246	0.294
	LR : Genotype	2	31.3	<0.001	0.4	0.304	1334	<0.001	0.346	0.304
	LR : Genotype	2	1.9	0.204	0.1	0.304	72	0.294	0.613	0.299
	S : Genotype	2	12.3	<0.001	0.2	0.304	649	0.008	3.542	0.080
	LR : C : Genotype	2	0.1	0.304	1.8	0.210	32	0.304	0.307	0.304
	LR : S : Genotype	2	0.4	0.303	1.1	0.274	23	0.304	2.981	0.114
	C : S : Genotype	2	0.3	0.304	0.7	0.294	119	0.260	0.436	0.303
	Residuals	197					14 670		145.5	
Nijmegen	Drought (D)	1	4.1	0.110	6.1	0.054	42	0.276	1.826	0.218
	Jasmonic acid (JA)	1	21.3	<0.001	6.0	0.055	618	0.004	0.438	0.297
	Salt (S)	1	5.0	0.080	0.1	0.304	178	0.131	0.224	0.303
	Genotype	2	26.7	<0.001	0.7	0.295	1758	<0.001	38.47	<0.001
	D : JA	1	5.8	0.060	0.4	0.298	155	0.151	0.81	0.278
	D : S	1	27.3	<0.001	0.1	0.304	925	<0.001	0.714	0.283
	JA : S	1	5.3	0.072	0.8	0.276	150	0.156	0.177	0.304
	D : Genotype	2	7.3	0.007	2.9	0.126	444	0.048	1.396	0.296
	JA : Genotype	2	8.5	0.003	0.8	0.290	271	0.140	3.796	0.204
	S : Genotype	2	1.6	0.231	1.6	0.228	166	0.227	1.294	0.298
	D : JA : Genotype	2	9.7	0.001	0.2	0.304	658	0.010	4.143	0.189
	D : S : Genotype	2	11.0	<0.001	1.0	0.278	1289	<0.001	0.641	0.304
	JA : S : Genotype	2	2.8	0.131	1.1	0.271	151	0.239	2.619	0.254
	Residuals	175					15 841		127.1	
Vienna	Low nutrients (N)	1	2.8	0.164	1.4	0.242	64	0.234	0.095	0.304
	Mild heat (MH)	1	0.0	0.304	2.3	0.193	0	0.304	0.543	0.256
	Intense heat (IH)	1	13.6	0.003	0.7	0.282	384	0.014	0.069	0.304
	Genotype	2	22.1	<0.001	1.8	0.211	1010	<0.001	58.65	<0.001
	N : MH	1	9.3	0.015	0.3	0.301	188	0.091	0.272	0.289
	N : IH	1	23.6	<0.001	0.1	0.304	281	0.038	0.787	0.224
	MH : IH	1	5.8	0.060	0.4	0.297	85	0.206	2.309	0.075
	N : Genotype	2	0.9	0.286	0.1	0.304	134	0.228	2.475	0.137
	MH : Genotype	2	6.3	0.013	1.0	0.281	455	0.021	3.844	0.054
	IH : Genotype	2	4.4	0.051	0.5	0.302	224	0.138	1.339	0.241
	N : MH : Genotype	2	0.6	0.299	0.6	0.300	10	0.304	0.448	0.303
	N : IH : Genotype	2	9.3	0.001	0.3	0.304	299	0.081	0.257	0.304
	MH : IH : Genotype	2	2.6	0.149	0.7	0.295	344	0.056	0.268	0.304
	Residuals	194					12 337		148.7	0.242

As the parental generation was grown in four different locations, the data were analyzed separately for each. Significant effects are highlighted, with P-values corrected for false discovery rate. P-values < 0.05 are shaded with gray.
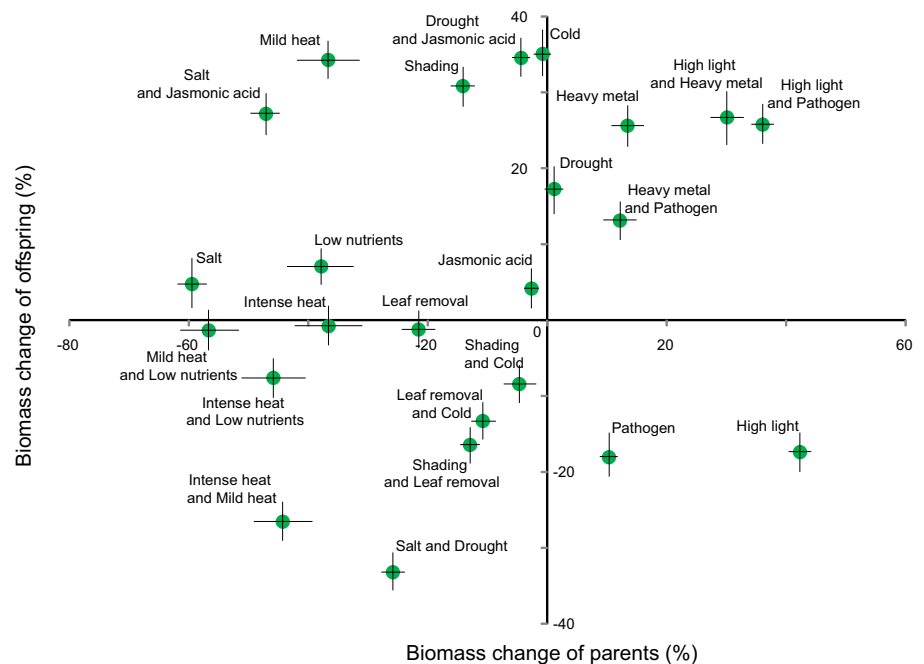
Fig. 2 Relation of offspring biomass of *Arabidopsis thaliana* production responses to parental treatments with responses of parents to the treatments. The responses are % changes (with SE) in biomass production of plants experiencing treatment (or offspring of parents of the treatments) in comparison with control plants (or offspring of control parents).

geographic origins (significant genotype effects in Table 1; see also Stinchcombe *et al.*, 2005), but it is hardly plastic across generations.

The extent and often also direction of parental effects strongly varied among the three genotypes in our study (Fig. 1). Our results thus demonstrate substantial genetic variation for parental effects among *Arabidopsis* genotypes, which supports previous studies with *Arabidopsis* and other plant species (e.g. Andalo *et al.*, 1999; Agrawal, 2001, 2002; Galloway, 2001; Riginos *et al.*, 2007; Bossdorf *et al.*, 2009; Groot *et al.*, 2017; Lampei, 2019) that also found genotype-specificity of parental effects. Compared with previous studies, our experiment included a much broader range of environmental stresses, and it thus demonstrates that G × E effects are extremely common across generations, just as they are for within-generation plasticity (Sultan, 2000; Pigliucci *et al.*, 2006). An important implication of this result is that generalization from simple studies is difficult. With evidence from only a single plant genotype – as in many previous studies on parental effects – one cannot draw general conclusions about the presence or absence of parental effects, let alone their direction and magnitude.

In summary, we find that parental effects are common and strong, but genotype-specific, in *A. thaliana*. Because of this genotype-specificity, and their effects particularly on fitness-related traits, we should expect parental effects to influence selection and evolution of the species.

Effects on parental vs offspring generation

Having demonstrated parental effects of a broad range of environmental stresses, we next asked whether the direction and magnitude of these cross-generation effects were related to the within-generation effects of the different stresses. Intuitively, we

expected that negative transgenerational effects would be caused by environmental stresses that also have negative effects on the same trait in mother plants, and vice versa. However, we found that the effects of environmental stresses on mother plants and their offspring were often very different, sometimes in opposite directions, or absent in one generation but present in the other. As a consequence, there was altogether no significant relationship between the within- and across-generation effects of environmental stresses. What surprised us most was that there were also cases, for example, for cold and drought stress, where the direct, within-generation effects were nearly zero, but there were nevertheless substantial transgenerational effects (Fig. 2). Environmental stresses with strong direct impacts but no parental effects have been reported previously (e.g. Sultan *et al.*, 2009; Lampei, 2019), but we are not aware of any previous studies that have shown the opposite. While a discussion of the biological mechanisms underlying the diverse results of different environmental stresses in our experiment is beyond the scope of this paper, an important take-home message is that the direction and magnitude of parental effects cannot be predicted from the parental responses to an environmental stress, and that sometimes seemingly ineffective environmental changes may nevertheless cause strong parental effects.

Parental effects of multiple simultaneous environmental stresses

Environmental change is usually multifactorial (Hof *et al.*, 2011; IPCC, 2021). It is therefore important to understand interactions between multiple drivers of environmental change, and their potential nonadditive effects on organisms (e.g. Shaw *et al.*, 2002; Niinemets, 2010; Shears & Ross, 2010; Zhang & Sonnewald, 2017; Zandalinas *et al.*, 2021). Our experiment

allowed us to address these questions for parental effects of a broad range of environmental stresses on *A. thaliana*. We found that in the majority of the cases, two environmental stresses acted nonadditively across generations when combined. In extreme cases, the parental effects of combinations of two stresses even had opposite directions to those of the individual stresses. Our results corroborate the findings of the few previous studies that tested for transgenerational effects of multiple stresses (Lau *et al.*, 2008; Latzel *et al.*, 2010; Lampei, 2019) and that found similar nonadditive effects as in our study. They clearly show that the nonadditivity – or context-dependency – of multiple environmental stresses is another challenge for predicting parental effects, particularly under realistic conditions.

Conclusions

Our study finds that a broad range of environmental stresses affects the growth and reproduction of *A. thaliana* plants across generations. Parental environmental effects are thus a very general phenomenon, and researchers planning common garden studies with seeds of different origins must generally assume that such effects exist and that they will impact plant phenotypes, genetic parameters, and tests for local adaptation. To reduce parental influences, it should remain a rule to cultivate one to two intermediate generations in a common environment before setting up the main experiment (Latzel, 2015).

The parental effects observed in our study were strongly genotype-dependent, multiple stresses often acted nonadditively, and the direction and magnitude of cross-generation effects were completely unrelated to how stresses acted directly on the parental plants. Altogether, parental environmental effects in *A. thaliana* appear so complex and difficult to predict that we must avoid generalizing from simple studies with single plant genotypes or only few individual environmental stresses. From all we know about the ubiquity of G × E interactions (e.g. Sultan, 2000; Nicotra *et al.*, 2010), it seems likely that all of this will also be true for other plant species. Therefore, understanding parental environmental effects appears to strictly require large experiments with multiple genotypes and multiple, interacting environmental drivers. This is an important insight not only for basic research but also because we urgently need to understand the dynamics of plants in changing environments, and besides phenotypic plasticity (Chevin *et al.*, 2010; Nicotra *et al.*, 2010; Hoffmann & Sgrò, 2011) and longer term adaptation (e.g. Jump & Penuelas, 2005), parental effects might be another important facet of how plants respond to global environmental changes.

Competing interests

None declared.

Author contributions

VL and OB designed the study. VL, MG, RG, CL and PV performed the experiments. YZ, MP, OB and VL analyzed the data. All authors contributed to the writing of the paper.

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Data availability

The data that support the findings of this study are openly available in Dryad repository doi: 10.5061/dryad.wm37pvmrc.

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Supporting Information

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

Table S1 Results of testing for direct, within-generation effects of individual stresses, or their combinations, on the biomass of three genotypes of *Arabidopsis thaliana*.

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