Transgenerational effects of temperature fluctuations in *Arabidopsis thaliana*

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ABSTRACT

Plant stress responses can extend into the following generations, a phenomenon called transgenerational effects. Heat stress, in particular, is known to affect plant offspring, but we do not know to what extent these effects depend on the temporal patterns of the stress, and whether transgenerational responses are adaptive and genetically variable within species. To address these questions, we carried out a two-generation experiment with nine *Arabidopsis thaliana* genotypes. We subjected the plants to heat stress regimes that varied in timing and frequency, but not in mean temperature, and we then grew the offspring of these plants under controlled conditions as well as under renewed heat stress. The stress treatments significantly carried over to the offspring generation, with timing having stronger effects on plant phenotypes than stress frequency. However there was no evidence that transgenerational effects were adaptive. The magnitudes of transgenerational effects differed substantially among genotypes, and for some traits the strength of plant responses was significantly associated with the climatic variability at the sites of origin. In summary, timing of heat stress not only directly affects plants, but it can also cause transgenerational effects on offspring phenotypes. Genetic variation in transgenerational effects, as well as correlations between transgenerational effects and climatic variability, indicate that transgenerational effects can evolve, and have probably already done so in the past.

INTRODUCTION

Plants encounter various environmental challenges in nature, such as episodes of stressful temperatures or low water availability. Many previous studies have investigated how plants respond to contrasting environmental conditions in terms of their fitness and functional traits (e.g. Sultan et al., 1998; Callahan and Pigliucci, 2002; Ibañez et al., 2017; Marais et al., 2017). Although plants generally show reduced fitness under stressful environments, different genotypes often vary in their fitness responses and thus their ability to maintain fitness under adverse environmental conditions (Sultan, 1987, 2000; Ghalambor et al., 2007). This variation in fitness responses is often related to underlying variation in the plasticity of functional traits. For instance, decreased fitness under warmer temperatures may be caused by advanced flowering in the annual *Arabidopsis thaliana* (Ibañez et al., 2017). More generally, there is usually intraspecific variation in plant responses to environmental treatments (i.e. genotype-by-environment interactions, G × E; Sultan, 2000; Pigliucci, 2001), and if such variation exists within populations, then natural selection can act on it, and the trait plasticity can evolve and adapt to local environmental conditions (Sultan, 2000; Groot et al., 2017). If past environments have influenced the evolution of plasticity, we should be able to detect plasticity-environment correlations to identify agents of selection shaping plasticity (Groot et al., 2017; Marais et al., 2017).

Organisms may not only respond directly to their current environments, but their phenotypes may also be influenced by the environmental conditions that their ancestors were exposed to (Uller, 2008; Latzel et al., 2014; Groot et al., 2017) – a phenomenon called ‘transgenerational plasticity’ or ‘transgenerational effects’. In plants, such transgenerational effects can be physiological and controlled by the mother plant (Herman and Sultan, 2011), for instance through endosperm or seed coat modifications, or they can be epigenetic (Whittle et al., 2009; Rasmann et al., 2012; Suter and Widmer, 2013) and therefore potentially transferable across even more than one generation (Suter and Widmer, 2013; Groot et al., 2017). Through transgenerational effects, plants could prepare (or ‘prime’) their phenotypes for particular environmental conditions, particularly when offspring are likely to experience similar conditions as their parents, thereby increasing local adaptation (i.e. adaptive transgenerational plasticity; Roach and Wulff, 1987; Mousseau and Fox, 1998ab; Agrawal, 2001; Galloway, 2005; Galloway and Etterson, 2007; Uller, 2008; Mousseau et al., 2009; Latzel et al., 2014). However, as with regular (within-generation) phenotypic plasticity, transgenerational effects can only evolve as an adaptation when there is genotypic variation in transgenerational effects and when offspring environmental conditions correlate with parental environmental conditions (Uller, 2008).

An increasing number of empirical studies with plants investigated how transgenerational effects may confer adaptation particularly under temperature stress (Sultan et al., 2009; Herman and Sultan, 2011; Latzel et al., 2014; Groot et al., 2017). For instance, in a single genotype of the model plant *Arabidopsis thaliana*, transgenerational effects of heat stress were observed even in the F3 generations where F3 offspring with the same heat stress in the P1 and F1 generations had a fitness advantage (Whittle et al., 2009). Recently, Groot and co-workers (2017) showed strong genotypic variation in parental and grandparental effects of heat stress in 14 *A. thaliana* genotypes.

So far most studies investigating plant responses to altered and/or stressful environmental conditions – including those studies investigating transgenerational effects – were performed under controlled conditions but usually with stable treatments that did not consider the temporal variability of environmental stress, which however plays an important role in natural ecosystems (Knapp et al., 2002; Schwinning et al., 2004; Shea et al., 2004). For instance, while global warming is expected to continue (Giorgi et al., 2004; Barros and Field, 2014), climate anomalies will increase too (e.g. European heat waves in 2003 and 2010), resulting in increasing temporal variability of temperature and, presumably, heat stress (Schär et al., 2004; Fischer and Schär, 2008; Barriopedro et al., 2011). During climatic extreme events, the variability aspect itself is often thought to be more important than the involved changes in means (Katz and Brown, 1992), and some ecosystems have even been found to be more sensitive to changes in environmental variability than to changes in environmental means (Knapp et al., 2002).

To date, only few studies have examined plant responses to changes in environmental variability, or genetic variation therein (Parepa et al., 2013, Scheepens et al., 2018), specifically with respect to the timing (Stone and Nicolas, 1995, 1996; Prasad et al., 1999; Wang et al., 2016) or frequency (Walter et al., 2009) of stress. To our knowledge, no previous study tested for transgenerational effects of stress timing and frequency.

To address these questions and to better understand the complexity of plant responses to climatic variability (Knapp et al., 2002; Reyer et al., 2013) we carried out a two-generation experimental study with *Arabidopsis thaliana* (L.) Heynh. that tested plant responses to altered timing and frequency of heat stress. To explore intraspecific variation and evolutionary potential, our study included multiple genotypes from different geographic and climatic origins. In the first generation (published in Scheepens et al., 2018) we found that the timing of heat stress had a much stronger effect on the plants than its frequency, that *A. thaliana* genotypes significantly differed in their responses to stress timing, and that this intraspecific variation correlated with the precipitation variability at the geographic origins, indicating a possible adaptive evolution of this type of phenotypic plasticity in more variable environments.

Here, we report on the results from the offspring generation where we grew plants from 9 of the 11 genotypes included in the parental-generation experiment and tested on the one hand for transgenerational effects of parental stress treatments in a simple common-garden experiment, and on the other hand we subjected a subset of the offspring plants to renewed stress to test the adaptive value of transgenerational effects (reciprocal experiment). As in the parental-generation experiment, we also tested for intraspecific variation in plant responses, correlated this variation with climates of origin, and tested whether increased trait plasticity correlates with fitness robustness, i.e. more stable fitness across treatments. Specifically, we asked the following questions: (1) Are there transgenerational effects of heat stress timing or frequency on the phenotypes of the offspring? (2) If yes, do transgenerational effects affect responses to current stress in an adaptive way? (3) Are there differences among *A. thaliana* genotypes in the magnitudes and/or direction of transgenerational effects? (4) If yes, does this intraspecific variation correlate with environmental conditions at the geographic origins and/or with fitness robustness?

MATERIALS AND METHODS

Parental generation experiment

The plant material used here came from a previous study (Scheepens et al., 2018) in which we tested for the direct effects of different temperature stress scenarios, varying in timing and frequency (**Figure 1**), on 11 *Arabidopsis thaliana* genotypes. The 11 genotypes were selected to maximize genetic diversity and came from the “core collection” of the Versailles *Arabidopsis* Stock Center (McKhann et al., 2004). After one week of cold-moist (4 °C) stratification, all seeds were planted into 5 × 5 × 4.5 cm pots with a 9:9:2 mixture of low-nutrient soil, regular potting soil and sterilized sand and placed in a growth chamber with 20/15 °C and a 16/8 h light/dark cycle until one week after germination. For the experimental treatments, we used two identical climate chambers, one set to 20/15 °C (‘control chamber’), the other set to 30/25 °C (‘stress chamber’), both with a 16/8 h light/dark cycle. A day temperature of 30 °C is known to be stressful for *A. thaliana* and to reduce its fitness (Groot et al., 2017; Scheepens et al., 2018). Light conditions (230 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and air humidity (40-60%) were identical in both chambers. The experimental treatments were created by moving different subsets of plants to the stress chamber at different times and intervals. Specifically, we varied the timing and frequency of heat stress periods experienced by the plants (**Figure 1**). To vary timing, we stressed plants either early in their life cycle (plants moved to stress chamber on day 8, right after the first week of seedling establishment), in the middle of most genotypes’ life cycle (starting on day 26) or late in the life cycle (starting on day 44). The timing treatment was crossed with a frequency/duration treatment, where heat stress was either applied at low frequency (2 times 6 days of stress, with 6 days in between) or high frequency (4 times 3 days of stress, each time with 2 days in between). Important to note is that in all stress scenarios the plants experienced the same total time in the stress chamber and therefore also the same mean temperature during the experiment (**Figure 1**). In each chamber, the spatial positions of all pots were completely randomized, and were re-randomized every week. We had eight replicate plants of each genotype in each treatment. Altogether, our parental-generation experiment included 11 genotypes × 6 treatments × 8 replicates = 528 plant individuals. The experiment ran for approximately 10 weeks. When plants began flowering, we placed their inflorescences into ARACON tubes (Betatech bvba, Gent, Belgium) to prevent cross-fertilization and collect the seeds for the next experimental generation.

Offspring generation experiments

We tested for transgenerational effects in two separate experiments, (1) a simple common-environment comparison of offspring from the six parental treatments under control condition (16/8 h light/dark at 20/15 °C), and (2) a reciprocal transplant where we used offspring from only two of the parental treatments, the early and mid-term stress at low stress frequency (**Figure 1**), re-created these two treatments and grew both types of offspring in both environments. We restricted the second experiment to these two treatments because they had the strongest effects in the parental generation (Scheepens et al., 2018). Since in the reciprocal experiment there were ‘local’ (same conditions as parents) versus ‘foreign’ (different conditions from parents) plants in each environment, this experiment allowed to test for adaptive transgenerational effects. In both offspring experiments we used nine of the 11 genotypes from the previous generation, because of limited numbers of seeds in the remaining two genotypes (**Table 1**; Scheepens et al., 2018), and we stratified and germinated seeds as in the parental experiment. In the first experiment, we had seven replicates per genotype and maternal treatment, for a total of 9 genotypes × 6 parental environments × 7 replicates = 378 plants. In the second experiment, there were eight replicates per genotype by treatment combination, with a total of 9 genotypes × 2 maternal environments × 2 offspring environments × 8 replicates = 288 plants. In both experiments, we watered all plants regularly, and re-randomized their spatial positions every week. On day 44, right after the intermediate stress treatment in the reciprocal experiment, we took leaf samples for molecular analyses (not reported here) from 3-4 randomly selected plants from each genotype by treatment combination in each of the two experiments (i.e. from roughly half of the plants). Throughout the experiment, we recorded flowering time as the number of days from germination to when the white petals of the first flower became visible. As in the parental experiment, we placed ARACON tubes over the flowering stems to prevent outcrossing and collect seeds. Each plant was harvested one week after its fruits had started to turn yellow. We estimated plant fecundity as the number of fruits >2 mm, and we counted the number of basal shoots and lateral shoots and calculated the ratio of lateral to basal shoot number as index of plant architecture, with lower values indicating more ‘shrubby’ plants. After that, we separated inflorescences and rosettes, dried them at 60 °C for 72 h and weighed them, and then calculated total aboveground biomass, as well as reproductive allocation as the ratio of reproductive to total aboveground biomass.

Statistical analysis

We used linear models to test for the effects of experimental treatments, plant genotypes, and their interactions, on each of the five measured traits: flowering time, plant architecture, aboveground biomass, reproductive allocation and fecundity. For the simple common-environment experiment, the models included plant genotype, timing of parental stress, frequency of parental stress, and all possible interactions, as fixed factors. For the reciprocal experiment, the models included plant genotype, timing of parental stress, timing of offspring stress, and their interactions. Additionally, to account for possible influences of the leaf sampling, all models also included leaf sampling (yes/no) as a fixed factor. To improve the normality of residuals and homogeneity of variance, the flowering time and aboveground biomass data were log-transformed prior to the analyses.

In those cases where we discovered a significant genotype by treatment interaction, i.e. genetic variation in plasticity, in either of the two experiments, we additionally tested whether trait plasticities of genotypes were associated with (1) their climates of origin and (2) their fitness robustness. As measure of trait plasticity we used the coefficient of variation (CV) of a trait (Valladares et al., 2006) across all treatments in an experiment (common environment: six parental environments; reciprocal experiment: four combinations of parental and offspring environments). For the climate-plasticity test we extracted climate data for each genotype origin from the WorldClim database (Hijmans et al., 2005), and we used on the one hand several existing bioclimatic variables that describe annual climatic variability [BIO_2 = Annual Mean Diurnal Range, BIO_3 = Isothermality, BIO_4 = Temperature Seasonality (SD), BIO_7 = Annual Temperature Range, BIO_{15} = Precipitation Seasonality (CV)], and on the other hand we calculated several climate variabilities for the specific growing season (see **Table 1**) of each genotype: the SDs of temperature, and the CVs of precipitation, evapotranspiration and climatological water deficit. Additionally, we included latitude from each genotype's origin. To test for relationships between climate variability of origin and the plasticity of *Arabidopsis* genotypes, we calculated Pearson correlations between trait plasticity and the bioclimatic variables, growing-season variabilities and latitude, respectively. For the plasticity-fitness test we calculated the fitness robustness of each genotype as the mean fitness (in terms of number of fruits) across treatments divided by the maximum fitness achieved in one of the six treatments, and calculated Pearson correlations between trait plasticity and fitness robustness.

All statistical analyses were done in JMP 12 (SAS Institute, Heidelberg).

RESULTS

Common-environment experiment

In the simple common-environment experiment, we found strong genotype differences in all measured traits (**Table 2**), confirming that there was substantial genetic diversity in the studied *A. thaliana* genotypes. The effects of parental stress treatments were much more moderate, and were largely confined to the timing of parental heat stress: Offspring from parents which experienced early stress generally showed an increased ratio of lateral to basal shoots compared to intermediate and late stress (**Figure 2**). For flowering time, the effect of stress timing depended on stress frequency (PT × PF interaction in **Table 2**): at high stress frequency, stress timing had an effect on flowering time, whereas at low stress frequency it did not (**Figure S1**). We found significant genotype by stress timing interactions for flowering time and plant architecture (G × PT interactions in **Table 2**; **Figure 3**), indicating genetic variation in these transgenerational responses. There were no main effects of stress frequency in any of the studied traits, and no genotype by stress frequency interactions. Only for aboveground biomass, there was a significant three-way interaction between plant genotype, parental stress timing and parental stress frequency for aboveground biomass (G × PT × PF interaction in **Table 2**), indicating complex relationships between these three factors.

Reciprocal experiment

When offspring from early and intermediate (low-frequency) stress parents were reciprocally subjected to the same treatments, there were strong effects of offspring environment on all measured traits except for flowering time (OT main effects in **Table 3**), whereas the parental heat stress timing affected only the flowering time of the plants (PT main effect in **Table 3**), with offspring from early-stress parents flowering earlier (**Figure 4**). However, a significant interaction between parental and offspring environment (PT × OT in **Table 3**) indicated that the expression of transgenerational effects on flowering time depended on the offspring environment: the differences between parental treatments were expressed only if the offspring was subjected to early heat stress, but not if heat stress occurred later (**Figure 4**).

As in the common-environment experiment, there were significant genotype differences in all of the studied traits (**Table 3**), and there were significant genotype by offspring environment interactions (G × OT in **Table 3**) in four out of the five measured traits, indicating genetic variation in (within-generation) phenotypic plasticity. In addition, we found a genotype by parental environment interaction (G × PT in **Table 3**), indicating genotype-specific transgenerational effects, for flowering time.

We did not find a significant parental by offspring environment interaction for plant fecundity (PT × OT in **Table 3**), as would have been predicted for adaptive transgenerational effects. However, there was a significant G × PT × OT interaction, indicating that these interactions are genotype-specific. We therefore tested for a significant PT × OT interaction separately for each genotype. Only in Mt-0 this interaction was significant ($F = 10.38$, $P = 0.003$; **Figure S2**), but the results did not confirm our hypothesis. In each offspring environment the plants from the respective *other* parental environment produced more fruits than the ones from the same parental environment, suggesting rather a maladaptive transgenerational effect.

Plasticity, climates of origin, and fitness robustness

We found no correlations between climates of origin and trait plasticity in the common-environment experiment (**Table S1**), but in the reciprocal experiment there were several significant climate-plasticity correlations (**Table S2**). The CV of fecundity was negatively correlated with temperature seasonality and annual temperature range, and positively correlated with isothermality (**Table S2**). Thus, genotypes from geographic origins with higher temperature seasonality displayed lower fecundity variation - and therefore greater fitness homeostasis - in response to different stress treatments (**Figure 5A**). The CV of fecundity was also positively correlated with the seasonal CV of evapotranspiration variability (**Table S2**). Moreover, we also found that the CV of aboveground biomass was positively correlated with isothermality and precipitation seasonality (**Figure 5B**), and negatively correlated with latitude and with seasonal CV of climatological water deficit. Finally, the CV of plant architecture correlated negatively with the annual mean diurnal range. Despite significant genotypic variation in the response of flowering time to parental or offspring stress timing, this variation in plasticity did not correlate with any of the climate variables. In both experiments we found that the plasticity of aboveground biomass, but not that of the other traits, was significantly negatively correlated with fitness robustness (**Table S3**; **Figure 6**).

DISCUSSION

Changes in the temporal variability of environmental stresses are an important aspect of climate change, but we so far know little about the evolutionary consequences for plants: whether plant responses can be transgenerational, if plants harbour intraspecific variation (and thus evolutionary potential) in this respect, and how such transgenerational responses relate to environmental adaptation and fitness. Our study demonstrates that changes in the temporal patterns of heat stress can carry over to the next generation in *Arabidopsis thaliana*, and that there is substantial genotypic variation in the magnitude and direction of these transgenerational effects. Thus, changes in heat stress patterns not only affect plants directly (Scheepens et al., 2018), but also across generations. Several previous studies have reported transgenerational responses to various environmental changes (e.g. Galloway and Etterson, 2007; Herman et al., 2012; Groot et al., 2017). For instance, Groot et al. (2017) subjected parental and grandparental plants of 14 *A. thaliana* genotypes to continuous heat stress and found transgenerational effects, as well as genotypic variation therein. The unique aspect of our study is that, while previous studies usually compared stressed and non-stressed plants, we only manipulated the temporal patterns of heat stress, i.e. when the stress occurred and how it was apportioned across time, whereas the total amount of stress (i.e. temperature sums) was identical in all parental environments.

Transgenerational effects of stress timing versus frequency

Overall, the timing of heat stress had much stronger transgenerational effects than its frequency, consistent with our observations in the parental plants (Scheepens et al., 2018). Variation in parental stress timing consistently affected the architecture, and, depending on the genotype and/or stress frequency, also the flowering time and biomass of offspring plants, whereas the transgenerational effects of stress frequency were only minor.

One possibility why stress frequency may play such a little role within and across generations is that plant physiological responses to heat stress may be triggered by the initial stress event, and simply remain ‘switched on’ afterwards, so that the number or duration of stress events does not matter, at least on the short time-scales of our experiment. A candidate mechanism for this would be heat shock proteins that plants produce to stabilize protein function (Nover et al., 2001; Sung et al., 2003; Swindell et al., 2007), and that may protect plants and their offspring against subsequent heat stress events.

In contrast to stress frequency, the timing of parental heat stress influenced several traits of the plant offspring. It is generally well-established that the susceptibility of many plant traits to environmentally-induced developmental changes depends on the life stage. For instance, heat stress during floral bud development determines peg number in peanut (Prasad et al., 1999), in wheat the maximum sensitivity to heat stress for protein accumulation is during the grain filling period (Stone and Nicolas, 1996), and in the herbaceous plants *Andropogon gerardii* and *Solidago canadensis* late-season heat stress causes the greatest reduction in photosynthetic productivity (Wang et al., 2016). The usual explanation for such results is that signaling pathways determining trait changes may only be active during certain developmental periods, but the precise underlying mechanisms are often unknown. Another explanation would be that no active developmental mechanism is involved, but plants are simply more sensitive at some life stages (analogous to ‘active’ versus ‘passive’ phenotypic plasticity; van Kleunen and Fischer, 2005). In our experiment, early heat stress occurred at a small seedling stage of *A. thaliana*, whereas in the intermediate treatment the plants were already much larger and well-established. In fact, some were already bolting and/or close to flowering. It is not surprising that heat stress effects differed between these plants. However, all arguments so far, as well as the empirical studies mentioned above, are about within-generation responses to heat stress, whereas in our study we observed transgenerational effects. Thus, signaling and developmental regulation alone cannot explain our results, and there must be additional, so far unknown, physiological (Herman and Sultan, 2011) and/or epigenetic (Whittle et al., 2009; Rasmann et al., 2012), mechanisms involved.

No evidence for adaptive transgenerational plasticity

In the reciprocal experiment we applied stress treatments to offspring plants to test if transgenerational effects can be adaptive. In our case, if the offspring whose parents experienced the same stress timing as the respective tested one had a higher fitness than the offspring of parents that experienced another stress timing, this would indicate an adaptive transgenerational effect. We found that responses in plant fecundity to current stress timing depended on parental stress timing, but in a highly genotype-specific manner. In fact, the majority of the parent-offspring interactions for separate genotypes were non-significant and only the genotype Mt-0 showed a significant interaction to parental and offspring heat stress timing. However, the pattern was maladaptive, i.e. in contrast to our expectation offspring from parents with the same stress timing had a *lower* fitness. This contrasts with observations of adaptive transgenerational plasticity from previous studies (Galloway and Etterson, 2007; Latzel et al., 2014). The virtual absence of significant interactions across genotypes in our study may have various explanations, such as limited within-population genetic variation in parental effects for the environment to select on, or a lack of selective pressure for adaptive responses under unpredictable temperature stress events.

Offspring plants that received early stress showed accelerated flowering when their parents had also experienced early stress compared to plants whose parents had experienced intermediate stress. Such advanced flowering may reflect an escape strategy (Franks, 2011), which could enhance the possibility of lineage survival under continuing high temperature conditions (Wahid et al., 2007). Although the induction of earlier flowering by environmental stress treatments is known from previous studies (Balasubramanian et al., 2006; Franks, 2011; Ibañez et al., 2017), its transgenerational aspect has so far been rarely studied. Suter and Widmer (2013) detected accelerated flowering in *Arabidopsis thaliana* under control conditions in the fourth generation after heat exposure, but this effect disappeared in the fifth generation after two generations without stress exposure. Groot and co-workers (2017) observed earlier flowering in response to grandparental heat stress, but only in late-flowering genotypes. Our own results confirm that stress exposure can induce earlier flowering also transgenerationally, and thereby contribute to an escape strategy, but that such effects may be restricted to situations with early stress exposure, where plants are still in sensitive developmental stages.

Genotypic variation in transgenerational plasticity

So far, few studies have investigated intraspecific variation in transgenerational plasticity under stress conditions (Gaudet et al., 2011; Suter and Widmer, 2013; Nolf et al., 2016; Groot et al., 2017), and our study provides novel evidence for it. Using nine genetically and morphologically diverse genotypes, we found significant genotype × parental treatment interactions both under control conditions and under renewed stress treatments in the offspring generation. Thus, intraspecific variation in environmentally-induced transgenerational responses exists in *A. thaliana*. This genotypic variation among widespread origins suggests evolutionary divergence among populations, which could result from adaptation, genetic drift, or both. We used only a single genotype per population, precluding assessment of within-population variation (or constancy) of responses to experimental treatments. However, the genetic diversity within populations is likely very restricted

(Bomblies et al., 2009) whereas genetic diversity is large among the selected populations (McKhann et al., 2004). Therefore, we are confident that the observed patterns reflect evolutionary divergence among populations.

Relationships with climates of origin and fitness robustness

We found that plasticity in response to heat stress correlated with a range of climate variables from the genotypes' geographic origins, suggesting that environmental variability at sites of origin might be an important selective factor (Endler, 1986) for the evolution not only of within-generation plasticity (Scheepens et al., 2018) but also of transgenerational plasticity. Interestingly, these relationships were only found under stressful conditions in the reciprocal experiment but not under stress-free conditions in the common-environment experiment.

One of the observed plasticity-climate relationships was a negative correlation between plasticity in fecundity and temperature seasonality at sites of origin. The same plasticity was also correlated to two other, closely related, climate variables, isothermality (positive correlation) and annual temperature range (negative correlation). Genotypes from origins with increasing temperature seasonality showed a reduced plasticity in fecundity and thus appear to have evolved a stronger fitness homeostasis in the face of fluctuating temperature conditions, whereas genotypes from origins with more stable temperature regimes evolved to respond more strongly to temperature stress, leading to reduced fitness in our experiments.

We also observed a positive relationship between plasticity in biomass and precipitation seasonality, i.e. plants from unpredictable precipitation environments responded more strongly to temperature stress. Since biomass and fecundity are strongly positively correlated in *A. thaliana* (Clauss and Aarssen, 1994), this plasticity-climate relationship seems to contrast with the above-mentioned negative correlation between plasticity in fecundity and temperature seasonality. However, precipitation seasonality and temperature seasonality are not correlated in the studied plant origins, so these plasticity-environment correlations may reflect independent evolutionary responses to different aspects of climate variability.

The strongest plasticity-environment correlation was between plasticity in aboveground biomass and latitude: plants from higher latitudes responded less to variation in temperature stress. Since increasing latitude is associated with decreasing precipitation seasonality, the latter may be the underlying driver of this relationship. High precipitation seasonality at low latitudes may have selected for strong biomass responses to temperature stress, potentially reflecting escape mechanisms under periods of drought (Franks, 2011).

In the parental experiment (Scheepens et al., 2018) we had previously found positive correlations between plasticity and precipitation variability at sites of origin in four out of five traits. We did not find the same relationship in the offspring generation in the current study, even though transgenerational effects were still present in three out of five traits. One possible explanation for this is that the plant responses in the parental generation were passive and/or maladaptive (cf. fitness robustness), and that transgenerational effects caused the offspring generation to respond less in order to retain fitness. We did find correlations between plasticity in plant architecture, aboveground biomass and fecundity and several other climate variables in the reciprocal experiment, indicating a possible adaptive function of these plant responses, and highlighting the general relevance of studying environmental variability for understanding transgenerational plant responses to temperature stress.

We found negative correlations between fitness robustness and plasticity in aboveground biomass, but not in other traits, in both experiments. This is similar to our results from the parental plants (Scheepens et al., 2018) and implies that more plastic genotypes show stronger fitness variation in response to (parental and/or offspring) treatments. However, the slopes of these relationships are flatter in offspring compared to parental plants, with fitness robustness values of 0.69-0.95 in the common-environment experiment and 0.72-0.97 in the reciprocal experiment, compared to values of 0.50-0.90 in the parental generation (Scheepens et al., 2018). Therefore, the offspring generation, even when under identical stress, shows an overall improved fitness robustness, which may reflect a transgenerational adaptive response to temperature stress.

Conclusions

Given that changes in temporal environmental variability are an important aspect of climate change, it is important to understand their effects on plants, both in terms of phenotypic plastic responses and of intraspecific evolutionary divergence. To our knowledge, no previous study has tested for transgenerational responses of plants to temporal variability of environmental stresses, rather than their mean changes. We found ample genotypic variation in transgenerational responses to temporal variation in heat stress, suggesting that selection can act on it, and plasticity-environment correlations indicate a possible adaptation to the environmental variability of plant origins.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

JFS and OB designed the study. YD and JFS performed the experiment. YD, JFS and OB analyzed the data. YD drafted the manuscript, and JFS and OB helped to improve it.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: [WEBLINK]

REFERENCES

Agrawal, A. A. (2001) Transgenerational consequences of plant responses to herbivory: An adaptive maternal effect? *American Naturalist* 157: 555-569. DOI: 10.1086/319932

Balasubramanian, S., Sureshkumar, S., Lempe, J., and Weigel, D. (2006) Potent induction of *Arabidopsis thaliana* flowering by elevated growth temperature. *PLoS Genetics* 2: 980-989. DOI: 10.1371/journal.pgen.0020106

Barriopedro, D., Fischer, E. M., Luterbacher, J., Trigo, R. M., and García-Herrera, R. (2011) The hot summer of 2010: redrawing the temperature record map of Europe. *Science* 332: 220-224. DOI: 10.1126/science.1201224

Barros, V. R., and Field, C. B. (eds.) (2014) Climate Change 2014 Impacts, Adaptation, and Vulnerability Part A: Global and Sectoral Aspects Working Group II Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Preface. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Bomblies, K., Yant, L., Laitinen, R. A., Kim, S. T., Hollister, J. D., Warthmann, N. et al. (2009) Local-scale patterns of genetic variability, outcrossing, and spatial structure in natural stands of *Arabidopsis thaliana*. *PLoS Genetics* 6: e1000890. DOI: 10.1371/journal.pgen.1000890

Callahan, H. S., and Pigliucci, M. (2002) Shade-induced plasticity and its ecological significance in wild populations of *Arabidopsis Thaliana*. *Ecology* 83: 1965-80. DOI: 10.1890/0012-9658(2002)083[1965:SIPAIE]2.0.CO;2

Clauss, M. J. and Aarssen, L. W. (1994) Phenotypic plasticity of size-fecundity relationships in *Arabidopsis thaliana*. *Journal of Ecology* 82: 447-455. DOI: 10.2307/2261254

Endler, J. A. (1986) *Natural selection in the wild*. Princeton University Press, Princeton, NJ.

Fischer, E. M., and Schär, C. (2008) Future changes in daily summer temperature variability: driving processes and role for temperature extremes. *Climate Dynamics* 33: 917. DOI: 10.1007/s00382-008-0473-8

Franks, S. J. (2011) Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. *New Phytologist* 190: 249-257. DOI: 10.1111/j.1469-8137.2010.03603.x

Galloway, L. F. (2005) Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytologist* 166: 93-99. DOI: 10.1111/j.1469-8137.2004.01314.x

Galloway, L. F., and Etterson, J. R. (2007) Transgenerational plasticity is adaptive in the wild. *Science* 318: 1134-1136. DOI: 10.1126/science.1148766

Gaudet, M., Pietrini, F., Beritognolo, I., Iori, V., Zacchini, M., Massacci, A. et al. (2011) Intraspecific variation of physiological and molecular response to cadmium stress in *Populus nigra* L. *Tree Physiology* 31: 1309-1318. DOI: 10.1093/treephys/tpr088

Ghalambor, C. K., McKay, J. K., Carroll, S. P,. and Reznick, D. N. (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* 21: 394-407. DOI: 10.1111/j.1365-2435.2007.01283.x

Giorgi, F., Bi, X. Q., and Pal, J. (2004) Mean, interannual variability and trends in a regional climate change experiment over Europe. II: climate change scenarios (2071-2100). *Climate Dynamics* 23: 839-858. DOI: 10.1007/s00382-004-0467-0

Groot, M. P., Kubisch, A., Ouborg, N. J., Pagel, J., Schmid, K. J., Vergeer, P. et al. (2017) Transgenerational effects of mild heat in *Arabidopsis thaliana* show strong genotype specificity that is explained by climate at origin. *New Phytologist* 215: 1221-1234. DOI: 10.1111/nph.14642

Herman, J. J., and Sultan, S. E. (2011) Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. *Frontiers in Plant Science* 2: 102. DOI: 10.3389/fpls.2011.00102

Herman, J. J., Sultan, S. E., Horgan-Kobelski, T., and Riggs, C. (2012) Adaptive transgenerational plasticity in an annual plant: grandparental and parental drought stress enhance performance of seedlings in dry soil. *Integrative and Comparative Biology*: 77-88. DOI: 10.1093/icb/ics041

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., and Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965-1978. DOI: 10.1002/joc.1276

Ibañez, C., Poeschl, Y., Peterson, T., Bellstädt, J., Denk, K., Gogol-Döring, A. et al. (2017) Ambient temperature and genotype differentially affect developmental and phenotypic plasticity in *Arabidopsis thaliana*. *BMC Plant Biology* 17:114. DOI: 10.1186/s12870-017-1068-5

Katz, R. W., and Brown, B. G. (1992) Extreme events in a changing climate: Variability is more important than averages. *Climatic Change* 21: 289-302. DOI: 10.1007/BF00139728

Knapp, A. K., Fay, P. A., Blair, J. M., Collins, S. L., Smith, M. D., Carlisle, J. D. et al. (2002) Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* 298: 2202-2205. DOI: 10.1126/science.1076347

Latzel, V., Janecek, S., Dolezal, J., Klimesova, J., and Bossdorf, O. (2014) Adaptive transgenerational plasticity in the perennial *Plantago lanceolata*. *Oikos* 123: 41-46. DOI: 10.1111/j.1600-0706.2013.00537.x

Marais, D. L. D., Lasky, J. R., Verslues, P. E., Chang, T. Z., and Juenger, T. E. (2017) Interactive effects of water limitation and elevated temperature on the physiology, development and fitness of diverse accessions of *Brachypodium distachyon*. *New Phytologist* 214: 132–44. DOI: 10.1111/nph.14316

McKhann, H. I., Camilleri, C., Berard, A., Bataillon, T., David, J. L., Reboud, X. et al. (2004) Nested core collections maximizing genetic diversity in *Arabidopsis thaliana*. *Plant Journal* 38: 193-202. DOI: 10.1111/j.1365-313X.2004.02034.x

Mousseau, T. A., and Fox, C. W. (1998a) The adaptive significance of maternal effects. *Trends in Ecology & Evolution* 13: 403-407. DOI: 10.1016/S0169-5347(98)01472-4

Mousseau, T. A., and Fox, C. W. (eds.) (1998b) *Maternal effects as adaptations*. Oxford University Press, New York.

Mousseau, T. A., Uller, T., Wapstra, E., and Badyaev, A. V. (2009) Evolution of maternal effects: past and present. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364: 1035-1038. DOI: 10.1098/rstb.2008.0303

Nolf, M., Rosani, A., Ganthaler, A., Beikircher, B., and Mayr, S. (2016) Herb hydraulics: inter- and intraspecific variation in three *Ranunculus* species. *Plant Physiology* 170: 2085-2094. DOI: 10.1104/pp.15.01664

Nover, L., Bharti, K., Doring, P., Mishra, S. K., Ganguli, A., and Scharf, K. D. (2001) *Arabidopsis* and the heat stress transcription factor world: how many heat stress transcription factors do we need? *Cell Stress & Chaperones* 6: 177-189. DOI: 10.1379/1466-1268(2001)006<0177:aathst>2.0.co;2

Parepa, M., Fischer, M., and Bossdorf, O. (2013) Environmental variability promotes plant invasion. *Nature Communications* 4: 1604. DOI: 10.1038/ncomms2632

Pigliucci, M. (2001) *Phenotypic plasticity: beyond nature and nurture.* Johns Hopkins University Press, Baltimore.

Prasad, P. V. V., Craufurd, P. Q., and Summerfield, R. J. (1999) Sensitivity of peanut to timing of heat stress during reproductive development. *Crop Science* 39: 1352-1357. DOI: 10.2135/cropsci1999.3951352x

Rasmann, S., De Vos, M., Casteel, C. L., Tian, D., Halitschke, R., Sun, J. Y. et al. (2012) Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant Physiology* 158: 854–863. DOI: 10.1104/pp.111.187831

Reyer, C. P. O., Leuzinger, S., Rammig, A., Wolf, A., Bartholomeus, R. P., Bonfante, A. et al. (2013) A plant's perspective of extremes: terrestrial plant responses to changing climatic variability. *Global Change Biology* 19: 75-89. DOI: 10.1111/gcb.12023

Roach, D. A., and Wulff, R. D. (1987) Maternal effects in plants. *Annual Review of Ecology and Systematics* 18: 209-235. DOI: 10.1146/annurev.es.18.110187.001233

Schär, C., Vidale, P. L., Luthi, D., Frei, C., Haberli, C., Liniger, M. A. et al. (2004) The role of increasing temperature variability in European summer heatwaves. *Nature* 427: 332-336. DOI: 10.1038/nature02300

Scheepens, J. F., Deng, Y., and Bossdorf, O. (2018) Phenotypic plasticity in response to temperature fluctuations is genetically variable, and relates to climatic variability of origin, in *Arabidopsis thaliana*. *AoB Plants* 10: ply043. DOI: 10.1093/aobpla/ply043

Schwinning, S., Sala, O. E., Loik, M. E., and Ehleringer, J. R. (2004) Thresholds, memory, and seasonality: understanding pulse dynamics in arid/semi-arid ecosystems. *Oecologia* 141: 191-193. DOI: 10.1007/s00442-004-1683-3

Shea, K., Roxburgh, S. H., and Rauschert, E. S. J. (2004) Moving from pattern to process: coexistence mechanisms under intermediate disturbance regimes. *Ecology Letters* 7: 491-508. DOI: 10.1111/j.1461-0248.2004.00600.x

Stone, P. J., and Nicolas, M. E. (1995) Effect of timing of heat stress during grain filling on two wheat varieties differing in heat tolerance. 1. Grain growth. *Australian Journal of Plant Physiology* 22: 927-934. DOI: 10.1071/PP9950927

Stone, P. J., and Nicolas, M. E. (1996) Effect of timing of heat stress during grain filling on two wheat varieties differing in heat tolerance. 2. Fractional protein accumulation. *Australian Journal of Plant Physiology* 23: 739-749. DOI: 10.1071/PP9960739

Sultan, S. E. (1987) Evolutionary implications of phenotypic plasticity in plants. *Evolutionary Biology* 21: 127-178. DOI: 10.1007/978-1-4615-6986-2_7

Sultan, S. E., Wilczek, A. M., Bell, D. L., and Hand, G. (1998) Physiological response to complex environments in annual *Polygonum* species of contrasting ecological breadth. *Oecologia* 115: 564-78. DOI: 10.1007/s004420050554

Sultan, S. E. (2000) Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science* 5: 537-542. DOI: 10.1016/S1360-1385(00)01797-0

Sultan, S. E., Barton, K., and Wilczek, A. M. (2009) Contrasting patterns of transgenerational plasticity in ecologically distinct congeners. *Ecology* 90: 1831-1839. DOI: 10.1890/08-1064.1

Sung, D. Y., Kaplan, F., Lee, K. J., and Guy, C. L. (2003) Acquired tolerance to temperature extremes. *Trends in Plant Science* 8: 179-187. DOI: 10.1016/S1360-1385(03)00047-5

Suter, L., and Widmer, A. (2013) Environmental heat and salt stress induce transgenerational phenotypic changes in *Arabidopsis thaliana*. *PLoS ONE* 8: e60364. DOI: 10.1371/journal.pone.0060364

Swindell, W. R., Huebner, M., and Weber, A. P. (2007) Transcriptional profiling of *Arabidopsis* heat shock proteins and transcription factors reveals extensive overlap between heat and non-heat stress response pathways. *Bmc Genomics* 8: 125. DOI: 10.1186/1471-2164-8-125

Uller, T. (2008) Developmental plasticity and the evolution of parental effects. *Trends in Ecology and Evolution* 23: 432-438. DOI: 10.1016/j.tree.2008.04.005

Valladares, F., Sanchez-Gomez, D., and Zavala, M. A. (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology* 94: 1103-1116. DOI: 10.1111/j.1365-2745.2006.01176.x

Van Kleunen, M., and Fischer, M. (2005) Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist* 166: 48-60. DOI: 10.1111/j.1469-8137.2004.01296.x

Wahid, A., Gelani, S., Ashraf, M., and Foolad, M. R. (2007) Heat tolerance in plants: An overview. *Environmental and Experimental Botany* 61: 199-223. DOI: 10.1016/j.envexpbot.2007.05.011

Walter, J., Jentsch, A., Beierkuhnlein, C., and Kreyling, J. (2009) Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. *Environmental and Experimental Botany* 94: 3-8. DOI: 10.1016/j.envexpbot.2012.02.009

Wang, D., Heckathorn, S. A., Mainali, K., and Tripathee, R. (2016) Timing effects of heat-stress on plant ecophysiological characteristics and growth. *Frontiers in Plant Science* 7: 1629. DOI: 10.3389/fpls.2016.01629

Whittle, C. A., Otto, S. P., Johnston, M. O., and Krochko, J. E. (2009) Adaptive epigenetic memory of ancestral temperature regime in *Arabidopsis thaliana*. *Botany* 87: 650-657. DOI: 10.1139/B09-030

Conflict of interest:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Table 1. *Arabidopsis thaliana* genotypes used in this study, and their geographical coordinates and natural growing season (in months; from Scheepens et al. 2018).

Name	Country	Latitude	Longitude	Growing season
Bur-0	Ireland	54.1	-6.2	5-8
Can-0	Spain	29.21	-13.48	11-2
Ct-1	Italy	37.51	15.09	12-3
JEA	France	43.68	7.33	3-6
Mt-0	Libya	32.34	22.46	11-2
N13	Russia	61.36	34.15	6-9
Oy-0	Norway	60.39	6.19	5-8
Sha	Tajikistan	38.59	68.79	2-5
St-0	Sweden	59.34	18.06	5-8

Table 2. Results of the common-environment experiment, testing the effects of leaf sampling, parental stress timing, parental stress frequency, genotype, and their interactions, on the flowering time, plant architecture, aboveground biomass, reproductive allocation and fecundity of *Arabidopsis thaliana* offspring. Significant effects ($P < 0.05$) are in bold; df = degrees of freedom.

		Flowering time		**Plant architecture**		**Aboveground biomass**		**Reproductive allocation**		**Fecundity**	
	df	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value
Leaf sampling	1	1.03	0.311	1.41	0.236	52.88	**<0.001**	20.54	**<0.001**	32.43	**<0.001**
Parental timing (PT)	2	0.85	0.429	5.96	**0.003**	0.25	0.777	1.35	0.261	1.33	0.267
Parental frequency (PF)	1	0.95	0.331	2.82	0.094	0.33	0.567	0.25	0.615	1.06	0.305
PT × PF	2	5.92	**0.003**	0.12	0.891	0.19	0.831	0.55	0.577	0.16	0.852

Genotype (G)	8	260.23	**<0.001**	99.12	**<0.001**	35.65	**<0.001**	174.37	**<0.001**	79.23	**<0.001**
G × PT	16	2.19	**0.006**	2.15	**0.007**	1.30	0.193	1.29	0.202	1.19	0.275
G × PF	8	0.40	0.920	0.54	0.829	1.22	0.287	0.88	0.536	1.30	0.242
G × PT × PF	16	0.97	0.494	1.01	0.441	1.99	**0.013**	1.47	0.109	1.10	0.353

Table 3. Results of the reciprocal experiment, testing the effects of leaf sampling, parental stress timing, offspring stress timing, genotype, and their interactions, on the flowering time, plant architecture, aboveground biomass, reproductive allocation and fecundity of *Arabidopsis thaliana* offspring. Significant effects ($P < 0.05$) are in bold; df = degrees of freedom.

		Flowering time		**Plant architecture**		**Aboveground biomass**		**Reproductive allocation**		**Fecundity**	
	df	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value
Leaf sampling	1	0.00	0.960	0.14	0.707	18.38	**<0.001**	7.90	**0.005**	11.88	**0.001**
Parental timing (PT)	1	9.92	**0.002**	0.00	0.970	0.21	0.651	0.14	0.708	2.07	0.152
Offspring timing (OT)	1	0.76	0.385	8.08	**0.005**	41.77	**<0.001**	114.43	**<0.001**	17.48	**<0.001**
PT × OT	1	4.74	**0.030**	0.01	0.914	0.23	0.630	0.84	0.360	0.21	0.643

Genotype (G)	8	184.29	**<0.001**	14.67	**<0.001**	12.13	**<0.001**	158.91	**<0.001**	57.10	**<0.001**
G × PT	8	3.50	**0.001**	0.50	0.856	0.90	0.517	1.17	0.317	0.86	0.549
G × OT	8	2.07	**0.039**	2.91	**0.004**	5.49	**<0.001**	1.59	0.128	3.97	**<0.001**
G × PT × OT	8	1.82	0.074	0.37	0.937	0.43	0.905	1.28	0.253	2.39	**0.017**

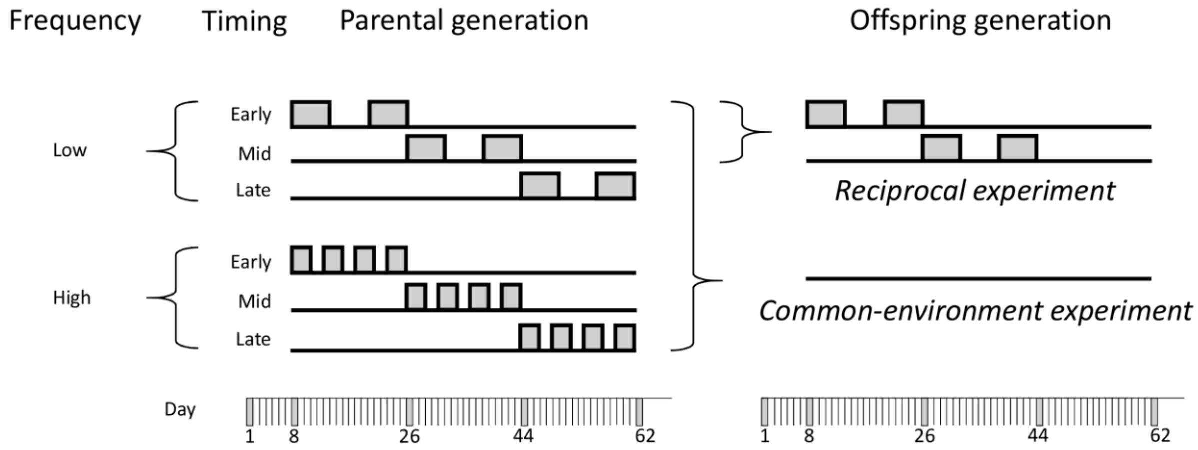


Figure 1. Experimental design of the parental-generation experiment (left) and the two offspring experiments (right) with *Arabidopsis thaliana*, with periods of 30 °C heat stress indicated in grey. In the offspring generation, plants from all parental treatments are grown in a constant control environment (common-environment experiment), and plants from two parental stress treatments are subjected to the same two treatments again (reciprocal experiment).

Figure 2. Effects of parental stress timing on plant architecture (number of lateral shoots / number of basal shoots) of *Arabidopsis thaliana* in the common-environment experiment.

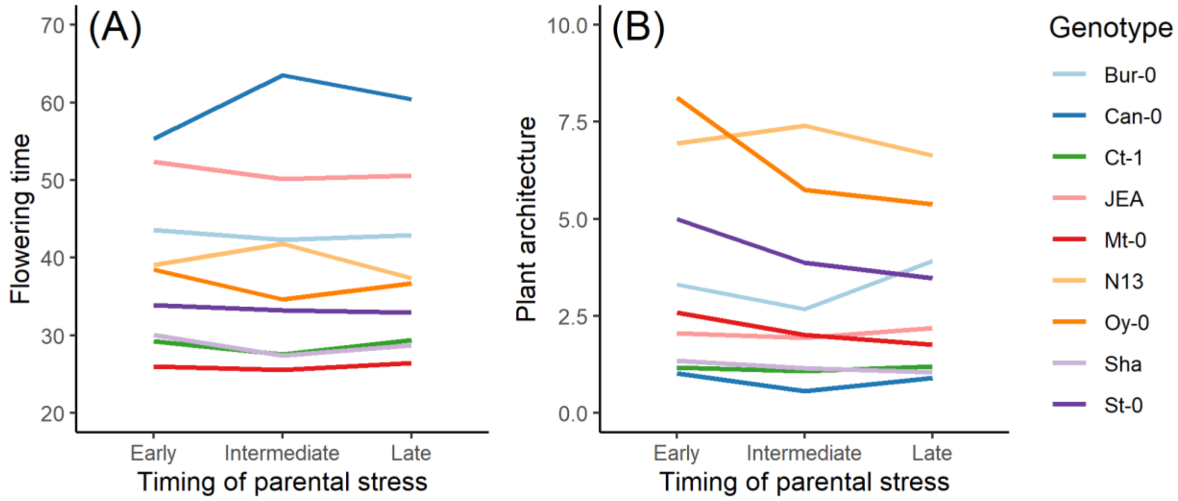


Figure 3. Genotypic variation in the transgenerational responses of flowering time (A) and plant architecture (number of lateral shoots / number of basal shoots; B) of nine *Arabidopsis thaliana* genotypes to different timing of parental heat stress in the common-environment experiment.

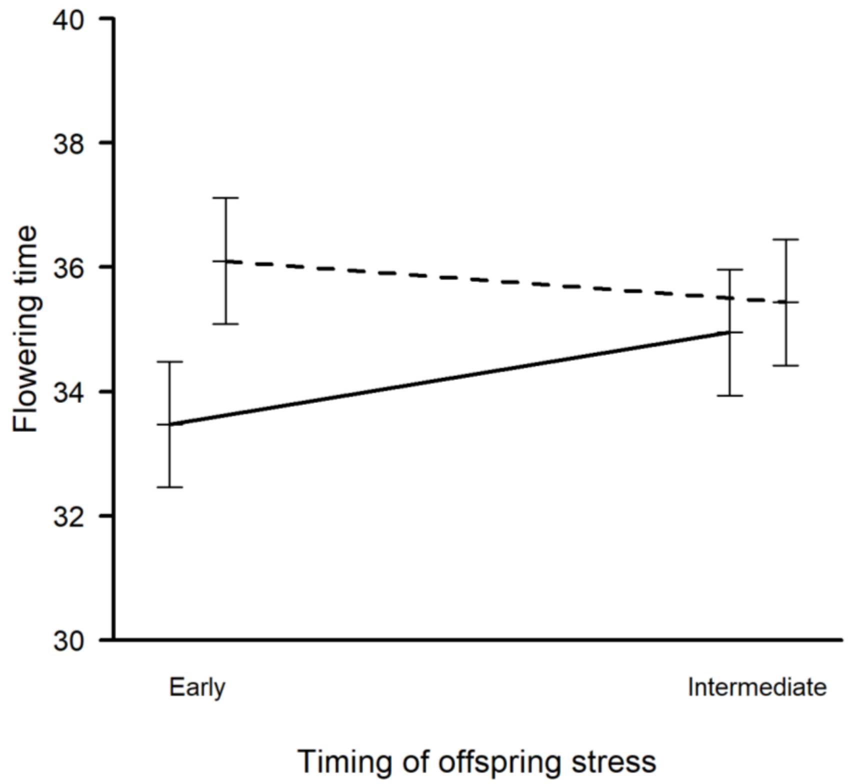


Figure 4. Effects of parental and offspring heat stress timing on flowering time in *Arabidopsis thaliana* in the reciprocal experiment. Solid line – early parental stress; dashed line – intermediate parental stress.

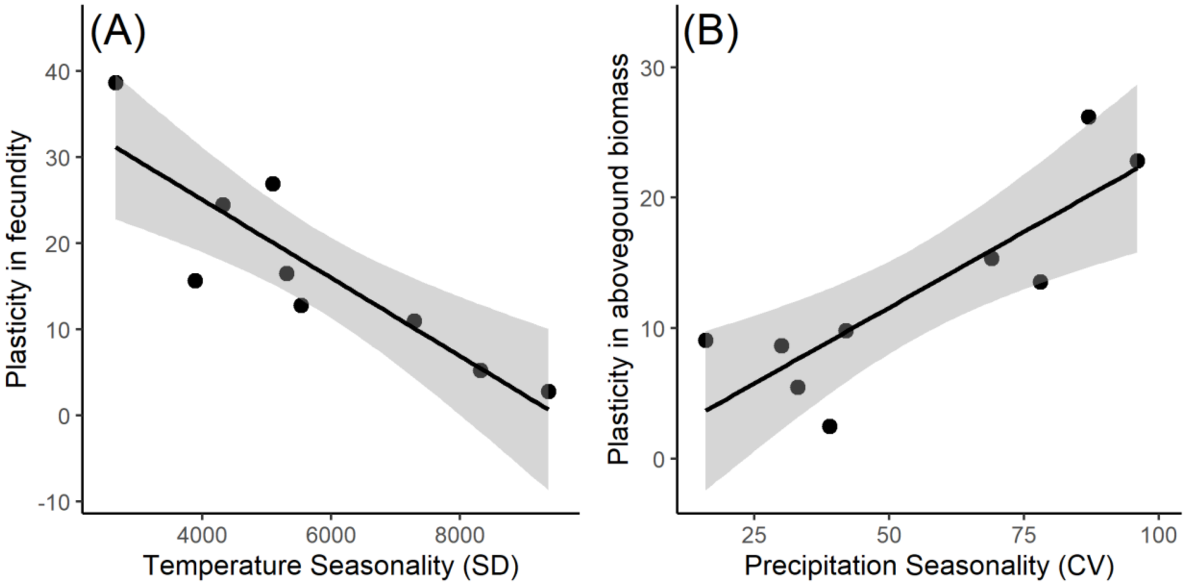


Figure 5. Relationships between trait plasticities and climates of origins for nine *Arabidopsis thaliana* genotypes in the reciprocal experiment. (A) Correlation between temperature seasonality (SD) and plasticity in fecundity. (B) Correlation between precipitation seasonality (CV) and plasticity in aboveground biomass. The plasticity values are coefficients of variation across experimental treatments. The grey areas indicate the 90% confidence intervals of the correlations.

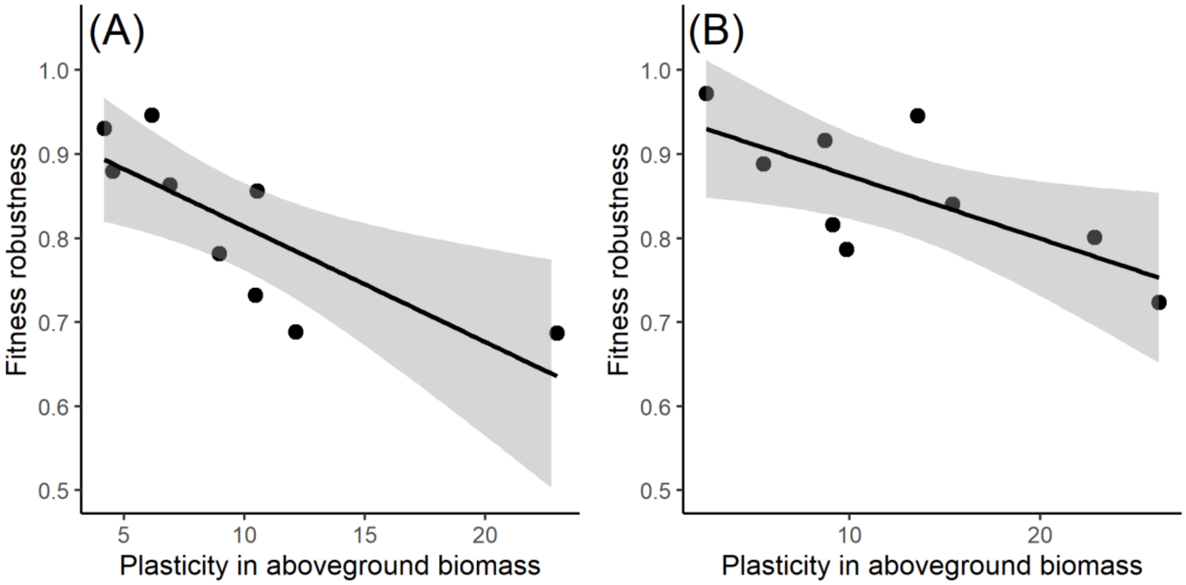


Figure 6. Relationships between fitness robustness across environments and plasticity in aboveground biomass for nine genotypes of *Arabidopsis thaliana* in the common-environment experiment (A) and in the reciprocal experiment (B). The grey areas indicate the 90% confidence intervals of the correlations.

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Correlations between climates of origin and phenotypic plasticity across nine *Arabidopsis thaliana* genotypes in the common-environment experiment. The *R*-values are Pearson correlation coefficients.

Supplementary Table S2. Correlations between climates of origin and phenotypic plasticity across nine *Arabidopsis thaliana* genotypes in the reciprocal experiment. The *R*-values are Pearson correlation coefficients. Significant correlations ($P < 0.05$) are in bold.

Supplementary Table S3. Correlations between trait plasticities (CV across all treatments) and fitness robustness across nine *Arabidopsis thaliana* genotypes. The *R*-values are Pearson correlation coefficients.

Supplementary Figure S1. The effects of timing and frequency of parental heat stress on the flowering time of *Arabidopsis thaliana* in the common-environment experiment.

Supplementary Figure S2. Genotypic variation in the effects of parental and offspring heat stress timing on fecundity in nine *Arabidopsis thaliana* genotypes in the reciprocal experiment. Solid line: early parental stress, dashed line: intermediate parental stress.

Supplementary Table S1. Correlations between climates of origin and phenotypic plasticity across nine *Arabidopsis thaliana* genotypes in the common-environment experiment. The *R*-values are Pearson correlation coefficients.

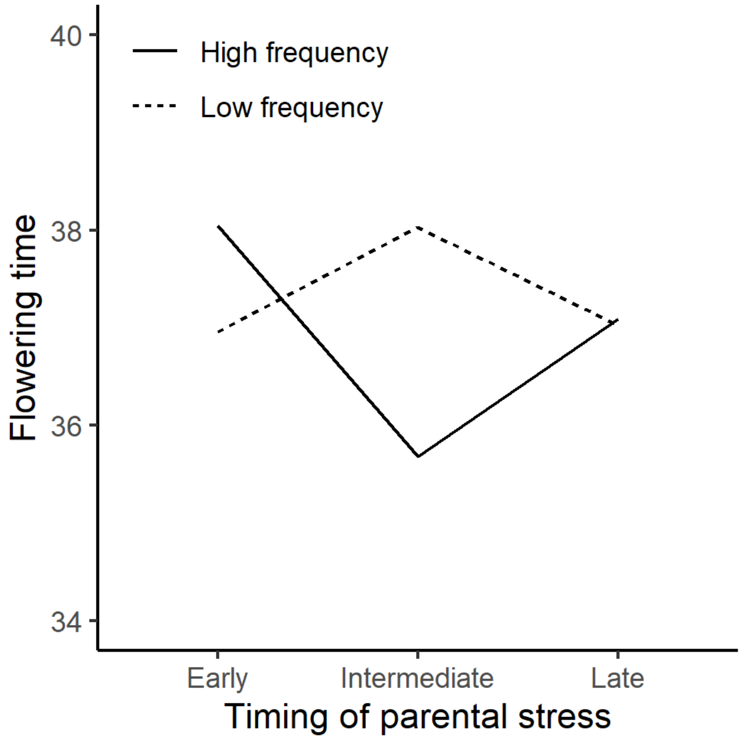
Climate variables	**Flowering time**		**Plant architecture**		**Aboveground biomass**	
	R	*P*-value	*R*	*P*-value	*R*	*P*-value
Growing season-based						
Temperature SD	-0.08	0.845	0.48	0.188	-0.12	0.755
Precipitation CV	0.37	0.333	0.00	0.990	-0.51	0.159
Evapotranspiration CV	0.54	0.133	0.15	0.708	0.45	0.222
Climatological Water Deficit CV	-0.19	0.624	-0.31	0.414	-0.02	0.966
Year-based						
Annual Mean Diurnal Range	-0.14	0.711	0.27	0.481	-0.56	0.113
Isothermality	0.25	0.511	0.37	0.322	-0.50	0.166
Temperature Seasonality (SD)	-0.26	0.492	-0.31	0.412	0.17	0.655
Annual Temperature Range	-0.22	0.567	-0.20	0.615	-0.01	0.983
Precipitation Seasonality (CV)	0.19	0.630	0.21	0.588	-0.39	0.300
Latitude	-0.28	0.470	-0.42	0.266	0.43	0.250

Supplementary Table S2. Correlations between climates of origin and phenotypic plasticity across nine *Arabidopsis thaliana* genotypes in the reciprocal experiment. The *R*-values are Pearson correlation coefficients. Significant correlations ($P < 0.05$) are in bold.

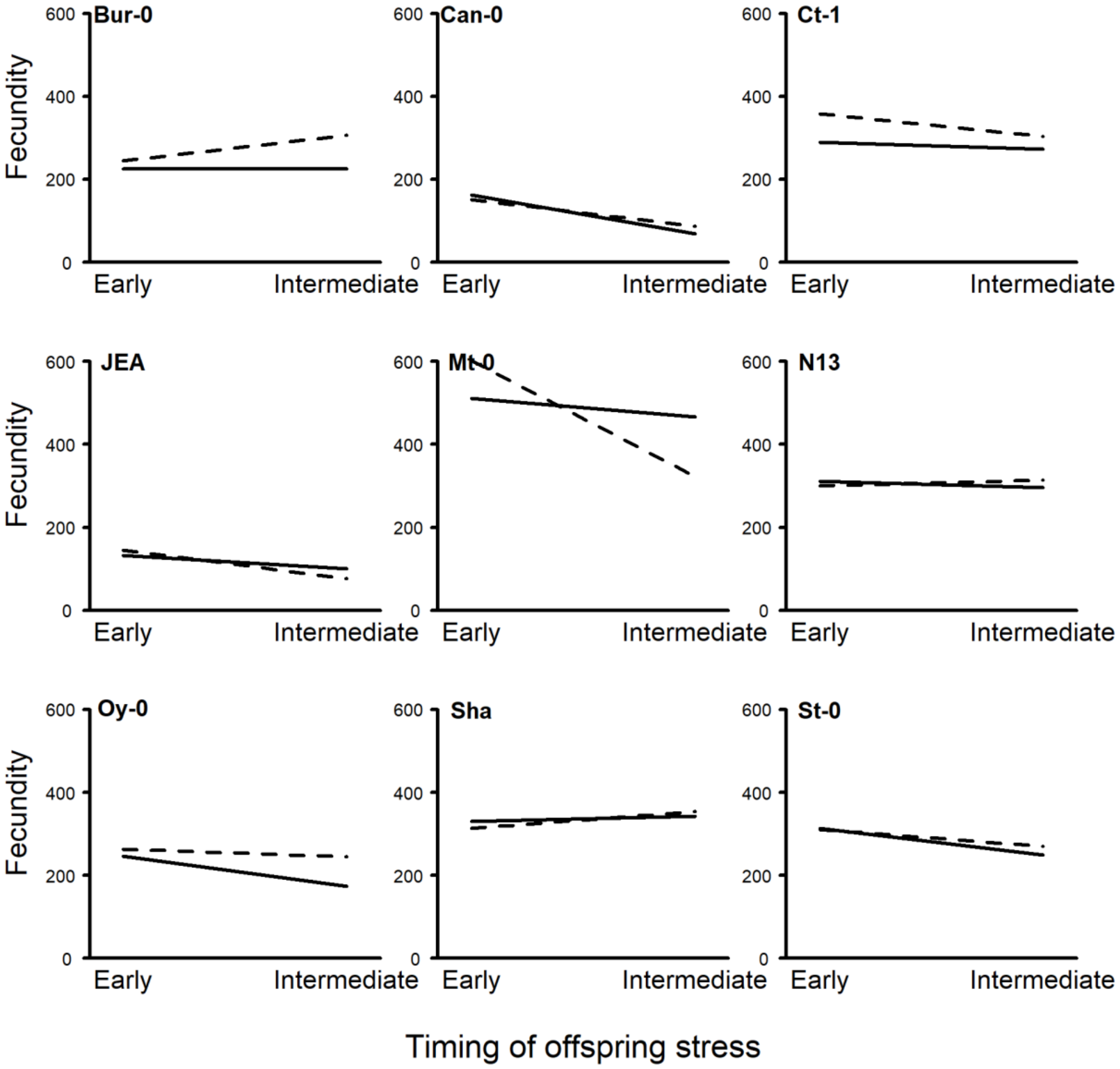
Climate variables	**Flowering time**		**Plant architecture**		**Aboveground biomass**		**Fecundity**	
	R	*P*-value	*R*	*P*-value	*R*	*P*-value	*R*	*P*-value
Growing season-based								
Temperature SD	0.20	0.605	-0.17	0.670	-0.20	0.609	-0.38	0.312
Precipitation CV	0.10	0.795	0.13	0.745	-0.01	0.974	0.13	0.747
Evapotranspiration CV	-0.19	0.629	0.50	0.175	0.47	0.200	0.73	**0.025**
Climatological Water Deficit CV	-0.14	0.713	0.27	0.475	-0.84	**0.005**	-0.58	0.099
Year-based								
Annual Mean Diurnal Range	0.21	0.589	-0.73	**0.027**	0.32	0.401	-0.24	0.526
Isothermality	0.45	0.225	-0.21	0.593	0.86	**0.003**	0.74	**0.022**
Temperature Seasonality (SD)	-0.37	0.325	-0.32	0.404	-0.63	0.066	-0.87	**0.002**
Annual Temperature Range	-0.28	0.469	-0.49	0.183	-0.45	0.222	-0.82	**0.007**
Precipitation Seasonality (CV)	0.05	0.889	-0.47	0.200	0.85	**0.004**	0.38	0.312
Latitude	-0.30	0.428	0.41	0.278	-0.92	**<0.001**	-0.58	0.104

Supplementary Table S3. Correlations between trait plasticities (CV across all treatments) and fitness robustness across nine *Arabidopsis thaliana* genotypes. The *R*-values are Pearson correlation coefficients.

	Flowering time		**Plant architecture**		**Aboveground biomass**	
	R	*P*-value	*R*	*P*-value	*R*	*P*-value
Common-environment experiment	-0.31	0.422	0.11	0.785	-0.79	**0.012**
Reciprocal experiment	-0.47	0.200	-0.15	0.702	-0.71	**0.031**



Supplementary Figure S1. The effects of timing and frequency of parental heat stress on the flowering time of *Arabidopsis thaliana* in the common-environment experiment.



Supplementary Figure S2. Genotypic variation in the effects of parental and offspring heat stress timing on fecundity in nine *Arabidopsis thaliana* genotypes in the reciprocal experiment. Solid line: early parental stress, dashed line: intermediate parental stress.