Developmental plasticity of *Arabidopsis thaliana* accessions across an ambient temperature range

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Abstract

The global increase in ambient temperature constitutes a significant challenge to wild and cultivated plant species. Yet, a comprehensive knowledge on morphological responses and molecular mechanisms involved is scarce. Studies published to date have largely focused on a few, isolated temperature-relevant phenotypes such as flowering time or hypocotyl elongation. To systematically describe thermomorphogenesis, we profiled more than 30 phenotypic traits throughout an entire life cycle in ten distinct accessions of *Arabidopsis thaliana* grown in four different ambient temperatures. We observed a uniform acceleration of developmental timing in the vegetative growth phase with a low contribution of genotype effects on variation indicating a passive effect of temperature. In contrast, reproduction-associated phenotypes and several quantitative growth traits were sensitive to both, genotype and temperature effects or could be attributed primarily to either factor. Therefore, the results argue against a general mechanism of passive temperature effects by thermodynamic processes. Temperature responses of several phenotypes rather implicate differential function of specific signaling components that might be targets of adaptation to specific environmental conditions.

Keyword index

ambient temperature, thermomorphogenesis, natural variation, phenotypic plasticity

Introduction

Recurrent changes in ambient temperature provide plants with essential information about time of day and seasons. Yet, even small changes in mean ambient temperature can profoundly affect plant growth and development which collectively can be summarized as thermomorphogenesis. In crops like rice, a season-specific increase in the mean minimum temperature of 1°C results in approximately a 10% reduction in grain yield (Peng et al., 2004). Similarly, up to 10% of the yield stagnation of wheat and barley in Europe over the past two decades can be attributed to climate trends (Moore and Lobell, 2015). Current projections indicate that mean global air temperatures will increase up to 4.8 °C by the end of the century (IPCC; Lobell and Gourdji, 2012). Global climate change will thus have significant implications on biodiversity and future food security.

Naturally, increased ambient temperatures also affect wild species and natural habitats. Long-term phenology studies of diverse plant populations have revealed an advance in first and peak flowering and alterations in the total length of flowering times (CaraDonna et al., 2014; Fitter and Fitter, 2002). Furthermore, estimates project that temperature effects alone will account for the extinction of up to one-third of all European plant species (Thuiller et al., 2005). As the impact of changes in ambient temperature on crop plants and natural habitats emerge, a comprehensive understanding of thermomorphogenesis and developmental temperature responses becomes paramount.

Our present knowledge on molecular responses to ambient temperature signaling has largely been gained from studies in *Arabidopsis thaliana.* Model thermomorphogenesis phenotypes such as hypocotyl elongation (Gray et al., 1998), hyponastic leaf movement (van Zanten et al., 2009), and alterations in flowering time have served in forward or reverse genetic approaches to identify some of the molecular signal transduction components involved in triggering

thermomorphogenic responses. So far, the main molecular players identified seem to function in response to both temperature and light stimuli and form a highly interconnected network of signaling elements. Prominent members of this network are PHYTOCHROME INTERACTING FACTOR 4 (PIF4, Franklin et al., 2011; Koini et al., 2009; Proveniers and van Zanten, 2013), the DE-ETIOLATED1-CONSTITUTIVELY PHOTOMORPHOGENIC1-ELONGATED HYPOCOTYL 5 (DET1-COP1-HY5) cascade (Delker et al., 2014; Toledo-Ortiz et al., 2014) and EARLY FLOWERING 3 (ELF3) as a component of the circadian clock (Box et al., 2015; Raschke et al., 2015). In addition, considerable naturally occurring variation in thermomophogenic traits like hypocotyl elongation and flowering time has been demonstrated (Balasubramanian et al., 2006; Delker et al., 2010). This variation might be attributed to local adaptation processes of diverse *A. thaliana* accessions and indicates a high variability regarding temperature-induced phenotypic plasticity.

The use of thermomophogenic model phenotypes has undoubtedly been useful for the identification of several molecular signaling components. Meeting future challenges in plant breeding will, however, require more extensive knowledge about temperature effects on plant development and morphology beyond commonly described traits. As such, it would be vital to determine (i) which phenotypes are sensitive to ambient temperature effects, (ii) which of these traits are robustly affected by temperature within a gene pool, and (iii) which phenotypic traits show natural variation in temperature responses and thus might be consequences of adaptation processes to cope with local climate or general environmental conditions. Robustly affected temperature response might indicate passive consequences of general thermodynamic effects. According to basic principles of thermodynamics, temperature-induced changes in free energy will affect the rates of biological reactions. As these effects should occur more

generally and non-selective, phenotypic responses can be expected to occur robustly and rather independently of genetic variation. However, natural variation in thermomorphogenesis could implicate the relevance of specific signaling elements showing natural genetic variation as a consequence of adaptation. Such genes would represent attractive candidates for targeted breeding approaches.

Here, we aim to address these questions by profiling of more than 30 developmental and morphological traits of ten *A. thaliana* accessions which were grown at 16, 20, 24, and 28°C. In addition, we provide accession-specific developmental reference maps of temperature responses that can serve as resources for future experimental approaches in the analysis of ambient temperature responses in *A. thaliana*.

Materials and methods

Plant material and growth conditions

A. thaliana accessions were obtained from the Nottingham Arabidopsis Stock Centre (Scholl et al., 2000). Detailed information on stock numbers and geographic origin are listed in Supplementary Tab. S1. For seedling stage analyses, surface-sterilized seeds were stratified for 3 days in deionized water at 4°C and subsequently placed on *A. thaliana* solution (ATS) nutrient medium (Lincoln et al., 1990). Seeds were germinated and cultivated in growth chambers (Percival) at constant temperatures of 16, 20, 24 or 28°C under long day photoperiods (16h light/8h dark) and a fluence rate of 90 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. We refrained from including a vernalization step because the primary focus of this study was to record morphology and development in response to different constant ambient temperature conditions.

Germination rates were assessed daily and hypocotyl, root length, and petiole angles were measured in 7 days old seedlings with ImageJ (http://imagej.nih.gov/ij/) and Root Detection (http://www.labutils.de/rd.html).

All other analyses were performed on soil-grown plants at a fluence rate of 140 µmol·m^{-2}·sec^{-1}. After imbibition for 3 days at 4°C, seeds were grown in individual 5 x 5 cm pots, which were randomized twice a week to minimize position effects. Relative humidity of growth chambers was maintained at 70% and plants were watered by subirrigation. Plants were photographed daily for subsequent determination of phenotypic parameters using Image J (http://imagej.nih.gov/ij/). Determination of developmental progression largely followed the stages defined in Boyes et al., (2001). At transition to the reproductive growth phase, the number of leaves was determined by manual counting in addition to recording the days after germination.

Spectrophotometric determination of chlorophyll content was performed as described in Porra et al., (1989). Rates of germination and seedling establishment were determined from ~100 individual seeds. Two different seed pools were generated by proportional merging of four different seed batches from individuals from one accession (1:1:1:1). Both sample pools were used in the actual experiments. Sterilized and stratified seeds were germinated on ATS medium without sucrose. Germination was determined in the first three days and seedling establishment data was recorded at day six. Morphological markers for germination and seedling establishment are described in Table1. Data were recorded from three independent germination experiments of which one representative set is shown.

Data analysis

Data visualization and statistical analyses of the data were performed using the software R (Team R Core, 2012). For visualization of the data set, box plots were generated using the *boxplot* function contained in the graphics package. For visualization of the statistical measures, heat maps were generated using the *heatmap.2* function contained in the gplots package, which is available on http://cran.r-project.org.

ANOVA for single factors

ANOVAs for a single factor (either accession or temperature) were done using the *anova* function contained in the R stats package. In case of temperature, the factor had four levels. In case of accession, the factor had ten levels. As post hoc test Tukey's 'Honest Significant Difference' test was used to determine the pairs of factor levels that are significantly different. To perform this test, the function *TukeyHSD* contained in the stats package was used.

Calculation of intraclass correlation coefficients λ

In order to quantify the distinct influences of genotype and temperature on a given phenotype, we determined intraclass correlation coefficients λ_{gen} and λ_{temp} using the ANOVA framework similar to (Donner and Koval, 1980). This involved the calculation of sum of squared differences *SSD* values, which are defined for a set of data points $M=\{x_1, x_2, \ldots, x_m\}$ as

$SSD(M)=\sum_{i=1}^{m}(x_i-\bar{x})^2$, where $\acute{x}=\frac{1}{m}\sum_{i=1}^{m}x_i$ is the mean of all values in *M*. In the case of λ_{temp} we split all data points *M* corresponding to a given phenotype and genotype into four groups M_{16}, M_{20}, M_{24}, and M_{28} according to the temperatures. The total variation of the data

given by the $SSD_{total}=SSD(M)$ is the composition of two components, namely the variation between the groups $SSD_{between}$ representing the effect of the temperature, and the variation inside of the groups SSD_{within} representing the accession-specific biological variability. The latter component can be calculated by adding up the *SSD* values computed separately for each groups, i.e., $SSD_{within}=SSD(M_{16}) + SSD(M_{20}) + SSD(M_{24}) + SSD(M_{28})$, while the former is given by $SSD_{between}= SSD_{total} - SSD_{within}$. We defined the value λ_{temp} to be the fraction of variation due to the temperature, i.e., $\lambda_{temp} = SSD_{between}/SSD_{total}$. Accordingly, the fraction of variation due to the genotype λ_{gen} was calculated by splitting the set of data points *M* corresponding to a given phenotype and temperature into ten groups according to the accessions.

Regression analysis

Linear regression analyses were conducted using the *lm* function contained in the stats package to get a trend of the temperature effect. The slope of the resulting regression line was used to determine the direction (and strength) of the effect caused by temperature (for a specific phenotype).

Results

To assess phenotypic plasticity in a range of ambient temperatures, *A. thaliana* plants were cultivated throughout an entire life cycle at four different temperatures (16, 20, 24 and 28 °C) under otherwise similar growth conditions (see Materials and methods for further details). More than 30 morphological and development-associated traits were recorded in the vegetative and reproductive growth phases (Tab. 1).

Temperature responses in the A. thaliana reference accession Col-0

In Col-0, almost all phenotypes analyzed in this study were affected by the cultivation in different ambient temperatures. Only seed weight and maximum height remained constant regardless of the growth temperature (Fig. 1A, Supplementary Fig. S1). Among the temperature sensitive traits were several growth-associated phenotypes in early vegetative stages. Primary root length, hypocotyl and petiole elongation all increased with elevated temperatures which concurs with previously published results (Gray et al., 1998; Zanten et al., 2009). As a further example, yield-related traits, such as the number of siliques per plant and the number of seeds per silique decreased with an increase in ambient temperature (Fig. 1A).

As reported previously, Col-0 plants showed a decrease in developmental time until flowering with increasing ambient temperatures (Balasubramanian et al., 2006). The transition from vegetative to reproductive phase occurred about 25 days earlier at 28°C than at 16°C (Fig. 1B). Similarly, the number of rosette leaves developed at time of bolting differed by 26 leaves between 28°C and 16 °C (Fig. 1A).

The fact that only a very limited number of phenotypes was insensitive to cultivation in different temperatures clearly illustrates the fundamental impact of ambient temperature on plant growth and development.

Natural variation of temperature responses

To assess whether the observed temperature responses in Col-0 are robust throughout the *A. thaliana* population or which of the responses are affected by natural variation, phenotypic profiling was performed in nine other *A. thaliana* accessions parallel to the analysis in Col-0 (Supplementary Tab. S1, Fig.S1-10). Although a panel of ten accession does of course not represent the world-wide *A. thaliana* gene pool in its entity, it is certainly sufficient to address the aim of this study, i.e. to identify and distinguish between traits that may be targets for

adaptation and those that are genetically fixed.

To approximate and to compare temperature sensitivity of traits among different accessions, we transformed individual trait values into temperature responses by linear regression of values across all four ambient temperature regimes (Fig. 2A). The slope values were then normalized to the respective trait median of all temperatures combined to allow comparison and cluster analysis of phenotypes with different dimensions of units (Fig. 2A).

Fig. 2B shows that hierarchical clustering of temperature responses (slopes) clearly separated seedling growth traits and chlorophyll content from all other phenotypes due to the strong increase of trait values with increasing temperatures. An additional cluster was constituted by phenotypes associated with the transition to reproductive development. Here, most of the accessions showed a temperature-induced reduction in time/development to flowering as indicated by negative slope values. However, in accordance with previously published results on natural variation of temperature-induced flowering (Balasubramanian et al., 2006) the strength of the response differed. Most striking in this respect was the temperature response of Rrs-7 and Got-7. In contrast to the other accessions, they showed a delay in flowering time with increasing temperature (Fig. 2B). Got-7 did not flower within the first 90 days of cultivation when grown in 24 or 28°C likely caused by the lack of vernalization (Supplementary Fig. S5). Thus, initiated leaf senescence at bolting stage prevented accurate determination of leaf number at the onset of flowering.

A third cluster is formed by traits associated with the timing of vegetative development. Negative slope values for germination and induction of rosette leaves indicate accelerated development in response to higher temperatures, which was uniformly observed in all analyzed accessions.

A direct comparison of leaf number and time of development corroborates a sudden increase

in variation at the transition to flowering. However, at 16°C and 20°C several accessions contribute to the overall variability in the graph, whereas at 24°C and 28°C, C24 and Rrs-7 are the main determinants of variation due to their massive number of leaves corresponding to an extension of the vegetative growth phase (Supplementary Fig. S11). This finding harbors several interesting aspects. First, natural variation in the transition to flowering is already observed at lower temperatures. As the flowering time differences of Rrs-7 and Got-7 (Fig. 2B) become pronounced primarily at temperatures above 24°C, the general variation in flowering time seems to be largely, independent of vernalization requirements. Furthermore, C24 contributes considerably to the variability of the reproductive traits, even though the general C24 temperature response follows the common pattern of earlier transition to flowering at higher temperatures (Fig.2B, Supplementary Fig. S3).

To further substantiate this analysis and to identify specific traits with adaptive potential, we aimed to dissect and quantify the individual effects of temperature and genotype on the observed variability of each trait/phenotype in the following.

Genotype contributions to phenotypic variation

For genotype effects, we compared the variation that occurs within each individual accession and compared it to the total variation occurring among all accessions for each phenotypic trait at each given temperature. As a measure for variability we made use of the sum of squared differences (SSD). While the SSD_{within} represents the biological variation within an individual accession (e.g. Ler-1 or Got-7, Fig.3A), $SSD_{between}$ describes the range of variability that is observed among the mean values across the ten analyzed accessions. Values of SSD_{within} and $SSD_{between}$ were subsequently used to obtain a unit-free measure of genotype effects on variation (λ_{gen}). While a λ_{gen} value = 1 indicates a strong genotype effect on the observed

variability, no effect of natural variation on the phenotypic differences can be assumed for $\lambda = 0$ (Fig. 3A).

Assessing the degree of genotype effects on the overall range of phenotypic variation observed at each temperature showed highly variable patterns. Regardless of the individual temperature, genotype effects on the developmental timing throughout the vegetative phase was generally very low. This objectively supports the above described initial impression of low natural variation observed in the general temperature sensitivities of traits (Fig. 2B). Similarly, strong genotype effects were observed for many reproductive traits. Other phenotypes show more differential or even gradual genotype effects at different temperatures. For example, effects of natural variation on plant height, silique production and silique length decreased with an increase in temperature, whereas opposite effects are observed for hypocotyl and petiole length as well as flowering time (number of leaves). Although in some cases, such as flowering time, a strong genotype effect seems to correlate also with a strong general temperature sensitivity (Fig. 3B and Fig. 2B), this differs in case of root length. Here, only low genotype effects were observed (Fig. 3B), even though the phenotype was highly sensitive to a change in ambient temperature (Fig. 2B).

Temperature contributions to phenotypic variation

To further dissect and differentiate genotype and temperature effects, we also computed the degree of temperature effects (λ_{temp}) on the total variation for each of the ten accessions (Supplementary Fig. S12A). The heatmap representation of λ_{temp} partially mirrors the λ_{gen} results, for instance in the strong temperature effect on the timing of vegetative development (Supplementary Fig. S12A). However, many traits exhibit highly differential temperature

responses among accessions. This is particularly obvious for yield-related traits such as total number of seeds per plant and silique as well as silique length. Here, temperature effects on total phenotype variation were low for Col-0, C24 and Bay-0, whereas higher λ_{temp} values were determined for the other accessions. Importantly, the latter could be of relevance for future breeding approaches. Similar distinct patterns of temperature effects were observed for a number of traits indicating a highly diverse and complex interplay of temperature and genotype effects on phenotypic plasticity.

Comparison of temperature and genotype effects

To identify global effects of both contributing factors, we computed mean values for λ_{gen} across all temperatures and λ_{temp} across all accessions (Supplementary Fig. S12B). A direct comparison of mean λ_{gen} and λ_{temp} pinpoints the predominant temperature effect on changes in the timing of leaf development (Fig. 3C Supplementary Fig. S12C). In contrast, the variation in quantitative growth phenotypes in the vegetative growth phase displayed considerably higher degrees of genotype effects with similarly high temperature effects. This combination of factorial effects is most prominent for phenotypes associated with shifts to reproductive development. Phenotypes associated with late developmental stages or senescence as well as seed phenotypes were generally less affected by both factors with a general tendency of slightly higher genotype than temperature effects (Fig. 3C, Supplementary Fig. S12C).

Several yield-associated phenotypes such as total number of seeds, seed size and seed weight showed varying degrees of temperature sensitivity, likely caused by the partially distinct temperature effects on individual accessions (Fig.2B, Supplementary Fig. S11A). A comparison of total seed numbers harvested from plants grown at 28°C or 16°C clearly illustrates that for most accessions higher temperatures cause a strong decrease in total yield

(Fig. 4A, Supplementary Fig. S13). However, Got-7 showed an opposite trend even though the overall yield was severely reduced at both temperatures (Supplemental Fig. S13). This illustrates that the extension of the vegetative growth phase might positively affect yield (it has to be noted that in the case of Got-7 this observation might be affected by the vernalization requirement). This would require further inspection using accessions, ideally those with less pronounced vernalization requirements.

The observed differences in yield and some of the seed size parameters prompted us to inspect potential trans-generational effects of ambient growth temperatures on the following generation. Therefore, we tested the rates of germination and seedling establishment of seeds collected from plants grown at 16°C and 28°C when cultivated again at the same or the respective other temperature. Germination rates ranged between 97 to 100% and were similar among all analyzed samples. Seedling establishment (= fully opened cotyledons) after 6 days, however, showed reproducible differences among the different samples. Seeds collected from plants grown at 16°C showed almost no differences in seedling establishment when germinated at 16 or 28°C (Fig. 4B). However, seeds collected from plants grown at 28°C seem to show higher seedling establishment rates when grown under the same temperature (28°C) compared to seeds germinated at 16°C (Fig. 4B). This improved development might indicate trans-generational priming of seeds for development at higher temperatures, putatively involving epigenetic processes. While these effects were repeatedly observed for individual seed pools, extensive analysis of seeds collected from independently cultivated parental lines need to be analyzed to substantiate these observations.

Correlation of phenotypic temperature responses

Finally, we analyzed putative correlations in temperature responses (28 vs. 16°C) among

different phenotypes. We used Pearson correlation coefficients for pairwise comparisons of trait ratios (28 vs. 16°C) among all accessions. As to be expected from the varying degrees of genotype and temperature effects on different traits, correlations among phenotypes covered a wide range (Supplementary Figure S14). Particularly high correlation values were observed among flowering time, hypocotyl length and seed production (Fig. 4C), indicating that traits with strong adaptive potential seem to be affected similarly. Moreover, these data reveal that model phenotypes used in classic forward genetic approaches (such as hypocotyl elongation) are at least partially indicative for general temperature responses in plants.

Discussion

Increased ambient temperatures have been shown to affect thermomorphogenesis for selected phenotypes (Gray et al. 1998, van Zanten et al. 2009). A systematic assessment of developmental plasticity across a complete life cycle has, to the best of our knowledge, been lacking so far. This study provides a solid base of temperature effects on plants by consecutive profiling of plant growth and development throughout a life cycle of *A. thaliana* grown in four different ambient temperatures. Furthermore, including several distinct *A. thaliana* accessions reduced potential genotype-specific biases in the data and allowed the analysis of temperature and genotype effects on the different phenotypic traits.

Of the 34 phenotypes analyzed, almost all were affected by different growth temperatures illustrating the fundamental impact of ambient temperature on plant physiology (Fig.1, Supplementary Fig. S1-S10).

Temperature-sensitive traits can be divided into two distinct groups. First, phenotypes that were similarly affected in all analyzed accessions. Second, phenotypes that showed natural variation in temperature responses. The induction of leaf development throughout the

vegetative growth phase was uniformly accelerated by increasing temperatures in all analyzed genotypes. This could indicate either a highly conserved regulation within *A. thaliana* or a regulation due to passive temperature effects. Indeed, thermomorphogenic responses are often speculated to be primarily caused by the effect of free energy changes on biological reactions (e.g. enzyme activities). The validity of the early proposed temperature coefficient (Q10) for plant development was demonstrated for germination rates and plant respiration (Atkin and Tjoelker, 2003; Hegarty, 1973). The strong temperature effect on the acceleration of developmental timing throughout the vegetative phase, which was only weakly affected by genotypes would certainly fit to this theory. When adopting the terms of "passive" and "active" temperature effects as proposed by Penfield and MacGregor (Penfield and MacGregor, 2014), timing of vegetative development would represent a passive temperature response that might be caused by thermodynamic effects on metabolic rates and enzyme activities.

On the other hand, phenotypes that show a high degree of genotype and temperature effects might rather be influenced by one or more specific genes that contribute to trait expression in a quantitative manner. As such, these phenotypes would represent "active" temperature effects (Penfield and MacGregor, 2014). Natural variation in thermomorphogenic responses could be caused by different polymorphisms of signaling or response genes ranging from alteration in gene sequence to expression level polymorphism (Delker and Quint, 2011) due to adaptation to local environmental conditions. As they provide keys to altered temperature responses that could be utilized in specific breeding approaches, these genes would thus be of high interest.

Several phenotypes analyzed here have the potential to contribute to adaptation to environmental conditions. Particularly hypocotyl and petiole elongation as well as hyponastic leaf movement (increased petiole angles) have previously been shown to improve leaf cooling

by increased transpiration rates (Bridge et al., 2013; Crawford et al., 2012). As such, variation in any of these traits could significantly impact on photosynthesis rates and affect further growth and development. In fact, the ratio of hypocotyl elongation showed a high correlation with the ratio of flowering induction and yield (28 vs. 16 °C, Fig. 4C). This could indicate that early seedling development significantly affects the timing of further development. Alternatively, these processes might involve similar signaling elements. In fact, PIF4 and ELF3 as central signaling elements that integrate multiple environmental stimuli have been shown to be involved in both, temperature induced hypocotyl elongation and the induction of flowering (Koini et al., 2009; Kumar et al., 2012).

In addition, natural allelic variation in the circadian clock components *ELF3* and in the regulation of *GIGANTEA* have recently been shown to directly affect PIF4-mediated hypocotyl elongation in response to elevated temperatures (Box et al., 2015; de Montaigu et al., 2015; Raschke et al., 2015). Therefore, PIF4 and PIF4-regulating components could be important targets of adaptation.

The increasing number of identified genes and allelic variations that contribute to specific phenotypic changes in response to elevated ambient temperatures argue against a general explanation of morphological and developmental changes due to passive effects by thermodynamic processes.

Exploiting natural genetic variation to identify genes that are involved in the regulation of temperature effects on specific traits (e.g., *ELF3* and *PIF4*) can provide new avenues in breeding. Specific approaches will depend on the focus on either yield- or biomass-associated traits. In addition, initial evidence for trans-generational effects require further analysis to account for potential epigenetic transduction of temperature cues on growth and development.

In conclusion, our work provides a data resource that allows the dissection of

thermomorphogenesis in phenotypic traits that are either robustly affected by temperature or traits that are differentially affected by temperature among different accessions; the latter might be a consequence of adaptive processes. While robust temperature-sensitive phenotypes might indeed be be caused by thermodynamic acceleration of metabolism, natural genetic variation of temperature responses implicate the relevance of specific regulatory cascades that might be targets of adaptation to local environmental conditions.

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Table

Tab. 1 Growth and development phenotypes analyzed for temperature sensitivity

Trait	Morphologic marker/time point	Units	# Trait
Developmental data			
Germination			
Germination time	radicle emergence	days	1
Seeding establishment	cotyledons opened fully	days	2
Leaf Production			
2 rosette leaves	rosette leaves >1 mm in length	days	3
3 rosette leaves	rosette leaves >1 mm in length	days	4
4 rosette leaves	rosette leaves >1 mm in length	days	5
5 rosette leaves	rosette leaves >1 mm in length	days	6
6 rosette leaves	rosette leaves >1 mm in length	days	7
7 rosette leaves	rosette leaves >1 mm in length	days	8
8 rosette leaves	rosette leaves >1 mm in length	days	9
9 rosette leaves	rosette leaves >1 mm in length	days	10
10 rosette leaves	rosette leaves >1 mm in length	days	11
11 rosette leaves	rosette leaves >1 mm in length	days	12
12 rosette leaves	rosette leaves >1 mm in length	days	13
13 rosette leaves	rosette leaves >1 mm in length	days	14
14 rosette leaves	rosette leaves >1 mm in length	days	15
Reproductive development			
Inflorescence emergence	First flower buds visible	days	16
Flowering time_days	Bolt>1cm	days	17
Flowering time_n leaves	Bolt>1cm	n° leaves	18
Flowering time_first flower	First flower full opened	days	19
Siliques production	First silique appear	days	20
Quantitative/morphometric phenotypes			
Vegetative stage			
Hypocotyl length	7 days old seedlings	pixels	21
Petiole angle	7 days old seedlings	º	22
Length of primary root	7 days old seedlings	pixels	23
Petiole length	20 days old seedlings	pixels	24
Chlorophyll content	14 days old seedlings	µg/mg leave	25
Foliar surface	Bolt>1cm	mm^2	26
Senescence			
Total number of siliques per plant	First silique shattered	Count	27
Max. Plant height	First silique shattered	cm	28
Seed phenotype			
Seed area		pixels	29
Seed length		pixels	30
Seed weight		µgr.	31
Total number of seeds per plant		Count	32
Total numer of seeds per silique		Count	33
Silique length		mm	34

Figures

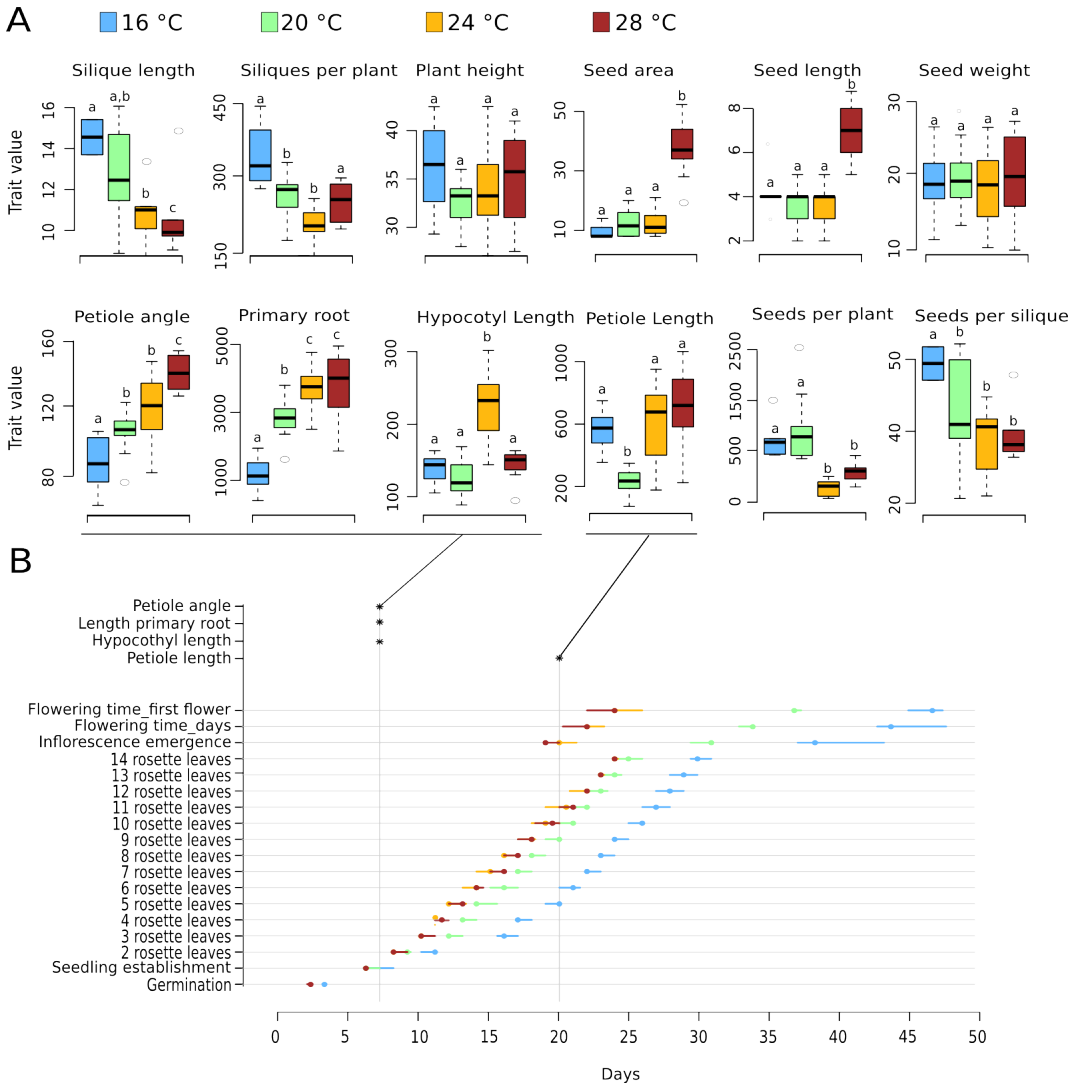

Fig. 1 Col-0 growth and development in response to different ambient temperatures
(A) Quantification of phenotypic traits recorded at different growth temperatures. Box plots show median and interquartile ranges (IQR), outliers (> 1.5 times IQR) are shown as circles. Units for each trait are specified in Table 1. Different letters denote statistical differences ($P > 0.05$) among samples as assessed by one-factorial ANOVA and Tukey HSD. (B) Summary of temperature effects on developmental timing. Circles denote medians, bars denote IQRs ($n > 15$). Time of phenotypic assessment for selected traits in (A) is indicated by asterisks.

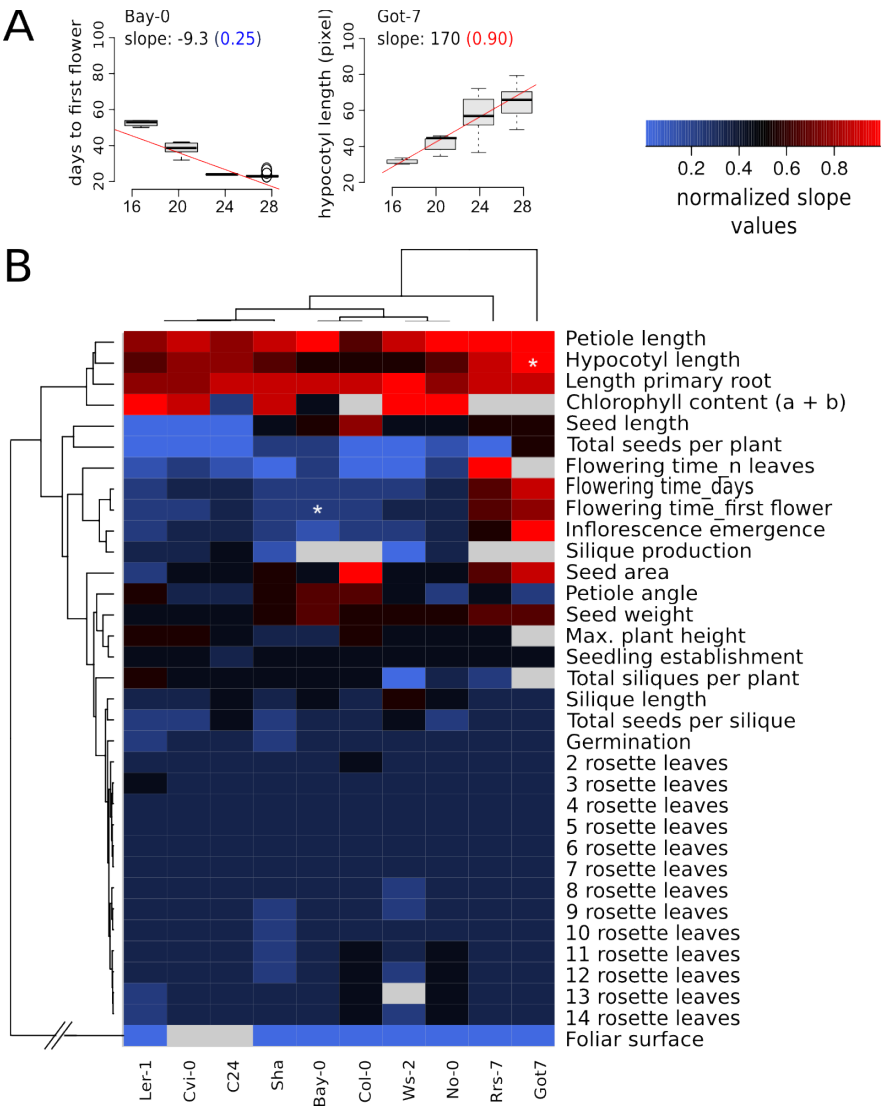

Fig. 2 Natural variation in temperature sensitivity of phenotypic traits
(A) Example graphs illustrating the origin of slope values (in black) for each phenotype and genotype combination. Median-normalized slope values are shown in red and blue for increasing and decreasing values, respectively and are highlighted by asterisks in (B). Corresponding figures for all other available combinations of phenotypes and genotypes are shown in Supplementary Fig.S1-S10. (B) Heatmap and hierarchical clustering of normalized slope values derived for each phenotype/genotype combination as indicated in (A).

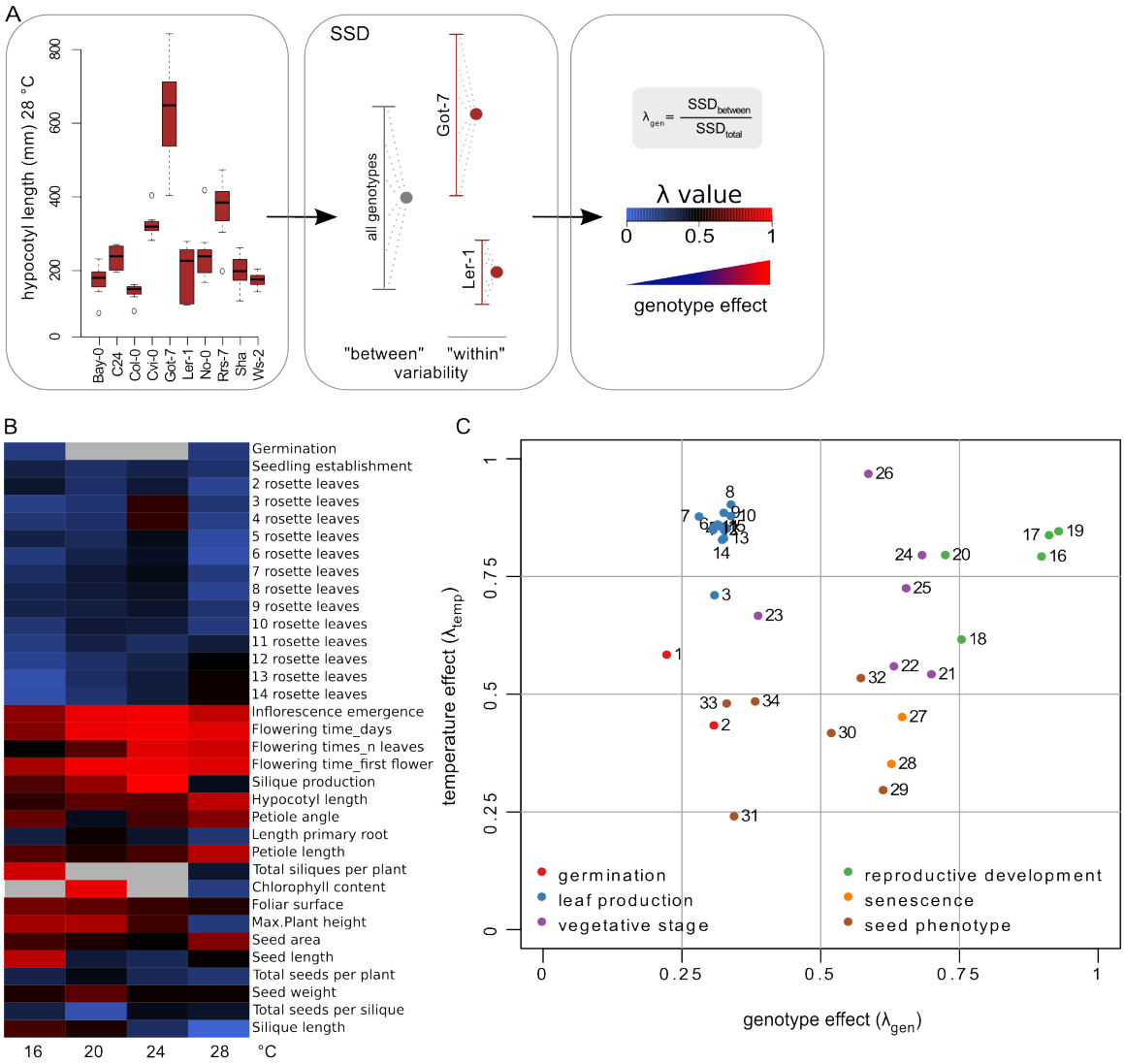

Fig. 3 Genotype and temperature effects on phenotypic variation

(A) Illustration of the concept of "within" and "between" variability and the calculation of genotype effects (λ_{gen}) taking hypocotyl elongation at 28°C as an example. Variation "within" a genotype was calculated as the sum of squared differences (SSD) between individual data points of one accession to the respective accession mean (SSD_{within}) as shown for Ler-1 and Got-7 as an example. Variation between genotypes was calculated by assessing the SSD of accession means to the global mean of values of all accessions combined ($SSD_{between}$). λ_{gen} provides a measure of genotype effects on the variation observed for individual phenotypes. (B) Heat map representation of the intraclass correlation coefficient λ_{gen} of all recorded phenotypes. Missing data is shown in grey. (C) Scatter plot of mean λ_{gen} and λ_{temp} values over all temperatures and accessions, respectively. Phenotypes are color-coded according to developmental stage. Heatmaps of individual $\lambda_{temp,}$ mean λ values and standard deviations are shown in Supplementary Fig. S12A-C.

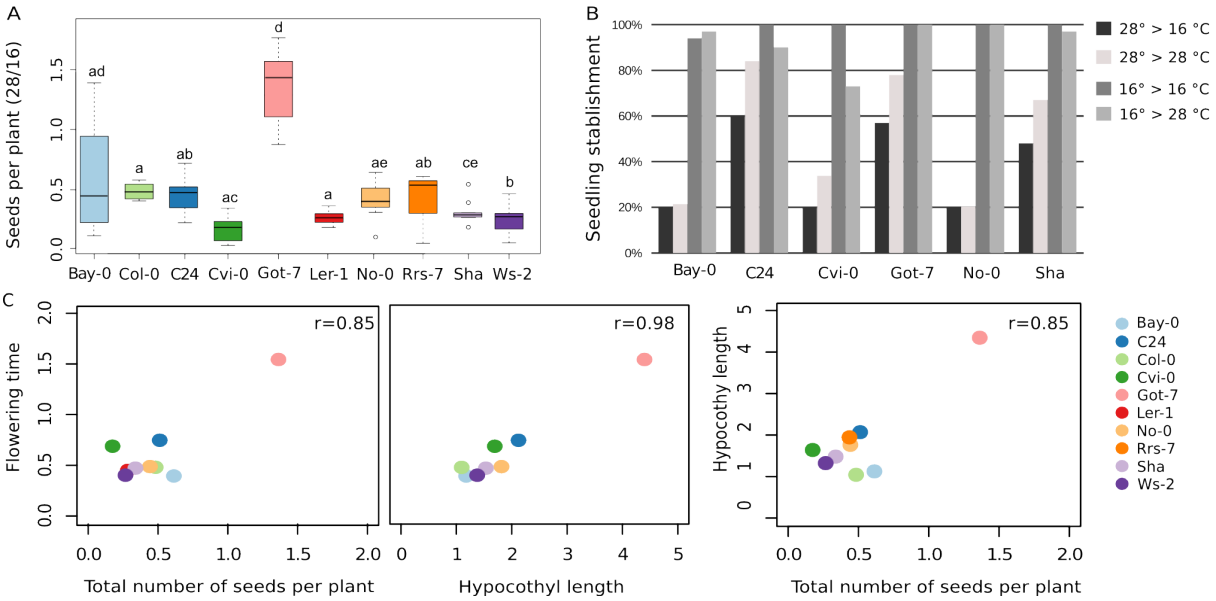

Fig. 4 Yield, trans-generational effects and phenotypic correlations
(A) Comparison of temperature sensitivities of accession yield. Box plots show relative seed numbers (28°C vs. 16°C median). Different letters denote significant differences ($P < 0.05$) as assessed by two-factorial ANOVA of absolute data shown in Supplementary Fig. S13. Got-7 was significantly less affected by high temperature but showed lower absolute yield values at all analyzed temperatures (Supplementary Fig. S13). (B) Rates of seedling establishment of 6 days old seedlings. Seeds were collected from plants grown at 16 or 28°C for an entire life cycle and were germinated either the same (16 > 16°C and 28 > 28°C) or the respective other growth temperature (16 > 28°C and 28 > 16°C). The experiment was performed three times with similar results of which one representative is shown. (C) Scatter plot of temperature response ratios (28 vs. 16°C) of selected phenotypes. Pearson correlation coefficients (r) of trait temperature response ratios (28 vs. 16°C) are shown in the upper right corners. See Supplementary Fig. S14 for complete set of pair-wise comparisons among traits.

Supplementary Data:

Tab. S1: Identity and geographic origin of analyzed *A. thaliana* accessions

Fig. S1: Summary of Col-0 thermomorphogenesis

Fig. S2: Summary of Bay-0 thermomorphogenesis

Fig. S3: Summary of C24 thermomorphogenesis

Fig. S4: Summary of Cvi-0 thermomorphogenesis

Fig. S5: Summary of Got-7 thermomorphogenesis