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Adaptive epigenetic memory of ancestral temperature regime in *Arabidopsis thaliana*[1]

C.A. Whittle, S.P. Otto, M.O. Johnston, and J.E. Krochko

Abstract: Although certain acquired nongenetic (i.e., epigenetic) traits are known to be heritable in plants, little is known currently about whether environmental parameters can induce adaptive epigenetic responses in plants and whether such effects can persist through generations. We used an experimental design based on classical genetics principles to assess whether plants respond to the environmental conditions of their ancestors in an adaptive epigenetic manner. An extensive examination of genetically identical *Arabidopsis thaliana* (L.) Heynh lines exposed to mild heat (30 °C) or cold (16 °C) treatments in the parental and F_1 generations revealed that the prior elevated temperature regime lead to a greater than fivefold improvement in fitness (seed production per individual) for plants exposed to heat in a later generation (F_3). The heat-specific fitness improvements among F_3 plants were observed even though the heat-treated parental and F_1 generations were followed by a generation grown at a normal temperature (F_2) and point towards a temperature-induced adaptive epigenetic phenomenon. No such adaptive responses were detected for cold-treated plants, indicating that there are distinctive biological processes inherent to these two temperature regimes. Overall, the data are consistent with the existence of an environmentally induced epigenetic and heritable adaptive response in plants.

Key words: adaptation, plants, epigenetic, environment, transgenerational, heat stress.

Résumé : Bien qu'on sache que certains caractères acquis non génétiques (i.e., épigénétiques) peuvent être hérités chez les plantes, on ne sait pas si des paramètres environnementaux peuvent induire des réactions épigénétiques adaptatives chez les plantes et si de tels effets peuvent persister au cours des générations. Les auteurs ont utilisé un dispositif expérimental basé sur les principes de la génétique classique pour évaluer si les plantes réagissent de façon épigénétique aux conditions environnementales subies par leurs ancêtres. Un examen extensif de lignées identiques de l'*Arabidopsis thaliana* (L.) Heynh exposées à une température chaude (30 °C) ou froide (16 °C), chez les générations parentales et la F_1, a révélé que le régime à température chaude a conduit à une amélioration de l'adaptation plus que cinq fois plus grande (production de graines par individu) chez les plantes exposées à la chaleur dans une génération ultérieure (F_3). On observe les améliorations de l'adaptation spécifique à la chaleur chez les plantes F_3 même si la lignée parentale traitée à la chaleur et les générations F1 ont été suivies d'une génération cultivée à une température normale (F_2) ce qui suggère l'existence d'un phénomène épigénétique adaptatif induit par la température. Les auteurs n'ont pas observé de telle réaction adaptative chez les plantes traitées par le froid, ce qui indique qu'il existerait des processus biologiques distincts inhérents à ces deux régimes de température. Dans l'ensemble, ces données sont congrues avec l'existence chez les plantes d'une réaction adaptative héritable et épigénétique induite par l'environnement,

Mots-clés : adaptation, plantes, épigénétique, environnement, transgénérationnel, stress thermique.

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Introduction

Epigenetic traits include all heritable phenotypic states not explained by genetic principles (Waddington 1942; Goldberg et al. 2007). Examples of epigenetic traits in plants include those affecting flower morphology and colour, kernel colour (*Zea mays* L.) and vernalization, as well as other phenotypes that can only be explained by nongenetic heritable factors (McClintock 1951; Brink 1958; Jorgensen 1995; Jacobsen and Meyerowitz 1997; Kakutani 2002; Zhang et al. 2003; Baulcombe 2004; Kalisz and Purugganan 2004; Sung and Amasino 2004; Gendrel and Colot 2005; Bonnet et al. 2006; Grant-Downton and Dickinson 2006; Sung et al. 2006). Many of the reported epigenetic phenomena in plants persist only across mitotic cell generations within an indi-

vidual (intraorganismal; e.g., vernalization), while some are stable across both mitosis and meiosis (transgenerational; e.g., *SUP* gene epiallele affecting flower morphology, Jacobsen and Meyerowitz 1997). Despite the growing number of studies supporting a major role for epigenetic processes in regulating specific plant phenotypes, minimal data are available regarding whether environmental parameters can lead to transgenerational epigenetically controlled phenotypes in plants. The current lack of data and clarity with respect to this possibility in plants is remarkable given that an ability to rapidly respond and adjust to changing conditions (i.e., nongenetically) across generations could be highly advantageous for these sessile organisms (Walbot and Cullis 1985; Grant-Downton and Dickinson 2006).

The limited data that are available to date regarding environmentally induced epigenetic responses in plants are largely derived from studies of intraorganismal phenomena. For example, growing evidence suggests that the process of vernalization is mediated by nongenetic changes affecting the *Flowering Repressor Locus* (*FLC*) during/following long periods of chilling (Zhang et al. 2003; Bastow et al. 2004; Sung and Amasino 2004; Sung et al. 2006). It also has been found that plants exposed to short periods of mild heat or cold stress early in development can later tolerate normally lethal hot or cold temperatures, respectively, which is an unexplained phenomenon possibly regulated by an epigenetic system (Sung et al. 2003). In addition, it has been reported that warm temperatures during the development of maternal and embryonic tissues in *Picea abies* (L.) Karst. result in improvements in the performance of juvenile offspring grown at the same elevated temperature (Johnsen et al. 2005), a result that has been attributed to an epigenetic system. Similar findings have been reported recently for *A. thaliana* (Blodner et al. 2007). In these studies the temperature treatment applied to the parental generation could have affected the offspring while they were retained on the parent plant (via maternal provisioning of the seed or via a direct impact of temperature on the developing embryo; Westoby 1981; Rakyan and Whitelaw 2003; Haughn and Chaudhury 2005; Johnsen et al. 2005); therefore, these findings need not involve the direct inheritance of epigenetic signals from parents to offspring. These observations are consistent with an intraorganismal process and do not necessarily demonstrate interorganismal inheritance. Nevertheless, these studies clearly indicate that temperature changes can induce epigenetic-related molecular changes, and support the possibility of multigenerational and (or) adaptive epigenetic phenomena.

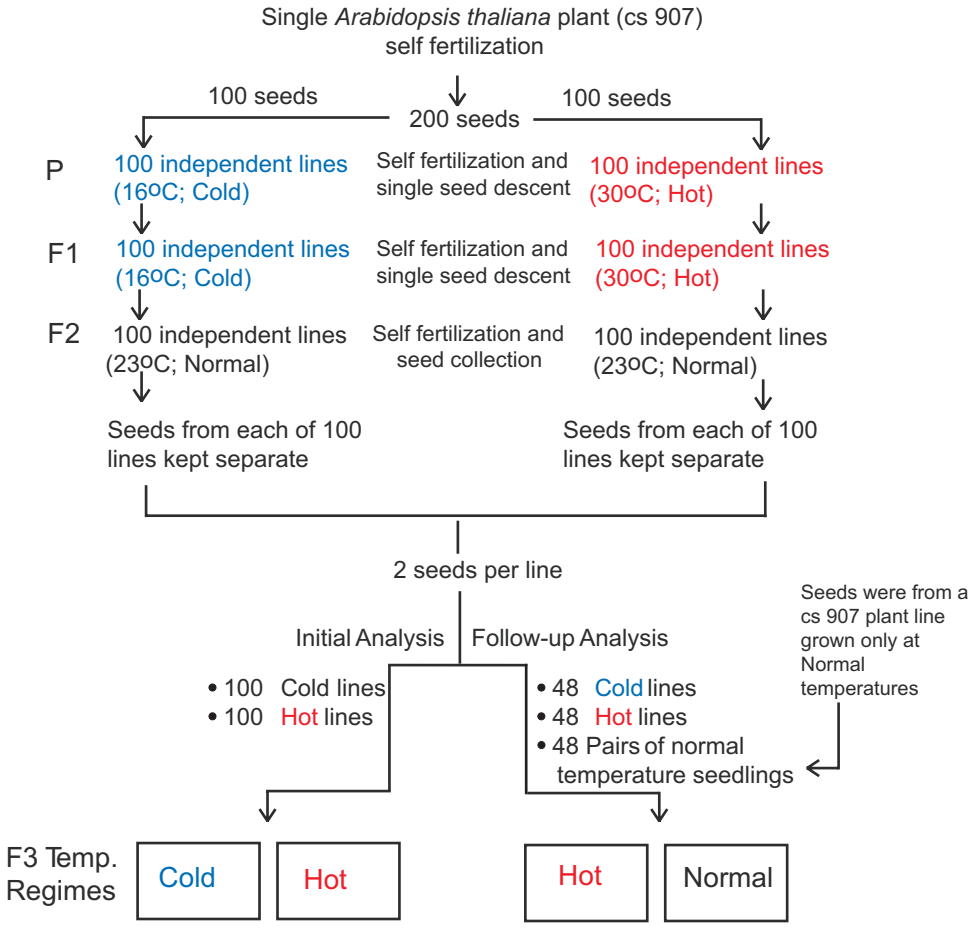
Although information about environmentally induced and transgenerational epigenetic traits has been very limited (Grant-Downton and Dickinson 2006), recent data from *A. thaliana* plants treated with UV-C (notably, an unnatural source of UV irradiation) and *A. thaliana* and *Nicotiana tabacum* L. plants treated with plant pathogens have shown that there is enhanced somatic homologous recombination (mitotic recombination between homologous chromosomes) in response to these treatments, and that this can persist across subsequent untreated generations (Molinier et al. 2006; Boyko et al. 2007). It also has been found that temperature modification during the early stages of development in maize leads to heritable changes in the activity level of an epiallele at the *r*-locus (kernel colour locus, Mikula 1995; Chandler and Stam 2004). These previous studies did not assess whether naturally occurring abiotic environmental factors can induce a nongenetic transgenerational memory, and most importantly, whether such phenomena can lead to nongenetic adaptations. The lack of data regarding epigenetic adaptation is surprising given the increasing recognition that epigenetic phenomena play an important role in within-plant signaling (Jorgensen 1995) and plant development, and that epigenetic signals can be inherited and alter traits associated with fitness (e.g., floral development) (Jacobsen and Meyerowitz 1997; Grant-Downton and Dickinson 2006). Altogether, given the present lack of data, it is evident that further study is needed to reveal whether environmental factors can give rise to transgenerational epigenetic traits that facilitate adaptation in plants (Kalisz and Purugganan 2004; Grant-Downton and Dickinson 2006).

In the present study, we assessed whether imposition of a mild heat or cold treatment (30 °C or 16 °C) during reproduction in *A. thaliana* lines altered the fitness, in an adaptive and epigenetic manner, in a succeeding generation grown under those same temperature conditions. The heat and cold treatments in the parental and F_1 generation were followed by a generation grown at normal temperatures to ascertain whether any epigenetic effects could persist across an untreated generation (i.e., through both mitosis and meiosis). To achieve this objective, namely to isolate epigenetic adaptive effects, we conducted our study using a set of inbred plant lines, all derived from the seeds of a single self-fertilized plant, with each line having a population size of one individual. By using a propagating population size of one inbred individual per line, there is little scope for novel mutations, genetic variation, and selection within these lines (Schultz et al. 1999), allowing us to focus upon epigenetic effects. In particular, only epigenetic effects that are shared among lines, and which arise and persist independently in each line, can explain consistent and detectable phenotypes across groups of lines for each temperature treatment. The data presented here demonstrate that plants maintain a nongenetic memory of the temperature conditions of their ancestors through to succeeding generations in a manner that enhances fitness.

Materials and methods

For this experiment, a total of 200 seedlings produced from the self fertilization of a single *A. thaliana* plant were used as the parental (P) generation (catalog No. cs907, Arabidopsis Biological Resource Center, Ohio; a plant line descended from Col-1 that is highly inbred). As shown in Fig. 1, each of these 200 seedlings comprised the beginning of a line of single-seed descent that was maintained for three generations. Specifically, five seeds of the original self-fertilized plant were sown in each of 200 pots, and within 5 d after sowing, all but one seedling was removed and the remaining seedling grown to maturity (P generation). The plant that was kept for each line was chosen completely arbitrarily (i.e., we systematically rotated between keeping the topmost, bottommost, leftmost, rightmost, or centrally positioned seedling from among the five seedlings per pot; this procedure was used throughout the

Fig. 1. Experimental design. A total of 200 sibling plants produced from the self fertilization of a single *A. thaliana* plant (cs 907) were used as the parental (P) generation. Lines were maintained using single seed descent and were grown at either a hot (30 °C, 100 lines) or cold (16 °C, 100 lines) temperature during reproduction (from bolting to senescence) for two generations (P and F_1). The F_2 generation was grown entirely at a normal temperature (23 °C, F_2). An untreated single seed descent plant line was also utilized. An initial and a follow-up analysis were conducted for F_3 plants grown at hot, cold or normal temperatures as described in the Materials and methods.



present study). Thus, there was little scope for researcher-imposed bias in selection among the five seedlings; this limitation on genetic selection within the experiment is underscored by the severe lack of genetic variation among seedlings per line. This same process was repeated for the F_1 and F_2 generations. In the P generation, the 200 lines were arbitrarily divided into two groups of 100 and placed in one of two identical Conviron growth chambers. All plants in both chambers in the P and F_1 generation were maintained at 23 °C under 18 h of fluorescent light per day (130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux; bulbs suspended 55 cm above plants) until the third week of development, coinciding with the initiation of bolting. Both the P and F_1 plants then underwent either a heat treatment at 30 °C (Hot; varied between 30 and 33 °C) or cold treatment at 16 °C (Cold) for the remainder of their lifespan (i.e., one temperature per growth room). In the F_2 generation, all lines were grown at a normal temperature for their entire lifespan (Normal; 23 °C). For all of these treatments, pots were randomly placed in trays and trays were randomly placed along the bench. Plants were maintained in 5 cm deep pots filled with Sunshine Mix (Sun Gro Horticulture Inc., Bellevue, Wash.), placed in trays measuring 25 cm × 40 cm, and watered from below. All seeds used in this study were stored under dry conditions at 4 °C and were under 6 months of age, a storage period thought not to affect viability in *Arabidopsis* seeds maintained under these conditions (Wilson 2000).

Initial evaluation of response to temperature treatment in the F_3 generation

For our initial analysis, plants derived from lines historically exposed to cold (100 lines) or heat (100 lines) were subjected to cold or hot temperatures in the F_3 generation. Specifically, two individuals per line (for each of the 200 lines, see Fig. 1) were grown for the F_3 heat and for the F_3 cold treatment, for a total of 800 plants. Individuals derived from the ancestral hot and cold treatments were grown side-by-side in trays as shown in the supplementary data[3],

[3] Supplementary data for this article are available on the journal Web site (http://botany.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3935. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/eng/ibp/cisti/collection/unpublished-data.html.

Fig. S1. Trays were arbitrarily placed in the growth chambers. F_3 plants were cultivated at the normal temperature until bolting, and then exposed to cold treatment or heat treatment, as appropriate. Upon maturation (senescence), the number of flowers per plant and the number of seeds per silique (from three siliques per plant) were determined.

Follow-up analysis of plant fitness and reproductive and developmental traits

To further evaluate the relationship between ancestral parental and F_1 heat stress on the fitness of heat-treated descendents (F_3), we conducted follow-up experiments using seeds from the F_2 generation and seeds from an *Arabidopsis* cs 907 line historically grown only at a normal temperature (see Fig. 1). The offspring of the normal temperature plant were used to reveal baseline phenotypes of temperature-treated *A. thaliana* plants. The offspring were grown side-by-side with plants ancestrally subjected to hot or cold temperatures. Specifically, for each of three temperature treatment categories (plants whose ancestors were grown at the cold, normal, or hot temperature), 2 seeds from each of 48 lines (or 96 seeds from the normal temperature line) were grown under heat treatment in the F_3 generation (for a total of 288 plants). The number of offspring per treatment category was determined, a priori, to be greater than the minimum number required to yield 0.95 power to detect a difference in fitness (at $\alpha = 0.05$), based on the means and standard deviations of total fitness values observed at the hot temperature in the F_3 generation in the initial analysis (i.e., $N_{0.95\ \text{power}} \geq 21$). Additional plants were grown in a similar manner for destructive sampling of reproductive tissues during the heat treatment (two individuals from each of 12 lines ($N = 24$) for each of the three ancestral treatment types; $N_{\text{Total}} = 72$). F_3 plants from each treatment category (ancestral exposure to hot, cold and normal temperatures) were also grown at normal temperatures (23 °C) for the measurement of fitness and morphological phenotypes and for destructive sampling (two sets of plants (one set for fitness/phenotype measurements and one set for destructive sampling); each set containing two individuals from each of 12 lines ($N = 24$) per ancestral treatment type; $N_{\text{Set}} = 72$). All plants were grown in trays containing 24 plants, with each tray containing four lines (8 plants) from each of the three treatment categories (see supplementary data,[3] Fig. S1). For the F_3 heat treatments, plants were grown at 23 °C until the initiation of bolting, after which the temperature was changed to 30 °C (ranged between 30 °C and 33 °C) for the remainder of their lifespan. F_3 plants grown at a normal temperature were maintained at 23 °C throughout their lifespan. Control plants are defined as those always grown at normal temperatures (i.e., the F_3 plants grown at a normal temperature that were also historically grown only at a normal temperature).

A series of measurements were conducted on fitness and developmental traits for F_3 plants grown under normal or elevated temperatures. Specifically, we measured the following: flower number per plant; number of seeds per silique and plant height; rosette diameter and plant dry mass (air dried for 3 weeks at room temperature). The total seed number per plant was calculated as the number of seed-producing siliques × average number of seeds per silique. Additionally, we measured reproductive traits for F_3-heat and F_3-normal temperature treatments for plants that were specifically grown for destructive sampling. These traits were measured between 10 to 15 d after the start of the heat treatment (for both the normal and heat treated F_3 plants). Specifically, three pistils per plant and all anthers from one flower per plant were manually opened on double-sided tape for each individual and examined under a stereomicroscope. Pistils/immature siliques were chosen from flowers that were 3 d past stage 12 (when petals first emerge from the flower) (Smyth et al. 1990) and the number of ovules per silique and the number of ovules/embryo abortions per silique were measured (for each pistil/immature silique). For the pollen measurements, unopened anthers were removed from stage 12 flowers and the number of pollen per flower was scored. The number of anthers per flower also was recorded. In addition to these measurements, one flower from each plant of one of the three heat-treated trays was manually crossed (before destructive sampling occurred) with freshly produced pollen from control plants (normal temperature for all generations) and the number of seeds per silique was measured (to assess the role of potential pollen limitation on seed production). No difference was detected in seed production for pistils crossed with pollen from control plants as compared to pollen from heat-treated plants. To confirm that the pollen from the control plants was viable at the time of application, pollen from several recently opened flowers from control plants was placed on germination media consisting of 0.1% boric acid, 0.7% bacto-agar, 0.07% calcium chloride, 3% polyethylene glycol, and 20% sucrose (Derksen et al. 2002) that was boiled and then poured into and solidified in Petri dishes. Dishes containing pollen were placed uncovered under high humidity at 27 °C in the dark for 2 d, after which time pollen germination was assessed under a stereomicroscope.

Statistical analysis

To assess whether ancestral hot or cold temperature treatments (in the parental and F_1 generations) alter fitness of plant lines grown later under these temperatures (in the F_3 generation) we conducted, for the initial analysis, a *t*-test for each combination of ancestral temperature treatment (Hot, Cold) and final temperature treatment (Hot, Cold) using the number of seeds per silique and the number of flowers per plant. A Bonferroni correction was applied across *t*-tests in the initial analysis. For the follow-up analysis of plants grown under heat treatment in the F_3 generation, we conducted a series of one-way ANOVAs between the plant lines historically exposed to cold, normal, and hot temperature treatments and the controls (lines always grown at normal temperature) for each of the following traits: the number of flowers per plant, number of seeds per silique, seed number per plant, number of ovule/embryo abortions per pistil/immature silique, number of pollen per flower, the number of anthers per flower, plant height, rosette diameter and plant dry mass. Holm-Sidak multiple comparison tests were conducted for the ANOVAs that were statistically significant ($P < 0.05$). A similar analysis was conducted for plants grown at the normal temperature in the F_3 generation relative to ancestral temperature regime (cold, normal, hot). To determine the probabilities of mutations arising among heat-related loci in our plant lines, the Bionomial distribu-

tion was utilized. All analyses were conducted using SigmaStat 3.5 (Systat Software Inc., San José, Calif.).

Results and discussion

As shown in Fig. 1, we used 200 genetically homogeneous and homozygous lines of *A. thaliana*, derived from the self fertilization of a single plant. The lines were studied over four generations. Lines were grown at either a hot (30 °C; $N = 100$) or cold temperature (16 °C; $N = 100$) during reproduction (from bolting to senescence) for two generations (P and F_1), followed by one generation at a normal temperature (23 °C, F_2). Fitness traits were then evaluated for plants grown at either the hot or cold temperature in the F_3. Plants were grown according to a systematic planting design whereby plants from each of the ancestral temperature regimes were grown in proximity to each other (see supplementary data,[3] Fig. S1). With this approach, the influence of ancestral temperature regime on fitness can be differentiated from position or temperature effects per se in the F_3 generation.

Data collected from the initial comparison of reproductive fitness in the F_3 generation for plant lines that had been grown at either hot or cold temperatures in both the parental and F_1 generations (Fig. 2A) show clearly the detrimental effects of the higher temperature on reproductive fitness. In particular, the number of seeds per silique and numbers of flowers produced per plant are drastically and statistically significantly lower for plants grown at 30 °C as compared with those grown at 16 °C in the F_3 generation (Fig. 2A), regardless of the ancestral stress treatments of those plant lines. The data also demonstrate, however, that plant lines historically exposed to heat treatments have a statistically significant and markedly higher fitness during heat exposure in the F_3 generation, as measured by seed number per silique and number of flowers per plant, than those historically grown under cold conditions (Fig. 2A), consistent with an epigenetic and adaptive memory of the ancestral temperature regime (note that this conclusion is also supported by interaction between ancestral and F_3 temperatures from two-way ANOVAs, i.e., seed number per silique $P = 8.2 \times 10^{-5}$; flower number per plant $P = 0.045$). No differences were found relative to ancestral temperature regime (Cold or Hot) in F_3 plants grown under cold conditions (Fig. 2A), perhaps because this temperature was not as stressful and did not impact fitness potential as much as the higher temperature treatment.

Given the above findings, we conducted a detailed follow-up analysis in the F_3 generation, focusing on heat-treatment effects, using seeds from the two ancestral temperature regimes (Cold, Hot; $N = 48$ pairs), as well as seeds from a line historically grown at normal temperatures to reveal *A. thaliana* baseline traits (see Fig. 1). The F_3 plants were grown at hot or normal temperatures after the initiation of bolting with plants from all three ancestral temperature regimes (Cold, Normal, Hot) organized in a side-by-side design (see supplementary data,[3] Fig. S1). In this experiment, a more complete set of reproductive and developmental traits were measured to more fully assess the impact of both the ancestral temperature regimes and the effects of contrasting temperature regimes (23 °C versus 30 °C) on fitness in

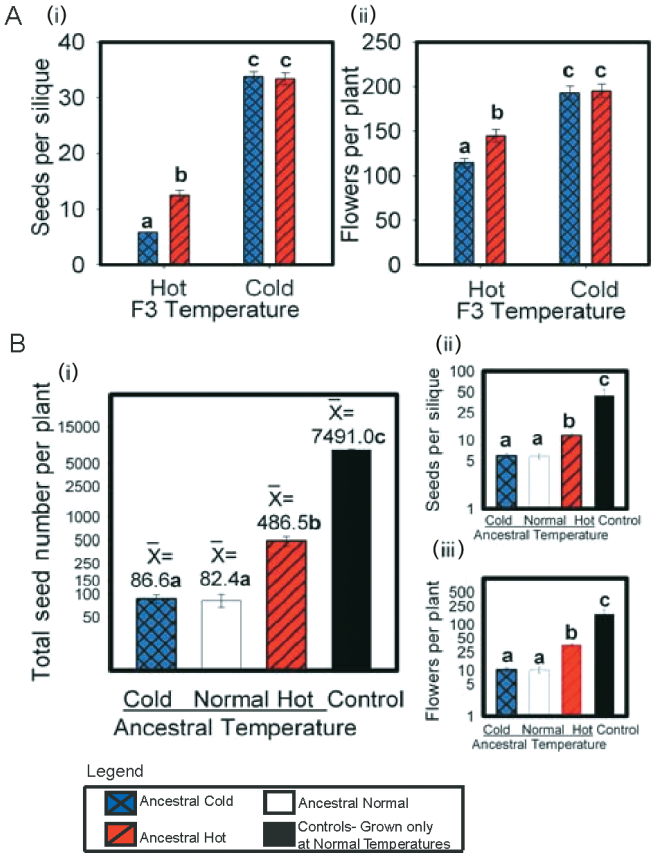


Fig. 2. (A) Initial fitness measurements for F_3 generation plants grown at either hot (30 °C) or at cold (16 °C) temperatures relative to ancestral temperature regime: (*i*) The number of seeds per silique; and (*ii*) the number of flowers per individual plant. (B) Fitness measurements for the follow-up analysis of heat-treated F_3 plants [including plants derived from the ancestral hot and ancestral cold treatments and the previously untreated (ancestral normal) line]. Data for control plants are also provided for comparison (i.e., plants grown only at normal temperatures in all generations, including the F_3): (*i*) total seed number per plant; (*ii*) number of seeds per silique; and (*iii*) number of flowers per plant. Note that the *y*-axes are on a log scale. Different letters within each graph indicate a statistically significant difference from *t*-tests (panel A, all *t*-tests remained statistically significant after Bonferroni correction) or from one-way ANOVAs and Holm-Sidak pairwise tests (panel B) ($\alpha = 0.05$). Data represent mean ± SE.

the F_3 generation plants (Fig. 2B; Table 1; supplementary data,[3] Tables S1 and S2). The results confirm our initial experimental result that total fitness during heat exposure in F_3 plants is improved by an ancestral exposure to heat treatments (Fig. 2B). Specifically, heat-treated F_3 plants that are derived from the lines historically grown at hot temperatures show statistically significantly higher total fitness, as measured by seed production per plant (mean, 486.5 ± 63.0; resulting from both a higher seed number per silique and flower number per plant), than those descended from the plant(s) historically grown at cold and at normal (baseline; the untreated plant line) temperatures (means, 86.6 ±10.9 and 82.4 ±16.0, respectively, Fig. 2B). This is consistent with the presence of transgenerationally transmitted adaptive features in plant lines grown previously under heat condi-

Table 1. Fitness traits of *Arabidopsis thaliana* (cs 907) plants grown at the normal temperature (23 °C) in the F_3 generation in the follow-up analysis.

	Ancestral temperature treatments			
	Cold	Normal (controls)	Hot	*P*-value
Total seed number per plant	7095.1 (610.4)	7491.0 (734.0)	8134.3 (612.1)	0.498
Flower number	154.5 (12.1)	165.5 (36.4)	178.0 (28.1)	0.298
Number of seeds per silique	45.6 (7.3)	44.3 (9.42)	45.8 (11.3)	0.909

Note: Cold, 16 °C; Normal, 23 °C; Hot, 30 °C. Controls are plants always grown at normal temperatures (ancestral and F_3 temperatures are each normal). Data represent means and standard errors. No statistically significant *P*-values were detected for the one-way ANOVAs.

tions. No such differences in fitness (seed or flower production) were detected with respect to the ancestral temperature regime (Cold, Normal, or Hot) in plants grown at the normal temperature in the F_3 generation (Table 1), suggesting that the adaptive response is specific to and (or) only evident under elevated heat. Further, additional data collected for a range of reproductive and growth traits in ancestral hot, cold and normally grown plants maintained at normal temperatures (23 °C) in the F_3 show no indications of any changes to fitness or growth habit among any of these three groups of lines at that temperature (supplementary data,[3] Table S1). By contrast, the relatively mild heat treatment used in the present study (an increase of 7 °C) had a marked negative impact on numerous additional plant traits measured in the F_3 generation plants (ancestral Hot, Cold, and Normal plant lines) including the ovule number per pistil, number of aborted ovules, pollen per flower, and number of anthers per flower (supplementary data,[3] Table S2). The severity of these detrimental effects was markedly similar among the plants examined, irrespective of the ancestral temperature regime (supplementary data,[3] Table S2). Thus, the transgenerational adaptive response reported for the previously heat-treated plant lines is primarily associated with increased seed output per plant, i.e., number of flowers produced and early post-fertilization seed survival, as it does not alter other specific male or female reproductive traits and does not preferentially or broadly affect other nonreproductive traits (supplementary data,[3] Table S2).

These data, derived using a classical genetics approach (Figs. 1 and 2), suggest that the positive effects of past exposure to heat treatment on fitness result from an epigenetic memory of the ancestral temperatures. This conclusion is supported as follows. First, the observed fitness effects due to the historical temperature regime (Hot) occur across a group of independent and genetically homogeneous lines (developed from a single well-established homozygous seed source, cs 907, see Materials and methods), wherein spontaneous mutations are expected to arise very rarely and sporadically (Drake et al. 1998; Schultz et al. 1999). In particular, the mutation rate in gene-coding non-neutral DNA in plants, including *A. thaliana*, has been estimated at between 0.1 and 0.9 mutations per haploid genome (i.e., portion containing functional genes wherein mutations affect phenotypes) per generation (Johnston and Schoen 1995; Drake et al. 1998; Schultz et al. 1999). Using the average of these values (0.5), it may be inferred that 1.5 mutations might arise per haploid effective genome per line in the present study between the P and F_2 generations (before the F_3). This represents a maximum total of three gene mutations among any of the 56 000 diploid genes ($2n$) in the *Arabidopsis* genome (The Arabidopsis Information Resource (TAIR) 2007) per line, an extremely low number of hits. Assuming that up to 2.1% of gene coding DNA is associated with tolerance to mild heat treatment (as indicated by differentially expressed genes at 37 °C, a greater temperature increase than used here) (Lim et al. 2006), the probability that a mutation occurs in a heat-tolerance related gene in every one of the 100 lines is essentially zero [8.6×10^{-121}; and the same is true for the follow-up analysis $2.3 \times 10^{-58} = (3 \times 0.021)^{48}$]. Even the probability of a mutational hit in a quarter of the lines is negligibly small (1.8×10^{-9} and 2.6×10^{-5} for the initial and follow-up analysis, respectively). Although high heat stress can marginally increase mutation rates in some organisms (Drake et al. 1998), the heat treatment in the present experiment was mild (7 °C above normal growth conditions); therefore, even if the mutation rate had increased by 10-fold this would still not generate a substantial probability of a single mutation occurring in a heat-tolerance-related gene in each line (8.6×10^{-21}). Second, each line consists of a single selfed plant, so selection among individuals within a line is severely limited owing to experimental design (Drake et al. 1998; Schultz et al. 1999). Although selection could have acted at the very early seedling stage (during thinning of the very young seedlings), this is unlikely given that germination rates were typically high, germination occurred at a normal temperature, and we chose one out of five seedlings to survive at a very early stage of development (within five days of seed sowing) in a predetermined fashion rather than based on growth characteristics. Gametophytic selection at the reproductive haploid stage, was also limited as evidenced by the fact that pollen and ovule/early seed number in heat-treated F_3 plants was not enhanced among plant lines ancestrally grown under the hot treatment as compared to those from the cold or from the normal (baseline) treatments (supplementary data,[3] Table S2). Most importantly, there would be insufficient acquired genetic variation for selection to be very effective at any stage (see mutation rates above). Third, the data reported in this study cannot be explained by physiological maternal effects on seed traits, such as seed mass or seed size, each of which has been shown to directly influence the fitness of immediate progeny (Mousseau and Fox 1998), as the experimental design included an interceding generation (F_2) between the heat treatments during which all plant lines were grown at normal temperatures. As well, it has been demonstrated previously that environmentally induced maternal effects on progeny are not transmitted through to the grand-

offspring in *A. thaliana* (Andalo et al. 1999). Overall, given the severe lack of genetic variation in these lines in the present study, the limitations on genetic selection and the absence of maternal effects, we conclude that our data are best explained by the existence of an epigenetic temperature-induced alteration that can be transmitted across generations (through both mitosis and meiosis) and that this phenomenon contributes to adaptation in *A. thaliana*. In particular, the data indicate that the adaptive memory of the ancestral temperature regime results from the inheritance of epigenetic factors affecting flower production and early-stage seed survival in plants grown at an elevated temperature.

The present data are consistent with a transgenerational phenomenon because all plant lines were cultivated at a normal temperature in the intervening F_2 generation; suggesting that the epigenetically induced effect can span at least one untreated generation in these plants (arises in the P–F_1, transmitted through the F_2, and is present in F_3). Although the efficiency of selection on genetic variation was severely limited in this study, it is notable that epigenetic selection (selection among epialleles) could have occurred within a plant (affecting flower production) or at the early seed stage and could have contributed to the observed epigenetic adaptation. We also note that the fitness effects in F_3 heat-treated plants relative to historical temperature regime show some variation among lines, and these effects were not universal across every plant in the compared treatments (i.e., not every ancestral heat-treated plant had improved fitness compared to every normal or cold-treated plant), suggesting the adaptive heat-response is not a dominant trait, but rather a continuous trait that varies among lines and perhaps with microclimate. The latter possibility is not unexpected as even the plants raised in the normal environment exhibited small, but measurable, variations in fitness (Table 1), presumably due to stochasticity in microclimate and developmental responses. Given that many/most loci can affect fitness, it is important to recognize that the adaptive response observed here could be mediated by epigenetic activity at one or at many loci (Drake et al. 1998; Schultz et al. 1999). It is also worthwhile to note that the present results also suggest that epigenetic factors could partially contribute to short term intergenerational heritable and seemingly genetically based responses to temperature already reported for certain plants (e.g., *Plantago lanceolata* L., Case et al. 1996). The transgenerational stability of epigenetic activity reported here could be partially facilitated by the fact that, unlike in many animals, certain molecular changes (e.g., methylation, small RNAs) that characterize epialleles in plants may persist across both mitosis and meiosis (Kakutani et al. 1999; Kakutani 2002; Takeda and Paszkowski 2006). To ascertain the underlying mechanisms of the present results, studies will be needed to track changes in epigenetic marks (e.g., changes in small RNAs, methylation, chromatin formation/structure) across generations and to assess changes in heat shock proteins, stress responses, and other processes crucial to fecundity and seed survival in response to heat. Future studies should focus on revealing the range of environmental parameters that lead to transgenerational epigenetic fitness effects, the number of loci regulated by and involved in the regulation of this process and the long-term persistence of this phenomenon across generations.

Conclusions

Altogether, the present results demonstrate that plants retain an adaptive epigenetic memory of the temperature conditions of their ancestors. This finding has important implications to our understanding of plant adaptation in dynamic environments and suggests that plant populations may rapidly and heritably respond to changing growth conditions. In addition, the result has significant and practical implications for understanding variation in agronomic productivity as it suggests that crop/seed yields could be greatly altered by ancestral climate regimes.

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