

Evolution of active and passive forms of plasticity: insights from artificially selected *Arabidopsis*

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ABSTRACT

Questions: Does artificial selection targeting a trait's plasticity to a given stimulus indirectly alter its plasticity to other stimuli? Is such indirect selection more effective for active forms of plasticity (i.e. enhancements of phenotype–environment matching that improve fitness) than for passive forms of plasticity (i.e. inevitable growth or performance reductions detrimental to fitness)?

Hypothesis: Artificial selection for active plasticity to a light signal will not indirectly alter passive plasticity to cold.

Study organism: Artificial selection lines of *Arabidopsis thaliana* differentiated for plasticity to a light signal, an active form of plasticity.

Study site: Split-family laboratory experiment.

Methods: Eight replicate juvenile plants from 108 families, 18 from each selection line, were exposed to either 12 days at 5°C or a control treatment. We scored five traits on each individual: number of rosette leaves at bolting, days to bolting, length of the largest leaf at bolting, inflorescence height, and number of fruits produced. Using mixed-model analyses of variance, we tested for significant variation among families and among selection lines.

Results: We observed plasticity to cold, and it was consistent with passive plasticity. There was among-family variation for cold-mediated plasticity of rosette leaf number, but not among-line variation for this plasticity. Passive, cold-mediated plasticity did not evolve via byproduct selection, which contrasts with a previous study documenting byproduct selection on active, photoperiod-mediated plasticity.

Keywords: adaptive phenotypic plasticity, *Arabidopsis thaliana*, artificial selection, cold acclimation, genetic constraint, genetic differentiation, red:far-red, shade avoidance.

INTRODUCTION

Organisms frequently express alternative phenotypes in response to variation in the external environment, a phenomenon referred to as phenotypic plasticity. The phenotypic plasticity of a trait induced by any given stimulus will involve two conceptually distinct components.

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On the one hand, active and adaptive responses may be triggered. Such responses enhance phenotype–environment matching and, therefore, fitness. Additionally, genetic analysis can typically trace active plastic responses to a specific signal perception–transduction pathway, essentially the ‘plasticity genes’ responsible for the response (Pigliucci, 1996). On the other hand, essentially passive and inevitable reductions in metabolism and growth may not be attributable to a particular biochemical pathway because they are mediated by myriad underlying shifts in physiology and gene expression. In the more than 20 years since Smith-Gill (1983) proposed the terms ‘developmental conversion’ and ‘phenotypic modulation’ to distinguish active and passive plasticity, many other authors have pointed out a similar distinction (Pigliucci, 2001). It has also been pointed out that any particular plastic response is likely to be a mixture of inevitable/passive and adaptive/active components, but that it is often possible to classify it as predominantly one or the other (Schlichting and Pigliucci, 1995; Sultan, 1995; Dorn *et al.*, 2000; van Kleunen and Fischer, 2005).

The distinction between active and passive plasticity is relevant to the issue of constraints on the evolution of plasticity (Dorn *et al.*, 2000), and here we investigate one such constraint: the possibility that artificial selection targeting the active plasticity of a trait to a given stimulus may indirectly alter the plasticity of that trait to other stimuli. We hypothesize that this is more likely to occur if the directly- and indirectly-selected plastic responses are both predominantly active and anticipatory. This is because the two plastic responses are more likely to be subject to shared physiological or developmental constraints, to involve overlap or cross-talk in the underlying biochemical pathways responsible for signal perception and transduction, or a combination of these mechanisms. We expect that this is much less likely to occur if the indirectly selected plasticity predominantly involves passive and inevitable reductions in metabolism, growth or performance.

For investigating this issue, a valuable tool is the genomically enabled model annual plant *Arabidopsis thaliana*, in which the transition from vegetative to reproductive growth and the onset of flowering can be considered a ‘model plastic trait’ (Callahan *et al.*, 1997). In nature, *Arabidopsis* populations flower during certain seasons, with the onset of flowering being influenced by a variety of environmental signals (e.g. light quantity, light quality, photoperiod, temperature regime) and developmental status (e.g. plant size, plant age). The complex developmental and environmental regulation of flowering time has been carefully investigated with respect to the underlying physiological mechanisms and biochemical pathways regulating its plasticity (Amasino, 2004; Baurle and Dean, 2006). Flowering time plasticity has also been the focus of evolutionary ecology research (e.g. Schmitt *et al.*, 2003; Shimizu and Purugganan, 2005). This is unsurprising since the onset of reproduction is a crucial life-history trait and a frequent target of natural selection (Stearns, 1992).

A second and useful tool for exploring the quantitative genetic architecture of complex traits is artificial selection (Scheiner, 2002; Callahan, 2005; Garland and Kelly, 2006). In a previous paper, we discussed the development of artificially selected lines derived from a wild base population of *Arabidopsis thaliana* originally collected in Kendalville, Michigan (Callahan and Pigliucci, 2005). The population harboured among-family variation for rosette leaf number at bolting, the developmental transition from vegetative to reproductive growth and therefore a key life-history trait. The population also harboured among-family variation for the red:far red- (R:FR-) induced plasticity of this trait. We created six experimental lines: two control lines selected randomly, two high plasticity selection lines that responded to selection with 1.6- and 2.4-fold increases in an index of R:FR-mediated plasticity of rosette leaf number, and two low plasticity selection lines that showed 1.4- and 1.1-fold decreases in this index.

The initial goal of developing these lines was to explore correlations between two forms of active flowering time plasticity – R:FR- and photoperiod-mediated plasticity of rosette leaf number at bolting. Here, we expand that work, examining plastic responses to cold temperatures.

In addition to our own work, the cold responsiveness of *Arabidopsis thaliana* and the Kendalville ecotype (Kin-0, ABRC Stock #CS1272) has been examined previously. The Kendalville ecotype's constitutively early flowering habit has been documented in several studies (Karlsson *et al.*, 1993; Pigliucci *et al.*, 1995) and is consistent with molecular population genetic surveys indicating that it has a non-functional allele at the *FRIGIDA (FRI)* locus that results in a failure to upregulate the downstream *FLOWERING LOCUS C (FLC)* gene that can repress and delay the transition to flowering in the absence of vernalization (Shindo *et al.*, 2005).

Although much *Arabidopsis* research has emphasized how flowering time is regulated by the impact of vernalization (extended cold exposure) and the *FRI-FLC* genetic mechanism, regulation of *FLC* involves multiple upstream genes, as well as cross-talk with other floral regulatory pathways. As a result there can be *FLC*-mediated variation in flowering time even in genotypes lacking a functional *FRI* allele. For example, the upstream gene *FVE* plays an important role not only in regulating *FLC* but also in regulation of C-repeat/dehydration responsive elements (*C/DRE*) (Amasino, 2004; Ausin *et al.*, 2004; Kim *et al.*, 2004). *C/DREs* are, in addition, known to be light regulated. In *A. thaliana* plants exposed to a reduction in R:FR or carrying genetic lesions that abolish or weaken responses mediated by phytochrome B, there is a decrease in *C/DRE*-mediated activation of the cold response (Kim *et al.*, 2002).

We therefore have mechanistic reasons for expecting variation in cold-response among our six selection lines, for which we have quantified cold-induced phenotypic plasticity in a suite of traits observed at the whole-plant level. Two of these traits quantify the developmental and chronological time of the vegetative–reproductive transition: rosette leaf number at bolting and the number of days of vegetative growth before bolting. The other three are: (1) size of the largest rosette leaf at bolting, (2) inflorescence height, and (3) fruit production. All traits were estimated on individual plants from 108 families, 18 from each of six selection lines. We evenly split replicates from each family between two treatments: a 12-day exposure to 5°C temperatures and a control treatment kept at 20–22°C throughout the life cycle. We examined each family's cold-induced reaction norm as well as each line's mean reaction norm. To determine if lines or families harboured genetic variation for cold-induced plasticity, we used analyses of variance to test the significance of line × treatment and family × treatment interaction terms. We interpreted the plasticity observed as predominantly passive, because it generally involved large decreases in performance traits (i.e. size, fruit production) across all families. The plasticity of bolting time traits, however, varied among families in both magnitude and pattern. This was in stark contrast to the varying magnitude but consistent pattern of the active plasticity in this trait induced by variation in R:FR or photoperiods. Finally, passive cold-mediated plasticity did not co-vary with active R:FR-mediated plasticity.

MATERIALS AND METHODS

Plant material and experimental design

Lines and families in this study were derived from a wild population of *Arabidopsis thaliana* collected in Kendalville, Michigan, USA and maintained as a bulk population by Lehle

Seed, Inc. (Round Rock, TX, USA). M. Camara kindly shared the seed used to initiate our research population. Callahan and Pigliucci (2005) provide details of the selection protocol. Briefly, given the almost completely selfing mating system in *Arabidopsis* (Abbott and Gomes, 1989), we chose not to impose outcrossing artificially. Our line-sorting selection protocol precluded recombination among genotypes, and involved three generations (two selection episodes). Responses to selection were modest compared with studies involving inter-family or inter-population crosses and more generations (e.g. Ward *et al.*, 2000), and certainly compared with mass selection experiments with obligate outcrossers such as *Drosophila*. Nonetheless, responses were statistically significant. Additional caveats associated with this selection protocol are discussed below.

Seed for the families in this study was drawn from the four selection lines and two control lines (high plasticity: HP1, HP2; low plasticity: LP1, LP2; control: C1, C2). The maternal seed donor for each seed family in the study was a plant that had grown in high R:FR conditions without exposure to chilling. Seeds from each family were stored dry in paper envelopes at room temperature for 5 months. For a total of 108 families (18 per line), we planted eight replicate plants per family, evenly split between the two treatments ($N = 864$ plants).

Growing conditions and treatments

Seeds were plated and germinated on $8 \text{ g} \cdot \text{l}^{-1}$ agar medium adjusted to pH 5.7 and containing $4.3 \text{ g} \cdot \text{l}^{-1}$ Murashige-Skoog basal salts, $0.5 \text{ g} \cdot \text{l}^{-1}$ MES buffer, and 1 ml 1000X vitamins diluted to $1.0 \text{ g} \cdot \text{l}^{-1}$ (Sigma Chemical Co., St. Louis, MO, USA) and imbibed for 7 days in dark, cold conditions (5°C). After 15 days under 12 h/12 h light/dark conditions, seedlings were transplanted into standard greenhouse flats with 36 pots ($5.1 \times 5.1 \times 5.9$ cm deep) filled with GroMix #2 (Fafard Peat Moss Co., Ltd., Agawam, MA, USA) and placed in a growth room (Beeline Cooling Ltd., Bronx, NY, USA) set on a 16 h/8 h light/dark cycle at 20°C and 70% humidity. All lights used in this experiment were 40-W Gro-Lux tubes (GTE Sylvania Inc., Danvers, MA, USA). There were four light tubes per shelf, approximately 10 cm from the surface of the soil, with four flats per shelf. Such artificial light conditions produce high R:FR well above natural sunlight (i.e. > 5 vs. 1–1.2). While plants, including *A. thaliana*, respond sensitively to decreases in R:FR ratios (i.e. 1–1.2 vs. 0.8 or below), perception of increasing R:FR ratios greater than natural conditions do not affect equilibrium between the two photoreversible forms of phytochrome, or plant response (Smith, 1986).

We bottom-watered plants every fourth or fifth day. To chill vegetative rosettes, we used an adjacent growth room with similar conditions, but at 5°C . The rosette-stage plants were in the cold room for 12 days; lighting conditions were maintained and plants were spot watered as needed. Plants in the control treatment stayed in the warmer growth chamber during this time and were watered regularly.

Data collection and analyses

For each plant, we estimated flowering time using rosette leaf number on the date of bolting because we were especially interested in the developmental timing of bolting, the focal trait for selection in the original selection experiment. We also recorded the number of days to bolting, excluding days in the chilling treatment because we observed negligible growth and development during this time. Excluding these days makes our assessment of cold-mediated

plasticity for this trait quite conservative. On the date of bolting, we also estimated the length of the largest rosette leaf (to the nearest millimetre) and rosette leaf number, the focal life-history trait and an estimate of the developmental stage at which plants make the transition from the vegetative to the reproductive phase. Six weeks after an individual plant had bolted, we counted the number of flowering stems it had produced and counted the number of seed-containing fruits produced as an estimate of fitness.

For the five traits that were response variables, we estimated a univariate mixed model for each trait in which treatment and line were fixed effects, and family within selection line was a random effect. For tests of family and the treatment \times family interaction, the denominator was the error mean square. Selection line and the treatment \times line interaction were tested over the mean square for family within line and the treatment \times family interaction. Treatment was also tested over the mean square for the treatment \times family interaction. Failed germination reduced the number of families in a few lines, and in some cases early mortalities or missing data reduced the number of replicates per family. These did not severely compromise the balance of the experiment, and are reflected in the error degrees of freedom. Raw data and residuals were approximately normally distributed and homoscedastic.

We calculated two plasticity indices using line and family means for flowering time with and without cold treatment (this experiment, H. Callahan, unpublished data), and line and family means for these traits in control and reduced light conditions [R:FR of ~1:1 and ~1:2, respectively (Callahan and Pigliucci, 2005)]. The cold-induced plasticity index was $1 - [\text{mean for trait after cold treatment} / \text{mean for trait without cold}]$; the R:FR-induced plasticity index was $1 - [\text{mean for trait in low R:FR} / \text{mean for trait in high R:FR}]$. This index-based approach to quantifying plasticity contrasts with use of a simple trait difference between the two treatments. It was used in the original selection experiment in an effort to avoid a selection protocol that would simultaneously select for greater plasticity and later flowering (or flowering with a greater number of rosette leaves) (e.g. Zhang and Lechowicz, 1994).

We examined Pearson correlation coefficients (r) between cold- and R:FR-induced plasticity indices using line means. We were searching for significant values of r (at the $P < 0.05$ level) as evidence consistent with 'byproduct selection' having resulted in among-line differentiation for trait plasticity.

RESULTS

Bolting time traits

The effect of the cold treatment was modest and only marginally significant, slightly increasing the mean number of leaves at bolting. Differences between the two environments ranged from about 8% more leaves in the C1 line to only about 1% more leaves in the L2 line. The other bolting time trait, days to bolting, showed only rather modest (and modestly significant) plasticity to the cold treatment, with cold slightly accelerating bolting (Fig. 1D,E, Table 1).

The results of analyses of variance support the significance of differentiation among lines for the mean of these two bolting time traits (Table 1), with similar patterns of variation among lines for both traits (Fig. 1E,D). Across both treatments, the HP lines flowered with the most leaves and after more days, the LP lines with the fewest leaves and earliest. Control lines were intermediate. We also found significant among-family variation for the mean of

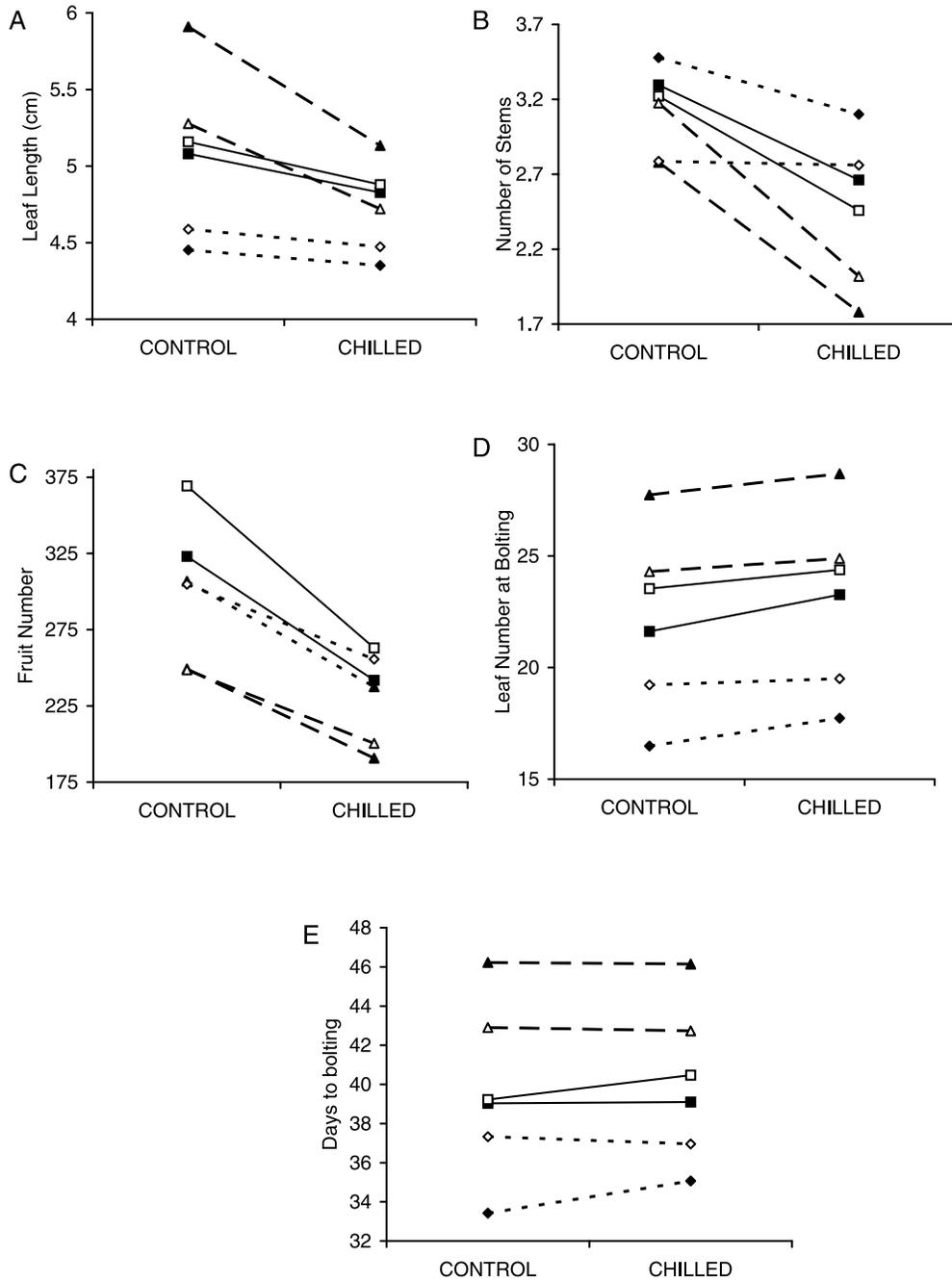


Fig. 1. Reaction norms depicting among-line and between-treatment variation for (A) leaf length, (B) number of stems, (C) fruit number, (D) rosette leaf number, and (E) days to bolting. HP1 (▲, dashed line) and HP2 (△, dashed line) are high plasticity lines; LP1 (◆, dotted line) and LP2 (◇, dotted line) are low plasticity lines; C1 (■, solid line) and C2 (□, solid line) are control lines, all from a previous artificial selection study (Callahan and Pigliucci, 2005).

Table 1. Results of univariate analysis of variance to test for effects of cold treatment and for variation among selection lines and families within lines, with least squares means within treatments (± 1 standard error)

	Line	Treatment	Treatment \times Line	Family (Line)	Treatment \times Family(Line)	Without cold	With cold
Degrees of freedom	5,95	1,95	5,95	95,551	95,551		
Rosette leaf number	8.31***	3.63 ⁺	0.21	8.56***	1.44**	22.4 \pm 0.429	23.4 \pm 0.390
Days to bolting	14.16***	5.64*	0.88	13.76***	1.45**	39.9 \pm 0.364	39.3 \pm 0.310
Leaf length	3.97**	30.27***	2.45*	7.62***	1.28	5.11 \pm 0.067	4.76 \pm 0.049
No. of stems	0.96	22.37***	1.93	4.02***	0.80	3.14 \pm 0.125	2.41 \pm 0.113
Fruit production	4.78***	47.98***	1.01	2.03***	0.96	301 \pm 8.6	231 \pm 6.1

Note: Asterisks indicate significance: $P < 0.001$ (***), $P < 0.01$ (**), $P < 0.05$ (*), $P < 0.07$ (+).

both traits, and significant variation among families for phenotypic plasticity (i.e. significant treatment \times family interaction term; Table 1).

Using estimates of line means from the current study and from our previous work (Callahan and Pigliucci, 2005), we found a very weak and non-significant correlation between the cold- and R:FR-induced plasticity indices of rosette leaf number (RLN) ($r = -0.06$, $P = 0.92$; Fig. 2A), indicating that the former had not become differentiated indirectly in response to previous artificial selection targeting the latter. The correlation between the cold-induced plasticity index of RLN and mean RLN was also weak and non-significant ($r = 0.29$, $P = 0.58$; Fig. 2B), in contrast to the correlation between the R:FR-induced plasticity index of RLN and mean RLN, which was stronger ($r = 0.68$, $P = 0.14$; Fig. 2C) but still non-significant.

Size and fitness traits

Leaf length (an estimate of rosette size) decreased plastically in response to the cold treatment (Fig. 1A, Table 1), as did stem number ($\sim 7\%$ drop) and fruit production ($\sim 38\%$ drop) (Fig. 1B,C, Table 1). There was also significant variation among the six selection lines for the mean of leaf length, with the two low lines showing especially short leaves across treatments (Fig. 1A, Table 1). There was no among-family variation for the plasticity of any of these traits, but for all three traits there was among-family variation for trait means (Table 1). Since the cold-treatment tended to increase slightly the number of leaves (Fig. 1D), above-ground vegetative size may have been about the same in the two environments. The tendency of the HP1 and HP2 lines to have lower fruit production despite having more and larger leaves at flowering (Fig. 1A,C,D) may be due to chronologically later flowering in these plants (Fig. 1E). Since the experiment was conducted in relatively small pots and without fertilizer, there may have been deterioration in environmental conditions such as nutrient levels with time.

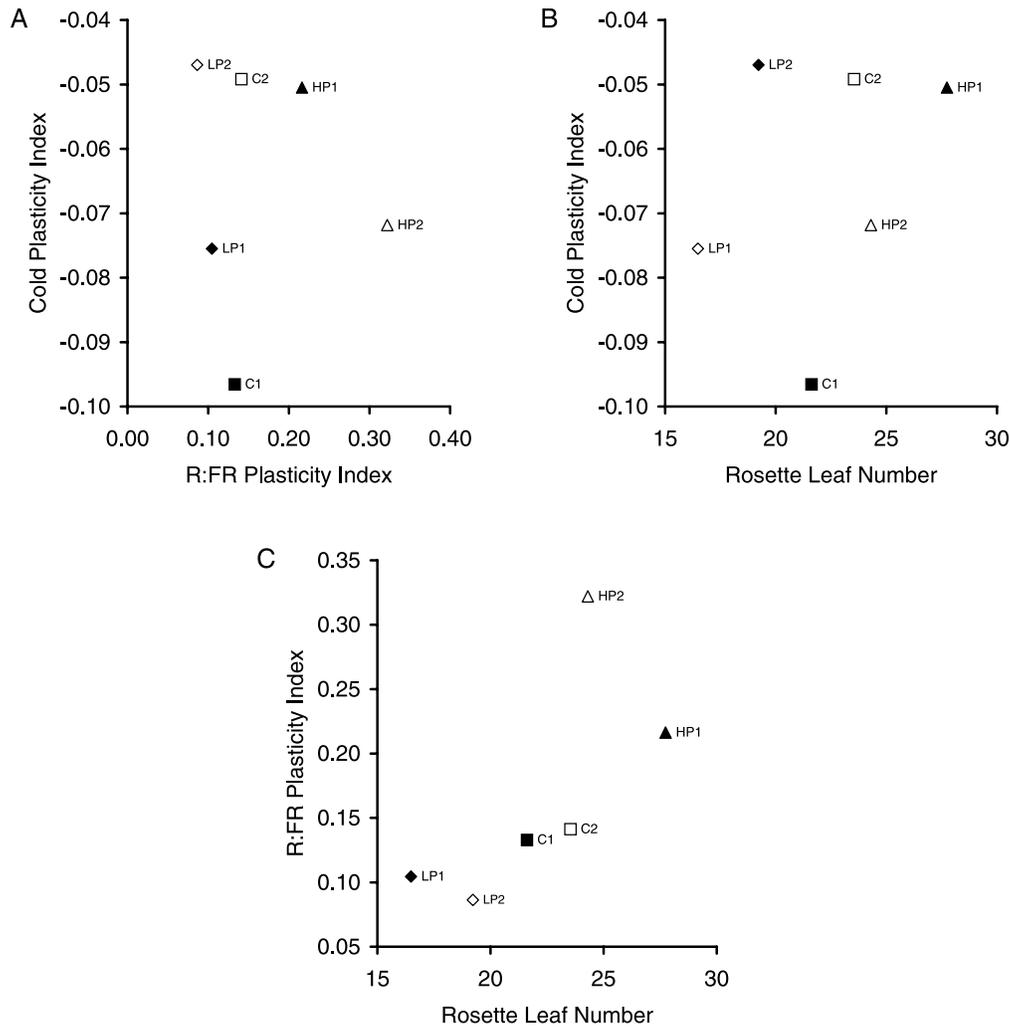


Fig. 2. Plasticity indices for mean number of rosette leaves at bolting. The indices quantifying cold-mediated responses were not correlated with plasticity indices quantifying R:FR-mediated responses (A) or with the mean number of rosette leaves at bolting (B). Plasticity indices quantifying R:FR-mediated responses do increase with the mean number of rosette leaves at bolting, but this is not significant (C). HP1 (▲) and HP2 (△) are high plasticity lines; LP1 (◆) and LP2 (◇) are low plasticity lines; C1 (■) and C2 (□) are control lines from a previous artificial selection study (Callahan and Pigliucci, 2005).

DISCUSSION

The evolution of plasticity, whether active or passive plasticity, requires genetic variation for plasticity and appropriate selection regimes (Via and Lande, 1985; Via *et al.*, 1995). In our six artificial selection lines of *Arabidopsis thaliana*, we found no evidence of line \times environment or family \times environment interactions for performance traits. Apparently, genetic variation for

the plasticity of these three performance traits to cold was rather limited in the Kendalville base population. Cold exposure resulted in plastic reductions in performance traits, consistent with inevitable rather than active forms of phenotypic plasticity (Smith-Gill, 1983; van Kleunen and Fischer, 2005).

For both bolting time traits, we found modest cold-induced plasticity. Across all six lines, the cold treatment resulted in flowering with slightly more leaves and in slightly fewer days. Along with this limited plasticity, we found limited among-line differentiation for plasticity, but significant variation for plasticity among families. In a selfing species, this among-family variation is the raw material necessary for cold-mediated plasticity to evolve, given an appropriately heterogeneous selection regime.

This returns us to our interest in whether or not differentiation for passive, cold-induced plasticity could have occurred as a byproduct of artificial selection that had altered active, R:FR-mediated plasticity. This could have occurred even in the absence of pleiotropy or developmental constraints, because genetic correlations may have existed in the Kendalville base population (i.e. due to past natural selection). In nature, this could have occurred because of past *ecological* links between patterns of variation in R:FR conditions and patterns of variation in temperature regimes. In a highly selfing species such as *Arabidopsis*, and in our lines which were developed using a line-sorting selection protocol, the two forms of plasticity could fail to respond independently to selection because of linkage disequilibrium (Lynch and Walsh, 1998). Yet this did not occur in our lines.

Overlap in the underlying genetic mechanisms that regulate R:FR- and cold-triggered plasticity could constrain their evolution. Molecular genetic studies in *A. thaliana* and other angiosperms have documented such overlap, and suggested integration of light- and cold-signalling via the C-repeat/dehydration responsive element (C/DRE) (Kim *et al.*, 2002, 2004). While our lines were differentiated for R:FR-mediated plasticity but not for cold-mediated plasticity, families within lines did show variation for cold-mediated plasticity. Perhaps the among-family variation for cold-mediated plasticity detected in our experiment reflects multiple genetic and physiological mechanisms other than those mediated by C/DRE (Xin and Browse, 2000).

We would also expect a constraint between two differently mediated forms of plasticity if the two are subject to a shared developmental constraint. This was the case in our earlier study, in which differentiation among lines for the active R:FR-mediated plasticity of rosette leaf number (RLN) resulted, indirectly, in differentiation for the active photoperiod-mediated plasticity of RLN, most likely because both types of plasticity were related to mean RLN (Callahan and Pigliucci, 2005). In the current study, we instead found no correlation between the line means for R:FR-plasticity and line means for cold-mediated plasticity. There was also no correlation between cold-mediated plasticity of RLN and mean RLN. Unlike the R:FR- and photoperiod-mediated plasticities of this trait, the R:FR- and cold-mediated plasticities of the trait appear, in the short term, to have the potential to respond independently to future selection.

The evolutionary ecology literature has often emphasized that many traits comprise the whole-organism phenotype, and that any given trait is unlikely to evolve independently of other traits (Lande and Arnold, 1983; Brodie *et al.*, 1995). Similarly, any given plasticity may fail to evolve independently, depending on how the trait's development results from detection of and response to multiple environmental inputs. Recognizing that some responses are passive and others active, and that the active and passive forms of plasticity are generally governed by quite different underlying mechanisms, we have provided a prediction about

the evolutionary trajectories of these contrasting forms of plasticity. Having supported this prediction with data from artificial selection lines, we urge other researchers using artificial selection to examine not only traits but also plasticities (Garland and Kelly, 2006), and specifically to address whether there is a contrast between the evolutionary trajectories of active and passive forms of plasticity. Clearly, much more data from *A. thaliana* and from other model and non-model organisms will be required before generalizing this prediction.

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