

A latitudinal cline and response to vernalization in leaf angle and morphology in *Arabidopsis thaliana* (Brassicaceae)

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Summary

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- Adaptation to latitudinal patterns of environmental variation is predicted to result in clinal variation in leaf traits. Therefore, this study tested for geographic differentiation and plastic responses to vernalization in leaf angle and leaf morphology in *Arabidopsis thaliana*.
- Twenty-one European ecotypes were grown in a common growth chamber environment. Replicates of each ecotype were exposed to one of four treatments: 0, 10, 20 or 30 d of vernalization.
- Ecotypes from lower latitudes had more erect leaves, as predicted from functional arguments about selection to maximize photosynthesis. Lower-latitude ecotypes also had more elongated petioles as predicted by a biomechanical constraint hypothesis. In addition, extended vernalization resulted in shorter and more erect leaves.
- As predicted by functional and adaptive hypotheses, our results show genetically based clinal variation as well as environmentally induced variation in leaf traits.

Key words: *Arabidopsis thaliana*, latitudinal cline, leaf angle, leaf morphology, population differentiation, vernalization.

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Introduction

Clinal variation in ecologically important traits is considered to be strong evidence of adaptation to geographically varying selection (Mayr, 1956; Endler, 1977; Caicedo *et al.*, 2004; Stinchcombe *et al.*, 2004; Stillwell *et al.*, 2007). Sessile organisms, such as plants, can experience considerable variation in natural selection across their range, and local adaptation to such selection can result in geographic differentiation of populations (Joshi *et al.*, 2001; Streisfeld & Kohn, 2005; Springer, 2007). Traits in leaves, the organs of photosynthesis and transpiration, are likely to experience clinal variation in selection as a result of latitudinal differences in day length, light intensity, solar angle, and temperature. One of the primary physiological constraints on plants is the need to maximize photosynthesis and minimize water loss. Extreme environments, such as deserts, have resulted in extreme morphological adaptations, such as cactus spines, but

more subtle environmental variation can also result in morphological adaptations to maximize photosynthesis while minimizing transpiration.

Leaf characteristics play a critical role in determining rates of photosynthesis and transpiration (Baldocchi *et al.*, 1985; Niklas, 1993; Smith & Whitlam, 1997). Leaf angle, the angle a leaf deviates from horizontal to ground, directly affects the flux of solar energy per unit leaf area, and is thus an important factor in determining the maximum photosynthetic ability of a plant (Ehleringer, 1988; Ridao *et al.*, 1996; Falster & Westoby, 2003). Photosynthesis models predict that optimal leaf angle at lower latitudes is more erect than optimal leaf angle at higher latitudes (Baldocchi *et al.*, 1985; Ehleringer, 1988; Herbert, 2003). Because the standard photosynthetic light response curve saturates at moderate light intensities, plants at low latitudes can actually maximize photosynthesis in the intensity of midday sun, and decrease water loss and the deleterious effects of high leaf temperatures, by increasing leaf

angle and avoiding direct vertical light on the leaf blade (Bjorkman & Powles, 1984; Ehleringer, 1988; Ridao *et al.*, 1996; Smith *et al.*, 1998; Falster & Westoby, 2003). In addition, more vertical leaves have greater photosynthetic ability at the beginning and end of the day when the sun is at shallower angles. Greater leaf angles decrease transpiration and heat damage by decreasing direct exposure to high-intensity light (Bjorkman & Powles, 1984; Ehleringer, 1988; Ridao *et al.*, 1996).

Functional studies, which predict that greater leaf angle in lower latitudes maximizes photosynthesis and decreases water loss, provide an adaptive explanation for observed latitudinal trends in leaf angle (McMillen & McClendon, 1979; Ehleringer, 1988; Halloy & Mark, 1996; Ridao *et al.*, 1996; Werner *et al.*, 1999; Barclay, 2001; Falster & Westoby, 2003; Kalapos & Csontos, 2003). Two field studies, focusing mostly on trees, have detected steeper leaf angles and smaller leaf surfaces in communities with either lower levels of precipitation or higher levels of available light (Ehleringer, 1988; Smith *et al.*, 1998). Studies of a single species across its range have also found latitudinal clines in leaf angle (Ezcurra *et al.*, 1991; Herbert, 1991, 2003). However, all of these studies were conducted by measuring traits on plants growing in the field, and therefore did not test whether the observed clines are a result of genetic differentiation, or environmentally induced plastic responses to geographic differences in growing conditions.

In contrast to the predictions about leaf angle variation, there are more complex hypotheses about how environmental variation influences selection on other aspects of leaf morphology. Previous studies have found that, in general, leaf size can increase with increasing average temperature (McDonald *et al.*, 2003; Thuiller *et al.*, 2004). Large leaves have thicker boundary layers of air around their surfaces which insulate and decrease water loss through transpiration (Parkhurst & Loucks, 1972; Schuepp, 1993; Westoby *et al.*, 2002). Furthermore, leaves from moister environments tend to be larger than leaves from drier climates (Parkhurst & Loucks, 1972; Schuepp, 1993; Thuiller *et al.*, 2004). Thus, temperature and moisture gradients across latitudinal transects may inform hypotheses about large-scale geographic variation in leaf traits. Alternatively, variation in leaf traits tends to be correlated, and thus a change in leaf size or morphology could be a result of a correlated effect of change in leaf angle. As leaf angle increases, the force required to hold up a leaf blade decreases and thus plants in lower latitudes may have elongated leaves because of a release from a biomechanical constraint (Niklas, 1999). To date, there have been few surveys of within-species variation in leaf morphology across a very large geographic range, and thus there is a need for further empirical evidence to elucidate the important factors affecting leaf size.

Variation in both leaf angle and leaf morphology observed across the range of a species could be a result of either genetic differentiation or plastic responses to environmental variation.

Temperature is one important environmental regulator of plant growth and development that varies across a latitudinal gradient. There are two important aspects of temperature: ambient temperature, the average temperature at which a plant undergoes most of its growth and reproduction, and vernalization, which refers to an extended period of cold. Studies have demonstrated a strong effect of ambient temperature on vegetative growth (Weinig, 2000; Heggie & Halliday, 2005). In particular, warmer temperatures have been shown to result in increased auxin production and thus hypocotyl elongation (Gray *et al.*, 1998). In addition, genetic pathways that have been shown to affect leaf morphology interact intricately with temperature-sensing pathways (Mazzella *et al.*, 2000; Halliday *et al.*, 2003; Halliday & Whitelam, 2003). Vernalization, or prolonged exposure to cold temperatures, has been shown to be necessary for the transition between vegetative and reproductive growth for some plants (Bastow *et al.*, 2004; Amasino, 2005; Trevaskis *et al.*, 2007), but, to date, there has been very little direct investigation of how vernalization, or over-wintering, affects leaf morphology (for an exception see Lee & Amasino, 1995). Studying how plant vegetative tissue (i.e. leaf angle and leaf length) responds to variation in vernalization may provide a more complete understanding of how plants may have adapted to variation in environmental conditions across a range of latitudes.

Here we test for latitudinal differentiation and the effect of vernalization in leaf angle and morphology in 21 European genotypes of the model species *Arabidopsis thaliana* grown under common controlled environmental conditions. *Arabidopsis thaliana* grows in a wide variety of climates across its native range (Hoffmann, 2002), and phenotypic clines suggestive of adaptive differentiation have recently been observed in several ecologically important traits (Li *et al.*, 1998; Maloof *et al.*, 2001; Stenoien *et al.*, 2002; Stinchcombe *et al.*, 2004; Lempe *et al.*, 2005). To date there have been no reports of clinal genetic differentiation in leaf angle in *A. thaliana* or other species. We also investigated two aspects of leaf morphology: leaf length, a general measure of leaf size, and leaf ratio, the ratio of leaf blade length to total leaf length. Specifically, we asked the following questions.

- Do ecotypes show variation in leaf angle and leaf morphology across a latitudinal gradient as predicted by functional arguments?
- Does the duration of vernalization affect leaf morphology and leaf angle?

Materials and Methods

Study organism

To investigate variation in leaf morphology and leaf angle across a latitudinal gradient we grew 21 genotypes of *Arabidopsis thaliana* (L.) Heynh. originating throughout Europe. Genotypes

Table 1 Stock number (from <http://www.arabidopsis.org>), name, latitude, and longitude of *Arabidopsis thaliana* genotypes used in experiment

Stock number	Name	Latitude (°N)	Longitude (°E)	Precipitation (Oct–April)	Temperature (Oct–April)
CS917	Da(1)-12	49.8	15.5	38.5	2.1
CS1352	Lu-1	55.7	13.2	53.7	2.9
CS1540	Su-0	53.7	-2.9	75.4	6.2
CS6616	Bla-1	41.7	2.8	64.6	10.8
CS6622	Bla-10	41.7	2.8	64.6	10.8
CS6626	Br-0	49.2	16.6	30.8	2.7
CS6659	Cal-0	53.3	-1.6	70.9	5.1
CS6665	Chi-1	54	34	377	-2.3
CS6669	Co-1	40.2	-8.4	97.8	12.6
CS6683	Do-0	50.7	8.2	57.1	3.6
CS6688	Edi-0	55.9	-3.2	60.4	5.0
CS6770	Le-0	52.2	4.5	67.5	5.8
CS6797	Ms-0	55.8	37.6	45.9	-2.3
CS6807	Nok-0	52.3	4.4	67.5	5.8
CS6825	Pa-1	38.1	13.4	72.4	13.3
CS6834	Pla-0	41.9	3.1	64.7	10.7
CS6839	Po-0	50.7	7.1	58.5	5.6
CS6854	Sap-0	49.8	14.4	33.5	2.9
CS6855	Sf-1	42.5	0.5	88.4	4.4
CS6867	Ta-0	49.4	14.7	38.0	2.3
CS6918	Ob-2	60.1	23.3	45.1	-1.2

Precipitation (mm) and temperature (°C) are mean values from the months of October through April for the years 1961–1990 estimated from climatological data presented in New *et al.* (2002).

were ordered from the Arabidopsis Biological Resource Center (<http://www.arabidopsis.org>) (Table 1). After germination, *A. thaliana* grows as a vegetative rosette until transitioning to reproductive growth. We took all measurements during vegetative growth.

Design and measurements

Twelve replicates with two seeds of each genotype were planted in 164-ml Ray Leach 'Cone-tainers'TM (Stuewe & Sons, Inc., Corvallis, OR, USA) filled with water saturated Scott's Metro Mix 360 Coir (Scott's-Sierra Horticultural Products, Marysville, OH, USA). A randomized block design with two replicates in each of six blocks was used. To investigate the effect of vernalization on vegetative development we planted four treatments of seeds that underwent 0, 10, 20, or 30 d of vernalization. We staggered plantings so that all vernalization treatments ended on the same day. To synchronize germination within treatments we stratified all seeds for 3 d in darkness at 4°C and then moved them into Conviron E7/2 growth chambers (Controlled Environments Inc., Pembina, ND, USA; 20°C, 10 : 14 h light:dark photoperiod) in the Brown University glasshouse for 3 d. At this point we exposed germinants to a vernalization treatment in the cold room (4°C, 10 : 14 h light:dark photoperiod). Within the cold room, plants were positioned in their randomized blocks. When the vernalization treatments were complete, we removed

all plants from the cold room, thinned to one plant per cone and moved them by block to a growth chamber compartment (20°C, 10 : 14 h light:dark photoperiod). The average photosynthetically active radiation (PAR) across all chambers was 255 $\mu\text{mol m}^{-2} \text{s}^{-1}$ as measured with an AccuPARTM model Par80 linear ceptometer (Decagon Devices, Inc., Pullman, WA, USA).

Eleven days after we removed seedlings from the vernalization treatment we used digital calipers to measure the most erect fully grown leaf, which corresponded to the most recent fully expanded leaf on each plant. Plants had 5–10 leaves at this point and we measured them in their randomized order, two blocks a day, over 3 d. Measurements were taken between 4 and 8 h after lights went on in the chambers each day to control for circadian variation (Mullen *et al.*, 2006). We measured leaf height (the distance from leaf tip to a point directly below the leaf tip and on the same horizontal plane as the apex of the rosette), leaf length (apex of rosette growth to leaf tip) and the blade length (end of the petiole to the tip of the leaf blade) (Fig. 1). Leaf ratio, the blade length divided by total leaf length, is a measure of how much of the leaf is composed of blade. A change in leaf ratio represents a disproportionate change in blade length relative to leaf length. We calculated leaf angle from the arcsine of the ratio of leaf height to leaf length.

We used three approaches to reduce sources of variation present in leaf measurements attributable to both unknown

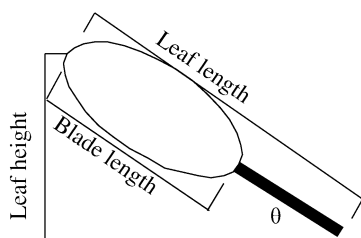


Fig. 1 Diagram of *Arabidopsis thaliana* leaf illustrating measurements of leaf length, blade length, leaf height, and leaf angle (θ). Leaf ratio = blade length/leaf length. Leaf angle (θ) = arcsine (leaf height/leaf length).

micro-environmental differences between plants in the chambers and the developmental stage of the plants (Coleman *et al.*, 1994). First, as already described, we always measured the youngest full-sized leaf on each plant, and thus measured comparably aged leaves on each individual. Secondly, to control for variation in growth and development caused by subtle environmental differences between blocks and other sources of within-accession variation, we estimated genotypic means for leaf number, leaf angle, leaf ratio, and leaf length (e.g. Rausher, 1992). Finally, to control for differences in developmental rates among accessions, we used rosette leaf number at the time of measurement as a covariate in our analyses (see following section); rosette leaf number in *A. thaliana* is highly correlated with flowering time and developmental timing (Ungerer *et al.*, 2002).

Statistical analysis

We performed a multivariate analysis of covariance (using the 'manova' statement of Proc GLM, SAS; SAS Institute, Cary, NC, USA) to test for the effects of vernalization treatment, latitude of origin, and developmental stage (leaf number) on mean leaf angle, leaf ratio, and leaf length. In these models, latitude of origin and leaf number were included as continuous variables, and vernalization treatment was a categorical variable. The data for these analyses were the genotypic means, which, as described

above, were calculated across all blocks; so there was no block effect in the MANOVA. We elected to use a MANOVA approach to account for the possibility that the response variables of interest were potentially correlated (Scheiner, 2001). Interactions between independent variables were originally included in the model but were not significant and were therefore excluded from our final analysis. For significant overall main effects, individual univariate ANCOVAs were performed to investigate how variation in each leaf trait is affected by each independent variable.

To examine the correlation between leaf traits across vernalization treatments, we first calculated least squares genotypic means of each leaf trait by treatment adjusting for latitude of origin, and number of leaves. Using these adjusted means, we estimated the pair-wise correlations between leaf phenotypes across vernalization treatments. For all statistical analyses, we report significance tests based on the original analysis as well as whether individual tests remained significant after Bonferroni adjustments.

Results

We found genetically based variation in leaf traits across the latitudinal range of *A. thaliana* included in our experiment (Table 2). All plants were grown in a common environment and analyses were performed on genotypic means of inbred lines, and thus significant effects indicate genetically based variation. The MANOVA results show an overall effect of both latitude and developmental stage (estimated as leaf number) on the measured leaf phenotypes. In particular, univariate ANCOVAs revealed a significant decrease in leaf angle with increasing latitude (Fig. 2a). Plants from higher latitudes held their leaves at a more horizontal angle relative to the ground than plants from lower latitudes. Leaf length decreased with increasing latitude (Fig. 2c), whereas leaf ratio increased with latitude (Fig. 2b). In other words, genotypes from higher latitudes have shorter leaves but proportionally larger leaf blades than plants from lower latitudes.

We performed simple correlation analyses (using Proc Corr of SAS) to determine how temperature and precipitation vary across the latitudinal transect of *A. thaliana* populations. The

Factor	MANOVA			Univariate ANOVAs					
	df	Error df	F	Leaf angle		Leaf ratio		Leaf length	
	df	Error df	F	df	F	F	F	df	F
Latitude	3	76	15.64**	1	35.70**	1	9.30*	1	17.70**
Treatment	9	185.11	5.79**	3	4.74*	3	1.12	3	12.35**
Leaf number	3	76	45.62**	1	9.07*	1	2.16	1	108.25**
Error				78		78		78	

* $P < 0.05$; ** $P < 0.0001$.

Table 2 Multivariate analysis of variance results, including F statistics for individual ANCOVAs

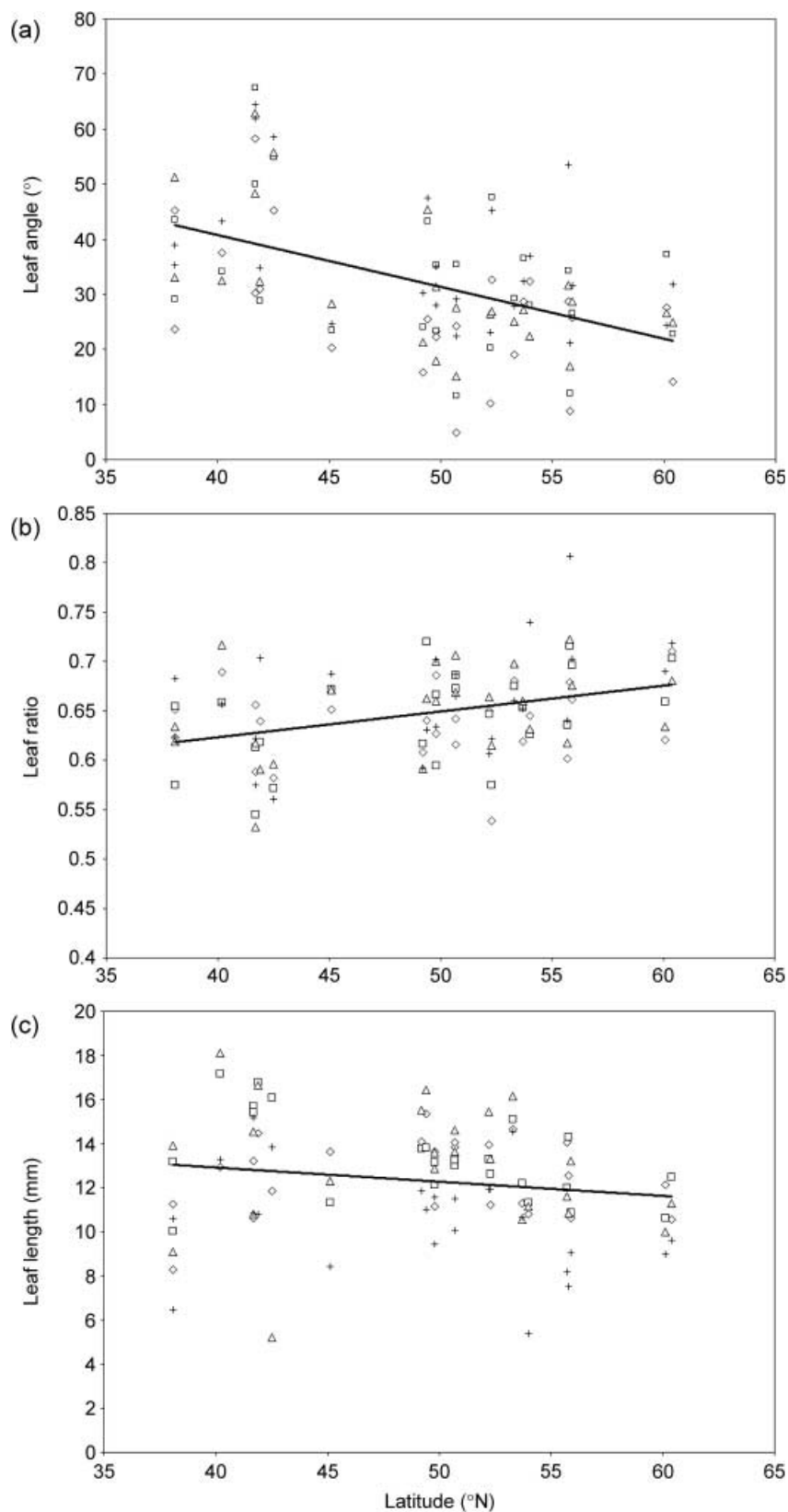


Fig. 2 The relationship between genotypic means of *Arabidopsis thaliana* leaf traits and latitude for vernalization treatment for 0 d (diamonds), 10 d (squares), 20 d (triangles), and 30 d (crosses). A solid line indicates a significant linear regression across all treatments. (a) Leaf angle shows a significant negative relationship with latitude. (b) Leaf ratio shows a positive relationship with latitude. (c) Leaf length shows a negative relationship with latitude.

	Least square mean	Treatment (days of vernalization)	0	10	20
Leaf angle (degrees)	26.079	0			
	35.382	10	0.0058		
	32.685	20	<i>0.0458</i>	0.4000	
	37.593	30	0.0006	0.4920	0.1284
Leaf ratio	0.636	0			
	0.637	10	0.9389		
	0.645	20	0.5154	0.5582	
	0.659	30	0.1112	0.1277	0.3416
Leaf length (mm)	13.330	0			
	13.003	10	0.5049		
	12.713	20	0.2068	0.5429	
	10.711	30	< 0.0001	< 0.0001	< 0.0001

Table 3 Pair-wise comparisons of least square means by vernalization treatment accounting for latitude and leaf number

Values in italics are significant at $P < 0.05$, while bold indicates a significant P -value after a sequential Bonferroni correction for multiple tests.

growing season for the rosette stage in winter annual cohorts of *A. thaliana* is generally between the months of October and April; we therefore calculated the mean temperature and mean precipitation across these months for each site of origin for each ecotype (data extracted from New *et al.*, 2002). Mean temperature and mean precipitation are strongly negatively correlated with latitude ($r(21) = -0.81$, $P < 0.0001$ and $r(21) = -0.48$, $P = 0.0278$, respectively) in our sample. There was no overall effect of temperature or precipitation when these factors were included in the above MANOVA (data not shown), so they were excluded from the final analyses.

Leaf angle also decreased significantly with increasing leaf number (i.e. developmental age) independent of the effects of latitude (Table 2, Fig. 3a). Total leaf length increased with leaf number, although leaf ratio showed no effect (Fig. 3b,c). The contrasting results for the effects of leaf number on leaf length and leaf ratio suggest that more rapidly developing accessions produce longer leaves, but that this trend is driven by changes in the petiole length rather than blade length.

Finally, our vernalization treatment had a strong effect on leaf traits, demonstrating environmentally inducible variation (Table 3). Vernalized plants had significantly more erect leaves than control plants. Longer vernalization (30 d) also resulted in significantly shorter leaves (Table 3). However, there was no detectable effect of vernalization on leaf ratio, and thus blade length and petiole length were affected proportionally by vernalization.

Discussion

Leaf traits are predicted to play an important role in adaptation to climate, but previous studies of latitudinal variation in leaf traits have not distinguished genetic variation from plastic responses to local environments. Isolating the genetic basis for a trait from a plastic environmental response is necessary to determine whether a trait is adaptive. Our common-garden

experiment with ecotypes originating from across a large geographic range enabled us to identify genetically based latitudinal variation in ecologically important leaf traits as well as to test for a plastic response to vernalization, an important environmental signal for many plant species. We found a strong genetically based latitudinal cline in leaf angle and leaf ratio and an environmental response to vernalization in leaf angle and total leaf length.

Leaf angle variation

Arabidopsis thaliana ecotypes displayed the latitudinal differentiation in leaf angle predicted from functional arguments; plants from higher latitudes had more horizontal leaves, and thus smaller leaf angles, than plants from lower latitudes. This cline was significant even after controlling for the effect of developmental stage. The pattern in leaf angle corresponds to the predicted leaf angles for optimal photosynthetic ability across the light intensity gradient associated with latitude (Falster & Westoby, 2003). These data are consistent with the hypothesis that natural selection has resulted in a close relationship between leaf angle variation and environmental variation. Further study will need to be carried out to determine the precise mechanism and fitness effects of this adaptation.

Leaf morphology variation

Leaf morphology displayed a more complicated relationship with latitude of origin. We observed latitudinal clines in leaf length as well as leaf ratio, but in opposite directions; although leaves were longer in lower-latitude ecotypes, the proportion of total length made up by blade decreased. Thus, longer leaves in lower latitudes are predominantly a result of elongated petioles and not of changes in blade length. Our results support the biomechanical constraint hypothesis,

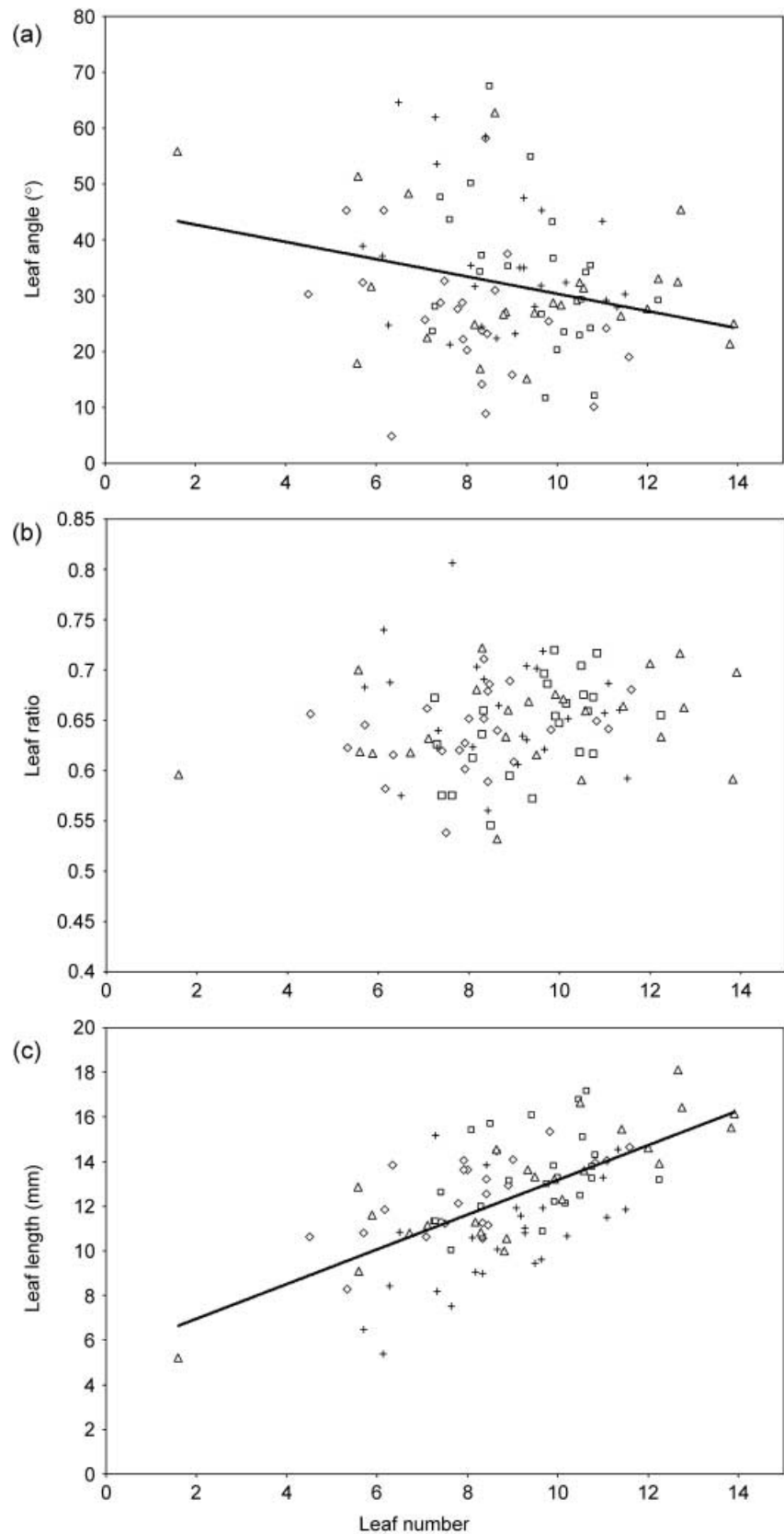


Fig. 3 The relationship between genotypic means of *Arabidopsis thaliana* leaf traits and developmental stage as measured in leaf number for vernalization treatment for 0 d (diamonds), 10 d (squares), 20 d (triangles), and 30 d (crosses). A solid line is a significant linear regression across all treatments. (a) Leaf angle shows a negative relationship with developmental stage. (b) Leaf ratio shows no relationship with leaf number. (c) Leaf length shows a positive relationship with leaf number.

which predicts that variation in leaf angle has the greatest influence on variation in leaf morphological traits (Niklas, 1993, 1999). Plants in higher latitudes with more horizontal leaves maximize midday light, but potentially suffer biomechanical constraints on petiole length as a result of the force required to hold an almost horizontal blade off the ground. Plants from lower latitudes, which have greater leaf angles, can elongate petioles to a greater extent as a result of a release from this constraint. Having longer petioles can be advantageous, particularly for plants that can grow at high density (such as *A. thaliana*) under high competition for light resources (Ballare *et al.*, 1991; Schmitt & Wulff, 1993).

Climate data from across the range of *A. thaliana* show that as latitude decreases temperature and precipitation increase, and thus adaptive hypotheses predict that as latitude decreases leaf size will increase (Parkhurst & Loucks, 1972; Schuepp, 1993; Thuiller *et al.*, 2004). Although leaf length did increase with decreasing latitude, this was attributable to petiole elongation and not an increase in blade size. Therefore, *A. thaliana* does not appear to conform to the functional hypothesis that larger leaves are more adaptive in humid, warm climates. There are clearly a number of ways to adapt to the various environmental gradients that can correlate with latitude, and our evidence shows that *A. thaliana* did not follow the predicted pattern for leaf size and humidity. Existing evidence for *A. thaliana* suggests that adaptation to water stress conditions is likely to be mediated by a combination of changes in flowering time, stomatal conductance, and transpirational efficiency (McKay *et al.*, 2003; Hausmann *et al.*, 2005; Juenger *et al.*, 2005) and not leaf size.

An advantage to working on a model system such as *A. thaliana* is the extensive genetic knowledge of the organism. Future work should investigate the molecular genetic basis of the latitudinal cline in leaf angle and leaf morphology. In particular, genes in the phytochrome family are likely candidates for influencing the differences we found in leaf characteristics. *Arabidopsis thaliana* with knockout mutations in the photoreceptor gene *PHYTOCHROME B* (*PHYB*) express leaf characteristics similar to those of low-latitude genotypes (elongated petioles and steeper leaf angles; Fankhauser & Casal, 2004; Kozuka *et al.*, 2005). It has been shown that low or no expression of *PHYB* results in elongated petioles and hypocotyls as well as more erect cotyledons (Fankhauser & Casal, 2004). In addition, *PHYTOCHROME C*, which has a similar knockout mutant phenotype to that for *PHYB*, has already been shown to be involved in a latitudinal cline in flowering time (Balasubramanian *et al.*, 2006).

Vernalization response

We also investigated the plastic response of leaf traits to vernalization, an important environmental cue for winter annuals such as *A. thaliana*. Both leaf angle and leaf morphology show a significant response to vernalization, indicating

that vegetative development is affected by extended cold temperatures. Our results indicate that, regardless of latitude of origin, without vernalization (treatment zero) leaves are more horizontal than with vernalization. After long periods of vernalization (30 d), elongation of both blade and petiole is suppressed. Our data support the findings from ambient temperature studies which suggest that light- and temperature-signaling pathways interact, resulting in suppressed elongation with exposure to cold temperatures (Gray *et al.*, 1998). Because we found reduced elongation in plants even after they had been removed from cold temperatures, our study indicates either that the temperature response may have a long degradation period, or that the vegetative response to vernalization is determined early and is not reversible (which would be similar to flowering time responses to vernalization mediated by epigenetic changes to regulatory genes; e.g. Sung *et al.*, 2006). Plants from all latitudinal origins responded equally to vernalization, and thus the observed clinal variation in leaf traits is not attributable to variation in response to vernalization. Smaller leaves may not be an adaptive response to long vernalization but rather may be a result of the cost of physiological maintenance for prolonged periods at cold temperatures.

Differentiation in *Arabidopsis thaliana*

Recently, Nordborg *et al.* (2005) and Schmid *et al.* (2006) have documented that *Arabidopsis thaliana* exhibits appreciable population structure at neutral loci, including some evidence of north–south differentiation. It is therefore possible that the latitudinal differentiation that we have detected is caused by nonselective mechanisms rather than adaptation to latitudinally varying cues. With sufficient data on polymorphism at neutral loci, it is possible to statistically control for background genetic differentiation while testing for clines (e.g. Korves *et al.*, 2007), and such an approach would provide the most rigorous test of whether the observed clines are adaptive. Although this approach was not feasible in our limited sample of genotypes, we suggest that neutral forces resulting in the observed variation of leaf traits is unlikely for two reasons. First, the detected clines in the present study are in quantitative traits, which are probably controlled by several to many genes and environmental factors, making the evolution of clines by purely stochastic factors less likely. Secondly, the observed clines in *A. thaliana* (leaf angle in this study; Maloof *et al.*, 2001; Stenoien *et al.*, 2002; Stinchcombe *et al.*, 2004, 2005; Lempe *et al.*, 2005) are in directions predicted *a priori* by functional arguments, while stochastic processes should be just as likely to produce clines that are either consistent or inconsistent with functional arguments.

Conclusions

Although our study does not show the fitness consequences of various leaf morphologies across environments, physiological

models have predicted the adaptive advantage of the patterns we found. We found genetically based clinal variation in leaf morphology and leaf angle that maximizes photosynthesis under varying light conditions. In addition, we reported an environment-induced response to vernalization duration consistent with previous studies investigating the effect of temperature on leaf traits.

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