

Arabidopsis thaliana populations show clinal variation in a climatic gradient associated with altitude

Alicia Montesinos-Navarro^{1,2}, Jennifer Wig^{1,3}, F. Xavier Pico² and Stephen J. Tonsor¹

¹Department of Biological Sciences, University of Pittsburgh, 162 Crawford Hall, 4249 Fifth Avenue, Pittsburgh, PA 15260, USA; ²Departamento de Ecología Integrativa, Estación Biológica de Doñana (EBD), Consejo Superior de Investigaciones Científicas (CSIC), Isla de La Cartuja, Av Americo Vesputio, s/n 41092 Sevilla, Spain; ³Department of Forest Ecosystems and Society, 321 Richardson Hall, Oregon State University, Corvallis, OR 97331, USA

Summary

Author for correspondence:
Alicia Montesinos-Navarro
Tel: +34 954466700 ext. 1462
Email: ali.montesinos@gmail.com

Received: 22 June 2010
Accepted: 11 August 2010

New Phytologist (2011) **189**: 282–294
doi: 10.1111/j.1469-8137.2010.03479.x

Key words: altitude, *Arabidopsis thaliana*, climate change, climatic gradient, clinal variation, genetic differentiation, local adaptation, natural variation.

- Understanding the adaptive basis of life history variation is a central goal in evolutionary ecology. The use of model species enables the combination of mechanistic knowledge with ecological and evolutionary questions, but the study of life history variation in natural environments is required to merge these disciplines.
- Here, we tested for clinal variation in life history and associated traits along an environmental and altitudinal gradient in the model species *Arabidopsis thaliana*. Seventeen natural populations of *A. thaliana* were geo-referenced in north-eastern Spain on a gradient in which precipitation increases but maximum spring temperature and minimum winter temperature decrease with altitude.
- One hundred and eighty-nine genotypes from the 17 populations were grown under uniform controlled conditions. Variations in traits related to biomass allocation, fecundity, phenology and vegetative growth were tested for relationships with the altitude and climatic variables associated with the home sites. Above-ground mass, number of rosette leaves at bolting, developmental time and seed weight increased with the home site's altitude. Root allocation, vegetative growth during winter and number of seeds decreased with altitude.
- We suggest that the differences among home sites provide clues to the variation in adaptive strategies associated with the climatic gradient. We compared these results with adaptations and clinal relationships reported for other species and with molecular mechanisms described in *Arabidopsis*.

Introduction

Adaptation is a process of genetic change that increases the likelihood of survival and reproduction in a given environment (Endler, 1986). An evolutionary response to novel selective pressure on traits such as phenology, biomass allocation, growth rate and life history can allow an organism to persist in newly encountered environments. Understanding the potential for and constraints on adaptation to altered environments has important implications in the areas of conservation biology, global change biology and the evolution of species' ranges.

The study of adaptation requires that we relate genotypic and associated phenotypic variation to the environment in which variants have evolved or survived. A cline in geneti-

cally based variation in ecologically important traits, when associated with an environmental gradient, is considered to be strong evidence of adaptation to geographically varying selection (Mayr, 1956; Endler, 1977, 1986; Caicedo *et al.*, 2004; Stinchcombe *et al.*, 2004; Stillwell *et al.*, 2007). Altitudinal gradients have been commonly used to study clinal adaptation (Clausen *et al.*, 1940; Williams & Black, 1993; Oleksyn *et al.*, 1998; Angert & Schemske, 2005; Brennan *et al.*, 2009; Gonzalo-Turpin & Hazard, 2009). One of the main advantages of altitudinal gradients is that they offer steep environmental gradients across short spatial distances, representing exciting biological experiments in nature (Körner, 2007). Of course, it is not altitude itself to which organisms adapt. Instead, a suite of environmental gradients accompanies changes in altitude. By using

altitudinal gradients it is possible, within a circumscribed region, to study a species' response to a suite of climatic conditions that are representative of the broader latitudinal range of a species.

An explanation of how a pattern of clinal adaptation arose can often lead to a broader understanding of adaptation and its constraints. This requires both an understanding of the network of molecular, physiological and morphological mechanisms that underlie adaptive phenotypic evolution (Lepetz *et al.*, 2009) and the functional effects of variation in these mechanisms in the environmental context in which the adaptations arise. Although studies of local adaptation abound (see Linhart & Grant, 1996; Reznick & Ghalambor, 2001), an understanding of the underlying mechanisms driving adaptation lags behind. This is partly a result of the difficulty of integrating multidisciplinary knowledge from the internal biology with an understanding of the natural environments in which the species has evolved.

For the model plant, the mouse-ear cress, *Arabidopsis thaliana* (L.) Heyhn. (Brassicaceae) (hereafter *A. thaliana*), detailed knowledge of internal biology (Meyerowitz & Somerville, 1994) and the molecular genetic basis of this biology is accumulating with extraordinary rapidity (reviewed in Sheldon *et al.*, 2000; Blazquez *et al.*, 2006; Holdsworth *et al.*, 2008; Ishida *et al.*, 2008), making this species ideal for the study of adaptive polymorphisms (Tonsor *et al.*, 2005; Metcalf & Mitchell-Olds, 2009). Further, latitudinal clines have been noted in functional genetic variation underlying several traits, including flowering time (Stinchcombe *et al.*, 2004; Wilczek *et al.*, 2009), heat shock protein expression (Tonsor *et al.*, 2008), response to vernalization (Hopkins *et al.*, 2008), freezing tolerance (Hannah *et al.*, 2006; Zhen & Ungerer, 2008), and size and growth rate (Li *et al.*, 1998). However, the study of the natural variation in ecologically important traits in the field (Donohue *et al.*, 2005a,b; Handley *et al.*, 2005; Hannah *et al.*, 2006; Boyd *et al.*, 2007; Lundemo *et al.*, 2009) and its ecology in natural populations (Arany *et al.*, 2005; Montesinos *et al.*, 2009; Bomblies *et al.*, 2010) is just beginning. An understanding of the process of adaptive evolution in *A. thaliana* in nature requires the identification and linkage of important adaptive traits to the key environmental features that have historically acted as selective sieves (Metcalf & Mitchell-Olds, 2009).

Interest in adaptation has stimulated efforts at inferring *A. thaliana*'s phylogeography across its native range in western Eurasia (Sharbel *et al.*, 2000; François *et al.*, 2008), its rapid worldwide expansion (Provan & Campanella, 2003), and has uncovered the extent of admixture from multiple Pleistocene refugia in much of its range (Mitchell-Olds & Schmitt, 2006). To understand the nature and causes of adaptation to divergent environments, it is highly

desirable to work in those areas in which recent migration and admixture are minimal.

The Iberian Peninsula served as the Mediterranean's Pleistocene refuge and *A. thaliana* has been present there for more than 10 000 yr (Sharbel *et al.*, 2000). *Arabidopsis thaliana* in Iberia shows strong genetic isolation by distance among regions associated with major geographic barriers that divide the Peninsula (Picó *et al.*, 2008). Populations in north-eastern Spain belong to a distinct genetic group and are strongly differentiated from populations in the other Iberian geographic regions, interpreted as a common ancestry within the region and a history of isolation from other regions (Picó *et al.*, 2008). Within the north-eastern region, isolation by distance is not detectable (Montesinos *et al.*, 2009).

In the altitudinal gradient of this study, the climatic conditions are based on the confluence of Atlantic and Mediterranean influences, as well as on elevation itself (Del Barrio *et al.*, 1990; Martínez *et al.*, 2007). Low altitudes are expected to be associated with moderate precipitation and cool temperatures in the winter and low precipitation and high maximum temperatures in spring. By contrast, higher altitudes are associated with increased precipitation and lower minimum temperatures in the winter and a prolonged cool and moist spring.

We predict that, at low altitudes, selection will favor the maximization of winter growth, as winter rosettes of many species are capable of net carbon gain when above freezing in winter (Regehr & Bazzaz, 1976). We also expected selection at low altitudes to favor mechanisms that increase allocation to roots as a response to low spring moisture, and the avoidance of or tolerance to heat and drought during late spring through early flowering. By contrast, at high altitudes, we hypothesized that selection would favor the maximization of growth at the earlier onset of favorable conditions in the autumn, tolerance of extreme cold during winter, and prolonged growth and delayed flowering in the spring.

Here, we report our test for the gradual quantitative genetic differentiation of 17 natural *A. thaliana* populations collected along an altitudinal gradient from 100 to 1600 m above sea level (asl). We grew plants under controlled conditions that recreated the average temperatures and photoperiod across the altitudinal gradient during the growing period of the species. Because we were interested in adaptation in both geographic and climatic terms, we structured the test in three sequential steps: (1) we characterized the altitudinal gradient based on seasonal precipitation and temperature; (2) we tested for a relationship between genetically based trait variation and the altitude of each genotype's origin; and (3) we described the relationship between genetically based trait variation and the climatic gradient associated with altitude.

In this approach, a significant relationship between phenotype in the common environment and the altitude/

environment of origin is sufficient evidence of a nonrandom evolutionary genetic cause for the cline in phenotype (i.e. not caused by genetic drift). However, two causes are hypothetically possible, one historical accident and one adaptive.

The first posits an historic artifact in the founding of the populations and consequent isolation by distance. In this scenario, high- and low-altitude sites could have been colonized by distinct lineages that were differentiated at the time of colonization. Colonization would have to have been followed by spread to intermediate altitudes from the high- and low-altitude colonization sites, followed by continued isolation-by-distance gene flow. In this scenario, natural selection would have played no role in establishing the relationship between altitude and phenotype.

By contrast, the entire landscape may have been colonized by either a single lineage or by multiple lineages. In this scenario, there would have been no relationship between altitude and the distribution of genetically based variation in phenotype at the time of colonization. Only over time, through the action of natural selection, would genotypes have sorted out along the altitudinal gradient, or have differentiated *de novo* along the gradient.

In this article, we measured phenotypes under controlled environmental conditions, testing for a relationship between multivariate trait variation and the altitude of origin of the lineages. In the discussion, we consider the two possible causes for the patterns outlined above, asking what our previously published patterns in neutral genetic variation can tell us about the first potential cause, and then placing the relationship between trait values and environment in the context of previous ecological work to determine how our growth chamber measures can be interpreted in terms of previously observed patterns of adaptive variation.

Materials and Methods

Study species, source populations and experimental design

Arabidopsis thaliana is a highly selfing annual plant (Abbott & Gomes, 1989) for which life history timing varies among populations from different parts of the world (Baskin & Baskin, 1972). Two distinct life cycles have been described. Winter annuals germinate in autumn, overwinter as a rosette, and flower and fruit the next spring. By contrast, spring annuals spend the winter as seeds, germinate, and flower and fruit the following spring, thus completing their entire life cycle during a single season. Both winter annual and spring annual *A. thaliana* occur in north-eastern Spanish populations (Montesinos *et al.*, 2009). Demographic surveys conducted in the same region revealed that the earliest germination of *A. thaliana* occurs in September. Flowering starts in March at low elevation and is delayed until April at higher altitudes. Seed production ends by mid-June (Montesinos *et al.*, 2009).

We sampled 17 populations distributed approximately evenly along an altitudinal gradient from 109 m to 1668 m asl (Fig. 1). All sites were located in different watersheds, avoiding a substructure of sites associated with local landscape features. We collected seed from individuals haphazardly chosen from multiple patches in each population on the day of final spring census. We collected seeds from 11 populations in 2006 and six populations in 2008 (Fig. 1). This made 189 lineages available for this experiment, each lineage descended from a single plant in the field. The mean number of lineages per population was 11.1 ± 4.8 (see Table 1 for details). For 111 of these lineages, it was possible to record all the traits measured in our experiment (see the 'Statistical analyses' section).

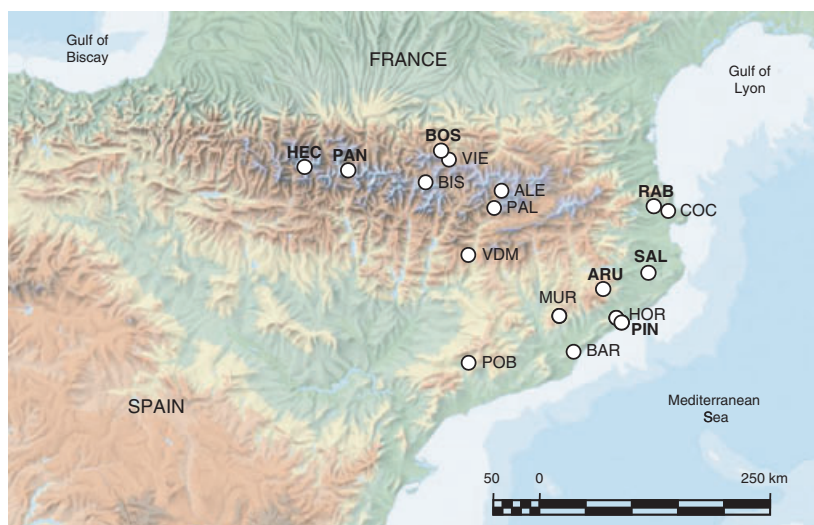


Fig. 1 Geographic locations of population sites used in the study. Figure adapted from Montesinos *et al.* (2009). Populations in bold are those newly collected in 2008. Colors indicate altitudes, going from low to high elevation: green, yellow, brown and white, respectively.

Table 1 Information on the source populations

Population	<i>n</i>	Altitude (m asl)	Year of seed collection	UTMX 30N	UTMY 30Y	Minimum <i>T</i> (°C) Sep–Feb	Maximum <i>T</i> (°C) Mar–Jun	Minimum precipitation (mm) (Mar–Jun)	Minimum precipitation (mm) (Sep–Feb)
PIN	7	109	2008	971.3	4627.4	3	25	32.2	52.3
RAB	13	110	2006	998.5	4709.5	3	24	40.9	50.5
SAL	7	332	2008	990.6	4658.7	1	24	42.6	50.3
BAR	11	429	2006	928.8	4599.8	4	20	19.7	24.7
HOR	13	431	2006	968.2	4628.1	2	24	36.7	56.1
ARU	9	440	2008	956.4	4643.8	2	23	48.9	54.1
COC	14	519	2006	1010.6	4703.1	2	23	45.1	48.7
POB	9	656	2006	836.9	4586.0	6	25	19.9	43.6
BOS	7	719	2008	802.0	4743.4	–2	20	58.0	64.0
MUR	5	836	2006	916.3	4625.9	–1	23	40.7	45.2
VDM	16	975	2006	831.7	4661.7	–4	24	35.4	41.4
ALE	16	1225	2006	855.7	4704.6	–4	22	37.5	33.9
HEC	2	1238	2008	688.3	4747.5	–3	21	64.4	100.8
PAL	16	1433	2006	854.1	4696.9	–6	20	37.7	32.0
BIS	17	1450	2006	790.7	4710.3	–5	20	70.1	71.2
VIE	19	1620	2006	808.7	4726.4	–5	18	74.7	71.8
PAN	8	1668	2008	726.6	4738.1	–5	16	81.5	115.8

n, number of individuals collected and used in this study. UTMX and UTM Y are the global positioning Universal Transverse Mercator (UTM) coordinates in the north–south and east–west directions, respectively.

Individuals collected in 2006 were passed through one generation of growth during 2007 by single-seed descent in controlled environmental chambers at the University of Pittsburgh to increase seed numbers (= bulked). Seeds collected in 2008 were sufficient in number and were used directly (= not bulked). We tested for two possible differences between the 2006 and 2008 seeds: differences caused by being bulked or not bulked and differences caused by year of collection. These potential effects would be completely confounded. We performed a multivariate analysis using all the traits measured in our experiment (see the 'Phenology monitoring and trait estimates' section) as dependent variables and year of collection/bulked vs not bulked as an independent variable. No significant differences were detected ($n = 111$; Wilks' lambda = 0.93; $F = 0.48$; NumDF = 16; DenDF = 93; $P = 0.95$). Thus, the effects of bulked/not bulked seed origin and year of collection were not considered in further analyses.

The climatic data of the 17 sites included in this study were extracted from the digital climatic atlas of the Iberian Peninsula (http://opengis.uab.es/wms/iberia/en_index.htm). This atlas uses 50-yr averages of monthly precipitation and minimum and maximum temperature from meteorological stations from Spain and Portugal. Spatial interpolation is employed to produce a surface of continuous values covering the entire Peninsula (Ninyerola *et al.*, 2005). We extracted monthly information associated with the Universal Transverse Mercator (UTM) Geographical Positioning System co-ordinates of the populations. Our goal was to extract historical values of climatic variables reflecting altitude-associated differences in climate during

the known growing season for *A. thaliana* at these sites. September–February are the months of germination and growth, whereas March–June are the months of flowering and fruiting (Montesinos *et al.*, 2009). The climatic variables chosen represent the extremes of climate that were expected to be strong selective forces on phenotype during critical periods in the life history of *A. thaliana* at these sites: maximum temperature during spring at low elevation; minimum temperature during winter at high elevation; and minimum precipitation in spring and autumn at both high and low elevation. From the atlas data, we therefore extracted two temperature and two precipitation variables: the maximum 50-yr monthly average temperature recorded between March and June; the minimum 50-yr monthly average temperature recorded from September to February; the minimum 50-yr monthly average precipitation from March to June; and the minimum 50-yr monthly average precipitation from September to February.

In addition to the historic data, we obtained field soil temperature data on an hourly basis over a 2-yr period from 2007 to 2009, using Hobo UA-002-08 temperature loggers (<http://www.OnSetcomp.com>) placed 5 cm below the soil surface at each site. We used these specific temperatures as a reference to set growing conditions in the controlled environment.

Growing conditions in the controlled environmental chamber

Our growing conditions represent a single environment based on measured conditions at the midpoint of the gradient.

Further, all seeds were germinated under simulated autumn conditions. A separate study (A. Montesinos-Navarro *et al.*, unpublished) was conducted to examine clines in seed dormancy and germination.

Seeds were placed on moistened filter paper in Petri dishes and stratified at 4°C in the dark for 4 d to promote germination. Following stratification, all Petri dishes were placed in a single diurnal cycle consisting of 10 h light (25°C) and 14 h dark (16°C). After 3 d, three to four germinated seeds per lineage were transferred to pots with Metromix-grade Turface (<http://www.turface.com>) topped with 1 cm of Sunshine germination mix (<http://www.sungro.com>) in which an aliquot of 1.5 ml of Nutricote controlled-release fertilizer (NPK 20-20-20; <http://www.sungro.com>) was placed *c.* 5 cm below the surface. After 1 wk, plants were thinned to one seedling per pot. A single individual from each field-collected lineage was used in the study, resulting in a total of 189 study individuals. The plants were randomized within eight racks which were, in turn, randomized in their location within the growth chamber every 4 d to average microenvironmental effects across racks. After 30 d of growth, all the plants were exposed to the same vernalization treatment: 10 h : 14 h light : dark and constant temperatures of 4°C for 42 d. After vernalization and until the plants produced seeds, they were grown in a Conviron PGW36 controlled environmental chamber (<http://www.conviron.com>) under 10 h : 14 h light : dark at temperatures ramped between 10 and 18°C night : day. This temperature range corresponds to the minimum and maximum values of the weekly mean temperature averaged across populations between April and May 2007 recorded directly in the field. The plants were watered every day to field capacity with an automated ebb-and-flood system. With these conditions, we exposed the plants to nonlimiting resources, allowing them to develop without stress caused by temperature, nutrient or water limitations. When each individual bolted, the main stem was inserted into a clear plastic cylinder (16 cm × 46 cm) through a small hole in the cylinder base. The top of the cylinder was covered with a screen cap. The entire inflorescence developed inside the cylinder, ensuring self-fertilization. When seeds were released, they were collected from the bottom of the cylinder and used in as yet unpublished studies (A. Montesinos-Navarro *et al.*, unpublished) of dormancy and germination.

Phenology monitoring and trait estimates

The 189 individuals were monitored every 2 d. For 111 individuals, we were able to record all traits. We recorded the timing of developmental stages as the number of days from the day of germination to: emergence of the first two true leaves (Germination–Two true leaves); elongation of the internodes and first flower bud production (Bolting);

opening of the first flower (Flowering); protrusion of the first fruit beyond the corolla (Immature fruit); and maturation of the first fruit, noted by the fruit's loss of chlorophyll (Mature fruit). These data were used to calculate the number of days between each two consecutive stages: Two true leaves–Bolting, Bolting–Flowering, Flowering–Immature fruit and Immature fruit–Mature fruit.

The number of leaves in the rosette was counted at the start and at the end of the vernalization treatment. These data were used to calculate the number of rosette leaves produced during the vernalization treatment (Rosette growth during vernalization) as [(rosette leaves after vernalization) – (rosette leaves before vernalization)]. The number of leaves was sometimes quite large. To ensure accuracy, the number of leaves per rosette was therefore counted twice within 5 d after bolting and the two counts were averaged to estimate the rosette leaf number at bolting (Rosette leaves at bolting).

When plants had senesced and released all seeds, the above- and below-ground parts were separated and weighed to obtain above-ground biomass (Above-ground mass) and below-ground biomass (Below-ground mass) after drying at 30°C for 3 d. From these data, the total mass was calculated as the sum, and the percentage of the total biomass allocated to roots (Percentage of root) was calculated as [(below-ground mass/total mass) × 100].

All the seeds collected per individual were pooled and weighed on a Cahn microbalance. For each individual, three samples of 50 seeds were counted and weighed and the values were averaged across the three samples (50 Seeds mass). The total number of seeds (Number of seeds) was calculated as the mass of all the seeds divided by the (50 Seeds mass)/50. The means, standard errors and sample size of all traits for each population are presented in Supporting Information Table S1.

Statistical analyses

We performed three sets of analyses. First, we characterized the climatic gradient associated with altitude in our study area. Second, we tested for clinal differentiation associated with altitude. Finally, we described the pattern of variation and the independent contributions of multiple traits to the multivariate correlation with altitude and its associated variation in climate.

The environmental gradient associated with altitude was characterized through univariate linear regressions of the population mean values of the climatic variables on altitude to visualize the relationship with individual climatic variables (Figs 2, 3). We also conducted univariate regressions between population trait means and altitude. Although they are not appropriate to test for associations among trait values and altitude because they do not take into account correlations among dependent variables, they allow the

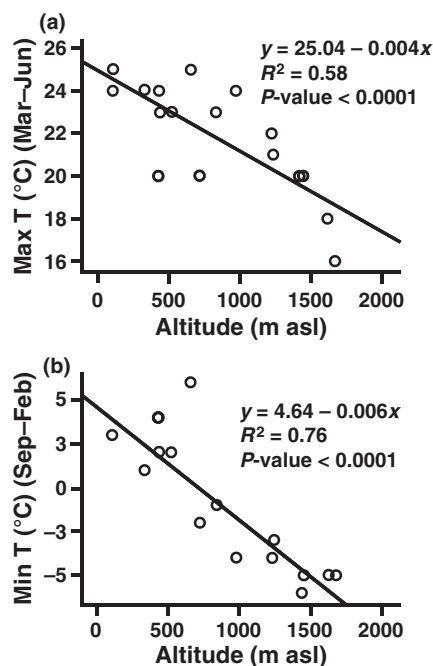


Fig. 2 Scatter plots and fitted regression lines using the mean temperatures of the population sites as dependent variables and the altitude of the site as the independent variable. (a) Maximum temperature (March–June). (b) Minimum temperature (September–February).

visualization of the clinal nature variation in life history traits along the altitudinal gradient. Univariate regressions were performed in SPSS (version 17.0; IBM Company Headquarters, Chicago, Illinois, USA).

The overall clinal genetic differentiation associated with altitude was tested using a multivariate regression of all the measured traits as dependent variables and altitude of the sites of origin as the independent variable. The analyses were performed using the 'MANOVA' statement of PROC GLM in SAS (version 9.2; SAS Institute Inc., Cary, North Carolina, USA). Each field-collected line with every trait measured was treated as a replicate in this analysis; although 189 lineages were grown, not all measures could be made in all lineages, reducing the multivariate analyses to a sample size of 111. In a previous study of 10 of our current 17 populations, we observed that 62% of all single nucleotide polymorphism (SNP)-based genetic variation was within populations, and 15–18% of loci differed among individuals (Montesinos *et al.*, 2009). Because of this high level of genetic variation within populations, we use individual lineages as replicates in multivariate analysis to best assess the proportion of all genetic variation that is associated with altitude and environment.

The independent contributions of the traits to the multivariate correlation with altitude and environmental factors were assessed using canonical correlation analysis (CCA). CCA establishes the linear combination of traits and envi-

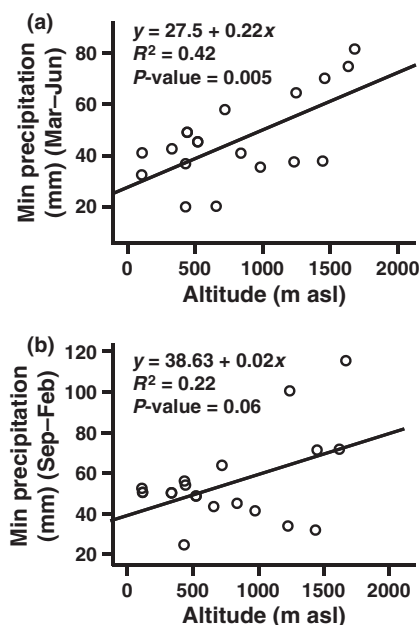


Fig. 3 Scatter plots and regression lines using the mean precipitation of population sites as dependent variables and the site altitudes as the independent variable. (a) Minimum value of the mean precipitation (March–June). (b) Minimum value of the mean precipitation (September–February).

ronmental variables that maximizes the Pearson product-moment correlation between two matrices, one combining traits and the other combining environmental variables. These analyses account for both among-trait and among-environmental variable correlations.

The magnitude of a trait's coefficient indicates the extent to which a trait is correlated with the environmental gradient after accounting for the correlations with the rest of the traits (Scheiner & Gurevitch, 2001). We used standardized canonical coefficients, thus allowing comparison of the relative magnitudes of the effects of each trait. The squared canonical correlation coefficient was used to describe the proportion of the total variation explained by each variate.

In CCA, a set of linear combinations (variates) is produced, each orthogonal to the others. The number of variates is equal to the number of variables contained in the smallest matrix, in this case the altitude or environmental matrices. As altitude is a single variable, only one canonical variate vector was produced in its analysis. We used the four previously described climatic variables: minimum temperature (September–February), maximum temperature (March–June), minimum precipitation (September–February) and minimum precipitation (March–June). Because this canonical correlation used four environmental variables, four canonical variates were produced. The analyses were performed using PROC CANCORR in SAS (version 9.2; SAS Institute Inc., Cary, North Carolina, USA). To test for similarity between altitude and the significant climatic

variates, Pearson's correlation coefficient was calculated between the standardized canonical coefficients in the two vectors.

Univariate Pearson's correlation coefficients among all pairs of traits were calculated using PROC CORR in SAS (version 9.2; SAS Institute Inc., Cary, North Carolina, USA) (Table S2).

Results

Altitudinal gradient in climatic conditions

The climatic conditions in our study region vary dramatically with altitude during the September–June window in which the plants develop (Table 1). Spring–summer maximum temperatures are higher at the sites of lower altitude populations (Fig. 2a) and autumn–winter minimum temperatures are lower at sites of high-altitude populations (Fig. 2b). The maximum temperature (March–June) along the altitudinal gradient ranges from 16°C to 25°C (Fig. 2a), whereas the minimum temperature (September–February) ranges from –6°C to 6°C (Fig. 2b). In both cases, the temperatures decrease with altitude ($P < 0.001$, $R^2 = 0.58$ and 0.76, respectively). Spring–summer precipitation increases with population altitude. The minimum value of the mean monthly precipitation when *A. thaliana* is flowering and fruiting (March–June) ranges from 19.7 to 81.5 mm, and increases significantly with altitude ($P = 0.005$, $R^2 = 0.42$, Fig. 3a). Across the source populations, the minimum value of the mean monthly precipitation in autumn–winter (September–February), when *A. thaliana* is germinating and growing, ranges from 24.7 to 115.8 mm, and shows a marginally significant increase with altitude ($P = 0.06$, $R^2 = 0.22$, Fig. 3b).

Genetically based differentiation associated with altitude and climate

Genetically based trait variation is significantly associated with altitude, as indicated by the highly significant multivariate regression of all traits combined on altitude ($n = 111$; Wilks' lambda = 0.45; $F = 11$; NumDF = 11; DenDF = 99; $P < 0.0001$). Altitude explained 55% of the

multivariate trait variation (squared canonical correlation = 0.55, $\lambda = 1.22$). A separate canonical correlation using the four climate variables explained a similar amount of trait variation ($r^2 = 0.58$, $P < 0.0001$; Table 2). Figs 2–4 show the results of univariate regressions, illustrating the clinal nature of the variation.

The CCAs using the climatic variables generated one significant variate (Table 2). The nonsignificant variates are not discussed. The significant variate describes a climatic gradient mainly characterized by conditions from September to February. This is indicated by the higher absolute value of the standardized canonical coefficients for minimum temperature (–0.76) and precipitation (0.61) from September to February, rather than the same climatic variables from March to June (–0.13 and 0.19 for maximum temperature and minimum precipitation, respectively) (Table 2). Along this axis, minimum temperature in September to February decreases, whereas minimum precipitation in September to February increases. This pattern of decreasing temperature and increasing precipitation is similar to the characterization of the climatic gradient associated with altitude in our study area, described in the previous section. The magnitude and sign of the standardized canonical coefficients for each trait are similar when the canonical correlation is performed using either altitude or the four climatic variables (correlation of coefficient from the altitude CCA with that from the climate CCA is 0.97; Table 3).

Our study plants have evolved more rapid development from germination to the production of two true leaves at higher altitudes (standardized canonical correlation coefficient –0.1; Table 3). Based on the sign of the standardized canonical correlation coefficients, in brackets, the high-altitude plants incorporate fewer leaves into the rosette during the vernalization treatment (our simulated winter) (–0.29), but show a prolongation of later developmental stages: from two leaves to bolting (0.13) and from bolting to flowering (0.26). They end up with more leaves in the rosette at bolting (0.31), and spend slightly more days in growing from flowering to the production of the first immature fruit (0.04) and a slightly longer time from fruit set to fruit maturation (0.02). The final above-ground biomass of high-altitude plants is greater (0.20) and their percentage

Table 2 Characteristics of the standardized canonical variates for climate variables

Variate	R^2	P value	Minimum T (°C) Sep–Feb	Maximum T (°C) Mar–Jun	Minimum precipitation (mm) (Mar–Jun)	Minimum precipitation (mm) (Sep–Feb)
1	0.58	< 0.0001	–0.76	–0.13	–0.19	0.61
2	0.17	0.24	1.22	–0.32	1.72	–0.83
3	0.10	0.61	0.02	1.14	0.64	0.28
4	0.04	0.84	–0.09	–0.52	–2.19	2.07

Squared canonical correlation (R^2), P value and loading coefficients for these variables. Only the first variate explains significant variation.

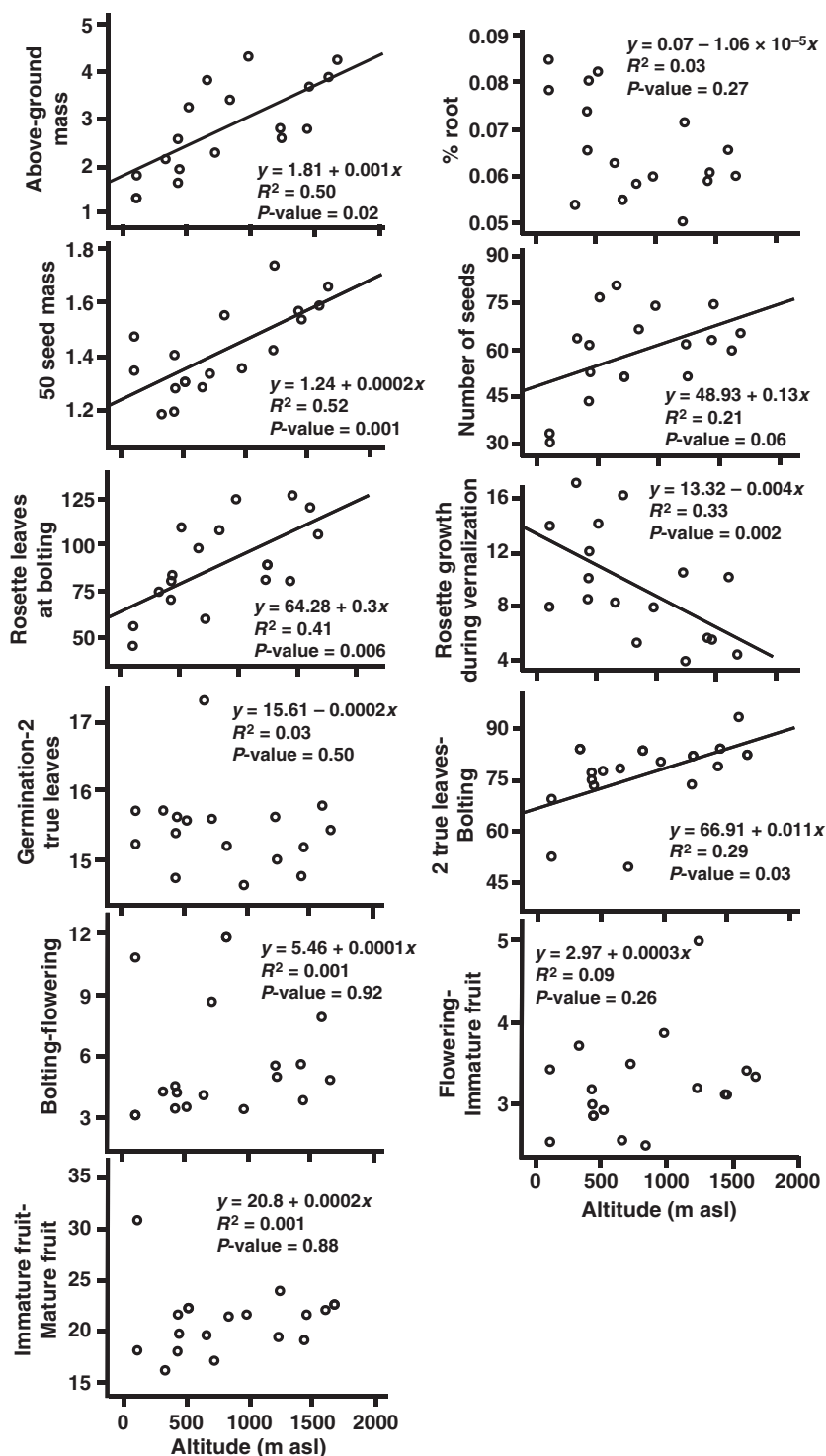


Fig. 4 Scatter plots of the mean population values for each trait (vertical axes) vs altitude of origin (horizontal axes). The line is only graphed if $P < 0.10$.

of biomass allocated to roots is lower (-0.16) than those of low-altitude plants. High-altitude plants produce fewer (-0.18) but heavier (0.57) seeds. The pattern is very similar when considering coefficients of the canonical correlation using climatic variables (Table 3).

Some traits exhibit contrasting standardized coefficient signs. Notably, above-ground mass and percentage of roots are negatively correlated in their clines (Table 3). In plants from lower elevation of origin, above-ground mass decreases, whereas the percentage of mass allocated to roots

Table 3 Standardized canonical coefficients for canonical correlation analyses (CCAs) of traits vs altitude and traits vs the four climate variables

	Altitudinal canonical variate	Climatic canonical variate 1
50 Seeds mass	0.57	0.59
Number of seeds	-0.18	-0.16
Above-ground mass	0.20	0.21
Percentage of root	-0.16	-0.05
Rosette leaves at bolting	0.31	0.36
Rosette growth during vernalization	-0.29	-0.28
Germination–Two true leaves	-0.10	-0.12
Two true leaves–Bolting	0.13	-0.01
Bolting–Flowering	0.26	0.32
Flowering–Immature fruit	0.04	0.14
Immature fruit–Mature fruit	0.02	0.05
Correlation of coefficients from altitudinal vs climatic CCAs		0.97

Only the first variate from the climatic CCA is statistically significant and presented here. Climatic variables used in the analyses: maximum temperature from March to June; minimum temperature from September to February; minimum precipitation from March to June; and minimum precipitation from September to February. The final row presents the correlation between the coefficients in the two CCAs.

increases (Table 3). This shift to greater below-ground allocation is associated with plants from sites with lower minimum monthly mean rain in September–February (Table 3). Plants at high altitude increase rosette leaf number before vernalization, but decrease leaf production during vernalization, compared with low-altitude plants. Plants from sites with lower minimum temperatures in September–February produce more leaves before vernalization (Table 2), but fewer leaves during vernalization (Table 3). We also found that plants from lower altitudes flower earlier and accumulate fewer rosette leaves by the time of bolting than plants from higher altitudes, shown by the positive correlations of both traits with altitude. In environmental terms, the number of leaves in the rosette at bolting increases with increasing values of canonical variate 1. Seed number and size exhibit opposing trends (Table 3): individuals from high-altitude populations produce fewer but heavier seeds. In environmental terms, plants from sites with lower minimum temperatures and higher minimum monthly mean rain from September to February produce fewer but heavier seeds.

Discussion

In north-eastern Spain, the altitude of our *A. thaliana* study population sites of origin is significantly associated with maximum temperatures in spring, minimum temperatures in winter and precipitation during the growing season. Biomass allocation, fecundity, phenology and vegetative

growth for 111 genetic lineages from these sites exhibit gradual changes with altitude of origin under controlled environmental conditions. Using CCAs that account for interdependences among traits and climatic variables, we tested directly for a relationship of trait values with the environmental conditions in the sites of origin. In cool and wet environments with cold winters and warm late springs, plants tend to reduce rosette growth during cold and extend their development time (days to flower and to produce fruit). Although high-altitude plants are genetically differentiated to produce fewer, heavier seeds, their greater overall size results in a larger number of heavier seeds. At low altitudes, plants allocate a greater percentage of biomass to roots, and shorten the number of days to flower and produce fruits. Thus, changes in allocation and the timing of life history transitions appear to be associated with adaptation to the climatic gradient associated with altitude.

The association of genetically based clines in trait values with altitude or other environmental gradients can result from adaptive clinal differentiation (Endler, 1986). However, an alternative explanation must also be considered.

As only a single regional gradient in altitude was included in this study, it is possible that two different lineages initially colonized low altitudes (say lineage 1) and high altitudes (say lineage 2). With time, these lineages could have spread, eventually coming into contact in mid-altitudes on our gradient. With continued gene flow, it is possible that a genetic cline would have been established with more or less pure 'lineage 1' at low altitudes, gradually transitioning to pure 'lineage 2' at high altitude, with the genotypes in between exhibiting characteristics that are dependent on the percentage ancestry from each of these founding lineages. In this scenario, there would be no necessary adaptive value to the cline. If this scenario were the cause of the pattern, we would see evidence of isolation by distance among the populations, and a gradual transition in neutral genetic marker frequencies from low to high elevation. Montesinos *et al.* (2009) reported population genetic structure for 10 of our 17 study populations. In that study, there was no evidence for isolation by distance based on spatial autocorrelation. Montesinos *et al.* (2009) used AMOVA to partition genetic variation between montane vs coastal regions (7%), populations within regions (31%) and variation within populations (62%). This pattern is consistent with high levels of isolation and drift among populations (Hartl & Clark, 2007). Furthermore, 15–18% of loci were singletons within populations. As no multilocus SNP-based genotype was found in more than one population, the rate of migration between the populations considered in this study is likely to be considerably lower than the rate of mutation at the 118 SNP loci. Thus, there is little evidence in support of the notion that dispersal and mixing of predifferentiated colonists at low and high elevation has led to the observed pattern of clinal variation in genetically based multitrait phenotypes.

The remaining explanation is that the cline results from adaptive differentiation along the altitudinal gradient.

We do not pretend that the conditions in our controlled environment chambers mimic the conditions in any specific population site. Neither do we assume that the phenotypes we have measured in the growth chambers are the phenotypes that would be manifested in the field. Furthermore, in order to expose every plant to the same conditions, we germinated all seeds in the simulated autumn that a winter annual would have experienced, then exposing rosettes to vernalization. Therefore, we make no claims regarding the fitness consequences in the wild that would be associated with the differentiation we observed in controlled environments. The syndromes described under controlled conditions can, however, be ecologically interpreted in the light of the environmental conditions of their sites of origin. Below, we interpret the observed association of genetic differentiation with environment from our experiments as evidence for adaptation, suggesting that the observed differences associated with the populations' locations along this gradient could be described as ecotypic differentiation.

Our observed altitudinal and climatic cline is broadly consistent with our *a priori* expectations. Regarding trait variation, our *a priori* expectation that low altitudes would favor increased allocation to roots, higher rates of winter growth, and avoidance of or tolerance to heat and drought during late spring, whereas high altitudes would favor high growth rates in the autumn, tolerance of winter cold and prolonged growth in the spring, was broadly consistent with the results. This suggests that divergent life history strategies have differentiated along the altitudinal gradient (Table 3 and Fig. 4).

Plants from lower altitudes experience moderate Mediterranean winters, allowing vegetative growth of the rosette during winter. Our results suggest that low-altitude populations have adapted to the mild winters by increasing growth at low temperatures relative to high-altitude populations. After the winter, the low-altitude plants accelerate their reproductive cycle, with fewer days to bolt, flower and produce fruits. We suggest that this adjustment avoids the onset of heat and drought in the late spring. This rapid life cycle in a warmer drier growing season and the negative correlation between greater allocation to roots and more limited above-ground productive capacity suggest an explanation for the lower total biomass observed in low-altitude populations.

By contrast, plants from higher altitudes of origin show greater prewinter growth and less vegetative growth during the winter months. They have slower developmental timing, and bolt later. Later bolting can potentially reduce the risk of frost damage to flowers and fruits in early spring and allow more leaves to be added to the rosette before bolting. Later bolting under benign conditions can lead to higher total fecundity (e.g. Tonsor & Scheiner, 2007). High-altitude plants can prolong growth in the moister and cooler late spring, and thus show relatively greater biomass accu-

mulation overall than low-elevation populations. These hypothetical strategies are supported by field censuses of flowering phenology in nine of our study populations (Montesinos *et al.*, 2009). The combination of rapid growth and early flowering as a response to higher temperature and lower precipitation is a widely documented pattern in the context of climate change (i.e. Dech & Nosko, 2004; Bertin, 2008; Gordo & Sanz, 2009). In another annual species, *Brassica rapa*, early flowering has been shown to be selected rapidly under these conditions (Franks *et al.*, 2007; Franks & Weis, 2008).

We observed lower lifetime accumulation of biomass in populations from low altitudes relative to high altitudes when grown under common conditions in our controlled environment experiments. This result is consistent with the suggestion that a reduction in total biomass could be an adaptive response to selection favoring reduced transpirational water loss in dry environments (Hermes & Mattson, 1992). Interactions between phenology and biomass-related traits have been reported previously. Mitchell-Olds (1996) observed a trade-off between flowering time and plant size at reproduction in *A. thaliana*, and McKay *et al.* (2003) showed that two flowering time-associated genes, *FRI* and *FLC*, have epistatic effects on water use efficiency.

Surprisingly, in the field, our low- vs high-elevation study populations exhibited no statistically significant differences in plant size using fruit number as a proxy for size (Montesinos *et al.*, 2009). This suggests that the growth environment at high elevations limits the genetic potential of high-elevation plants to accumulate more biomass, compared with the uniform environmental conditions that all populations received in our experiment.

The increase in biomass allocation to roots in low-elevation populations relative to high-elevation populations is in keeping with previous studies. In *Polygonum persicaria* (Heschel *et al.*, 2004) and *Boechera holboellii* (Knight *et al.*, 2006), individuals from sites with low soil moisture exhibited adaptation to drought through an increased allocation to roots.

The lower rate of rosette growth during the winter for plants from high altitude may result from a balance between tolerance to below-freezing temperatures and the ability to grow at low temperatures (Körner, 2003). Physiologically, *A. thaliana* genotypes that exhibit enhanced freezing tolerance show correlated down-regulation of photosynthesis, and cold tolerance is associated with the latitude of genotype origin (Hannah *et al.*, 2006). In addition, Li *et al.* (1998) found that growth rates decrease with latitude in *A. thaliana*.

Arabidopsis thaliana individuals in this study showed the classic negative genetic correlation between seed mass and seed number (Harper, 1977; De Jong & Klinkhamer, 2005), here associated with the altitudinal gradient and, more specifically, with temperature and conditions during

autumn–winter. There is an apparent conflict between the observed trade-off between seed number and seed size from the CCA and the regressions shown for seed number and seed mass in Fig. 4, in which both seed size and seed number increase with altitude. This conflict is resolved by recognizing that plant mass increases with the altitude of origin and seed number is highly correlated with plant mass. This is the same issue as illustrated in Sterns (1992) (Fig. 4). An increase in size results in increases in both seed number and seed size, disguising the underlying negative correlation. A genetic correlation between seed mass, seed number and flowering time was reported for the Landsberg erecta × Cape Verde Islands recombinant inbred line population (Alonso-Blanco *et al.*, 1999). In the gradient we studied, seed mass increases with altitude, whereas seed number decreases. An increase in seed mass has been suggested as a strategy for survival under intense seedling competition (Salisbury, 1942; Turnbull *et al.*, 2004). Theory also predicts that seed mass will be higher and seed number lower in regions in which unpredictable periods of stressful conditions make increased dormancy advantageous (Venable & Brown, 1988; Pake & Venable, 1996). Previous field studies of our populations revealed that high-altitude populations show higher dormancy in autumn, higher densities of seedlings in October and higher mortality during the winter (Montesinos *et al.*, 2009). Therefore, the production of fewer but heavier seeds at high altitudes could be an adaptive mechanism to facilitate dormancy, survive intense competition in populations with high densities of seedlings germinating in the autumn, or provide larger seedlings with greater tolerance to autumn and winter stresses experienced at high altitude. These scenarios are not mutually exclusive.

As altitude increases, plants exhibit faster developmental rates in early stages and slower rates in later stages, matching our hypothesis of a benefit of accelerating prewinter growth and lengthening the period of growth before flowering in the cooler springs of higher altitudes. However, the variance explained by the timing of development in this experiment is small. The low canonical correlations of developmental timing traits are likely to have been influenced by our experimental design in which we forced all seed to germinate in the simulated autumn, constraining them to a winter annual life history. In the population sites, we see germination in pulses starting in autumn and ending in spring. This variation in germination is a major factor determining the timing of life history transitions (Boyd *et al.*, 2007; Chiang *et al.*, 2009).

Although we have presented strong plausibility arguments for an adaptive interpretation of the clinal patterns observed in our controlled environment studies, field studies will be necessary to resolve the adaptive effects of the field phenotypes associated with the observed clines in seed, morphological and life history traits reported here.

The use of individual lineages as replicates in multivariate analysis was justified on the basis of the high level of genetic

variation within populations shown in previous studies, but there is still the possibility of some pseudoreplication in these analyses. However, the univariate regressions presented in the figures are based on population means. The significance of these regressions, in general, makes it clear that the significant pattern observed in the multivariate analyses is not simply the result of pseudoreplication, but represents a real pattern of clinal differentiation.

Conclusion

Arabidopsis thaliana shows clinal variation associated with altitude. Altitude is a proxy for a climatic gradient: precipitation increases with altitude; minimum winter temperature and maximum spring temperature decrease with altitude. Vegetative growth during winter and the number of seeds produced decrease in higher altitude sites where the minimum temperatures in autumn–winter are lower. Above-ground mass, number of leaves in the rosette at bolting, age at flowering and seed weight increase in sites with low minimum temperatures in autumn–winter and higher rain in autumn–winter, associated with high altitudes. Smaller plant size, early flowering and greater root allocation have been associated with adaptations to drought stress in other ecological studies, and high maximum temperatures in spring–summer and less rain during autumn–winter are associated with low elevation in the altitudinal gradient studied. Decreased vegetative growth during winter, delayed flowering time and heavier seeds have been associated with overwintering dormancy and high densities of seedlings, conditions that are found in sites with low temperatures in autumn–winter and spring–summer, associated with high altitude in this gradient. Some of these traits have been shown to have partially shared molecular genetics causes. In this study, we have identified how multiple traits correlate with an environmental gradient, suggesting that certain combinations of traits have been favored by natural selection, locally adapting plants to particular environments. The results presented here indicate that this study system could be useful for improving our understanding of adaptation to environment across a species' range. The multidisciplinary knowledge accumulated in model species, such as *A. thaliana*, combined with ecological studies in natural environments, can contribute to exciting increases in the understanding of the underlying mechanisms driving adaptation in the field.

Acknowledgements

S. Kalisz thoroughly read and provided valuable input that considerably improved the manuscript. We are also especially grateful to T. Elnaccash, M. Wolfe, T. Helbig, M. Simon, R. Spigler, M. Groner, PEER discussion group, P. Jordano's group, A. Valiente-Banuet and P. Rey for their

critical and constructive comments and constant discussion on the manuscript. The comments of three anonymous referees have contributed considerably to improvement of the manuscript. D. Yarnott, S. Valle and E. York contributed to plant maintenance. Funding was provided by NSF IOS- 0809171 to S.J.T. and Ministerio de Ciencia e Innovacion CGL2009-07847/BOS to F.X.P. A.M.-N. was supported by a fellowship from Fundacion Caja Madrid.

References

- Abbott RJ, Gomes MF. 1989. Population genetic-structure and outcrossing rate of *Arabidopsis-thaliana* (L.) Heynh. *Heredity* 62: 411–418.
- Alonso-Blanco C, Blankestijn-de Vries H, Hanhart CJ, Koornneef M. 1999. Natural allelic variation at seed size loci in relation to other life history traits of *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* 96: 4710–4717.
- Angert AL, Schemske DW. 2005. The evolution of species' distributions: reciprocal transplants across the elevation ranges of *Mimulus cardinalis* and *M. lewisii*. *Evolution* 59: 1671–1684.
- Arany AM, De Jong TJ, Van der Meijden E. 2005. Herbivory and abiotic factors affect population dynamics of *Arabidopsis thaliana* in a sand dune area. *Plant Biology* 7: 549–555.
- Baskin JM, Baskin CC. 1972. Ecological life-cycle and physiological ecology of seed-germination of *Arabidopsis thaliana*. *Canadian Journal of Botany* 50: 353–360.
- Bertin RI. 2008. Plant phenology and distribution in relation to recent climate change. *Journal of the Torrey Botanical Society* 135: 126–146.
- Blazquez MA, Ferrandiz C, Madueno F, Parcy F. 2006. How floral meristems are built. *Plant Molecular Biology* 60: 855–870.
- Bomblies K, Yant L, Laitinen RA, Kim S-T, Hollister JD, Warthmann N, Fitz J, Weigel D. 2010. Local-scale patterns of genetic variability, outcrossing, and spatial structure in natural stands of *Arabidopsis thaliana*. *Plos Genetics* 6: e1000890.
- Boyd EW, Dorn LA, Weinig C, Schmitt J. 2007. Maternal effects and germination timing mediate the expression of winter and spring annual life histories in *Arabidopsis thaliana*. *International Journal of Plant Sciences* 168: 205–214.
- Brennan AC, Bridle JR, Wang AL, Hiscock SJ, Abbott RJ. 2009. Adaptation and selection in the Senecio (Asteraceae) hybrid zone on Mount Etna, Sicily. *New Phytologist* 183: 702–717.
- Caicedo AL, Stinchcombe JR, Olsen KM, Schmitt J, Purugganan MD. 2004. Epistatic interaction between *Arabidopsis* FRI and FLC flowering time genes generates a latitudinal cline in a life history trait. *Proceedings of the National Academy of Sciences, USA* 101: 15 670–15 675.
- Chiang GCK, Barua D, Kramer EM, Amasino RM, Donohue K. 2009. The major flowering time gene, FLOWERING LOCUS C, regulates seed germination in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* 106: 11661–11666.
- Clausen J, Keck DD, Hiesey WM. 1940. *Experimental studies on the nature of species. I. Effect of varied environment on Western North American plants*. Washington, DC, USA: Carnegie Institution of Washington 520.
- De Jong T, Klinkhamer P. 2005. *Evolutionary ecology of plant reproductive strategies*. Cambridge, UK: Cambridge University Press.
- Dech JP, Nosko P. 2004. Rapid growth and early flowering in an invasive plant, purple loosestrife (*Lythrum salicaria* L.), during an El Nino spring. *International Journal of Biometeorology* 49: 26–31.
- Del Barrio G, Creus J, Puigdefàbregas J. 1990. Thermal seasonality of the high mountain belts of the Pyrenees. *Mountain Research and Development* 10: 227–233.
- Donohue K, Dorn L, Griffith C, Kim E, Aguilera A, Polisetty CR, Schmitt J. 2005a. Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. *Evolution* 59: 740–757.
- Donohue K, Dorn L, Griffith C, Kim E, Aguilera A, Polisetty CR, Schmitt J. 2005b. The evolutionary ecology of seed germination of *Arabidopsis thaliana*: variable natural selection on germination timing. *Evolution* 59: 758–770.
- Endler JA. 1977. *Geographic variation, speciation, and clines*. Princeton, NJ, USA: Princeton University Press.
- Endler JA. 1986. *Natural selection in the wild*. Princeton, NJ, USA: Princeton University Press.
- François O, Blum MGB, Jakobsson M, Rosenberg NA. 2008. Demographic history of European populations of *Arabidopsis thaliana*. *PLOS Genetics* 4: e100075.
- Franks SJ, Sim S, Weis AE. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences, USA* 104: 1278–1282.
- Franks SJ, Weis AE. 2008. A change in climate causes rapid evolution of multiple life-history traits and their interactions in an annual plant. *Journal of Evolutionary Biology* 21: 1321–1334.
- Gonzalo-Turpin H, Hazard L. 2009. Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *Journal of Ecology* 97: 742–751.
- Gordo O, Sanz JJ. 2009. Long-term temporal changes of plant phenology in the Western Mediterranean. *Global Change Biology* 15: 1930–1948.
- Handley R, Ekbom B, Agren J. 2005. Variation in trichome density and resistance against a specialist insect herbivore in natural populations of *Arabidopsis thaliana*. *Ecological Entomology* 30: 284–292.
- Hannah MA, Wiese D, Freund S, Fiehn O, Heyer AG, Hinch DK. 2006. Natural genetic variation of freezing tolerance in *Arabidopsis*. *Plant Physiology* 142: 98–112.
- Harper JL. 1977. *The population biology of plants*. London, UK, New York, NY, USA: Academic Press.
- Hartl DL, Clark AG. 2007. *Principles of population genetics, 4th edn*. Sunderland, MA, USA: Sinauer Associates.
- Herms DA, Mattson WJ. 1992. The dilemma of plants—how to grow or defend. *Quarterly Review of Biology* 67: 478–478.
- Heschel MS, Sultan SE, Glover S, Sloan D. 2004. Population differentiation and plastic responses to drought stress in the generalist annual *Polygonum persicaria*. *International Journal of Plant Science* 165: 817–824.
- Holdsworth MJ, Bentsink L, Soppe WJJ. 2008. Molecular networks regulating *Arabidopsis* seed maturation, after-ripening, dormancy and germination. *New Phytologist* 179: 33–54.
- Hopkins R, Schmitt J, Stinchcombe JR. 2008. A latitudinal cline and response to vernalization in leaf angle and morphology in *Arabidopsis thaliana* (Brassicaceae). *New Phytologist* 179: 155–164.
- Ishida T, Kurata T, Okada K, Wada T. 2008. A genetic regulatory network in the development of trichomes and root hairs. *Annual Review of Plant Biology* 59: 365–386.
- Knight CA, Vogel H, Kroymann J, Schumate A, Witsenboer H, Mitchell-Olds T. 2006. Expression profiling and local adaptation of *Boechera holboellii* populations for water use efficiency across a naturally occurring water stress gradient. *Molecular Ecology* 15: 1229–1237.
- Körner C. 2003. *Alpine plant life, 2nd edn*. Heidelberg, Germany: Springer.
- Körner C. 2007. The use of 'altitude' in ecological research. *Trends in Ecology and Evolution* 22: 569–574.
- Lepetz V, Massot M, Schmeller DS, Clobert J. 2009. Biodiversity monitoring: some proposal to adequately study species' responses to climate change. *Biodiversity and Conservation* 18: 3185–3203.
- Li B, Suzuki JI, Hara T. 1998. Latitudinal variation in plant size and relative growth rate in *Arabidopsis thaliana*. *Oecologia* 115: 293–301.

- Linhart YB, Grant MC. 1996. Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* 27: 237–277.
- Lundemo S, Falahati-Anbaran M, Stenoien HK. 2009. Seed banks cause elevated generation times and effective population sizes of *Arabidopsis thaliana* in northern Europe. *Molecular Ecology* 18: 2798–2811.
- Martínez MD, Lana X, Burqueño A, Serra C. 2007. Spatial and temporal daily rainfall regime in Catalonia (NE Spain) derived from four precipitation indices, years 1950–2000. *International Journal of Climatology* 27: 123–138.
- Mayr E. 1956. Geographical character gradients and climatic adaptation. *Evolution* 10: 105–108.
- McKay JK, Richards JH, Mitchell-Olds T. 2003. Genetics of drought adaptation in *Arabidopsis thaliana*. I. Pleiotropy contributes to genetic correlations among ecological traits. *Molecular Ecology* 12: 1137–1151.
- Metcalfe CJE, Mitchell-Olds T. 2009. Life history in a model system: opening the black box with *Arabidopsis thaliana*. *Ecology Letters* 12: 593–600.
- Meyerowitz EM, Somerville CR. 1994. *Arabidopsis*. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press.
- Mitchell-Olds T. 1996. Genetic constraints in life-history evolution: quantitative-trait loci influencing growth and flowering in *Arabidopsis thaliana*. *Evolution* 50: 140–145.
- Mitchell-Olds T, Schmitt J. 2006. Genetic mechanisms and evolutionary significance of natural variation in *Arabidopsis*. *Nature* 441: 947–952.
- Montesinos A, Tonsor SJ, Alonso-Blanco C, Picó FX. 2009. Demographic and genetic patterns of variation among populations of *Arabidopsis thaliana* from contrasting native environments. *PLoS ONE* 4: e7213.
- Ninyerola M, Pons X, Roure JM. 2005. *Atlas Climático Digital de la Península Ibérica. Metodología y aplicaciones en bioclimatología y geobotánica*. Bellaterra, Spain: Universidad Autónoma de Barcelona.
- Oleksyn J, Modrzyński J, Tjoelker MG, Zytowski R, Reich PB, Karolewski P. 1998. Growth and physiology of *Picea abies* populations from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. *Functional Ecology* 12: 573–590.
- Pake CE, Venable DL. 1996. Seed banks in desert annuals: implication for persistence and coexistence in variable environments. *Ecology* 77: 1427–1435.
- Picó FX, Méndez-Vigo B, Martínez-Zapater JM, Alonso-Blanco C. 2008. Natural genetic variation of *Arabidopsis thaliana* is geographically structured in the Iberian Peninsula. *Genetics* 180: 1009–1021.
- Provan J, Campanella JJ. 2003. Patterns of cytoplasmic variation in *Arabidopsis thaliana* (Brassicaceae) revealed by polymorphic chloroplast microsatellites. *Systematic Botany* 28: 578–583.
- Regehr DL, Bazzaz FA. 1976. Low temperature photosynthesis in successional winter annuals. *Ecology* 57: 1297–1303.
- Reznick DN, Ghalambor CK. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica* 112: 183–198.
- Salisbury EJ. 1942. *The reproductive capacity of plants*. London, UK: G. Bell and Sons.
- Scheiner SM, Gurevitch J. 2001. *Design and analysis of ecological experiments, 2nd edn*. Oxford, UK: University Press.
- Sharbel TF, Haubold B, Mitchell-Olds T. 2000. Genetic isolation by distance in *Arabidopsis thaliana*: biogeography and postglacial colonization of Europe. *Molecular Ecology* 9: 2109–2118.
- Sheldon CC, Finnegan EJ, Rouse DT, Tadege M, Bagnall DJ, Helliwell CA, Peacock WJ, Dennis ES. 2000. The control of flowering by vernalization. *Current Opinion in Plant Biology* 3: 418–422.
- Stearns, Stephen C. 1992. *The evolution of life histories*. Oxford, UK: Oxford University Press.
- Stillwell RC, Morse GE, Fox CW. 2007. Geographic variation in body size and sexual size dimorphism of a seed-feeding beetle. *The American Naturalist* 170: 358–369.
- Stinchcombe JR, Weinig C, Ungerer M, Olsen KM, Mays C, Halldorsdottir SS, Purugganan MD, Schmitt J. 2004. Latitudinal cline in flowering time in *Arabidopsis thaliana* modulated by the flowering time gene FRIGIDA. *Proceedings of the National Academy of Sciences, USA* 101: 4712–4717.
- Tonsor SJ, Alonso-Blanco C, Koornneef M. 2005. Gene function beyond a single trait: natural variation, gene effects, and evolutionary ecology in *Arabidopsis thaliana*. *Plants, Cell & Environment* 28: 2–20.
- Tonsor SJ, Scheiner SM. 2007. Plastic trait integration across a CO₂ gradient in *Arabidopsis thaliana*. *American Naturalist* 169: E119–E140.
- Tonsor SJ, Scott C, Boumaza I, Liss TR, Brodsky JL, Vierling E. 2008. Heat shock protein 101 effects in *A. thaliana*: genetic variation, fitness and pleiotropy in controlled temperature conditions. *Molecular Ecology* 17: 1614–1626.
- Turnbull LA, Coomes D, Hector A, Rees M. 2004. Seed mass and the competition/colonization trade-off: competitive interactions and spatial patterns in a guild of annual plants. *Journal of Ecology* 92: 97–109.
- Venable DL, Brown JS. 1988. The selective interactions of dispersal, dormancy and seed size as adaptations for reducing risk in variable environments. *American Naturalist* 131: 360–384.
- Wilczek AM, Roe JL, Knapp MC, Cooper MD, Lopez-Gallego C, Martin LJ, Muir CD, Sim S, Walker A, Anderson J *et al.* 2009. Effects of genetic perturbation on seasonal life history plasticity. *Science* 323: 930–934.
- Williams DG, Black RA. 1993. Phenotypic variation in contrasting temperature environments: growth and photosynthesis in *Pennisetum setaceum* from different altitudes on Hawaii. *Functional Ecology* 7: 623–633.
- Zhen Y, Ungerer MC. 2008. Clinal variation in freezing tolerance among natural accessions of *Arabidopsis thaliana*. *New Phytologist* 177: 419–427.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Trait means per population and sample size per trait

Table S2 Pearson correlation coefficient and *P* values for the pair-wise correlations between traits using the population mean trait values

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.