Heredity (2015) 114, 220–228

www.nature.com/hdy

ORIGINAL ARTICLE

# The roles of genetic drift and natural selection in quantitative trait divergence along an altitudinal gradient in *Arabidopsis thaliana*

Y Luo[1,2], A Widmer[2] and S Karrenberg[2,3]

Understanding how natural selection and genetic drift shape biological variation is a central topic in biology, yet our understanding of the agents of natural selection and their target traits is limited. We investigated to what extent selection along an altitudinal gradient or genetic drift contributed to variation in ecologically relevant traits in *Arabidopsis thaliana*. We collected seeds from 8 to 14 individuals from each of 14 *A. thaliana* populations originating from sites between 800 and 2700 m above sea level in the Swiss Alps. Seed families were grown with and without vernalization, corresponding to winter-annual and summer-annual life histories, respectively. We analyzed putatively neutral genetic divergence between these populations using 24 simple sequence repeat markers. We measured seven traits related to growth, phenology and leaf morphology that are rarely reported in *A. thaliana* and performed analyses of altitudinal clines, as well as overall $Q_{ST}$-$F_{ST}$ comparisons and correlation analyses among pair-wise $Q_{ST}$, $F_{ST}$ and altitude of origin differences. Multivariate analyses suggested adaptive differentiation along altitude in the entire suite of traits, particularly when expressed in the summer-annual life history. Of the individual traits, a decrease in rosette leaf number in the vegetative state and an increase in leaf succulence with increasing altitude could be attributed to adaptive divergence. Interestingly, these patterns relate well to common within- and between-species trends of smaller plant size and thicker leaves at high altitude. Our results thus offer exciting possibilities to unravel the underlying mechanisms for these conspicuous trends using the model species *A. thaliana*.
*Heredity* (2015) **114,** 220–228; doi:10.1038/hdy.2014.89; published online 8 October 2014

## INTRODUCTION

Local adaptation is an important process both for the generation of biological diversity and for future adaptation to changing conditions. Adaptive divergence within species has been extensively investigated over the past decades and has become a current focus in evolutionary biology (reviewed in Leinonen *et al.*, 2013; Savolainen *et al.*, 2013). Local adaptation often leads to the formation of clinal or ecotypic variation that can evolve over surprisingly short distances (e.g., Brady *et al.*, 2005). Clinal variation can establish fast, repeatedly and with ongoing gene flow, as has for example been shown for cyanogenesis clines in white clover (Kooyers and Olsen, 2012) and for climate-associated morphological clines in invasive plants (Monty and Mahy, 2009). Understanding local adaptation requires the identification of genetically based traits under divergent selection. Here we contribute an analysis of neutral and adaptive divergence in ecologically relevant traits along an altitude in *A. thaliana*.

Variation in phenotypic traits and in the underlying genes is shaped by random genetic drift, gene flow, selection or a combination thereof. Selectively neutral genetic divergence can be caused by restricted gene flow between populations or by extinction-recolonization dynamics (e.g., Vasemägi, 2006). A widely used approach to draw inferences about the role of selection and neutral divergence in shaping phenotypic trait differentiation compares overall quantitative trait differentiation among populations ($Q_{ST}$) with neutral genetic differentiation (e.g., $F_{ST}$) where $Q_{ST} > F_{ST}$ and $Q_{ST} < F_{ST}$ suggest divergent selection and uniform selection, respectively, and $Q_{ST} = F_{ST}$ suggests neutral divergence (Merilä and Crnokrak, 2001; Whitlock, 2008; Leinonen *et al.*, 2013). Clinal variation can further be analyzed using correlations of pair-wise (between population pairs) $Q_{ST}$, $F_{ST}$ and geographic distances or environmental differences (e.g., Kawakami *et al.*, 2011). $Q_{ST}$ -$F_{ST}$ comparisons have been criticized based on theoretical grounds but recent method improvements (O'Hara and Merilä, 2005; Whitlock, 2008; Karhunen *et al.*, 2013) as well as empirical support (Porcher *et al.*, 2006) suggest that $Q_{ST}$-$F_{ST}$ comparisons remain one of the best exploratory methods to identify potentially adaptive traits (Karrenberg and Widmer, 2008; Leinonen *et al.*, 2013).

Whereas latitudinal and longitudinal climatic clines cover large geographic areas, altitudinal clines involve dramatic climatic changes over short geographic distances that are associated with decreases in temperature and increases in precipitation in temperate regions (Körner, 2003, 2007). High-altitude species typically have a smaller stature and thicker leaves than low altitude species (Körner, 2003, 2007). Similar trends have been reported as genetically based trait divergence within species, even when plants are sampled from regional altitudinal gradients where gene flow between populations can be high

[1]Institute of Integrative Plant Biology, School of Life Sciences, Jiangsu Normal University, Xuzhou, Jiangsu, China; [2]ETH Zurich, ETH Zurich, Plant Ecological Genetics, Institute of Integrative Biology (IBZ), Universitätstrasse 16, Zurich, Switzerland and [3]Department of Ecology and Genetics, Uppsala University, Uppsala, Sweden
Correspondence: Dr S Karrenberg, Department of Ecology and Genetics, Uppsala University, Evolutionary Biology Centre, Norbyvägen 18D, 75236 Uppsala, Sweden.
E-mail: sophie.karrenberg@ebc.uu.se
Received 21 March 2014; revised 29 July 2014; accepted 19 August 2014; published online 8 October 2014

(Monty and Mahy, 2009). For example, plant height or size and specific leaf area often decrease, whereas seed size often increases with altitude of origin within species when plants are grown in a common environment (Monty and Mahy, 2009; Montesinos-Navarro *et al.*, 2011). The underlying mechanisms generating these high-altitude morphologies are still unclear, however, Körner (1999) proposed that small size at high altitude may allow plants to benefit from higher temperatures closer to the soil and thicker leaves may allow higher photosynthetic activity. In many cases, however, it is not known to what extent such genetically based trait differentiation within species is due to adaptive or neutral divergence, however, traits related to growth and leaf morphology are clear potential targets for selection along altitudinal gradients.

*Arabidopsis thaliana* is a common species in the central European part of its range, where it occurs from sea level of to about 2000 m above sea level (Hoffmann, 2002). Populations of *A. thaliana* from even higher altitudes have been reported from the Eastern part of its distribution range but are largely unknown from the European Alps. *Arabidopsis thaliana* is an annual species that exhibits either a winter-annual life history with flowering after vernalization or a summer-annual life history where germination and flowering occur in the same year (Donohue, 2005; Montesinos-Navarro *et al.*, 2012; Picó, 2012). The winter-annual life history is considered to be most common but recent evidence shows that the summer-annual life history is more successful in mountainous regions and that both life histories can be expressed in the same population (Picó, 2012). Ecologically relevant variation in *A. thaliana* and the genomic signature thereof has received substantial interest in recent years and numerous traits and genes displaying clinal variation along latitude have been identified (reviewed in Bergelson and Roux, 2010). Altitudinal clines in *A. thaliana* have thus far been described for populations from Spain, where population means of growth, flowering and seed traits changed with altitude of origin (Mendez-Vigo *et al.*, 2011; Montesinos-Navarro *et al.*, 2011). Such trait–altitude correlations are compatible with adaptive divergence along altitude; however, it is important to note that clines in trait means can also be caused by selectively neutral processes such as genetic drift through geographic isolation or by extinction-recolonization dynamics (Vasemägi 2006; Kawakami *et al.* 2011).

In the present study, we investigated the roles of natural selection and neutral divergence for population differentiation in ecologically relevant traits along an altitudinal gradient. We grew newly collected seeds of *A. thaliana* from an altitudinal gradient in the central European Alps (800–2700 m) with and without vernalization. Vernalization treatments correspond the two alternative life histories: winter-annual with vernalization and summer-annual without vernalization. We estimated neutral genetic differentiation between these populations ($F_{ST}$) using 24 simple sequence repeat (SSR) loci as well as genetically based differentiation ($Q_{ST}$) in seven traits related to growth, phenology and leaf morphology. We analyzed clinal trait variation along the altitudinal gradient, tested whether overall phenotypic trait differentiation exceeds overall neutral genetic differentiation (indicating adaptive divergence) and investigated associations of pair-wise (between population) $Q_{ST}$ estimates with pair-wise $F_{ST}$ and/or altitudinal differences. An association of pair-wise $Q_{ST}$ with altitude differences suggests adaptive divergence along altitude whereas an association of $Q_{ST}$ with $F_{ST}$ suggests neutral trait divergence. Our study questions were: (i) Is there evidence for adaptive trait divergence along altitude? (ii) Do patterns of trait divergence differ between vernalized and non-vernalized plants?

## MATERIALS AND METHODS

### Collection sites and plant material

Fourteen natural populations of *A. thaliana* were sampled in the Swiss Alps at altitudes ranging from 800 to 2700 m above sea level (Supplementary Table S1, Supplementary Figure S1). Fifty-year averages (1950–2000) of mean annual temperature and total annual precipitation extracted from the WorldClim database (resolution 1 $km^2$, www.worldclilm.org, Hijmans *et al.*, 2005) ranged from 7.4 °C (NAT, 850 m above sea level) and 1085 mm (NAT, 1000 m above sea level) to −0.38 °C and 1847 mm (ZIN, 2700 m above sea level, Supplementary Figure S2). In our set of sampling sites, annual temperature strongly decreased with altitude (Pearson correlation, $r=-0.96$, $t_{12}=-12.23$ $P=3.9\times10^{-8}$, Supplementary Figure S2a) and annual precipitation strongly increased with altitude ($r=0.91$, $t_{12}=7.65$ $P=5.9\times10^{-6}$, Supplementary Figure S2b) as is expected for temperate regions (Körner, 2007).

In each population, seeds were collected from plants approximately 1 m apart, where possible. Selfed seed families for use in experiments were produced from field-collected seeds in a greenhouse to reduce maternal effects on trait expression. All experiments were conducted at the ETH Zurich experimental station in Eschikon, Switzerland.

### Growth conditions and trait measurements

Eight to 14 seed families from each of the 14 study populations (compare Supplementary Table S1) were grown with and without vernalization (see below) with three to four replicate plants per family. For pre-cultivation, seeds were sown in 150-cell trays, stratified at 4 °C for 7 days, and then germinated and grown for 7 days under short-day conditions (8 h/16 h (day/night)). For the vernalization treatment, plants were exposed to 4 °C in a dark chamber for 6 weeks. To synchronize the growth of vernalized and non-vernalized plants, the latter were sown 14 days before the end of the vernalization treatment. All plants were subsequently transplanted into single pots. Plants were arranged in a randomized block design with four blocks, each receiving one vernalized and one non-vernalized plant of each family and grown in a greenhouse under long-day conditions with 16 h of light. Plants were irrigated when necessary and rotated within the greenhouse every other day to minimize environmental variation.

Seven traits were scored on both vernalized and non-vernalized plants. Plants were visited daily and the number of days from germination to flowering (first flower open, excluding the days for vernalization treatment) was recorded as days to flowering. Rosette diameter was measured (mm) as the maximal distance between two rosette leaves after 3 weeks in the vegetative stage and at flowering. Rosette leaf number was counted in 3-week-old plants. The eighth fully expanded true leaf from each plant was collected and fresh and dry mass (g) were determined using an analytical balance. Dry, pressed leaves were scanned and leaf area ($cm^2$) was determined using ImageJ (http://rsb.info.nih.gov/ij/). Specific leaf area was calculated as leaf area/dry mass ($cm^2\ g^{-1}$) and leaf succulence was calculated as (fresh mass-dry mass)/leaf area (g $H_2O$ $cm^{-2}$) (Reiman and Breckle, 1995). Rosette diameter and roesette leaf number after 3 weeks, days to flowering and rosette diameter at flowering were scored in three to four plants per family in 8 to 14 families per population comprising a total of 720 vernalized and 644 non-vernalized individuals. Leaf areas, specific leaf area and leaf succulence were scored in two to three individuals per seed family for 8 to 11 families per population; for these traits, we collected data on 353 vernalized and 342 non-vernalized individuals (compare Supplementary Table S1).

### Genetic analysis

Genomic DNA was extracted from one individual of 8–28 families of each population, 258 individuals in total (Table 1). Twenty-four SSR markers in the genome of *A. thaliana* were selected from the INRA microsatellite database (http://www.inra.fr/internet/Produits/vast/msat.php) with three to six SSRs per chromosome (Supplementary Table S2). The average physical distance between markers was 4916 ± 594 kbp (mean with standard error (s.e.), compare Supplementary Table S2). After PCR amplification with fluorescently labeled primers, PCR products were subjected to fragment analysis using a 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Fragment size analysis proceeded with GeneMapper Version 4.1 (Applied Biosystems).

**Table 1 Population genetic analysis of *Arabidopsis thaliana* populations from the Swiss Alps using 24 microsatellite markers**

| *Pop.* | N | *% Poly-morphic loci* | *Unique multilocus genotypes* | *No. hetero-zygotic ind.*[a] | *No. alleles per locus*[b] | *Allelic diversity*[c] | $F_{IS}$[b] | *Nei's gene diversity*[b] | *% Sign. pair-wise LD (no. tests)* |
|---|---|---|---|---|---|---|---|---|---|
| BRI | 18 | 79 | 14 | 5 | 2.208 | 2.023 | 0.735 | 0.24 | 2.34 (171) |
| NAT | 22 | 96 | 12 | 3 | 2.458 | 1.926 | 0.962 | 0.162 | 0.00 (225) |
| EGG | 22 | 100 | 17 | 3 | 3.625 | 3.142 | 0.989 | 0.518 | 44.16 (274) |
| AUS | 15 | 42 | 13 | 4 | 1.583 | 1.558 | 0.985 | 0.191 | 4.44 (45) |
| GEI | 8 | 4 | 2 | 0 | 1.042 | 1.042 | 1 | 0.011 | — (0) |
| RI1 | 17 | 21 | 10 | 4 | 1.208 | 1.173 | 0.778 | 0.046 | 0.00 (10) |
| RI2 | 12 | 92 | 11 | 1 | 2.5 | 2.469 | 0.97 | 0.491 | 1.73 (231) |
| BI2 | 22 | 13 | 7 | 1 | 1.125 | 1.093 | 0.925 | 0.028 | 0.00 (3) |
| BI1 | 23 | 33 | 20 | 5 | 1.333 | 1.281 | 0.886 | 0.098 | 3.70 (27) |
| SAF | 28 | 92 | 26 | 6 | 2.375 | 2.107 | 0.962 | 0.337 | 22.83 (219) |
| GRA | 20 | 75 | 19 | 6 | 2.083 | 1.908 | 0.932 | 0.214 | 0.00 (151) |
| BAE | 10 | 79 | 10 | 2 | 2.292 | 2.274 | 0.978 | 0.393 | 0.00 (153) |
| SAO | 17 | 79 | 15 | 6 | 2.5 | 2.206 | 0.929 | 0.258 | 2.40 (167) |
| ZIN | 24 | 17 | 10 | 4 | 1.167 | 1.144 | 0.77 | 0.042 | 0.00 (6) |

Abbreviation: LD, linkage disequilibrium.
[a]individuals with >1 heterozygous locus.
[b]averaged over loci.
[c]after rarefaction to $N=8$.

## Data analysis

*Population genetics.* Unique multi-locus genotypes were identified using the package *allelematch* for R (Galpern *et al.*, 2012) with no mismatch allowed. We calculated the following population genetic parameters: allele number per population and per marker, allelic richness with rarefaction to the smallest sample size ($N=8$), Nei's gene diversity $H_e$, linkage disequilibrium between pairs of loci within populations and the inbreeding coefficient $F_{IS}$ after Weir and Cockerham (1984) using FSTAT 2.9.3.2. (Goudet, 1995) and GENEPOP 4.0 (Rousset, 2008). We tested whether allelic diversity or Nei's gene diversity were associated with the altitude of origin using Pearson correlations. Population differentiation was estimated as overall and pair-wise (between populations) $F_{ST}$ after Weir and Cockerham (1984). The 95% confidence interval (CI) of overall $F_{ST}$ was calculated with bootstrapping over loci and significance of pair-wise $F_{ST}$ was tested using permutation tests with 999 permutations. We corrected pair-wise (between loci) linkage disequilibrium tests and the significance tests of pair-wise $F_{ST}$ for multiple comparisons using false discovery rate control at an overall $\alpha=0.05$ (Verhoeven *et al.*, 2005).

*Trait analyses: vernalization response, correlations and altitudinal clines.* We first tested for trait differences between populations and vernalization response using a mixed model with family identity as a random effect and population identity, vernalization treatments and the interaction of these factors as fixed effects. We extracted trait means for each population, separately for vernalized and non-vernalized plants, using mixed models with family identity as a random effect and the intercept removed (Schielzeth, 2010). We first tested for correlations of population means for each trait in vernalized vs non-vernalized plants. Correlations among population means for the different traits within vernalized and non-vernalized plants were analyzed as bivariate Pearson correlations and using principal components analysis (PCA) on trait correlation matrices in the package *psych* for R (Revelle, 2013; Wolfe and Tonsor, 2014). Significance of principal components (PCs), that indicates significant overall correlation structure, was inferred by comparing eigenvalues with eigenvalues of 1000 simulated datasets using the command *fa.parallel* (Revelle, 2013). PCAs were further conducted on all individuals with data for all traits (352 vernalized and 318 non-vernalized individuals) to explore joint trait divergence within and between populations.

For the above and further analyses, we transformed responses to yield satisfactory model fit and distribution of residuals, where needed (Venables and Ripley, 2002): days to flowering and leaf succulence were 1/Y transformed and rosette diameter after 3 weeks and rosette diameter at flowering were $\log_e$-transformed. Trait variation along altitude was analyzed using a regression of back-transformed population means of individual traits or PCs on altitude of origin. For several traits, residual analysis of the trait-altitude regression analysis indicated deviations from normality and sub-optimal model fit (leaf succulence, vernalized; leaf area, non-vernalized, rosette diameter after 3 weeks and specific leaf area, both conditions), however, this is difficult to assess with 14 data points and we thus retained the analyses. We corrected for pair-wise multiple comparisons across traits and correlations within vernalization treatments using false discovery rate control at an overall $\alpha=0.05$ (Verhoeven *et al.*, 2005).

*Phenotypic differentiation ($Q_{ST}$) and broad-sense heritability ($H^2$).* We estimated overall and pair-wise (between population) $Q_{ST}$ for significant PCs and individual traits for this highly inbreeding species using hierarchical random effects model as $Q_{ST}=V_{pop}/(V_{pop}+V_{fam})$ where $V_{pop}$ is the between population variance component and $V_{fam}$ is the between family (within populations) variance component (Bonnin *et al.*, 1996; Le Corre, 2005; Mendez-Vigo *et al.*, 2013). We further calculated $H^2$ of traits across all populations using a model with family identity as the only random effect as $H^2=V_{popfam}/(V_{popfam}+V_{within})$ (compare Bonnin *et al.*, 1996; Le Corre, 2005; Mendez-Vigo *et al.*, 2013). Here, $V_{popfam}$ is family variance component and the $V_{within}$ is the within-family variance component. We extracted variance components from mixed models with responses transformed as described above and obtained 95% CIs for $Q_{ST}$ and $H^2$ using parametric bootstrapping 10 000 bootstrap samples (*bootMer* function) using the *lme4* package (Bates *et al.*, 2013). This method has been shown to be one of the least biased methods for CI calculations of $Q_{ST}$ (O'Hara and Merilä, 2005).

*$Q_{ST}$—$F_{ST}$ comparisons and Mantel tests.* We inferred statistical differences between overall $Q_{ST}$ (for PCs and individual traits) and $F_{ST}$ when there was no overlap between their CIs (Merilä and Crnokrak, 2001; O'Hara and Merilä, 2005). Using pair-wise (between population) data and Mantel tests with 10 000 permutations with the *vegan* package for R (Oksanen *et al.*, 2009) we assessed (i) whether neutral genetic differentiation between populations ($F_{ST}$) correlated with differences in their altitude of origin (similar to an isolation by distance pattern), (ii) whether $Q_{ST}$ correlated with $F_{ST}$ as would be expected if trait differentiation is due to selectively neutral processes and (iii) whether $Q_{ST}$ correlated with altitude differences as would be expected if trait differentiation results from adaptive divergence along altitude. Moreover, we conducted a partial Mantel test for the association between $Q_{ST}$ and altitude differences while controlling for neutral genetic differentiation ($F_{ST}$). For each series of Mantel tests across the seven traits, we corrected for multiple

comparisons using false discovery rate control at an overall $\alpha = 0.05$ (Verhoeven *et al.*, 2005).

## RESULTS

### Population genetics

Populations contained between 2 and 26 unique multi-locus genotypes (Table 1) corresponding to 25 –100% of the sampled individuals (mean with s.e.: 72.0 ± 6.7%). Within populations, between 4% (one SSR locus) and 100% of the 24 SSR loci were polymorphic and average allele numbers per locus ranged from 1.04 to 3.62 (Table 1). All populations except GEI with the smallest sample size ($N = 8$) contained one or more individuals with one or more heterozygous loci (Table 1). Allelic richness ranged from 1.04 to 3.14 (mean and s.e.: 1.81 ± 0.17) and Nei's gene diversity $H_e$ from 0.011 to 0.518 (mean and s.e.: 0.22 ± 0.05; Table 1). The inbreeding coefficient $F_{IS}$ ranged from 0.735 to 1 (mean and s.e.: 0.914 ± 0.024). For 12 of the populations, less than 5% of the possible tests for pair-wise between loci linkage disequilibrium were significant while 22.8% and 44.2% of the two-locus pairs exhibited significant linkage disequilibrium for the remaining populations SAF and EGG, respectively (Table 1). Neither allelic richness nor $H_e$ were associated with the altitude of origin (allelic richness: $r = -0.150$, $t_{12} = -0.525$, $P = 0.609$; $H_e$: $r = -0.119$, $t_{12} = -0.415$, $P = 0.685$). Populations were significantly differentiated with an overall $F_{ST}$ of 0.73 (95% CI: 0.663–0.771). Pair-wise between-population $F_{ST}$ ranged from 0.386 to 0.985 (Supplementary Table S3) and was significantly different from zero after false discovery rate control in all cases.

### Effects of vernalization and altitude of origin on trait values

Trait values differed significantly between populations (all traits) and were significantly affected by vernalization (all traits except leaf area) and the interaction between vernalization treatment and population (all traits, Supplementary Table S4). Population means of rosette diameter after 3 weeks of vegetative growth ranged from 30 to 53 mm and decreased after vernalization in the majority of populations (Figure 1a). Population means of rosette diameter after 3 weeks decreased marginally significantly with altitude in vernalized plants ($P = 0.03$, not significant after false discovery rate control, Table 2). Rosette leaf number after 3 weeks of vegetative growth ranged from 8.2 to 12.2 and exhibited variable responses to vernalization (Figure 1b). Rosette leaf number after 3 weeks decreased significantly with altitude for both vernalized and non-vernalized plants (Table 2, Figure 1b). Plants flowered after 25 to 50 days when vernalized and after 30 to 70 days without vernalization (Figure 1c). Vernalization strongly reduced days to flowering in all populations (Figure 1c). Rosette diameter at flowering ranged from 60 to 122 mm and decreased with vernalization in most but not all populations (Figure 1d). Study plants had leaf areas ranging from 2.2 to 4.0 $cm^2$ and leaf area responses to vernalization were highly variable (Figure 2a). Specific leaf area ranged from 320 to over 600 $cm^2\,g^{-1}$ for vernalized plants and from 290 to 410 $cm^2\,g^{-1}$ for non-vernalized plants (Figure 2b). Vernalization increased specific leaf area in all populations, however, the extent of this increase varied strongly between populations (Figure 2b). Leaf succulence ranged from 0.022 to 0.038 g $H_2O$ $cm^{-2}$ and most but not all populations decreased leaf

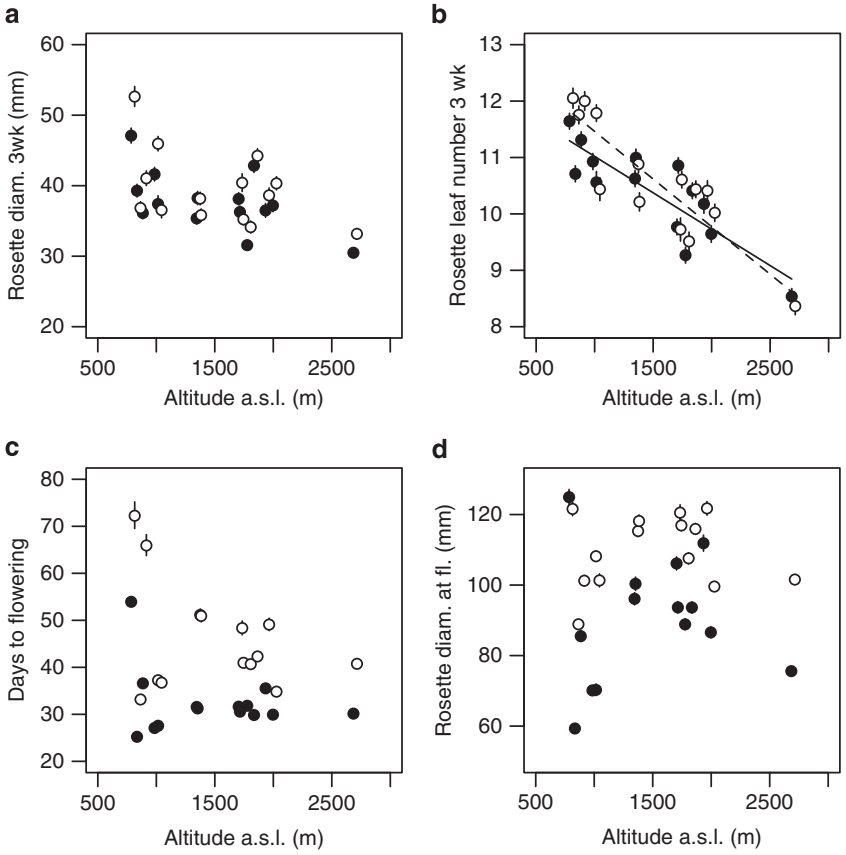


**Figure 1** Population means with s.e. of rosette diameter after 3 weeks (**a**), rosette leaf number after 3 weeks (**b**), days to flowering (**c**) and rosette diameter at flowering (**d**) in 14 *Arabidopsis thaliana* populations from the Swiss Alps that were grown in a greenhouse with and without vernalization. Means are plotted against altitude of origin, significant regression lines are indicated. Vernalized: full symbols and entire line, non-vernalized: empty symbols and dashed line.

**Table 2 Regression analyses of growth, phenology and leaf traits, as well as of PC, in *Arabidopsis thaliana* from the Swiss Alps (14 populations, population means used) against altitude of origin (800–2700 m above sea level); plants were grown in a greenhouse with and without vernalization**

| | *Vernalized* | | | | *Non-vernalized* | | | |
|---|---|---|---|---|---|---|---|---|
| | b[a] | *s.e.* (b)[b] | $t_{1,13}$ | P-*value* | b[a] | *s.e.* (b)[b] | $t_{1,13}$ | P-*value* |
| PC1 | 0.835 | 0.799 | 1.04 | 0.3170 | 0.204 | 0.590 | −0.345 | 0.7360 |
| PC2 | **1.411** | **0.255** | **5.53** | **0.0001** | **1.453** | **0.301** | **4.83** | **0.0004** |
| PC3 | — | — | — | — | 0.284 | 0.299 | 0.95 | 0.3620 |
| Rosette diam. 3 wk (mm) | *−4.49* | *1.79* | *−2.50* | *0.0278* | −4.46 | 2.42 | −1.85 | 0.0898 |
| Rosette leaf number 3 wk | **−1.29** | **0.23** | **−5.63** | **0.0001** | **−1.69** | **0.25** | **−6.72** | **0.00002** |
| Days to flowering | −3.01 | 3.49 | −0.86 | 0.4053 | −7.20 | 5.58 | −1.29 | 0.2211 |
| Rosette diam. at fl. (mm)[c] | 2.82 | 9.21 | 0.31 | 0.7650 | 2.29 | 5.26 | 0.43 | 0.6715 |
| Leaf area ($cm^2$) | 0.33 | 0.27 | 1.22 | 0.2464 | −0.01 | 0.19 | −0.03 | 0.9761 |
| Specific leaf area ($cm^2\ g^{-1}$) | −71.25 | 42.69 | −1.67 | 0.1210 | −36.82 | 17.75 | −2.07 | 0.0602 |
| Leaf succulence (g $H_2O$ $cm^{-2}$) | **0.006** | **0.002** | **3.52** | **0.0042** | **0.005** | **0.001** | **3.97** | **0.0019** |

Abbreviation: PC, principal component.
Bold type, significant after correction for multiple comparisons; italic type, *P*-value<0.05.
[a]Regression coefficient b (slope) × 1000 to increase readability.
[b]s.e. of b.
[c]Rosette diameter at flowering.

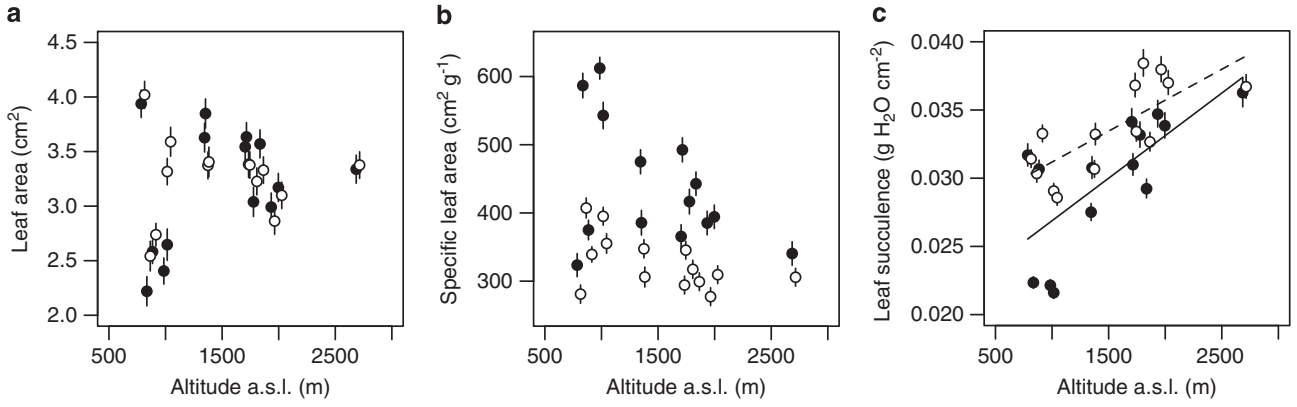


**Figure 2** Population means with standard errors of leaf area (**a**), specific leaf area (**b**) and leaf succulence (**c**) in 14 *Arabidopsis thaliana* populations from the Swiss Alps that were grown in a greenhouse with and without vernalization. Means are plotted against altitude of origin, significant regression lines are indicated. Vernalized: full symbols and entire line, non-vernalized: empty symbols and dashed line.

succulence when vernalized. Population means of leaf succulence were significantly associated with altitude for both vernalized and non-vernalized plants (Table 2,Figure 2c).

### PCAs, trait correlations and $H^2$

Trait expression was generally similar in vernalized and non-vernalized plants as indicated by significant correlation across vernalization treatments for all traits (Supplementary Table S5). PCA of population trait means within vernalization treatments yielded two significant PCs in both cases, explaining 54.4 and 30.2% of the variation in vernalized plants and 40.1 and 33.4% of the variation in non-vernalized plants (Supplementary Figures S3). These significant PCs indicate that an overall correlation structure is present in the data (Revelle, 2013; Wolfe and Tonsor, 2014). In the analysis of vernalized plants, PC1 was mainly associated with leaf traits, rosette diameter at flowering and days to flowering, whereas PC2 was associated with rosette leaf number and rosette diameter after 3 weeks; this is in accordance with the bivariate correlations (Supplementary Figure S3,Supplementary Table S4). In the analysis of non-vernalized plants, the traits contributed more evenly to the first two PCs, and none of the bivariate correlations were significant after correction for multiple testing (Supplementary Figure S3, Supplementary Table S4). PCAs of individuals rather than population means were generally similar to PCAs of population means; however, a third significant PC was present in the PCA for non-vernalized plants (Supplementary Figures S5). For both vernalized and non-vernalized plants, only population means of PC2 were significantly associated with altitude (Table 2).

Overall $H^2$ was generally higher in vernalized than in non-vernalized plants (Supplementary Table S6). The highest $H^2$ values were observed for days to flowering for both vernalized and non-vernalized plants (0.92 and 0.83, respectively) whereas leaf traits for non-vernalized plants had the lowest $H^2$ values ranging from 0.12 for leaf area to 0.31 for specific leaf area (Supplementary Table S6).

### Overall differentiation in phenotypic traits vs neutral markers: $Q_{ST}$-$F_{ST}$ comparisons

Overall $Q_{ST}$ estimates for significant PCs overlapped with the CI of $F_{ST}$ for vernalized plants (PC1: 0.89 (CI, 0.76–0.95), PC2: 0.80 (CI, 0.55–0.81)). For non-vernalized plants, in contrast, overall $Q_{ST}$ for

PC1 and PC2 was significantly higher than $F_{ST}$ (PC1: 0.96 (CI, 0.80–1.00), PC2: 0.96 (CI, 0.79–1.00), PC3: 0.78 (CI, 0.67–1.00)).

Overall $Q_{ST}$ estimates for individual traits ranged from 0.703 to 0.955 and their CIs never included zero indicating that that our study populations were significantly differentiated for all traits (Figure 3). $Q_{ST}$ was significantly higher than $F_{ST}$ for rosette leaf number after 3 weeks in non-vernalized plants, specific leaf area in vernalized and leaf succulence in both vernalized and non-vernalized plants (Figure 3). For the remaining traits, $Q_{ST}$ CI overlapped with the CI of $F_{ST}$ (Figure 3) suggesting neutral differentiation.

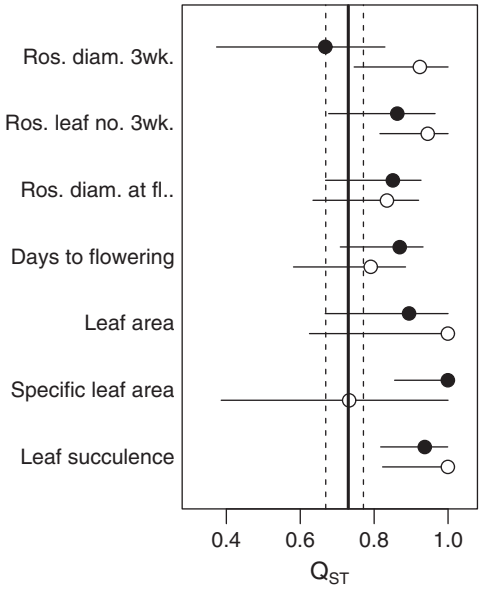


**Figure 3** $Q_{ST}$ estimates with 95% CIs for growth, phenology and leaf traits in 14 *Arabidopsis thaliana* populations from the Swiss Alps that were grown in a greenhouse with and without vernalization. Full symbols, vernalized; empty symbols, non-vernalized. $F_{ST}$ (full line) with its 95% CI (dashed lines) is indicated.

**Pair-wise (between-population) differentiation: Mantel tests**

A Mantel test for isolation by distance along altitude did not meet the significance threshold of 0.05 (Mantel $r = 0.225$, $P = 0.0839$). Pair-wise analyses of PCs differed strongly between vernalized and non-vernalized plants. For vernalized plants, PC1 and PC2 both were significantly associated with neutral genetic divergence and with altitude differences and; a partial Mantel test was not significant for PC1, whereas the test for PC2 was close to significance ($P = 0.0661$). For non-vernalized plants, we did not detect any associations of PCs with neutral genetic divergence and PC2 was significantly associated with altitude differences, whereas the association of PC1 and altitude differences had a *P*-Value of 0.0688 (Table 3). In partial Mantel tests, PC2 for non-vernalized plants remained highly significantly associated with altitude differences (Table 3).

Pair-wise $F_{ST}$ and $Q_{ST}$ were not significantly correlated for any of the individual traits after false discovery control (Table 3). Mantel tests further detected significant associations of pair-wise $Q_{ST}$ with altitude differences for rosette leaf number after 3 weeks and leaf area measured on vernalized plants, as well as for rosette leaf number after 3 weeks and leaf succulence in non-vernalized plants (Table 3). In partial Mantel tests controlling for the effect of neutral genetic divergence ($F_{ST}$), the association of $Q_{ST}$ with altitude differences remained significant only for rosette leaf number after 3 weeks for both vernalized and non-vernalized plants. The significance tests presented above are conservative, particularly as corrections for multiple comparisons depend on the number of traits studies. For several further traits, *P*-values below 0.05 are pointing toward potentially interesting relationships, for example, the partial Mantel test of leaf area under vernalized conditions ($P = 0.0185$) and of leaf succulence under non-vernalized conditions ($P = 0.0134$, Table 3).

**Table 3 Mantel tests and partial Mantel tests on pair-wise $Q_{ST}$ vs $F_{ST}$ and $Q_{ST}$ vs difference in altitude of origin (Alt. diff.), as well as partial Mantel tests on $Q_{ST}$ vs Alt. diff. controlled for $F_{ST}$ for growth, phenology and leaf traits, as well as PC, for 14 *Arabidopsis thaliana* populations from the Swiss Alps that were grown in a greenhouse with and without vernalization**

| *Trait* | *$Q_{ST}$ vs $F_{ST}$* | | *$Q_{ST}$ vs Alt. diff.* | | *$Q_{ST}$ vs Alt. diff\| $F_{ST}$* | |
|---|---|---|---|---|---|---|
| | *Mantel* r | P-*value* | *Mantel* r | P-*value* | *Mantel* r | P-*value* |
| *Vernalized* | | | | | | |
| PC1 | **0.405** | **0.0208** | **0.226** | **0.0463** | 0.151 | 0.1603 |
| PC2 | **0.389** | **0.0321** | **0.299** | **0.0144** | 0.235 | 0.0661 |
| Rosette diam. 3 wk (mm) | 0.310 | 0.0961 | 0.273 | 0.0463 | 0.219 | 0.1101 |
| Rosette leaf number 3 wk | 0.233 | 0.1178 | **0.366** | **0.0023** | **0.331** | **0.0069** |
| Days to flowering | 0.262 | 0.0978 | 0.263 | 0.0243 | 0.217 | 0.0647 |
| Rosette diam. at fl. (mm)[c] | 0.363 | 0.0343 | 0.250 | 0.0309 | 0.186 | 0.1003 |
| Leaf area ($cm^2$) | 0.206 | 0.1065 | **0.306** | **0.0100** | 0.273 | 0.0185 |
| Specific leaf area ($cm^2\ g^{-1}$) | 0.385 | 0.0267 | 0.201 | 0.0725 | 0.127 | 0.2123 |
| Leaf succulence (g $H_2O\ cm^{-2}$) | 0.084 | 0.3047 | 0.186 | 0.0706 | 0.172 | 0.0956 |
| *Non-vernalized* | | | | | | |
| PC1 | 0.124 | 0.2288 | 0.176 | 0.0688 | 0.153 | 0.1141 |
| PC2 | 0.199 | 0.1527 | **0.334** | **0.0009** | **0.303** | **0.0054** |
| PC3 | 0.150 | 0.2622 | −0.14 | 0.7827 | −0.181 | 0.8482 |
| Rosette diam. 3 wk (mm) | 0.455 | 0.0105 | 0.195 | 0.0914 | 0.106 | 0.2579 |
| Rosette leaf number 3 wk | 0.302 | 0.0154 | **0.377** | **0.0028** | **0.332** | **0.0074** |
| Days to flowering | 0.146 | 0.2134 | 0.233 | 0.0273 | 0.208 | 0.0559 |
| Rosette diam. at fl. (mm)[c] | 0.187 | 0.1138 | 0.079 | 0.2375 | 0.038 | 0.3571 |
| Leaf area ($cm^2$) | 0.022 | 0.4262 | 0.190 | 0.0335 | 0.190 | 0.0696 |
| Specific leaf area ($cm^2\ g^{-1}$) | −0.227 | 0.8605 | 0.164 | 0.1219 | 0.227 | 0.0836 |
| Leaf succulence (g $H_2O\ cm^{-2}$) | 0.141 | 0.1768 | **0.281** | **0.0058** | 0.259 | 0.0134 |

Abbreviation: PC, principal component.
Bold type, significant after correction for multiple comparisons; italic type, *P*-value$<0.05$.

## DISCUSSION

Our study substantially extends knowledge on clinal divergence along altitude using the model species *A. thaliana*. We included populations from very high altitudes up to 2700 m above sea level, report ecologically relevant traits that have not been considered in this context previously and dissect evolutionary processes driving trait divergence using analyses of altitudinal clines for population trait means, overall $Q_{ST}$-$F_{ST}$ comparisons, as well as pair-wise (between population) correlation analyses among $Q_{ST}$, $F_{ST}$ and altitude of origin differences.

In multivariate analyses, we found stronger evidence for adaptive population differentiation along altitude for traits expressed in the summer-annual life history (non-vernalized plants) as compared with traits expressed in the winter-annual life history (vernalized plants). In non-vernalized plants, PC2 was significantly associated with altitude, $Q_{ST}$ for PC2 exceeded $F_{ST}$, pair-wise $Q_{ST}$ was associated with altitude differences but not with neutral genetic divergence ($F_{ST}$) and the association between $Q_{ST}$ and altitude differences remained highly significant when controlled for $F_{ST}$ in partial Mantel tests. In vernalized plants, in contrast, PC2 was associated with both altitude differences and $F_{ST}$, and partial Mantel tests were not significant. This suggests that genetic drift impacted population differentiation in traits expressed in the winter-annual life history but not traits expressed in the summer-annual life history. The summer-annual life history is generally rare in *A. thaliana* (Donohue, 2005; Montesinos-Navarro *et al.*, 2012) but has recently been described to be the common in high elevation population in Spain (Montesinos-Navarro *et al.*, 2012; Picó, 2012). Our study populations were collected as very small mature plants with only a few siliques suggesting that these plants completed their life cycle within one season (summer-annual) and this is also supported by field experiments recently conducted in the Swiss Alps (N. Quèbre, A. Widmer and S. Karrenberg, unpublished results). Thus, our study is consistent with a predominance of the summer-annual life history in high-altitude populations of *A. thaliana* and further suggests that traits expressed in that life history are shaped by natural selection along an altitudinal gradient, whereas, at the same time, traits expressed in the winter-annual life history diverged through genetic drift. It is important to note though that trait expression was strongly correlated across vernalization treatments and this may give rise to similar patterns of trait divergence in vernalized and non-vernalized plants.

Here we consider cases with all analyses pointing toward adaptive divergence, as presented above for PC2 for non-vernalized plants, as the strongest evidence for adaptive divergence. This could be overly conservative at times, as power to detect $Q_{ST} > F_{ST}$ is generally limited for species with high neutral genetic divergence (Porcher *et al.*, 2006; Le Corre and Kremer, 2012). Moreover, several improved estimation approaches are currently not applicable for selfing species with high neutral divergence (personal communication M. Karhunen; Whitlock, 2008; Karhunen *et al.*, 2013). Nonetheless, $Q_{ST}$-$F_{ST}$ comparisons have been experimentally shown to be valid in *A. thaliana* (Porcher *et al.*, 2006). Overall $F_{ST}$ was 0.73 in this study, a value in the upper range of regional $F_{ST}$ of *A. thaliana* reported for Scandinavia, France and Spain and calculated from either SSRs or SNPs (Kuittinen *et al.*, 1997; Le Corre, 2005; Brachi *et al.*, 2013; Mendez-Vigo *et al.*, 2013). However, $F_{ST}$ calculated using SSRs, as we do here, can be lower than $F_{ST}$ calculated using SNPs because of the high mutation rate of SSRs combined with very low migration rates (Edelaar *et al.*, 2011; Mendez-Vigo *et al.*, 2013), but it is not clear whether this is a general pattern (Leinonen *et al.*, 2013). Pair-wise analyses, of $Q_{ST}$, $F_{ST}$ and altitude differences, in contrast, are not as impacted by high neutral genetic differentiation and thus are given particular weight here. Together, these considerations show that it is important to consider joint evidence of different analyses in studies of trait divergence.

Analyses of individual traits provide further evidence for the nature of natural selection along altitude. For one trait, rosette leaf number after 3 weeks of vegetative growth in non-vernalized plants, altitudinal cline analysis, $Q_{ST}$-$F_{ST}$ comparisons and pair-wise analyses all point toward adaptive divergence along altitude rather than genetic drift underlying a significant rosette leaf number decrease with altitude. This is well in accordance with the multivariate analysis as rosette leaf number was associated mainly with the second PC that exhibited evidence for selective divergence along altitude. Rosette leaf number for plants under vernalized conditions, also significantly decreased with altitude and pairwise $Q_{ST}$ was significantly associated with altitude differences even when controlled for $F_{ST}$; however, overall $Q_{ST}$ was not significantly different from $F_{ST}$. This inconsistency could be due to limitations in detecting $Q_{ST} > F_{ST}$ or to trait correlations across vernalization treatments (see above).

An adaptive decrease of rosette leaf number in the vegetative state with increasing altitude is consistent with phenotypic clines in the rate of leaf production during winter along altitude as reported for Iberian *A. thaliana* populations (Montesinos-Navarro *et al.*, 2011). Similarly, leaf production and plant size decreased along latitude that is correlated with a decrease in temperature at a larger scale (Li *et al.*, 1998). Note that in these studies and in our study, rosette leaf number and rosette diameter after a defined period of time are used as measures of growth; in our study, rosette leaf number and rosette diameter after 3 weeks were not correlated with flowering time (Supplementary Table S5). Most other *A. thaliana* studies report rosette leaf number and rosette diameter at flowering, measures that often are highly correlated with flowering time and represent investment into growth vs reproduction (e.g., Mendez-Vigo *et al.*, 2011; Montesinos-Navarro *et al.*, 2011; Mendez-Vigo *et al.*, 2013). The reduced rosette leaf number with higher altitudes in our study is likely associated with above ground biomass accumulation during vegetative growth as neither rosette diameter after 3 weeks nor leaf area decreased with altitude. Smaller stature or size of plants growing at high altitude is one of the most conspicuous altitudinal pattern across species (Körner, 1999, 2003) and has also been detected within other species, for example, in *Senecio inaequidens* (Monty and Mahy, 2009) and in *Festuca eskia* (Gonzalo-Turpin and Hazard, 2009). The physiological mechanism giving rise to this pattern is, however, unclear, although different hypotheses have been proposed, for example, that small plants benefit from the warmer temperatures close to the soil in cold conditions at high altitude (Körner, 1999).

Population differentiation in leaf traits exhibited less clear patterns. We found a significant association of pairwise $Q_{ST}$ with altitude differences for leaf area in vernalized plants but this was not supported by an altitudinal cline in population means or significant $Q_{ST} > F_{ST}$ and we thus do not have conclusive evidence for this trait. Leaf traits were measured on fewer individuals than the other traits we report here and this could have reduced statistical power. For specific leaf area in vernalized plants, in contrast, $Q_{ST} > F_{ST}$ was significant, indicating possible adaptive divergence. However, this divergence could not be attributed to altitude, as population means were not significantly related to altitude and pairwise $Q_{ST}$ were not associated with altitude differences. Thus, considering the entire set of populations used in our study, selective agents other than climatic conditions along altitude and possibly occurring during the winter-annual life history, at least in some populations, may have driven the divergence in specific leaf area. Indeed, in 12 of 14 populations, specific leaf area in vernalized plants did decrease strongly with altitude (Figure 2b).

The two populations from the lowest altitudes did not fit into that pattern and exhibited equally low specific leaf area than did populations from the highest altitudes. In *A. thaliana*, specific leaf area decreases with latitude of origin (Li *et al.*, 1998). In other species, specific leaf area was shown to decrease with either altitude or drought or with both factors (e.g., Hovenden and Vander Schoor, 2004; Gonzalo-Turpin and Hazard, 2009; Ramirez-Valiente *et al.*, 2010; Scheepens *et al.*, 2010) and this is also the pattern observed between species (Körner, 1999, 2003; Niinemets, 2001; Poorter *et al.*, 2009). Thus, it could be that both of these selective pressures act on specific leaf area in different populations of *A. thaliana*. For leaf succulence under both vernalized and non-vernalized conditions, overall $Q_{ST}$ significantly exceeded $F_{ST}$ and population means significantly increased with altitude. Pair-wise $Q_{ST}$ for leaf succulence was significantly associated with altitude but these associations did not remain significant when controlled for neutral genetic divergence ($F_{ST}$). Our data thus give only partial evidence for selective divergence in leaf succulence along altitude. We measured leaf succulence as g $H_2O$ per leaf area and an increased leaf succulence at high altitude could thus be related to higher leaf thickness at high altitude, a pattern that has previously been reported in *Nothofagus cunninghamii* (Hovenden and Vander Schoor, 2004) and is suspected to underlie the commonly described within- and between-species pattern of specific leaf area decrease with altitude (Körner, 1999; Niinemets, 2001; Körner, 2003; but see Gonzalo-Turpin and Hazard, 2009). A higher leaf thickness in high-altitude species or populations has been related to leaf anatomical modification resulting in a higher photosynthetic activity (Körner, 1999). Overall, our data are consistent with an adaptive increase along altitude in leaf succulence, however, further experiments are needed to elucidate the possible adaptive advantage of higher leaf succulence at high altitude.

For the remaining traits, rosette diameter after 3 weeks and at flowering as well as days to flowering, we did not find any indication of adaptive divergence or trait association with altitude. Overall $Q_{ST}$ values for these traits were not distinguishable from $F_{ST}$. $Q_{ST} = F_{ST}$ is often interpreted as divergence driven by selectively neutral processes (Merilä and Crnokrak, 2001; Whitlock, 2008; Leinonen *et al.*, 2013). However, neutral divergence would also be expected to lead to pairwise $Q_{ST}$-$F_{ST}$ associations (Hangartner *et al.*, 2011; Kawakami *et al.*, 2011), but these were not significant indicating that we have no evidence for neutral divergence in these traits. Our findings differ from other studies on Iberian populations reporting flowering time increase as one of the most prominent patterns of adaptive divergence with altitude (Mendez-Vigo *et al.*, 2011, 2013; Montesinos-Navarro *et al.*, 2011). Populations in those studies originated from about 100 to 1700 m above sea level, whereas ours originated from 600 to 2700 m. This shift in regional altitude ranges and thus climatic conditions could be related to the different conclusions on flowering times divergence along altitude. Very low altitude regions in Spain have extreme drought conditions in summer selecting for early flowering time after vernalization (Mendez-Vigo *et al.*, 2011, 2013; Montesinos-Navarro *et al.*, 2011; Wolfe and Tonsor, 2014). The very-high-altitude conditions in our study (half of the populations were from above 1700 m, exceeding the altitudinal range of the Iberian populations) include extended and cold winters that *A. thaliana* probably cannot survive as a vegetative plant but only as seed. This could have led to selection pressure for fast cycling and early flowering at a small size without vernalization. Indeed, we assume that only summer-annual *A. thaliana* populations are viable at such high altitudes (see above).

Despite the limitations of $Q_{ST}$-$F_{ST}$ comparisons, our study detected indications for adaptive divergence in ecologically relevant traits, particularly when expressed in the summer-annual life history. Of the individual traits, rosette leaf number after 3 weeks showed the strongest evidence of adaptive divergence along altitude, and weaker evidence was detected for leaf succulence. To our knowledge, this is the first time that some of these ecologically relevant traits are reported in studies of phenotypic and genetic population divergence in *A. thaliana*. Patterns of reduced leaf number and increased leaf succulence with higher altitude as reported here conform to commonly observed patterns within and between species. Detecting these patterns in the model species *A. thaliana* provides possibilities for exciting research on the underlying genetic and physiological mechanisms leading to some of the most conspicuous ecological trends along altitude.

## DATA ARCHIVING

SSR data, as well as phenotypic measurement data available from the Dryad Digital Repository: doi:10.5061/dryad.r867b.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## ACKNOWLEDGEMENTS

We thank Franziska Berger-Glarner for leaf measurements, Beatrice Blattmann for help with genotyping and Maja Frei for taking care of the plants. This work was supported by the Competence Center Environment and Sustainability (CCES) of the ETH-Domain in the framework of the 'GeneMig' project (Nr. 35-01). The Genetic Diversity Centre (GDC) of ETH Zurich supported molecular analyses.

Bates D, Maechler M, Bolker B, Walker S (2013). *lme4*: Linear mixed-effects models using Eigen and S4. *R package version*: 1.0-4.

Bergelson J, Roux F (2010). Towards identifying genes underlying ecologically relevant traits in *Arabidopsis thaliana*. *Nat Rev Genet* **11**: 867–879.

Bonnin I, Prosperi JM, Olivieri I (1996). Genetic markers and quantitative genetic variation in *Medicago truncatula* (Leguminosae): A comparative analysis of population structure. *Genetics* **143**: 1795–1805.

Brachi B, Villoutreix R, Faure N, Hautekeete N, Piquot Y, Pauwels M *et al.* (2013). Investigation of the geographical scale of adaptive phenological variation and its underlying genetics in *Arabidopsis thaliana*. *Mol Ecol* **22**: 4222–4240.

Brady KU, Kruckeberg AR, Bradshaw HD (2005). Evolutionary ecology of plant adaptation to serpentine soils. *Annu Rev Ecol Evol Syst* **36**: 243–266.

Donohue K (2005). The evolutionary ecology of seed germination of *Arabidopsis thaliana*: Variable natural selection on germination timing. *Evolution* **59**: 758–770.

Edelaar P, Burraco P, Gomez-Mestre I (2011). Comparisons between $Q_{ST}$ and $F_{ST}$—how wrong have we been? *Mol Ecol* **20**: 4830–4839.

Galpern P, Manseau M, Hettinga P, Smith K, Wilson P (2012). ALLELEMATCH: an R package for identifying unique multilocus genotypes where genotyping error and missing data may be present. *Mol Ecol Resour* **12**: 771–778.

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*. *J Ecol* **97**: 742–751.

Goudet J (1995). FSTAT (Version 1.2): a computer program to calculate F-statistics. *J Hered* **86**: 485–486.

Hangartner S, Laurila A, Raesaenen K (2011). Adaptive divergence in moor frog (*Rana arvalis*) populations along an acidification gradient: inferences from qst-fst correlations. *Evolution* **66**: 867–881.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005). Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* **25**: 1965–1978.

Hoffmann MH (2002). Biogeography of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). *J Biogeogr* **29**: 125–134.

Hovenden MJ, Vander Schoor JK (2004). Nature vs nurture in the leaf morphology of Southern beech, *Nothofagus cunninghamii* (Nothofagaceae). *New Phytol* **161**: 585–594.

Karhunen M, Ovaskainen O, Herczeg G, Merila J (2013). Bringing habitat information into statistical tests of local adaptation in quantitative traits: a case study of nine-spined sticklebacks. *Evolution* **68**: 559–568.

Karrenberg S, Widmer A (2008). Ecologically relevant genetic variation from a non-*Arabidopsis* perspective. *Curr Opin Plant Biol* **11**: 156–162.

Kawakami T, Morgan TJ, Nippert JB, Ocheltree TW, Keith R, Dhakal P *et al.* (2011). Natural selection drives clinal life history patterns in the perennial sunflower species. *Mol Ecol* **20**: 2318–2328.

Kooyers NJ, Olsen KM (2012). Rapid evolution of an adaptive cyanogenesis cline in introduced North American white clover (*Trifolium repens* L.). *Mol Ecol* **21**: 2455–2468.
Kuittinen H, Mattila A, Savolainen O (1997). Genetic variation at marker loci and in quantitative traits in natural populations of *Arabidopsis thaliana*. *Heredity* **79**: 144–152.
Körner C (1999). Alpine plants: stressed or adpated? In: Press MC, Scholes JD, Barker MG (eds) *Physiological Plant Ecology*. Blackwell: Oxford, UK. pp 297–312.
Körner C (2003). *Alpine Plant Life—Functional Plant Ecology Of High Mountain Ecosystems*. Springer: Heidelberg, Germany.
Körner C (2007). The use of 'altitude' in ecological research. *Trends Ecol Evol* **22**: 569–574.
Le Corre V (2005). Variation at two flowering time genes within and among populations of *Arabidopsis thaliana:* comparison with markers and traits. *Mol Ecol* **14**: 4181–4192.
Le Corre V, Kremer A (2012). The genetic differentiation at quantitative trait loci under local adaptation. *Mol Ecol* **21**: 1548–1566.
Leinonen T, McCairns RJ, O'Hara RB, Merilä J (2013). $Q_{ST}$-$F_{ST}$ comparisons: evolutionary and ecological insights from genomic heterogeneity. *Nat Rev Genet* **14**: 179–190.
Li B, Suzuki JI, Hara T (1998). Latitudinal variation in plant size and relative growth rate in *Arabidopsis thaliana*. *Oecologia* **115**: 293–301.
Mendez-Vigo B, Gomaa NH, Alonso-Blanco C, Pico FX (2013). Among- and within-population variation in flowering time of Iberian *Arabidopsis thaliana* estimated in field and glasshouse conditions. *New Phytol* **197**: 1332–1343.
Mendez-Vigo B, Pico FX, Ramiro M, Martinez-Zapater JM, Alonso-Blanco C (2011). Altitudinal and climatic adaptation is mediated by flowering traits and *FRI*, *FLC*, and *PHYC* genes in *Arabidopsis*. *Plant Physiol* **157**: 1942–1955.
Merilä J, Crnokrak P (2001). Comparison of genetic differentiation at marker loci and quantitative traits. *J Evol Biol* **14**: 892–903.
Montesinos-Navarro A, Pico FX, Tonsor SJ (2012). Clinal variation in seed traits influencing life cycle timing in *Arabidopsis thaliana*. *Evolution* **66**: 3417–3431.
Montesinos-Navarro A, Wig J, Pico FX, Tonsor SJ (2011). *Arabidopsis thaliana* populations show clinal variation in a climatic gradient associated with altitude. *New Phytol* **189**: 282–294.
Monty A, Mahy G (2009). Clinal differentiation during invasion: *Senecio inaequidens* (Asteraceae) along altitudinal gradients in Europe. *Oecologia* **159**: 305–315.
Niinemets U (2001). Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology* **82**: 453–469.
Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson GL, Solymos P *et al*. (2009). *vegan*: Community Ecology Package. R package version 1.15-4 http://CRAN.R-project.org/package=vegan.
O'Hara RB, Merilä J (2005). Bias and precision in $Q_{ST}$ estimates: problems and some solutions. *Genetics* **171**: 1331–1339.
Picó FX (2012). Demographic fate of *Arabidopsis thaliana* cohorts of autumn- and spring-germinated plants along an altitudinal gradient. *J Ecol* **100**: 1009–1018.
Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytol* **182**: 565–588.
Porcher E, Giraud T, Lavigne C (2006). Genetic differentiation of neutral markers and quantitative traits in predominantly selfing metapopulations: confronting theory and experiments with *Arabidopsis thaliana*. *Genet Res* **87**: 1–12.
Ramirez-Valiente JA, Sanchez-Gomez D, Aranda I, Valladares F (2010). Phenotypic plasticity and local adaptation in leaf ecophysiological traits of 13 contrasting cork oak populations under different water availabilities. *Tree Physiol* **30**: 618–627.
Reiman C, Breckle S-W (1995). Salt tolerance and ion relations of *Salsola kali* L.: differences between ssp. *tragus* (L.) Nyman and ssp. *ruthenica* (Iljin) Sod. *New Phytol* **130**: 37–45.
Revelle W (2013). *psych*: Procedures for Psychological, Psychometric, and Personality Research. version 1.3.10, http://CRAN.R-project.org/package=psych.
Rousset F (2008). GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Mol Ecol Resour* **8**: 103–106.
Savolainen O, Lascoux M, Merila J (2013). Ecological genomics of local adaptation. *Nat Rev Genet* **14**: 807–820.
Scheepens JF, Frei ES, Stöcklin J (2010). Genotypic and environmental variation in specific leaf area in a widespread Alpine plant after transplantation to different altitudes. *Oecologia* **164**: 141–150.
Schielzeth H (2010). Simple means to improve the interpretability of regression coefficients. *Methods Ecol Evol* **1**: 103–113.
Vasemägi A (2006). The adaptive hypothesis of clinal variation revisited: single-locus clines as a result of spatially restricted gene flow. *Genetics* **173**: 2411–2414.
Venables WN, Ripley BD (2002). *Modern Applied statistics with R*. Springer: New York, NY, USA.
Verhoeven KJF, Simonsen KL, McIntyre LM (2005). Implementing false discovery rate control: increasing your power. *Oikos* **108**: 643–647.
Weir BS, Cockerham CC (1984). Estimating F-statistics for the analysis of population-structure. *Evolution* **38**: 1358–1370.
Whitlock MC (2008). Evolutionary inference from $Q_{ST}$. *Mol Ecol* **17**: 1885–1896.
Wolfe MD, Tonsor SJ (2014). Adaptation to spring heat and drought in northeastern Spanish *Arabidopsis thaliana*. *New Phytol* **201**: 323–334.

Supplementary Information accompanies this paper on Heredity website (http://www.nature.com/hdy)