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RESEARCH IN CONTEXT

Climate as a driver of adaptive variations in ecological strategies in *Arabidopsis thaliana*

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• **Background and aims** The CSR classification categorizes plants as stress tolerators (S), ruderals (R) and competitors (C). Initially proposed as a general framework to describe ecological strategies across species, this scheme has recently been used to investigate the variation of strategies within species. For instance, ample variation along the S–R axis was found in *Arabidopsis thaliana*, with stress-tolerator accessions predominating in hot and dry regions, which was interpreted as a sign of functional adaptation to climate within the species.
• **Methods** In this study the range of CSR strategies within *A. thaliana* was evaluated across 426 accessions originating from North Africa to Scandinavia. A position in the CSR strategy space was allocated for every accession based on three functional traits: leaf area, leaf dry matter content (LDMC) and specific leaf area (SLA). Results were related to climate at origin and compared with a previous study performed on the same species. Furthermore, the role of natural selection in phenotypic differentiation between lineages was investigated with Q_{ST}–F_{ST} comparisons, using the large amount of genetic information available for this species.
• **Key Results** Substantial variation in ecological strategies along the S–R axis was found in *A. thaliana*. By contrast with previous findings, stress-tolerator accessions predominated in cold climates, notably Scandinavia, where late flowering was associated with traits related to resource conservation, such as high LDMC and low SLA. Because of trait plasticity, variations in CSR classification in relation to growth conditions were also observed for the same genotypes.
• **Conclusions** There is a latitudinal gradient of ecological strategies in *A. thaliana* as a result of within-species adaptation to climate. Our study also underlines the importance of growth conditions and of the methodology used for trait measurement, notably age versus stage measurement, to infer the strength and direction of trait–environment relationships. This highlights the potential and limitations of the CSR classification in explaining functional adaptation to the environment.

Key words: *Arabidopsis thaliana*, adaptive differentiation, climate, CSR classification, ecological strategy, functional trait, genetic diversity, Grime triangle, latitudinal gradient, Q_{ST}–F_{ST}, trait–environment relationships.

INTRODUCTION

Screening approaches allow species comparison on the basis of key functional traits, i.e. traits representative of major functions, such as growth, stress resistance, defence and reproduction (Keddy, 1992; Violle *et al.*, 2007). Trait-based approaches in plant ecology have a long history of classifying plant species into functional groups according to the combination of phenotypic traits they exhibit (Garnier *et al.*, 2016). Such approaches have been mainly applied for comparative analyses at the interspecific level to identify general patterns of trait variation and covariation. However, recent comparative analyses argue for a better integration of intraspecific variability for understanding the role of trait covariation in plant adaptation, ecosystem functioning and community assembly (Albert *et al.*, 2010, 2011; Violle *et al.*, 2012; Siefert *et al.*, 2015).

Amongst the prominent examples of plant species classification, Grime (1977) defined ecological strategies based on the idea that there are two main ecological drivers of plant diversification: (1) the effect of stress related to the shortage of resources (e.g. nutrient, water and light); and (2) the effect of disturbance. Stress is viewed in this context as any environmental factors or combination of factors that reduce plant growth, although the shortage of nutrients, water or light can each affect specific traits (Grime and Hunt, 1975; Grime, 1977; Hodgson *et al.*, 1999). By contrast, disturbance is viewed as factors that cause the partial or total destruction of plant biomass, which include grazing, trampling and mowing, but also extreme climatic events such as severe drought, frost and fire (Grime and Hunt, 1975). Differences in disturbance and stress intensity are expected to result in quantitative variation in three ecological strategies: (1) stress tolerators (S) in stressed, resource-poor habitats with low disturbance, which invest resources to protect tissue from stress damages; (2) ruderals (R) in resource-rich environments associated with repeated disturbance, which

invest resources in rapid reproduction and propagule dispersal; and (3) competitors (C) in highly productive habitats with low stress intensity and disturbance, which invest resources in the rapid growth of large organs to outcompete neighbours. The S–R axis is traditionally viewed as an axis of resource-use variations at the leaf level (Pierce *et al.*, 2013), where ruderality is associated with acquisitive resource use (characterized by short-lived, flimsy leaves with high nutrient concentration and high net photosynthetic rate), and stress tolerance is associated with conservative resource use (characterized by long-lived, tough leaves with low nutrient concentration and low net photosynthetic rate). By contrast, variation in competitive ability along the C axis is thought to reflect variation in plant and organ size, and it is expected to operate where the impacts of stress and disturbance are low (Grime, 1977; Hodgson *et al.*, 1999).

Originally designed in the context of temperate herbaceous vegetation, the CSR scheme has been extended to other types of vegetation (Caccianiga *et al.*, 2006; Navas *et al.*, 2010; Schmidtlein *et al.*, 2012), including a recent worldwide application (Pierce *et al.*, 2017). An algorithm has recently been developed to quantify the CSR scores of diverse plant species based on the measurement of three leaf traits: leaf area (LA); specific leaf area (SLA); and leaf dry matter content (LDMC) (Pierce *et al.*, 2013, 2017). Albeit less precise than methods that consider whole-plant traits, which are more closely associated with stress response, competitive ability and ruderality (Hodgson *et al.*, 1999), classification tools based on a few leaf traits have the advantage that many measurements can be performed with minimal effort. This makes it possible to compare very ecologically disparate species (Pierce *et al.*, 2017), or many genotypes and populations within species (May *et al.*, 2017).

Arabidopsis thaliana is a small, rosette-shaped species that is widely used in molecular biology and quantitative genetics. It has recently gained renewed interest in evolutionary ecology due to the large collection of natural accessions collected from various climates and genotyped at high density (Weigel, 2012). Furthermore, *A. thaliana* has been shown to exhibit a significant range of phenotypic variation in relation to climate, making it possible to investigate the genetic and evolutionary drivers of functional diversification (Vasseur *et al.*, 2018). For instance, Q_{ST}–F_{ST} analysis has been proposed as a powerful way to discriminate adaptive and non-adaptive processes at the origin of phenotypic differentiation between genetic groups, populations or lineages (Leinonen *et al.*, 2013). Indeed, this method allows one to compare the level of phenotypic differentiation (Q_{ST}) with the genetic differentiation (F_{ST}) expected under the neutral model of population divergence. In plants, this has been used to investigate the role of selection at the origin of between-population phenotypic differences related to resource-use traits (Brouillette *et al.*, 2014), drought resistance (Ramírez-Valiente *et al.*, 2018), life history traits (Moyers and Rieseberg, 2016) and functional adaptation to an elevation gradient (Luo *et al.*, 2015).

Arabidopsis thaliana is generally described as a ruderal species that, like most annual plants, reproduces quickly and preferentially invests resources in the production and dispersal of propagules (Díaz *et al.*, 2016; Pierce *et al.*, 2017). In a recent paper, May *et al.* (2017) used the CSR framework to investigate intraspecific variation in ecological strategies within this species. Using 16 accessions originating from contrasted climates in Europe, they found that *A. thaliana* actually exhibits a wide range of variation from ruderals to stress tolerators, with most accessions being classified as intermediate (SR) and none as a competitor. Interestingly, May *et al.* (2017) also found that ruderality was negatively correlated with the temperature at the site where the accession originated. For instance, stress tolerators originated predominantly from sites in hot climates (Libya, Sicily and Cape Verde Islands). However, May *et al.* (2017) used a relatively low number of accessions, which prevents examination of the evolutionary and adaptive bases of CSR variations with the environment.

In the present study, we analysed CSR variations in a set of 426 *A. thaliana* accessions originating from contrasting climates in Europe, North Africa and East Asia. Using the classification method based on three leaf traits (LA, SLA and LDMC) (Pierce *et al.*, 2017), we tested the range of ecological strategies exhibited by these accessions. We investigated whether the variation in strategies can be attributed to adaptive processes, using the genetic data available in this species to perform Q_{ST}–F_{ST} analysis. We also examined how CSR strategies measured with leaf traits correlated with whole-plant traits related to competitive ability (rosette size) and propagule dispersal (fruit number). Finally, we compared our results with the findings of May *et al.* (2017), and discuss the possible causes of differences between studies, such as the direction of trait–environment relationships.

MATERIALS AND METHODS

Plant material

Two experiments were performed in this study: the first one in the PHENOPSIS automaton (see below) and the second one in a greenhouse. In the first experiment we used a total of 400 natural accessions of *Arabidopsis thaliana* representative of a geographical sampling from the worldwide lines of the RegMap population (Horton *et al.*, 2012) (n = 214) and from French local populations (Brachi *et al.*, 2013) (n = 186). In the second experiment, we used a total of 200 accessions from a random sampling from the worldwide lines of the RegMap population. Overall, 426 accessions ranging latitudinally from North Africa to Scandinavia were phenotypically characterized, 172 of which were common to the two experiments (Supplementary Data Tables S1 and S2).

Experimental design

In Experiment 1 (PHENOPSIS), plants were grown in the high-throughput phenotyping platform PHENOPSIS (Granier *et al.*, 2006) in 2014, using one replicate plant per accession, except for Col-0 for which there were ten replicates. Seeds were stratified in the dark at 4 °C for at least 1 week before sowing to ensure homogeneous germination among genotypes. Four to six seeds were sown at the soil surface in 225-mL pots filled with a 1:1 (v:v) mixture of loamy soil and organic compost. Prior to sowing, the soil surface was moistened with one-tenth strength Hoagland solution, and pots were kept in the dark for

48 h under controlled environmental conditions (20 °C, air relative humidity 70 %). Pots were then placed in the PHENOPSIS automaton growth chamber at 20 °C, daylength12 h, relative humidity 70 % and photosynthetic photon flux density (PPFD) 175 µmol m^{-2} s^{-1}. Pots were sprayed with deionized water three times per day until germination, and soil water content was then adjusted to 0.35 g H_2O g^{-1} dry soil (soil water potential –0.07 MPa) to ensure optimal growth (Aguirrezábal *et al.*, 2006; Vile *et al.*, 2012; Vasseur *et al.*, 2014). After emergence of the fourth leaf, seedlings were thinned to keep only one plant in each pot.

In Experiment 2 (greenhouse), plants were grown in four replicates per accession in a greenhouse between December 2015 and May 2016. Seeds were sown on organic soil and stratified at 4 °C for 4 d. At the emergence of the first two true leaves, plants were transplanted into 300-mL individual pots filled with a 1:1 (v:v) mixture of loamy soil and organic compost. Pots were randomly distributed among four blocks that were rotated every day in the greenhouse. All pots were watered twice a week. To reduce environmental heterogeneity in the greenhouse, walls were painted white and a semi-transparent curtain was installed below the glass roof. Additional light was provided to reach ~65 µmol m^{-2} s^{-1} PPFD. Photoperiod and temperature were kept constant at day length 12 h and day/night temperatures of 18/16 °C, respectively.

Trait measurement

In both experiments, traits were measured following standardized protocols (Perez-Harguindeguy *et al.*, 2013) at a fixed phenological stage when flower buds were macroscopically visible [i.e. bolting stage, used as measurement of flowering time (FT)]. The lamina of a fully expanded, adult, non-senescent leaf exposed to light was detached from the rosette, kept in deionized water at 4 °C for 24 h for water saturation, and then weighed (mg). After determination of water-saturated mass, individual leaves were scanned for determination of LA (mm^2) using ImageJ (https://imagej.nih.gov/ij/). Dry mass of the leaf lamina was obtained after drying for 72 h at 65 °C. We calculated LDMC (mg g^{-1}) and SLA (mm^2 mg^{-1}) as the ratio of lamina dry and water-saturated masses and the ratio of lamina area to lamina dry mass, respectively (Perez-Harguindeguy *et al.*, 2013). In the PHENOPSIS, plants were harvested at first opened flower and rosette fresh mass (mg) was measured. In the greenhouse, plants were harvested after full senescence and the total number of fruits was manually counted on the inflorescence. Overall, out of the 400 and 200 accessions in PHENOPSIS and the greenhouse, respectively, 357 and 198 accessions were completely phenotyped for all traits (Supplementary Data Tables S1 and S2), with 152 accessions common to both experiments.

We calculated CSR scores (i.e. percentages along the C, S and R axes) for all accessions in the PHENOPSIS and the greenhouse based on the three traits LA, LDMC and SLA, using the recent method developed by Pierce *et al.* (2017). The method is based on an algorithm that combines data for three leaf traits (LA, SLA and LDMC) that were shown to reliably position the species in the CSR scheme. We calculated CSR scores for each accession using the average trait value per experiment using the calculation table provided in the supplementary information of Pierce *et al.* (2017).

Re-analysis of published data

In our study there were several accessions in common with a previously published analysis of CSR variations in *A. thaliana* (May *et al.*, 2017). Ten accessions were common between May *et al*. and the PHENOPSIS experiment and six accessions in common with the greenhouse experiment. In May *et al*., CSR scores were calculated based on six traits with a method previously proposed by Hodgson *et al.* (1999). To compare the two datasets, we first recalculated CSR scores from data given by May *et al*. with Pierce's method, using LA, LDMC and SLA provided for their 16 accessions (May *et al.*, 2017), and compared them with the CSR scores they measured with Hodgson's method.

Genetic analysis and Q_{ST}–F_{ST} *comparisons*

Genetic groups in *A. thaliana* were determined by clustering of 395 accessions for the PHENOPSIS dataset and 198 accessions for the greenhouse dataset, both using the 250 K single nucleotide polymorphism (SNP) data available in Horton *et al.* (2012). Clustering was performed with ADMIXTURE (Alexander *et al.*, 2009) after linkage disequilibrium pruning ($r^2 < 0.1$ in a 50-kb window with a step size of 50 SNPs) with PLINK (Purcell *et al.*, 2007), resulting in 24 562 independent SNPs. We assigned each genotype to a group if >60 % of its genome derived from the corresponding cluster. The accessions not matching this criterion were labelled 'admixed' and were not used for the F_{ST} and Q_{ST} calculation. Cross-validation for different numbers of genetic clusters revealed that the PHENOPSIS dataset was composed of six genetic groups (group 1, 74 accessions; group 2, 48; group 3, 18; group 4, 55; group 5, 5; group 6, 71; admixed, 123), while the greenhouse dataset was composed of four genetic groups (group 1, 38 accessions; group 2, 16; group 3, 83; group 4, 7; admixed, 54). Consistent with the hypothesis of genetic divergence because of isolation by distance, these genetic groups were geographically clustered (Supplementary Data Fig. S1). We calculated Weir and Cockerham F_{ST} value for all 24 562 SNPs, and Q_{ST} as the between-group phenotypic variance divided by the total phenotypic variance, using mixed-effect models with group as random factor. We used a parametric bootstrap method to generate 95 % confidence intervals (CIs) around Q_{ST} values with the package MCMCglmm in R (R Core Team, 2014) (100 000 iterations).

Statistical analyses

Genotypic means in the greenhouse experiment were estimated as the fitted genotypic values from the linear models, using the lsmeans function. The genotype effect on trait variation and broad-sense heritability (H^2) were assessed using

individual data from the greenhouse experiment (Supplementary Data Table S3). The genotype effect was tested with one-way ANOVA following linear modelling, using genotype and block as explanatory variables. We measured H^2 as the ratio of phenotypic variance attributable to genotypic effect to total phenotypic variance, using mixed-effect models with block as fixed factor and genotype as random factor, using the package nlme in R.

Climate variables at the collection points of each accession were extracted from the Worldclim database (http://www.worldclim.org/bioclim), with resolution 2.5 arc-min. Trait–trait, trait–environment and trait–CSR relationships were examined with Spearman's rank coefficients of correlation (ρ) and associated P-values, using the function cor.test (Supplementary Data Table S4). Pearson coefficients of correlation (r) between traits and climatic variables were also calculated (Supplementary Data Table S5). Regression lines were drawn from Standard Major Axis (SMA), using the package smatr. All analyses were performed in R 3.2.3 (R Core Team, 2014).

RESULTS

Trait variation and covariation

All traits varied significantly among accessions (all $P < 0.001$; Supplementary Data Table S3). We found that FT ranged between 30 and 101 d (57 d on average) in the PHENOPSIS and between 25 and 115 d (61 d on average) in the greenhouse. Trait variation was mainly due to genetic variability among accessions, as measured by the high amount of phenotypic variance accounted for by the genotype effect (H^2 ranged between 0.58 for LA and 0.73 for SLA, 0.88 for FT; Supplementary Data Table S3). Most traits were correlated with each other (Supplementary Data Fig. S2, Tables S4 and S5): SLA and LDMC were negatively correlated (Spearman's $\rho = -0.94$ and -0.88 in the PHENOPSIS and the greenhouse, respectively; both $P < 0.001$) (Supplementary Data Fig. S2F), and FT was positively correlated with LDMC ($\rho = 0.63$ and 0.86; $P < 0.001$) (Supplementary Data Fig. S2B) and negatively with SLA ($\rho = -0.73$ and -0.92; both $P < 0.001$) (Supplementary Data Fig. S2D).

CSR classification

Arabidopsis thaliana accessions mainly varied along the S–R axis, between purely ruderals (R) and moderate stress tolerators (S/SR) (Fig. 1). We found only three accessions (together <1 %) classified as CS, CR or CSR. The accessions were mainly R-oriented: R, R/CR, R/CSR and R/SR represented 84 and 91 % of all accessions in the PHENOPSIS and the greenhouse, respectively (Table 1). Although we calculated CSR scores with only three leaf traits using Pierce's method, whole-plant traits were consistent with our classification. For instance, the C-axis is expected to be related to plant size and height, while the R-axis is expected to be related to FT and seed dispersal (Grime, 1977; Hodgson *et al.*, 1999). Accordingly, we found that the C- and R-axes were positively but poorly correlated with rosette fresh mass and the total number of fruits, respectively ($\rho < 0.50$, $P < 0.05$; Supplementary Data Fig. S3).

The CSR scores were significantly correlated between the PHENOPSIS and greenhouse experiments, as measured across the 152 accessions common to both experiments ($\rho = 0.34$, 0.41 and 0.54 for C, S and R, respectively, all $P < 0.001$; Supplementary Data Fig. S4). However, they were also significantly different between the two experiments ($P < 0.01$ for all the three scores). Accordingly, 78 accessions (51 %) were classified in different CSR groups between the two experiments ('plastic' accessions hereafter). Globally, plastic accessions shifted towards more ruderal strategies in the greenhouse compared with the PHENOPSIS, as reflected by the differences in S and R scores between experiments (Fig. 2). Twenty-two percent of the plastic accessions were classified as R in the PHENOPSIS and R/CR in the greenhouse (inversely, 18 % were classified as R/CR in the PHENOPSIS and R in the greenhouse). Comparatively, C scores did not differ a lot between the two experiments (Fig. 2B).

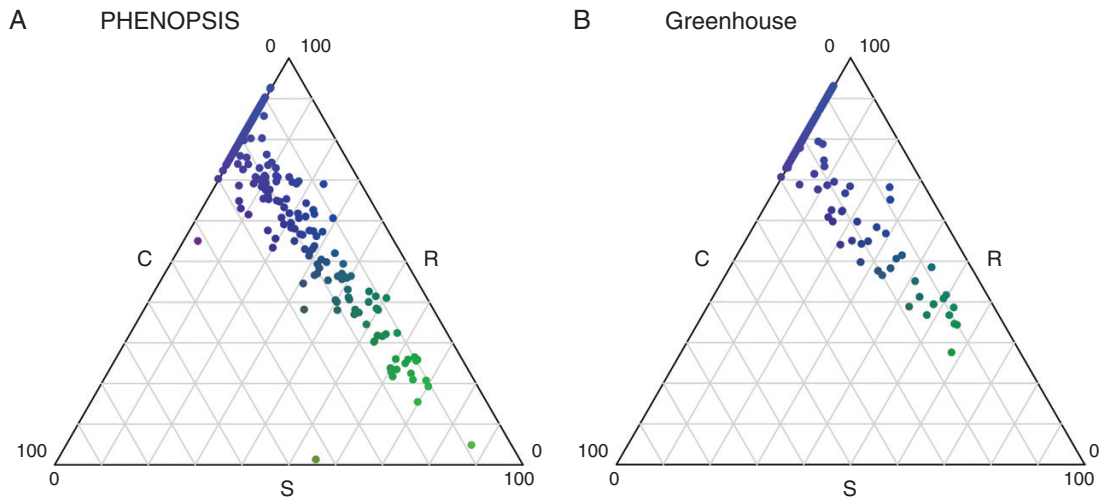

FIG. 1. CSR variation (%) in *A. thaliana*. (A) CSR representation of the 357 accessions from the PHENOPSIS. (B) CSR representation of the 198 accessions from the greenhouse. Dots are coloured according to CSR score following the colour code provided in Pierce *et al.* (2017).

Table 1. *Proportion (%) of ecological strategies among* A. thaliana *accessions.*

	PHENOPSIS	Greenhouse	Original scores from May *et al.* (2017)	Recalculated scores with data from May *et al.* (2017)
R	25.5	24.2		31.3
R/CR	47.6	58.6	12.5	
R/CSR	8.7	7.1		
R/SR	2.2	1.5	6.2	6.2
SR/CSR	8.4	4.5		12.5
SR	0.6	3.5	56.3	25.0
S	0.2			
S/CSR	3.1	0.6		18.7
S/SC			25.0	
S/SR	2.8			6.3
CSR	0.3			
CS	0.3			
CR	0.3			

Relationships between CSR scores, flowering time and climate

Ruderality was positively correlated with SLA and mean annual temperature (MAT, °C) at the collection point of the accessions, but negatively with FT and LDMC (Fig. 3; Supplementary Data Tables S4 and S5). Thus, our results suggest that ruderality is typical of early-flowering plants with leaf traits representative of fast resource acquisition, as reflected by low LDMC and high SLA values (Wright *et al.*, 2004; Shipley *et al.*, 2006). Inversely, stress tolerators were characterized by late flowering, with resource-conservative trait values such as high LDMC and low SLA, which were negatively correlated with MAT (Supplementary Data Fig. S5). Consistently, S and R strategies were positively and negatively correlated with latitude, respectively (Supplementary Data Table S4).

The Q_{ST}–F_{ST} analysis suggested that the latitudinal variations in CSR strategies resulted from adaptive processes such as natural selection acting on leaf traits. Indeed, a value of Q_{ST} significantly higher than F_{ST} at neutral loci is generally considered a signature of diversifying selection on the underlying traits (Leinonen *et al.*, 2013). Here, we used the 95th quantile of the F_{ST} distribution genome-wide as a threshold of significance for phenotypic differentiation above neutral expectation. In the greenhouse, both S and R scores were significantly above neutral F_{ST} (Q_{ST} = 0.95, 95 % CI 0.72–1.00 for S; Q_{ST} = 0.82, 95 % CI 0.62–1.00 for R; mean F_{ST} = 0.09 and F_{ST} 95th quantile = 0.35; Fig. 4A). In the PHENOPSIS, only R scores were above, but not significantly above, neutral F_{ST} (Q_{ST} = 0.37 versus F_{ST} 95th quantile = 0.33). The S scores were slightly, and non-significantly, below the neutral expectation (Q_{ST} = 0.29, 95 % CI 0.10–0.80; Fig. 4C). By contrast, in both the greenhouse and the PHENOPSIS, Q_{ST} values of C scores were close to 0, suggesting that this axis of plant strategies did not vary under the influence of adaptive processes in *A. thaliana*. The lower Q_{ST} values reported for the PHENOPSIS experiment can be explained by the absence of individual replicates in this experiment. By contrast, using the genotypic mean in the greenhouse across four replicates made it possible to reduce intra-genotypic variance and thus total phenotypic variance compared with phenotypic variance between genetic groups. Consistent with these results, plotting the distribution of *A. thaliana* ecological strategies across Europe (Fig. 4B, D) revealed that accessions with S-oriented strategies (S, SR, SR/CSR, S/CSR, S/SC, SC and SC/CSR) originated from northern regions, Sweden in particular.

Comparison with observations from May et al. *(2017)*

In contrast with our results, the 16 accessions in the study published by May *et al.* were mainly categorized as S-oriented: S/SC, S/SR, S/CSR, SR and SR/CSR (Table 1; Supplementary Data Fig. S6). May *et al.* used Hodgson's method to calculate

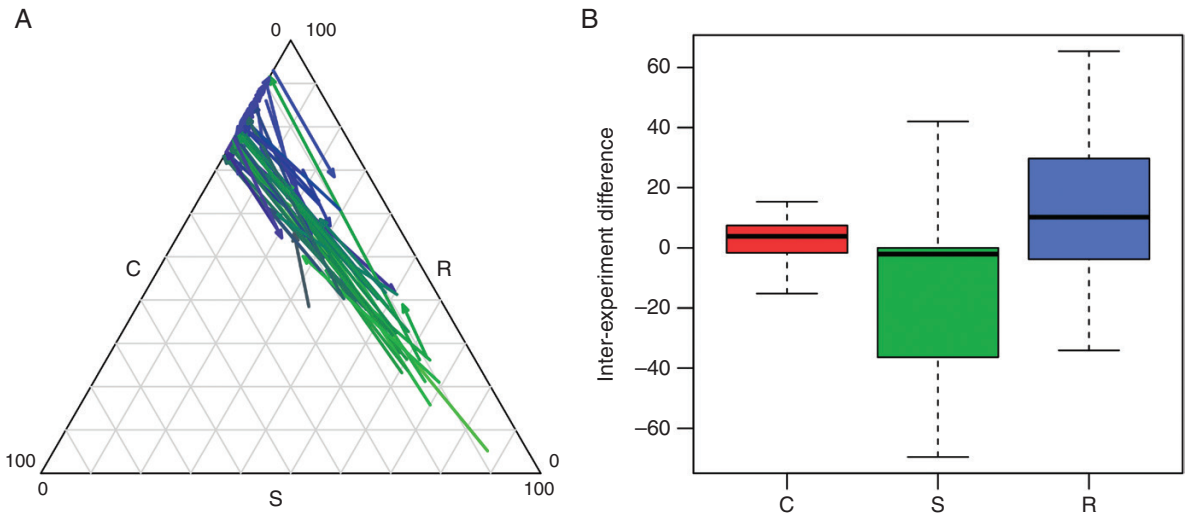

Fig. 2. Plasticity of CSR classification in *A. thaliana*. (A) The 78 plastic accessions that have a different CSR classification (%) between the PHENOPSIS and greenhouse experiments are plotted. Arrows start at the greenhouse position and end at the PHENOPSIS position, and are coloured according to CSR scores in the PHENOPSIS, following the colour code provided in Pierce *et al.* (2017). (B) Boxplot representing the difference in CSR scores (%) between experiments (greenhouse values minus PHENOPSIS values).

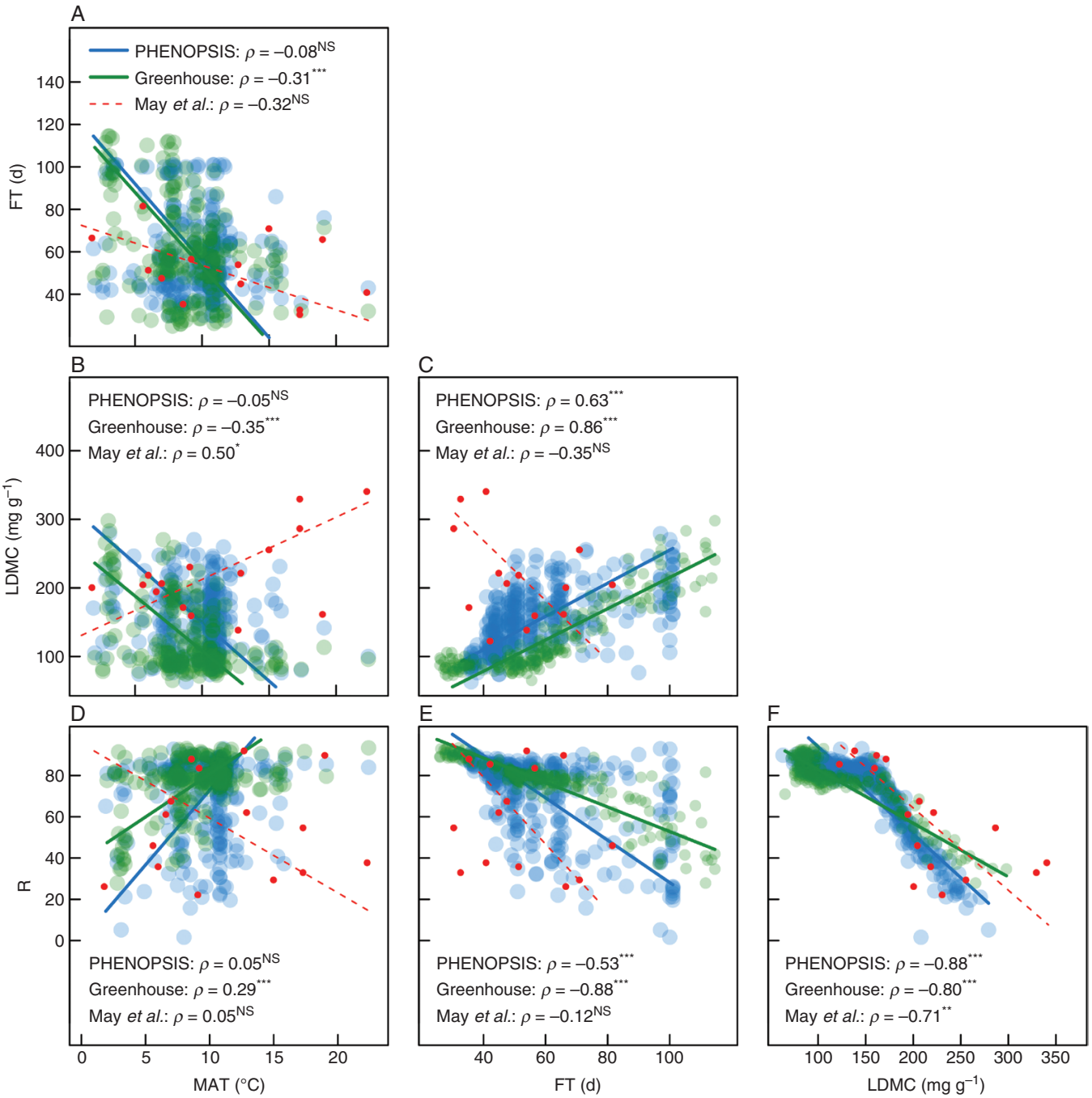

FIG. 3. Relationships between ruderality, traits and environment in *A. thaliana*. Leaf trait and flowering time data were obtained from May *et al.* (2017) (red dots, $n = 16$), the PHENOPSIS (green dots, $n = 357$) and the greenhouse (blue dots, $n = 198$). Ruderality (R) was calculated with Pierce's method (2017) for all data. Mean annual temperature (MAT, °C) was extracted at the collection point of the accessions using Worldclim. NS, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Regression lines were drawn using standard major axis (SMA).

CSR scores with seven traits, including FT and duration, two important components of ruderality (Hodgson *et al.*, 1999). To compare the two classification methods, we used the trait values for LA, SLA and LDMC provided by May *et al.* to calculate CSR scores with Pierce's method and compared them with those calculated with Hodgson's method. The CSR scores calculated with the two methods were positively correlated (ρ = 0.77, 0.79 and 0.73 for C, S and R, respectively; all $P < 0.01$; Supplementary Data Fig. S7), suggesting that the two methods return similar categorizations (Table 1). However, and despite the significant correlations, CSR scores varied substantially between the two methods (Supplementary Data Fig. S7). This showed that the traits related to ruderality (FT and duration) and competition (plant height and lateral spread) used in

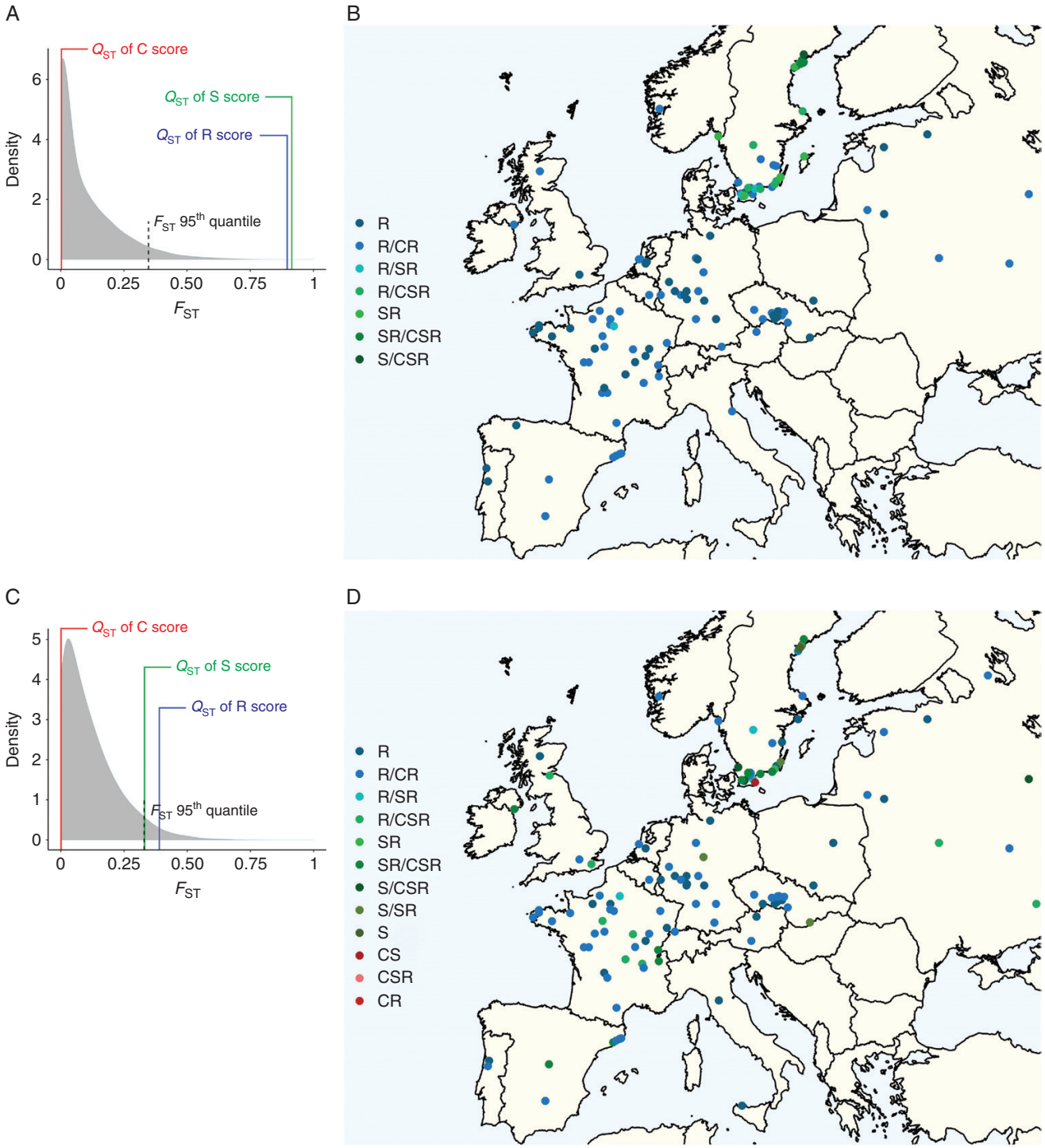

FIG. 4. Q_{ST}–F_{ST} analysis and geographical location of CSR strategies in *A. thaliana*. Distribution (in grey) of F_{ST} values across the 24 562 SNPs with the 95th quantile threshold of non-neutral expectation (dashed line), and Q_{ST} values for the C, S and R scores measured as the ratio of phenotypic variance between genetic groups over total phenotypic variance. (A, C) Analysis was performed independently on the greenhouse dataset (A) and the PHENOPSIS dataset (C). (B, D) Geographical location of CSR strategies with the greenhouse dataset (B, $n = 198$) and PHENOPSIS dataset (D, $n = 357$). Note that not all CSR strategies are found equally across Europe (for frequencies see Table 1).

Hodgson's method impacted the inference of plant ecological strategies compared with leaf traits alone.

Values of FT measured in this study were strongly positively correlated with those measured by May *et al.* under controlled conditions ($n = 10$ and 6 in the PHENOPSIS and the greenhouse, respectively, both $r = 0.96$, $P < 0.01$; Supplementary Data Fig. S8A). By contrast, LDMC measured by May *et al.* was negatively correlated with our measurements (Supplementary

Data Fig. S8D), possibly because of three individuals with early FT and extremely high LDMC values (>250 mg g^{-1}) compared with our measurement (<110 mg g^{-1}) on the same accessions (red dots in Fig. 3C). As a result, FT and LDMC were negatively, albeit non-significantly, correlated in the May *et al.* study (ρ = −0.35; Fig. 3C). Furthermore, there was a positive correlation between LDMC and MAT in May *et al.* ($P < 0.05$), while we found the opposite in both the PHENOPSIS and the greenhouse (Fig. 3B). By construction of the CSR classification method, LDMC participates strongly in the S and R axes (Fig. 3F; Supplementary Data Fig. S5). Consequently, the positive correlation between LDMC and MAT found in May *et al.* was associated with a positive correlation between S and MAT (Supplementary Data Fig. S5D), and inversely a negative correlation between R and MAT (Fig. 3D), although these two relationships were not significant with the 16 accessions from May *et al.* when using Pierce's method of CSR classification.

DISCUSSION

Functional adaptation to climate in A. thaliana

The relationship between CSR and climate at the interspecific level is still not well established (Pierce *et al.*, 2017). More broadly, trait–environment relationships remain a central question in functional ecology and functional biogeography (Poorter *et al.*, 2009; Violle *et al.*, 2014; Borgy *et al.*, 2017; Butler *et al.*, 2017; Šímová *et al.*, 2018). By contrast, adaptation to climate has been widely studied within species, notably genetic adaptation along latitudinal or altitudinal gradients in annual plants, and in *A. thaliana* in particular (Johanson *et al.*, 2000; Picó *et al.*, 2008; Banta *et al.*, 2012; Guo *et al.*, 2012; Brachi *et al.*, 2013; Wolfe and Tonsor, 2014; Bloomer and Dean, 2017; Tabas-Madrid *et al.*, 2018). Indeed, *A. thaliana* has been the model species in molecular biology, plant genetics and evolution in recent decades (Bergelson and Roux, 2010; Weigel, 2012). It is widely distributed in various climates, but is generally considered as a ruderal species that grows fast, reproduces early and dies right after seed dispersal (Pierce *et al.*, 2017). As expected, we found in this study that *A. thaliana* was predominantly ruderal, secondly a stress tolerator and poor competitor. However, we showed an important range of CSR variation among *A. thaliana* accessions along the S–R axis and associated with FT variation.

Consistent with previous studies, FT was positively correlated with latitude (Caicedo *et al.*, 2004; Lempe *et al.*, 2005; Banta *et al.*, 2012). For instance, northern accessions exhibit late flowering and a long life cycle even when they are grown under controlled conditions in a growth chamber or greenhouse (Vasseur *et al.*, 2018). Our results showed that FT was positively correlated with LDMC, and that values for the two traits were higher in accessions originating from higher latitudes and lower temperatures. Thus, northern accessions exhibit a suite of traits associated with resource conservation and longevity, such as late flowering, high LDMC and low SLA (Wright *et al.*, 2004; Shipley *et al.*, 2006; Vasseur *et al.*, 2012). The Q_{ST}–F_{ST} analysis revealed that these latitudinal variations result from the adaptive diversification of leaf traits. These adaptive shifts can be explained because, in cold regions, biomass production during the growing season is limited by various stresses. Low temperatures directly limit plant growth rate by slowing metabolic processes. Furthermore, cold indirectly limits plant growth rate because of the reduction in the availability of water and nutrients. In these conditions, a slow-growing genotype with a long life cycle, associated with high LDMC, low SLA and low metabolic activities, can be an efficient strategy. Interestingly, stress tolerance has been shown to be selected at both ends of the geographical range of *A. thaliana*, but is expressed under different temperature conditions (Exposito-Alonso *et al.*, 2018; Vasseur *et al.*, 2018).

Conversely, ruderal strategies were more abundant in temperate and hot environments. Ruderal plants are typically associated with a short life cycle, low LDMC and high SLA, and presumably high metabolic rate and low tissue protection (Grime, 1977). In temperate climates with a relatively long growing season and high resource availability, these characteristics may allow *A. thaliana* individuals to complete their growth cycle early and avoid competition with taller species. Furthermore, in hot and dry climates with a shorter growing period (e.g. the Mediterranean climate), fast-growing strategies may allow *A. thaliana* individuals to complete their growth cycle and disperse before the onset of drought, which operates as a disturbance rather than a stress, and should therefore be more favourable to ruderality (Madon and Médail, 1997; Volaire, 2018). This result is consistent with interspecific studies at global scale that reported a positive relationship between SLA and temperature in herbaceous species (Borgy *et al.*, 2017; Šímová *et al.*, 2018). This can be interpreted as a sign of selection for fast-growth ruderal strategies in hot and stressing environments at both intra- and interspecific levels (Anderegg *et al.*, 2018).

The lack of adaptive differentiation between genetic groups along the C axis, as reflected by the low Q_{ST} values compared with neutral F_{ST}, can be explained by the low variation in competitive ability among *A. thaliana* accessions. Additionally, it could suggest that competitive environments can be found in various climates as long as stress does not dominate vegetation processes. This would also explain the lack of a clear geographical pattern and latitudinal gradient of competitive ability across plant populations and species (Damgaard and Weiner, 2017).

Influence of classification methodologies, trait measurement and growth conditions on trait–environment relationships

Trait–trait, trait–CSR and trait–environment relationships were sometimes opposite between May *et al.* (2017) and our study. For instance, May *et al.* reported a positive correlation between stress tolerance and mean temperature, while we found the opposite. A first explanation of these differences is the methods used to calculate CSR scores among accessions. Although Pierce's and Hodgson's scores were all positively correlated when performed on the same set of traits and accessions, scores obtained from the two methods varied substantially. For instance, an accession had an S score of 35 % with Hodgson's method but 0 % with Pierce's method (Supplementary Data Fig. S7B). The re-analysis of the data of May *et al.* (2017) made

by the authors of that paper (A. Wingler, University College Cork, Ireland, pers. comm.) indicated that the three accessions with very high values for S identified using Hodgson's method (Mt-0, Cvi-0 and Ct-1) were no longer in the top three ranked accessions for S when using Pierce's method, which led to a lack of correlation of S and R with temperature when using this method. This can be explained because life history traits at whole-plant level, notably FT and plant size, are important components of ruderality and competitive ability in herbaceous species (Violle *et al.*, 2009; Hodgson *et al.*, 2017), but they are not included in Pierce's method of CSR classification. Here, we found that C and R axes calculated with leaf traits were positively, but poorly, correlated with rosette fresh mass and fruit number, respectively. Additionally, many early-flowering accessions were similarly classified as purely ruderal (R = 100 %), although they displayed variations in leaf traits and FT, and consequently in their level of ruderality. This was translated into no or small differences in CSR strategies between accessions from temperate and Mediterranean climates (Fig. 4), although Mediterranean accessions can be very short-lived and thus more ruderal than accessions from less stressing environments (Vasseur *et al.*, 2018). Together, this suggests that classification methods based on leaf traits can be a powerful means of screening large databases or performing many measurements at global scale, but might be of limited value in the examination of subtle variations within species and/or in specific taxa. For instance, including other, easily measurable traits might be necessary to better describe ruderality in annual plants, such as phytomer miniaturization and the number of juvenile phytomers, because each promotes early maturity (Hodgson *et al.*, 2017).

A second explanation of the opposite trait–environment relationships found between this study and May *et al.* (2017) is the difference in the protocols used for trait measurement. In our experiments, we followed the recommended procedures to phenotype traits of all individuals at the same *ontogenetic stage* (Reich *et al.*, 1999; Perez-Harguindeguy *et al.*, 2013). Specifically, LDMC and SLA were measured at the transition to flowering (i.e. bolting stage). By contrast, leaf traits were measured in a growth chamber at the same *age* by May *et al.* (61 d for LDMC), although FT in the growth chamber varied from 30 to 82 d (and some accessions did not flower at all), and although it is widely recognized that leaf traits strongly vary during plant ontogeny (Walters *et al.*, 1993; Hérault *et al.*, 2011; Pantin *et al.*, 2012). In other words, LDMC was measured 30 d after flowering for the earliest accessions and before flowering for the latest ones. With such a procedure, the leaves compared might have been in contrasted physiological stages. In particular, leaves measured on the early-flowering accessions might have been – at least in part – senescing, which may result in much higher LDMC values – and lower SLA values – in these accessions (Fig. 3C). In agreement with this hypothesis, the LDMC values measured on the early flowering accessions in our experiment were approximately half of the values estimated by May *et al*. As LDMC strongly participates in the S–R axis, this could explain the opposite correlations between CSR and environment between the two studies. Furthermore, we found that FT was positively correlated with LDMC, consistently with previous studies in a smaller set of accessions (Vile *et al.*, 2012), as well as in recombinant inbred lines (El-Lithy *et al.*, 2010; Vasseur *et al*., 2012, 2014). Previous studies have notably reported that early-flowering genotypes have resource-acquisitive strategies, characterized by high SLA but low LDMC and short lifespan (El-Lithy *et al.*, 2010; Vasseur *et al*., 2012, 2014, 2018; Blonder *et al.*, 2015).

Finally, opposite correlations between studies might also result partly from trait plasticity to growth conditions. In *A. thaliana* FT is expected to vary with light conditions and temperature (Mouradov *et al.*, 2002). For instance, *A. thaliana* does not generally flower under short-day conditions. In our study, traits were measured in controlled and constant conditions, on plants grown in a 12-h photoperiod and without cold exposure (i.e. vernalization). However, we could expect FT and leaf traits, and thus CSR–environment relationships, to be different when measured on plants grown outside, as in May *et al.* (2017), after vernalization or in short- or long-day conditions. Consistent with this idea, we found that half the accessions common to the PHENOPSIS and the greenhouse did not have the same position in the CSR space: plants grown in the greenhouse were generally shifted towards the R end of the spectrum compared with plants grown in the PHENOPSIS. This can be explained by the relative low light intensity provided by artificial lamps in the greenhouse compared with the phenotyping platform (65 versus 175 µmol m^{-2} s^{-1} PPFD). In addition, plants were grown in the greenhouse at higher density than in the PHENOPSIS, which could have increased competition for light between plants. The shade-avoidance syndrome has been described as a suite of leaf trait responses to low light and competition (Kim *et al.*, 2005; Mullen *et al.*, 2006). This includes an increase in leaf angle and SLA, associated with a reduction in LDMC and FT (Kim *et al.*, 2005; Vasseur *et al.*, 2011). This is consistent with a shift towards resource-acquisitive strategies in the greenhouse. Importantly and more broadly, controlled conditions are very different from the natural conditions that plants experience in the wild, and where plants should ideally be measured to properly infer their ecological strategies. However, it remains difficult to take into account genotype × environment interactions when screening genotypes in natural conditions. Consequently, trait-based approaches for the functional classification of plants were initially proposed as a tool to infer the adaptive significance of traits in controlled conditions (Grime and Hunt, 1975).

Conclusions

Intraspecific variation in functional strategies varied substantially along the S–R axis in *A. thaliana*. Tolerance to stress seems to be favoured in cold environments at higher latitudes while ruderality is predominant in temperate and hot climates. However, CSR categorization within species, specifically in a herbaceous species like *A. thaliana*, is sensitive to several parameters, such as the type of traits used to classify accessions and the protocols used for trait measurement. Furthermore, our results suggest that phenotypic plasticity to growth conditions can significantly impact trait values and thus the determination of plant ecological strategies. This suggests that the use of trait databases for local or global analyses of trait–environment relationships at species level might suffer from biases due to both phenotypic plasticity and intraspecific trait variation. In a

recent analysis, ruderality has been demonstrated to correlate positively with the probability of naturalization of alien species (Guo *et al.*, 2018). In future studies it will be interesting to examine in more detail the response of traits, trait combinations and strategies to environmental conditions. For instance, analysing the plasticity of CSR strategies to different temperatures and water stresses could reveal whether S-related strategies are constitutive or stress-induced, and whether invasive species show greater plasticity in ecological strategies than other species.

SUPPLEMENTARY DATA

Supplementary Data are available online at https://academic.oup.com/aob and consist of the following. Table S1: phenotypic traits measured in the PHENOPSIS experiment. Table S2: phenotypic traits measured in the greenhouse experiment. Table S3: heritability and genetic effects on traits measured in the greenhouse experiment. Table S4: Spearman's pairwise correlations between traits and environments. Table S5: Pearson's pairwise correlations between traits and environments. Fig. S1: geographical location of the genetic groups defined by SNP clustering. Fig. S2: trait–trait relationships in *A. thaliana.* Fig. S3: correlations between C and R axes, plant biomass and fruit number. Fig. S4: correlations between CSR scores in the PHENOPSIS and greenhouse experiments. Fig. S5: CSR–trait and CSR–environment relationships in *A. thaliana.* Fig. S6: CSR representation of the 16 accessions from May *et al.* (2017). Fig. S7: correlation between Hodgson's and Pierce's methods for quantifying CSR. Fig. S8: correlations between traits measured by May *et al.* (2017) and the present study.

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