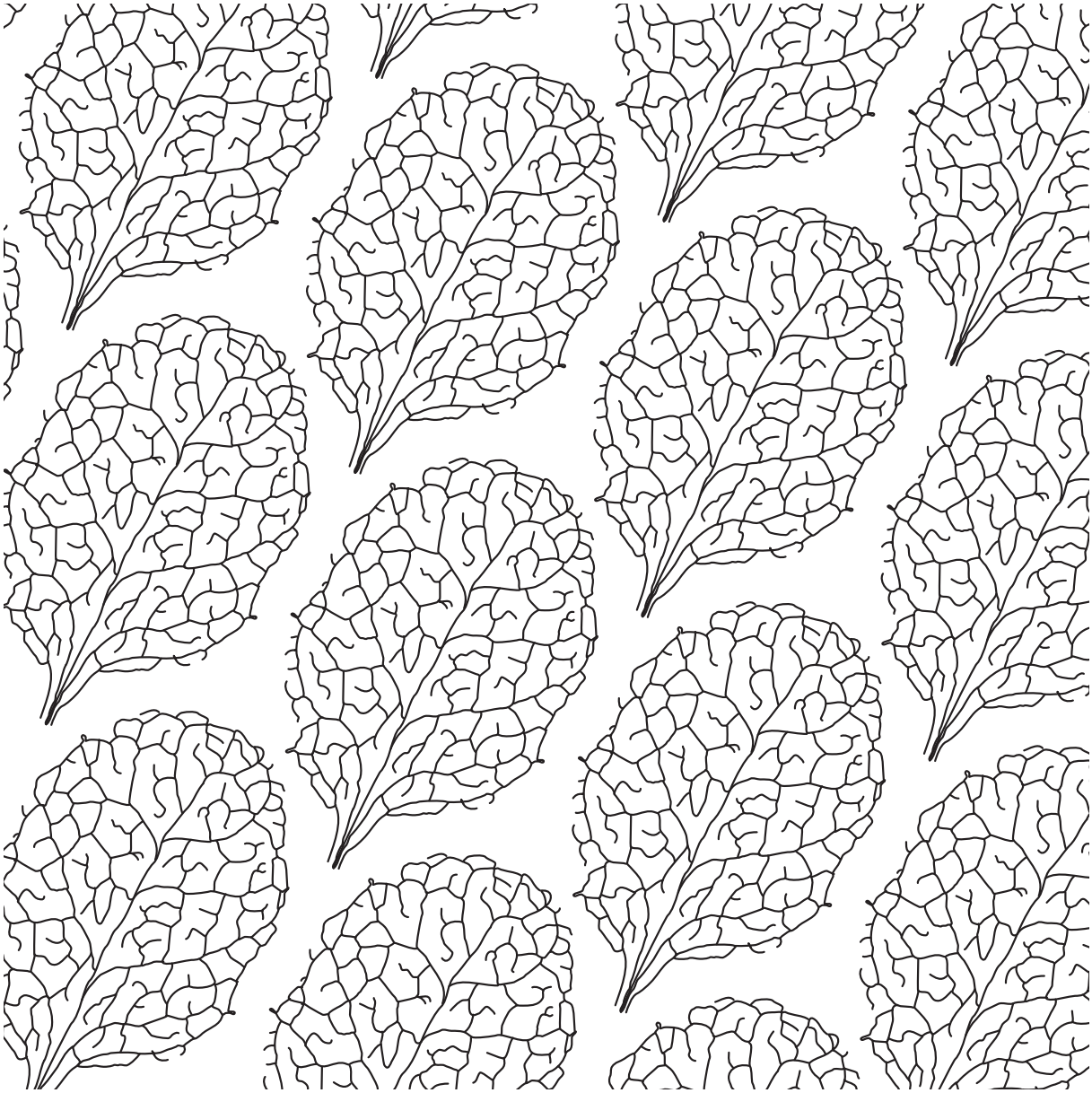

Exploration de la valeur adaptative de l’architecture des nervures.

A within-species analysis reveals the adaptive value of vein density along climatic gradients

En préparation.

Summary

Interspecific variability in vein density (VD) has been attributed to the diversity of the strategies of plant adaptation to their environment. However, the lack of within-species variability studies impedes a thorough evaluation of the role of VD in plant adaptation. This study brings new evidences of the role of vein architecture in plant adaptation to climate. We explored the morphological, genetic and environmental determinants of natural variation of leaf vein density among 169 Arabidopsis thaliana genotypes grown under controlled experimental conditions. Contrary to the global interspecific pattern, vein density was mainly explained by leaf size variation and increased with decreasing temperature. Nonetheless, we detected a genetic basis for VD variation with genes previously associated with VD variation as well as new genes not previously identified in VD studies. We further detected selection cues on the genome that collocated with the loci associated with VD variation. The model species Arabidopsis thaliana proves to be an attractive model to test hypotheses regarding the implication of functional traits for the plant adaptation to climate, merging approaches such as functional ecology, evolutionary biology and omics.

Keywords

Genetic differentiation, intraspecific variability, local adaptation, leaf vein density, leaf area

Introduction

Functional ecology holds promise for revealing the phenotypic determinants of the adaptation of organisms to their local environment thanks to the comparison of plant functional traits across species or genotypes on a physiological basis (Calow, 1987; Keddy, 1992; Violle *et al.*, 2007; Garnier *et al.*, 2016). In that respect, plant ecology has gained tremendous progress in our understanding of local adaptation through structure-function analyses at the leaf level (e.g., REF Kikuzawa, wright et al. 2004, un papier de Reich). Notably, leaf vein density (VD) has a strong physiological basis, and has been advanced as a key trait to explain both leaf-level variation in metabolism (Brodribb *et al.*, 2007; Brodribb & Feild, 2010) and plant-level adaptation to climate (Blonder *et al.*, 2018). This echoes macroevolution studies that have long emphasized the complexification of leaf vein architecture as a major morphological innovation in the success of Angiosperms over Gymnosperm and ferns (Roth-Nebelsick *et al.*, 2001; Boyce C. Kevin *et al.*, 2009; de Boer *et al.*, 2012; Simonin & Roddy, 2018). In addition, leaf vein traits have been proposed as a palaeoclimate and palaeoenvironmental proxy (Uhl & Mosbrugger, 1999; Blonder *et al.*, 2014). However, most of these studies are based on the environment-leaf venation-fitness linkage that is in fact still poorly known.

Establishing trait-environment relationships (TERs) is a foundation stone of functional ecology and functional biogeography (Violle *et al.*, 2014; Garnier *et al.*, 2016), but many of them have been hardly examined in plants (Shipley *et al.*, 2016). This is especially true for VD-climate relationships even if theoretical expectations have been provided. Physiological models hypothesized that VD is positively linked to growing season temperature, based on an assumed coupling between leaf transpiration rate and the maximum potential water demand of the environment (potential evapotranspiration) (Blonder & Enquist, 2014). Higher VD strategies should also be advantageous in arid environments given the putative role of leaf venation in withstanding hydraulic continuity failure (i.e. embolism) resulting from soil water depletion (Brodribb *et al.*, 2016). Some studies indeed highlighted a positive relationship between VD and temperature or aridity (Zhu *et al.*, 2012)(Blonder *et al.*, 2018)(Schneider *et al.*, 2017). However, many others also showed a lack of climate signal on VD variation (e.g., Jordan *et al.*, 2013). Overall, VD-climate relationships appeared to be taxon-specific, which can blur cross-species explorations. The comparison of intraspecific and interspecific TERs has gained momentum in functional ecology (Siefert *et al.*, 2015), and has allowed to reveal physiological mechanisms at play. More generally, cross-species TERs are sensitive to many sampling bias

(Borgy *et al.*, 2017), and phylogeny-controlled TERs can only partly capture their underlying mechanisms (Violle *et al.*, 2014).

TERs are classically built trait-by-trait, i.e. by overlooking trait covariation. Such a single-trait approach can lead to spurious physiological response curves if one or several traits covary with the trait and the environmental factor under scrutiny (Wüest *et al.*, 2018). This could be particularly true for VD that is computed as the total path length of vein conduit divided by leaf area. By construction, it is a leaf area-based trait, which makes it a scale-dependent parameter (Price *et al.*, 2014a). If VD is under the control of leaf area, TERs can be quite complex because the latter is already known to display a strong environmental signal at both local and global scales (Moles *et al.*, 2014; Wright *et al.*, 2017). In this context, the scaling relationship for vascular architecture with leaf size is pivotal to examine (Sack *et al.*, 2012). Sack et al. (2012) advocated that the relationship between VD and leaf area depends on the developmental stage of the leaves, and by consequence, on the different orders of veins that are used to compute VD. For full mature leaves, the authors did not expect any relationship between the density of minor veins as well of total VD and leaf area. Global cross-species analyses indeed revealed a lack of relationship between total VD and leaf area (Price *et al.*, 2012; Sack *et al.*, 2012). On the opposite, a negative relationship was found in several taxa and in more local studies (Roth-Nebelsick *et al.*, 2001). The divergence between global interspecific patterns and local and/or intraspecific patterns is common in functional ecology (Price *et al.*, 2014b; Messier *et al.*, 2017; Anderegg *et al.*, 2018; Osnas *et al.*, 2018). It can translate differential actions of evolutionary, ecophysiological and biophysical constraints at different scales and biological organizational levels.

Within-species studies are required to go deeper into the characterization of the constraints at the origin of trait-trait relationships. In particular, allometric scaling relationships are expected to hold within species if they result from fundamental biophysical (Witting, 1998; Shoval *et al.*, 2012) and/or evolutionary (Donovan *et al.*, 2011) constraints. More broadly, it is the time for functional ecology to meet molecular ecology in order to reveal the adaptive meaning of plant functional traits. In particular, model species for which genetic information is available are the best candidates to examine whether VD is linked to local adaptation. The co-localization (or lack of) of genes involved in both VD and leaf area could further be very promising to identify pleiotropic effects and explain the interdependency of both traits. Among model species, *Arabidopsis thaliana* has been used for decades in molecular biology due to its

short life cycle and small genome (Krämer, 2015). The natural distribution of the species covers large climatic gradients that led to a strong genetic structure of the populations, suggesting underlying local adaptation (Lasky *et al.*, 2012). Recently, *A. thaliana* has been used in functional ecology to quantify the heritability of functional traits and plant ecological strategies (Vasseur *et al.*, 2018b; Kazakou *et al.*, 2019) as well as to identify the mechanisms at the origin of functional tradeoffs and allometric scaling relationships (Vasseur *et al.*, 2012, 2018a; Blonder *et al.*, 2015; Sartori *et al.*, 2019). Surprisingly, the analysis of the natural variability of VD in *A. thaliana* remains scarce (Rishmawi *et al.*, 2017), as well as the exploration of VD-environment linkage and the underlying local adaptation (Stewart *et al.*, 2015, 2016). The combined use of the unique genetic data available for the species and the newly developed fast and efficient genome analysis methods (Zhou & Stephens, 2012; Luu *et al.*, 2017) allows an unprecedented exploration of the VD genetic determinism and adaptive value across the distribution range of *A. thaliana*. Specifically, following a recent study that highlights drought resistance-associated alleles at both Northern and Southern extremes of *A. thaliana* distribution (Exposito-Alonso *et al.*, 2018), we expect genes conferring high vein density to be under selection at these margins, too.

Material and Methods

Plant material and growth conditions

We selected a set of 169 *A. thaliana* genotypes covering the natural distribution of the species (Fig. 1) and loaded the genetic sequences from the 1001 genome website (1001genomes.org). Seeds were sown in moist organic compost and stratified in a cold chamber at 4 °C for four days. Four seedlings per genotype were then transferred in individual pots filled up with organic compost (Neuhaus N2). Pots were randomly distributed on four tables, i.e. blocks, with one replicate per genotype per table. Tables were placed in a greenhouse with temperature maintained at 18°C during the day and 16°C during the night and with a supplemental lighting to maintain a constant 12.5 h day length. Plants were watered twice a week. Tables were rotated daily to reduce block effects within the greenhouse. The experiment lasted 140 days, from sowing (1st of December 2015) to the last harvest (19th of April 2016).

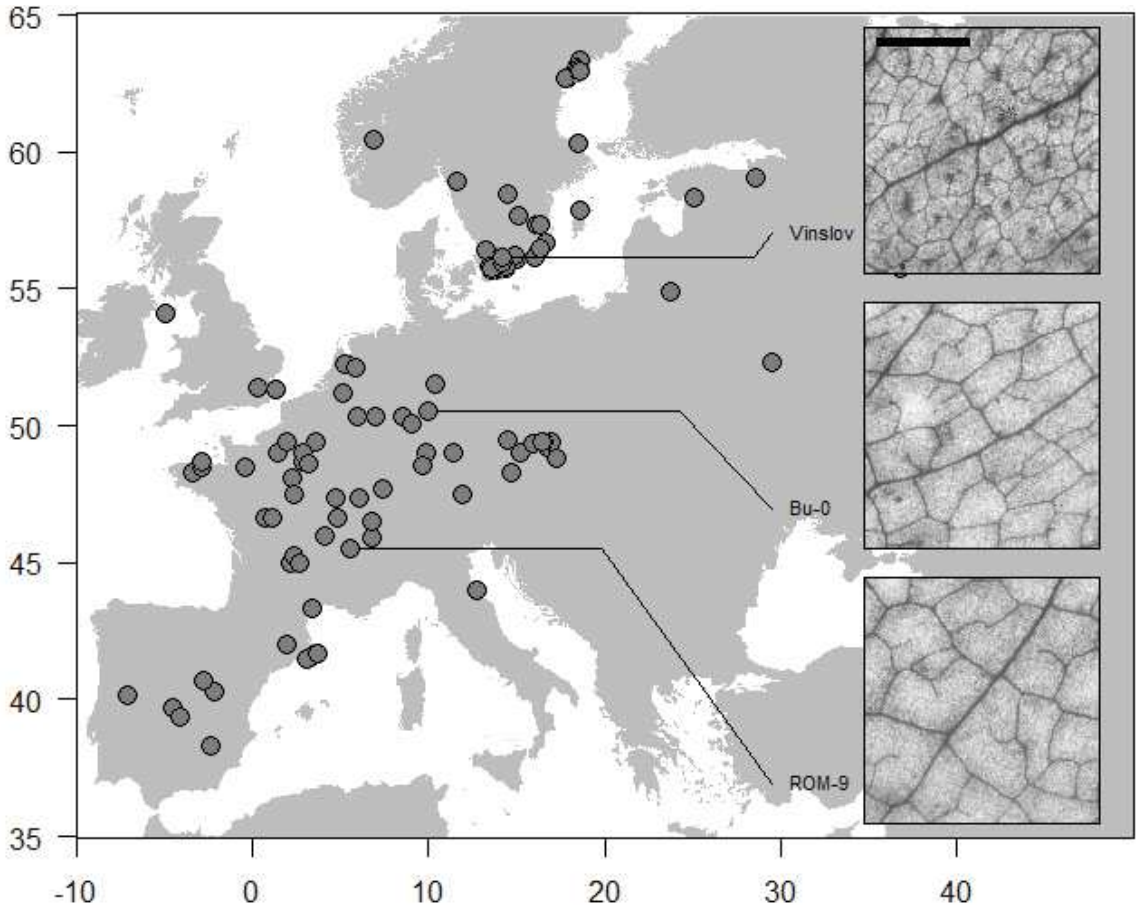

Figure 1: Geographic distribution of the European genotypes grown in this study and examples of contrasted leaf vein densities measured ($VD_{Vinslov}$ ~ 7mm mm^{-2}, VD_{BU-0} ~ 5 mm mm^{-2}, VD_{ROM-9} ~ 4 mm mm^{-2}). Scale bar on leaf vein network image is 2mm long.

Vein density measurement

We harvested the last developed leaf which was fully expanded and fully exposed to light, at the bolting stage of each individual plant. Doing so, we aimed to measure traits expressed by adult leaves and avoid bias potentially caused by ontogenic trait variations (Vasseur *et al.*, 2018b). Leaf tissues were fixed by placing the leaves in individual micro-tubes filled with Formalin–Acid–Alcohol for at least two days. Leaves were then cleared by a solution of 95% ethanol and 5% glacial acetic acid for 24 h. To ensure a high contrast between the vein network and other leaf tissues, the solution was supplemented with 0.001% of safranin powder. Then, leaves were dipped one hour in pure glycerol before mounting between two glass blades. We took pictures of the samples using a backlight device and a digital camera (Nikon D300s) equipped with a macro lens, at a 100 pixel per millimeter resolution. Leaf veins were manually

traced using the Gimp software (The GIMP Development Team, 2019) and the vein networks were analyzed using MATLAB (Thompson & Shure, 1995) with the code provided in Blonder *et al.* (2018). Vein density was computed as the ratio of total vein length to leaf area. Leaf area were measured using the *ImageJ* software (Schneider *et al.*, 2012). We compared the VD range in our dataset to the range reported in a previous *A. thaliana* study and global interspecific study using published datasets (Sack *et al.*, 2012; Rishmawi *et al.*, 2017).

Statistical analyses

Statistical analyses were performed with the R software (R Core Team, 2019, version 3.6.1). We calculated the genotype means of both VD and LA by estimating the marginal means of the variables from linear mixed models. The linear mixed models included the genotype as a random effect and the experimental block as a fixed effect. The linear mixed models were performed with the *lme* function from the *nlme* package (Pinheiro *et al.*, 2020). The marginal means were computed with the *emmeans* function from the *emmeans* package (Searle *et al.*, 1980). We evaluated the part of VD variation explained by LA and by the genotypes using a partial regression model, performed using the *varpart* function from the *vegan* package We explored the relationships between leaf traits and mean annual temperature and precipitation using spearman correlation test. When the correlation was significant but the distribution of the trait were asymmetric, we fitted 5^{th} and 95^{th} quantile linear regressions. The mean annual temperature and mean annual precipitation of genotypes' collecting sites were extracted from the CHELSA database (chelsa-climate.org). The 5^{th} and 95^{th} quantile regressions and their slope comparison were performed using the *quantreg* package.

Genotype-phenotype associations

Taking advantage from the genetic data available for *A. thaliana*, we estimated the narrow sense heritability (h2) of the traits, i.e. the phenotypic variance due to the additive effect of the alleles. We used the Bayesian sparse linear mixed model using a Markov chain Monte Carlo method performed by the GEMMA software (Zhou *et al.*, 2013). The model takes the genetic structure into account by computing the relatedness matrix of the genotypes. The model compute the overall additive effect of all the single nucleotide polymorphisms (SNPs) on the phenotype. To look for specific association between particular SNPs and the phenotype, and detect candidate genes, we performed genome wide association studies (GWAs) for both VD and LA. We used a linear mixed model performed with GEMMA (Zhou & Stephens, 2012) that

controls for the genetic structure of the dataset by using the genetic relatedness matrix. In this case, we calculated the relatedness matrix using the *-gk* GEMMA function. We ensured that the tests were not liberal looking at the histograms of significance values (François *et al.*, 2016) and calculated q-values (corrected p-values for the false discovery rates) using the Bioconductor's q value R package when required (Storey, 2002). Using a Bonferroni significance threshold, we spotted sequences significantly associated with the phenotype (quantitative trait loci, QTL). To account for the effect of linkage disequilibrium, we extended the targeted sequences by 10 Kb upstream and downstream significant sequences (Kim *et al.*, 2007). Using the Arabidopsis Information Resource database (www.arabidopsis.org), we extracted the list and functions of genes carried by these QTL. The final filter consisted in considering the relevance of the gene functions for the studied phenotype.

Selection cues

We scanned the genome for selection cues using the *PCAdapt* R package (Luu *et al.*, 2017). The algorithm assesses the importance of each SNP on the genetic structure of the dataset. It postulates that SNPs under selection contribute more to the population structure than expected by neutral processes, such as genetic drift. The method uses a multivariate analysis that is well suited to study the large regional continuous populations of *A. thaliana* (Horton *et al.*, 2012). It identifies the principal components (PC) of genetic variation, i.e. the genetic structure, and measures the contribution of each SNPs on their construction. The contributions of the SNPs are processed as p-values and filtered for false discovery rates. Using this method, we conducted an analysis with a genotype set independent from the GWAS' genotype sets that are limited by the available phenotypic data. We started with the full set of 1135 genotype sequences available on the 1001 genomes project website (1001genomes.org) containing 12,883,854 variant loci. The distribution of *P*-values computed by *PCAdapt* from this dataset did not show the expected uniform distribution due to correlations between genotypes and between SNPs. We reduced the dataset until the uniform distribution assumption was correct (François *et al.*, 2016). We pruned the data by keeping only the Single Nucleotide Polymorphisms, and loci and genotypes having less than 10% of missing data, which resulted in a 1,032 genotypes by 6,385,774 SNPs dataset. We then filtered the genotypes by the genetic distance using a 0.075 correlation coefficient limit. Final data contained 222 genotypes and 6,385,774 SNPs. As for GWAs, the p-values produced by PCAdapt were controlled for false discovery rates using the Bioconductor's q value R package and the subsequent q-values were used to scan for adaptive peaks using a Bonferroni threshold.

Results

Vein density variability among genotypes

The mean (± sd) leaf VD was 5.27 ± 0.85 mm mm^{-2} and varied by 2.5-fold among the genotype set, from 3.4 to 8.3 mm mm^{-2} (Fig. 1, Fig. 2a). It is higher in mean but similar in variance than previously shown in *A. thaliana* (between 1.54 and 3.46 mm mm^{-2}, Rishmawi *et al.*, 2017). Our range of VD is located at the low part of the VD variability recorded in large interspecific comparisons (0.5 to 25 mm mm^{-2}, Sack *et al.*, 2012). The mean (± sd) of LA was 423.41 (± 123.31) mm^2 on average and varied from 44.3 to 715.3 mm^2. LA was significantly negatively correlated with VD (R^2 = -0.77, $P < 0.01$; Fig. 2b). The variance partitioning showed that 41% of VD variation was explained by LA versus 17% by the genotype, and 20% was explained jointly by VD and LA.

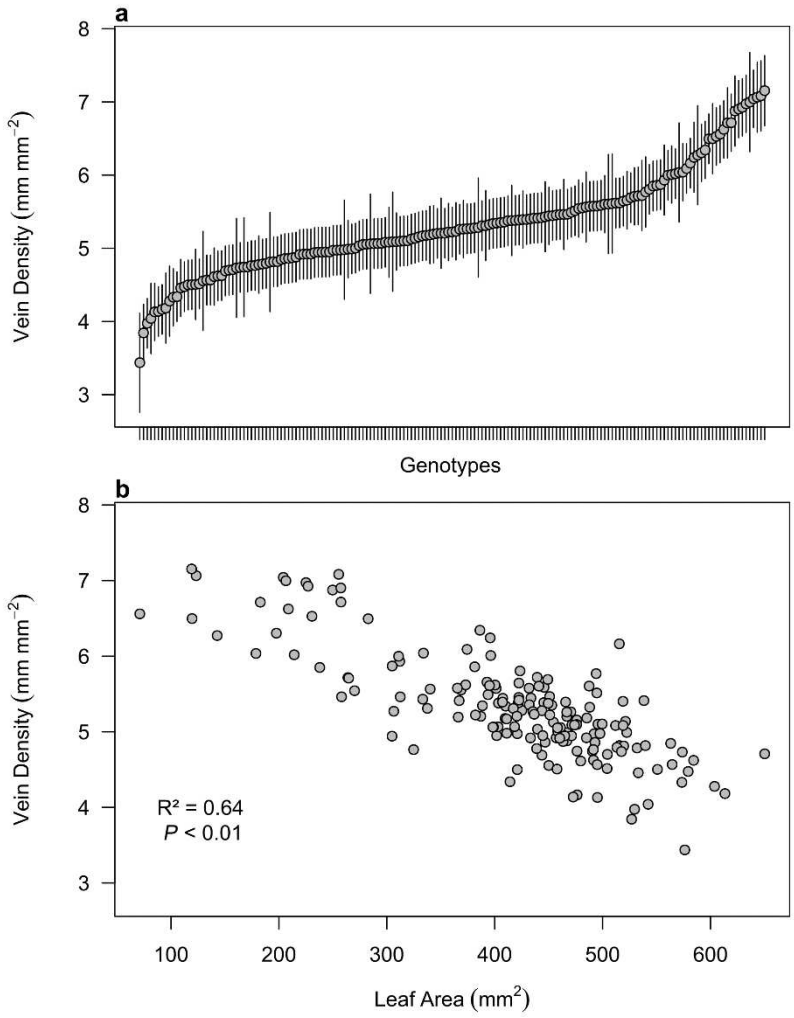

Figure 2: Leaf area variation explained a large part of the vein density variation among Arabidopsis thaliana genotypes. Variation of vein density across genotypes (a) and relationship between vein density and leaf area (a). Each dot represent one genotype (n = 169) and straight bars represent standard errors.

Traits-climate relationships

VD decreased with increasing mean annual temperature (MAT) at genotype's collecting site (r = -0.24, $P < 0.01$, Fig. 3a). LA increased on average with increasing MAT (r = 0.22, $P < 0.01$, Fig. 3a). However, LA distribution was asymmetric and the quantile regression revealed a triangular relationship between LA and MAT (Fig. 3c). The 5th and the 95th quantile regression slopes were significantly different ($P < 0.01$). Large to small leaves were encountered at low temperatures while rather large leaves at higher temperatures (Fig. 3c). VD and LA varied independently from the mean annual precipitations (MAP) at the genotype's collecting site ($P > 0.05$, Fig. 3b,d).

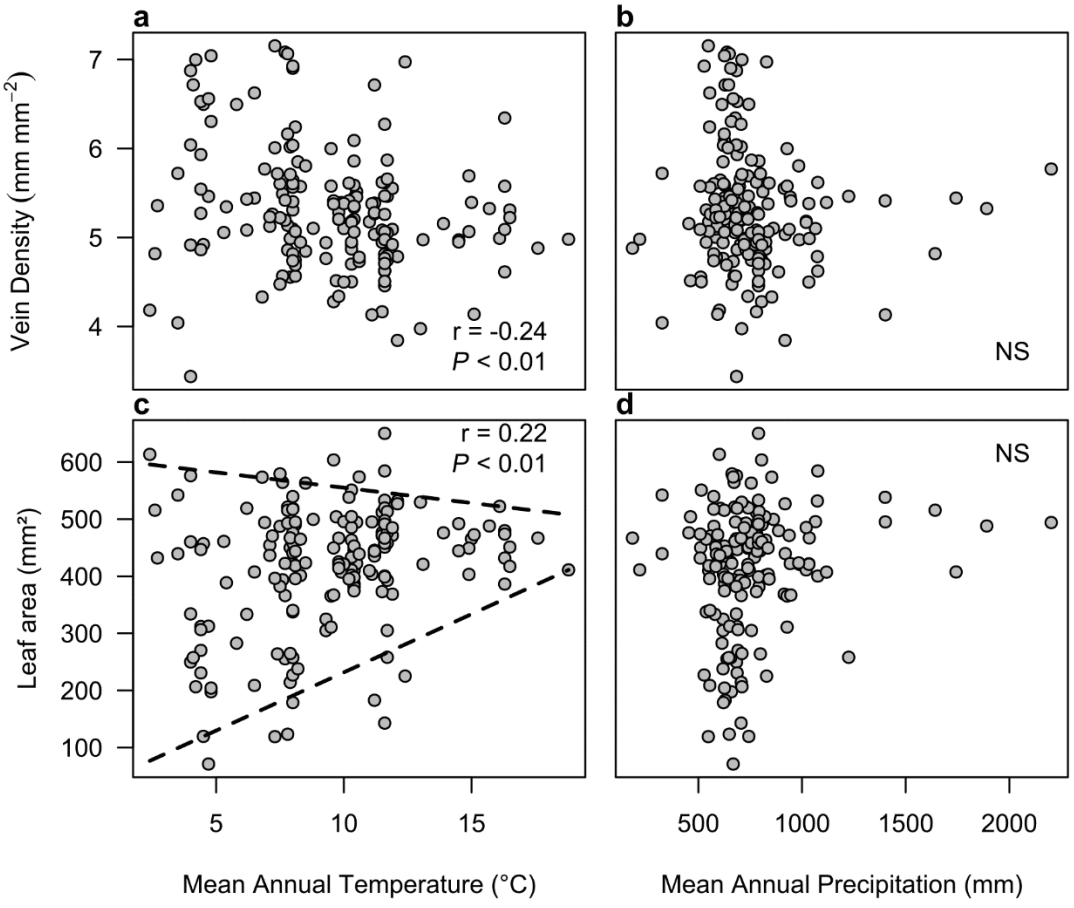

Figure 3: Traits-climate relationships show influence of temperature on vein density and leaf area. Relationships between vein density and mean annual temperature (a) and mean annual precipitation (b), and relationships between leaf area and mean annual temperature (c) and mean annual precipitation (d). Each dot represent one genotype (n=169), dashed lines represent 5th and 95th quantile regression fits.

Genetic determinism of VD

The cumulative allelic additive effect explained 26% of the natural variation of VD in our dataset. The genome wide association study revealed that four QTLs were significantly associated with VD variation. The significant QTLs are illustrated by the four association peaks on the Manhattan plot (Fig. 4a,b): three peaks were located on the first chromosome and the last peak was located on the fourth chromosome. The first QTL covers a region gathering three genes involved in cell wall modification; XTH8, PMEPCRA and PME19. In the second QTL, three genes attracted our attention: AT1G12440, CDI3 and DDF1. AT1G12440 is a gene from the zinc finger family reported to interact with the vascular-specific adaptor proteins VIT and VIK that influence leaf venation patterning (Ceserani *et al.*, 2009). CDI3, is involved in water homeostasis, regulation of stomatal closure and opening, response to abscisic acid, response to carbon dioxide and response to humidity (Saito & Uozumi, 2019). DDF1, is a transcription factor that causes dwarfism and delays flowering when overexpressed (Kang *et al.*, 2011). Overexpression of this gene is triggered in response to freezing, heat, salt stress and water deprivation. The third QTL covers a region including LSM1A, a gene involved in cold acclimation and response to water deprivation (Perea-Resa *et al.*, 2016), and ARF11, a gene involved in the auxin-activated signaling pathway. Finally, the fourth QTL covers a region including two genes involved in response to stress: REIL1, involved in acclimation to cold and ABC1K1 involved in the response to water deprivation, and a gene involved in cell wall deposition (FLA5), reported to contribute to the biomechanical resistance of vascular tissues (MacMillan *et al.*, 2010).

Testing the specificity of the QTL associated with VD

The cumulative allelic additive effect explained 59% of the natural variation of LA in our dataset. The genome wide association study revealed that two QTLs were significantly associated with LA variation. As illustrated by the Manhattan plot (Fig. 4c,d), the QTLs highlighted in this analysis corresponded to the first and third QTL associated with VD variation. Thus, the effects of the second and fourth QTLs were specific to VD variation.

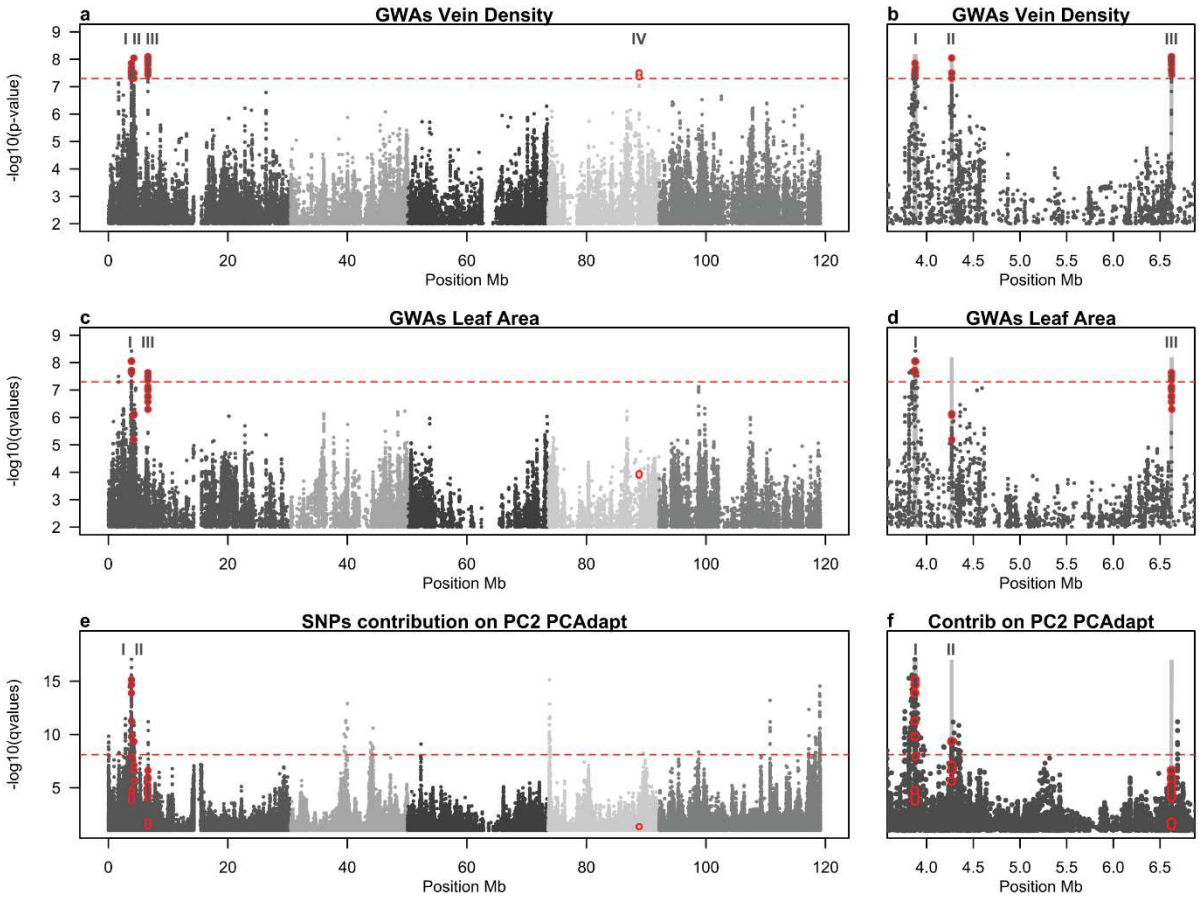

Figure 4: Manhattan plots highlighting the QTL significantly associated with vein density, leaf area, and the North-South differentiation of *A. thaliana* populations. Genome wide association study (GWAs) of vein density (VD) (a), and a zoom on the first chromosome (b). GWAs of leaf area (c), and a zoom on the first chromosome (d). Contribution of each SNPs on the construction of the second axis of *A. thaliana* genetic differentiation (e), and a zoom on the first chromosome (f). Each grey dot represent a single nucleotide polymorphism (SNP), dots surrounded by red are the significant SNPs of the vein density genome wide association study. The five shades of grey materialize the five *A. thaliana* chromosomes. The red dashed lines represent the Bonferroni significance threshold.

Adaptation cues of VD variation

The analysis of the genetic structure of the *1001 genomes* accession set showed that three principal components (PCs) structured the dataset. Given the high number of PCs (6,385,774), the proportion of variance explained by each principal component was small, around 0.6% for the three firsts. Yet, the three first PCs captured 47% of the variance explained by the ten first components (Fig. S1). The PC 1, 2 and 3 were representative of the population differentiation along an East-West axis, a South-North axis going from central Europe to Scandinavia and a North-South axis going from central Europe to Spain, respectively (Fig. S2). Among the SNPs that contributed more than expected to the construction of the three PCs, a

few of them were located on the QTL identified by the GWAs performed on VD. More precisely, the first and second QTL associated with VD were strong contributors to the population differentiation along the South-North axis going from central Europe to Scandinavia (Fig S3).

Discussion

Global patterns of vein architecture reported a fifty-fold magnitude variation of vein density across species and sites. In addition, vein density was independent from leaf size despite its scale-dependent nature, suggesting that natural selection operates independently on leaf area and vein density (Sack *et al.*, 2012). By contrast, our results indicated a 2.5-fold variation in vein density and a strong negative relationship between vein density and leaf area in *A. thaliana*. This inconsistency with the global pattern might be explained by the natural history of the species. Despite its large phenotypic variability and its wide distribution, *A. thaliana* is mainly a ruderal-stress tolerant species (May *et al.*, 2017; Vasseur *et al.*, 2018b) that explores a few diverse environmental contexts. In addition, the rosette body plan reduces the constraints on the structural role of the vein architecture, compare to erected plant form exposed to wind. Thus, the evolutionary constraints acting on vein density and leaf area might not be conflicting, if not parallel. Beyond that, this result suggests that the leaf vein density is not under stabilizing selection and might be correlated with environmental drivers. Moreover, a significant part of the vein density variation was explained by the genotype suggesting underlying genetic structure that might arise from adaptation to climate.

We initially expected higher vein densities to occur in both the Mediterranean and the Scandinavian extremes of the *A. thaliana* distribution, where drought is more likely to occur due to low amounts of precipitation and low temperature, respectively (Exposito-Alonso *et al.*, 2018). However, our data did not support any relationship between vein density and precipitation, or any indices of aridity (data not shown), and Mediterranean genotypes did not exhibit high leaf vein densities. By contrast, the main climatic driver of vein density across *A. thaliana* populations was temperature: vein density increased linearly with decreasing mean annual temperature. Interestingly, the response of leaf area to temperature corresponded to the pattern observed in large-scale interspecific studies (Wright *et al.*, 2017): large sized leaves were favored at high temperature while small to large leaves occurred at low temperature. This result indicates that high vein densities are selected under low temperatures in both large and small leaves, despite the overall coordination between leaf area and vein density. The water

viscosity increases at low temperatures, which further slowdown the flux of water from soil to leaves and through the leaves (Richardson, 2000). In this context, three mechanisms may favor high vein densities under cold climates. Firstly, high vein density reduces the distance between veins and transpiration sites, which might compensate for such reduction of hydraulic conductance. Secondly, high vein density makes leaf water potential less negative, reducing the tensions in the vascular network (Roth-Nebelsick *et al.*, 2001). These tensions are susceptible to cause embolism, i.e. generate air bubbles obstructing vessels and tracheids (Tyree *et al.*, 1994). Finally, a denser leaf vein network guaranties the hydraulic continuity in case of embolism events by providing alternative routes (Brodribb *et al.*, 2016). This might be of particular evolutionary advantage for the Scandinavian *A. thaliana* populations that grow during the cold season, in some cases under snow covers (Lewandowska-Sabat *et al.*, 2017).

The cumulative allelic additive effect on vein density was weak in our dataset, around 26%, while it explained 59% of leaf area variation. Nonetheless, the genome wide association study uncovered four loci significantly associated with vein density variation, including the two associated with leaf area variation. This result presupposes a strong effect of a few genes on the genetic vein density variation. By contrast, leaf area is determined by numerous small effects that did not reached the detecting threshold of the method. Not surprisingly, leaf area is the result of many developmental processes including cell growth and cell division, which implies complex gene expression patterns (Tsiantis & Hay, 2003). Two loci associated with vein density variation were also among the few loci excessively related to the differentiation of *A. thaliana* populations toward the North of its distribution. The first one refers to the gene cluster of cell wall modifiers, common to both vein density and leaf area genome wide association studies. This result gives strong support to the crucial role of cell wall in shaping the major physiological constraints in leaves and the plant adaptation to their environment (Onoda *et al.*, 2017). The second one was specific to vein density; it refers to a factor influencing leaf patterning identified in previous molecular study (Ceserani *et al.*, 2009). This is a rare convergence of findings between a pure genomics study comparing mutants to a reference genotype and a large-scale study screening the natural variability of a trait.

Among the loci identified in our dataset, the first one gathered three genes involved in cell wall modification. Their proximity suggests the existence of a cluster of genes performing a similar function and tightly linked along the genome. Previous work has shown how variation in cell wall thickness alters the development and final features of vein architecture (Bourquin,

2002). The modification of cell wall properties could influence the mechanical structure of the leaf through reinforcement of vascular tissues. This property has been described by (MacMillan *et al.*, 2010) for FLA5, a gene identified here in the fourth QTL. Our results show how cell wall properties and vein architecture can be genetically and developmentally linked, bringing evidences of the role of cell wall on leaf hydraulic and mechanic properties through vein network alteration. The second QTL contained an unlabeled gene coding for a zinc finger protein (At1g12440). The role of this protein family is to make tandem contact with other molecules, promoting or repressing their activity. Ceserani et al. (2009) reported a strong interaction signal between the protein encoded by this gene and the VH1/BRL2 receptor-like kinase. Mutations of VH1/BRL2 cause vein density reduction and vein gapping. While Ceserani et al. (2009) did not retain At1g12440 as a major promotor of VH1/BRL2 in Col-0 mutants; our results suggest reconsidering its importance on the natural variation of vein density. Interestingly, a set of genes located in the second, third and fourth loci were associated with plant resistance to drought. For instance, the CDI3 gene, involved in guard cells homeostasis, regulates the stomatal closure and opening. Stomata and veins share a common evolutionary history: modification of stomata properties in leaves and thus the evaporative demand implies a coordinated modification of the leaf water supply and conductivity, achieved by vein density adjustments (Schneider *et al.*, 2017). The role of vein density variation in drought adaptation might explain this coordination. The genome wide association studies highlighted both genes directly influencing venation patterns and co-varying genes involved in drought adaptation.

The species *Arabidopsis thaliana* appears to be an attractive model to merge disciplines such as population genetics, molecular biology and functional ecology through innovative approaches. The large distribution of the species covering contrasted environments coupled to an extensive genetic characterization of the populations allow an unprecedented exploration of how functional constraints guide the genetic differentiation of plant species (Krämer, 2015; Baron *et al.*, 2015; Vasseur *et al.*, 2018a; Sartori *et al.*, 2019). Next steps would be to extend the exploration of the natural variation and functional roles of vein architecture to other metrics. Notably, the differentiation of vein orders might be of differential importance depending on leaf size (Sack *et al.*, 2012). In addition, the functional role of free ending veinlets remains to be elucidated. Nonetheless, interpretation of our results should be made considering the peculiarities of the species. As a ruderal species, *Arabidopsis thaliana* is characterized by a small size, simple organization, fast growing and short life cycle. We still need intraspecific explorations of the importance of vein density for tree adaptation.

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Supplemental Information

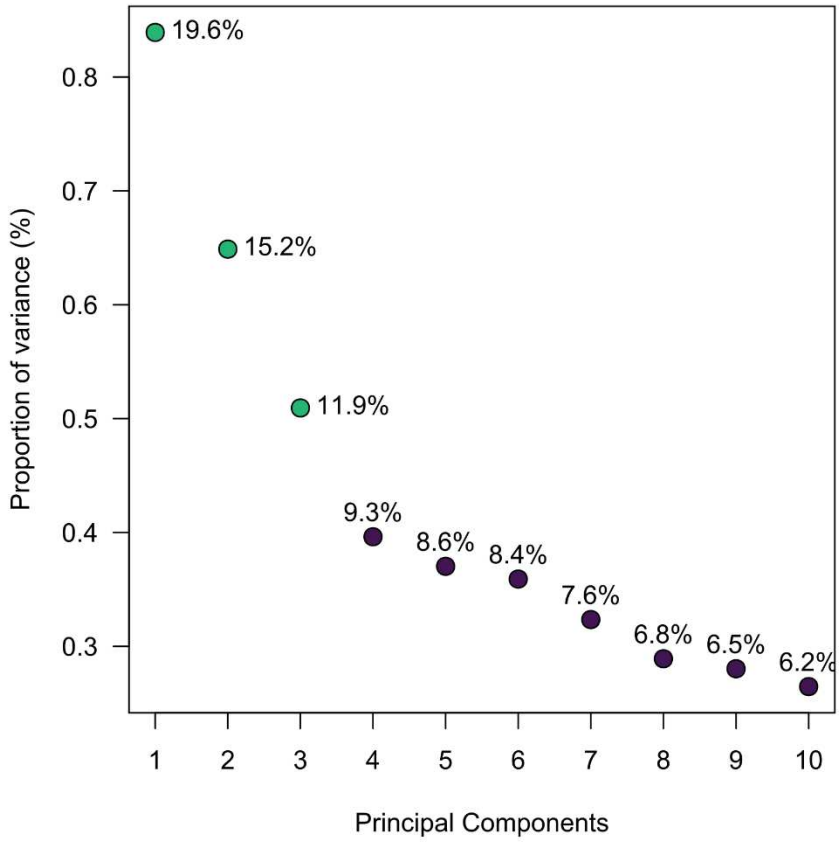

Figure S1: Proportion of genetic variance explained by the ten first principal components (PCs). The broken stick method (blue vs purple) indicates that the three first PCs are valuable to explain the A. thaliana genetic structure. Percentages on the plot indicate the proportion of variance explained by each PC relatively to the proportion of variance explained by the ten firsts PCs.

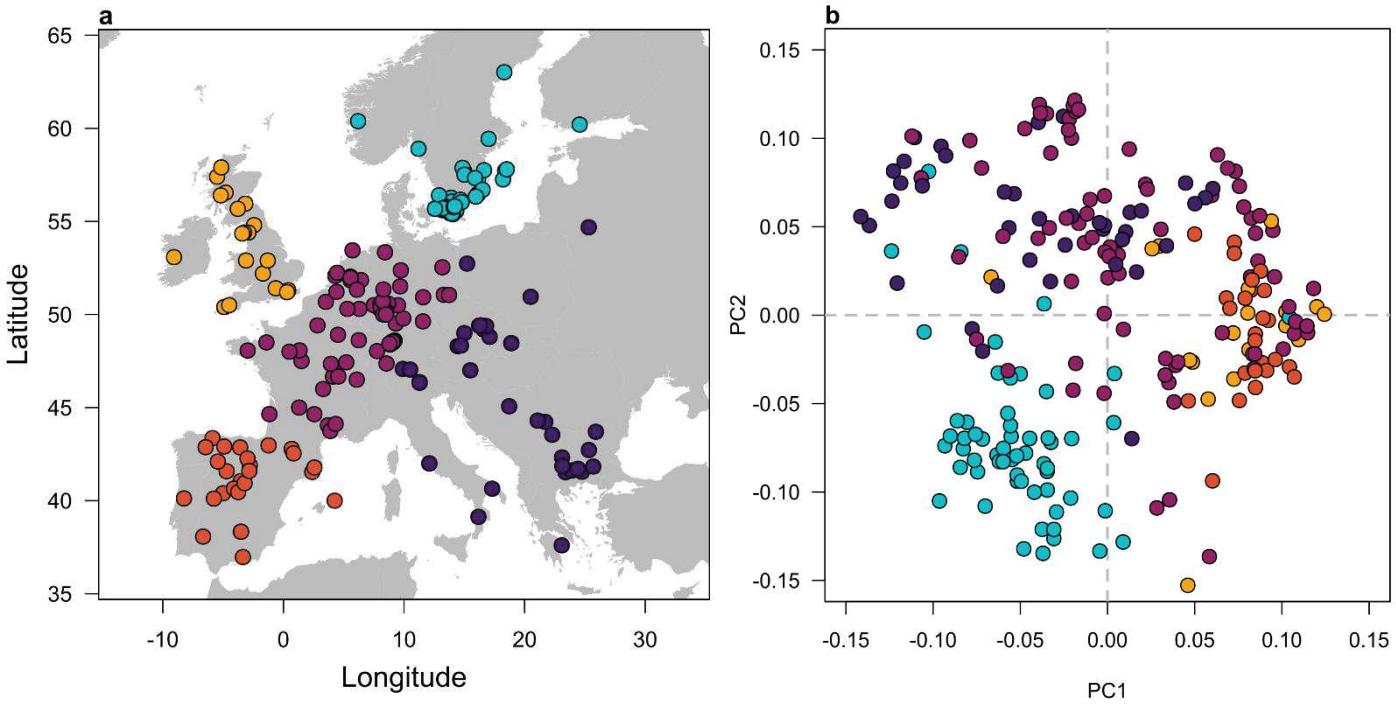

Figure S2: The genetic differentiation echoes the geographic distribution of Arabidopsis thaliana genotypes. Position of the genotypes on the map of Europe (a). The colors define geographic groups attributed using the 1001 GENOMES genotype information and allow visualizing the genotype origin on the multivariate analysis (b): Iberian Peninsula; orange, Western Europe; purple, central Europe; dark blue, Ireland and United Kingdom; yellow and Scandinavia; light blue. Position of 222 A. thaliana genotypes representatives of the genetic diversity of the species on a plane constituted by the two principal components (PC) of genetic variability (b).

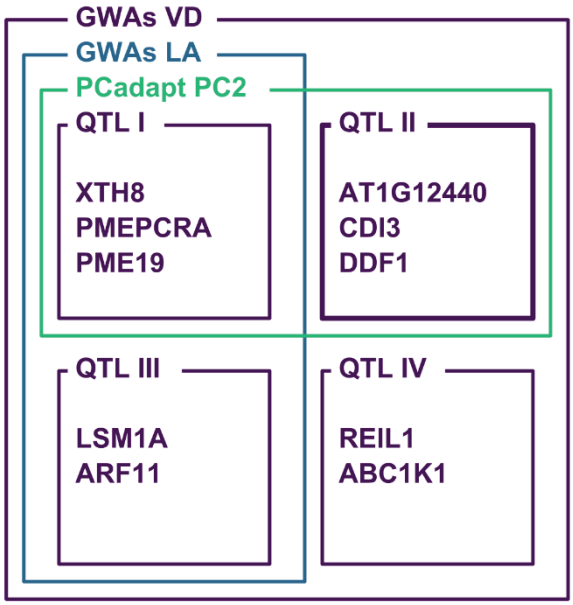

Figure S3: Summary of the results from the genome wide association studies and the scan for selection. QTLs associated with vein density variation and the genes they code for (purple), including the QTLs associated with leaf area variation (blue) and QTLs under selection (green). QTL II was specific to vein density variation and adaptive along the South-North axis going from central Europe to Scandinavia (PCadapt principal component 2).