Investigating the evolution of complex phenotypes and the underlying molecular bases of their variation is critical to understand how organisms adapt to their environment. Applying classical quantitative genetics on a segregating population derived from a Can-0xCol-0 cross, we identify the MADS-box transcription factor FLOWERING LOCUS M (FLM) as a player of the phenotypic variation in plant growth and color. We show that allelic variation at FLM modulates plant growth strategy along the leaf economics spectrum, a trade-off between resource acquisition and resource conservation, observable across thousands of plant species. Functional differences at FLM rely on a single intronic substitution, disturbing transcript splicing and leading to the accumulation of non-functional FLM transcripts. Associations between this substitution and phenotypic and climatic data across Arabidopsis natural populations, show how noncoding genetic variation at a single gene might be adaptive through pleiotropic effects.
Evolution is a continuous and complex process requiring coordinated changes in many traits according to environmental selective pressure, in theory resulting in an optimal fitness in a given habitat at a given time point. Owing to the numerous traits that need to evolve coordinately, mathematical models predicted that complex organisms would evolve more slowly than simple organisms toward a fitness optimum when considering mutations of same effect size. This was called the “cost of complexity” or the “cost of pleiotropy” since those models assumed that every gene is able to affect every trait.

Pleiotropy can have several meanings but overall states that one gene or variant controls several traits. The nature of pleiotropy strongly depends on the traits measured (molecular, physiological, metabolic etc.) and the level of organization considered (from cell to population). Moreover, one needs to be cautious about the relevance of the observed pleiotropy according to the method used to assess it. For instance, studying knockout mutants may not reflect the diversity of mutational effects selected in nature. In addition, when classical quantitative genetics is used to study pleiotropy, a nontrivial point is to distinguish real pleiotropy from genetic linkage. Indeed, several independent quantitative trait loci (QTLs) in the same region may control different traits because mapping QTL in segregating populations has limited resolution with respect to the trait’s genetic architecture. Finally, many related traits may be correlated just owing to a cause-and-effect relationship, in other words an indirect effect.

Reverse genetics and QTL mapping approaches in various organisms suggested that pleiotropy is relatively rare. Moreover, a high degree of pleiotropy (i.e., the diversity of traits controlled by one variant/gene) is thought to limit the potential of adaptation because it would be more likely to affect negatively some functions while improving others (antagonistic pleiotropy), thus changing the optimal balance selected over the course of evolution. From this perspective, adaptation speed would rather depend on the degree and the modularity of pleiotropy (the combination of traits controlled by one variant/gene) than on the complexity of organisms. Inspection of phenotypic data from yeast, nematode, and mice mutants revealed that an intermediate degree of pleiotropy seems the best trade-off to reach the highest rate of adaptation. A recent study revealed that indeed, a combination of QTLs having an intermediate degree of pleiotropy, with QTLs affecting specifically one trait were able to promote a fast adaptation.

In plants, phenotypic traits involved in the strategies for resource acquisition and use, called functional traits, have been widely investigated across species. Several trait–environment relationships have been identified and are assumed to be representative of species adaptation to various environmental conditions. Ecologists have long recognized that functional traits are often correlated with each other, and that adaptation to new environments requires a simultaneous change in these traits due to resources limitation and biophysical constraints. For instance, the rate of carbon fixation through photosynthesis is linked to the structure of the leaves, such as LMA and leaf dry matter content. Leaf structure and growth is also related to leaf chemical composition and its lifespan. Together, these traits shape a trade-off between resource acquisition and resource conservation, bound by physiological constraints and observable across thousands of plant species. This trade-off has been called the “Worldwide Leaf Economics Spectrum,” although it has also been observed between genotypes within species. For instance in the model species Arabidopsis thaliana, variations in the leaf economics spectrum (LES) are associated with life history traits’ variation between accessions, such as flowering time.

Major developmental switches during plant growth cycle interact with plant physiology and fitness, among which the timing to flowering is expected to play a crucial adaptive role to ensure plant reproductive success. Some flowering time genes were shown to have pleiotropic functions during development. Consistently, a previous study has shown tight relationships between flowering time and the vegetative growth dynamics in Arabidopsis thaliana. In A. thaliana, the molecular control of flowering time is orchestrated by a network of genes integrating several signals that converge towards a few regulators. Among them, FLOWERING LOCUS C (FLC) is a MADS-box transcription factor known as a repressor of flowering. Interestingly, FLC also plays a role in germination, another critical phase in plant development as well as vegetative development. Furthermore, FLC was shown to bind to promoters of hundreds of genes involved in stress-related and hormonal pathways, activating or repressing their expression.

Natural variation of FRIGIDA, another major regulator of flowering time, modulates drought tolerance strategy through a pleiotropy exerted on several physiological traits and with different effects on plant water status if studied at a specific time point or integrated over the plant lifetime. Indeed, the role of pleiotropy in drought adaptation was studied by assessing physiological traits that are correlated in nature such as water use efficiency (WUE) and flowering time. Another example is that of ERECTA and its effect on functions like transpiration efficiency as well as inflorescence development.

Examples of genes responsible for the natural variation of single traits are increasing (see for example), however these studies are about a specific type of adaptation essentially having a simple genetic architecture in most studied populations. Studies aiming at the dissection of the genetic basis of phenotypic integration of several complex traits such as plant growth or fitness are scarcer and rarely reach the stage of the functional validation of the candidate genes and even less the identification of the causal natural variants. Thus, dissecting the genetic architecture and identifying the molecular variants behind complex and/or correlated traits is crucial to fill this gap and to better understand how adaptation of organisms is constrained at the genetic level and the type of molecular variants selected (for instance regulatory versus protein coding).

To understand how phenotypic integration regulates plant adaptation to contrasted environments, we examine the genetic underpinnings of relationships between life history, leaf physiology, and climate. To do so, we seek to decompose complex traits into individual genetic and molecular components using a segregating population originating from a genetically divergent cross. We screen this population with a high-throughput phenotyping platform designed to allow great precision in measuring diverse traits, including highly integrative descriptors of plant growth. Growth is used as a model integrative trait to dissect relationships between traits’ variation and their adaptive potential. We identify the molecular basis of a QTL controlling both leaf vegetative growth and time to flowering in Arabidopsis thaliana. Through a combination of quantitative genetics, molecular biology, ecophysiology, and population genetics, we show how modulating the transcriptional output of a gene can have a pleiotropic effect on multiple functions affecting life history and plant physiology, with potential impacts on ecological strategies. These results are exploited to distinguish pleiotropy from linkage in a concrete case, and to connect general theories about adaptation and pleiotropy.

Results

From several QTLs to a pleiotropic gene. Using the Phenoscope phenotyping platform for Arabidopsis thaliana, we measured plant growth through projected rosette area 29 days after sowing (PRA29) during the vegetative phase of growth on 360 individuals of a recombinant inbred line (RIL) population derived from...
the cross between Can-0 and Col-0. We performed a QTL analysis to assess the genetic bases of variation in plant growth. In addition, several other image-derived traits such as leaf color, a surrogate for pigment concentration, were investigated. QTLs for several of the measured traits co-localize at the bottom of chromosome 1 with mild effect on growth and large effect on leaf color (Supplementary Fig. 1A). We then followed a classical fine-mapping approach to identify the gene(s) underlying these trait variations. To this end, we selected the F7 RIL line 19Rv337 with a residual heterozygous region covering the expected locus to build the heterogeneous inbred family (HIF) 19HV337 by selecting progenies homozygous for each of the parental allele at the candidate region. The phenotypic comparison of homozygous siblings confirmed that the allele originating from Can-0 (Can) was promoting PRA29 with respect to the allele originating from Col-0 (Col), and also influenced leaf color (Supplementary Fig. 1B). Phenotyping 12 selected recombinant HIF lines (rHIF) derived from 19HV337 confirmed the association of phenotypic variation with a candidate region containing ten genes (Supplementary Fig. 1C). Since the Can allele at this locus conferred both faster vegetative growth and earlier bolting with respect to the Col allele, we focused on the candidate gene coding for FLOWERING LOCUS M (FLM). We then used the null mutant flm-3 in the Col-0 background. Compared to its wild-type (WT) control, this mutant displayed similar phenotypic effects on plant growth and color as rHIF099-Can compared to rHIF099-Col (our ultimate mutant displayed similar phenotypic effects on plant growth and color as rHIF099-Can compared to rHIF099-Col (our ultimate mutant displayed similar phenotypic effects on plant growth and color)). Finally, we genetically transformed the flm-3 mutant with a genomic fragment containing 3 kb of the promoter and the full FLM genomic region (including introns and the 3’UTR) from either FLM-Can or FLM-Col. FLM-Col fully complemented flm-3 to WT level in terms of flowering time, unlike FLM-Can, confirming that this gene is hypofunctional in Can-0 (Fig. 1d). Altogether, these data show that allelic variation at FLM is underlying the plant growth and color QTLs segregating in Can-0 × Col-0 at the lower end of chromosome 1.

**Molecular bases of the natural variation at FLM.** Since de novo sequencing of PCR-amplified FLM-Can and FLM-Col alleles did not show any polymorphism within the coding sequence between these alleles (Supplementary Data 1 and Fig. 2), we assessed their expression with qRT-PCR. FLM-dependent thermosensitivity of flowering relies on a differential alternative splicing decreasing the production of the repressive isoform FLM-β when
temperature increases. Quantitative PCR showed that accumulation of exon2-containing transcripts (typical of FLM-β isoform) is strongly reduced in rHIF099-Col compared to rHIF099-Col (Fig. 2a).

Considering the complexity of the isoforms produced at the FLM gene according to a previous publication, we thus set out to clone all the isoforms present in our samples. In Col-0, we found a majority of FLM-β isoform (34 out of 52 clones sequenced) as previously reported and discovered two isoforms including one which is potentially functional (Fig. 2b). In Can-0, we observed that most of the isoforms produced (26/27) contain an insertion of 17bp before exon3 (Fig. 2b). Interestingly, a polymorphism changing a “G” to an “AG” is located right before this additional stretch of DNA in Can-0 at the position 28,958,437. We deduced that this substitution in Can-0 (hereafter named “SNP28958”) is creating a consensus sequence of a splice acceptor site, which modifies FLM splicing and leads to the incorporation of these additional 17 nucleotides, producing a premature stop codon in the exon3. We showed that SNP28958 abolishes FLM function in term of flowering time, PRA29 and leaf color as the flm-3 mutant was not complemented by an FLM-Col construct substituted with this SNP (Supplementary Fig. 3A). Moreover, we detected the exon3-containing isoform size shift in these transgenic plants (Supplementary Fig. 3B). Overall, it demonstrates that this single substitution is indeed creating a hot acceptor splice site generating nonfunctional FLM isoforms.

**Pleiotropic effects of FLM on leaf physiology.** We then wanted to describe how FLM-Can and flm-3 alleles were affecting growth throughout the growth period under long day photoperiod, by comparison to the Col allele, using parallel PRA and leaf number measurements. As expected, we observed that both weak alleles promoted PRA in a first phase of vegetative growth (Fig. 3 and Supplementary Fig. 4). However, the growth of all lines slowed down and then stopped maximum 1 week after bolting, which means that the lines carrying a functional allele of FLM continued to grow over a longer period of time to reach the same (if not higher) final rosette sizes (Fig. 3 and Supplementary Fig. 4). Leaf number was rather similar in a first phase but lines carrying a functional FLM allele progressively developed more rosette leaves.

As FLM affected plant growth and color, we hypothesized that it might modulate leaf physiology through pleiotropic effects during vegetative growth. To test this hypothesis, we measured several traits related to leaf physiology and resource-use across a set of segregating lines and flm mutant. Leaf temperature measurements showed that leaves of rHIF099-Can and flm-3 alleles were cooler compared to their respective controls, indicating a higher water flux through the plant (Supplementary Fig. 5). We also measured net photosynthetic rate per unit dry mass (\(A_{\text{mass}}\)) and leaf dry mass per area (LMA), two core traits underlying variations in leaf physiology across and within species and illustrating physiological constraints observed in the plant kingdom. flm-3 or FLM-Can were associated with significantly lower LMA when compared to their controls respectively in the Col-0 and rHIF099 genetic backgrounds, while \(A_{\text{mass}}\) was significantly higher only in the rHIF099 genetic background, although a similar trend was observed in the flm-3/Col-0 comparison (Fig. 4a). We also observed that FLM functional variation is associated with a negative correlation between these two traits, as observed across species and within A. thaliana natural accessions (Fig. 4b).

Regression slopes were not significantly different (SMA test \(p > 0.05\)) when comparing our FLM dataset (rHIF099-Can/Col and flm-3/Col-0 together) with the variation observed across 340 natural A. thaliana (SMA slope in FLM = \(-1.80, 95\% \text{ confidence interval} = [\-2.11;\-1.54], r^2 = 0.17\); SMA slope in natural Arabidopsis accessions = \(-2.04, 95\% \text{ confidence interval} = [\-2.16;\-1.92], r^2 = 0.54\)). By contrast, regression slopes were significantly different (SMA test \(p < 0.05\)) between our FLM dataset and interspecific measurements (SMA slopes in all species from the GLOPNET database = \(-1.32, 95\% \text{ confidence interval} = [\-1.39;\-1.25], r^2 = 0.50\); SMA slope in
Can-like IP strains do not cluster geographically and are scattered across Spain and Portugal (Supplementary Fig. 7). We used climatic variables (http://www.worldclim.org/) collected over 30 years to know whether the location of the Can-like IP strains was associated with particular environmental parameters. We found a significant association between the causal polymorphism and isothermality \((p = 0.0017)\), which corresponds to the mean of the monthly temperature range during the day, divided by the maximal variation of temperature over the year (Fig. 5c). This suggests that the IP strains carrying the FLM-Can-like allele are rather located in areas where diurnal monthly temperatures are less homogeneous and could be interpreted as an adaptive response. This difference seems to be driven mainly by the monthly mean diurnal range for which a similar tendency is observed \((p = 0.052)\).

**FLM variation relevance in nature.** To better understand the implications of the polymorphism identified, we screened all *A. thaliana* strains with data available for SNP28958\(^{41}\). Interestingly, out of the available 50 strains carrying this SNP, 45 come from the Iberian Peninsula (hereafter called IP strains), 2 from France, 2 from Italy and Can-0 from the Canary Islands (Supplementary Data 2). To evaluate the impact of SNP28958 on FLM expression in natural IP strains, two groups of nine IP strains carrying either the Can or the Col allele were selected for qRT-PCR experiments. A clear association between the allelic form and the accumulation level of exon2-containing transcripts was observed (Fig. 5a). Moreover, a shift in exon3-containing transcript size was only detected in the Can-like IP accessions indicating that this SNP most likely retain the same hypoactive molecular function as the Can allele (Supplementary Fig. 6).

We then wanted to know if the functional impact of SNP28958 on multiple traits could play a substantial role in IP strains adaptation to their habitat. We used a set of 186 IP strains for which we were able to gather geographic and phenotypic data from previous publications, including the 46 strains carrying SNP28958\(^{42}\) (Supplementary Data 3). We used this dataset to fit a general linear model corrected by a kinship matrix to avoid bias due to population structure (Supplementary Table 1). Consistent with our previous observations, we found significant associations between the FLM causal polymorphism and leaf production rate with or without vernalization (Fig. 5b).
Discussion

Plant growth is a complex trait integrating variation from underlying features called functional traits such as net photosynthetic rate, nitrogen metabolism or WUE, themselves also affected by environmental cues throughout the plant life cycle. Plant growth variability is thus the output of the joint action of multiple genes at different levels of organization and is of potential adaptive significance. Likewise, studying this trait is of interest to better understand the genetic architecture and the degree of pleiotropy of traits under complex selective pressures. Here, we intended to map the genetic bases of multiple traits related to rosette growth in a RIL population using our phenotyping platform and focused on QTLs co-localized at the end of chromosome 1 having mild effects on plant growth. We found that this is the result of pleiotropic functions of the FLM gene highlighting that variation at FLM plays a much wider role than initially described. This makes an interesting study case to work on the relationships between trait complexity and pleiotropy in the light of adaptation.

Further phenotyping experiments showed that FLM is also controlling leaf temperature, photosynthetic rate by unit of dry mass (\( A_{\text{mass}} \)) and leaf mass per area (LMA). Interestingly, these are core traits of the LES, a universal trade-off between resource acquisition and resource conservation observed across thousands of plant species. The tight relationship between \( A_{\text{mass}} \) and LMA is supposed to reflect fundamental physiological constraints among plant species worldwide, shaping their diversification and local adaptation. At one end of the spectrum are plants with resource-conservative strategies, characterized by long-lived, tough leaves with low nutrient concentration and low net photosynthetic rate. At the other end are plants with resource-acquisitive strategies, characterized by short-lived, flimsy leaves with high nutrient concentration and high net photosynthetic rate. The adaptive significance of the LES was recently shown using intraspecific data in A. thaliana suggesting that the LES is reflecting physiological constraints at the origin of growth variation and local adaptation at the leaf level. From this perspective, we tested whether (1) phenotypic variability in our A. thaliana accessions were reflected in the LES and (2) the relationship between \( A_{\text{mass}} \) and LMA was consistent across different species.
Fig. 5 The variant at position 28,958,437 bp in Can-O modulates FLM function and may play a role in adaptation. **a** Accumulation of exon2-containing isoforms (typical of FLM-β) measured by qRT-PCR using the primer pair qFLM-F3 + qFLM-R3b (see Fig. 2) in nine Arabidopsis strains from the Iberian Peninsula (IP) carrying either the Can (blue) or the Col (brown) genotype at position 28,958,437 bp. Each data point represents one IP strain. The asterisks are the mean expression of each variant. **b** Boxplots represent leaf production rate with or without vernalization in Iberian Peninsula strains categorized according to their genotype at position 28,958,437 bp. The data were obtained from a previous publication42 and are gathered in Supplementary Data 3. Significance of the differences was calculated according to a mixed-effect model using the genotype as a random factor and the kinship matrix as covariates (see Supplementary Table 1). **c** Boxplots represent isothermality in IP strains categorized according to their genotype at position 28,958,437 bp. Isothermality is calculated as follows: mean of monthly (max temp − min temp)/(max temperature of warmest month − min temperature of coldest month) × 100, and was obtained from http://www.worldclim.org/ and are gathered in Supplementary Data 3. Significance of the differences was calculated according to a mixed-effect model using the genotype as a random factor and the kinship matrix as covariates (see Supplementary Table 1). Source data underlying Fig. 5a are provided as a Source Data file.
data is also bound by the physiological constraints underlined by LES, and (2) whether FLM might contribute to phenotypic variation within the LES, using comparisons between our dataset and the generic interspecific GLOPNET database\textsuperscript{12}, as well as the global variation measured among \textit{A. thaliana} natural accessions\textsuperscript{14}. Our results revealed that FLM functional variation is constrained by a relationship between photosynthetic rate per unit of leaf dry mass and leaf mass per area, similarly to what is observed in the inter- and intraspecific datasets. However, slopes differed between \textit{A. thaliana} (in our FLM dataset and among natural accessions) and interspecific measurements (in all or only herbaceous GLOPNET species). This suggests that the physiological constraints described by the LES might at least slightly differ between species, which can be explained by important evolutionary transitions and metabolic differences between plant species, such as deciduous–evergreen, herbaceous–woody, and annual–perennial. Second, our results show that functional variation at FLM is associated with coordinated changes in functional traits that modulate physiology along the resource acquisition/conservation axis of the LES. Thus, variation at a single flowering time gene has consequences on at least two core traits of the LES ($A_{\text{mass}}$ and LMA), which is consistent with previous findings on flowering time genes in \textit{A. thaliana}\textsuperscript{31}. Strikingly, the impact of the FLM function in \textit{A. thaliana} may represent about 3% of the worldwide variation in $A_{\text{mass}}$ and LMA along the LES\textsuperscript{12}. Two different scenarios may explain the range of phenotypic consequences caused by FLM. On the one hand, as physiological traits are highly interconnected\textsuperscript{23}, FLM would modulate directly one of them e.g., photosynthetic rate of the plant, which in turn would indirectly change leaf mass per area, plant growth, and flowering time. This type of correlation was previously observed\textsuperscript{31} but the relationship(s) between leaf economics and flowering time is still unclear. On the other hand, FLM may activate or repress a set of target genes, selected through evolution owing to physiological constraints along the spectrum of leaf economics. Investigating the transcriptional network controlled by FLM might provide some answers as previously performed for FLC\textsuperscript{32}. In parallel, it may be worth looking at the transcriptome response according to FLM-$\beta$ expression level.

Although it is difficult to distinguish between a direct causality between traits due to FLM variation versus a synergistic pleiotropic effect along the LES axis, the associations between the FLM genotype and either growth strategy (leaf production rate) or the environment (isothermality) in natural populations would suggest an adaptive role for this variation. Interestingly, the knockout (flm-$\beta$) and the knockdown (Can) alleles of FLM used here displayed many features of the drought escape strategy, one of the strategies developed by the plants to overcome water shortage\textsuperscript{45}. Indeed, these genotypes have a more rapid vegetative development, earlier flowering and higher photosynthetic rate, which would help them to complete their life cycle before a severe drought season\textsuperscript{45}. To further validate this hypothesis, it would be interesting to investigate the adaptive value of FLM by combining water availability, temperature fluctuation, and neighbor-competition using our \textit{Arabidopsis} transgenic lines differing only at SNP28958 or the Iberian natural accessions of each FLM group. Common garden experiments in locations differing in temperature fluctuations and precipitations could also be considered to quantify the adaptive effect of the FLM alleles in natural environments\textsuperscript{32}.

FLM is a well-known transcription factor whose splicing variation in response to temperature change modulates the time to flowering\textsuperscript{35,36}. FLM-$\beta$ is the repressive variant of this gene, and its downregulation along increasing temperature triggers flowering\textsuperscript{38,40,46,47}. The FLM coding sequences of Col-0 and Can-0 do not differ, but the typical FLM-$\beta$ isoform is almost undetectable in Can-0 compared to Col-0. We identified a single nucleotide substitution in the 2nd intron of this gene ("SNP28958"), which is creating a seemingly hot acceptor splice site outcompeting other sites in Can-0 as well as in other related natural strains. This leads to the production of transcripts containing a premature stop codon. These aberrant variants may then be targeted by nonsense-mediated mRNA decay, as previously reported as a mechanism repressing FLM activity under higher temperature\textsuperscript{39}. We have specifically validated the impact of the SNP28958 by testing its ability to change the functionality of FLM-Col in complementing the flm-$\beta$ mutant phenotype.

Intronic polymorphisms were already shown to modulate FLM-$\beta$ accumulation level in \textit{Arabidopsis} natural strains and to correlate with flowering time\textsuperscript{36,47}. This type of modification might be a way to modulate FLM function according to the thermosensitivity required in a given natural habitat, as the response to subtle changes in ambient temperature is crucial to set seeds at the appropriate time of the season. Loss-of-function mutations were proposed to play a substantial role in plant adaptation\textsuperscript{48,49}. However, the selective value of such natural mutations was rarely demonstrated so far\textsuperscript{30–32}. Even if it is challenging, this illustrates the value to identify causal genes as well as the causal polymorphisms involved in the natural variation of traits, as this type of subtle genetic variants are more difficult to reveal using large-scale approaches while they might play an important role in the course of evolution.

Many examples in human showed how natural variation in alternative splicing is altering gene functions, triggering numerous
sensitivity of splicing in a key clock gene was proposed to drive species, supporting a similar hypothesis. It is noteworthy that strains carrying SNP28958, i.e., the weak allele of FLM, which might thus be less responsive to ambient temperature change, are overall located in environments where the amplitude in temperature variation is more pronounced. Therefore, it is conceivable that this FLM allele is advantageous by desensitizing plants against fluctuating temperature to avoid the inappropriate triggering of flowering. Likewise, genetic variation affecting thermosensitivity of splicing in a key clock gene was proposed to drive thermal adaptation from equatorial to temperate climate in Dro sophila species, supporting a similar hypothesis.

The history of Arabidopsis migrations is regularly revisited as more data become available for population genetic studies. IP is consistently mentioned as a location where highly diverged Arabidopsis lines (called relics) are found in preserved and ancestral habitats. We identified SNP28958 in Can-0 and we found this substitution in several IP strains, i.e., in 2 out of the 5 relict groups initially identified. Interestingly, the frequency of SNP28958 is enriched 2.5 times among the relict IP strains compared to all IP strains. Furthermore, even among the non-relict IP strains carrying SNP28958, the genotype probability to be relict is specifically rising at the FLM locus, further supporting its relict origin. Clearly, the alleles of FLM described here have distinct admixed origins. Finally, we found SNP28958 in the 11 native Madeiran lines which are considered as some of the most divergent Arabidopsis strains so far. Arabidopsis migrations were associated with climate disruptions such as temperature rise or precipitation fluctuation. Regarding the pleiotropic functions of SNP28958 towards more acquisitive strategy, it is conceivable that this knockdown allele was adapted to ancestral habitats and was then competed and wiped out by the FLM-Col allele during the last expansion of non-relict Arabidopsis in Europe.

Since Darwin’s studies, the classical view in evolutionary biology is that adaptive evolution occurs through gradual steps and is therefore rather slow and highly polygenic. However, an increasing number of studies showed that evolution of organisms can happen through more step-like examples for due to polyploidization events or to mutations in genes of large effect. Under strong selective pressure, it seems logical that adaptation must be accelerated through large effect alleles for simple traits or by targeting pleiotropic genes if the given trait is more complex. While pleiotropy (and its role in adaptation) has been studied mainly theoretically for years, some empirical works relying on published mutant data or QTL analysis showed that pleiotropic genes are relatively rare in a range of organisms. Recent works demonstrated that a combination of intermediate pleiotropic loci and loci having a specific function may constitute an optimal balance for a relatively fast adaptation and/or to manage complex traits, but the functional validation of candidate genes was not yet done. Our study is in line with those works, and provide an important case study, as natural variation at FLM-coordinated traits is quantitatively associated with contrasted life strategy and leaf functions, and is associated with the environment. The determination of the causality relationships between traits with respect to variation at FLM still requires some work that will help us understand the patterns of selection for the different traits, and how this would be responsive in the face of climatic challenges.

**Methods**

**Genetic material.** A segregating population (named 19RV) derived from the cross Can-0 (accession 163AV) and Col-0 (accession 186AV) was described here. Can-0 was fixed in a segregating progeny from the following RIL (19RV337) with a residual segregating region covering position 27,401,322 bp to 29,693,733 bp on chromosome 1 (see Supplementary Data 4). For fine mapping, 3360 progenies of heterozygous 19RV337 were genotypically screened by multiplex SNP-PCR by crossing each RIL allelic line to either the fmi-3 mutant or its WT background (Col-0) and testing its phenotype directly in the F1 plants.

**Phenotyping and growth conditions.** Initial RILs phenotyping, fine mapping, and mutant characterization were first obtained on the Phenoscope robots in well-watered conditions (60% soil water content saturation) as previously described. The RIL set and the parental accessions have been phenotyped in two independent Phenoscope experiments, with one individual plant per RIL per experiment. The QTL values can be found in Supplementary Data 5. The growth room is set at an 8 h short days photoperiod (230 µmol m$^{-2}$ s$^{-1}$) with days at 21 °C/65%RH and nights at 17 °C/65% RH. Growth-related traits are extracted from daily images after segmentation (FRA = Projected Rosette Area = cumulative growth; RER = Rosette Expansion Rate = relative growth rate), as well as parameters describing the rosette color expressed in the HSV (Hue/Saturation/Value) scale.

Leaf temperature was measured on the Phenoscope with FLIR infrared cameras targeting the whole rosette as well as a wet paper control for room temperature. Normalized leaf temperature was calculated by subtracting room temperature to the average rosette temperature. The growth chambers used for the experiments carried out at 16 and 23 °C was set at 16 h long day photoperiod of 150 µmol m$^{-2}$ s$^{-1}$, 65% RH. For the in vitro culture, seeds were surface-sterilized for 10 min in 70% EtOH, 0.1% TritonX-100, followed by one wash with 95% EtOH for another 10 min. The seeds were then resuspended in a 0.1% agar solution and stratified in the dark at 4 °C for 3 days. Ten seedlings per tray for different growth conditions were kept at 21 °C, 65% RH. QTL mapping. QTL mapping was performed using Multiple QTL Mapping algorithm implemented in the R/qtl package. At first, genotypic missing data were augmented. Then, one marker every three markers were selected as cofactors and significant ones were selected through backward elimination (backward selection of cofactors). QTL was moved along the genome using these preselected markers as cofactors, except for the markers in the 25.0 cm window around the region of interest. QTL were identified based on the most informative model through maximum likelihood. According to permutation tests results, a conservative LOD threshold of 2.4 was applied to identify significant QTL.

**Measurement of physiological traits in greenhouse.** fmi-3 knockout mutant and its WT (Col-0), as well as one recombinant HIF segregating for FLM (HIF999) were grown in greenhouse at the Center d’Ecologie Fonctionnelle et Evolutive (CEFÉ, Montpellier, France) in fall 2017. Plants were grown in 32 replicates per genotype. Seeds were sown in 260 ml individual pots filled with a 1:1 (v:v) mixture of loamy soil and organic compost, and stratified at 4 °C for 3–10 days. At the emergence of the first two true leaves, plants were thinned to keep only one plant per pot. Pots were randomly distributed among four trays that were rotated every day in the greenhouse. All pots were watered twice a week. A reduction of CO$_2$ assimilation per dry mass (A$_{\text{CO}_2}$ nmolCO$_2$ g$^{-1}$ s$^{-1}$) was measured using a whole-plant chamber designed for Arabidopsis (Li-Cor 6400-17, Li-Cor Inc., Lincoln, NE, USA) connected to a gas exchange system designed for a whole-plant chamber.
Cloning procedures. FLM genomic fragments from Col-0 and Can-0 were amplified using Phusion high-fidelity Taq polymerase (Finnzymes, http://www.thermoscientific.com/finzymes/) with a couple of primers including Gateway sites and flanking positions –2219 to +4737 relative to the FLM start codon of Col-0 annotated in TAIR10 (Supplementary Table 3). The pDONR207 entry vector containing the gFLM from Col-0 was mutagenized by PCR to substitute the nucleotide at the position +2759 by an “A”. Gateway-compatible fragments were cloned into the pDONR207 entry vector (Invitrogen) via BP recombination and subsequently transferred into the binary vector pFAST27 via a LR reaction following the Gateway cloning procedure (Invitrogen, www.invitrogen.com). After a verification done by Sanger sequencing, expression constructs were transformed into Agrobacterium tumefaciens strain C58C1. fn-3 mutants were then transformed by floral dipping and transgenic plants were isolated using seed fluorescence. We selected four independent transgenic lines carrying the Col-0 and the Can-0 fragment of FLM, and two independent transgenic lines carrying the mutated version of FLM.

FLM transcripts variants of Col-0 and Can-0 were amplified using cDNAs obtained from pools of 3 rosettes grown at 23 °C with primers including start and stop codons using Phusion high-fidelity Taq polymerase. PCR products were cloned with the Zero Blunt TOPO PCR cloning kit (Invitrogen) following manufacturer specifications. Colony PCR was used to assess the size and the frequency of the different isoforms.

Gene expression analysis. The RNeasy Plant Mini kit (Qiagen) was used for RNA extractions followed by a DNase treatment (Fermentas). RT-PCR was performed on 500 ng of RNA using RevertAid H Minus reverse transcriptase (Fermentas) with oligo(dT) in 20 μl reactions. Then, 5 μl of tenfold diluted cDNAs was used for qRT-PCRs using a CFX96 real-time PCR machine (BioRad) with a SYBR solution. The data used for Fig. 4b can be found in Wright et al.12

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Author contributions
M.H. and O.L. designed the research. M.H., F.V., E.M., E.G., J.B., and I.G. performed experiments. M.H. and F.V. analyzed the data. M.H., F.V., C.V., and O.L. wrote the paper with contributions from all authors.

Competing interests
The authors declare no competing interests.

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