Patterns of phenotypic plasticity and local adaptation in the wide elevation range of the alpine plant *Arabis alpina*

Pierre de Villemereuil[*, †], Médéric Mouterde[*], Oscar E. Gaggiotti[*, ‡, 1], and Irène Till-Bottraud[*, §, 1]

[*] *Université Grenoble Alpes, CNRS, LECA, UMR 5553, 2233 rue de la piscine, 38400 Saint Martin d'Hères, France*
[†] *School of Biological Sciences, University of Auckland, City campus, Auckland, New Zealand*
[‡] *Scottish Oceans Institute, University of St Andrews, Fife, KY16 8LB, United Kingdom*
[§] *Université Clermont Auvergne, CNRS, GEOLAB, F-63000 Clermont-Ferrand, France*
[1] *These two authors are co-last authors*

Abstract

1. Local adaptation and phenotypic plasticity are two important characteristics of alpine plants to overcome the threats caused by global changes. Among alpine species, *Arabis alpina* is characterised by an unusually wide altitudinal amplitude, ranging from 800m to 3100m of elevation in the French Alps. Two non-exclusive hypotheses can explain the presence of *A. alpina* across this broad ecological gradient: adaptive phenotypic plasticity or local adaptation, making this species especially useful to better understand these phenomena in alpine plant species.

2. We carried out common garden experiments at two different elevations with maternal progenies from 6 sites that differed in altitude. We showed that *(i)* key phenotypic traits (morphotype, total fruit length, growth, height) display significant signs of local adaptation, *(ii)* most traits studied are characterised by a high phenotypic plasticity between the two experimental gardens, and *(iii)* the two populations from the highest elevations lacked morphological plasticity compared to the other populations.

3. By combining two genome scan approaches (detection of selection and association methods), we isolated a candidate gene (SPS1). This gene was associated with height and local average temperature in our studied populations, consistent with previous studies on this gene in *A. thaliana*.

Synthesis Given the nature of the traits involved in the detected pattern of local adaptation and the relative lack of plasticity of the two most extreme populations, our findings are consistent with a scenario of a locally adaptive stress response syndrome in high elevation populations. Due to a reduced phenotypic plasticity, an overall low intra-population genetic diversity of the adaptive traits and weak gene flow, populations of high altitude might have difficulties to cope with e.g. a rise of temperature.

Keywords: local adaptation, phenotypic plasticity, common garden, RAD sequencing, *Arabis alpina*, alpine ecology

Corresponding author: Pierre de Villemereuil, E-mail: bonamy@horus.ens.fr

Published in: *Journal of Ecology*

DOI: 10.1111/1365-2745.12955

Accepted version after peer-review.

Introduction

Local adaptation arises when populations, possibly in contact through moderate gene flow, experience contrasted environmental conditions: if the environment imposes strong constraints and if some adaptive potential exists in the populations, selection is expected to favour trait values that increase the fitness of individuals in their local environment. As a result, individuals have a better fitness in their local environment than individuals from other populations (Kawecki & Ebert, 2004). Local adaptation has important implications for conservation and response to global change (Aitken et al., 2008; Alberto et al., 2013). Indeed, it participates to the preservation of the adaptive potential of the species because it maintains polymorphism, especially *adaptive* polymorphism, at the level of the metapopulation (Hedrick et al., 1976; Hedrick, 1986). Hence, when environmental conditions are changing (e.g. under the influence of global change) and provided that gene flow is sufficient, pre-adapted variants can invade the population through migration and selection, hopefully allowing for a relatively quick evolutionary response (this is the concept behind genetic rescue through assisted gene flow, see e.g. Aitken & Whitlock, 2013). Another efficient mechanism to cope with environmental changes is phenotypic plasticity (Charmantier et al., 2008; Alberto et al., 2013), i.e. the ability of a given genotype to express different phenotype according to some environmental cues or circumstances. This mechanism is quicker than local adaptation, but its maintenance is assumed to be associated with costs (DeWitt et al., 1998; Van Buskirk & Steiner, 2009) and it can sometimes be maladaptive (Langerhans & DeWitt, 2002; Ghalambor et al., 2007).

Because montane and alpine habitats are characterised by strong environmental differences over small geographic distances (Körner, 2003), alpine species, especially sessile organisms such as plants, are likely to undergo local adaptation or be highly plastic. Mountains harbour resource gradients along which environmental conditions harshen dramatically as elevation increases (e.g. lower temperatures, shorter reproductive seasons, less fertile soils, Körner, 2003), although many other factors modulate the effects of altitude (Körner, 2007). In the Alps, from 400m to 2000m, environmental change is well depicted by the transition from deciduous to coniferous forest. Above 2000m, the gradient is even more striking from the treeline

to the alpine meadows and to the sparse high-alpine vegetation around 3000m. Elevation has the strongest impact above treeline on the specific and functional diversity of plant communities, with a switch from interspecific competition to facilitation (Choler et al., 2001), as well as on plant physiology and morphology, with, for example, the extreme case of cushion plants in the alpine and nival zones (Körner, 2003; Boucher et al., 2012). As a result, very different species are typical of these various environmental conditions. In this context, climate change is generally thought to induce an upward migration of species (Theurillat & Guisan, 2001; Alberto et al., 2013) with the potential impossibility for high altitude plants to move further up. Indeed, Pauli et al. (2012) found an upward shift of European alpine species distribution (2.7 m elevation gain between 2001 and 2008) mostly driven by leading edge expansions following an increase in temperatures and resulting in an increase in species richness in temperate regions. However in the Mediterranean Alpine, the reduction of precipitations induces a rear-edge retraction and a reduction of species richness. Moreover, trends are not uniform across continents, depend on the reference flora (Malanson & Fagre, 2013) or could be due to a release of anthropogenic pressure (Kammer et al., 2007). Species might also be able to survive (or subsist longer) *in situ* by taking advantage of the thermal microhabitat mosaic (Scherrer & Körner, 2010) or through niche construction (Bråthen et al., 2017). Overall, local adaptation and phenotypic plasticity of alpine species are key features for their persistence (Theurillat & Guisan, 2001; Grassein et al., 2010; Alberto et al., 2013; Münzbergová et al., 2017; Delnevo et al., 2017), although phenotypic plasticity can be maladaptive as in *Campanula thyrsoides* where warmer temperatures lead to earlier flowering, but also to a reduced seed set (Scheepens & Stöcklin, 2013).

Among the characteristic plants of the French Alps, *Arabis alpina* is remarkable for its wide altitudinal amplitude ranging from 800m to 3100m of elevation (Poncet et al., 2010). This range extends from the bottom of the montane zone to the top of the alpine zone, consisting of widely different habitat types, especially in terms of the resources mentioned above. This begs the question of how *A. alpina* is able to grow, survive and reproduce along such a wide altitudinal range, and especially to cope with the associated resource gradient and how it will respond to climate change. As explained above, two main mechanisms might explain this: phenotypic plasticity and local adaptation. Of course, these hypotheses are not mutually exclusive and both might contribute to the wide altitudinal range of this species. *A. alpina* is thus an interesting system to study the relative importance of local adaptation and phenotypic plasticity in alpine plants for coping with heterogeneous mountain environments. From a genomic perspective, *A. alpina* offers the power of both a properly assembled genome (Willing et al., 2015) and good orthology with the model species *Arabidopsis thaliana* (Lobréaux et al., 2014). Finally, its biology and ecology is starting to be well understood with a wealth of studies focusing on this species, from phylogenetic and historical aspects (Koch et al., 2006; Assefa et al., 2007; Ansell et al., 2011), to population genetics and ecology (Ansell et al., 2008; Manel et al., 2010; Poncet et al., 2010; Buehler et al., 2012; Buehler et al., 2013; Toräng et al., 2015) with a strong focus on phenology (R. Wang et al., 2009; R. Wang et al., 2011; Albani et al., 2012) and resistance to frost (Wingler et al., 2012; Kolaksazov et al., 2013; Kolaksazov et al., 2014; Wingler et al., 2015). To study local adaptation in *A. alpina*, we conducted a common garden experiment using six populations covering the altitudinal range of the species in the French Alps. Because individuals from diverse origins are grown in the same environment, common gardens allow to compare phenotypes of different populations without the confounding effect of phenotypic plasticity (Kawecki & Ebert, 2004; Savolainen et al., 2013). In order to minimise the remaining possibility of confounding genotype-by-environment interactions and to assess the extent of phenotypic plasticity, we performed the experiment in two contrasted common gardens. To characterise the process of local adaptation (Savolainen et al., 2013) in this species, we combined phenotypic, genotypic (genome representation genotyping) and *in situ* measures of environmental data in a statistical model accounting for the effects of population structure and genetic drift developed by Ovaskainen et al. (2011). Finally, we conducted genome scan analyses to detect selection, and association studies to search for potential candidate genes associated with our patterns of local adaptation. Combining these genomic analyses with information available on the closely related species *Arabidopsis thaliana* allowed us to detect a candidate gene potentially involved in local adaptation.

Material & Methods

Species and population

Species *A. alpina* is a common arctic-alpine plant. It is a pioneer species (Whittaker, 1993) and a bad competitor, hence it is most often found in open rocky habitats, mostly resource-poor and unstable. The plant is perennial but short-lived (1.82 years on average, Andrello et al., 2016), with entomogamous pollination and autochorous seed dispersal. In the French Alps where this study was conducted, it is also characterised by a high level of selfing (around 84% of selfed offspring, Buehler et al., 2012) with a resulting measured F_{IS} of 0.533 (Ansell et al., 2008). As a consequence, gene flow is limited in this area, with most pollen dispersal very close to the individuals, though long-distance dispersal has also been observed (Buehler et al., 2012).

Populations and sites The six studied sites and corresponding populations (see Figure S1 in Supplementary Information) covered much of the natural altitudinal range of the species (900m to 3000m) and were localised in two different massifs: three populations were from the Vercors

Table 1: Summary of the characteristics of the 6 studied sites. *Avg. Temp.*: average daily mean temperature; *Temp. Range*: average daily temperature range; *Avg. Hum.*: average daily mean humidity index; *Season Length*: average length of the reproductive season (in days); *Freezing days*: average number of days below 0℃ during the growing season.

ID	Longitude	Latitude	Massif	Elevation	Aspect	Avg. Temp.	Temp. Range	Avg. Hum.	Season Length	Freezing days	Comments
BRU	45,15065	5,61112	Vercors	930m	South	9.1℃	7.3℃	102.6%	251.6	18.6	Chalky scree
CHA	45,07117	5,59267	Vercors	1480m	North	10℃	11.2℃	87%	238.3	31.8	Chalky scree, meadow
VIL	45,01809	5,57083	Vercors	1980m	South	8.2℃	11.7℃	96.6%	217.5	47	Chalky rocks & scree
LAU	45,02846	6,39034	Lautaret	2090m	North	8.2℃	6.1℃	100%	136.2	6.1	Schistose river
GAL	45,06049	6,40375	Lautaret	2590m	North	6.2℃	6.6℃	92.4%	120.7	16.3	Chalky scree
PIC	45,06385	6,38426	Lautaret	2930m	South	6.8℃	15.2℃	86.5%	158.2	62.1	Schistose scree

massif (900m to 2000m) and three populations were located near the Lautaret pass (2000m to 3000m). Sites characteristics are summarised in Table 1.

Environmental data Using the GPS location points, we estimated the elevation and aspect (North- or South-facing slope) of each site. Environmental data was collected using temperature and humidity sensors (iButton® from Maxim Integrated™). Data collection was performed every three hours in each quadrat of a demographic survey (Andrello et al., 2016, 2-4 quadrats per site) taking place from 2008 to 2015. The sensors were placed approx. 20 cm above the ground (around the canopy height of *A. alpina*) protected by a small wooden plate from direct exposure to the sun, limiting temperature inflation by direct irradiation during the day. Using these semi-continuous data, we were able to define, for each site and each year, the start of the growing season as the date where positive degree-days (i.e. reference 0℃) started to accumulate (i.e. increase after a flat or decreasing trend during winter) and the end of the growing season as the date were positive degree-days stopped accumulating. From the semi-continuous series, we summarised the environmental conditions in each site with five variables (see Table 1):

Average Temperature Average of the daily mean temperature during the growing season.

Temperature Range Average of the daily temperature range during the growing season.

Average Humidity Average of the daily mean humidity during the growing season.

Season Length Number of days between the start and the end of the growing season.

Freezing days Number of days for which a negative temperature was recorded during the growing season.

In order to describe the spatio-temporal environmental variations, we performed a Principal Component Analysis (PCA) and a discriminant analysis on the yearly fluctuations of these variables using either the site or the year as a discriminant factor. The significance of those discriminant factors was tested using a permutation test. These analyses were conducted using the ade4 R package (Dray & Dufour, 2007).

Common garden experiment

Plant collection We collected maternal progenies from the six natural populations. To do so, during the summer of 2012, we put fine-mesh nets around maturing infructescences of 20 plants located in these populations. The plants were chosen to be as close as possible to the demographic quadrats while being separated from each other by at least 1m. The bags were collected when fruits were ripe during July and August 2012. During the spring of 2013, we germinated seeds in the lab in germination plates (½ potting compost, ½ plain soil) for Vercors and Lautaret. We then transferred and planted the two-week old seedlings in the two different gardens (during May 2013 at Vercors and July 2013 at Lautaret, some plants were planted late at Vercors during July 2013 to compensate for a low germination rate and a high juvenile mortality).

Experimental setting One garden was located near the Vercors Regional Natural Park House (5,58733°E, 45,12972°N, elevation 996m) at the edge of a grove. The experimental garden was shady, with a fertile and moist clay soil. At this location, mean annual temperature is 7.2℃ with on average 141.8 freezing days a year. The second garden was located in the experimental site of the Joseph Fourier Alpine Station at the Lautaret pass (6,40007°E, 45,03635°N, elevation 2100m). The experimental garden was largely exposed to sunlight, with a stony and less fertile soil. To regulate soil moisture and avoid severe drought, the garden was automatically irrigated every evening. At this location, mean annual temperature is 3.1℃ with on average 175.4 freezing days per year. We planted 4.1 offspring per family and 7.7 families per population at Vercors, and 4.6 offspring per family and 9.2 families per population at Lautaret. The two experimental gardens were composed of three and five blocks of 100 plants, respectively. To avoid border effects, we planted 54 *A. alpina*

individuals around the 100 monitored individuals within each block.

Phenotypic traits We phenotyped the individuals from the common gardens for different traits at the height of the reproductive season. We measured the total fruit length as the total number of fruits multiplied by the average fruit length (measured over 5 fruits). At Vercors, the plants were too big to record the actual number of fruits, so we estimated the total number of fruits as the number of reproductive stems multiplied by the average number of fruits per stem (measured on 10 stems). We recorded different morphological measurements: basal height (height to the highest leaf of the "rosette" part of the plant), vegetative height (height of the highest leaf) and reproductive height (height of the highest flower corolla). We estimated individual surface area by measuring, from above, two orthogonal diameters and approximating the area to an ellipse. We estimated growth rate as the ratio between individual area in 2015 and 2014 (only for individuals at Lautaret). We categorised the vegetative habit of the individuals into four different morphotypes: "sparse", "intermediate", "numerous", "compact" (see Section S2 in Supplementary Information for more details). Because of a large discrepancy in size between the individual morphotypes at Vercors clearly separating "compact" individuals from the others, no "numerous" morphotype was recorded. We computed flowering time as the number of weeks between the disappearance of snow cover in the garden and the first observation of an open flower. Because the season started particularly early in 2015, many plants were already flowering at the time of our first visit at Lautaret.

All of these traits were recorded during the summer of 2014 at Vercors and during the summer of 2014 and the summer of 2015 at Lautaret, except for morphotype, which was only recorded once during the summer of 2014. At the end of 2014 for Vercors and 2015 for Lautaret, we pulled the surviving plants out to weight their aerial biomass and dried them to measure their dry biomass. Finally, we recorded survival at the beginning and the end of each summer, from transplantation to biomass measurement. We considered that plants that were pulled out for biomass measurement would have survived until the following year.

During the summer of 2014, at Lautaret, an outbreak of white rust (*Albugo candida*, Baka, 2008) severely infected the individuals with dramatic consequences on their growth, reproduction and survival. We recorded plants displaying symptoms of sickness during this summer. The white rust targeted more specifically the local populations (i.e. the three populations from the Lautaret massif, $F_{1,442} = 23.8, p < 2.10^{-6}$).

Genotyping

During July 2014, leaf samples were collected on 204 surviving individuals at Lautaret. Note that this involves a slight bias in terms of the genotyped progenies, which is mitigated by the fact that the sampling of maternal plants is unbiased by this. We extracted DNA from these samples using the Qiagen DNeasy Plant Mini kit with minor modifications (i.e. cell lysis and protein digestion over night). We then used a double digest RAD sequencing protocol (Peterson et al., 2012), with minor modifications (see Supplementary Information), using the *ecoRI* and *mspI* restriction enzymes. Fragments between 150bp and 600bp were pair-end sequenced on 125bp using an Illumina HiSeq sequencer. Reads were analysed using the pipeline Stacks (Catchen et al., 2011; Catchen et al., 2013). After the cleaning process using the `process_radtags` function (`-c` and `-q` flags), we mapped reads on *A. alpina* reference genome (NCBI, GenBank, Accession JNGA00000000, Version 1, http://www.ncbi.nlm.nih.gov/nuccore/JNGA00000000.1, Willing et al., 2015) using the Burrows-Wheeler aligner (bwa, Li & Durbin, 2009, using the `mem` function and default parameters). Reads with an alignment score below 35 were excluded. Mapped reads were grouped into "stacks" corresponding to a RAD-tag using the `pstacks` function (only reads with a read depth of 2 or above were allowed to be considered a "stack"), then catalogued using `cstacks` and `sstacks`. The final RADseq output was created with the `populations` function while filtering for read depth above 5 for all individuals, missing rate below 30% and minor allelic frequencies above 1%. Furthermore, individuals with high rates of missing values due to overall low read depth were removed (42 individuals missing more than 2,000 RAD-tags). Individuals with aberrant clustering were also removed (i.e. between-massif hybrids and individuals with no clustering signal, 10 individuals). We used these SNP data to perform genome scans for selection and association studies. For neutral population structure inference, to avoid issues due to strong linkage between SNP on the same reads, we used multiallelic sequence polymorphism of the 125bp RAD-tags, which we hereafter refer to as "RAD haplotypes". In the end, we retained 3,528 RAD-tags (14,714 SNPs) loci for 152 individuals, with on average 25.3 individuals per population, 3.1 individuals per family and 9.2 families per population.

Statistical analyses of quantitative genetic variation

Population and family structures We checked population structure using the unsupervised clustering algorithm sNMF (Frichot et al., 2014), which infers the ancestry coefficients in a faster but similar fashion to ADMIXTURE (Alexander et al., 2009). The software Genepop (Rousset, 2008) was used to infer the Weir & Cockerham (1984) F_{ST} and F_{IS} estimates. Nucleotide diversity and percentage of polymorphic sites were estimated using the `populations` function of Stacks (Catchen et al., 2013). To study the ancestral additive genetic variance of the traits, we used the model of Ovaskainen et al. (2011). Simply put, this model decomposes the ancestral additive genetic variance of the traits into between- and within-population vari-

ances. It does so by decomposing the relatedness matrix **A** of all individuals into a population-level relatedness **B** and a within-population relatedness **W**. The matrix **B** was estimated using the admixture F-model implemented in the R package RAFM (Karhunen & Ovaskainen, 2012). We ran RAFM separately on each massif dataset and combined the matrix estimates for both massifs into a composite matrix assuming a coancestry of 0 between massifs. We did so because of the particular hierarchical structure of our data, which the F-model has difficulty to account for (Excoffier et al., 2009, but see Foll et al., 2014). The diagonal elements of the matrix yielded by RAFM are linked to the level of drift experienced by the populations since the split from the hypothetical ancestral population, and as such, are related to the population F_{ST} in the F model (Gaggiotti & Foll, 2010; Karhunen & Ovaskainen, 2012). To construct the matrix **W**, we inferred the sibship structure (paternal and maternal progenies) from molecular data, separately for each population. To do so, we used the COLONY software (Jones & J. Wang, 2010; J. Wang, 2011; J. Wang, 2012), including mother identity (known from sampling), using the hybrid full- and pairwise-likelihood score (FPLS, medium run length and high likelihood precision) and accounting for partial selfing and hermaphroditism.

Analysis of phenotypic traits Since only a subset of the individuals was genotyped, focusing only on these individuals might result in a great loss of power. To minimise this problem, we performed two analyses. The first, hereafter referred to as "Subset analysis", includes only the genotyped individuals, hence only individuals at Lautaret. The second analysis, hereafter referred to as "Full analysis", includes all individuals from both gardens.

The estimated random effect variances in the "Subset analysis" included the between-population genetic variance V_B (inferred using the covariance matrix **B**), the within-population genetic variance V_W (inferred using the covariance matrix **W**), the maternal effect variance V_M (inferred using maternal identity) and the block effect variance V_{block}. Because matrix **B** was inferred with uncertainty from molecular data, we integrated over this uncertainty by performing 100 runs using 100 outputs from the RAFM posterior distribution. The runs were then combined into one posterior distribution. This process is akin to integrating over phylogenetic uncertainty in phylogenetic comparative analysis (Huelsenbeck et al., 2000; Huelsenbeck & Rannala, 2003; de Villemereuil et al., 2012).

The estimated random effect variances in the "Full analysis" included the between-population genetic variance V_B again, the family effect variance V_F (which, in the absence of genotypic information, includes both V_W and V_M with unknown weighting), a garden-by-population interaction variance $V_{G\times E}$ and again a block effect variance V_{block}. The garden-by-population effect could not be estimated for growth rate since we only had data for the Lautaret garden individuals.

For both analyses, we also tested potentially confounding effects (included as fixed effects). The "garden" effect tested whether the phenotypes were different in the two gardens, the "year" effect tested whether phenotypes differed between measurements in 2014 and in 2015 at Lautaret, the "white rust" effect tested the effect of the white rust on the phenotype and the "late" effect tested the effect of a late planting date for plants Vercors (only for the full analysis). The most complete model for the "Full analysis" can thus be written as (indices and residuals are omitted for the sake of simplicity):

$$y \sim \mu + \text{garden} + \text{year} + \text{rust} + \text{late} + b + f + i + l + e \quad (1)$$

where the random effects (b for between-population effect, f for family effect, i for G×E interaction and l for block effect) were assumed to have the following multivariate Normal distributions:

$$\begin{aligned} \mathbf{b} &\sim \mathcal{N}(0, \mathbf{B}V_B) \\ \mathbf{f} &\sim \mathcal{N}(0, \mathbf{I}V_F) \\ \mathbf{i} &\sim \mathcal{N}(0, \mathbf{I}V_{G\times E}) \\ \mathbf{l} &\sim \mathcal{N}(0, \mathbf{I}V_{block}) \\ \mathbf{e} &\sim \mathcal{N}(0, \mathbf{I}V_R) \end{aligned} \quad (2)$$

were **I** is the identity matrix. The most complete model for the "Subset analysis" can be written:

$$y \sim \mu + \text{year} + \text{rust} + b + w + m + l + e \quad (3)$$

where the random effects (b for between-population effect, w for within-population effect, m for maternal effect and l for block effect) were assumed to have the following multivariate Normal distributions:

$$\begin{aligned} \mathbf{b} &\sim \mathcal{N}(0, \mathbf{B}V_B) \\ \mathbf{w} &\sim \mathcal{N}(0, \mathbf{W}V_W) \\ \mathbf{m} &\sim \mathcal{N}(0, \mathbf{I}V_M) \\ \mathbf{l} &\sim \mathcal{N}(0, \mathbf{I}V_{block}) \\ \mathbf{e} &\sim \mathcal{N}(0, \mathbf{I}V_R) \end{aligned} \quad (4)$$

The error distributions were chosen to fit each trait: *(i)* a log-Gaussian model was used for growth, basal, vegetative and reproductive heights, individual surface area, dry biomass and the fresh-to-dry biomass ratio, *(ii)* a Poisson (with a log link function) model was used for total fruit length (with values rounded up to integer values) and flowering time and *(iii)* a threshold model was used for the ordinal traits morphotype and survival. In all the analyses, morphotypes were ordered from the sparsest to the more compact ("sparse" = 0 to "compact" = 3) and survival was ordered according to the year of death (0 for a death in 2013 to 3 for a survival up to 2015).

Using the "Subset analysis", we computed Q_{ST} values as:

$$Q_{ST} = \frac{V_B}{V_B + 2V_W} \quad (5)$$

but did not test them against F_{ST} values. Since some of the traits were non-Gaussian, the heritabilities of the traits were computed based on the framework of de Villemereuil

et al. (2016). The component V_W is the within-population additive genetic variance on the latent scale. We computed the phenotypic variance on the latent scale $V_{P,lat}$ as the sum of within-population variances of non-experimental origins (i.e. V_W, V_M and V_R). To obtain the (narrow-sense) heritability on the observed data scale, we transformed the latent phenotypic variance $V_{P,lat}$ to the observed data scale phenotypic variance $V_{P,data}$ and computed the parameter Ψ relating the latent to the data scale additive genetic variance using the QGglmm package (see de Villemereuil et al., 2016, for more details). We then computed the heritability on the observed data scale (h^2) as:

$$h^2 = \frac{\Psi^2 V_W}{V_{P,data}} \quad (6)$$

For the threshold models, the estimates were computed on the more convenient liability scale (again see de Villemereuil et al., 2016):

$$h^2 = \frac{V_W}{V_{P,lat} + 1} \quad (7)$$

Note that these computations are based on the strong assumption that the within-population additive genetic variance is comparable across populations.

All the analyses were conducted using the MCMCglmm R package (Hadfield, 2010). Significance of fixed effects was tested using the pMCMC value yielded by MCMCglmm. Point estimates given in this paper are the mean for the fixed effects and the median for variances and variance ratios. For all models, convergence was checked graphically and using the Heidelberger and Welch's test (Heidelberger & Welch, 1981), and length of runs and thinning were set up so as to obtain an effective size above 1000 for all parameters of the model. We used default priors for fixed effects and extended parameters priors for variance components, with `V=1`, `nu=1` and `alpha.V=1000` for all traits, except for traits analysed using a threshold model for which used `V=1`, `nu=1000` and `alpha.V=1` as per de Villemereuil et al. (2013). Residual variance priors were set to `V=1` and `nu=0.02`, except threshold models where it was fixed to 1.

Testing for patterns of local adaptation In order to detect patterns consistent with local adaptation, we tested whether each of the five *in situ* local environmental variables had an effect on the traits measured in the common garden(s). Our null model included only statistically significant confounding effects and all random effects. Because the matrix **B** captures the effect of genetic drift and migration, this null model accounted for the hypothesis of migration-drift equilibrium. We decided to use only association with environmental variables as a test for local adaptation since our restricted number of populations would make most other test frameworks overly conservative (e.g. the *S*-test, Ovaskainen et al., 2011). This association was tested by including each environmental variable separately in the null model and testing whether its effect was significant (pMCMC< 0.05). In order to assess the impact of multiple testing on our results, we conducted a Monte Carlo analysis (100 simulations) where environmental variables were drawn with a similar variance-covariance structure as in our analysis and significant association with our phenotypic traits were tested (using both the Full and Subset analysis, exactly the same models). The overall p-value of the analysis was computed as the proportion of occurrence of results with the same variable being significant in both the Full and Subset analysis at least as often as in our analysis. A more detailed description the Monte Carlo simulation can be found in the Appendix.

Genome scans and association studies

Genome scans to detect selection To detect loci that show a signature of local adaptation we used LFMM (Frichot et al., 2013), which tests for a linear pattern between individual genotypes and an environmental variable, while accounting for population structure using latent factors. We set the number of factors K to 6 (our number of populations and preferred number of clusters) and performed 10 runs of the algorithm. We only considered environmental variables contributing to local adaptation (i.e. with a significant association with a phenotypic trait measured in the common gardens as tested above). The z-values yielded by the different runs were combined using Stouffer's method (Stouffer et al., 1949). A genomic inflation factor (GIF, a scaling factor used to correct for deviations of p-values from a uniform distribution, Devlin & Roeder, 1999) was computed using the resulting z-values, which were then transformed into corrected p-values when GIF was higher than 1. To control for false discovery rate, the distribution of p-values were further transformed into q-values using the Storey & Tibshirani (2003) algorithm. Correction of p-values using the genomic inflation factor before calculation of q-values allows for a better behaviour of false discovery rate control (François et al., 2016). We used a significance threshold of 0.05 for the q-values. Quantile-quantile plots (QQplot) of the p-values were used to assess the false positive rate.

Association studies We performed an association study to link our genotypic markers to the detected traits with adaptive patterns. Because relatedness-based mixed models such as EMMA (Kang et al., 2008) are poorly suited for the study of strongly differentiated populations, we decided to use LFMM (Frichot et al., 2013), which can account for population structure with strong drift-induced genetic differentiation between populations. As a consequence, our test framework does not use the genotypes to predict the phenotypic traits, but the reverse. To be used as predictors, traits were mean-centred and scaled to a variance of 1. Ordered categorical traits were transformed into integer values before centring and scaling. The settings of the analysis and post-analysis were identical to the above, e.g. we used K=6 and 10 runs, controlled for genomic inflation and

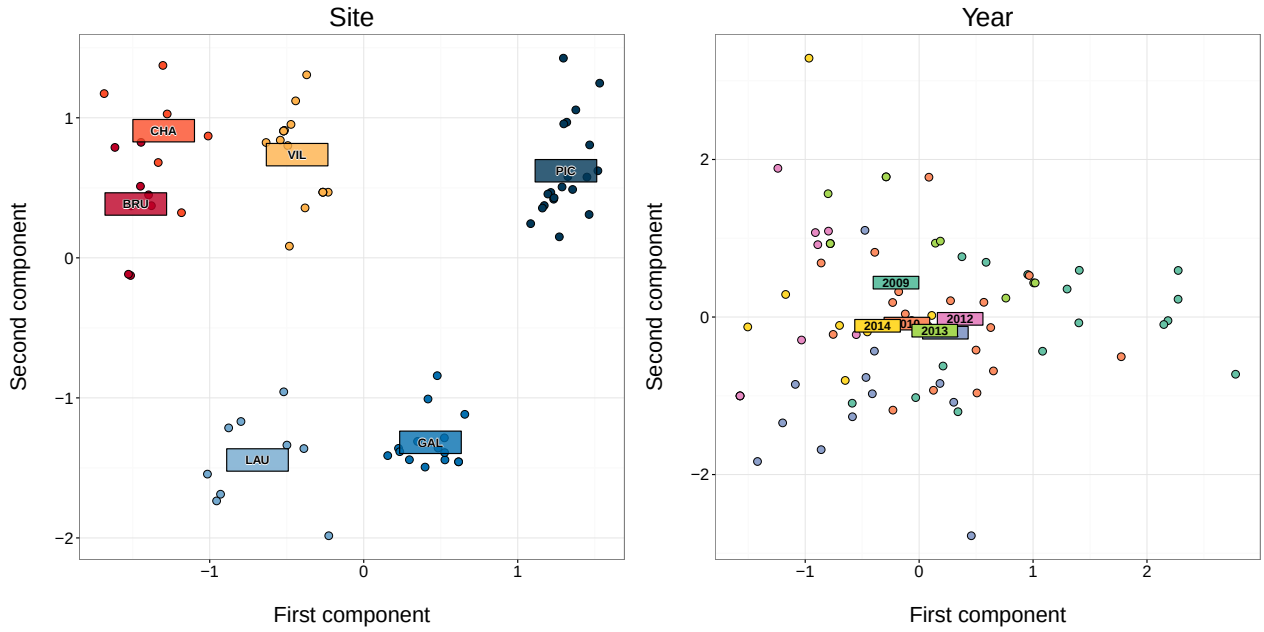

Figure 1: Projections of the environmental conditions at the site of origin after discriminant analysis according to the site (left) or the year of measurement (right) on the two first axes. Each point corresponds to one year in one quadrat within the site. For the left graph, BRU, CHA and VIL in red-yellow tones are the Vercors massif sites while LAU, GAL and PIC in blue tones are the Lautaret massif sites.

transformed the p-values into q-values. Again, we used a significance threshold of 0.05 for the q-values and QQplots of the p-values to assess the false positive rate. Because it was not possible to use phenotypic values from both years, we used data from 2014 in Lautaret for which more measurements were available.

Finding candidate genes Loci that were found associated to one of the traits with adaptive patterns and with a selective environmental factor were considered as candidates. Combining these different tests allows for a more stringent false discovery control, but can be a very conservative approach (de Villemereuil et al., 2014). SNPs within a distance of 5000bp were regrouped into the same genomic region. When these loci were located within an annotated gene in the *A. alpina* genome, we performed Blast queries (Altschul et al., 1997) against the *A. thaliana* protein database (Lamesch et al., 2012). We considered only significant query hits as homologous when the maximum "bit score" was above 200 and the percentage of identity above 60%. If several genes validated these criteria, they were all shown. We only considered as candidate a gene with molecular homology with a gene with demonstrated effect on this kind of phenotype in *A. thaliana*.

Results

Analysis of *in situ* environmental variables

The PCA on the *in situ* environmental variables separated the temperature amplitude variables (temperature amplitude and freezing days) on the first axis (40.1% of explained variance), the temperature trend variables (average temperature and length of season) on the second axis (31.3% of explained variance) and the average humidity on the third axis (19.7% of explained variance). The first two axes could also be respectively related to aspect (correlation $\rho = 0.65$, $p = 3.13.10^{-10}$) and elevation (correlation $\rho = 0.89$, $p < 2.10^{-16}$), though the first axis was also related to elevation (correlation $\rho = 0.36$, $p = 0.0019$).

Discriminant analyses show that environmental conditions varied widely across sites (Figure 1, left), but much less so across years (Figure 1, right). However permutation tests were significant ($p < 0.001$ for both analyses, with 1000 randomisations), indicating a non-random clustering according to both sites and years. The discriminant analysis on the sites (Figure 1, left) shows a greater environmental differentiation between the sites at high elevation near

Table 2: Genetic characteristics of the studied populations. AFM: Diagonal elements of the **B** yielded by RAFM (measure the strength of drift since the hypothetical ancestral population). F_{IS}: Weir & Cockerham (1984) F_{IS}. π: nucleotide diversity. % poly: percentage of polymorphic sites.

ID code	Massif	AFM	F_{IS}	π	% poly
BRU	Vercors	0.673	0.340	0.166	2.52
CHA	Vercors	0.241	0.497	0.172	2.94
VIL	Vercors	0.344	0.594	0.180	2.90
LAU	Lautaret	0.637	0.390	0.252	2.70
GAL	Lautaret	0.353	0.412	0.171	3.03
PIC	Lautaret	0.563	0.272	0.320	2.62

Table 3: Results for the "Full analysis" (left) and "Subset analysis" (right) for the morphotype, total fruit length (TFL), survival, growth, flowering time (FT), basal height (H. base), vegetative height (H. veg.), reproductive height (H. repro.), area, dry biomass (Biom. dry) and the dry/fresh biomass ratio (Biom. ratio). The table shows the significant confounding and environmental effects. Estimates of slopes are given with highest posterior density (HPD) intervals within parenthesis (in case of interaction with the garden, estimates are separated by garden).

Trait	Full analysis				Subset analysis			
	Confounding effects	Environment effects	Estimate (HPD interval)	pMCMC	Confounding effects	Environment effects	Estimate (HPD interval)	pMCMC
Morphotype	Late	Avg. Temp.	-1.37 (-2.18,-0.57)	0.00339	—	Avg. Temp.	-1.24 (-2.24,-0.53)	0.0018
TFL	Garden, White rust, Late	Avg. Temp.	0.78 (0.03,1.55)	0.0438	White rust	Avg. Temp.	0.77 (0,1.6)	0.0496
Survival	Garden	—	—	—	—	—	—	—
Growth	—	Avg. Temp.	0.34 (0.07,0.61)	0.0217	—	Avg. Temp.	0.43 (0.09,0.77)	0.0211
FT	Year	—	—	—	Year	—	—	—
H. base	Garden, Year, Late	—	—	—	Year	Aspect (Southern)	-0.22 (-0.41,-0.02)	0.0326
H. veg.	Garden, Year, Late	Avg. Temp.	0.24 (0.04,0.46)	0.0288	Year	Avg. Temp.	0.22 (0,0.46)	0.0473
H. repro.	Garden, Year, Late	Avg. Temp.	0.21 (0.03,0.41)	0.0300	Year	—	—	—
Area	Garden, Year, Late	Garden : Season length	Vercors: 0.45 (0.02,0.9) Lautaret: 0.08 (-0.35,0.52)	0.0431 (Vercors)	Year	—	—	—
Biom. dry	Late	—	—	—	—	—	—	—
Biom. ratio	Garden, Late	—	—	—	—	—	—	—

the Lautaret pass (LAU, GAL, PIC), whereas sites from the lower Vercors massif (BRU, CHA, VIL) were more similar to each other, with long growing seasons and high average temperature (Table 1). In relation with its high elevation and Southern aspect, PIC was strongly characterised by a wide daily temperature range and a high number of freezing days. LAU and GAL were characterised by both a narrow daily temperature range and a short growing season. BRU and LAU were also characterised by a high humidity (Table 1).

Neutral population structure

We found a strong population clustering, with the most likely number of population being $K = 6$ (S4 in SI) and little sign of gene flow between populations (Figure S5 in SI) based on the cross-entropy criterion used in sNMF (Frichot et al., 2014). The variance-covariance matrix **B** estimated by RAFM (Karhunen & Ovaskainen, 2012) is well-aligned with these results, with very little coancestry between populations (Figure S6, off-diagonal elements ranging from 2.6×10^{-5} to 1.1×10^{-2} with an average of 2.5×10^{-3}).

Consistent with these results, neutral genetic differentiation between populations as measured by the Weir & Cockerham (1984) estimator of F_{ST} was very strong: 0.60. Furthermore, populations exhibited important differences (Table 2), with some population being more strongly differentiated (high AFM, e.g BRU and LAU), and others more inbred (high F_{IS}, e.g. CHA and VIL). However, these genetic characteristics do not appear to be linked to altitude or temperature (Table S1).

Linkage disequilibrium between RAD haplotype markers was low overall (Figure S8 in SI) with an average value of 0.0468. The marker density in our study was relatively high with 9.41 markers *per* Mbp (compared to a median of 4.08 in a recent meta-analysis, Lowry et al., 2016).

Analysis of the common garden phenotypic traits

Full analysis For the analysis using all the individuals (Full analysis), in both gardens, total fruit length (pMCMC $= 0.0438$), growth (pMCMC $= 0.0217$), reproductive (pMCMC $= 0.03$) and vegetative (pMCMC $= 0.0288$) height increased significantly with average temperature at the site of origin, while morphotype (pMCMC $= 0.00339$) was less compact with higher average temperature at the site of origin (Table 3). Area increased with season length at the site of origin, but only so in the Vercors garden (pMCMC $= 0.0431$, Table 3).

Comparison of the intra-class correlation coefficients (ICC, Figure 2, top panels) for the between-population variance V_B with or without environmental effect showed that average temperature at the site of origin explained a large amount of the between-population variance for morphotype ($\frac{V_B}{V_T} = 0.0016$ with the environment effect, $\frac{V_B}{V_T} = 0.01$ without), growth ($\frac{V_B}{V_T} = 0.014$ with the environment effect,

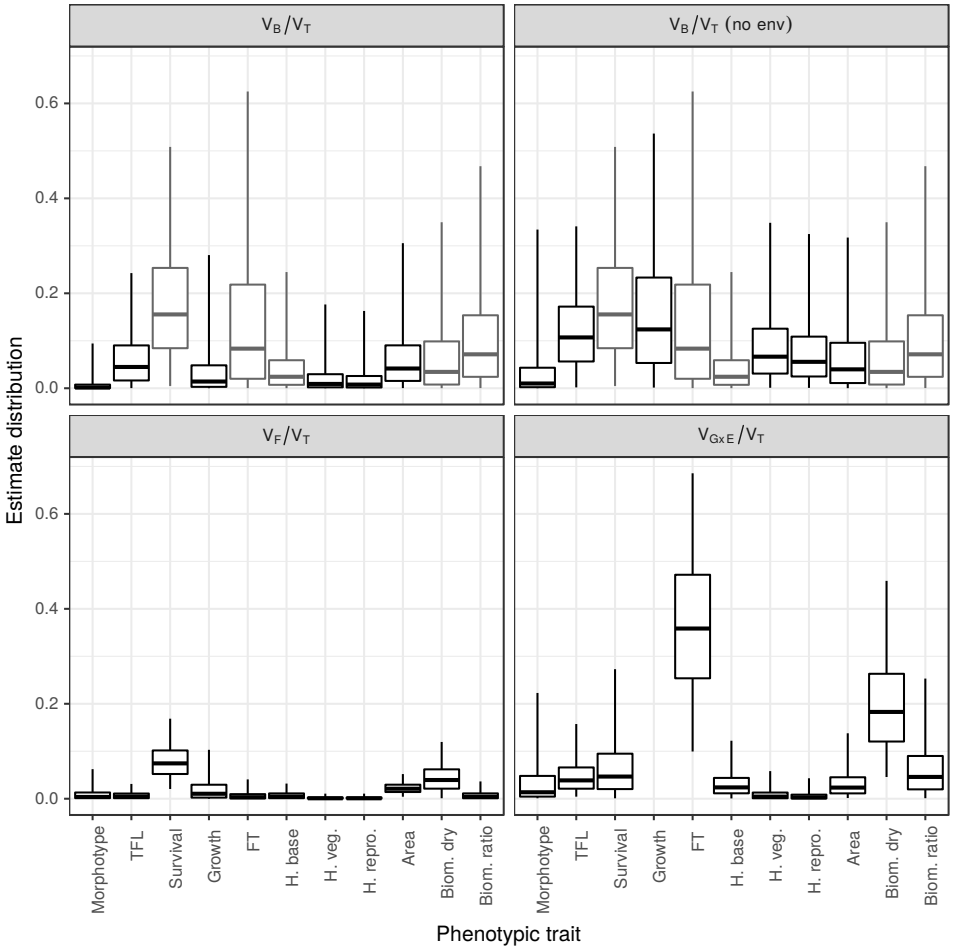

Figure 2: Results from the "Full analysis": intraclass correlation coefficients (ICC, i.e. ratio of the effect variance to the total variance V_T) for the 11 phenotypic traits. ICCs corresponding to the between-population genetic variance V_B is shown with (top-left panel) or without (top-right panel) the environmental effect. When no environmental effect was significant, both estimates are identical and thus displayed in grey. Bottom panels show the ICCs corresponding to the family effect variance V_F (bottom-left panel) and to the garden-by-population effect variance $V_{G\times E}$ (bottom-right panel). Since "Growth" was measured only in the Lautaret garden, its $V_{G\times E}$ estimate is not available. The boxes and whiskers correspond to the 50% and 95% inter-percentile intervals respectively, the middle corresponds to the point estimate.

$\frac{V_B}{V_T} = 0.12$ without), vegetative ($\frac{V_B}{V_T} = 0.0091$ with the environment effect, $\frac{V_B}{V_T} = 0.067$ without) and reproductive ($\frac{V_B}{V_T} = 0.0077$ with the environment effect, $\frac{V_B}{V_T} = 0.056$ without) heights. The data thus depicts bigger, less compact plants that are growing faster and reproducing more with higher temperatures and season length at the site of origin. Graphical representation of population average phenotypic values supports these statistical trends (Figure S9 in SI).

Subset analysis The results of the analysis using only genotyped individuals (thus only the Lautaret garden) were similar to those of the full analysis. Total fruit length (pMCMC $= 0.0496$), growth (pMCMC $= 0.0211$) and vegetative height (pMCMC $= 0.0473$) increased significantly with average temperature at the site of origin, and morphotype was less compact with higher average temperature at the site of origin (pMCMC $= 0.0018$,Table 3). Basal height was shorter in populations for which the site of origin has a Southern aspect (pMCMC $= 0.0326$, Table 3). As shown by the Q_{ST} estimates computed with or without environmental variable (Figure 3, bottom panels), the effect of the environment at the site of origin explained a large amount of the total additive genetic variance for morphotype and growth, but almost none for total fruit length.

A strong phenotypic differentiation among populations was found for survival as indicated by the high ICC values corresponding to V_B (0.46) and the high Q_{ST} (0.78, Figure 3). However, survival was not linked to any of the environmental variables tested. Despite a strong signal of local adaptation, the morphotype was one of the phenotypic traits with the greatest proportion of variance explained by within-population genetic variance (Figure 3, top-middle panel). The dry biomass and biomass ratio variances were

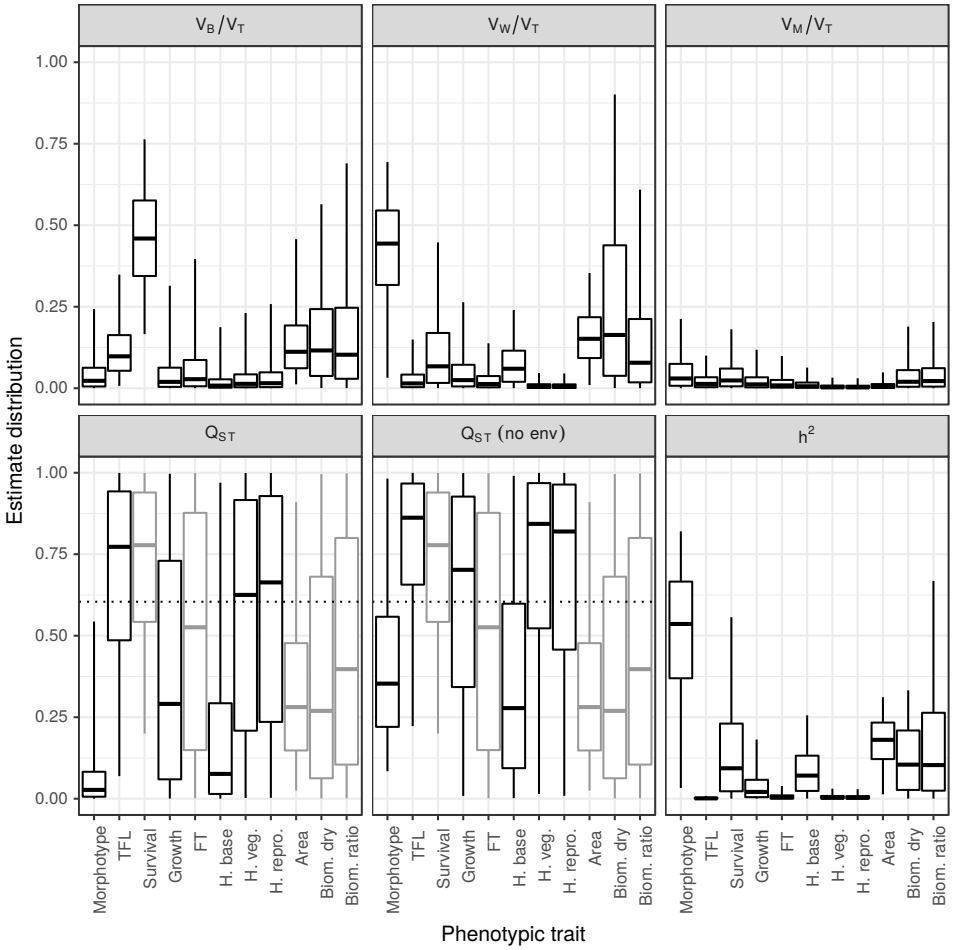

Figure 3: Results for the "Subset analysis": Intraclass correlation coefficients (ICC, i.e. ratio of the effect variance to the total variance V_T), Q_{ST} and h^2 estimates for the 11 phenotypic traits. ICCs shown correspond to the between-population genetic variance V_B (top-left panel), the within-population genetic variance V_W (top-middle panel) and the maternal effect variance V_M (top-right panel). Bottom panels show the Q_{ST} estimates when environmental effects are fitted in the model (bottom-left panel), without environmental effect (bottom-centre panel) and the h^2 estimates (bottom-right panel). When no environmental effect was significant, both estimates are identical and thus displayed in grey. The boxes and whiskers correspond to the 50% and 95% inter-percentile intervals respectively, the middle corresponds to the point estimate. The dotted line correspond to the estimated value of the F_{ST}.

equally explained by the within- (ICC resp. 0.16 and 0.078) and by the between-population (ICC resp. 0.12 and 0.10) genetic variance components resulting in a relatively small Q_{ST} value (resp. 0.27 and 0.40, Figure 3), but the uncertainty around this estimate is too large to conclusively suggest potential balancing selection.

For all traits, maternal effects explained very little of the total variance (Figure 3, top-right panel). Finally, the morphotype heritability was high (0.54) and the only estimate with a lower bound of the 95% credible interval clearly distinct from zero (0.067 against 1.6×10^{-11} for the second highest value), while the heritabilities of total fruit length (0.001), growth (0.021), flowering time (0.0035) and reproductive (0.0029) and vegetative (0.0027) heights were extremely low. Despite a small 95% credible interval lower bound, the heritability estimates of survival (0.093), area (0.18), dry biomass (0.1) and biomass ratio (0.1) where mildly high.

Using a criterion of the same variable found significant in at least 4 traits in both the Full and Subset analysis (note that this is slightly more permissive, but a simpler criterion, than our actual results in Table 3), we found a family-wise p-value of our whole analysis of 0.05 (see Section S8 in SI).

Phenotypic plasticity and garden-by-population interaction

All traits showed signs of phenotypic plasticity either through a significant garden effect (total fruit length, survival, basal, reproductive and vegetative height, area; Table 3) or through a notable garden-by-population interaction (flowering time, dry biomass and, to a lesser extent, biomass ratio, Figure 2). We could not estimate the phe-

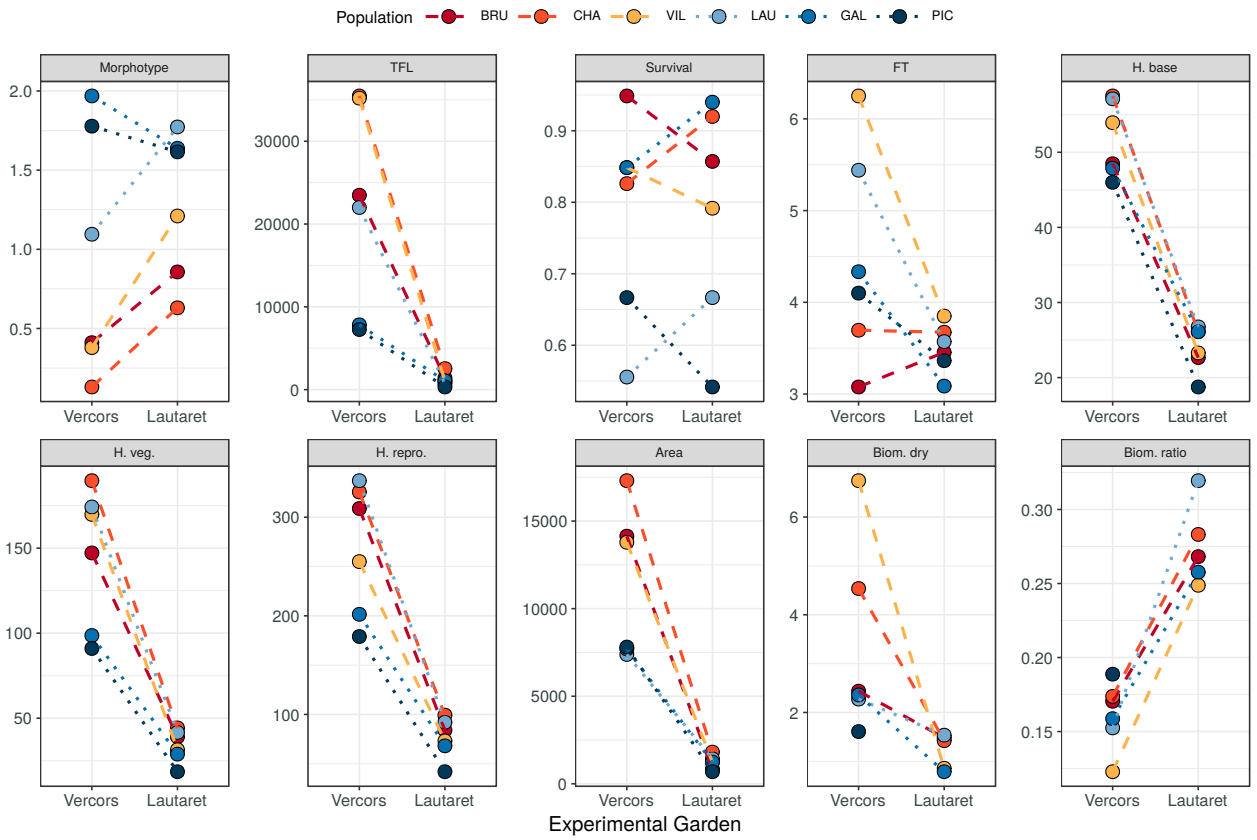

Figure 4: Reaction norms of 10 of the studied traits (Growth is excluded): mean phenotype of each population (different color dots and lines) in the two gardens (Vercors and Lautaret). Values for "numerous" and "compact" morphotypes have been merged to facilitate the comparison between gardens ("numerous" morphotype absent at Vercors). Survival is expressed as survival from one year to the next (survival: 1, death: 0). TFL: Total Fruit Length (mm). FT: Flowering Time (number of weeks). H. base: basal height (mm). H. veg.: vegetative height (mm). H. repro: reproductive height (mm). Area: individual surface area (mm²). Biom. dry: dry biomass (g). Biom. ratio: fresh-to-dry biomass ratio (no unit).

notypic plasticity of growth, because this trait was measured only at Lautaret. Two of the traits for which the garden effect was non-significant (flowering time and dry biomass) were the ones displaying strong signal of garden-by-population interaction. Hence, the absence of significant garden effect might be due to (or compensated by) the presence of large garden-by-population effects (Figure 2, bottom-right panel). Indeed, running a model without garden-by-population resulted in a significant Garden effect for both (results not shown).

The Vercors garden was warmer, more humid and had a more fertile soil which resulted in plants that were 9.83 times larger at Vercors than at Lautaret (average individual area $12,442\text{mm}^2$ and $1,266\text{mm}^2$, respectively, for the year 2014, $F_{1,522} = 406$, $p < 2.10^{-6}$, see also Figure 4).

Plasticity between gardens was low (Figure 4) for the populations from the two highest elevation sites (GAL and PIC) for many traits (morphotype, total fruit length, vegetative and reproductive heights, individual surface area and dry biomass). Two traits, while not following this pattern, still displayed considerable garden-by-population interaction with crossing reaction norms: survival and flowering time.

Genome scans

Genome scans to detect selection LFMM detected 142 SNPs (0.97% of the total) associated with average temperature at the site of origin and 63 associated with aspect at the site of origin. In total, 201 SNPs (1.4%) were significantly associated with at least one environmental variable, without much overlap between temperature and aspect. The QQplots (Figure S11) show that the tests were too liberal. This was mitigated by using the GIF correction (GIF $= 1.47$ for aspect, GIF $= 2.24$ for average temperature), but only to a limited extent. There was a slight enrichment of significant SNPs in genic regions (3.4% for non-genic regions and 4.1% for genic regions, $\chi^2_1 = 4.4$, $p = 0.035$).

Association studies The association study identified between 0 and 79 SNPs (0.54%) significantly associated with the phenotypic traits identified as involved in adaptation (Table 4). Despite GIF values being mostly below 1 (GIF $=$

Table 4: Association study: number of significant SNPs associated to variation for the 6 phenotypic traits we identified as involved in adaptation.

	Morpho type	TFL	Growth	H. base	H. veg.	H. repro.
Nb. SNPs	7	1	79	0	10	16

1.05 for morphotype, ranging from 0.63 to 0.71 for the heights, GIF $= 0.79$ for total fruit length and GIF $= 0.55$ for growth), the QQplots (Figure S12) show that the tests were enriched for large numbers of significant p-values. Particularly, the number of significant SNPs associated with growth was the highest (79) compared to the other traits. This was most likely due to the presence of four atypical individuals (with growth rates of 11.9, 13.1, 23.8 and 28.3, compared to an overall average of 3.12) from the two lowest populations (BRU and CHA). In total, 106 SNPs (0.72%) were significantly associated with at least one trait with adaptive pattern, among which 36 (0.24%) were located in 17 different genic regions (1.4% of the genes associated to at least one SNP). There was no enrichment of significant SNPs in genic regions (0.77% for non genic regions and 0.62% for genic regions, $\chi^2_1 = 0.95, p = 0.33$).

Candidate genes To minimise issues with the false positive rate, we combined the results from association studies and genome scans, selecting only loci significant in both. This allowed us to draw up a small list of 5 genomic regions which comprised 2 genes, both of which had significant homologues in the *A. thaliana* genome (and both consistent with existing annotations in *A. alpina* genome). The first gene (gene 3899) was homologous to AT1G60500 (a.k.a. DRP4C), which is a GTP binding related protein only expressed in egg cell, which function has yet to be established (Hong et al., 2003). Blasting the second gene (gene 26269) returned multiple hits of genes from the Sucrose-Phosphate Synthase (SPS) family. To investigate further, we aligned this gene with the four genes (SPS1F, SPS2F, SPS3F and SPS4F) of the family in *A. thaliana* using the ClustalX aligner (Larkin et al., 2007). This analysis showed that gene 26269 clusters with the SPS2F gene in *A. thaliana* (Figure S13). This suggests an orthology between gene 26269 in *A. alpina* and SPS2F (AT5G11110, a.k.a. SPS1) in *A. thaliana*. Our SNP was situated at position 4392 on gene 26269, which aligned on position 4136 on SPS2F. This position is located within a small intronic region (103bp) and does not correspond to a described SNP in *A. thaliana*. Gene 26269 was associated with height in our analysis in *A. alpina* and an overexpression of the SPS genes (including SPS2F) was shown to result in increased growth rate and higher stem height in *A. thaliana* (Park et al., 2007). It is thus the only gene satisfying all of our candidate criteria, including validated functional homology in *A. thaliana*. The shift in allelic frequency of the SNP corresponding to this gene is very strong: going from 0 for the four coldest populations to frequencies over 0.93 for the two warmest populations (Figure S14). It is clearly distinct from a massif effect, because the coldest population of the Vercors massif (VIL) has a population allelic frequency of zero.

Discussion

Patterns of local adaptation

The major genetic difference between our populations of *A. alpina* was that individuals from cold condition sites were significantly smaller, more compact and had a lower reproductive effort (total annual fruit length) and slower growth than individuals from milder conditions. These patterns are major components of the genetic differentiation between populations, as the variance explained by the relationship with environmental conditions of the site of origin accounted for much of the inter-population variance for all traits showing an adaptive pattern (except for total fruit length). Interestingly, humidity never appeared as an explanatory factor for population differences, possibly because the variation in humidity was relatively small between sites and air humidity is a limited proxy for the moisture condition of plants. Basal height was associated with aspect (shorter basal height in populations from a Southern aspect site) at Lautaret and plants originating from sites with long growing seasons had a larger area at Vercors.

A definitive proof of local adaptation would require the measurement of fitness in reciprocal transplant experiments (Kawecki & Ebert, 2004). However, our results are strongly suggestive of a pattern of local adaptation, especially as our study accounts for: *(i)* phenotypic plasticity by using a common garden approach, *(ii)* neutral evolution (i.e. migration and drift) by using genetic information and a model of neutral evolution and to some extent *(iii)* genotype-by-environment (here in the form of a garden-by-population) interaction created by using two contrasted common gardens (differing in altitude, soil characteristics and exposition). Adaptive maternal effects are another possible origin of the detected signal. As we used field-collected seeds rather than seeds produced in a common environment, they could explain the results along with a scenario of local adaptation (Roach & Wulff, 1987). Although this cannot be totally excluded, the fact that maternal effects variance was negligible for all traits is a strong indication that this might not be the case. The scenario of local adaptation is rendered even more likely by the fact that the spatial environmental variability among the sites is much greater than the temporal one, as shown by the discriminant analyses. This preponderance of spatial over temporal variability is indeed a prerequisite for local adaptation (Kawecki & Ebert, 2004). Of course, the small number of populations we studied limits the extent to which our results can be generalised to the whole *A. alpina* species, especially since the definition of local adaptation is very sensitive to the geographical scale chosen (Brachi et al., 2013).

The results are consistent with a previous *in situ* study

of the same 6 natural populations (Andrello et al., 2016), which found that growth rate positively correlates with average temperature late in the reproductive season and that the population from the coldest site (GAL) displayed a low reproductive effort when compared to the other populations, although the overall association between temperature and reproductive effort was not significant (Andrello et al., 2016). A similar pattern involving reproductive effort was found in a latitudinal study in Spanish and Swedish populations of *A. alpina* (lower reproductive effort in populations from colder Scandinavian sites, Toräng et al., 2015). However, this pattern was not found on a latitudinal gradient in *Arabidopsis lyrata* (non-clinal adaptive pattern in reproductive output; Vergeer & Kunin, 2013) and was found in the opposite direction in *A. thaliana* (negative correlation between temperature and seed weight, Manzano-Piedras et al., 2014).

Factors other than those studied here may help explain genetic divergence between our populations. For example, the positive relationship between area and breeding/growing season length at the site of origin is consistent with the pattern of smaller, more compact plants in cold sites because season length is strongly correlated with average temperature ($\rho = 0.82$, $p < 0.048$). But the relationship between basal height and aspect at the site of origin indicates the presence of another gradient of selection involving environmental variables decoupled from elevation. A large fraction of the inter-population variance in reproductive effort also remains unexplained and the high Q_{ST} value for this trait suggests that other selective factors might be involved. Finally, despite trends suggesting local adaptation in survival (e.g. large Q_{ST} value), none of the environmental variables we tested had a significant effect. This might be because survival does not vary monotonically with elevation: survival was very low in LAU (2000m above sea level, asl) and PIC (3000m asl) but comparable to the other populations in GAL (2500m asl). Once again, these results can be related to findings on *in situ* survival (Andrello et al., 2016), for which a very strong negative association with the average temperature was found for all populations except LAU and PIC and are an indication that other environmental variables are important.

Local adaptation can also originate from biotic factors. This can take the special form of co-evolution between a host and its parasite (Thompson & Burdon, 1992; Thompson, 1994). Such forms of selection are local adaptation in the sense that the selective factor is typical of the local environment and the host demonstrates an evolutionary response, but since the selective factor (the parasite) itself adapts to this response, the expected effect in terms of local vs. alien fitness is not necessarily obvious. In our case, the outbreak of white rust in the Lautaret garden seemed to have preferentially targeted plants originating from the Lautaret area. Although this is difficult to test due to a lack of a more in-depth knowledge regarding this outbreak, such a scenario of co-evolution could explain why the locals rather than the aliens were targeted: the parasite (assuming it originates from the neighbouring area) would also be adapted to the hosts of the area and thus more efficiently infect plants originated from the Lautaret area rather than plants from Vercors.

Instead of using the Q_{ST}-F_{ST} framework (Spitze, 1993; Leinonen et al., 2013), we used a rigorous model-based approach that explicitly incorporates the effects of genetic drift and migration under an island model (Ovaskainen et al., 2011). This approach is better from the following angles. It accounts for population differences in drift and migration rates (Ovaskainen et al., 2011) and should thus generally be a better fit for realistic scenarios of wild populations history and demography. Especially, since this approach is based on between-population relatedness, we were able to include the matrix of these relatedness in a mixed model framework to test for an association between the phenotypic values measured in our gardens and environmental values at the site of origin while accounting for neutral evolution (e.g. migration and drift). By using the additional information of *in situ* environment, this allowed us to detect significant signatures of local adaptation despite a small number of populations and a very strong neutral genetic differentiation between populations ($F_{ST} = 0.60$). Finally, accounting for the uncertainty of the Q_{ST} and F_{ST} estimates is relatively challenging and often overlooked (O'Hara & Merilä, 2005). Here we used Bayesian posterior distributions to propagate uncertainty in our estimation of ancestral between-population relatedness based on molecular markers to our model for quantitative traits as suggested by Karhunen et al. (2013).

Overall high phenotypic plasticity, but more limited at high elevation

Overall, grouping together the Garden and G×E effects as *sensu lato* "phenotypic plasticity", every studied trait showed clear signs of plasticity. When compared to plants at Lautaret, plants in the warmer, more humid and more fertile garden at Vercors were *c.* 10 times larger in area and produced *c.* 16 times more fruits. The Garden effect was significant for all traits but three. We also found significant differences between years at Lautaret for five traits, among which four morphological traits, so this is most certainly due to the expected year to year growth of the plants. We identified a strong garden-by-population interaction effect for two traits for which the Garden effect was not significant. This suggests that a strong interaction is masking a slight effect of the environment alone.

Despite the high phenotypic plasticity, it was still possible to detect patterns of local adaptation linked to average temperature. This indicates that both local adaptation and phenotypic plasticity play a role in allowing the wide habitat range of *A. alpina*. Because distinguishing both phenomena in natural populations is utterly complicated, it is extremely difficult to quantify their relative role, which might be further confounded by drift.

The two populations from the upper edge of the distribution range of the species (GAL and PIC, resp. 2500m and

3000m) showed a reduced phenotypic variability between gardens for some morphological traits and for reproductive effort. For these two populations, this reduced response to the environment of the more favourable experimental garden (lower elevation, Vercors) suggests that they harbour low phenotypic plasticity for some traits compared to the other populations. Given that we found no relationship between elevation and the genetic characteristics of the populations (Table 2), this lack of phenotypic plasticity cannot be explained by a lack of genetic diversity.

Direct selection due to thermic stress or response to resource gradient?

In this study, the average local temperature comes out as the main environmental factor driving the observed pattern, though many other environmental factors of possible strong influence, such a soil fertility and stability, were unmeasured. Local temperature is not totally confounded with elevation as our warmest population is second to the one with lowest elevation and our coldest population is second to the highest elevation one. This illustrates the importance of accounting for local conditions rather than larger scale gradients such as elevation. In *A. alpina*, colder temperature seems to select for smaller and more compact plants, with a slow growth and with a diminished production of fruits. Such a trend has often been described for inter-specific variations along an altitudinal gradient. Short and compact stature, for example, is known to help plants to decouple their temperature from atmospheric temperatures, hence helping to keep photosynthetic activity sufficiently efficient, as is illustrated by cushion plants (Körner, 2003). Slow growth, lower productivity and higher survival are also typical traits that allow alpine plant species to adapt to colder conditions (Körner, 2003). Our results are well aligned with these ecological expectations, with the notable exception of survival. Another possible interpretation that would better explain these results is that average temperature is a close proxy for the resource gradient linked to both elevation and aspect. Colder sites (usually at high elevation and of Northern aspect) are also often associated with many characteristics other than low temperature (Körner, 2007): they have a shorter growing season, lower partial CO_2 pressure and lower soil fertility (Körner, 2003). It has been suggested that genetic variation for growth and overall height are pleiotropically linked to the stress response syndrome (SRS) to resource limitation (Chapin et al., 1993), allowing for an evolutionary response of the whole syndrome at once. SRS are widespread in alpine species; de Bello et al. (2013) showed that high elevation species tend to be smaller, with thicker leaves and reduced reproductive effort. At the intra-specific scale, the evolution of SRS-like signals along an elevation gradient has been discovered using common garden experiments, in at least two other alpine species: Gonzalo-Turpin & Hazard (2009) showed that *Festuca eskia* had increased survival and lowered fertility along an elevation gradient and Hautier et al. (2009) showed that *Poa alpina* at high elevation were smaller with a reduced reproductive output. However, our findings on *A. alpina*, stand out by the amplitude of the elevation gradient and the maximal elevation involved, reaching the alpine, rather than the sub-alpine stratum.

Adaptive SRS is a better explanation of our results than the direct and sole influence of temperature. First, not only can it explain the relationships between growth and height and temperature, but it also provides a relevant prediction on reproductive effort (i.e. total fruit length in our case). Second, SRS evolution theory predicts that populations in extreme environments evolve through a loss of phenotypic plasticity (Chapin et al., 1993), a phenomenon we observed for the highest populations, PIC and GAL. Such a loss of phenotypic plasticity might stem from the relationship between plasticity and growth rate in herbaceous plants (Lambers & Poorter, 1992) or from the costs associated with plasticity (DeWitt et al., 1998), or both. Note that SRS generally explains why lower phenotypic plasticity might be observed in environmentally marginal populations, despite inverse theoretical expectation (Chevin & Lande, 2011) and empirical findings (Lázaro-Nogal et al., 2015; Orizaola & Laurila, 2016). This would be the case when, as in our study and several others (Volis et al., 1998; Mägi et al., 2011; Grassein et al., 2014; Paccard et al., 2014), marginal populations are associated with chronic and predictable stressful conditions and source-sink-type gene flow is low enough.

Detection of candidate genes for local adaptation

We were able to isolate loci significantly linked to phenotypic traits displaying adaptive patterns (association studies) and to identify loci significantly associated with selective environmental variables (genome scan methods to detect selection). Combining these two analyses resulted in five genomic regions that were both associated with "adaptive" traits and selective environmental variables. Among these five regions, two were within genes, which had homologous counterparts in the genome of *A. thaliana*, but only one had a confirmed functional homology and was thus retained as a candidate. This gene (gene 26269) appears to be orthologous to SPS2F (AT5G11110) in *A. thaliana* which is involved in sucrose metabolism and its regulation. Park et al. (2007) showed that in *A. thaliana* its over-expression results in faster growth and increased stem height. This is in agreement with our results in *A. alpina* showing that this gene is involved in adaptive regulation of height and growth. Of course, the three intergenic regions should not be merely discarded as false positives. It is indeed possible for such intergenic polymorphism to be involved in adaptive evolution, as was shown in the collared flycatchter (*Ficedula albicollis*), where 44% of the conserved elements (hence seemingly under purifying selection) were intergenic (Craig et al., 2017). They are, however, more difficult to functionally assess using data from the literature, as was performed for the two genic re-

gions.

This low number of candidates may be related to the use of a genome representation technique instead of whole-genome sequencing. Also, we used very stringent criteria to identify the candidate genes. Many more candidate genes are likely to be discovered using a much more thorough sequencing method and a more powerful setting (e.g. with more individuals and populations). Nevertheless, we were able to identify a solid candidate that warrants further investigation (e.g. functional validation in *A. alpina*).

This candidate has not been previously found in molecular ecology studies of *Brassicacea*'s adaptation to elevation (Buehler et al., 2013; Fischer et al., 2013; Kubota et al., 2015; Günther et al., 2016). However, there is little, if any, overlap between the genes detected in these studies (Kubota et al., 2015) and even replicated studies on the same species over a wider area did not identify the same genes for the most part (Buehler et al., 2014; Rellstab et al., 2017). Adaptive studies are also strongly sensitive to the geographical scale considered (Brachi et al., 2013). The lack of reproducibility of those studies can be partially explained by the high false positive rates of genome scans for selection (Lotterhos & Whitlock, 2014; de Villemereuil et al., 2014; Hoban et al., 2016). In this study, we mitigated the issue of false positives in three ways: we used a method accounting for population structure (Frichot et al., 2013), we used genomic control by correcting p-values using a genomic inflation factor (Devlin & Roeder, 1999; François et al., 2016) and we selected loci which combined significant tests for both association with a selective environmental factor, a corresponding trait with significant adaptive pattern, and a known and consistent functional homology. However, false positives are not the only explanation for the lack of reproducibility in genome-scan studies. Another obvious issue is that different methods (e.g. F_{ST}-based, haplotype-based, environmental association) are sensitive to different signals of selection meaning that "genome scan results" might not be very easy to compare. Finally, the same selective pressure can lead to evolution of different response traits (divergent phenotypic evolution). Even in the case where the same trait evolved in different areas, most evolutionary relevant traits have most likely a highly polygenic structure (Rockman, 2012): this means that the same trait can evolve through the effect of many different genes (convergent phenotypic evolution with divergent molecular evolution).

Although previous study combined common garden and population genomics methods (Hancock et al., 2011; Fournier-Level et al., 2011; De Kort et al., 2014; Yoder et al., 2014), this study is more holistic in terms of combining signal from locally-measured *in situ* environmental data (in contrast to climate variable mapping from databases) of the population of origin, phenotypic measurement in common garden and use of molecular markers to infer relationships between selective factors, phenotypic traits and their underlying genetic basis (although the study of Fournier-Level et al., 2011, is similar in combining the significance of both phenotypic and environmental association, with a much higher marker density). Another difference from e.g. De Kort et al. (2014) is the availability of an annotated genome to analyse our candidates and genomic proximity to the model plant *A. thaliana* (though the studies of Hancock et al., 2011, and Fournier-Level et al., 2011, are on *A. thaliana* itself).

Adaptive potential in the face of climate change

Studies on a common plant such as *A. alpina* can inform us on the response of alpine plants to global change, especially as this species exhibits a considerable elevation amplitude. Our results show several characteristics of *A. alpina* that might be important in the context of climate change. First, populations along an elevation gradient exhibit greater between- than within-population genetic diversity, both for neutral and selected genes (as supported by the high F_{ST} estimates and the high Q_{ST} compared to h^2 estimates) and with no sign of a specific enrichment or exhaustion of genetic diversity at higher elevations. This suggests that genetic drift within population is sufficiently strong to erode the genetic diversity of complex traits, but weak enough so that populations at the margins do not suffer from a strong loss of genetic diversity. Second, populations appear to respond to differential selection linked directly to temperature, or indirectly to a stress caused by resource limitation. This suggests that climate factors are important drivers of *Arabis alpina*'s eco-evolutive system. Third, they are characterised by a high phenotypic plasticity. However, despite a noticeable ability of high elevation plants to survive and reproduce to some extent at lower elevation (i.e. higher temperature), they show lower phenotypic plasticity compared to the other populations. *A. alpina* is not presently threatened by climate change. However, these characteristics suggest that the high elevation populations might not be able to completely cope with a raise of temperature, because climate change will create environmental conditions in which such populations will be maladapted and might not develop an evolutionary response rapidly enough (Scheepens & Stöcklin, 2013). Rescue from lower elevation populations might be hindered by restricted gene flow and high selfing rate (Ansell et al., 2008; Tedder et al., 2015), further limiting local adaptation to the changing local environment, although pollination events may take place with low probability over considerable distances (> 1km, Buehler et al., 2012). Moreover, in situations with strong local adaptation, gene flow from other populations might first trigger outbreeding depression, before the onset of a noticeable adaptive response (Edmands, 2007; Aitken & Whitlock, 2013). Finally, *A. alpina* populations seem to be unstable and subject to bottlenecks or possibly extinctions over short-term periods (Andrello et al., 2016), making "rescue" due to pollination possibly too slow compared to the timespan of a population persistence. Seed bank size is however an unknown factor that might counteract this problematic factor of population in-

stability. As seed dispersal is autochorous, it is biased toward dispersal to lower elevation, making recolonisation of higher sites possibly slow. Combined together, these factors point to either a scenario of adaptive "rescue" of the higher elevation populations due to gene flow from pollination or, slightly more likely, an extinction of these populations followed by a (possibly slow) recolonisation from lower elevation populations due to seed dispersal. In any case, the result of such processes would be a loss of polymorphism at the level of the meta-population, rendering the species more susceptible to further changes.

Acknowledgements

We are indebted to Fabrice Roux who suggested to record the morphotype as a phenotypic trait, as well as to Jinliang Wang, Matthew Wolak, Markku Karhunen and Otso Ovaiskainen for their help during the analysis. We are also grateful to Delphine Rioux and Thibaut Capblancq who were of great help for the genotyping, and to Lucile Sage for identifying the white rust. We thank all the people who helped with the common garden experiment including Aymeric Pilleux, Pascal Salze, Amandine Deschamps, Quentin Rousseau, Perrine Augrit, Sofia Karabatsos, Charles Pouchon, Thomas Goeury, Mónica Cecilia Corona, Tristan Cumer, Florian Alberto, Loïc Chalmandrier, Alex Fajardo and Justine Bisson. We finally thank Anna Santure and five anonymous reviewers whose comments have helped greatly increase the quality of this paper. PdV was supported by a doctoral studentship from the French *Ministère de la Recherche et de l'Enseignement Supérieur*. OEG was supported by the Marine Alliance for Science and Technology for Scotland (MASTS). This research was partially conducted at the Maison du *Parc Naturel Régional du Vercors* and at the *Station Alpine J. Fourier*, a member of the AnaEE network (Infrastructure for Analysis and Experimentation on Ecosystems).

Data Accessibility

The data used in this article can be retrieved from the Dryad database: https://dx.doi.org/10.5061/dryad.9rd5f.

Authors' contributions

PdV, OEG and ITB conceived the experiment and analysis. PdV performed the common garden experiments, with help from ITB and MM (as well as many other volunteers). MM performed the genotyping, with help from PdV for the bioinformatics analysis. PdV performed the statistical analysis with help from OEG and led the writing of the article.

References

Aitken, S. N. & Whitlock, M. C. (2013). Assisted gene flow to facilitate local adaptation to climate change. Annual Review of Ecology, Evolution, and Systematics, 44, 367–388. DOI: 10.1146/annurev-ecolsys-110512-135747.

Aitken, S. N. et al. (2008). Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolutionary Applications, 1, 95–111. DOI: 10.1111/j.1752-4571.2007.00013.x.

Albani, M. C. et al. (2012). PEP1 of *Arabis alpina* is encoded by two overlapping genes that contribute to natural genetic variation in perennial flowering. PLoS Genet, 8, e1003130. DOI: 10.1371/journal.pgen.1003130.

Alberto, F. et al. (2013). Potential for evolutionary responses to climate change – evidence from tree populations. Global Change Biology, 19, 1645–1661. DOI: 10.1111/gcb.12181.

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. Genome Research, 19, 1655–1664. DOI: 10.1101/gr.094052.109.

Altschul, S. F. et al. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research, 25, 3389–3402. DOI: 10.1093/nar/25.17.3389.

Andrello, M. et al. (2016). Population dynamics of *Arabis alpina* in the French Alps: evidence for demographic compensation? bioRxiv, (en preparation), 070847. DOI: 10.1101/070847.

Ansell, S. W. et al. (2008). Genetic discontinuity, breeding-system change and population history of *Arabis alpina* in the italian peninsula and adjacent Alps. Molecular Ecology, 17, 2245–2257. DOI: 10.1111/j.1365-294X.2008.03739.x.

Ansell, S. W. et al. (2011). The importance of Anatolian mountains as the cradle of global diversity in *Arabis alpina*, a key arctic–alpine species. Annals of Botany, 108, 241–252. DOI: 10.1093/aob/mcr134.

Assefa, A. et al. (2007). Pleistocene colonization of afro-alpine 'sky islands' by the arctic-alpine *Arabis alpina*. Heredity, 99, 133–142. DOI: 10.1038/sj.hdy.6800974.

Baka, Z. (2008). Occurrence and ultrastructure of Albugo candida on a new host, *Arabis alpina* in Saudi Arabia. Micron, 39, 1138–1144. DOI: 10.1016/j.micron.2008.06.001.

Boucher, F. C. et al. (2012). Reconstructing the origins of high-alpine niches and cushion life form in the genus *Androsace s.l.* (*Primulaceae*). Evolution, 66, 1255–1268. DOI: 10.1111/j.1558-5646.2011.01483.x.

Brachi, B. et al. (2013). Investigation of the geographical scale of adaptive phenological variation and its underlying genetics in *Arabidopsis thaliana*. Molecular Ecology, 22, 4222–4240. DOI: 10.1111/mec.12396.

Bråthen, K. A., Gonzalez, V. T., & Yoccoz, N. G. (2017). Gatekeepers to the effects of climate warming? Niche construction restricts plant community changes along a temperature gradient. Perspectives in Plant Ecology, Evolution and Systematics, DOI: 10.1016/j.ppees.2017.06.005.

Buehler, D. et al. (2013). An outlier locus relevant in habitat-mediated selection in an alpine plant across independent regional replicates. Evolutionary Ecology, 27, 285–300. DOI: 10.1007/s10682-012-9597-8.

Buehler, D. et al. (2014). Validation of outlier loci through replication in independent data sets: a test on *Arabis alpina*. Ecology and Evolution, 4, 4296–4306. DOI: 10.1002/ece3.1300.

Buehler, D. et al. (2012). Contemporary gene flow and mating system of *Arabis alpina* in a Central European alpine landscape. Annals of Botany, 109, 1359–1367. DOI: 10.1093/aob/mcs066.

Catchen, J. M. et al. (2011). Stacks: Building and Genotyping Loci De Novo From Short-Read Sequences. G3: Genes, Genomes, Genetics, 1, 171–182. DOI: 10.1534/g3.111.000240.

Catchen, J. M. et al. (2013). Stacks: an analysis tool set for population genomics. Molecular Ecology, 22, 3124–3140. DOI: 10.1111/mec.12354.

Chapin, F. S., Autumn, K., & Pugnaire, F. (1993). Evolution of suites of traits in response to environmental stress. The American Naturalist, 142, S78–S92.

Charmantier, A. et al. (2008). Adaptive phenotypic plasticity in response to climate change in a wild bird population. Science, 320, 800–803. DOI: 10.1126/science.1157174.

Chevin, L.-M. & Lande, R. (2011). Adaptation to marginal habitats by evolution of increased phenotypic plasticity. Journal of Evolutionary Biology, 24, 1462–1476. DOI: 10.1111/j.1420-9101.2011.02279.x.

Choler, P., Michalet, R., & Callaway, R. M. (2001). Facilitation and competition on gradients in alpine plant communities. Ecology, 82, 3295–3308. DOI: 10.1890/0012-9658(2001)082[3295:FACOGI]2.0.CO;2.

Craig, R. J. et al. (2017). Natural selection beyond genes: Identification and analyses of evolutionarily conserved elements in the genome of the collared flycatcher (*Ficedula albicollis*). Molecular Ecology, DOI: 10.1111/mec.14462.

De Kort, H. et al. (2014). Landscape genomics and a common garden trial reveal adaptive differentiation to temperature across Europe in the tree species *Alnus glutinosa*. Molecular Ecology, 23, 4709–4721. DOI: 10.1111/mec.12813.

de Bello, F. et al. (2013). Hierarchical effects of environmental filters on the functional structure of plant communities: a case study in the French Alps. Ecography, 36, 393–402. DOI: 10.1111/j.1600-0587.2012.07438.x.

de Villemereuil, P., Gimenez, O., & Doligez, B. (2013). Comparing parent-offspring regression with frequentist and Bayesian animal models to estimate heritability in wild populations: a simulation study for Gaussian and binary traits. Methods in Ecology and Evolution, 4, 260–275. DOI: 10.1111/2041-210X.12011.

de Villemereuil, P. et al. (2012). Bayesian models for comparative analysis integrating phylogenetic uncertainty. BMC Evolutionary Biology, 12, 102. DOI: 10.1186/1471-2148-12-102.

de Villemereuil, P. et al. (2014). Genome scan methods against more complex models: when and how much should we trust them? Molecular Ecology, 23, 2006–2019. DOI: 10.1111/mec.12705.

de Villemereuil, P. et al. (2016). General methods for evolutionary quantitative genetic inference from generalised mixed models. Genetics, 204, 1281–1294. DOI: 10.1534/genetics.115.186536.

de Villemereuil, P. et al. (2018). Patterns of phenotypic plasticity and local adaptation in the wide elevation range of the alpine plant *Arabis alpina*. Journal of Ecology, DOI: 10.5061/dryad.9rd5f.

Delnevo, N. et al. (2017). Plastic and genetic responses to shifts in snowmelt time affects the reproductive phenology and growth of *Ranunculus acris*. Perspectives in Plant Ecology, Evolution and Systematics, DOI: 10.1016/j.ppees.2017.07.005.

Devlin, B. & Roeder, K. (1999). Genomic control for association studies. Biometrics, 55, 997–1004. DOI: 10.1111/j.0006-341X.1999.00997.x.

DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. Trends in Ecology & Evolution, 13, 77–81. DOI: 10.1016/S0169-5347(97)01274-3.

Dray, S. & Dufour, A.-B. (2007). The ade4 package: implementing the duality diagram for ecologists. Journal of statistical software, 22, 1–20.

Edmands, S. (2007). Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Molecular Ecology, 16, 463–475. DOI: 10.1111/j.1365-294X.2006.03148.x.

Excoffier, L., Hofer, T., & Foll, M. (2009). Detecting loci under selection in a hierarchically structured population. Heredity, 103, 285–298. DOI: 10.1038/hdy.2009.74.

Fischer, M. C. et al. (2013). Population genomic footprints of selection and associations with climate in natural populations of *Arabidopsis halleri* from the Alps. Molecular Ecology, 22, 5594–5607. DOI: 10.1111/mec.12521.

Foll, M. et al. (2014). Widespread signals of convergent adaptation to high altitude in Asia and America. The American Journal of Human Genetics, 95, 394–407. DOI: 10.1016/j.ajhg.2014.09.002.

Fournier-Level, A. et al. (2011). A map of local adaptation in *Arabidopsis thaliana*. Science, 334, 86–89. DOI: 10.1126/science.1209271.

François, O. et al. (2016). Controlling false discoveries in genome scans for selection. Molecular Ecology, 25, 454–469. DOI: 10.1111/mec.13513.

Frichot, É. et al. (2013). Testing for associations between loci and environmental gradients using latent factor mixed models. Molecular Biology and Evolution, 30, 1687–1699. DOI: 10.1093/molbev/mst063.

Frichot, É. et al. (2014). Fast and efficient estimation of individual ancestry coefficients. Genetics, 196, 973–983. DOI: 10.1534/genetics.113.160572.

Gaggiotti, O. E. & Foll, M. (2010). Quantifying population structure using the F-model. Molecular Ecology Resources, 10, 821–830. DOI: 10.1111/j.1755-0998.2010.02873.x.

Ghalambor, C. K. et al. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology, 21, 394–407. DOI: 10.1111/j.1365-2435.2007.01283.x.

Gonzalo-Turpin, H. & Hazard, L. (2009). Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. Journal of Ecology, 97, 742–751. DOI: 10.1111/j.1365-2745.2009.01509.x.

Grassein, F., Lavorel, S., & Till-Bottraud, I. (2014). The importance of biotic interactions and local adaptation for plant response to environmental changes: field evidence along an elevational gradient. Global Change Biology, 20, 1452–1460. DOI: 10.1111/gcb.12445.

Grassein, F., Till-Bottraud, I., & Lavorel, S. (2010). Plant resource-use strategies: the importance of phenotypic plasticity in response to a productivity gradient for two subalpine species. Annals of Botany, 106, 637–645. DOI: 10.1093/aob/mcq154.

Günther, T. et al. (2016). Genomic and phenotypic differentiation of *Arabidopsis thaliana* along altitudinal gradients in the North Italian Alps. Molecular Ecology, 25, 3574–3592. DOI: 10.1111/mec.13705.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. Journal of Statistical Software, 33, 1–22.

Hancock, A. M. et al. (2011). Adaptation to climate across the *Arabidopsis thaliana* genome. Science, 334, 83–86. DOI: 10.1126/science.1209244.

Hautier, Y. et al. (2009). Changes in reproductive investment with altitude in an alpine plant. Journal of Plant Ecology, 2, 125–134. DOI: 10.1093/jpe/rtp011.

Hedrick, P. W. (1986). Genetic polymorphism in heterogeneous environments: a decade later. Annual Review of Ecology and Systematics, 17, 535–566.

Hedrick, P. W., Ginevan, M. E., & Ewing, E. P. (1976). Genetic polymorphism in heterogeneous environments. Annual Review of Ecology and Systematics, 7, 1–32.

Heidelberger, P. & Welch, P. D. (1981). A spectral method for confidence interval generation and run length control in simulations. Communications of the ACM, 24, 233–245. DOI: 10.1145/358598.358630.

Hoban, S. et al. (2016). Finding the genomic basis of local adaptation: pitfalls, practical solutions, and future directions. The American Naturalist, 188, 379–397. DOI: 10.1086/688018.

Hong, Z. et al. (2003). A unified nomenclature for *Arabidopsis* dynamin-related large GTPases based on homology and possible functions. Plant Molecular Biology, 53, 261–265. DOI: 10.1023/B:PLAN.0000007000.29697.81.

Huelsenbeck, J. P. & Rannala, B. (2003). Detecting correlation between characters in a comparative analysis with uncertain phylogeny. Evolution, 57, 1237–1247.

Huelsenbeck, J. P., Rannala, B., & Masly, J. P. (2000). Accommodating phylogenetic uncertainty in evolutionary studies. Science, 288, 2349–2350. DOI: 10.1126/science.288.5475.2349.

Jones, O. R. & Wang, J. (2010). COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources, 10, 551–555. DOI: 10.1111/j.1755-0998.2009.02787.x.

Kammer, P. M., Schöb, C., & Choler, P. (2007). Increasing species richness on mountain summits: Upward migration due to anthropogenic climate change or recolonisation? Journal of Vegetation Science, 18, 301–306.

Kang, H. M. et al. (2008). Efficient control of population structure in model organism association mapping. Genetics, 178, 1709–1723. DOI: 10.1534/genetics.107.080101.

Karhunen, M. & Ovaskainen, O. (2012). Estimating population-level coancestry coefficients by an admixture F model. Genetics, 192, 609–617. DOI: 10.1534/genetics.112.140871.

Karhunen, M. et al. (2013). DRIFTSEL: an R package for detecting signals of natural selection in quantitative traits. Molecular Ecology Resources, 13, 746–754. DOI: 10.1111/1755-0998.12111.

Kawecki, T. J. & Ebert, D. (2004). Conceptual issues in local adaptation. Ecology Letters, 7, 1225–1241. DOI: 10.1111/j.1461-0248.2004.00684.x.

Koch, M. A. et al. (2006). Three times out of Asia Minor: the phylogeography of *Arabis alpina* L. (*Brassicaceae*). Molecular Ecology, 15, 825–839. DOI: 10.1111/j.1365-294X.2005.02848.x.

Kolaksazov, M. et al. (2013). Effect of chilling and freezing stresses on jasmonate content in *Arabis alpina*. Bulgarian Journal of Agricultural Science, 19, 15–17.

Kolaksazov, M. et al. (2014). Effect of frost stress on chlorophyll a fluorescence and modulated 820 nm reflection in *Arabis alpina* population from Rila mountain. Genetics and Plant Physiology, 1–2.

Körner, C. (2003). Alpine plant life: functional plant ecology of high mountain ecosystems. Berlin, Heidelberg (Germany): Springer, July 14, 2003.

– (2007). The use of 'altitude' in ecological research. Trends in Ecology & Evolution, 22, 569–574. DOI: 10.1016/j.tree.2007.09.006.

Kubota, S. et al. (2015). A genome scan for genes underlying microgeographic-scale local adaptation in a wild *Arabidopsis* species. PLOS Genet, 11, e1005361. DOI: 10.1371/journal.pgen.1005361.

Lambers, H. & Poorter, H. (1992). Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecological Research*. Ed. by M. B. Fitter & A. H. Vol. 23. Academic Press, 1992, pp. 187–261.

Lamesch, P. et al. (2012). The *Arabidopsis* Information Resource (TAIR): improved gene annotation and new tools. Nucleic Acids Research, 40, D1202–D1210. DOI: 10.1093/nar/gkr1090.

Langerhans, R. B. & DeWitt, T. J. (2002). Plasticity constrained: over-generalized induction cues cause mal-

adaptive phenotypes. Evolutionary Ecology Research, 4, 857–870.

Larkin, M. A. et al. (2007). Clustal W and Clustal X version 2.0. Bioinformatics, 23, 2947–2948. DOI: 10.1093/bioinformatics/btm404.

Lázaro-Nogal, A. et al. (2015). Environmental heterogeneity leads to higher plasticity in dry-edge populations of a semi-arid Chilean shrub: insights into climate change responses. Journal of Ecology, 103, 338–350. DOI: 10.1111/1365-2745.12372.

Leinonen, T. et al. (2013). QST–FST comparisons: evolutionary and ecological insights from genomic heterogeneity. Nature Reviews Genetics, 14, 179–190. DOI: 10.1038/nrg3395.

Li, H. & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics, 25, 1754–1760. DOI: 10.1093/bioinformatics/btp324.

Lobréaux, S., Manel, S., & Melodelima, C. (2014). Development of an *Arabis alpina* genomic contig sequence data set and application to single nucleotide polymorphisms discovery. Molecular Ecology Resources, 14, 411–418. DOI: 10.1111/1755-0998.12189.

Lotterhos, K. E. & Whitlock, M. C. (2014). Evaluation of demographic history and neutral parameterization on the performance of FST outlier tests. Molecular Ecology, 23, 2178–2192. DOI: 10.1111/mec.12725.

Lowry, D. B. et al. (2016). Breaking RAD: An evaluation of the utility of restriction site associated DNA sequencing for genome scans of adaptation. Molecular Ecology Resources, DOI: 10.1111/1755-0998.12596.

Mägi, M. et al. (2011). Limited phenotypic plasticity in range-edge populations: a comparison of co-occurring populations of two *Agrimonia* species with different geographical distributions. Plant Biology, 13, 177–184. DOI: 10.1111/j.1438-8677.2010.00342.x.

Malanson, G. P. & Fagre, D. B. (2013). Spatial contexts for temporal variability in alpine vegetation under ongoing climate change. Plant Ecology, 214, 1309–1319. DOI: 10.1007/s11258-013-0253-3.

Manel, S. et al. (2010). Common factors drive adaptive genetic variation at different spatial scales in *Arabis alpina*. Molecular Ecology, 19, 3824–3835. DOI: 10.1111/j.1365-294X.2010.04716.x.

Manzano-Piedras, E. et al. (2014). Deciphering the adjustment between environment and life history in annuals: lessons from a geographically-explicit approach in *Arabidopsis thaliana*. PLOS ONE, 9, e87836. DOI: 10.1371/journal.pone.0087836.

Münzbergová, Z. et al. (2017). Genetic differentiation and plasticity interact along temperature and precipitation gradients to determine plant performance under climate change. Journal of Ecology, 105, 1358–1373. DOI: 10.1111/1365-2745.12762.

O'Hara, R. B. & Merilä, J. (2005). Bias and precision in QST estimates: problems and some solutions. Genetics, 171, 1331–1339. DOI: 10.1534/genetics.105.044545.

Orizaola, G. & Laurila, A. (2016). Developmental plasticity increases at the northern range margin in a warm-dependent amphibian. Evolutionary Applications, 9, 471–478. DOI: 10.1111/eva.12349.

Ovaskainen, O. et al. (2011). A new method to uncover signatures of divergent and stabilizing selection in quantitative traits. Genetics, 189, 621–632. DOI: 10.1534/genetics.111.129387.

Paccard, A., Fruleux, A., & Willi, Y. (2014). Latitudinal trait variation and responses to drought in *Arabidopsis lyrata*. Oecologia, 175, 577–587. DOI: 10.1007/s00442-014-2932-8.

Park, J.-Y. et al. (2007). Over-expression of an *Arabidopsis* family A sucrose phosphate synthase (SPS) gene alters plant growth and fibre development. Transgenic Research, 17, 181–192. DOI: 10.1007/s11248-007-9090-2.

Pauli, H. et al. (2012). Recent plant diversity changes on Europe's mountain summits. Science, 336, 353–355. DOI: 10.1126/science.1219033.

Peterson, B. K. et al. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS ONE, 7, e37135. DOI: 10.1371/journal.pone.0037135.

Poncet, B. N. et al. (2010). Tracking genes of ecological relevance using a genome scan in two independent regional population samples of *Arabis alpina*. Molecular Ecology, 19, 2896–2907. DOI: 10.1111/j.1365-294X.2010.04696.x.

Rellstab, C. et al. (2017). Local adaptation (mostly) remains local: reassessing environmental associations of climate-related candidate SNPs in *Arabidopsis halleri*. Heredity, 118, 193–201. DOI: 10.1038/hdy.2016.82.

Roach, D. A. & Wulff, R. D. (1987). Maternal effects in plants. Annual Review of Ecology and Systematics, 18, 209–235.

Rockman, M. V. (2012). The QTN program and the alleles that matter for evolution: all that's gold does not glitter. Evolution, 66, 1–17. DOI: 10.1111/j.1558-5646.2011.01486.x.

Rousset, F. (2008). Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources, 8, 103–106. DOI: 10.1111/j.1471-8286.2007.01931.x.

Savolainen, O., Lascoux, M., & Merilä, J. (2013). Ecological genomics of local adaptation. Nature Reviews Genetics, 14, 807–820. DOI: 10.1038/nrg3522.

Scheepens, J. F. & Stöcklin, J. (2013). Flowering phenology and reproductive fitness along a mountain slope: maladaptive responses to transplantation to a warmer climate in *Campanula thyrsoides*. Oecologia, 171, 679–691. DOI: 10.1007/s00442-012-2582-7.

Scherrer, D. & Körner, C. (2010). Infra-red thermometry of alpine landscapes challenges climatic warming projections. Global Change Biology, 16, 2602–2613. DOI: 10.1111/j.1365-2486.2009.02122.x.

Spitze, K. (1993). Population structure in Daphnia obtusa: quantitative genetic and allozymic variation. Genetics, 135, 367–374.

Storey, J. D. & Tibshirani, R. (2003). Statistical significance for genomewide studies. Proceedings of the National Academy of Sciences, 100, 9440–9445. DOI: 10.1073/pnas.1530509100.

Stouffer, S. A. et al. (1949). The American soldier, Vol.1: adjustment during army life. Princeton University Press. Princeton, New Jersey (US), 1949.

Tedder, A. et al. (2015). Evolution of the selfing syndrome in *Arabis alpina* (*Brassicaceae*). PLoS ONE, 10, e0126618. DOI: 10.1371/journal.pone.0126618.

Theurillat, J.-P. & Guisan, A. (2001). Potential impact of climate change on vegetation in the European Alps: a review. Climatic Change, 50, 77–109. DOI: 10.1023/A:1010632015572.

Thompson, J. N. (1994). The coevolutionary process. University of Chicago Press, 1994. 398 pp.

Thompson, J. N. & Burdon, J. J. (1992). Gene-for-gene coevolution between plants and parasites. Nature, 360, 360121a0. DOI: 10.1038/360121a0.

Toräng, P. et al. (2015). Large-scale adaptive differentiation in the alpine perennial herb *Arabis alpina*. New Phytologist, 206, 459–470. DOI: 10.1111/nph.13176.

Van Buskirk, J. & Steiner, U. K. (2009). The fitness costs of developmental canalization and plasticity. Journal of Evolutionary Biology, 22, 852–860. DOI: 10.1111/j.1420-9101.2009.01685.x.

Vergeer, P. & Kunin, W. E. (2013). Adaptation at range margins: common garden trials and the performance of *Arabidopsis lyrata* across its northwestern European range. New Phytologist, 197, 989–1001. DOI: 10.1111/nph.12060.

Volis, S. et al. (1998). Phenotypic variation and stress resistance in core and peripheral populations of *Hordeum spontaneum*. Biodiversity & Conservation, 7, 799–813. DOI: 10.1023/A:1008844504010.

Wang, J. (2011). Unbiased relatedness estimation in structured populations. Genetics, 187, 887–901. DOI: 10.1534/genetics.110.124438.

Wang, J. (2012). Computationally efficient sibship and parentage assignment from multilocus marker data. Genetics, 191, 183–194. DOI: 10.1534/genetics.111.138149.

Wang, R. et al. (2009). PEP1 regulates perennial flowering in *Arabis alpina*. Nature, 459, 423–427. DOI: 10.1038/nature07988.

Wang, R. et al. (2011). Aa TFL1 confers an age-dependent response to vernalization in perennial *Arabis alpina*. The Plant Cell, 23, 1307–1321. DOI: 10.1105/tpc.111.083451.

Weir, B. S. & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. Evolution, 38, 1358–1370. DOI: 10.2307/2408641.

Whittaker, R. J. (1993). Plant population patterns in a glacier foreland succession: pioneer herbs and later-colonizing shrubs. Ecography, 16, 117–136. DOI: 10.1111/j.1600-0587.1993.tb00064.x.

Willing, E.-M. et al. (2015). Genome expansion of *Arabis alpina* linked with retrotransposition and reduced symmetric DNA methylation. Nature Plants, 1, 14023. DOI: 10.1038/nplants.2014.23.

Wingler, A. et al. (2012). Interactions between temperature and sugars in the regulation of leaf senescence in the perennial herb *Arabis alpina*. Journal of Integrative Plant Biology, 54, 595–605. DOI: 10.1111/j.1744-7909.2012.01145.x.

Wingler, A. et al. (2015). Adaptation to altitude affects the senescence response to chilling in the perennial plant *Arabis alpina*. Journal of Experimental Botany, 66, 355–367. DOI: 10.1093/jxb/eru426.

Yoder, J. B. et al. (2014). Genomic signature of adaptation to climate in *Medicago truncatula*. Genetics, 196, 1263–1275. DOI: 10.1534/genetics.113.159319.