

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

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Abstract

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.

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.

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*.

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

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 meta-population (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

35 adaptation, but its maintenance is assumed to be associated with costs (DeWitt et al., 1998; Van Buskirk & Steiner, 2009)
36 and it can sometimes be maladaptive (Langerhans & DeWitt, 2002; Ghalambor et al., 2007).

37 Because montane and alpine habitats are characterised by strong environmental differences over small geographic
38 distances (Körner, 2003), alpine species, especially sessile organisms such as plants, are likely to undergo local adaptation
39 or be highly plastic. Mountains harbour resource gradients along which environmental conditions harshen dramatically
40 as elevation increases (e.g. lower temperatures, shorter reproductive seasons, less fertile soils, Körner, 2003), although
41 many other factors modulate the effects of altitude (Körner, 2007). In the Alps, from 400m to 2000m, environmental
42 change is well depicted by the transition from deciduous to coniferous forest. Above 2000m, the gradient is even more
43 striking from the treeline to the alpine meadows and to the sparse high-alpine vegetation around 3000m. Elevation has
44 the strongest impact above treeline on the specific and functional diversity of plant communities, with a switch from
45 interspecific competition to facilitation (Choler et al., 2001), as well as on plant physiology and morphology, with, for
46 example, the extreme case of cushion plants in the alpine and nival zones (Körner, 2003; Boucher et al., 2012). As a
47 result, very different species are typical of these various environmental conditions. In this context, climate change is
48 generally thought to induce an upward migration of species (Theurillat & Guisan, 2001; Alberto et al., 2013) with the
49 potential impossibility for high altitude plants to move further up. Indeed, Pauli et al. (2012) found an upward shift
50 of European alpine species distribution (2.7 m elevation gain between 2001 and 2008) mostly driven by leading edge
51 expansions following an increase in temperatures and resulting in an increase in species richness in temperate regions.
52 However in the Mediterranean Alpine, the reduction of precipitations induces a rear-edge retraction and a reduction
53 of species richness. Moreover, trends are not uniform across continents, depend on the reference flora (Malanson &
54 Fagre, 2013) or could be due to a release of anthropogenic pressure (Kammer et al., 2007). Species might also be able to
55 survive (or subsist longer) *in situ* by taking advantage of the thermal microhabitat mosaic (Scherrer & Körner, 2010) or
56 through niche construction (Bråthen et al., 2017). Overall, local adaptation and phenotypic plasticity of alpine species
57 are key features for their persistence (Theurillat & Guisan, 2001; Grassein et al., 2010; Alberto et al., 2013; Münzbergová
58 et al., 2017; Delnevo et al., 2017), although phenotypic plasticity can be maladaptive as in *Campanula thyrsooides* where
59 warmer temperatures lead to earlier flowering, but also to a reduced seed set (Scheepens & Stöcklin, 2013).

60 Among the characteristic plants of the French Alps, *Arabis alpina* is remarkable for its wide altitudinal amplitude
61 ranging from 800m to 3100m of elevation (Poncet et al., 2010). This range extends from the bottom of the montane
62 zone to the top of the alpine zone, consisting of widely different habitat types, especially in terms of the resources
63 mentioned above. This begs the question of how *A. alpina* is able to grow, survive and reproduce along such a wide
64 altitudinal range, and especially to cope with the associated resource gradient and how it will respond to climate change.
65 As explained above, two main mechanisms might explain this: phenotypic plasticity and local adaptation. Of course,
66 these hypotheses are not mutually exclusive and both might contribute to the wide altitudinal range of this species.
67 *A. alpina* is thus an interesting system to study the relative importance of local adaptation and phenotypic plasticity in
68 alpine plants for coping with heterogeneous mountain environments. From a genomic perspective, *A. alpina* offers the

69 power of both a properly assembled genome (Willing et al., 2015) and good orthology with the model species *Arabidopsis*
70 *thaliana* (Lobréaux et al., 2014). Finally, its biology and ecology is starting to be well understood with a wealth of studies
71 focusing on this species, from phylogenetic and historical aspects (Koch et al., 2006; Assefa et al., 2007; Ansell et al., 2011),
72 to population genetics and ecology (Ansell et al., 2008; Manel et al., 2010; Poncet et al., 2010; Buehler et al., 2012; Buehler
73 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.,
74 2012) and resistance to frost (Wingler et al., 2012; Kolaksazov et al., 2013; Kolaksazov et al., 2014; Wingler et al., 2015).
75 To study local adaptation in *A. alpina*, we conducted a common garden experiment using six populations covering the
76 altitudinal range of the species in the French Alps. Because individuals from diverse origins are grown in the same
77 environment, common gardens allow to compare phenotypes of different populations without the confounding effect
78 of phenotypic plasticity (Kawecki & Ebert, 2004; Savolainen et al., 2013). In order to minimise the remaining possibility
79 of confounding genotype-by-environment interactions and to assess the extent of phenotypic plasticity, we performed
80 the experiment in two contrasted common gardens. To characterise the process of local adaptation (Savolainen et al.,
81 2013) in this species, we combined phenotypic, genotypic (genome representation genotyping) and *in situ* measures of
82 environmental data in a statistical model accounting for the effects of population structure and genetic drift developed
83 by Ovaskainen et al. (2011). Finally, we conducted genome scan analyses to detect selection, and association studies
84 to search for potential candidate genes associated with our patterns of local adaptation. Combining these genomic
85 analyses with information available on the closely related species *Arabidopsis thaliana* allowed us to detect a candidate
86 gene potentially involved in local adaptation.

87 **Material & Methods**

88 **Species and population**

89 **Species** *A. alpina* is a common arctic-alpine plant. It is a pioneer species (Whittaker, 1993) and a bad competitor, hence
90 it is most often found in open rocky habitats, mostly resource-poor and unstable. The plant is perennial but short-lived
91 (1.82 years on average, Andrello et al., 2016), with entomogamous pollination and autochorous seed dispersal. In the
92 French Alps where this study was conducted, it is also characterised by a high level of selfing (around 84% of selfed
93 offspring, Buehler et al., 2012) with a resulting measured F_{IS} of 0.533 (Ansell et al., 2008). As a consequence, gene flow
94 is limited in this area, with most pollen dispersal very close to the individuals, though long-distance dispersal has also
95 been observed (Buehler et al., 2012).

96 **Populations and sites** The six studied sites and corresponding populations (see Fig. S1 in Supplementary Informa-
97 tion) covered much of the natural altitudinal range of the species (900m to 3000m) and were localised in two different
98 massifs: three populations were from the Vercors massif (900m to 2000m) and three populations were located near the
99 Lautaret pass (2000m to 3000m). Sites characteristics are summarised in Table 1.

100 **Environmental data** Using the GPS location points, we estimated the elevation and aspect (North- or South-facing
101 slope) of each site. Environmental data was collected using temperature and humidity sensors (iButton[®] from Maxim
102 Integrated[™]). Data collection was performed every three hours in each quadrat of a demographic survey (Andrello et al.,
103 2016, 2-4 quadrats per site) taking place from 2008 to 2015. The sensors were placed approx. 20 cm above the ground
104 (around the canopy height of *A. alpina*) protected by a small wooden plate from direct exposure to the sun, limiting
105 temperature inflation by direct irradiation during the day. Using these semi-continuous data, we were able to define, for
106 each site and each year, the start of the growing season as the date where positive degree-days (i.e. reference 0°C) started
107 to accumulate (i.e. increase after a flat or decreasing trend during winter) and the end of the growing season as the date
108 where positive degree-days stopped accumulating. From the semi-continuous series, we summarised the environmental
109 conditions in each site with five variables (see Table 1):

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

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

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

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

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

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

119 **Common garden experiment**

120 **Plant collection** We collected maternal progenies from the six natural populations. To do so, during the summer of
121 2012, we put fine-mesh nets around maturing infructescences of 20 plants located in these populations. The plants were
122 chosen to be as close as possible to the demographic quadrats while being separated from each other by at least 1m. The
123 bags were collected when fruits were ripe during July and August 2012. During the spring of 2013, we germinated seeds
124 in the lab in germination plates (½ potting compost, ½ plain soil) for Vercors and Lautaret. We then transferred and
125 planted the two-week old seedlings in the two different gardens (during May 2013 at Vercors and July 2013 at Lautaret,
126 some plants were planted late at Vercors during July 2013 to compensate for a low germination rate and a high juvenile
127 mortality).

128 **Experimental setting** One garden was located near the Vercors Regional Natural Park House (5,58733°E, 45,12972°N,
129 elevation 996m) at the edge of a grove. The experimental garden was shady, with a fertile and moist clay soil. At this

130 location, mean annual temperature is 7.2°C with on average 141.8 freezing days a year. The second garden was located in
131 the experimental site of the Joseph Fourier Alpine Station at the Lautaret pass (6,40007°E, 45,03635°N, elevation 2100m).
132 The experimental garden was largely exposed to sunlight, with a stony and less fertile soil. To regulate soil moisture and
133 avoid severe drought, the garden was automatically irrigated every evening. At this location, mean annual temperature
134 is 3.1°C with on average 175.4 freezing days per year. We planted 4.1 offspring per family and 7.7 families per population
135 at Vercors, and 4.6 offspring per family and 9.2 families per population at Lautaret. The two experimental gardens were
136 composed of three and five blocks of 100 plants, respectively. To avoid border effects, we planted 54 *A. alpina* individuals
137 around the 100 monitored individuals within each block.

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

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

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

162 Genotyping

163 During July 2014, leaf samples were collected on 204 surviving individuals at Lautaret. Note that this involves a slight
164 bias in terms of the genotyped progenies, which is mitigated by the fact that the sampling of maternal plants is unbiased
165 by this. We extracted DNA from these samples using the Qiagen DNeasy Plant Mini kit with minor modifications (i.e.
166 cell lysis and protein digestion over night). We then used a double digest RAD sequencing protocol (Peterson et al.,
167 2012), with minor modifications (see Supplementary Information), using the *ecoRI* and *mspI* restriction enzymes. Frag-
168 ments between 150bp and 600bp were pair-end sequenced on 125bp using an Illumina HiSeq sequencer. Reads were
169 analysed using the pipeline Stacks (Catchen et al., 2011; Catchen et al., 2013). After the cleaning process using the `pro-`
170 `cess_radtags` function (`-c` and `-q` flags), we mapped reads on *A. alpina* reference genome (NCBI, GenBank, Acces-
171 sion JNGA00000000, Version 1, <http://www.ncbi.nlm.nih.gov/nuccore/JNGA00000000.1>, Willing
172 et al., 2015) using the Burrows-Wheeler aligner (bwa, Li & Durbin, 2009, using the `mem` function and default parame-
173 ters). Reads with an alignment score below 35 were excluded. Mapped reads were grouped into “stacks” corresponding
174 to a RAD-tag using the `pstacks` function (only reads with a read depth of 2 or above were allowed to be considered
175 a “stack”), then catalogued using `cstacks` and `sstacks`. The final RADseq output was created with the `popu-`
176 `lations` function while filtering for read depth above 5 for all individuals, missing rate below 30% and minor allelic
177 frequencies above 1%. Furthermore, individuals with high rates of missing values due to overall low read depth were re-
178 moved (42 individuals missing more than 2,000 RAD-tags). Individuals with aberrant clustering were also removed (i.e.
179 between-massif hybrids and individuals with no clustering signal, 10 individuals). We used these SNP data to perform
180 genome scans for selection and association studies. For neutral population structure inference, to avoid issues due to
181 strong linkage between SNP on the same reads, we used multiallelic sequence polymorphism of the 125bp RAD-tags,
182 which we hereafter refer to as “RAD haplotypes”. In the end, we retained 3,528 RAD-tags (14,714 SNPs) loci for 152
183 individuals, with on average 25.3 individuals per population, 3.1 individuals per family and 9.2 families per population.

184 Statistical analyses of quantitative genetic variation

185 **Population and family structures** We checked population structure using the unsupervised clustering algorithm
186 sNMF (Frichot et al., 2014), which infers the ancestry coefficients in a faster but similar fashion to ADMIXTURE (Alexan-
187 der et al., 2009). The software Genepop (Rousset, 2008) was used to infer the Weir & Cockerham (1984) F_{ST} and F_{IS} es-
188 timates. Nucleotide diversity and percentage of polymorphic sites were estimated using the `populations` function
189 of Stacks (Catchen et al., 2013). To study the ancestral additive genetic variance of the traits, we used the model of
190 Ovaskainen et al. (2011). Simply put, this model decomposes the ancestral additive genetic variance of the traits into
191 between- and within-population variances. It does so by decomposing the relatedness matrix **A** of all individuals into
192 a population-level relatedness **B** and a within-population relatedness **W**. The matrix **B** was estimated using the admix-
193 ture F-model implemented in the R package RAFM (Karhunen & Ovaskainen, 2012). We ran RAFM separately on each
194 massif dataset and combined the matrix estimates for both massifs into a composite matrix assuming a coancestry of 0

195 between massifs. We did so because of the particular hierarchical structure of our data, which the F-model has difficulty
196 to account for (Excoffier et al., 2009, but see Foll et al., 2014). The diagonal elements of the matrix yielded by RAFM
197 are linked to the level of drift experienced by the populations since the split from the hypothetical ancestral population,
198 and as such, are related to the population F_{ST} in the F model (Gaggiotti & Foll, 2010; Karhunen & Ovaskainen, 2012).
199 To construct the matrix \mathbf{W} , we inferred the sibship structure (paternal and maternal progenies) from molecular data,
200 separately for each population. To do so, we used the COLONY software (Jones & J. Wang, 2010; J. Wang, 2011; J. Wang,
201 2012), including mother identity (known from sampling), using the hybrid full- and pairwise-likelihood score (FPLS,
202 medium run length and high likelihood precision) and accounting for partial selfing and hermaphroditism.

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

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

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

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

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

225 where the random effects (b for between-population effect, f for family effect, i for $G \times E$ interaction and l for block

226 effect) were assumed to have the following multivariate Normal distributions:

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

227 where \mathbf{I} is the identity matrix. The most complete model for the “Subset analysis” can be written:

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

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

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

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

236 Using the “Subset analysis”, we computed Q_{ST} values as:

$$Q_{ST} = \frac{V_B}{V_B + 2V_W} \tag{5}$$

237 but did not test them against F_{ST} values. Since some of the traits were non-Gaussian, the heritabilities of the traits were
238 computed based on the framework of de Villemereuil et al. (2016). The component V_W is the within-population additive
239 genetic variance on the latent scale. We computed the phenotypic variance on the latent scale $V_{P,\text{lat}}$ as the sum of within-
240 population variances of non-experimental origins (i.e. V_W , V_M and V_R). To obtain the (narrow-sense) heritability on the
241 observed data scale, we transformed the latent phenotypic variance $V_{P,\text{lat}}$ to the observed data scale phenotypic variance
242 $V_{P,\text{data}}$ and compute the parameter Ψ relating the latent to the data scale additive genetic variance using the QGglmm

243 package (see de Villemereuil et al., 2016, for more details). We then computed the heritability on the observed data scale
244 (h^2) as:

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

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

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

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

249 All the analyses were conducted using the MCMCglmm R package (Hadfield, 2010). Significance of fixed effects
250 was tested using the pMCMC value yielded by MCMCglmm. Point estimates given in this paper are the mean for the
251 fixed effects and the median for variances and variance ratios. For all models, convergence was checked graphically and
252 using the Heidelberger and Welch's test (Heidelberger & Welch, 1981), and length of runs and thinning were set up so
253 as to obtain an effective size above 1000 for all parameters of the model. We used default priors for fixed effects and
254 extended parameters priors for variance components, with $V=1$, $\nu=1$ and $\alpha \cdot V=1000$ for all traits, except for
255 traits analysed using a threshold model for which used $V=1$, $\nu=1000$ and $\alpha \cdot V=1$ as per de Villemereuil et al.
256 (2013). Residual variance priors were set to $V=1$ and $\nu=0.02$, except threshold models where it was fixed to 1.

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

271 **Genome scans and association studies**

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

285 **Association studies** We performed an association study to link our genotypic markers to the detected traits with
286 adaptive patterns. Because relatedness-based mixed models such as EMMA (Kang et al., 2008) are poorly suited for
287 the study of strongly differentiated populations, we decided to use LFMM (Frichot et al., 2013), which can account for
288 population structure with strong drift-induced genetic differentiation between populations. As a consequence, our test
289 framework does not use the genotypes to predict the phenotypic traits, but the reverse. To be used as predictors, traits
290 were mean-centred and scaled to a variance of 1. Ordered categorical traits were transformed into integer values before
291 centring and scaling. The settings of the analysis and post-analysis were identical to the above, e.g. we used $K=6$ and
292 10 runs, controlled for genomic inflation and transformed the p -values into q -values. Again, we used a significance
293 threshold of 0.05 for the q -values and QQplots of the p -values to assess the false positive rate. Because it was not
294 possible to use phenotypic values from both years, we used data from 2014 in Lautaret for which more measurements
295 were available.

296 **Finding candidate genes** Loci that were found associated to one of the traits with adaptive patterns and with a se-
297 lective environmental factor were considered as candidates. Combining these different tests allows for a more stringent
298 false discovery control, but can be a very conservative approach (de Villemereuil et al., 2014). SNPs within a distance of
299 5000bp were regrouped into the same genomic region. When these loci were located within an annotated gene in the
300 *A. alpina* genome, we performed Blast queries (Altschul et al., 1997) against the *A. thaliana* protein database (Lamesch
301 et al., 2012). We considered only significant query hits as homologous when the maximum "bit score" was above 200
302 and the percentage of identity above 60%. If several genes validated these criteria, they were all shown. We only con-
303 sidered as candidate a gene with molecular homology with a gene with demonstrated effect on this kind of phenotype

304 in *A. thaliana*.

305 **Results**

306 **Analysis of *in situ* environmental variables**

307 The PCA on the *in situ* environmental variables separated the temperature amplitude variables (temperature amplitude
308 and freezing days) on the first axis (40.1% of explained variance), the temperature trend variables (average temperature
309 and length of season) on the second axis (31.3% of explained variance) and the average humidity on the third axis
310 (19.7% of explained variance). The first two axes could also be respectively related to aspect (correlation $\rho = 0.65$,
311 $p = 3.13 \cdot 10^{-10}$) and elevation (correlation $\rho = 0.89$, $p < 2 \cdot 10^{-16}$), though the first axis was also related to elevation
312 (correlation $\rho = 0.36$, $p = 0.0019$).

313 Discriminant analyses show that environmental conditions varied widely across sites (Fig. 1, left), but much less
314 so across years (Fig. 1, right). However permutation tests were significant ($p < 0.001$ for both analyses, with 1000
315 randomisations), indicating a non-random clustering according to both sites and years. The discriminant analysis on
316 the sites (Fig. 1, left) shows a greater environmental differentiation between the sites at high elevation near the Lautaret
317 pass (LAU, GAL, PIC), whereas sites from the lower Vercors massif (BRU, CHA, VIL) were more similar to each other,
318 with long growing seasons and high average temperature (Table 1). In relation with its high elevation and Southern
319 aspect, PIC was strongly characterised by a wide daily temperature range and a high number of freezing days. LAU and
320 GAL were characterised by both a narrow daily temperature range and a short growing season. BRU and LAU were
321 also characterised by a high humidity (Table 1).

322 **Neutral population structure**

323 We found a strong population clustering, with the most likely number of population being $K = 6$ (Fig. S4 in SI) and
324 little sign of gene flow between populations (Fig. S5 in SI) based on the cross-entropy criterion used in sNMF (Frichot
325 et al., 2014). The variance-covariance matrix \mathbf{B} estimated by RAFM (Karhunen & Ovaskainen, 2012) is well-aligned with
326 these results, with very little coancestry between populations (Fig. S6, off-diagonal elements ranging from 2.6×10^{-5} to
327 1.1×10^{-2} with an average of 2.5×10^{-3}).

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

333 Linkage disequilibrium between RAD haplotype markers was low overall (Fig. S8 in SI) with an average value of

334 0.0468. The marker density in our study was relatively high with 9.41 markers *per* Mbp (compared to a median of 4.08
335 in a recent meta-analysis, Lowry et al., 2016).

336 **Analysis of the common garden phenotypic traits**

337 **Full analysis** For the analysis using all the individuals (Full analysis), in both gardens, total fruit length (pMCMC =
338 0.0438), growth (pMCMC = 0.0217), reproductive (pMCMC = 0.03) and vegetative (pMCMC = 0.0288) height in-
339 creased significantly with average temperature at the site of origin, while morphotype (pMCMC = 0.00339) was less
340 compact with higher average temperature at the site of origin (Table 3). Area increased with season length at the site
341 of origin, but only so in the Vercors garden (pMCMC = 0.0431, Table 3).

342 Comparison of the intra-class correlation coefficients (ICC, Fig. 2, top panels) for the between-population variance
343 V_B with or without environmental effect showed that average temperature at the site of origin explained a large amount
344 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),
345 growth ($\frac{V_B}{V_T} = 0.014$ with the environment effect, $\frac{V_B}{V_T} = 0.12$ without), vegetative ($\frac{V_B}{V_T} = 0.0091$ with the environment
346 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.
347 The data thus depicts bigger, less compact plants that are growing faster and reproducing more with higher temperatures
348 and season length at the site of origin. Graphical representation of population average phenotypic values supports these
349 statistical trends (Fig. S9 in SI).

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

358 A strong phenotypic differentiation among populations was found for survival as indicated by the high ICC values
359 corresponding to V_B (0.46) and the high Q_{ST} (0.78, Fig. 3). However, survival was not linked to any of the environmental
360 variables tested. Despite a strong signal of local adaptation, the morphotype was one of the phenotypic traits with the
361 greatest proportion of variance explained by within-population genetic variance (Fig. 3, top-middle panel). The dry
362 biomass and biomass ratio variances were equally explained by the within- (ICC resp. 0.16 and 0.078) and by the
363 between-population (ICC resp. 0.12 and 0.10) genetic variance components resulting in a relatively small Q_{ST} value
364 (resp. 0.27 and 0.40, Fig. 3), but the uncertainty around this estimate is too large to conclusively suggest potential
365 balancing selection.

366 For all traits, maternal effects explained very little of the total variance (Fig. 3, top-right panel). Finally, the morphotype

367 heritability was high (0.54) and the only estimate with a lower bound of the 95% credible interval clearly distinct from
368 zero (0.067 against 1.6×10^{-11} for the second highest value), while the heritabilities of total fruit length (0.001), growth
369 (0.021), flowering time (0.0035) and reproductive (0.0029) and vegetative (0.0027) heights were extremely low. Despite
370 a small 95% credible interval lower bound, the heritability estimates of survival (0.093), area (0.18), dry biomass (0.1)
371 and biomass ratio (0.1) were mildly high.

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

375 **Phenotypic plasticity and garden-by-population interaction**

376 All traits showed signs of phenotypic plasticity either through a significant garden effect (total fruit length, survival,
377 basal, reproductive and vegetative height, area; Table 3) or through a notable garden-by-population interaction (flow-
378 ering time, dry biomass and, to a lesser extent, biomass ratio, Fig. 2). We could not estimate the phenotypic plasticity
379 of growth, because this trait was measured only at Lautaret. Two of the traits for which the garden effect was non-
380 significant (flowering time and dry biomass) were the ones displaying strong signal of garden-by-population interaction.
381 Hence, the absence of significant garden effect might be due to (or compensated by) the presence of large garden-by-
382 population effects (Fig. 2, bottom-right panel). Indeed, running a model without garden-by-population resulted in a
383 significant Garden effect for both (results not shown).

384 The Vercors garden was warmer, more humid and had a more fertile soil which resulted in plants that were 9.83 times
385 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,
386 $F_{1,522} = 406$, $p < 2.10^{-6}$, see also Fig. 4).

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

391 **Genome scans**

392 **Genome scans to detect selection** LFMM detected 142 SNPs (0.97% of the total) associated with average tempera-
393 ture at the site of origin and 63 associated with aspect at the site of origin. In total, 201 SNPs (1.4%) were significantly
394 associated with at least one environmental variable, without much overlap between temperature and aspect. The QQ-
395 plots (Fig. S11) show that the tests were too liberal. This was mitigated by using the GIF correction (GIF = 1.47 for
396 aspect, GIF = 2.24 for average temperature), but only to a limited extent. There was a slight enrichment of significant
397 SNPs in genic regions (3.4% for non-genic regions and 4.1% for genic regions, $\chi_1^2 = 4.4$, $p = 0.035$).

398 **Association studies** The association study identified between 0 and 79 SNPs (0.54%) significantly associated with the
399 phenotypic traits identified as involved in adaptation (Table 4). Despite GIF values being mostly below 1 (GIF = 1.05 for
400 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
401 QQplots (Fig. S12) show that the tests were enriched for large numbers of significant p -values. Particularly, the number
402 of significant SNPs associated with growth was the highest (79) compared to the other traits. This was most likely due to
403 the presence of four atypical individuals (with growth rates of 11.9, 13.1, 23.8 and 28.3, compared to an overall average
404 of 3.12) from the two lowest populations (BRU and CHA). In total, 106 SNPs (0.72%) were significantly associated with
405 at least one trait with adaptive pattern, among which 36 (0.24%) were located in 17 different genic regions (1.4% of the
406 genes associated to at least one SNP). There was no enrichment of significant SNPs in genic regions (0.77% for non genic
407 regions and 0.62% for genic regions, $\chi^2_1 = 0.95$, $p = 0.33$).

408 **Candidate genes** To minimise issues with the false positive rate, we combined the results from association studies
409 and genome scans, selecting only loci significant in both. This allowed us to draw up a small list of 5 genomic regions
410 which comprised 2 genes, both of which had significant homologues in the *A. thaliana* genome (and both consistent with
411 existing annotations in *A. alpina* genome). The first gene (gene 3899) was homologous to AT1G60500 (a.k.a. DRP4C),
412 which is a GTP binding related protein only expressed in egg cell, which function has yet to be established (Hong et al.,
413 2003). Blasting the second gene (gene 26269) returned multiple hits of genes from the Sucrose-Phosphate Synthase (SPS)
414 family. To investigate further, we aligned this gene with the four genes (SPS1F, SPS2F, SPS3F and SPS4F) of the family in
415 *A. thaliana* using the ClustalX aligner (Larkin et al., 2007). This analysis showed that gene 26269 clusters with the SPS2F
416 gene in *A. thaliana* (Fig. S13). This suggests an orthology between gene 26269 in *A. alpina* and SPS2F (AT5G11110, a.k.a.
417 SPS1) in *A. thaliana*. Our SNP was situated at position 4392 on gene 26269, which aligned on position 4136 on SPS2F. This
418 position is located within a small intronic region (103bp) and does not correspond to a described SNP in *A. thaliana*. Gene
419 26269 was associated with height in our analysis in *A. alpina* and an overexpression of the SPS genes (including SPS2F)
420 was shown to result in increased growth rate and higher stem height in *A. thaliana* (**park_over-expression_2007**).
421 It is thus the only gene satisfying all of our candidate criteria, including validated functional homology in *A. thaliana*.
422 The shift in allelic frequency of the SNP corresponding to this gene is very strong: going from 0 for the four coldest
423 populations to frequencies over 0.93 for the two warmest populations (Fig. S14). It is clearly distinct from a massif effect,
424 because the coldest population of the Vercors massif (VIL) has a population allelic frequency of zero.

425 Discussion

426 Patterns of local adaptation

427 The major genetic difference between our populations of *A. alpina* was that individuals from cold condition sites were
428 significantly smaller, more compact and had a lower reproductive effort (total annual fruit length) and slower growth
429 than individuals from milder conditions. These patterns are major components of the genetic differentiation between

430 populations, as the variance explained by the relationship with environmental conditions of the site of origin accounted
431 for much of the inter-population variance for all traits showing an adaptive pattern (except for total fruit length). Inter-
432 estingly, humidity never appeared as an explanatory factor for population differences, possibly because the variation
433 in humidity was relatively small between sites and air humidity is a limited proxy for the moisture condition of plants.
434 Basal height was associated with aspect (shorter basal height in populations from a Southern aspect site) at Lautaret
435 and plants originating from sites with long growing seasons had a larger area at Vercors.

436 A definitive proof of local adaptation would require the measurement of fitness in reciprocal transplant experiments
437 (Kawecki & Ebert, 2004). However, our results are strongly suggestive of a pattern of local adaptation, especially as
438 our study accounts for: (i) phenotypic plasticity by using a common garden approach, (ii) neutral evolution (i.e. mi-
439 gration and drift) by using genetic information and a model of neutral evolution and to some extent (iii) genotype-by-
440 environment (here in the form of a garden-by-population) interaction created by using two contrasted common gardens
441 (differing in altitude, soil characteristics and exposition). Adaptive maternal effects are another possible origin of the
442 detected signal. As we used field-collected seeds rather than seeds produced in a common environment, they could
443 explain the results along with a scenario of local adaptation (Roach & Wulff, 1987). Although this cannot be totally ex-
444 cluded, the fact that maternal effects variance was negligible for all traits is a strong indication that this might not be the
445 case. The scenario of local adaptation is rendered even more likely by the fact that the spatial environmental variability
446 among the sites is much greater than the temporal one, as shown by the discriminant analyses. This preponderance of
447 spatial over temporal variability is indeed a prerequisite for local adaptation (Kawecki & Ebert, 2004). Of course, the
448 small number of populations we studied limits the extent to which our results can be generalised to the whole *A. alpina*
449 species, especially since the definition of local adaptation is very sensitive to the geographical scale chosen (Brachi et al.,
450 2013).

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

460 Factors other than those studied here may help explain genetic divergence between our populations. For example, the
461 positive relationship between area and breeding/growing season length at the site of origin is consistent with the pattern
462 of smaller, more compact plants in cold sites because season length is strongly correlated with average temperature
463 ($\rho = 0.82$, $p < 0.048$). But the relationship between basal height and aspect at the site of origin indicates the presence

464 of another gradient of selection involving environmental variables decoupled from elevation. A large fraction of the
465 inter-population variance in reproductive effort also remains unexplained and the high Q_{ST} value for this trait suggests
466 that other selective factors might be involved. Finally, despite trends suggesting local adaptation in survival (e.g. large
467 Q_{ST} value), none of the environmental variables we tested had a significant effect. This might be because survival does
468 not vary monotonically with elevation: survival was very low in LAU (2000m above sea level, asl) and PIC (3000m asl)
469 but comparable to the other populations in GAL (2500m asl). Once again, these results can be related to findings on
470 *in situ* survival (Andrello et al., 2016), for which a very strong negative association with the average temperature was
471 found for all populations except LAU and PIC and are an indication that other environmental variables are important.

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

481 Instead of using the Q_{ST} - F_{ST} framework (Spitze, 1993; Leinonen et al., 2013), we used a rigorous model-based ap-
482 proach that explicitly incorporates the effects of genetic drift and migration under an island model (Ovaskainen et al.,
483 2011). This approach is better from the following angles. It accounts for population differences in drift and migration
484 rates (Ovaskainen et al., 2011) and should thus generally be a better fit for realistic scenarios of wild populations history
485 and demography. Especially, since this approach is based on between-population relatedness, we were able to include
486 the matrix of these relatedness in a mixed model framework to test for an association between the phenotypic values
487 measured in our gardens and environmental values at the site of origin while accounting for neutral evolution (e.g.
488 migration and drift). By using the additional information of *in situ* environment, this allowed us to detect significant
489 signatures of local adaptation despite a small number of populations and a very strong neutral genetic differentiation
490 between populations ($F_{ST} = 0.60$). Finally, accounting for the uncertainty of the Q_{ST} and F_{ST} estimates is relatively
491 challenging and often overlooked (O’Hara & Merilä, 2005). Here we used Bayesian posterior distributions to propagate
492 uncertainty in our estimation of ancestral between-population relatedness based on molecular markers to our model
493 for quantitative traits as suggested by Karhunen et al. (2013).

494 **Overall high phenotypic plasticity, but more limited at high elevation**

495 Overall, grouping together the Garden and G×E effects as *sensu lato* “phenotypic plasticity”, every studied trait showed
496 clear signs of plasticity. When compared to plants at Lautaret, plants in the warmer, more humid and more fertile garden

497 at Vercors were *c.* 10 times larger in area and produced *c.* 16 times more fruits. The Garden effect was significant for
498 all traits but three. We also found significant differences between years at Lautaret for five traits, among which four
499 morphological traits, so this is most certainly due to the expected year to year growth of the plants. We identified
500 a strong garden-by-population interaction effect for two traits for which the Garden effect was not significant. This
501 suggests that a strong interaction is masking a slight effect of the environment alone.

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

506 The two populations from the upper edge of the distribution range of the species (GAL and PIC, resp. 2500m and
507 3000m) showed a reduced phenotypic variability between gardens for some morphological traits and for reproductive
508 effort. For these two populations, this reduced response to the environment of the more favourable experimental garden
509 (lower elevation, Vercors) suggests that they harbour low phenotypic plasticity for some traits compared to the other
510 populations. Given that we found no relationship between elevation and the genetic characteristics of the populations
511 (Table 2), this lack of phenotypic plasticity cannot be explained by a lack of genetic diversity.

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

513 In this study, the average local temperature comes out as the main environmental factor driving the observed pattern,
514 though many other environmental factors of possible strong influence, such a soil fertility and stability, were unmea-
515 sured. Local temperature is not totally confounded with elevation as our warmest population is second to the one with
516 lowest elevation and our coldest population is second to the highest elevation one. This illustrates the importance of
517 accounting for local conditions rather than larger scale gradients such as elevation. In *A. alpina*, colder temperature
518 seems to select for smaller and more compact plants, with a slow growth and with a diminished production of fruits.
519 Such a trend has often been described for inter-specific variations along an altitudinal gradient. Short and compact
520 stature, for example, is known to help plants to decouple their temperature from atmospheric temperatures, hence help-
521 ing to keep photosynthetic activity sufficiently efficient, as is illustrated by cushion plants (Körner, 2003). Slow growth,
522 lower productivity and higher survival are also typical traits that allow alpine plant species to adapt to colder conditions
523 (Körner, 2003). Our results are well aligned with these ecological expectations, with the notable exception of survival.
524 Another possible interpretation that would better explain these results is that average temperature is a close proxy for
525 the resource gradient linked to both elevation and aspect. Colder sites (usually at high elevation and of Northern as-
526 pect) are also often associated with many characteristics other than low temperature (Körner, 2007): they have a shorter
527 growing season, lower partial CO₂ pressure and lower soil fertility (Körner, 2003). It has been suggested that genetic
528 variation for growth and overall height are pleiotropically linked to the stress response syndrome (SRS) to resource lim-
529 itation (Chapin et al., 1993), allowing for an evolutionary response of the whole syndrome at once. SRS are widespread

530 in alpine species; de Bello et al. (2013) showed that high elevation species tend to be smaller, with thicker leaves and
531 reduced reproductive effort. At the intra-specific scale, the evolution of SRS-like signals along an elevation gradient
532 has been discovered using common garden experiments, in at least two other alpine species: Gonzalo-Turpin & Hazard
533 (2009) showed that *Festuca eskia* had increased survival and lowered fertility along an elevation gradient and Hautier
534 et al. (2009) showed that *Poa alpina* at high elevation were smaller with a reduced reproductive output. However, our
535 findings on *A. alpina*, stand out by the amplitude of the elevation gradient and the maximal elevation involved, reaching
536 the alpine, rather than the sub-alpine stratum.

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

548 **Detection of candidate genes for local adaptation**

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

562 This low number of candidates may be related to the use of a genome representation technique instead of whole-

563 genome sequencing. Also, we used very stringent criteria to identify the candidate genes. Many more candidate genes
564 are likely to be discovered using a much more thorough sequencing method and a more powerful setting (e.g. with more
565 individuals and populations). Nevertheless, we were able to identify a solid candidate that warrants further investigation
566 (e.g. functional validation in *A. alpina*).

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

585 Although previous study combined common garden and population genomics methods (Hancock et al., 2011; Fournier-
586 Level et al., 2011; De Kort et al., 2014; Yoder et al., 2014), this study is more holistic in terms of combining signal from
587 locally-measured *in situ* environmental data (in contrast to climate variable mapping from databases) of the population
588 of origin, phenotypic measurement in common garden and use of molecular markers to infer relationships between
589 selective factors, phenotypic traits and their underlying genetic basis (although the study of Fournier-Level et al., 2011,
590 is similar in combining the significance of both phenotypic and environmental association, with a much higher marker
591 density). Another difference from e.g. De Kort et al. (2014) is the availability of an annotated genome to analyse our
592 candidates and genomic proximity to the model plant *A. thaliana* (though Hancock et al., 2011; Fournier-Level et al.,
593 2011, are on *A. thaliana* itself).

594 **Adaptive potential in the face of climate change**

595 Studies on a common plant such as *A. alpina* can inform us on the response of alpine plants to global change, especially as
596 this species exhibits a considerable elevation amplitude. Our results show several characteristics of *A. alpina* that might
597 be important in the context of climate change. First, populations along an elevation gradient exhibit greater between-
598 than within-population genetic diversity, both for neutral and selected genes (as supported by the high F_{ST} estimates
599 and the high Q_{ST} compared to h^2 estimates) and with no sign of a specific enrichment or exhaustion of genetic diversity
600 at higher elevations. This suggests that the amount of genetic drift within population is sufficiently high to erode the
601 genetic diversity of complex traits, but high enough so that populations at the margins do not suffer from a strong loss of
602 genetic diversity. Second, populations appear to respond to differential selection linked directly to temperature, or indi-
603 rectly to a stress caused by resource limitation. This suggests that climate factors are important drivers of *Arabis alpina*'s
604 eco-evolutionary system. Third, they are characterised by a high phenotypic plasticity. However, despite a noticeable abil-
605 ity of high elevation plants to survive and reproduce to some extent at lower elevation (i.e. higher temperature), they
606 show lower phenotypic plasticity compared to the other populations. *A. alpina* is not presently threatened by climate
607 change. However, these characteristics suggest that the high elevation populations might not be able to completely cope
608 with a raise of temperature, because climate change will create environmental conditions in which such populations will
609 be maladapted and might not develop an evolutionary response rapidly enough (Scheepens & Stöcklin, 2013). Rescue
610 from lower elevation populations might be hindered by restricted gene flow and high selfing rate (Ansell et al., 2008;
611 Tedder et al., 2015), further limiting local adaptation to the changing local environment, although pollination events
612 may take place with low probability over considerable distances (> 1km, Buehler et al., 2012). Moreover, in situations
613 with strong local adaptation, gene flow from other populations might first trigger outbreeding depression, before the
614 onset of a noticeable adaptive response (Edmands, 2007; Aitken & Whitlock, 2013). Finally, *A. alpina* populations seem
615 to be unstable and subject to bottlenecks or possibly extinctions over short-term periods (Andrello et al., 2016), making
616 “rescue” due to pollination possibly too slow compared to the timespan of a population persistence. Seed bank size is
617 however an unknown factor that might counteract this problematic factor of population instability. As seed dispersal
618 is autochorous, it is biased toward dispersal to lower elevation, making recolonisation of higher sites possibly slow.
619 Combined together, these factors point to either a scenario of adaptive “rescue” of the higher elevation populations due
620 to gene flow from pollination or, slightly more likely, an extinction of these populations followed by a (possibly slow)
621 recolonisation from lower elevation populations due to seed dispersal. In any case, the result of such processes would be
622 a loss of polymorphism at the level of the meta-population, rendering the species more susceptible to further changes.

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635 **Data Accessibility**

636 The data used in this article can be retrieved from the Dryad database: <http://dx.doi.org/10.5061/dryad.9rd5f>.
637

638 **Authors' contributions**

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

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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°C 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°C	7.3°C	102.6%	251.6	18.6	Chalky scree
CHA	45,07117	5,59267	Vercors	1480m	North	10°C	11.2°C	87%	238.3	31.8	Chalky scree, meadow
VIL	45,01809	5,57083	Vercors	1980m	South	8.2°C	11.7°C	96.6%	217.5	47	Chalky rocks & scree
LAU	45,02846	6,39034	Lautaret	2090m	North	8.2°C	6.1°C	100%	136.2	6.1	Schistose river
GAL	45,06049	6,40375	Lautaret	2590m	North	6.2°C	6.6°C	92.4%	120.7	16.3	Chalky scree
PIC	45,06385	6,38426	Lautaret	2930m	South	6.8°C	15.2°C	86.5%	158.2	62.1	Schistose scree

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	—	—	—	—	—	—	—

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

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

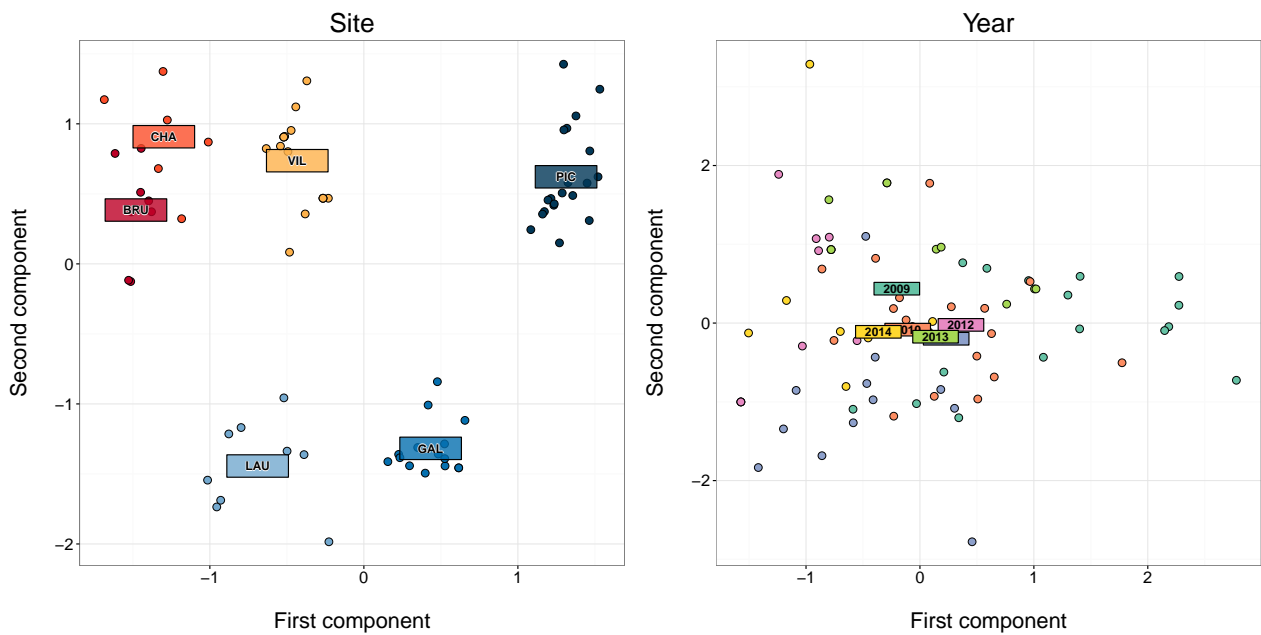


Figure 1: Projections of the environmental conditions at the site of origin after discriminant analysis according to the site (left) or the year of measurement (right) on the two first axes. Each point corresponds to one year in one quadrat within the site. For the left graph, BRU, CHA and VIL in red-yellow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

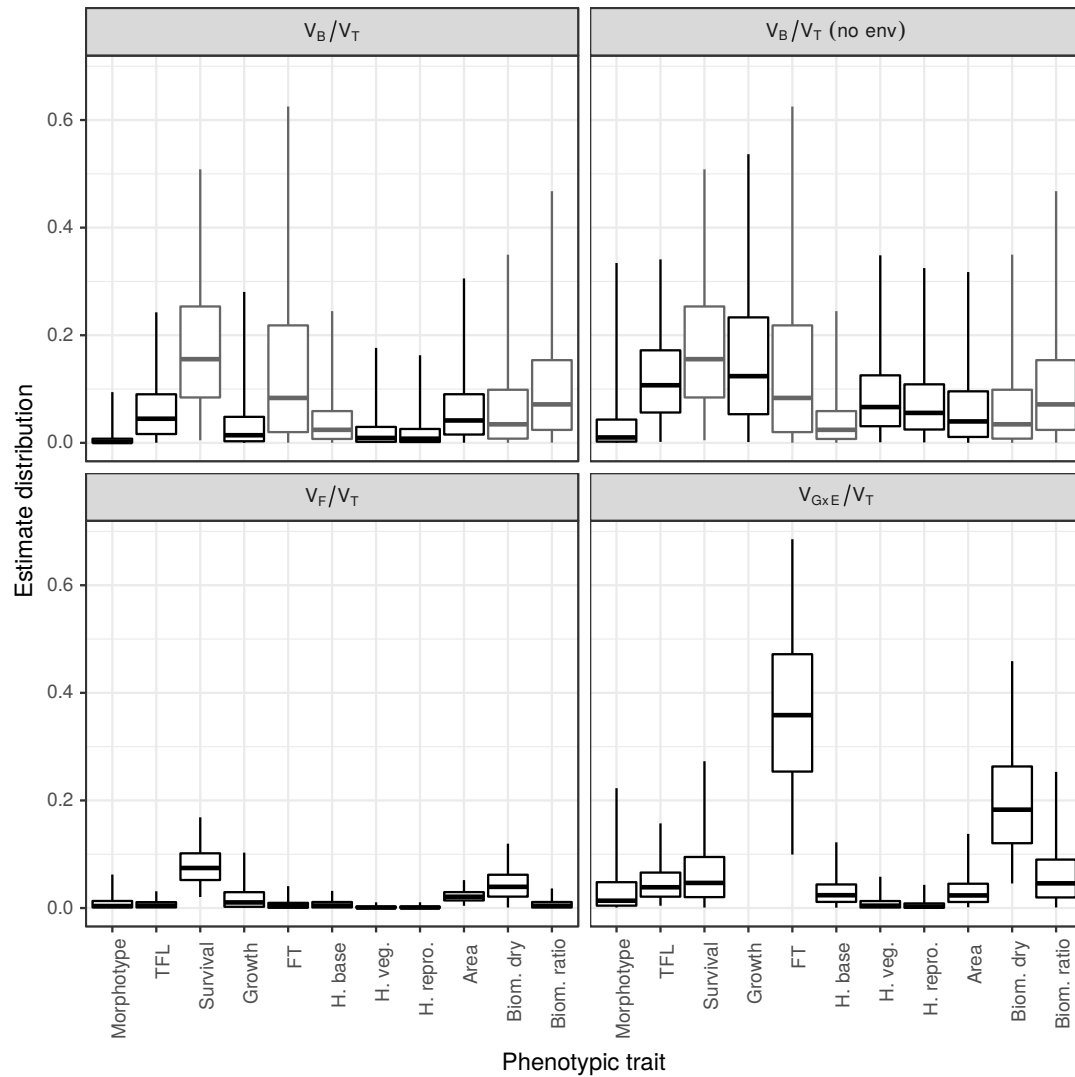


Figure 2: Results from the “Full analysis”: intraclass correlation coefficients (ICC, i.e. ratio of the effect variance to the total variance V_T) for the 11 phenotypic traits. ICCs corresponding to the between-population genetic variance V_B is shown with (top-left panel) or without (top-right panel) 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.

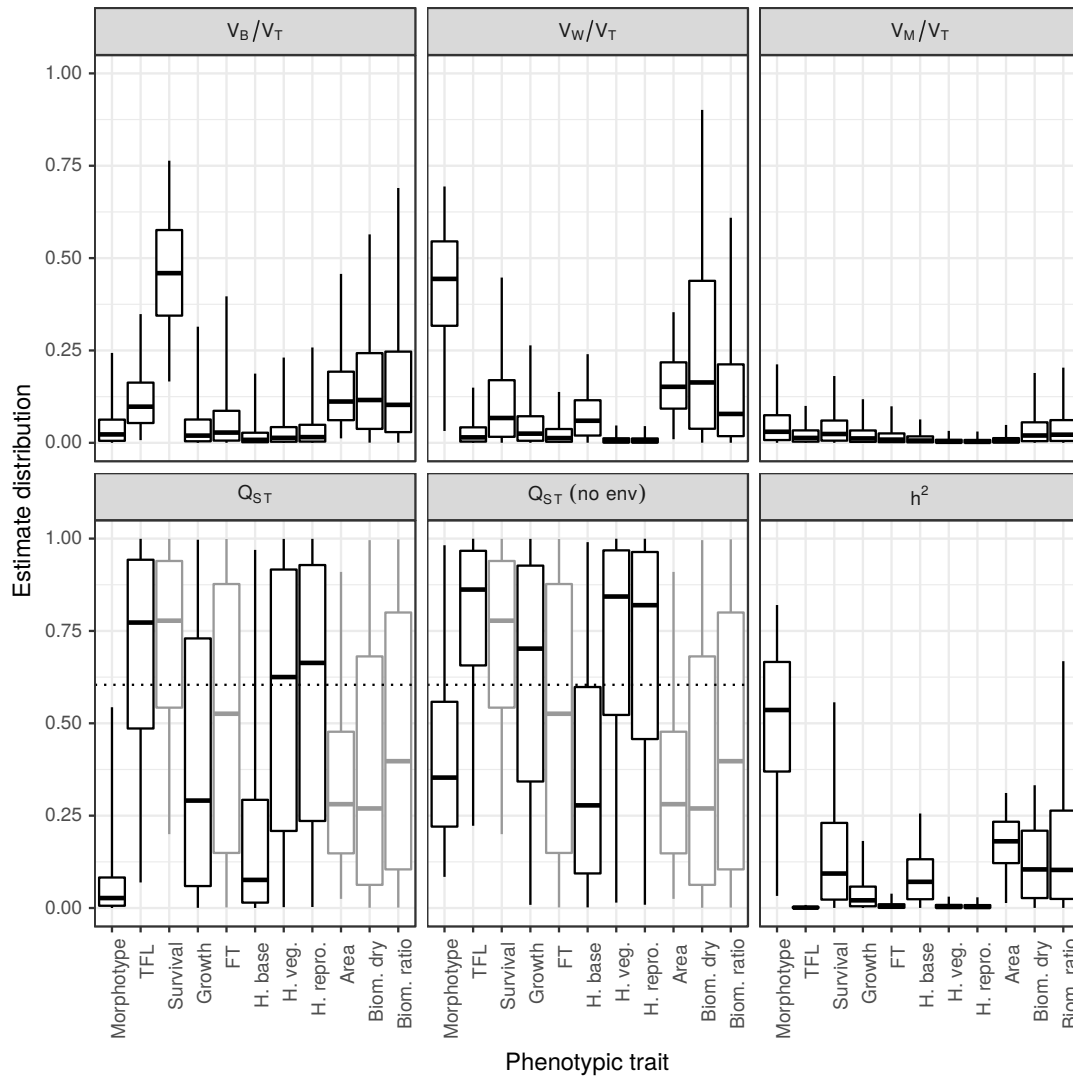


Figure 3: Results for the “Subset analysis”: Intraclass correlation coefficients (ICC, i.e. ratio of the effect variance to the total variance V_T), Q_{ST} and h^2 estimates for the 11 phenotypic traits. ICCs shown correspond to the between-population genetic variance V_B (top-left panel), the within-population 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-middle 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} .

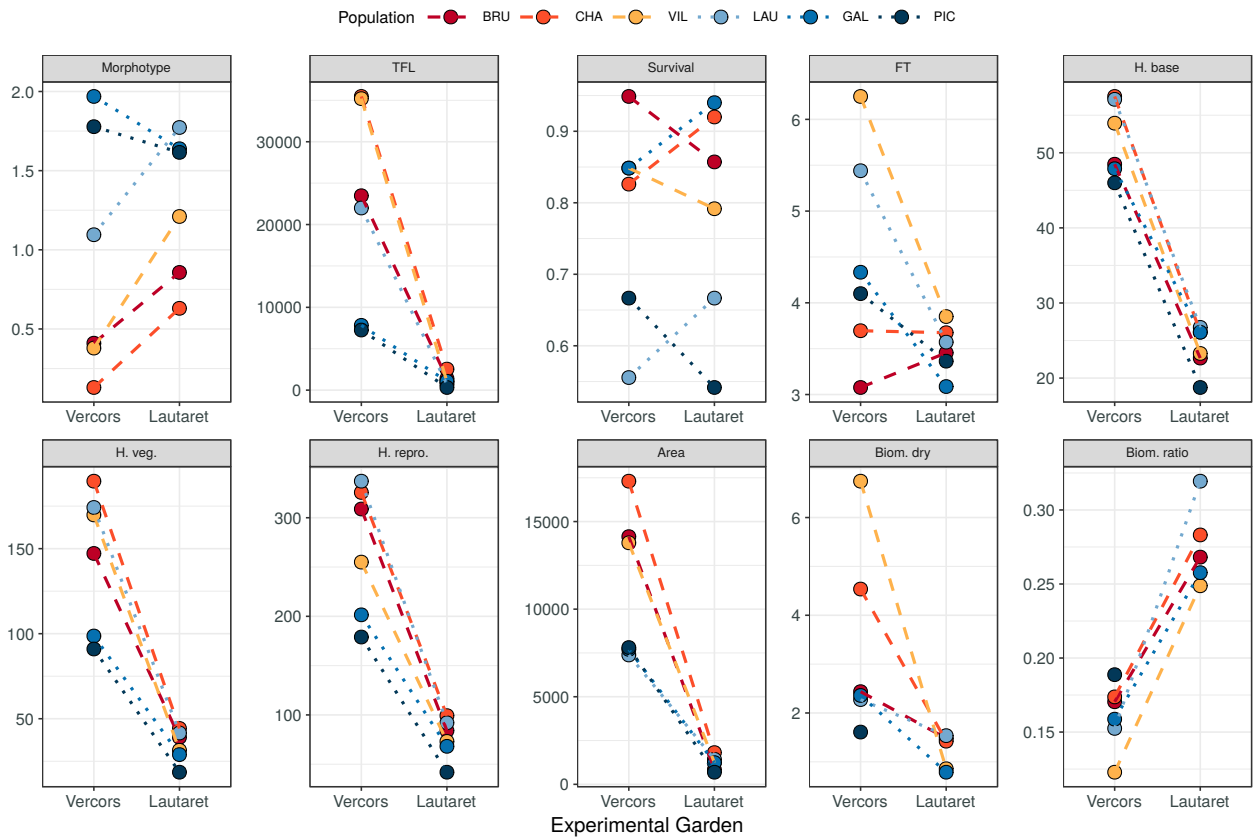


Figure 4: Reaction norms of 10 of the studied traits (Growth is excluded): mean phenotype of each population (different color dots and lines) in the two gardens (Vercors and Lautaret). Values for “numerous” and “compact” morphotypes have been merged to facilitate the comparison between gardens (“numerous” 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).