

1 **Title:** Trait divergence and trade-offs among Brassicaceae species differing in
2 elevational distribution

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14 **Title**

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16

17 **Abstract**

18 Species have restricted geographic distributions and the causes are still largely unknown.

19 Temperature has long been associated with distribution limits, suggesting that there are ubiquitous

20 constraints to the evolution of the climate niche. Here we investigated the traits involved in such

21 constraints by macroevolutionary comparisons involving around 100 Brassicaceae species differing

22 in elevational distribution. Plants were grown under three temperature treatments (regular frost,

23 mild, regular heat) and phenotyped for phenological, morphological and thermal resistance traits.

24 Trait values were analysed by assessing the effect of temperature and elevational distribution, by

25 comparing models of evolutionary trajectories, and by correlative approaches to identify trade-offs.

26 Analyses pointed to size, leaf morphology and growth under heat as among the most discriminating

27 traits between low- and high-elevation species, with high-elevation species growing faster under the

28 occurrence of regular heat bouts, at the cost of much reduced size. Mixed models and evolutionary

29 models supported adaptive divergence for these traits, and correlation analysis indicated their

30 involvement in moderate trade-offs. Finally, we found asymmetry in trait evolution, with

31 evolvability across traits being 50% less constrained under regular frost. Overall, results suggest

32 that trade-offs between traits under adaptive divergence contribute to the disparate distribution of

33 species along the elevational gradient.

34

35 **Key words**

36 heat and frost stress, macroevolution, Ornstein-Uhlenbeck, phylogenetic signal, range limits,

37 thermal niche

38

39

40 INTRODUCTION

41 Species have restricted geographic distributions, but the causes behind this phenomenon are still
42 unsolved (MacArthur 1972; Gaston 2003; Connallon and Sgrò 2018; Willi and Van Buskirk 2019).
43 From an ecological point of view, range limits reflect dispersal limitation or limits of the ecological
44 niche, with the niche being defined as the abiotic and biotic conditions that allow a species to persist
45 (i.e., the realized niche *sensu* Hutchinson 1957; Leibold 1995). From an evolutionary point of view,
46 range limits reflect limits to the evolution of the ecological niche. But why is it that species fail to
47 adapt to environmental conditions beyond their current range? MacArthur (1972) suggested that a
48 possible reason is exclusive divergent adaptation across habitats. He envisioned that specialization
49 to one environment imposes high demographic costs under colonization of a new environment, or in
50 other words, a trade-off. Trade-offs are a key concept in evolution, likely affecting all aspects of
51 ecological specialization (Rosenzweig 1995) and including species distribution limits, but they have
52 been rarely studied in this context.

53 Among the many ecological factors that may affect the persistence of organisms, climate is
54 known to be critical in controlling large-scale distribution (MacArthur 1972). Many past studies
55 noticed coincidences between geographic or elevational range limits and temperature isotherms
56 (Salisbury 1926; Iversen 1944; Dahl 1951; Root 1988). More recently, the field of species
57 distribution modelling confirmed the good agreement between range limits and climate variables
58 (e.g., Normand et al. 2009; Lee & Yaw et al. 2016). Further studies looked into phenotypic patterns
59 associated with the most limiting aspects of climate at range limits, particularly at the cold end of
60 distribution. Loehle (1998) suggested that the northern range limit of North American tree species
61 was determined by cold tolerance. Phenotypic data supported that species from higher latitudes
62 were usually more tolerant to the cold than those from lower latitudes (Addo-Bediako et al. 2000;
63 Hawkins et al. 2014; Wen et al. 2018; Sunday et al. 2019). Similarly, abiotic stress appeared to be
64 linked with the upper elevational range limit for some mountainous plant species, suggesting a
65 predominant role of negative temperatures (Vetaas 2002; Macek et al. 2009; Körner et al. 2016).

66 Also, the warm end of distribution may be strongly affected by climate even though the prevailing
67 hypothesis has emphasized the importance of negative species interactions (MacArthur, 1972;
68 Gaston 2003; Louthan et al. 2015). So far, no clear evidence exists that e.g., competition explains
69 the southern range limit of species on a global scale. Some studies supported the hypothesis (Loehle
70 1998; Pither 2003), while others did not (Cahill et al. 2014). Probably because of the general
71 dismissal of climate as a factor determining warm-end limits, few studies focused on how
72 organisms cope with heat in the context of species distribution limits (e.g., Sunday et al. 2012;
73 Kellermann et al. 2012;), particularly in plants (e.g., Kappen 1981; Vos and Willi 2015).

74 What are the sources of constraints in the evolution of the climate niche? According to
75 simple evolutionary principles, genetic variation and selection are needed for a response to selection
76 and adaptation (Falconer and Mackay 1996). Genetic constraints may involve low genetic variation
77 of traits under selection. However, microevolutionary studies have shown that there is commonly
78 ample genetic variation in single traits, and natural selection acting on populations is often strong
79 (Mousseau & Roff 1987, Houle 1992, Kingsolver & Diamond 2011). These findings suggest
80 generally rapid and ubiquitous adaptation through highly evolvable traits. Another type of genetic
81 constraint is trade-offs in fitness-relevant traits, often seen as an obstacle to adaptive evolution by
82 limiting the rate of adaptation (Futuyama and Moreno 1988; Bennett and Lenski 2007; Walker
83 2007). Negative genetic correlations among traits in regard to their fitness consequences appear
84 mainly due to two non-exclusive causes. The first is that both the environment and the genetics of
85 traits exert a limitation on trait values through differential allocation of limited amounts of resources
86 (Bell 1984; van Noordwijk and de Jong 1986). The second cause is purely genetic; pleiotropic
87 antagonism occurs when an allele increases the fitness via a first trait but reduces it via a second
88 (Rose 1983). If we translate this into a thermobiology context, it is reasonable to assert that thermal
89 extremes impose selection on some traits, resulting in a better thermal performance under one type
90 of extreme, paid at the price of a reduction in performance in a contrasting environment or a
91 contrasting aspect of the biology of the species. In ectothermic animals, relatively common trade-

92 offs involve thermal resistance on the one hand, and growth, starvation resistance, longevity or
93 reproduction on the other hand (Luckinbill 1998; Norry and Loeschcke 2002; Hoffmann et al. 2005;
94 Stoks and De Block 2011; Casanueva et al. 2012), or cold and heat tolerance (Norry et al. 2007).
95 Temperature can also mediate trade-offs between traits, e.g., between lifespan and reproduction
96 (Mockett and Sohal 2006), or longevity and body size (Norry and Loeschcke 2002), or it can
97 reverse the sign of a correlation (reviewed in Sgrò and Hoffmann 2004). In plants, trade-offs were
98 discovered between cold tolerance and frost resistance (e.g., *Raphanus raphanistrum*; Agrawal *et*
99 *al.* 2004), and between speed of development and frost tolerance (Koehler et al. 2012; Molina-
100 Montenegro et al. 2012; Bucher et al. 2019).

101 While micro-evolutionary studies can shed-light on trade-offs, those involving traits related
102 to the climate niche have not revealed any cohesive patterns (e.g., Williams et al. 2012; Kelly et al.
103 2013). However, in the last decades, the field of comparative phylogenetics has developed macro-
104 evolutionary models that allow the study of adaptive evolution of more than one trait while
105 accounting for the shared history among species (summarized in Garamszegi 2014). Based on
106 comparative models, the phylogenetic signal of traits can be estimated and interpreted in the context
107 of niche conservatism (Cooper et al. 2010). Furthermore, the contribution of different evolutionary
108 processes and constraints to respond to selection can be inferred (Butler and King 2004). Three
109 evolutionary processes are typically modelled. A first is *genetic drift*, by which inherited characters
110 slowly change in random direction and accumulate differences over time. The process is typically
111 modelled by Brownian motion (BM). A second process is *stabilizing selection*, a likely result of
112 dependencies among characters under opposing selection (Wagner and Schwenk 2000). It is
113 modelled by Ornstein-Uhlenbeck (OU1) diffusion, which constrains BM toward an optimal trait
114 value. Recent improvements allow variation in the direction of OU diffusion across lineages,
115 depicting the third process of *divergent selection* (OUM, Beaulieu et al. 2012). This approach has
116 been used in evolutionary studies linking traits with the climate niche, particularly on plants, and
117 they highlighted a link between life-form or growth strategy, and adaptation (or exposure) to a cold

118 environment (Boucher et al. 2012; Kostikova et al. 2013; Tonnabel et al. 2018). Examples
119 emphasize the great potential the approach has in detecting traits of adaptation to climate and
120 revealing potential trade-offs in such adaptation or indicating general evolutionary constraints.

121 Here we studied trait divergence associated with the predominant elevational distribution of
122 plant species and analysed trait data for patterns of trade-offs in a macroevolutionary context. The
123 study of elevational gradients is promising in the context for at least two reasons. On the one hand,
124 elevation provides a steep climatic gradient in most mountainous regions, where over a short
125 geographic distance a reduction of the mean temperature of 0.5 K every 100 m of elevation is found
126 rather consistently (Körner 2003). On the other hand, species often occupy narrow elevational
127 ranges (Körner 2003), making elevational gradients unique systems for studying adaptation to
128 thermal stress and constraints in such evolution. Our study involved 100 Brassicaceae species
129 occurring in the central Alps of Europe, with median elevational occurrence varying from 400 to
130 2800 m a.s.l. Seeds of the species were raised in climate chambers under three different temperature
131 regimes (regular frost, mild, regular heat), and over a dozen traits depicting growth, leaf
132 morphology and coping with thermal extremes were measured. Four main hypotheses were tested.
133 (i) Species differ in trait expression depending on their elevational distribution. (ii) Traits differ in
134 the signature of past evolutionary processes having acted on them. (iii) Phylogenetic conservatism
135 in traits depends on the growth (thermal) environment. And (iv) there are trade-offs among traits
136 associated with adaptation to elevation.

137

138 **MATERIAL AND METHODS**

139 **Plant species**

140 One hundred taxa (i.e., species and subspecies) belonging to the Brassicaceae family and naturally
141 occurring in the Swiss Alps (and Jura) from the colline to the alpine life zone were selected. Apart
142 from a good representation of the elevational gradient, other criteria were level of ploidy (diploid
143 taxa preferred) and good representation of the phylogeny (list in Supplementary material A1). In the

144 general area, around 180 species of Brassicaceae occur, of which 28 are strictly high-elevation
145 species. On a global scale, Brassicaceae is an angiosperm family composed of 3'700 species
146 (including important agricultural cultivars) subdivided into three main lineages (Al-Shehbaz et al.
147 2006).

148 For this study, seeds were collected from March to September during the years 2015-2017 at
149 two different sites for each species within Switzerland. The sites were around the most common
150 elevation for each species, at least 50 km apart from each other and preferentially from different
151 biogeographic regions (Jura, Plateau, northern Prealps, western and eastern Central Alps and
152 southern Prealps). For plants with very restricted distributions, only one population was sampled,
153 but the number of individuals was doubled. At each site, seeds were collected from 10 to 30
154 different mother plants over an area of usually 50 m² and spaced out from each other by 5 m. For
155 endangered species on the Red List 2002 for Switzerland (Moser et al. 2002), authorization for
156 sampling was obtained from the respective Cantonal authority. Sampled seeds of each mother plant
157 were stored in separate paper bags (80 g m⁻², 60 × 90 / 12 mm, ELCO AG, Brugg, Switzerland)
158 under cold (4 °C), dark and dry (added silica gel) conditions until sowing.

159

160 **Raising of plants under three growth treatments and trait assessment**

161 *Design* - The experimental design involved the raising of 100 taxa, each represented by 2
162 populations and 3 maternal lines per population, i.e. 6 maternal lines per species. The experiment
163 was split into 6 blocks, with a different maternal line per species in a block. Within block, plants of
164 a maternal line were exposed to 3 temperature treatments (regular frost, mild, regular heat). The
165 final design resulted in 1'800 individuals (100 taxa × 6 maternal lines each in a different block × 3
166 treatments = 1'800 individuals). Maternal lines of a population were selected randomly, and seeds
167 of a maternal line were selected haphazardly. A first round of sowing (S1) was done without the use
168 of gibberellic acid (GA₃), resulting in some species (20) not germinating and some heterogeneity in
169 the timing of germination. In a second round of sowing (S2), seeds were treated with gibberellic acid

170 (GA₃), resulting in the germination of 14 additional species (but 5 were now lacking that
171 germinated in S1) and a more similar timing of germination.

172 *Plant rearing* - Seeds were germinated in climate chambers under controlled conditions,
173 with similar procedures in S1 and in S2 (S2 described in detail below). Two seeds were placed in a
174 1.5 ml eppendorf tube filled with 500 µl of GA₃ solution (500 ppm, Merck KGeA, Dornstadt,
175 Germany), with 3 tubes per maternal line. Seeds were incubated for 1 week in dark and cold (4 °C
176 constant in Climecabs; Kälte 3000, Landquart, Switzerland) and then sown in multipot-trays (0.06
177 L, 54 pots per tray with Ø 4.4 cm each, BK Qualipot; gvz-rossat.ch, Otelfingen, Switzerland). Each
178 pot had been filled with a mixture of soil (bark compost, peat and perlite, Aussaat- und Pikiererde;
179 Oekohum, Herrenhof, Switzerland) and sand (0-4mm) in a ratio of 2:1. Multi-pot trays were
180 covered with a garden fleece (Windhager, Hünenberg, Switzerland) and set up in blocks within
181 growth chambers (MobyLux GroBanks; CLF Plant Climatics, Wertingen, Germany). Growth
182 chambers were located inside a PlantMaster (CLF Plant Climatics) with managed humidity and
183 temperature. Trays were kept at 18 °C during daytime (8 h) and 15 °C during nighttime (16 h), at
184 75% relative humidity (RH), and a light intensity of 150 µmol m⁻² s⁻¹ (fluorescent white lamps and
185 red-LED). Twice a week, blocks were moved to a different chamber, with re-randomized
186 positioning of trays. After 3 weeks, excess seedlings were used to fill pots with no germination with
187 the following priority: use of the same maternal line within block, or the same population, or the
188 same species. In week 4, germinated plants were moved back to climate chambers and entire trays
189 were subjected to one of three temperature treatments.

190 *Treatment* - The three temperature treatments were: "frost" (F), "mild/control" (M) and
191 "heat" (H). Conditions of the treatments were the following: frost: 20 °C (daytime), then -2 °C for 1
192 h (-4.8 K h⁻¹; nighttime) and back to 20 °C (+7.3 K h⁻¹; night); mild/control: 20 °C constant; and
193 heat: 20 °C (beginning of day), then 40 °C for 1 h (+5 K h⁻¹; day), back to 20 °C (-8.3 K h⁻¹; day),
194 20 °C (night). All treatments were conducted at cycles of 12:12 h light:dark and a light intensity of
195 about 300 µmol m⁻² s⁻¹ (LED white lamp) and 75% RH. Plants were acclimated two days before the

196 beginning of treatment by exposing them to milder extremes, 2 °C for the frost treatment, and 35 °C
197 for the heat treatment. We selected extreme temperatures based on records in the field during the
198 vegetative period (Larcher and Wagner 1976; Sutinen et al. 2001; Körner 2003), while for the mild
199 treatment we used a common standard temperature. Trays were randomized daily within each
200 block, while blocks were moved to different climate chambers twice a week. Plants were kept
201 under these conditions until the 9th week after sowing, when trait assessments were performed.
202 Mean species numbers across blocks that were assessed for a particular trait within the treatments
203 ranged from 82.1 ± 3.6 (heat) to 85.5 ± 3.5 (mild) in S2 (N = 1406 plants), and from 52.1 ± 24.0
204 (heat) to 74.6 ± 1.1 (mild) in S1 (N = 862 plants).

205 *Traits* - Two traits were assessed before treatment started: seed size (SSIZ, in mm²) and days
206 to germination (TGER). Five traits depicted the trajectory of plant growth based on leaf length: the
207 initial growth rate (IGR, in mm day⁻¹), parameters of a 3-parameter logistic model including the
208 maximal growth rate (MGR, scale⁻¹), the mid-point until final size was reached (XMID, in days)
209 and asymptotic size (ASYM, in mm), and finally the number of plants on day 35 of treatment
210 (NLEA). Since smaller values of XMID meant that a plant achieved mid-size faster, values were
211 multiplied by -1 ([-]XMID) to represent speed of growth. Five leaf functional traits were assessed:
212 leaf area (LA, in mm²), specific leaf area (SLA, area over dry weight in mm² mg⁻¹), leaf dry matter
213 content (LDMC, ratio of dry weight over fresh weight in mg g⁻¹), leaf dissection index (LDI, no
214 unit), and leaf thickness (LTh, in mm). Resistance of leaves to thermal extremes was assessed under
215 -10 °C (*minus*T2) and -5 °C (*minus*T1), and +45 °C (*plus*T1) and +50 °C (*plus*T2). Resistance to T1
216 was tested only on non-acclimated plants (i.e., plants of the mild growth treatment), while T2 was
217 tested on non-acclimated and acclimated plants (i.e., plants pre-exposed to frost for assessing frost
218 resistance, and plants pre-exposed to heat for assessing heat resistance). Tolerance to repeated frost
219 or heat during the growth phase was calculated as MGR, -XMID or ASYM under frost or heat
220 treatment minus the respective estimate in the mild treatment, divided by the estimate in the mild
221 treatment. We used the term frost/heat tolerance *sensu lato* (*s.l.*) to refer to tolerance and resistance

222 together. Full details are given in Supplementary material A2. For analyses, means of replicate trait
223 measures per plant were calculated, on which species means per treatment and sowing round and
224 finally species means per treatment across sowing rounds were calculated.

225

226 **Statistical analysis**

227 *Trait expression differing with temperature treatment during growth and elevational distribution -*

228 The effect of temperature treatment, median elevation of species distribution, and their interaction
229 on traits was tested using generalised linear mixed models based on Markov Chain Monte Carlo
230 techniques with the ‘brm’ function of the R package {brms} (Bürkner 2017). The fixed effect of
231 treatment was coded as a categorical variable, and contrasts were performed against the “mild”
232 treatment or, for tolerance, against “frost”. The fixed effect of median elevation of species
233 distribution was calculated based on reported species occurrences of a nation-wide species
234 inventory (infoflora.ch). Median elevation was mean-centred prior to analyses. Random effects
235 were the round of sowing (i.e., S1 and S2) and the relatedness among species. A phylogeny
236 produced based on several dozen chloroplast genes (Patsiou et al. 2021) was pruned to species
237 included in this study with the function ‘treedata’ of package {geiger} (Harmon et al. 2008). The
238 final matrix was obtained with the function ‘vcv’ {ape} (Paradis and Schliep 2018) and called with
239 the ‘cov_ranef’ argument in brm. For each model, the contribution of the phylogenetic effect was
240 tested by comparing the model that included it as a random effect to one that did not. Model
241 comparisons were performed using the leave-one-out cross validation (i.e., LOO), which was
242 calculated with the ‘add_criterion’ {brms} function combined with the expected log pointwise
243 predictive density (i.e., ELPD) with the ‘loo_compare’ {brms} function. Resistance traits were
244 modelled by a beta distribution because of their constrained nature between 0 and 1 (i.e., 100%), -
245 XMID and tolerances by a gaussian distribution, and the remaining traits by a log-normal
246 distribution because values could only be positive. Sampling behaviour of MCMC was inspected
247 visually, and number of iterations, warmup and sampling interval adapted to each model to retain an

248 effective sampling size of 1'000. Significance was tested by probability of direction calculated with
249 the 'p_direction' function in {bayestestR} (Makowski et al. 2019). All analyses and figures were
250 done with the statistics software R v. 4.0.3 (R Core Team 2014), and calculations were performed at
251 sciCORE (<http://scicore.unibas.ch/>) scientific computing center of the University of Basel.

252 *Past evolutionary forces* - Further analyses were performed on species trait means averaged
253 across rounds of sowing. Phylogenetic analyses on the evolutionary processes that had shaped trait
254 divergence among species were run separately for the three temperature treatments, and by
255 considering variance in trait means of species of the two rounds of sowing. We tested five
256 evolutionary models using the R package {geiger} and {mvMORPH} (Clavel et al. 2015): White
257 noise (WN) with trait evolution independent of phylogeny, BM, BMM with different speeds of the
258 different regimes, OU1 and OUM. For BMM and OUM, the contrasting environmental regimes
259 were low- vs. high-elevation distribution of species. Assignment to one of the two classes was made
260 using the InfoFlora (infoflora.ch) distribution information, with a threshold at 1500 m a.s.l.
261 (splitting species of the foothills/hills from those of sub-/alpine areas). For less frequent species on
262 Swiss territory, the assignment was verified by data on the entire Alps and neighbouring mountain
263 massifs (based on Aeschimann et al 2004). Ancestral state reconstruction and model comparison are
264 described in Supplementary material A2. Validation of the results was performed by simulations on
265 synthetic data and analyses after the random removal of species (A2).

266 Phylogenetic half-life, i.e. the time required for a trait to evolve halfway toward its adaptive
267 optimum, was calculated for all traits assessed in the three growth environments and in each
268 simulation described above. Values were extracted from an OU1 model, except when elevation had
269 a significant effect – either in mixed models or evolutionary analysis; in those cases, values were
270 derived from an OUM process. Small values of half-life indicate fast adaptation toward the optima
271 and a lack of phylogenetic inertia, while high values indicate that traits retain the influence of the
272 ancestral states for a longer time. We tested for an effect of growth environment (a factor with 3
273 levels, with 'mild' as baseline) on the evolutionary lability of traits with a generalised linear mixed

274 model with ‘brm’ (as specified above). Phylogenetic half-life was modelled assuming a lognormal
275 distribution (only positive values), and trait was a random effect.

276 *Multi-trait relationships and trade-offs* - To identify putative trade-offs between pairs of
277 traits, Pearson correlation coefficients were calculated using the ‘rcorr’ function of the package
278 {Hmisc} (Harrell 2019). Before performing correlations, some traits were log₁₀-transformed (i.e.,
279 SSIZ, MGR, NLEA, LA, LDI and RESpT2), and all traits were centred to a mean of zero and
280 scaled to the variance. Then, highly collinear traits were removed from the dataset using the
281 ‘vifstep’ function {usdm} with threshold of 10, which resulted in the drop of 10 traits (i.e.,
282 ASYM_{Frost}, ASYM_{Heat}, NLEA_{Mild}, NLEA_{Heat}, LA_{Frost}, LA_{Heat}, SLA_{Frost}, LDI_{Mild}, LDI_{Heat} and
283 TOL_IGR_{Frost}; correlation matrix in Supplementary material A5). To further reduce the number of
284 traits while maintaining the most discriminating ones depending on the elevation of origin of
285 species, discriminant analysis of principal components (DAPC) was performed with ‘dapc’ of the
286 package {adegenet} (Jombart 2008). The optimal number of PCs to retain was selected based on
287 stratified cross-validation with ‘xvalDapc’ function of the package {adegenet} and 10’000
288 simulations for each level of PC retention. Traits contributing with a loading higher than 0.024 (i.e.,
289 the third quartile of the variables contribution) were selected and used for correlation analysis.

290

291 **RESULTS**

292 **Trait expression differing with temperature treatment during growth and with elevational** 293 **distribution**

294 Results on trait expression differing between growth treatments and species depending on their
295 elevational distribution are summarized in Table 1, Fig. 1 and Supplementary material A3. A high
296 fraction of traits (~70%) responded to temperature. Under regular frost compared to mild
297 conditions, plants reached the midpoint of growth earlier (A3 Fig. 1E), but they had smaller
298 asymptotic size (Fig. 1B) and fewer and smaller leaves (A3 Fig. 1G, H). Their leaves had less
299 surface area per dry mass and were thicker (smaller SLA and LTh; Fig. 1C and A3 Fig. 1I, K).

300 However, frost resistance of leaves was not significantly different after pre-exposure to frost during
301 growth (A3 Fig. 1R). Under regular heat during growth compared to mild conditions, the maximal
302 growth rate of plants was significantly higher (Fig. 1A), the time to maximal growth shorter (A3
303 Fig. 1E) and plants had smaller asymptotic size (Fig. 1B, A3 Fig. 1F) and smaller leaves (Fig. A3
304 Fig. 1H). Furthermore, leaves had more surface area per dry mass and less dry mass per wet weight
305 (larger SLA, smaller LDMC; A3 Fig. 1I, J and Fig. 1C). Finally, tolerance to heat was generally
306 higher compared to tolerance to frost for maximal growth rate and asymptotic size (Fig. 1E, G).

307 Median elevation of species distribution alone explained only significant variation in the
308 general expression of three traits (Tab. 1). Species occurring at higher elevation had smaller leaves
309 (A3 Fig. 1H), lower dry-matter content (Fig. 1C) and lower heat resistance under no acclimation
310 (RES(+)_{T2}; Fig. 1D). A considerable fraction of traits was significantly affected by an interaction
311 between median elevation of distribution and treatment, but only in the comparison between mild
312 conditions and the heat treatment. The only notable exception was that higher-elevation species had
313 increased frost resistance (after acclimation), but only for the first round of sowing (A3 Fig. 1R).
314 When exposed to heat, higher- compared to lower-elevation species had faster growth (Fig. 1A),
315 reached maximal growth earlier (higher -XMID, Fig. A3 Fig. 1E), but ended up being smaller (Fig.
316 1B), with smaller and less dissected leaves (A3 Fig. 1H, L). In line, higher-elevation species
317 showed heightened tolerance to heat – compared to frost – by having a faster maximum growth
318 (Fig. 1E), which was reached earlier (Fig. 1F), but they also showed lower tolerance to heat by
319 ending up being smaller (Fig. 1G). Comparisons between models with and without considering the
320 phylogeny revealed that its inclusion improved the model for about 70% of traits (Tab. 1, A3).

321

322 **Past evolutionary forces**

323 Table 2 summarizes results on analyses of evolutionary processes having acted on traits, for each
324 growth environment (for a full account see Supplementary material A4). The comparison between
325 the two evolutionary switch models (i.e., ‘ER’, under which low and high elevation are predicted to

326 change at equal rate; or ‘ARD’, under which for- and backward rates between states can take
327 different values) indicated a slightly better performance of the more parameterised model (AIC_{ER}
328 106.898; AIC_{ARD} 103.111), with a fitted value of Q from low \rightarrow high of .030 and low \leftarrow high of
329 .910.

330 Several of the traits found to differ between low- and high-elevation species in mixed-model
331 analyses were confirmed to support a scenario of adaptive evolution with two optima. These traits
332 included: maximal growth rate, asymptotic size, leaf dry matter content, heat resistance and
333 tolerances in growth parameters (Tab. 2, A4). The optimum for high-elevation species was at a
334 lower MGR under control conditions, at a smaller asymptotic size under all growth conditions and
335 at a lower LDMC under regular frost. Furthermore, high-elevation species had an optimum at lower
336 heat resistance when raised under mild conditions, but at higher heat resistance when raised under
337 regular heat. Finally, high-elevation species had optima at higher tolerance values to heat based on
338 MGR and -XMID; they had been selected to accelerate the speed of growth more under heat stress.
339 But they had optima for tolerance to frost and heat based on asymptotic size that were lower. These
340 results appear to be robust, as they did not deviate significantly from the results obtained from
341 bootstrap simulations (A4).

342 Simulations performed on the phylogeny but with synthetic data (A4 Fig. 2) revealed that
343 adaptive divergence between low- and high-elevation species was identified correctly when trait
344 variance was low (<100) and the difference between optima (thetas) large. False positives for the
345 adaptive model were rare, while false negatives in favour of OU or WN were frequent. Simulations
346 that randomly removed a third of the species generally resulted in increased support for OUM (A4).
347 Specifically, to the traits already mentioned above, high- compared to low-elevation species also
348 differed in having optima at slower initial growth under control conditions, but at higher maximum
349 growth rate under stress (in frost and heat treatments). Furthermore, optima differed for leaf size
350 under frost (i.e., at smaller leaf size for high-elevation species) and leaf dry matter content under
351 heat (i.e., at lower LDMC for high-elevation species). No further differences were found for traits

352 related to resistance, while tolerance to regular frost based on maximal growth rate had separate
353 optima, with the one of high-elevation species being at lower tolerance to frost.

354 Measures of phylogenetic half-life (i.e., $\ln(2) \alpha^{-1}$; Tab. 3, A4) were rarely
355 significantly larger than 0 (25-38% depending on treatment, Tab. 3), The most constrained traits
356 were associated with size and morphology, e.g., ASYM with a half-life of 10-16 Mya, LA_{Heat} with
357 25 Mya, LTh with 7-10 Mya and LDI_{Frost} with 15 Mya. Mixed-effects analysis with bootstrap
358 simulations revealed that the evolution of trait values under regular frost was less constrained
359 compared to mild conditions or regular heat (i.e., Frost vs Mild: -.605 [-.616, -.593]; Heat vs Mild:
360 .192 [.180, .204]), resulting in a reduction of average half-life of about 50% (A4 Fig. 3A, B).

361

362 **Multi-trait relationships and trade-offs**

363 A principal component analysis on trait values of all trait-growth treatment combinations revealed
364 their correlation structure. The first PC axis explained 15.7% of the total variance and depicted the
365 relationship between timing of plant growth, especially in the heat treatment, and plant size under
366 mild conditions. The second PC (10.5%) was primarily influenced by LTh, and to a lesser extent by
367 basal resistance and tolerance components, depicting a distinction between these two strategies
368 (Supplementary material A5). The optimal number of principal components to retain (i.e., lowest
369 MSE and highest mean success) based on cross-validation was 35 (accounting for 99% of trait
370 variation, A5).

371 With these PCs, taxa could be assigned to their elevation of origin, either low or high, with
372 an accuracy of 98% and 94.4% respectively (A5). In multivariate space, the trait with the greatest
373 weight was leaf area under mild conditions, while the other traits that contributed most to
374 differentiating low- and high-elevation species were associated to leaf morphology under mild and
375 warm conditions (i.e., LTh, LDMC, SLA), speed of growth under heat (i.e., TOL_IGR, TOL_-
376 XMID) and tolerance under frost (i.e., LDI, TOL_ASYM, A5). Pearson correlations were
377 significantly negative between specific leaf area under heat and leaf area (LA_{Mild} , Fig 2A) or leaf

378 dry matter content ($LDMC_{Mild}$, Fig. 2C; $LDMC_{Heat}$, Fig. 2E), with the latter correlation being likely
379 driven by non-independence of calculating estimates. Furthermore, leaf area under mild conditions
380 was negatively correlated with heat tolerance based on the mid-point of growth (TOL_{-XMID} , Fig.
381 2B), suggesting a trade-off between maintaining large size and speeding up growth under heat.
382 Tolerance under warm based on midpoint of growth was also negatively associated with leaf
383 dissection index under frost (Fig. 2F), which in turn was negatively associated with leaf thickness
384 under mild conditions (Fig. 2D). However, these two correlations did not involve traits linked to
385 elevational distribution.

386

387 **DISCUSSION**

388 Past studies in ecology and biogeography have indicated that temperature is a limiting factor of
389 species distribution, suggesting that there are ubiquitous constraints to the evolution of the climate
390 niche. To improve our understanding of such constraints, we studied approximately 100 species
391 differing in elevational distribution and presumably with different climate niches. More specifically,
392 we investigated which traits differed with elevational distribution, whether those traits had been
393 under divergent selection over the elevational gradient, and potential sources of constraints in their
394 adaptive divergence. The species were found to systematically differ in few traits. Most
395 importantly, higher-elevation plants were found to have smaller and less robust leaves. Further
396 differences emerged when growing conditions included regular heat bouts. Then higher-elevation
397 species accelerated growth more, at the cost of a considerable reduction in size. The same or similar
398 traits were found to be under divergent selection over the elevational gradient, and some were
399 involved in moderate trade-offs, notably the ability to speed up growth under heat and plant size.
400 The discussion focuses on traits under divergent selection, evidence for evolutionary constraints,
401 and hypotheses on the selection environment and adaptive strategies.

402

403 *Trait differences between low- and high-elevation species*

404 Generalized linear models and evolutionary models mainly overlapped in pointing to differences in
405 traits depending on whether species had low- or high elevation distributions (Table 4). The traits
406 that were consistently different between low- and high-elevation species in the two types of models
407 depicted plant size (i.e., ASYM, LA), leaf morphology (i.e., LDMC, SLA), the response of speed of
408 growth to stress, and thermal resistance.

409 Across growth environments, alpine species had smaller leaves and less dry matter content
410 in leaves (Tab. 4, Fig. 1B, C and A3 Fig. 1H, J). Evolutionary models supported that optima for
411 plant size were at smaller values for high-compared to low-elevation species under all growth
412 conditions. Furthermore, they supported an optimum at lower LDMC under growth conditions with
413 regular frost, and as a trend an optimum at higher SLA, which is typically inversely related to
414 LDMC, under mild conditions or conditions with regular heat. Results for size are in line with
415 previous studies on multi-species comparisons, which reported a reduction in leaf size with
416 increasing elevational distribution (Qi et al. 2014; Zhong et al. 2014). In contrast, previous studies
417 reported either higher LDMC and smaller SLA (Körner et al. 1986; Qi et al. 2014; Rosbakh et al.
418 2014; Midolo et al. 2019), or the contrary (Zhong et al. 2014) as found for the Brassicaceae. Lower
419 LDMC and higher SLA are typically associated with a strategy of fast assimilation and growth but
420 weak hardiness and short leaf life-span (Pérez-Harguindeguy et al. 2013).

421 The other type of trait that generally differed between low- and high-elevation species was
422 the response to heat during the growth phase. Both heat and frost caused plants and their leaves to
423 be smaller, indicating that conditions were generally stressful. Furthermore, plants speeded up
424 growth under these conditions; the time to reach the midpoint of asymptotic size was shorter (-
425 XMID), and under the regular occurrence of heat bouts, also the maximum growth rate was higher
426 (MGR). An important finding of this study is that higher- compared to lower-elevation species
427 could accelerate growth under conditions with regular heat bouts even more (MGR, -XMID; Fig.
428 1A, E, F), at the cost that their leaves were more reduced (Fig. 1B, G). Evolutionary models too
429 provided evidence that tolerance for speeding up growth (TOL_MGR, TOL_-XMID) under heat

430 had an optimum at higher values in high-elevation species. Evolutionary models pointed also to an
431 optimum at higher values for tolerance of speeding up growth under frost (as a trend). Results
432 suggest general selection for escape strategies under stress, and that high-elevation species seem to
433 have adapted to exploit heat phases better by growing faster when they occur. The finding is novel
434 and needs verification in more plant families.

435 Interestingly, low- and high-elevation species also differed in thermal resistance, though not
436 in the direction that was previously advocated. Our mixed-effects analysis supported that heat
437 resistance decreased with median elevational of species distribution. Evolutionary models supported
438 a lower optimum for basal heat resistance in high-elevation species, but a higher optimum of
439 acclimation-based heat resistance. However, increased frost resistance (after acclimation) in high-
440 elevation species was only reported for the first round of sowing but not the second (-12 °C S1 vs -
441 10 °C S2, A3 Fig. 1R), and the result was significant only when phylogeny was not considered (A3,
442 model 15). In contrast, a number of earlier studies documented rather consistently that high-
443 elevation tree species were more frost resistant (Körner 2003; Taschler and Neuner 2004; Neuner
444 2014; Neuner et al. 2020; Schrieber et al. 2020). The discrepancy may have two potential reasons.
445 First, the latter studies did not account for phylogeny in their analysis, which could have produced
446 increased type I error (Li and Ives 2017). Second, there may be fundamental differences between
447 trees and herbaceous plants in the role of frost resistance on distribution limits because of
448 differences in the life history or the plant architecture and functioning.

449 In summary, the picture that emerges is that high- compared to low-elevation species are fast
450 growers when it is warm, but have reduced size, have less hardy leaves and are neither particularly
451 heat- nor frost-resistant.

452

453 *Trade-offs and evolutionary inertia*

454 We detected trade-offs among traits that contributed most to the differentiation between low- and
455 high-elevation species (Tab. 4, A5). Specific leaf area under heat was negatively related with leaf

456 area (LA_{Mild} , Fig 2A). In turn, leaf area under mild conditions was negatively correlated with heat
457 tolerance based on the mid-point of growth (TOL_{-XMID} , Fig. 2B). Before discussing the two
458 results in a more general context, it is to note that when analyses were done, a fraction of traits,
459 which were actually calculated on the basis of trait-growth environment combinations, had already
460 been excluded because of redundancy in information. The phenotypic aspect that the remaining
461 traits represented was therefore probably larger. Based on this reasoning, we can say that an
462 important trade-off was between assimilation efficiency combined with less leaf hardness (high
463 SLA) under heat and (plant) size. Another was between size and the capacity to speed up growth
464 under heat. In other words, there is good macroevolutionary evidence that fast growth under heat,
465 small size and assimilation potent leaves with less dry mass come as a syndrome of high-elevation
466 species, shaped by trade-offs. Whether these trade-offs occur on a within-species level and may
467 constrain adaptive evolution and niche expansion at range edges remains to be tested.

468 Trade-offs involving thermal resistance or tolerance were also found, but may have little
469 impact on species distribution. Weak to moderate negative relationships were detected between
470 non-acclimated resistances (to cold or heat) and assimilatory capacity (SLA, number of leaves; A5).
471 But, resistance did not figure among the nine most relevant traits in differentiating low- and high-
472 elevation species in a multivariate space (Tab. 4; Fig. 2; A5).

473 Considerable evolutionary half-lives of traits important in driving elevational distribution
474 were found. The highest value of phylogenetic inertia was found for leaf area, one of the two most
475 discriminating traits between low- and high-elevation species (Fig. 2, A5). The half-life was
476 estimated to be ~26Mya when leaf area was expressed under the regular occurrence of heat (Tab. 3,
477 A4). Also asymptotic size and leaf dissection index (under regular occurrence of frost) had
478 considerable half-lives, between 11 and 15 Mya. The remaining traits (i.e., IGR, SLA, LTH,
479 TOL_{IGR} and $-TOL_{XMID}$) had lower, but still considerable values ranging from ~1.5 Mya for
480 heat tolerance based on the time until fastest growth, to 9.5 Mya for leaf thickness under cold
481 conditions. This considerable half-lives generally indicate constraints to adaptive evolution.

482

483 *Selection environment, adaptive strategies and evolutionary constraints*

484 Insights discussed above and further ones gained from analyses evoke novel hypotheses on the

485 causes of limits to niche evolution and disparate elevational or climatic distribution.

486 Evidence for divergent adaptation between low- and high-elevation species was more

487 common for traits recorded under mild and heat conditions compared to the regular occurrence of

488 frost (Tab. 3, Tab. 4). This strongly supports that high-elevation species have adaptively diverged

489 on exploiting warm conditions and not (so much) to resist the cold. This is a very important insight,

490 and also – as a side note – warrants attention that the detection of traits under selection is

491 environment-dependent. Comparative studies typically rely on measurements taken in the field or

492 on collection material (e.g., Luxbacher and Knouft 2009; Edwards and Smith 2010), or after raising

493 organisms under standard conditions (e.g., Kellermann et al. 2012; Mason and Donovan 2015).

494 While the former brings the problem of the inability of separating the effects of genetics and the

495 environment on trait differences, the latter has the flaw that the adaptive potential of a trait may not

496 be detected as the environment is not the one in which divergence is expressed. Again, for

497 Brassicaceae along the elevational gradient, it is mild and heat conditions that are likely to have

498 played more of a role in adaptive divergence.

499 Several insights speak in favour that high-elevation conditions select for faster growth at the

500 cost of small size and possibly a shorter life, with the environmental driver being the short growing

501 season. On the one hand, our study showed that plants of high elevations were not better at coping

502 with cold, but they had evolved to better exploit warm conditions for fast growth. In line, previous

503 eco-physiological studies reported higher photosynthetic rate in alpine herbaceous species

504 cultivated at warmer temperature (Mächler and Nösberger 1977) or during daily warm spells in the

505 wild (Körner and Diemer 1987), pointing to faster resource acquisition under warm conditions.

506 Furthermore, niche-modelling suggested that upper ranges were constrained not primarily by the

507 direct effect of cool temperatures but the brevity of the growing season (Morin et al. 2007; Patsiou

508 et al. 2021). These studies too pointed to speed of growth or development being under selection
509 under higher-elevation conditions. Based on the two sets of insights, we propose that whether a
510 species (of Brassicaceae) can live at high elevation depends on the ability to cope with the short
511 growing season, which is achieved by maximising growth during short thermal windows when the
512 temperature is relatively high. Superficially, the geographic pattern may resemble counter-gradient
513 variation (Conover and Schultz 1995), where high-elevation genotypes grow faster while their
514 environment may generally cause growth to be slow. One distinction is that the heightened
515 acceleration of growth is expressed only under warmer conditions, and a second is that the relevant
516 environmental difference seems to be the shorter growing season.

517 Analyses on trade-offs pointed to leaf morphology being coupled with fast growth under
518 heat and reduced plant size. Speeding up growth under heat was negatively correlated with leaf size,
519 while small life size implied higher SLA (related with lower LDMC) – generally thinner leaves
520 with higher assimilation capacity (Fig. 2A, B). The importance of the leaf morphology in this
521 context adds a further notion of a constraint of fast growth. According to the world-wide leaf
522 economics spectrum (Wright et al. 2004), species either follow a strategy of quick return on
523 investment, with nutrient-rich leaves, high photosynthetic rates, and short life-spans *versus* a
524 strategy of slow return, with expensive but long-lived leaves. In a broader context, the continuum of
525 fast production versus slowness is also reflected in the concept of r/K selection (Pianka 1970),
526 where r-selected species grow more rapidly, but to a smaller size and they reproduce earlier, while
527 K-selected species grow more slowly, but to larger size and they reproduce later. For plants, the
528 concept was expanded, with now three strategies – stress-tolerant (S), competitive (C), ruderal (R) –
529 being positioned along three axes of environmental gradients, of abiotic stress, competition and
530 disturbance (Grime 1977). Pierce et al. (2013) showed how these strategies can be correctly
531 attributed with the use of the same leaf-traits that show the main trade-offs in our work, i.e., leaf
532 area, leaf dry matter content and specific leaf area. However, and in contrary to their reports,
533 LDMC and LA did not form separate axes in our study. Nonetheless, following their sorting

534 suggests that alpine (Brassicaceae) species primarily follow an r-strategy, whereas lowland species
535 follow a C/S (or K) strategy, at least in relation to temperature responses.

536 Finally, also phylogenetic inertia of traits was found to depend on the environment in which
537 they were expressed (Tab. 3, A4 Fig. 1A, B). In our study, the mild and heat treatments were not
538 only the more discriminating among low- and high-elevation species, they were also those in which
539 traits had on average higher phylogenetic inertia. The phylogenetic half-life of traits expressed
540 under mild and heat was 50% higher compared to trait expression under the regular occurrence of
541 frost. Results therefore suggest that adaptation to exploit or live under generally warmer conditions
542 is more constrained. The result is in line with a recent large-scale phylogenetic analysis, showing
543 that across plants and animals, the rate of adaptation to warm was much slower than to cold, both in
544 endotherms and ectotherms (Bennet et al. 2021).

545

546 **CONCLUSION**

547 Our study highlights that the most discriminating traits separating high- from low-elevation
548 Brassicaceae species are their ability to speed up growth under conditions with heat bouts, at the
549 cost of much reduced leaf and plant size, and possibly a more ephemeral lifestyle with less
550 investment into leaves. Results suggest a general trade-off between exploiting the short vegetation
551 period at high elevation and being less enduring in general or under certain thermal extremes or
552 under competition. The trade-off could be a result of multivariate selection differing among low-
553 and high-elevation sites and/or negative genetic correlations. In parallel, we found that thermal
554 resistance did not play a strong role in differentiating species along the elevational gradient. Finally,
555 we found evidence that divergent adaptation under conditions with regular heat was more
556 pronounced compared to conditions with regular frost, and that adaptation to heat was more
557 constrained.

558

559

560

561 **Declaration of authorship**

562 AM and YW conceived the study and conducted the field work. AM executed the experimental
563 work, run the statistical analyses and wrote the first draft of the manuscript. YW contributed to
564 writing. All authors gave final approval for publication.

565

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573

574 **Conflict of interest**

575 The authors declare that they have no conflict of interest.

576

577 **SUPPLEMENTARY MATERIAL**

578 A1 – List of species and populations used in this study

579 A2 – Additional information on traits measurement and methods for validating evolutionary models

580 A3 – Mixed-effects model on the relationship between traits, treatment and elevational distribution
581 and data distribution.

582 A4 – Model of trait evolution (WN, BM, BMM, OU, OUM), optimal trait values, AICc
583 distribution, phylogenetic half-life and simulations

584 A5 – Multivariate analysis, and Pearson's correlation

585

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861 **Table 1** Results of mixed-effects models on the relationship between median elevation of species
862 distribution, treatment during plant growth (regular frost [F], mild conditions [M], and regular heat
863 [H]) and their interaction on plant traits
864
865

Trait	Trait ID	Posterior median						
		Treatment (F M H)		Elevation	Treatment (F M H) × elevation			
		F vs M	H vs M		Slope of elevation under F vs M	Slope of elevation under H vs M		
Seed size ϕ	SSIZ			.058 [-.166, .028]				
Time to germination ϕ	TGER			.042 [-.017, .101]				
Growth	Initial growth rate	IGR		-0.006 [-.033, .021]	-0.019 [-.047, .008]	-0.009 [-.027, .011]	.012 [-.015, .040]	.015 [-.011, .044]
	Maximal growth rate	MGR	Fig. 1A	-0.043 [-.126, .037]	.421 [1.338, .500] ***	-0.034 [-.093, .020]	.012 [-.072, .089]	.128 [.046, .211] *
	(-)Time to fastest growth ϕ	(-)XMID		1.033 [.447, 1.594] **	2.114 [1.534, 2.722] ***	-0.702 [-1.292, -.105] (.)	.357 [-.276, .961]	1.491 [.899, 2.067] ***
	Asymptotic size ϕ	ASYM	Fig. 1B	-1.139 [-.185, -.091] ***	-1.153 [.200, -.103] ***	-0.040 [-.111, .031]	.025 [-.072, .023]	-1.134 [-.184, -.086] ***
	Number of leaves s_2, ϕ	NLEA		-.064 [-.111, -.012] *	.005 [-.043, .055]	-0.013 [-.107, .086]	-0.008 [-.057, .042]	-0.025 [-.071, .025]
Leaf traits	Leaf area ϕ	LA		-.162 [-.253, -.086] **	-.255 [-.338, -.170] ***	-.349 [-.483, -.218] ***	.032 [-.051, .120]	-.117 [-.203, -.028] *
	Specific leaf area ϕ	SLA		-.130 [-.184, -.075] ***	.112 [.056, .167] ***	.018 [-.036, .075]	-0.030 [-.083, .025]	.018 [-.040, .072]
	Leaf dry matter content ϕ	LDMC	Fig. 1C	.042 [.006, .075] (.)	-.182 [-.216, -.146] ***	-.066 [-.106, -.025] **	.008 [-.027, .045]	-0.019 [-.55, .018]
	Leaf thickness s_2, ϕ	LTh		.077 [.030, .126] **	-0.017 [-.064, .031]	.026 [-.016, .066]	-0.011 [-.058, -.040]	.004 [-.049, .050]
	Leaf dissection index ϕ	LDI		-0.009 [-.032, .012]	-0.017 [-.041, .005]	.040 [-.002, .077]	.019 [-.003, .042]	-.045 [-.069, -.022] **
Thermal tolerance s.l.	Frost resistance							
	...acclimated (1h at -6 °C) s_1	RES(-)T1					-0.081 [-.209, .057]	
	...non acclimated (1h at -5 °C) s_2, ϕ	RES(-)T1					.033 [-.042, .106]	
	...acclimated (1h at -11 °C)	RES(-)T2					.124 [.021, .234] (.)	
	...non acclimated, (1h at -10 °C) s_2	RES(-)T2					.044 [-.064, .160]	
	Heat resistance							
	...acclimated (1h at +47 °C) s_1, ϕ	RES(+)T1					-0.030 [-.174, .121]	
	...non acclimated (1h at +45 °C) s_2, ϕ	RES(+)T1					.043 [-.046, .133]	
	...acclimated (1h at +51 °C) ϕ_N	RES(+)T2					.029 [-.134, .183]	
	...non acclimated, (1h at +50 °C) s_2, ϕ	RES(+)T2	Fig. 1E				-.177 [-.317, -.038] *	
			H vs F	Elevation		Slope of elevation under H vs F		
Tolerance IGR ϕ_N	TOL_IGR		.003 [-.038, .041]	.013 [-.015, .042]		.012 [-.029, .051]		
Tolerance MGR ϕ	TOL_MGR	Fig. 1F	1.494 [1.287, 1.701] ***	.085 [-.128, .293]		.312 [.119, .523] *		
Tolerance (-)XMID ϕ	(-)TOL_XMID	Fig. 1G	.017 [-.011, .049]	-0.008 [-.019, .032]		.048 [-.018, .077] **		
Tolerance ASYM ϕ	TOL_ASYM	Fig. 1H	.411 [.288, .540] ***	.013 [-.107, .142]		-.304 [-.427, -.176] ***		

Posterior medians of fixed effects are reported, relative to the baseline of average elevation and mild growth conditions (full details in Supplementary material A3, including results on random effects). For tolerance traits, the coefficients express differences between estimates under heat compared to those under frost. Traits with a significant effect of elevation or its interaction are written in bold (posterior 90% high density interval [HDI] not overlapping with 0, and probability of direction > 97.5% [(.) pd > 95, * pd > 97.5%, ** pd > 99.5%, *** pd > 99.95]). Traits for which the model accounting for phylogeny was better supported (ϕ) or not (ϕ_N) are indicated; when nothing is reported, no statistical difference between models was found. If not specified, a trait was assessed both in S1 and S2.

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870 **Table 2** List of traits measured in the three growth environments (regular frost, mild and regular
 871 heat) for which the best supported evolutionary model was Ornstein-Uhlenbeck with two optima
 872 (OUM), and the suggested trait optima (θ) for low- and high-elevation species
 873

Trait ID	Treatment								
	Frost			Mild			Heat		
	Best model	θ_{LOW}	θ_{HIGH}	Best model	θ_{LOW}	θ_{HIGH}	Best model	θ_{LOW}	θ_{HIGH}
Seed size
Time to germination
Initial growth rate
Maximal growth rate	.	.	.	OUM _{4,12}	.21	.18	.	.	.
(-)Time to fastest growth
Asymptotic size	OUM _{1,88}	57.15	30.44	'OUM' _{1,84}	64.85	38.72	OUM _{2,75}	70.16	23.65
Number of leaves s_2
Leaf area
Specific leaf area	.	.	.	'OUM' _{3,36}	22.84	25.44	'OUM' _{5,67}	25.16	28.87
Leaf dry matter content	OUM _{18,33}	20.64	16.83
Leaf thickness s_2
Leaf dissection index
Frost resistance
...acclimated (1h at -6 °C) S_1
...non acclimated (1h at -5 °C) S_2
...acclimated (1h at -11 °C)
...non acclimated, (1h at -10 °C) S_2
Heat resistance
...acclimated (1h at +47 °C) S_1
...non acclimated (1h at +45 °C) S_2
...acclimated (1h at +51 °C)	OUM _{13,89}	13.16	21.42
...non acclimated, (1h at +50 °C) S_2	.	.	.	OUM _{0,84}	11.68	6.62	.	.	.
Tolerance IGR
Tolerance MGR	OUM _{57,5}	1.52	2.44
Tolerance (-)XMID	'OUM' _{96,22}	.02	.04	.	.	.	OUM _{32,88}	.00	.11
Tolerance ASYM	OUM _{126,02}	-.06	-.14	.	.	.	OUM _{76,5}	.78	-.30

874 The table shows when the best fitting model (lowest mean AICc value) obtained for each trait-treatment combination based on the consensus of 100
 875 simulations on the full phylogeny was OUM (full details in Supplementary material A4). When OUM was supported with $\Delta AICc \leq 2$, it is indicated
 876 by brackets ('OUM'), when it was not the best supported model, it is indicated by a dot (.). Values in subscript are $|\Delta AICc|$ between models of adaptive
 877 vs drift evolution (i.e., OUM vs Brownian motion). If not specified, a trait was assessed both in S_1 and S_2 .

878 **Table 3** Half-life of trait evolution toward the optimum in Mya
879

Trait ID	Treatment		
	Frost	Mild	Heat
	$t_{1/2} \pm SD$	$t_{1/2} \pm SD$	$t_{1/2} \pm SD$
Seed size		33.25 ± 28.42	
Time to germination		11.85 ± 11.41	
Growth			
Initial growth rate	5.40 ± 2.77 *	6.51 ± 2.34 *	6.66 ± 2.06 *
Maximal growth rate	.75 ± 2.74	0.14×10 ⁴ ± 9.46×10 ⁴	25.75 ± 667.31
(-)Time to fastest growth	1.21 ×10 ⁴ ± 70.84 ×10 ⁴	0.55 ×10 ⁴ ± 30.15×10 ⁴	2.89×10 ⁴ ± 142.19×10 ⁴
Asymptotic size	10.90 ± 4.99 *	15.49 ± 6.88 *	14.31 ± 9.51
Number of leaves _{S2}	2.58 ± 2.58	3.53 ± 3.36	3.52 ± 2.87
Leaf traits			
Leaf area	12.09 ± 12.09	14.23 ± 11.59	25.49 ± 11.98 *
Specific leaf area	7.48 ± 2.33 *	5.27 ± 1.78 *	5.25 ± 1.94 *
Leaf dry matter content	2.62 ± 3.19	2.86 ± 2.71	349.66 ± 2.51×10 ⁴
Leaf thickness _{S2}	9.55 ± 2.56 *	7.33 ± 1.57 *	7.81 ± 1.29 *
Leaf dissection index	15.07 ± 6.87 *	23.36 ± 16.50	11.44 ± 8.89
Thermal tolerance s.l.			
Frost resistance			
...acclimated (1h at -6 °C) _{S1}	.75 ± 1.49		
...non acclimated (1h at -5 °C) _{S2}		6.24 ± 4.47	
...acclimated (1h at -11 °C)	3.43 ± 2.70		
...non acclimated, (1h at -10 °C) _{S2}		1.74 ± 2.38	
Heat resistance			
...acclimated (1h at +47 °C) _{S1}			2.14 ± 2.54
...non acclimated (1h at +45 °C) _{S2}		4.15 ± 3.32	
...acclimated (1h at +51 °C)			1.69 ± 1.77
...non acclimated, (1h at +50 °C) _{S2}		24.86×10 ⁴ ± 475.96×10 ⁴	
Tolerance IGR	5.74 ± 2.47 *		5.51 ± 1.75 *
Tolerance MGR	.16 ± .26		.88 ± .70
(-)Tolerance XMID	.16 ± .39		1.46 ± .81 *
Tolerance ASYM	.09 ± .15		.71 ± .49

880 Values of phylogenetic half-life (from OUI, or OUM if elevation was significant) are based on an ARD model and 100 independent stochastic
881 character maps (full details in Supplementary material A4). Values are means ± standard deviation (SD) of phylogenetic half-life in Mya for traits
882 within treatments, calculated based on bootstrap replicates (i.e., the random removal of a third of the species, with N = 10'000 simulations *per* trait
883 within environment). Significance in half-life (*) was calculated by mean - 1.64SD > 0. If not specified, a trait was assessed both in S1 and S2.

884 **Table 4** Summary of results on trait differences between low- and high-elevation species in the
 885 three growth treatments (regular frost [F], mild conditions [M], and regular heat [H]) across types of
 886 analyses (mixed models [brms], testing for two evolutionary optima [OUM], half-life of trait
 887 evolution, discriminant analysis of principal components [DAPC], and (negative) correlations [ρ])

Trait ID	Effect of elevation						Phylogenetic inertia			Trade-offs						
	Brms			OUM			Half-life			DAPC			ρ			
	Elev	F	M	H	F	M	H	F	M	H	F	M	H	F	M	H
Seed size
Time to germination
Initial growth rate		c						✓	✓	✓						
Maximal growth rate		c	+✓			↓✓										
(-)Time to fastest growth		c	+✓													
Asymptotic size		c	-✓		↓✓	↓✓	↓✓	✓	✓		x	x				
Number of leaves _{S2}			c									x	x			
Leaf area	-✓		c	-✓						✓	x	✓	x			✓
Specific leaf area			c			↑✓	↑✓	✓	✓	✓	x	✓	✓			✓
Leaf dry matter content	-✓		c		↓✓						✓	✓				✓
Leaf thickness _{S2}			c					✓	✓	✓	✓					✓
Leaf dissection index			c	-✓				✓			✓	x	x			✓
Frost resistance																
...acclimated (1h at -6 °C) _{S1}			
...non acclimated (1h at -5 °C) _{S2}			
...acclimated (1h at -11 °C)			
...non acclimated, (1h at -10 °C) _{S2}			
Heat resistance																
...acclimated (1h at +47 °C) _{S1}			
...non acclimated (1h at +45 °C) _{S2}			
...acclimated (1h at +51 °C)			
...non acclimated, (1h at +50 °C) _{S2}	-✓		.	.	.	↓✓
Tolerance IGR			c	.	+✓	.	.	✓	.	✓	x	.	✓	.	.	.
Tolerance MGR			c	.	+✓	.	.	✓	.	✓	.	.	✓	.	.	.
(-)Tolerance XMID			c	.	+✓	↑✓	.	↑✓	.	↑✓	.	.	✓	.	.	✓
Tolerance ASYM			c	.	-✓	↓✓	.	↓✓	.	↓✓	.	✓

888 A significant role of elevation is indicated (✓) when a significant effect (positive or negative) of elevation or an interaction between elevation and
 889 growth environment (relative to contrast, c environment) was found in the analysis with brms, and when OUM was the best supported evolutionary
 890 model with the optimum at lower (negative) or higher (positive) trait values for high- compared to low-elevation species. Phylogenetic inertia is
 891 indicated (✓) when the half-life of trait evolution under OUM (or 'OUM') was > 5 Mya. A trade-off is indicated (✓) when discriminant analysis of
 892 principal components (DAPC) included the trait in the loads after a threshold was applied, and when selected traits after DAPC were involved in
 893 significant (negative) correlations (ρ). Additional signs indicate: a trait was not assessed in a particular environment (.), or was excluded from analysis
 894 (x). Lines in bold highlight traits that showed a significant effect of elevation and were involved in negative relationships with others. If not specified,
 895 a trait was assessed both in S1 and S2.

896

897 **Figure 1** Boxplot showing the distribution of species-mean trait values for which species differed
898 depending on their median elevation (low- vs high-elevation), either across growth treatments or in
899 a particular growth treatment (frost, mild, or heat). For simplicity, only data of the second round of
900 sowing are included and traits for which mixed-effects models and evolutionary models produced
901 concordant results (panels for both rounds of sowing and all traits are in Supplementary material
902 A3). Colours inside boxes represent the treatments (blue for frost, greyscale for control and red for
903 heat), while the intensity represents median elevation of species occurrence (darker colours for low
904 elevation and lighter colour for high elevation). The thick horizontal line is the median, the lower
905 and upper hinges are the 25th and 75th percentiles; whiskers extends from the hinges to the smallest
906 (largest) value at most (no further than) $1.5 * IQR$ of the hinges, and dots are values beyond that
907 range.

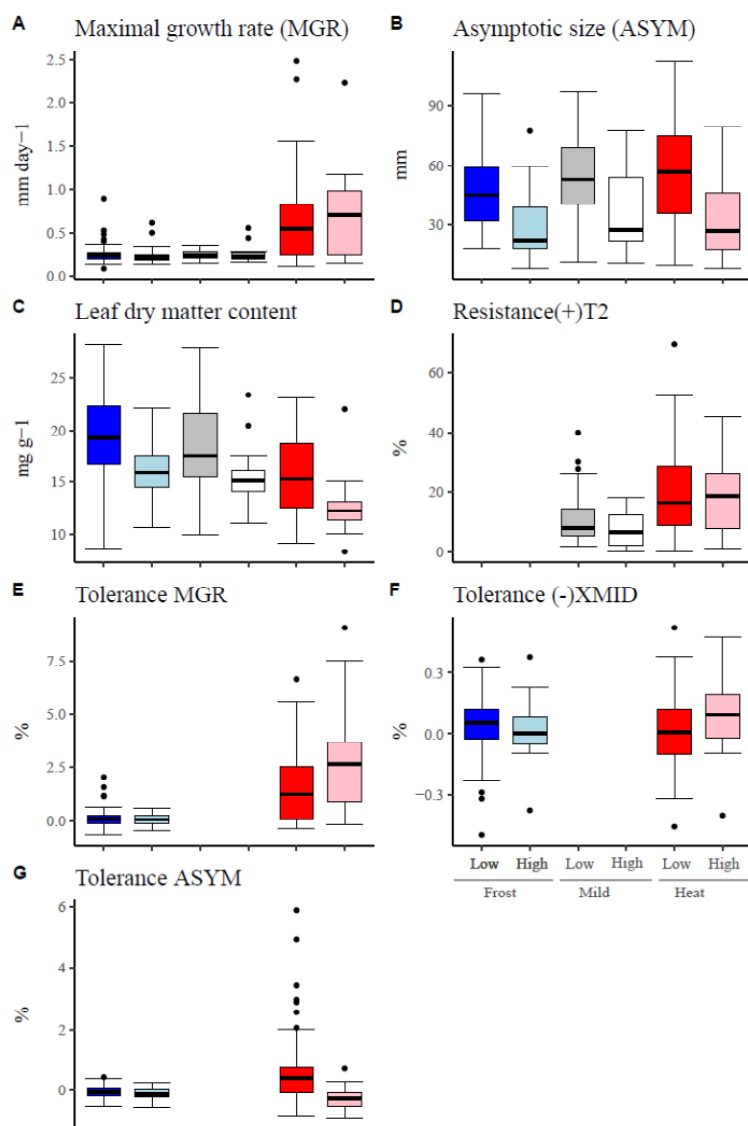
908

909 **Figure 2** Trait differentiation between low- and high-elevation species, as revealed by discriminant
910 analyses and multi-trait correlations. Each point represents a species. The median elevation of
911 origin is represented by a colour scale ranging from green (low elevation) to brown (high elevation).
912 The black line reflects the relationship between pairs of traits and the associated correlation
913 coefficient is reported (full details in Supplementary material A5). Traits values are centred and
914 scaled to unit variance.

915

916

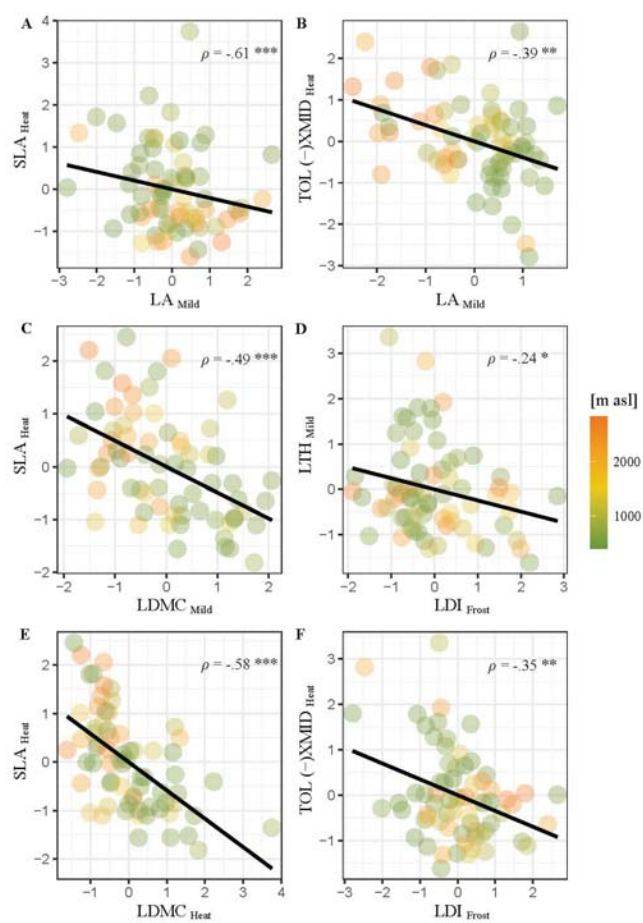
917 Fig. 1



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919

920 Fig. 2



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