

1 **Potential adaptive divergence between subspecies and populations of snapdragon plants**
2 **inferred from Q_{ST} – F_{ST} comparisons**

3 Sara Marin^{1,2§}, Anaïs Gibert^{1§}, Juliette Archambeau³, Vincent Bonhomme⁴, Mylène Lascoste²
4 and Benoit Pujol^{1,2*}

5
6 ¹ PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, Université de Perpignan,
7 52 Avenue Paul Alduy, 66860, 66360 Perpignan Cedex, France.

8 ² Laboratoire Évolution & Diversité Biologique (EDB UMR 5174), Université Fédérale de
9 Toulouse Midi-Pyrénées, CNRS, IRD, UPS, Toulouse, France

10 ³ BIOGECO, INRA, University of Bordeaux, Pessac, France

11 ⁴ Institut des Sciences de l'Évolution (ISEM), équipe "Dynamique de la biodiversité, anthropo-
12 écologie", UMR 5554, Université de Montpellier, CNRS, IRD, EPHE, Place Eugène Bataillon,
13 Cc 065, 34095 Montpellier cedex 05, France

14

15 § Both authors share first authorship of this work

16 * Author for correspondence: benoit.pujol@univ-perp.fr

17

18 **ABSTRACT**

19 Phenotypic divergence among natural populations can be explained by natural selection or by
20 neutral processes such as drift. Many examples in the literature compare putatively neutral (F_{ST})
21 and quantitative genetic (Q_{ST}) differentiation in multiple populations to assess their potential
22 evolutionary signature and identify potential traits involved with local adaptation. Investigating
23 these signatures in closely related or recently diversified species has the potential to shed light
24 on the potential divergence processes acting at the interspecific level. Here, we conducted this
25 comparison in two subspecies of snapdragon plants (eight populations of *Antirrhinum majus*

26 *pseudomajus* and five populations of *A. m. striatum*) in a common garden experiment. We also
27 tested whether altitude was potentially involved with population phenotypic divergence. Our
28 results identified candidate phenological and morphological traits involved with local
29 adaptation. Most of these traits were identified in one subspecies but not the other. Phenotypic
30 divergence increased with altitude for a few biomass-related traits, but only in *A. m. striatum*.
31 These traits therefore potentially reflect *A. m. striatum* adaptation to altitude. Our findings imply
32 that adaptive processes potentially differ at the scale of *A. majus* subspecies.

33

34 **KEYWORDS**

35 Local adaptation, altitudinal gradient, quantitative genetics, subspecies, *Antirrhinum majus*

36

37

38 **INTRODUCTION**

39 Local adaptation - the evolutionary response to selection that makes populations fitter in their
40 own local habitat than in other populations' local habitats - is widespread in both plant and
41 animal species (Kawecki and Ebert, 2004; Leinonen et al., 2013; Halbritter et al., 2018). There
42 is evidence for its role in the adaptive divergence of plant species (Leimu and Fischer, 2008;
43 Hereford, 2009; Halbritter et al., 2018). For example, empirical studies have demonstrated
44 differential adaptation in plant sister species or hybridizing species, for instance between pairs
45 of *Silene* species (Favre et al., 2017), *Senecio* species (Abbott and Brennan, 2014), *Mimulus*
46 species (Angert and Schemske, 2005). These studies compared local adaptation for sister
47 species confronted to different ecological requirements. Different species may also respond
48 similarly to a same type of environmental gradient. Recently, Halbritter et al. (2018) combined
49 studies of multiple plant species along elevation gradients. They found significant evidence for
50 adaptation to different elevations in terms of survival and biomass. Their results also showed
51 variation across species in plant responses to elevation. The study of local adaptation in
52 populations of closely related taxa exposed to environmental gradients, e.g. altitude, is an
53 opportunity to investigate the conditions promoting or impeding the consistency of adaptive
54 responses.

55
56 An indirect approach to investigate whether local adaptation might potentially be involved in
57 the phenotypic divergence of populations is the Q_{ST} - F_{ST} comparison (Spitze, 1993; Merilä and
58 Crnokrak, 2001; McKay and Latta, 2002). The comparison of population genetic differentiation
59 estimated for putatively neutral molecular markers with the population quantitative genetic
60 differentiation estimated for phenotypic traits can be used to identify potential candidate traits
61 playing a role in local adaptation (Whitlock, 2008). This is done by estimating whether trait
62 quantitative genetic differentiation among populations is more likely the potential result of

63 divergent selection ($Q_{ST} > F_{ST}$), stabilizing selection ($Q_{ST} < F_{ST}$), or neutral evolutionary
64 divergence ($Q_{ST} = F_{ST}$, e.g., as a result of drift). Some debate around the accuracy of $Q_{ST} - F_{ST}$
65 comparisons resulted in a variety of methodological adjustments (Whitlock, 2008; Edelaar et
66 al., 2011; Ovaskainen et al., 2011; Whitlock and Gilbert, 2012). In plants, reciprocal transplants
67 directly comparing fitness between the native habitat and the foreign habitats are often preferred
68 to $Q_{ST} - F_{ST}$ approaches conducted in common gardens because they allow to evaluate the effect
69 of environmental conditions (Etterson, 2004; Angert and Schemske, 2005; Kim and Donohue,
70 2013). When the conditions for the reciprocal transplant cannot be easily met, $Q_{ST} - F_{ST}$
71 comparisons represent an opportunity for exploring local adaptation hypotheses.

72

73 In our study, we investigated patterns of potential local adaptation in two closely related plant
74 subspecies by using $Q_{ST} - F_{ST}$ comparisons estimated in a common garden experiment, and
75 evaluated whether altitudinal gradients might play a role in the potential adaptive divergence of
76 populations. This evaluation was conducted in snapdragon plants (*Antirrhinum majus* L.,
77 Plantaginaceae). We studied eight populations of magenta-flowered *A. m. pseudomajus* and five
78 populations of yellow-flowered *A. m. striatum* sampled along altitudinal gradients. These two
79 species are interfertile (Andalo et al., 2010). They are distributed parapatrically, with the
80 geographic range of *A. m. striatum* surrounded by the range of *A. m. pseudomajus*, and come
81 frequently into contact at the margins of their ranges where there is evidence for gene exchanges
82 (Khimoun et al., 2011; Ringbauer et al., 2018). Their geographic separation is not explained by
83 actual climatic differences, as illustrated by the substantial overlap of environmental conditions
84 between the two species (Khimoun et al., 2013). This system is therefore promising to explore
85 potential differential adaptive responses between closely related subspecies, in particular
86 regarding the potential role played by altitude in their adaptive divergence.

87

88 There is poor support in the literature for adaptive changes in reproductive traits along
89 altitudinal gradients (Halbritter et al., 2018). In contrast, adaptive differentiation along
90 altitudinal gradients is expected for biomass-related traits and height, with a trend toward
91 smaller plants for populations from high altitude compared to plants from lowland sites
92 (Halbritter et al., 2018). We tested this hypothesis for five morphological traits (the basal stem
93 diameter, the number of branches on the plant, the number of vegetative nodes on the main
94 stem, and the total height of the plant). We also studied three additional traits: a phenological
95 trait (the germination date), a developmental trait (the average internode length) and a
96 functional trait (specific leaf area, SLA). We expected populations from higher altitudes to
97 germinate later, over a shorter period (Gimenez-Benavides et al., 2006; Donohue et al., 2010).
98 This is because germinating later over shorter periods allows plants to track the late arrival and
99 the shorter-term availability of suitable climatic conditions for growth at higher altitudes
100 (Körner, 1999). Because the internode length is a trait related to both plant height and growth
101 rates, we had no clear expectations. Finally, SLA refers to leaf construction cost and captures
102 information about leaf economic strategies (Wright et al., 2004); low SLA suggests high leaf
103 construction cost and high stress tolerance. Selective pressures associated with lower
104 temperatures at higher elevations are expected to promote leaf trait syndromes associated with
105 superior stress tolerance but inferior competitiveness (Read et al., 2014). These relationships
106 are generally stronger among species than among populations of the same species (Read et al.,
107 2014). Therefore, we expected no correlation or a negative correlation between SLA and
108 elevation among populations.

109

110 In this study, we estimated neutral genetic differentiation (F_{ST}), and quantitative genetic
111 differentiation (Q_{ST}) based on trait heritability (h^2) in *A. majus*. Previous studies of genetic
112 differentiation between populations and subspecies at putatively neutral microsatellite markers

113 brought evidence that gene flow was limited between populations (Debout et al., 2012; Pujol et
114 al., 2017), which sets the stage for local adaptation. We then tested for the hypothesis that traits
115 were potentially involved with local adaptation by comparing Q_{ST} and F_{ST} . Finally, we
116 investigated whether quantitative genetic differentiation increased with altitudinal difference,
117 with the hypothesis that environmental changes associated with altitude, which include a suite
118 of climatic variables, drove adaptive responses. Other environmental variables (e.g.,
119 atmospheric pressure) can also change with altitude. Our study also ultimately participates in
120 evaluating whether Q_{ST} - F_{ST} comparisons can be used as a tool to identify candidate traits
121 involved with the potential adaptation of populations to altitudinal gradients, and thereby
122 climate differences.

123

124 **MATERIAL AND METHODS**

125 **Study system**

126 *Antirrhinum majus* L. (Plantaginaceae) is a hermaphroditic, self-incompatible, short-lived
127 perennial species, characterized by a patchy distribution in southern Europe centred over the
128 Pyrenees Mountains (Khimoun et al., 2011). This species occurs from sea level to an altitude
129 of 1900 m (Andalo et al., 2010), on limestone or siliceous substrates and in habitats with
130 contrasted moisture regimes (rainfall 500-1000 mm per year), where it forms restricted patches
131 mostly in rocky outcrops and screes. *A. majus* thrives in disturbed habitats, and is especially
132 common along roadside and railway embankments (Khimoun et al., 2011).

133

134 **The subspecies level**

135 *A. majus* produces annual inflorescences with zygomorphic flowers. The colour of flowers is
136 either magenta or yellow and distinguishes two interfertile subspecies *A. m. ssp. pseudomajus*
137 and *A. m. ssp. striatum* respectively (Andalo et al., 2010). At the genetic level, ~1% genetic

138 differentiation was found between *A. m. ssp. pseudomajus* and *A. m. ssp. striatum* on the basis
139 of putatively neutral microsatellite loci, which was one order of magnitude lower than the ~10%
140 differentiation found among these populations (Pujol et al., 2017). There is evidence for gene
141 exchange between subspecies in multiple populations across contact zones (Khimoun et al.,
142 2011). Genome scans across a particular contact zone in the Pyrenees also revealed little to
143 negligible differentiation between the two subspecies, with the exception of loci underlying
144 flower colour differences between the two subspecies that were characterized by high
145 differentiation (Whibley, 2006; Tavares et al., 2018). At the environmental level, the separation
146 between the geographic distribution of *A. m. ssp. pseudomajus* and *A. m. ssp. striatum* is not
147 explained by habitat differences, as illustrated by the substantial overlap of environmental
148 conditions between the two species (Khimoun et al., 2013).

149

150 **Collection sites and plant material**

151 Thirteen wild populations of *A. majus* were sampled in 2011 across the geographic range
152 (between north-eastern Spain and south-western France) to represent the overall diversity of
153 the species, with eight populations of *A. m. ssp. pseudomajus* and five populations of *A. m. ssp.*
154 *striatum* (Figure 1, Table S1, Supporting Information). For each subspecies, we sampled
155 populations from low and high altitude habitats in different parts of the species geographic
156 range. Populations sampled along elevation gradients are likely to be confronted to contrasted
157 environmental conditions. Fifty-year averages (1950-2000) of mean annual temperature and
158 annual average rainfall were extracted from the WorldClim database (resolution 1km²,
159 www.worldclim.org, Hijmans et al., 2005). They ranged from 14.8°C and 52mm (BAN, 61m
160 above sea level) to 6.1°C and 94mm (MON, 1564m above sea level) (Figure S1, Supporting
161 Information). The sampling of populations in different valleys or on different summits limits

162 spatial autocorrelation in the data and shared phylogeographic history between populations
163 from similar altitudes.

164

165 In each wild population, seeds were randomly collected from plants across their entire spatial
166 distribution in October 2011. These seeds sampled in the wild were used to grow plants in 2012,
167 in a greenhouse at the CNRS Experimental Ecology Station in Moulis, France. Seeds were sown
168 in spring in individual pots ($9 \times 9 \times 10$ cm) filled with universal compost. Plants germinated
169 and grew with no nutrient addition under an average temperature from 15 to 28°C and weekly
170 watering. Mature plants were hand-pollinated during the summer 2012. These plants were not
171 measured. Crosses were conducted within populations where mates from different families
172 were assigned randomly. The seed collection of full sib families produced by these plants was
173 stored at room temperature, in the dark, under dry conditions until they were used to produce
174 the plants measured in our experiment. This intermediate generation of plants grown in
175 controlled conditions from seeds collected in the wild allowed us to reduce potential maternal
176 effects caused by natural habitat differences on trait measurements.

177

178 **Common garden experiment**

179 Nine to 42 seed families from each of the 13 study populations were grown outdoor in spring
180 2014 in a common garden at ENSFEA (Toulouse, France). Two plants per family were grown.
181 Some plants died before measurements were done, which resulted in some families being
182 represented by only one plant (Table S1, Supporting Information). Plants were grown in
183 individual pots ($9 \times 9 \times 10$ cm) filled with universal compost, with no nutrient addition, under
184 outdoor climatic conditions (average month temperatures ranging from 20.6 to 21.5°C and
185 cumulative monthly rainfall ranging from 28.3 to 73.4mm). Plants were arranged in a
186 randomized block design (40 plastic containers, $600 \times 400 \times 120$ mm) with each containing 24

187 randomly chosen plants. The bottom of each container was covered with an irrigation sheet
188 (400 g.m⁻²) that allowed to regulate the moisture of the compost. Plants were supplied with
189 water in case of prolonged drought. Damage caused by herbivorous insects were contained by
190 using a wintering veil. This veil also limited pollination.

191

192 **Phenotypic data**

193 We measured several vegetative traits on each individual: a phenological trait (the germination
194 date), a functional trait (the specific leaf area, SLA), a developmental trait (the average
195 internode length) and multiple morphological traits. Morphological traits included the basal
196 stem diameter, the number of branches on the plant, the number of vegetative nodes on the main
197 stem, and the total height of the plant. The SLA refers to leaf construction cost and captures
198 information about species leaf economic strategies (Wright et al., 2004). It was calculated as
199 the ratio between the cumulated area of five mature but non-senescent fresh leaves and their
200 oven-dried mass (Pujol, Salager, et al., 2008; Pérez-Harguindeguy et al., 2016). Leaf area was
201 measured by using the R package Momocs v. 1.2.9 (Bonhomme et al., 2014).

202

203 **Molecular analyses**

204 To infer genetic diversity estimates in each population and to compute F_{ST} , we genotyped the
205 637 plants. DNA was extracted from silica gel dried leaf samples using the Biosprint 15 DNA
206 Plant kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Individuals
207 were genotyped for 23 putatively neutral microsatellite markers that were developed for
208 population genetic studies (Debout et al., 2012; Pujol et al., 2017). To compute F_{ST} , we used
209 population pairwise F_{ST} estimates and the overall F_{ST} estimate amongst populations from the
210 study by Pujol et al. (2017). We used the GenoDive 3.0 software (Meirmans and Van Tienderen,

211 2004) to compute the complementary parameters required for this study, e.g., the genetic
212 diversity at each locus.

213

214 **Statistical analysis**

215 All statistical analyses were performed using the R.3.5.0 software (R Core Team, 2018).

216

217 *Phenotypic traits*

218 First, to test for phenotypic differences between subspecies, hierarchical generalized linear
219 models were conducted with population nested in subspecies. Second, for each subspecies
220 linear mixed models were conducted to test for phenotypic differences among populations, with
221 population as a fixed effect and the plastic container as a random effect. Estimates of marginal
222 means for each trait in each population were extracted using the emmeans package (Lenth et
223 al., 2019). These linear mixed-effects models were implemented in R via the lme4 package
224 (Bates et al., 2015). Traits change with altitude was analysed by using a linear regression of the
225 marginal means by altitude. Finally, mean phenotypic traits were also generated, and provided
226 in the Supplementary materials (Fig S1).

227

228 *Calculation of h^2 and phenotypic differentiation indices (Q_{ST}).*

229 For each subspecies, narrow-sense heritabilities (h^2) were estimated for each phenotypic trait
230 across all populations using a model with population, family and plastic containers as random
231 factors as $h^2 = 2 V_w / (V_w + V_{res})$, with V_w is family variance component and V_{res} is the residual
232 variance component corresponding to the within-population variance component. We
233 multiplied V_w by two in the calculation of h^2 because we used a full-sib crossing design (Roff,
234 1997). Caution must be taken when considering h^2 values because its estimation can be biased
235 by the estimation of $2V_w$. Indeed, h^2 was calculated based on all the families, without

236 considering the differences of h^2 between different populations. Confidence intervals of h^2 were
237 calculated following a parametric bootstrap method adapted from O'Hara and Merilä (2005).

238

239 For each trait and each subspecies, quantitative trait divergence indices (Q_{ST}) were generated
240 among populations (overall Q_{ST}) and for each population pair (population pairwise Q_{ST}), based
241 on mixed model analyses. In these models, population, family and plastic containers were
242 random factors. Variance components were extracted from these analyses for each trait and
243 used for estimating Q_{ST} using the following formula (Spitze, 1993): $Q_{ST} = V_b / (V_b + 2 h^2 V_w)$
244 with V_b being the trait genetic variance among populations. h^2 was calculated based on all the
245 families and populations by subspecies. Here, no environmental sources of phenotypic variance
246 due to the ecological conditions of the location of origin of populations could in theory bias Q_{ST}
247 estimates because data was obtained from a common garden experiment (Pujol, Wilson, et al.,
248 2008). When a variance component was non-significant, it was considered as null in further
249 calculations. When necessary (as for population pairwise Q_{ST} calculation), data was linearized
250 by using a square root transformation. All variance components were estimated by using the
251 linear mixed model approach implemented in the R package lme4 v. 1.1.17 (Bates et al., 2015).
252 Confidence intervals of Q_{ST} values were calculated following a parametric bootstrap method
253 adapted from O'Hara and Merilä (2005).

254

255 *Overall Q_{ST} - F_{ST} comparisons.*

256 We compared overall Q_{ST} and F_{ST} for each trait to investigate if divergence was compatible
257 with a scenario of genetic drift (overall $Q_{ST} = F_{ST}$), or whether it was more likely explained by
258 directional selection (overall $Q_{ST} > F_{ST}$) or by stabilizing selection (overall $Q_{ST} < F_{ST}$).
259 Comparisons between overall Q_{ST} and F_{ST} values were performed for each trait based on two
260 methods: i) a comparison of confidence intervals, the Q_{ST} is considered non significantly

261 different from neutral differentiation when the confidence interval of the overall Q_{ST} for a trait
262 overlaps the mean F_{ST} value, ii) a bootstrapping method developed by Whitlock and Guillaume
263 (2009). This last approach aims at comparing the observed difference between the overall Q_{ST}
264 and the F_{ST} with the expected simulated distribution of this difference under a scenario of
265 neutral evolution. We generated 100 000 bootstrap replicates of the expected $Q_{ST} - F_{ST}$
266 difference under the neutrality hypothesis for each trait, and built the corresponding
267 distribution. In this approach, P values were estimated by assessing whether the observed value
268 of the $Q_{ST} - F_{ST}$ difference overlapped its expected distribution under neutrality. We used the
269 modification by Lind *et al.* (2011) of the approach of Whitlock and Guillaume (2009) to
270 estimate the variance components of the simulated values of the $Q_{ST} - F_{ST}$ difference.

271

272 *Mantel tests*

273 Mantel tests (Mantel, 1967) were used to analyse correlations between geographic distances,
274 environmental distances (altitudinal), neutral genetic differentiation (population pairwise F_{ST}),
275 and quantitative genetic differentiation (population pairwise Q_{ST}). First, a correlation test
276 between population pairwise F_{ST} and population pairwise geographic distance matrices was
277 performed to test for an isolation by distance relationship. Second, a correlation test between
278 population pairwise F_{ST} and population pairwise Q_{ST} was performed for each trait to test if
279 neutral genetic differentiation explained divergence in quantitative traits. Third, a correlation
280 test between population pairwise Q_{ST} and population pairwise altitudinal differences was
281 performed for each trait to test whether divergence in quantitative traits was related to altitudinal
282 differences. Finally, we conducted partial mantel tests to test for the association between
283 population pairwise Q_{ST} and population pairwise altitude differences while controlling for
284 neutral genetic differentiation (F_{ST}). All mantel and partial mantel tests were performed in R,
285 with a significance $\alpha=0.05$, using the vegan package (Oksanen *et al.*, 2009).

286 RESULTS

287 Phenotypic differentiation between subspecies and populations

288 The two subspecies - *A. m. pseudomajus* and *A. m. striatum* - were significantly differentiated
289 by several phenotypic traits (Table 1 a, Figure S2). When grown in a common garden, plants
290 of *A. m. pseudomajus* were on average taller, with more branches and nodes than plants from
291 *A. m. striatum*. Their average germination date, internode length and SLA were however similar.
292 Differentiation between subspecies (c. 2.8 %) was lower than the differentiation estimated
293 among populations (c. 9.3 %, see mean R^2 in Table 1 a). Most of the traits showed a phenotypic
294 divergence among populations within each subspecies (see LRT in Table 1 b). Germination
295 date was the only trait that showed no significant difference among populations of *A. m.*
296 *pseudomajus* (see LRT in Table 1 b).

297

298 Neutral genetic differentiation

299 Population neutral genetic differentiation was low but significant. Overall F_{ST} among
300 populations of *A. m. pseudomajus* was 0.109 ($P < 0.001$), and ranged from 0.06 to 0.159 across
301 population pairs (see Table S2, and see Pujol et al., 2017 for more details on population pairwise
302 neutral genetic differentiation). F_{ST} among populations of *A. m. striatum* was 0.097 ($P < 0.001$),
303 and ranged from 0.055 to 0.131 (Table S2). There was no significant relationship between
304 population pairwise F_{ST} and population pairwise geographic distance for both subspecies
305 (Figure 2 a and b, *A. m. pseudomajus* Mantel $r = 0.04$, $P = 0.405$, *A. m. striatum* Mantel $r = -$
306 0.18 , $P = 0.595$). Similarly, there was no significant relationship between population pairwise
307 F_{ST} and population pairwise altitude difference for both subspecies (Figure 2 c), although the
308 Mantel tests showed a relationship close to significance levels in *A. m. pseudomajus* (*A. m.*
309 *pseudomajus* Mantel $r = 0.23$, $P = 0.052$, *A. m. striatum* Mantel $r = -0.3$, $P = 0.943$).

310

311 **Changes in phenotypic traits with altitude**

312 We found significant correlations between trait values (i.e. population estimates of marginal
313 means) and altitude for two traits across *A. m. striatum* populations. Plants from populations at
314 low altitude had more nodes and branches than plants from populations at high altitude of *A. m.*
315 *striatum* (Figure 3, see population arithmetic means in Figure S2 and population estimates of
316 marginal means for other traits in Figure S3). No phenotypic changes associated with altitude
317 were found significant in *A. m. pseudomajus*.

318

319 **Inheritance of quantitative traits**

320 Heritability estimates were comprised between 0.07 to 0.58 for *A. m. pseudomajus*, and 0.01 to
321 0.56 for *A. m. striatum* (Table S3, Supporting Information). The highest heritability estimates
322 were found for the internode length in *A. m. pseudomajus* (0.58) and the number of nodes in *A.*
323 *m. striatum* (0.56). Several traits had close to similar heritabilities between subspecies (stem
324 diameter, number of nodes), as illustrated by their overlapping confidence intervals. However,
325 other traits appeared to be different, with no CI overlap (germination date, number of branches,
326 plant height, internode length, SLA, Table S3).

327

328 **Q_{ST} - F_{ST} comparisons.**

329 Overall Q_{ST} was higher than mean F_{ST} for one trait in *A. m. pseudomajus* (number of branches,
330 Figure 4 a), and for three traits in *A. m. striatum* (number of branches, plant height and internode
331 length, Figure 4 b), as illustrated by their non-overlapping confidence intervals. Overall Q_{ST}
332 was lower than mean F_{ST} for the germination date in *A. m. pseudomajus* (Figure 4 a). We also
333 rejected the hypothesis of neutrality for these two traits in *A. m. pseudomajus* (number of
334 branches, germination date), and for three traits in *A. m. striatum* (number of branches, plant
335 height and internode length) on the basis of the bootstrapping method developed by Whitlock

336 and Guillaume (2009). For these traits, observed values of overall $Q_{ST} - F_{ST}$ differences were
337 either in the tail of the expected probability distribution under the hypothesis of neutrality, or
338 did not overlap with this distribution (Figure S4 et S5).

339 Mantel tests showed that population pairwise quantitative genetic differentiation (Q_{ST}) was not
340 correlated with population pairwise neutral genetic differentiation (F_{ST}) for all traits excepted
341 the germination date in *A. m. striatum* (Table 2). Population pairwise Q_{ST} for the germination
342 date was significantly correlated with population pairwise F_{ST} . They showed no support for the
343 overall $Q_{ST} < F_{ST}$ for the germination date in *A. m. pseudomajus*, almost certainly because of
344 three comparisons diverging abnormally from the overall pattern (Figure 5 b).

345

346 **Increased quantitative genetic differentiation with altitude difference**

347 Mantel tests showed a significant correlation between population pairwise Q_{ST} and population
348 pairwise altitudinal difference for two traits in *A. m. striatum*: the number of nodes and the
349 number of branches (Table 2, Figure 5). For both traits, the increase in pairwise population
350 differentiation associated with an increase in altitudinal difference was higher for the Q_{ST} than
351 for the F_{ST} (Figures 5 c and e). Partial mantel tests showed that population pairwise Q_{ST} was
352 significantly correlated with differences in altitude for the number of nodes (and marginally
353 significant for the number of branches, see Q_{ST} vs Alt. diff. / F_{ST} in Table 2) while controlling
354 for neutral genetic differentiation (F_{ST} matrix). This result is expected under the hypothesis that
355 the divergence among populations of *A. m. striatum* in the number of nodes is a result of
356 altitude-mediated divergent selection. In contrast, none of the seven traits showed a significant
357 correlation between population pairwise Q_{ST} and population pairwise altitude difference in *A.*
358 *m. pseudomajus*.

359

360 **DISCUSSION**

361 Our results support the hypothesis of differential adaptation between *A. m. pseudomajus* and *A.*
362 *m. striatum* subspecies. We detected phenotypic differentiation in a common garden among the
363 populations of *A. m. pseudomajus*, among the populations of *A. m. striatum*, and among
364 subspecies. For both subspecies, local adaptation and neutral evolution explained the extent to
365 which populations diverged over their geographic range, with slight differences between
366 subspecies. Potential divergence along altitude was also detected, but only for one subspecies:
367 *A. m. striatum*.

368

369 Our findings comforted the idea that Q_{ST} - F_{ST} comparisons are a good first step for exploring the
370 potential roles of divergent natural selection and neutral evolutionary processes in phenotypic
371 divergence (Whitlock, 2008; Edelaar et al., 2011; Ovaskainen et al., 2011; Whitlock and
372 Gilbert, 2012). They highlighted how traits can be used to identify the potential ecological
373 pressures underlying natural selection, with some traits potentially involved with *A. majus*
374 adaptation to the conditions of populations' local sites of origin, and a subsample of these traits
375 potentially playing a role in *A. m. striatum* adaptation to altitude.

376

377 **Adaptive evolution of *A. m. striatum* populations along the altitudinal gradient**

378 Our results brought indirect evidence supporting potential adaptive divergence between *A. m.*
379 *pseudomajus* and *A. m. striatum*. They imply that the quantitative genetic basis of two of the
380 seven traits under study (number of nodes, and marginally significant for the number of
381 branches) was shaped by divergent selection between populations from different altitudes in *A.*
382 *m. striatum* but not in *A. m. pseudomajus*. Most studies on plant adaptation to altitude report
383 the selection of smaller plants at higher altitudes (Körner, 1999; Halbritter et al., 2018). In
384 agreement with this expectation, we found that *A. m. striatum* plants at higher altitudes had less

385 branches and less nodes. There is also evidence for changes in leaf traits with elevation (Read
386 et al., 2014; Halbritter et al., 2018), with a decrease of SLA with elevation gradients. Our results
387 did not support a potential scenario of selection based on SLA at play in *A. m. striatum*.

388

389 **Support for different subspecies scenarios of adaptation to local sites of origin**

390 Our results showed that quantitative genetic differentiation was higher than what could be
391 explained by neutral evolutionary divergence among *A. m. pseudomajus* populations for one of
392 the seven studied traits (number of branches), and among *A. m. striatum* populations for three
393 of the seven traits (number of branches, plant height and internode length). They imply that
394 adaptation to local sites of origin potentially shaped the phenotypic diversity of populations for
395 both subspecies across their geographic range, with potentially different intensities reflected by
396 different signatures between subspecies. We used classical overall $Q_{ST} - F_{ST}$ comparisons to
397 detect potential adaptation to local sites conditions (Leinonen et al., 2008) and also more recent
398 methods to insure that our findings were robust against a range of neutral evolution scenarios
399 for these traits (Whitlock, 2008). Furthermore, our approach minimized the possibility that
400 phenotypic differences between populations were generated by environmental effects by using
401 a common garden experiment, and including trait heritability estimates in Q_{ST} calculations
402 (Spitze, 1993; Pujol, Wilson, et al., 2008). In contrast, four of the seven studied traits
403 (germination date, diameter, number of nodes and SLA) did not show departure from plausible
404 baseline scenarios of neutral evolutionary divergence, using overall $Q_{ST} - F_{ST}$ comparisons. One
405 particular trait (germination date) was in fact more similar among populations than expected
406 under neutrality in *A. m. pseudomajus*. A scenario of stabilizing selection is classically
407 extrapolated in the case of similar results (Lamy et al., 2012) but another plausible explanation
408 is that population similarity might have been caused by convergent phenotypic responses to the
409 common garden environmental similarity. Caution must be taken when interpreting different

410 Q_{ST} - F_{ST} patterns between subspecies as the signature of different adaptive processes. Here we
411 found different patterns between subspecies, which supports the hypothesis of their potential
412 adaptive divergence. Our results cannot be interpreted as direct proof for their adaptive
413 divergence, but only as evidence that this hypothesis has some potential.

414

415 **The ecological significance of adaptation to local sites of origin in *A. majus***

416 In the absence of environmental measures included in the overall Q_{ST} - F_{ST} analysis, it is
417 impossible to identify the potential environmental agents of local selection that shape the
418 quantitative genetic variation of traits. The functions behind the traits that have diverged can
419 nevertheless be used to discuss plausible evolutionary scenarios of natural selection. Our results
420 imply that adaptation to local sites of origin has potentially shaped the vegetative architecture
421 of plants that is specific to each *A. majus* population. The quantitative genetic variation of
422 several phenotypic traits characterising the vegetative growth and development of plants (plant
423 height, internode length, number of branches) has likely diverged among populations as a result
424 of adaptation to local sites of origin. Divergence in the genetic variation underlying the shape
425 and size of plants was already found at the level of *Antirrhinum* species but its adaptive
426 significance was not tested for (Langlade et al., 2005). In southern France and northern Spain,
427 under the Mediterranean climate, dryer locations are expected to select for plants with a bushier
428 vegetative architecture, i.e. plants with smaller leaves and more branches that have a better
429 water use efficiency and resilience to drought stress (Langlade et al., 2005). It is difficult to
430 identify exactly which environmental pressures underlay selection at local sites because several
431 combinations of environmental parameters (vegetation cover, wind, disturbance, temperature,
432 water availability, etc.) can interact to affect phenotypic traits.

433

434 **Gene flow, ecological and reproductive isolation**

435 Our findings imply that the most likely evolutionary scenario applying to *A. majus* requires
436 invoking a history of adaptation to local sites in a complex background of gene flow, ecological
437 heterogeneity and reproductive isolation. Pyrenees mountains are widely acknowledged to
438 constitute a heterogeneous landscape promoting complex patterns of population connectivity
439 and prone to generate local adaptation (Alberto et al., 2010). Q_{ST} - F_{ST} comparisons reflected a
440 potential scenario of population divergent adaptation to contrasting environmental conditions
441 between their local sites of origins. Our findings also suggested that evolutionary signatures of
442 local adaptation differed between *A. m. pseudomajus* and *A. m. striatum*, which includes the
443 potential adaptation to altitude of *A.m. striatum* populations. One might speculate that this
444 divergence might be related to the distribution of *A. m. striatum* populations across a narrower
445 range of climatic conditions, even if both subspecies share to a large extent the same ecological
446 niche (Khimoun et al., 2013). However, caution must be taken with this explanation because
447 the state of the environment in the past, when divergence might have occurred, is unknown and
448 might have differed. Contrasting hypotheses might be interesting to consider, e.g., different
449 evolutionary potentials in the presence of similar environmental pressures. These scenarios are
450 not exclusive and can reinforce each other through a feedback loop between reproductive
451 isolation, neutral divergence and selection.

452

453 Restricted gene flow or strong selection pressures are required for evolutionary divergence.
454 Genetic drift, or foundation events by different gene pools, might have shaped differentially the
455 genetic background of *A. majus* populations and to some extent subspecies at the scale of their
456 global geographic range. There is evidence for the genetic signature of restricted gene
457 exchanges in *A. majus* (Pujol et al., 2017). No genetic isolation by distance was found but
458 ecological barriers characterizing the mountain landscape of the Pyrenees likely participate to
459 isolate populations (Pujol et al., 2017). At first sight, *A. majus* subspecies divergence might not

460 be expected because both subspecies are interfertile (Andalo et al., 2010), and no genome wide
461 barrier to gene flow was found between them at the scale of a hybrid zone across c. 2km in the
462 Pyrenees (Ringbauer et al., 2018). There is also evidence for gene exchanges between the two
463 subspecies in several contact zone locations across at the periphery of their geographic ranges
464 (Khimoun et al., 2011). Yet, subspecies flower color differences attest that flower color genes
465 are under frequency dependent selection and generate reproductive isolation between
466 subspecies (Tastard et al., 2012; Ringbauer et al., 2018). This reproductive isolation might
467 participate to the subspecies phenotypic divergence of other traits that we detected here.

468

469 **CONCLUSION**

470 Our findings corroborate the utility of $Q_{ST} - F_{ST}$ approaches conducted in common garden
471 experiments to explore potential adaptive evolutionary divergence among populations and
472 between subspecies in plants. They also illustrate the limit of this approach that identifies traits
473 that might be involved with local adaptation but does not bring direct evidence for their
474 response to selection. Here, our common garden results for *A. m. pseudomajus* and *A. m.*
475 *striatum* populations identified vegetative traits that might play a role in the local adaptation
476 and the differential adaptation of *A. m. pseudomajus* and *A. m. striatum* along altitudinal
477 gradients. They suggest that the adaptation to climate variables of otherwise interfertile
478 subspecies might differ as a result of reproductive isolation.

479

480 **ACKNOWLEDGMENTS**

481 We thank Jessica Côte for helpful discussions on the methods and David Field for helpful
482 comments on the manuscript. This project has received funding from the European Research
483 Council (ERC) under the European Union's horizon 2020 research and innovation program
484 (grant agreement No ERC-CoG-2015-681484-ANGI) awarded to BP. This work was supported

485 by funding from the French “Agence Nationale de la Recherche” (ANR-13-JSV7-0002
486 “CAPA”) to BP. This project was also supported by the ANR funded French Laboratory of
487 Excellence projects “LABEX TULIP” and “LABEX CEBA” (ANR-10-LABX-41, ANR-10-
488 LABX-25-01).

489

490 TABLE 1
 491 Effects of subspecies and populations on phenotypic traits. a) R² and P-value from hierarchical
 492 generalized linear models (GLM) with subspecies alone and populations nested in subspecies,
 493 implemented in JMP® (Version X ,SAS Institute Inc., Cary, NC, 1989-2019). b) Likelihood
 494 Ratio Tests (LRT) comparing the maximum-likelihood fit between a model where populations
 495 were pooled and a model estimating the effect of the population of origin. A significant P- value
 496 means the model including populations effect fitted the data better than the null model.
 497 Significant results (P-value < 0.05) are in bold.

a)	Subspecies		Populations in subspecies	
	R ²	P-value	R ²	P-value
Germination date	0.0005	0.587	0.02	0.260
Diameter	0.007	0.00028	0.05	0.00068
Nodes	0.06	<0.0001	0.13	<0.0001
Branches	0.03	<0.0001	0.06	0.00001
Plant height	0.09	<0.0001	0.21	<0.0001
Internode length	0.0002	0.708	0.13	0
SLA	0.005	0.066	0.05	0.0003
Mean	0.028		0.093	

b)	<i>A. m. pseudmomajus</i>		<i>A. m. striatum</i>	
	LRT	P-value	LRT	P-value
Germination date	6	0.570	12	0.021
Diameter	18	0.001	23	<0.0001
Nodes	30	<0.0001	20	<0.0001
Branches	70	<0.0001	26	<0.0001
Plant height	32	<0.0001	81	<0.0001
Internode length	64	<0.0001	37	<0.0001
SLA	21	0.004	15	0.004

498

499

500 TABLE 2

501 Mantel tests and partial Mantel tests on pairwise Q_{ST} vs F_{ST} and Q_{ST} vs difference in altitude of
 502 origin (Alt. diff.), as well as partial Mantel tests on Q_{ST} vs Alt. diff. controlled for F_{ST} , for
 503 phenology traits in a) eight populations of *A. m. pseudomajus* and b) five populations of *A. m.*
 504 *striatum*, that were grown in a common garden. Significant values are indicated in bold.

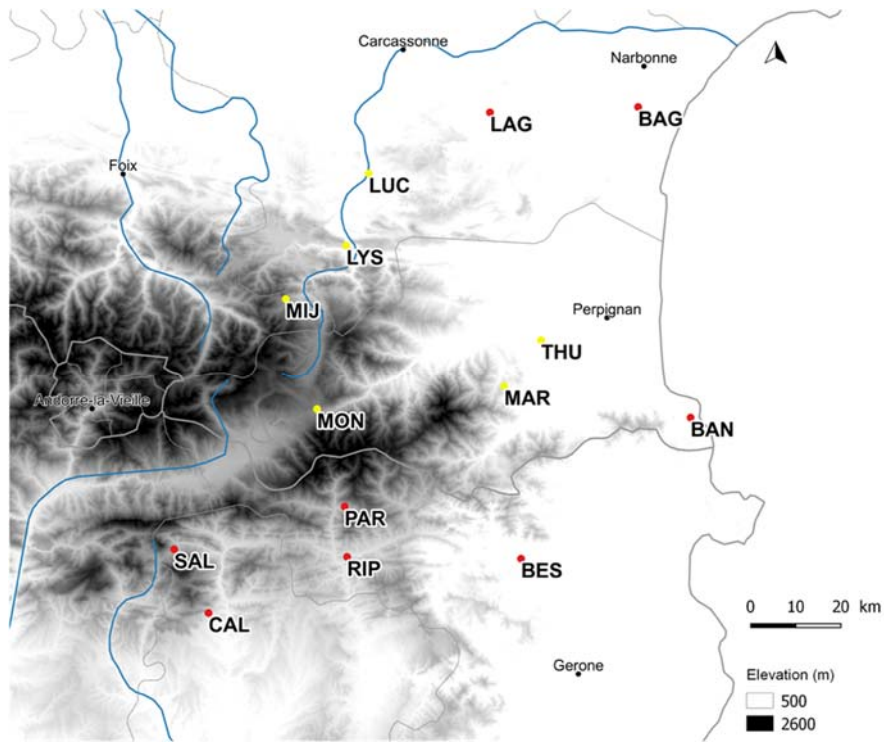
Traits	a) <i>A. majus pseudomajus</i>					
	Q_{ST} vs F_{ST}		Q_{ST} vs Alt. diff.		Q_{ST} vs Alt. diff. / F_{ST}	
	Mantel r	P-value	Mantel r	P-value	Mantel r	P-value
Germination date	-0.37	0.931	-0.13	0.737	-0.06	0.593
Diameter	-0.09	0.636	-0.15	0.812	-0.13	0.780
Nodes	0.07	0.426	-0.16	0.820	-0.18	0.888
Branches	0.07	0.329	-0.14	0.750	-0.17	0.820
Height	0.23	0.191	-0.13	0.751	-0.19	0.911
Internode length	0.24	0.184	0.03	0.335	-0.02	0.442
SLA	0.23	0.229	0.02	0.379	-0.04	0.529

Traits	b) <i>A. majus striatum</i>					
	Q_{ST} vs F_{ST}		Q_{ST} vs Alt. diff.		Q_{ST} vs Alt. diff. / F_{ST}	
	Mantel r	P-value	Mantel r	P-value	Mantel r	P-value
Germination date	0.53	0.042	0.05	0.333	0.26	0.267
Diameter	-0.01	0.508	-0.02	0.458	-0.03	0.517
Nodes	-0.3	0.842	0.95	0.008	0.94	0.008
Branches	-0.18	0.750	0.89	0.033	0.90	0.058
Height	-0.58	0.883	0.08	0.283	-0.12	0.6
Internode length	0.34	0.267	-0.02	0.492	0.09	0.258
SLA	-0.69	0.883	0.07	0.367	-0.2	0.858

505

506

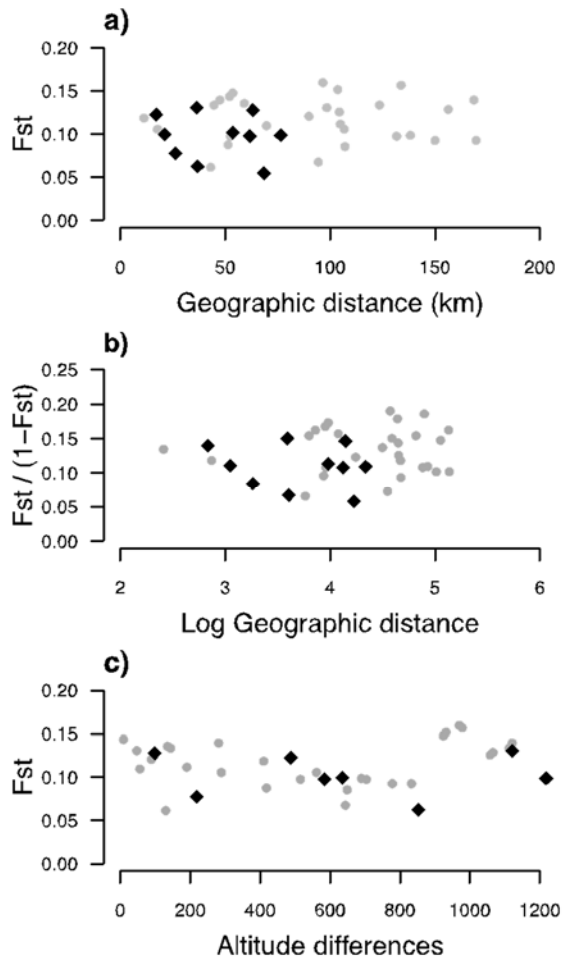
507 **FIGURE**



508

509 **FIGURE 1**

510 Map of *Antirrhinum majus* populations that were sampled across the geographic range of the
511 species in Southern France. Red dots represent *A. m. pseudomajus* populations, yellow dots
512 represent *A. m. striatum* populations. Population names and description can be found in Table
513 S1.

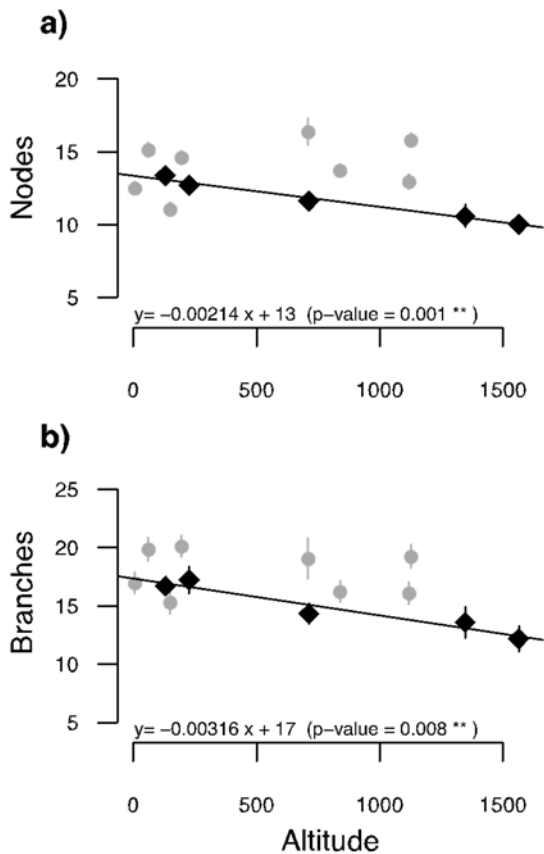


514

515 FIGURE 2

516 Pairwise neutral genetic differentiation F_{ST} plotted against pairwise geographic distances or
 517 altitudinal differences among eight *Antirrhinum majus pseudomajus* populations pairs (grey
 518 dots), and five *A. m. striatum* populations pairs (black diamonds). There were non-significant
 519 linear regression between a) F_{ST} and geographic distance in *A. m. pseudomajus* ($y = 1e-05 x +$
 520 0.116 , P -value = 0.92 ns) and in *A. m. striatum* ($y = -0.00019 x + 0.106$, $P = 0.67$ ns), b) $F_{ST} / (1 -$
 521 $F_{ST})$ and the log of geographic distance in *A. m. pseudomajus* ($y = 0.0022 x + 0.124$, $P = 0.83$
 522 ns) and in *A. m. striatum* ($y = -0.0105 x + 0.148$, $P = 0.63$ ns), c) F_{ST} and altitude differences in
 523 *A. m. pseudomajus* ($y = 2.e-05 x + 0.109$, $P = 0.26$ ns) and in *A. m. striatum* ($y = -2e-05 x + 0.111$,
 524 $P = 0.39$ ns).

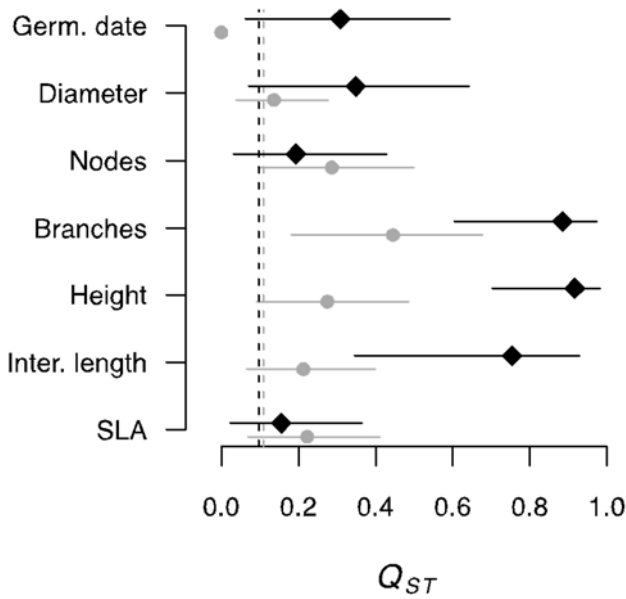
525



526

527 FIGURE 3

528 Population estimates of marginal means with standard errors of two phenotypic traits (a. number
 529 of nodes, b. number of branches) in populations of two subspecies of *Antirrhinum majus* grown
 530 in a common garden. Means are plotted against altitude of origin. Lines refer to the linear
 531 regression between trait mean estimates and altitude. Grey dots represent *A. m. pseudomajus*
 532 populations, black diamonds represent *A. m. striatum* populations. Equation of non-significant
 533 linear regressions were a) $y = 0.00125x + 12$ ($P = 0.43$ ns) and b), $y = -0.00048x + 18$ ($P = 0.78$
 534 ns) for *A. m. pseudomajus*.



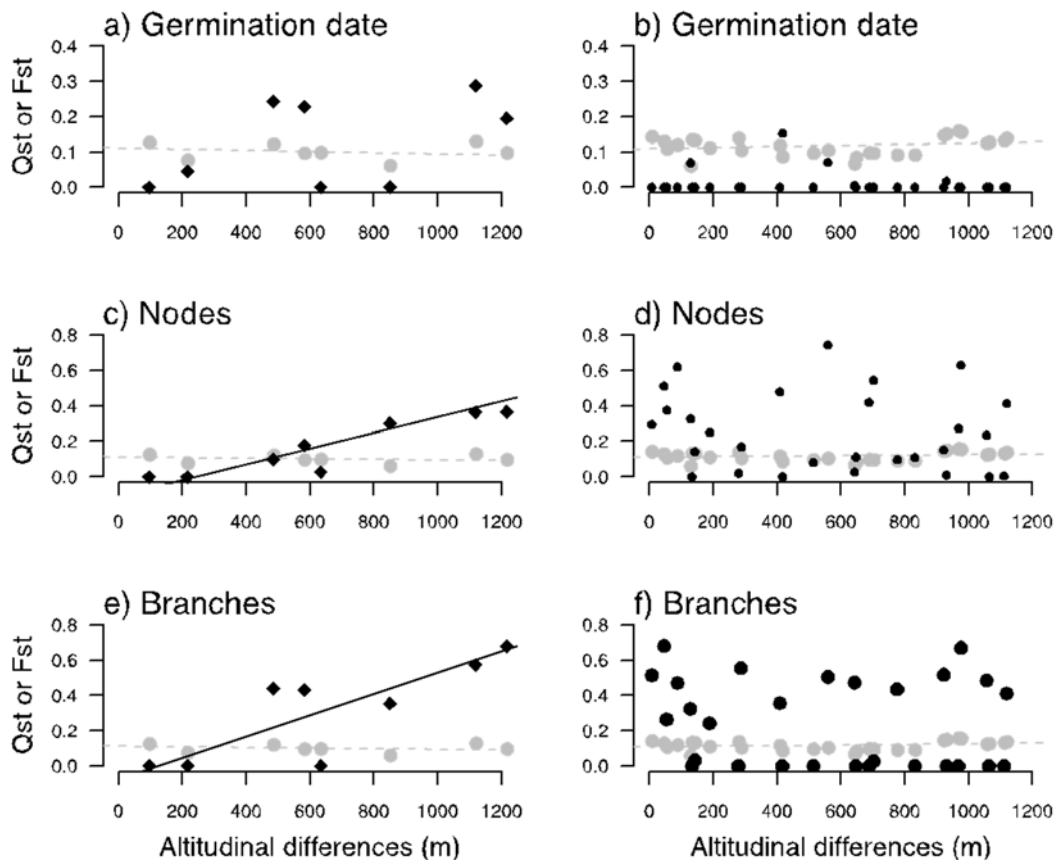
535

536 FIGURE 4

537 Overall Q_{ST} estimates with their 95% CI characterizing seven phenotypic traits in eight
 538 *Antirrhinum majus pseudomajus* populations (grey dots) and five *A. m. striatum* populations
 539 (black diamonds) that were grown in a common garden. Average population F_{ST} is represented
 540 by the dashed grey line for *A. m. pseudomajus*, and the dashed black line for *A. m. striatum*.
 541 Germ.date = germination date, Diameter= stem diameter, Nodes = number of nodes, Branches
 542 = number of branches, Height= plant height, Inter. Length= internodes length, SLA= specific
 543 leaf area.

544

545



546

547 FIGURE 5

548 Population pairwise quantitative trait differentiation (Q_{ST}) for the germination date, the number

549 of branches and the number of nodes in *Antirrhinum majus striatum* (a, c and e, black diamonds)

550 and *A. m. pseudomajus* (b, d and f, black dots). Dashed line indicates the trend and P the level

551 of significance of the linear regression between the population pairwise Q_{ST} and population

552 pairwise altitudinal differences (m). Grey dots and dashed line refer to population neutral

553 genetic differentiation (F_{ST}). Equation of linear regressions were a) $y = 1e-05 x + 0.09$ ($P = 0.9$

554 ns), b) $1e-05 x + 0.018$ ($P = 0.5$ ns), c) $y = 0.00044 x - 0.107$ ($P = 0$ ***), d) $y = -9e-05 x + 0.303$

555 ($P = 0.42$ ns), e) $y = 6e-04 x - 0.076$ ($P = 5e-04$ ***), f) $y = -1e-04 x + 0.302$ ($P = 0.46$ ns).

556

557

558

559 **Reference List**

- 560 Abbott, R. J., and A. C. Brennan. 2014. Altitudinal gradients, plant hybrid zones and
561 evolutionary novelty. *Philosophical Transactions of the Royal Society B: Biological Sciences*
562 369: 20130346.
- 563 Alberto, F., J. Niort, J. Derory, O. Lepais, R. Vitalis, D. Galop, and A. Kremer. 2010. Population
564 differentiation of sessile oak at the altitudinal front of migration in the French Pyrenees: genetic
565 diversity of oaks in the Pyrenees. *Molecular Ecology* 19: 2626–2639.
- 566 Andalo, C., M. B. Cruzan, C. Cazettes, B. Pujol, M. Burrus, and C. Thébaud. 2010. Post-
567 pollination barriers do not explain the persistence of two distinct *Antirrhinum* subspecies with
568 parapatric distribution. *Plant Systematics and Evolution* 286: 223–234.
- 569 Angert, A. L., and D. W. Schemske. 2005. The evolution of species' distributions: reciprocal
570 transplants across the elevation ranges of *Mimulus cardinalis* and *M. lewisii*. *Evolution* 59:
571 1671–1684.
- 572 Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models
573 using **lme4**. *Journal of Statistical Software* 67.
- 574 Bonhomme, V., S. Picq, C. Gaucherel, and J. Claude. 2014. **Momocs**: outline analysis using *R*.
575 *Journal of Statistical Software* 56.
- 576 Debout, G. D. G., E. Lhuillier, P.-J. G. Malé, B. Pujol, and C. Thébaud. 2012. Development
577 and characterization of 24 polymorphic microsatellite loci in two *Antirrhinum majus* subspecies
578 (Plantaginaceae) using pyrosequencing technology. *Conservation Genetics Resources* 4: 75–
579 79.
- 580 Donohue, K., R. Rubio de Casas, L. Burghardt, K. Kovach, and C. G. Willis. 2010.
581 Germination, postgermination adaptation, and species ecological ranges. *Annual Review of*
582 *Ecology, Evolution, and Systematics* 41: 293–319.
- 583 Edelaar, P., P. Burraco, and I. Gomez-Mestre. 2011. Comparisons between Q_{ST} and F_{ST} —how
584 wrong have we been? *Molecular Ecology* 20: 4830–4839.
- 585 Etterson, J. R. 2004. Evolutionary potential of *Chamaecrista fasciculata* in relation to climate
586 change. II. Genetic architecture of three populations reciprocally planted along an
587 environmental gradient in the great plains. *Evolution* 58: 1459–1471.
- 588 Gimenez-Benavides, L., A. Escudero, and J. M. Iriondo. 2006. Local adaptation enhances
589 seedling recruitment along an altitudinal gradient in a high mountain mediterranean plant.
590 *Annals of Botany* 99: 723–734.
- 591 Halbritter, A. H., S. Fior, I. Keller, R. Billeter, P. J. Edwards, R. Holderegger, S. Karrenberg,

592 et al. 2018. Trait differentiation and adaptation of plants along elevation gradients. *Journal of*
593 *Evolutionary Biology* 31: 784–800.

594 Hereford, J. 2009. A quantitative survey of local adaptation and fitness trade-offs. *The American*
595 *Naturalist* 173: 579–588.

596 Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. *Ecology Letters* 7:
597 1225–1241.

598 Khimoun, A., M. Burrus, C. Andalo, Z.-L. Liu, C. Vicédo-Cazettes, C. Thébaud, and B. Pujol.
599 2011. Locally asymmetric introgressions between subspecies suggest circular range expansion
600 at the *Antirrhinum majus* global scale. *Journal of Evolutionary Biology* 24: 1433–1441.

601 Khimoun, A., J. Cornuault, M. Burrus, B. Pujol, C. Thebaud, and C. Andalo. 2013. Ecology
602 predicts parapatric distributions in two closely related *Antirrhinum majus* subspecies.
603 *Evolutionary Ecology* 27: 51–64.

604 Kim, E., and K. Donohue. 2013. Local adaptation and plasticity of *Erysimum capitatum* to
605 altitude: its implications for responses to climate change. *Journal of Ecology* 101: 796–805.

606 Körner, C. 1999. Alpine plant life. Springer Berlin Heidelberg, Berlin, Heidelberg.

607 Lamy, J.-B., C. Plomion, A. Kremer, and S. Delzon. 2012. $Q_{ST} < F_{ST}$ as a signature of
608 canalization. *Molecular Ecology* 21: 5646–5655.

609 Langlade, N. B., X. Feng, T. Dransfield, L. Copsey, A. I. Hanna, C. Thebaud, A. Bangham, et
610 al. 2005. Evolution through genetically controlled allometry space. *Proceedings of the National*
611 *Academy of Sciences* 102: 10221–10226.

612 Leimu, R., and M. Fischer. 2008. A meta-analysis of local adaptation in plants. *PLoS ONE* 3:
613 e4010.

614 Leinonen, T., R. J. S. McCairns, R. B. O’Hara, and J. Merilä. 2013. Q_{ST} - F_{ST} comparisons:
615 evolutionary and ecological insights from genomic heterogeneity. *Nature Reviews Genetics* 14:
616 179–190.

617 Leinonen, T., R. B. O’Hara, J. M. Cano, and J. Merilä. 2008. Comparative studies of
618 quantitative trait and neutral marker divergence: a meta-analysis: Q_{ST} - F_{ST} meta-analysis.
619 *Journal of Evolutionary Biology* 21: 1–17.

620 Lenth, R., H. Singmann, J. Love, P. Buerkner, and M. Herve. 2019. Package ‘emmeans’:
621 Estimated marginal means, aka least-squares means. Version 1.4.3.01. *CRAN*.

622 Lind, M. I., P. K. Ingvarsson, H. Johansson, D. Hall, and F. Johansson. 2011. Gene flow and
623 selection on phenotypic plasticity in an island system of *Rana temporaria*. *Evolution* 65: 684–
624 697.

625 Mantel, N. 1967. The detection of disease clustering and a generalized regression approach.

626 *Cancer Research* 27: 209–220.

627 McKay, J. K., and R. G. Latta. 2002. Adaptive population divergence: markers, QTL and traits.
628 *Trends in Ecology & Evolution* 17: 285–291.

629 Meirmans, P.G., and P.H. Van Tienderen. 2004. GENOTYPE and GENODIVE: two programs
630 for the analysis of genetic diversity of asexual organisms, *Molecular Ecology Notes* 4: 792-794.

631 Merilä, J., and P. Crnokrak. 2001. Comparison of genetic differentiation at marker loci and
632 quantitative traits: Natural selection and genetic differentiation. *Journal of Evolutionary*
633 *Biology* 14: 892–903.

634 O’Hara, R. B., and J. Merilä. 2005. Bias and precision in Q_{ST} estimates: problems and some
635 solutions. *Genetics* 171: 1331–1339.

636 Oksanen, J., R. Kindt, P. Legendre, B. O’Hara, G. Simpson, P. Solymos, M. Stevens, and H.
637 Wagner. 2009. The VEGAN Package: community ecology package.

638 Ovaskainen, O., M. Karhunen, C. Zheng, J. M. C. Arias, and J. Merilä. 2011. A new method to
639 uncover signatures of divergent and stabilizing selection in quantitative traits. *Genetics* 189:
640 621–632.

641 Pérez-Harguindeguy, N., S. Díaz, E. Garnier, S. Lavorel, H. Poorter, P. Jaureguiberry, M. S.
642 Bret-Harte, et al. 2016. Corrigendum to: New handbook for standardised measurement of plant
643 functional traits worldwide. *Australian Journal of Botany* 64: 715.

644 Pujol, B., J. Archambeau, A. Bontemps, M. Lascoste, S. Marin, and A. Meunier. 2017.
645 Mountain landscape connectivity and subspecies appurtenance shape genetic differentiation in
646 natural plant populations of the snapdragon (*Antirrhinum majus* L.). *Botany Letters* 164: 111–
647 119.

648 Pujol, B., J.-L. Salager, M. Beltran, S. Bousquet, and D. McKey. 2008. Photosynthesis and leaf
649 structure in domesticated Cassava (Euphorbiaceae) and a close wild relative: have leaf
650 photosynthetic parameters evolved under domestication? *Biotropica* 40: 305–312.

651 Pujol, B., A. J. Wilson, R. I. C. Ross, and J. R. Pannell. 2008. Are $Q_{ST} - F_{ST}$ comparisons for
652 natural populations meaningful? *Molecular Ecology* 17: 4782–4785.

653 R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for
654 Statistical Computing, Vienna, Austria.

655 Read, Q. D., L. C. Moorhead, N. G. Swenson, J. K. Bailey, and N. J. Sanders. 2014. Convergent
656 effects of elevation on functional leaf traits within and among species. *Functional Ecology* 28:
657 37–45.

658 Ringbauer, H., A. Kolesnikov, D. L. Field, and N. H. Barton. 2018. Estimating barriers to gene
659 flow from distorted isolation-by-distance patterns. *Genetics* 208: 1231–1245.

660 Roff, D. A. 1997. *Evolutionary Quantitative Genetics*. Springer US, Boston, MA.

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

663 Tastard, E., J.-B. Ferdy, M. Burrus, C. Thébaud, and C. Andalo. 2012. Patterns of floral colour
664 neighbourhood and their effects on female reproductive success in an *Antirrhinum* hybrid zone:
665 flower colour and selection in a hybrid zone. *Journal of Evolutionary Biology* 25: 388–399.

666 Tavares, H., A. Whibley, D. L. Field, D. Bradley, M. Couchman, L. Copsey, J. Elleouet, et al.
667 2018. Selection and gene flow shape genomic islands that control floral guides. *Proceedings of*
668 *the National Academy of Sciences* 115: 11006–11011.

669 Whibley, A. C. 2006. Evolutionary paths underlying flower color variation in *Antirrhinum*.
670 *Science* 313: 963–966.

671 Whitlock, M. C. 2008. Evolutionary inference from Q_{ST} . *Molecular Ecology* 17: 1885–1896.

672 Whitlock, M. C., and K. J. Gilbert. 2012. Q_{ST} in a hierarchically structured population.
673 *Molecular Ecology Resources* 12: 481–483.

674 Whitlock, M. C., and F. Guillaume. 2009. Testing for spatially divergent selection: comparing
675 Q_{ST} to F_{ST} . *Genetics* 183: 1055–1063.

676 Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-
677 Bares, et al. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821–827.

678

679

680 **Supplementary material**681 **TABLES**682 TABLE S1 Description of *Anthrimum majus* populations grown in the common garden683 experiment. *Nfam*= number of families, *N*= number of plants

Acronym	Latitude	Longitude	Location	Elevation (m)	Subspecies	Description	<i>Nfam</i>	<i>N</i>
BAG	43.10	2.98	Bages	6	<i>pseudomajus</i>	Dunes on seaside (rocky / herbaceous)	40	67
BAN	42.49	3.12	Banyuls-sur-Mer	61	<i>pseudomajus</i>	Rockside bank (rocky)	32	54
THU	42.64	2.72	Thuir	130	<i>striatum</i>	Roadside bank (herbaceous)	34	60
LAG	43.09	2.58	Lagrasse	149	<i>pseudomajus</i>	Roadside bank (rocky / herbaceous)	32	55
BES	42.21	2.67	Besalú	195	<i>pseudomajus</i>	Stone walls in village	39	69
LUC	42.97	2.26	Luc-sur-Aude	227	<i>striatum</i>	Roadside bank and river-side bank (rocky)	19	29
RIP	42.21	2.20	Ripoll	709	<i>pseudomajus</i>	Roadside bank (herbaceous)	9	16
LYS	42.83	2.20	'Pierre-Lys' gorge	713	<i>striatum</i>	Roadside bank (rocky / herbaceous)	32	53
CAL	42.10	1.83	Berga	838	<i>pseudomajus</i>	Roadside bank (herbaceous)	42	69
PAR	42.31	2.20	Pardines	1118	<i>pseudomajus</i>	Roadside bank (herbaceous)	32	58
SAL	42.23	1.74	Saldes	1126	<i>pseudomajus</i>	Banks in pasture (herbaceous)	30	55
MIJ	42.73	2.04	Mijanès	1347	<i>striatum</i>	Roadside bank (herbaceous)	10	18
MON	42.51	2.12	Mont-Louis citadelle	1564	<i>striatum</i>	Stone walls on fortifications	21	34
<i>All populations</i>							372	637

684 TABLE S2

685 Population pairwise F_{ST} for a) *Anthirinum majus pseudomajus* and b) *A. m. striatum*.

a) *Anthirinum majus pseudomajus*

Obs.	BAG	BAN	BES	CAL	LAG	PAR	RIP	SAL
BAG	0.000000	0.109661	0.111560	0.093282	0.134302	0.134404	0.097873	0.140489
BAN	0.109661	0.000000	0.136375	0.093343	0.120889	0.125510	0.086353	0.128994
BES	0.111560	0.136375	0.000000	0.068186	0.131472	0.147507	0.098488	0.152424
CAL	0.093282	0.093343	0.068186	0.000000	0.099317	0.139897	0.062174	0.106419
LAG	0.134302	0.120889	0.131472	0.099317	0.000000	0.159517	0.106148	0.156761
PAR	0.134404	0.125510	0.147507	0.139897	0.159517	0.000000	0.119122	0.144271
RIP	0.097873	0.086353	0.098488	0.062174	0.106148	0.119122	0.000000	0.088219
SAL	0.140489	0.128994	0.152424	0.106419	0.156761	0.144271	0.088219	0.000000

b) *Anthirinum majus striatum*

Obs	LUC	LYS	MIJ	MON	THU
LUC	0.000	0.123	0.131	0.102	0.128
LYS	0.123	0.000	0.100	0.063	0.098
MIJ	0.131	0.100	0.000	0.078	0.099
MON	0.102	0.063	0.078	0.000	0.055
THU	0.128	0.098	0.099	0.055	0.000

686

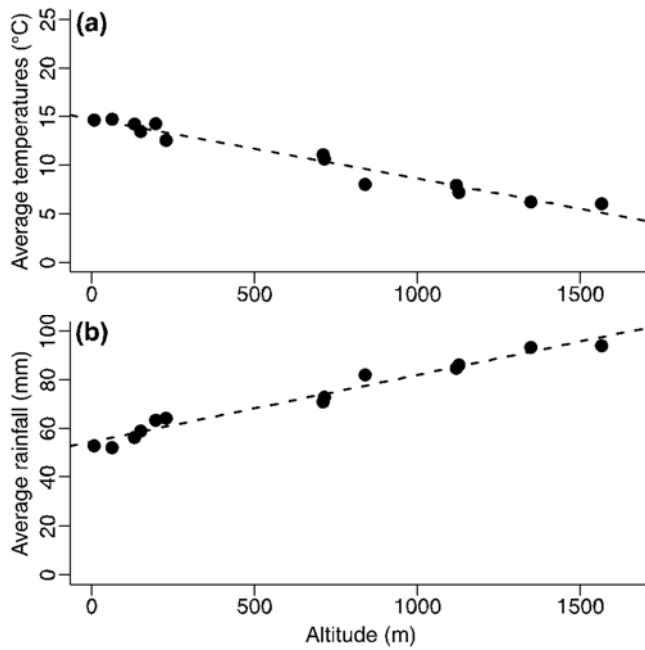
687

688 TABLE S3

689 Quantitative genetics parameters for phenotypic traits among eight populations of *Anthrinum*
 690 *majus pseudomajus* and five populations of *Anthrinum majus striatum* grown in a common
 691 garden. Values for trait heritability (h^2), family variance (V_w), among-population variance (V_b),
 692 residual variance corresponding to the within-population variance (V_{res}). The degrees of
 693 freedom used in the bootstrapping procedures are seven for the among-population component
 694 (V_b) for *A.m. pseudomajus* and four for *A. m. striatum*. Degrees of freedom are given in this
 695 table for the within-population component (df V_w).

Traits	h^2	h^2 .CI	V_w	V_b	V_{res}	df V_w
a) <i>A. m. pseudomajus</i>						
Germination date	0.35	0.3; 0.42 0.14;	14.05	0	65.13	246
Diameter	0.17	0.20 0.39;	0.06	0.02	0.69	248
Nodes	0.45	0.52 0.06;	3.03	2.62	10.37	248
Branches	0.07	0.09 0.26;	1.54	2.83	42.11	248
Plant height	0.32	0.38 0.51;	19.71	16.1	105.45	184
Internode length	0.58	0.67 0.10;	0.08	0.05	0.19	248
SLA	0.12	0.15	128.26	78.01	1973.77	248
b) <i>A. m. striatum</i>						
Germination date	0.11	0.09; 0.15	4.37	4.49	71.98	110
Diameter	0.20	0.26 0.46;	0.06	0.08	0.57	111
Nodes	0.56	0.69 0.01;	2.94	1.55	7.6	111
Branches	0.01	0.01 0.04;	0.14	3.52	26.14	111
Plant height	0.06	0.08 0.04;	2.48	92.94	84	82
Internode length	0.05	0.06 0.27;	0.01	0.05	0.23	111
SLA	0.34	0.43	397.54	155.9	1942.69	111

696 **Figures**

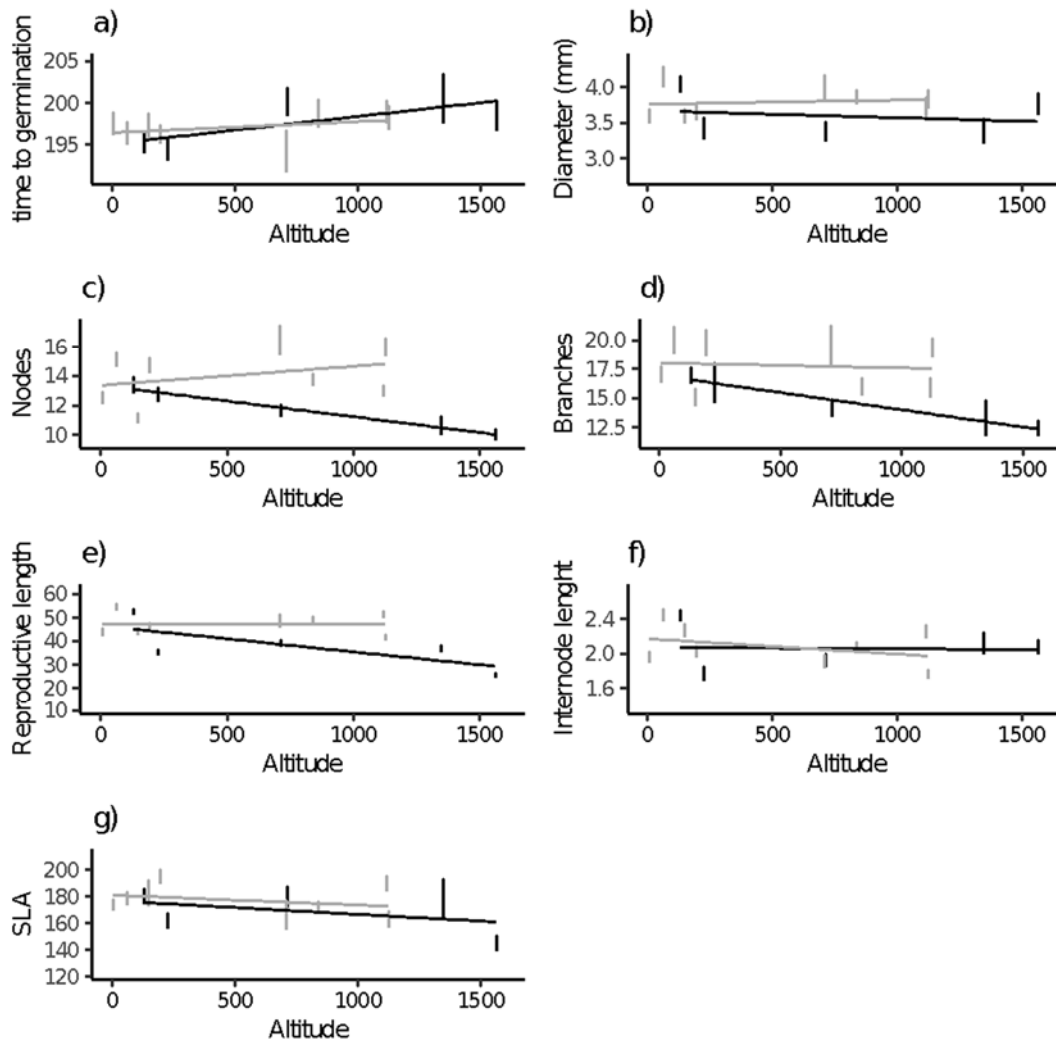


697

698 **FIGURE S1.**

699 Annual average temperatures and rainfall of 13 *Antirrhinum majus* populations from the
700 Southern France. Population average temperature (a) and average rainfall (b) as a function of
701 altitude. Bioclimatic data was extracted from the *WorldClim* database (www.worldclim.org).

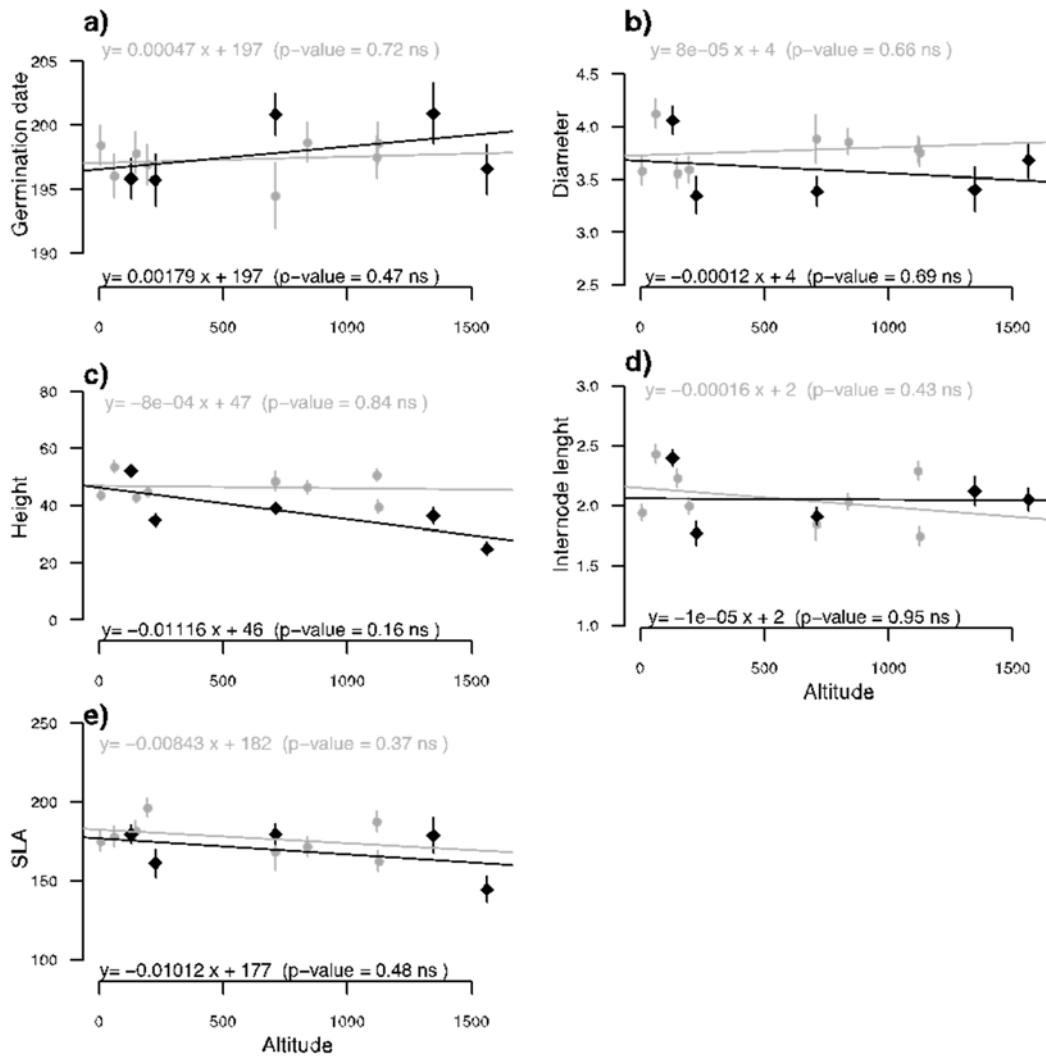
702



703

704 FIGURE S2.

705 Population arithmetic means with standard errors of seven phenotypic traits in populations of
 706 two subspecies of *Antirrhinum majus* grown in a common garden. Means are plotted against
 707 altitude of origin. Grey dots represent *A. m. ssp. pseudomajus* populations, black diamonds
 708 represent *A. m. ssp. striatum* populations.

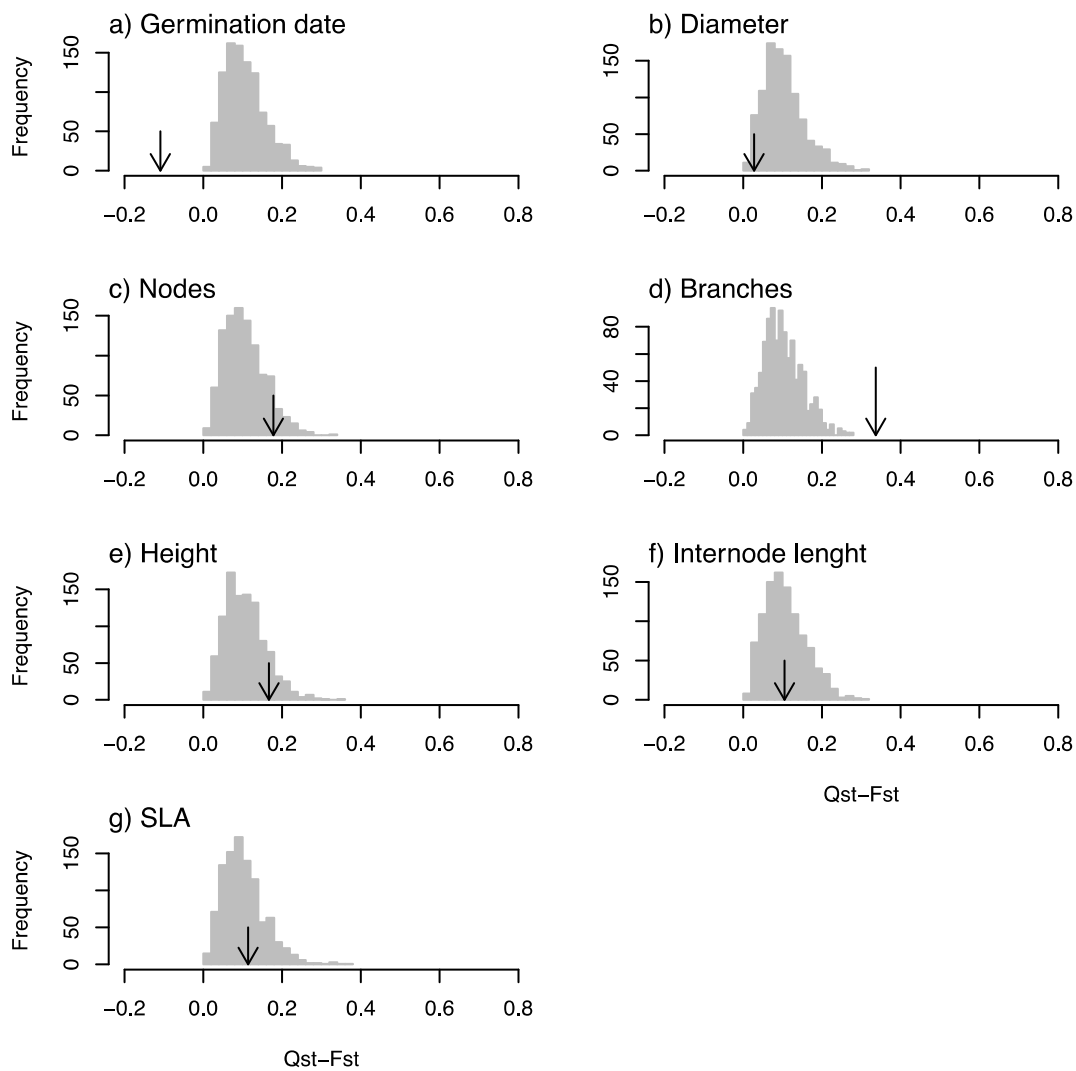


709

710 FIGURE S3. Population estimates of marginal means with standard errors of five phenotypic
 711 traits in populations of two subspecies of *Antirrhinum majus* grown in a common garden. Means
 712 are plotted against altitude of origin. Lines refer to the linear regression between traits means
 713 estimates and altitude. Grey dots and lines represent *A. m. pseudomajus* populations, black
 714 diamonds and lines represent *A. m. ssp. striatum* populations.

715

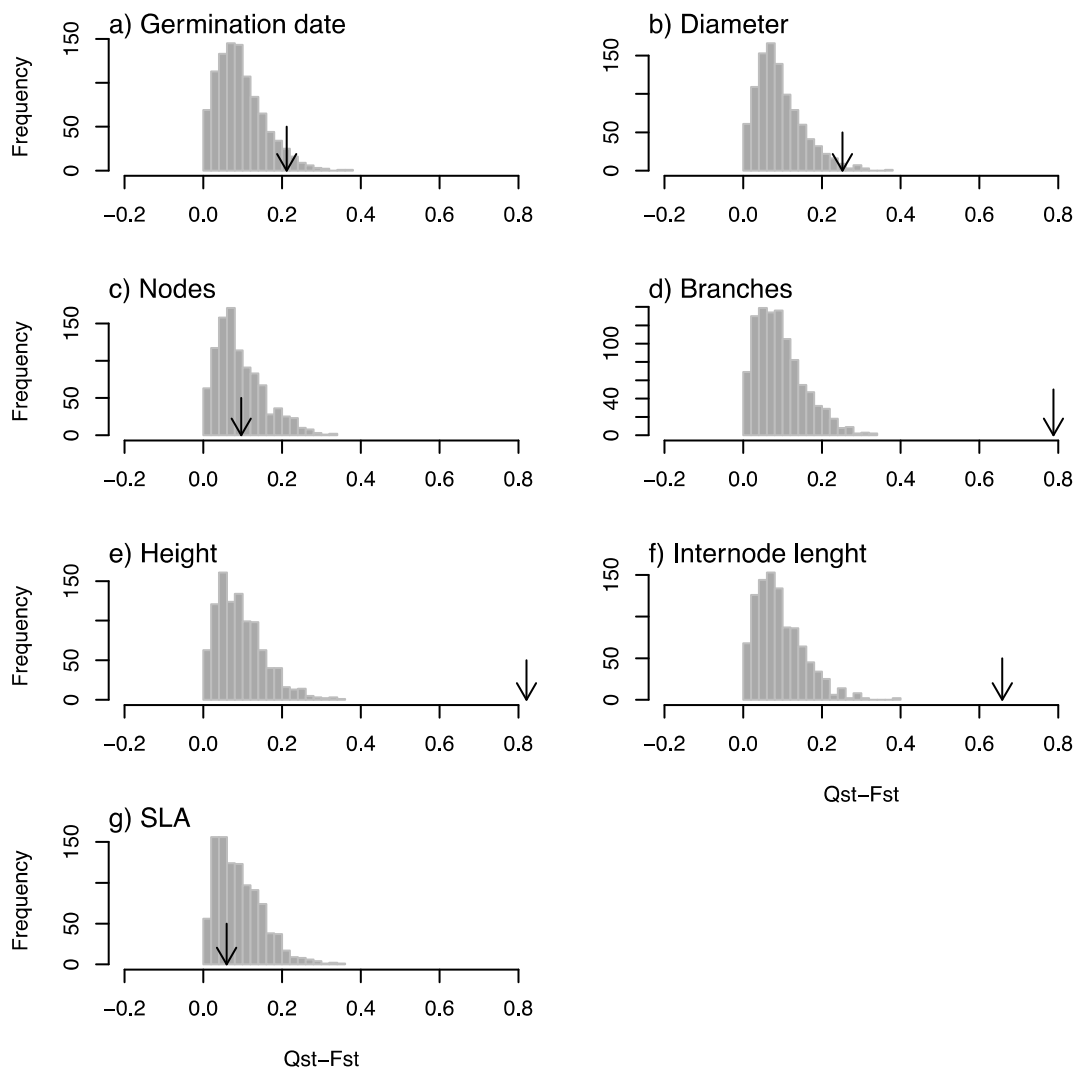
716



717

718 FIGURE S4. The simulated distribution of $Q_{ST} - F_{ST}$ for a neutral trait, and the observed point
 719 estimates of $Q_{ST} - F_{ST}$ differences in seven phenotypic traits measured for the eight *Antirrhinum*
 720 *majus pseudomajus* populations from the Southern France. The distribution of $Q_{ST} - F_{ST}$
 721 differences for a neutrally evolving trait was simulated following Whitlock and Guillaume
 722 (2009) based upon the observed population differentiation in neutral markers (F_{ST}) and the
 723 within-population variance in each trait. The arrow indicates the observed $Q_{ST} - F_{ST}$.

724



725

726 FIGURE S5. The simulated distribution of $Q_{ST} - F_{ST}$ for a neutral trait, and the observed point
 727 estimates of $Q_{ST} - F_{ST}$ differences in seven phenotypic traits measured for the five *Antirrhinum*
 728 *majus striatum* populations from the Southern France. The distribution of $Q_{ST} - F_{ST}$ differences
 729 for a neutrally evolving trait was simulated following Whitlock and Guillaume (2009) based
 730 upon the observed population differentiation in neutral markers (F_{ST}) and the within-population
 731 variance in each trait. The arrow indicates the observed $Q_{ST} - F_{ST}$.

732