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No evidence of direct contribution of adult plant stages to climate adaptation in snapdragon plants

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ABSTRACT

Signatures of local adaptation have been found at all life stages in plants. Yet, the contribution of later plant stages is rarely disentangled from the influence of early-life stages. Here, we investigate the direct contribution of adult plant stages to climate adaptation in two subspecies of snapdragon plants (*Antirrhinum majus*), while growth conditions have been homogenized at early-life stages. We compared genetic variation in fitness proxies (*i.e.* flowering, number of flowers and survival) and phenotypic traits in adult plants of eight populations of *A. m. pseudomajus* and seven populations of *A. m. striatum*, in two common garden experiments at high and low elevations. We found no evidence of adaptation to elevation in adult plants in both subspecies. Populations of low-elevation habitats outperformed populations of high-elevation habitats in all gardens. The effects of phenotypic traits on fitness components were similar at high and low elevation, suggesting no divergent selection along elevation. Different traits had an effect on fitness proxies in *A. m. striatum* and *A. m. pseudomajus*, suggesting that different mechanisms are at play in these two subspecies. Our findings suggest that there is no direct contribution of adult plant stages to climate adaptation in snapdragon plants and that adaptive processes potentially differ at the scale of *A. majus* subspecies.

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1. Introduction

Local adaptation is the microevolutionary response to local selection that makes populations fitter in their own local habitat than in any other population local habitats (Kawecki and Ebert 2004). This mechanism is expected to allow species to cope with ongoing climate changes (Jump and Penuelas 2005; Hoffmann and Sgro 2011; Franks et al. 2014; Kelly 2019). Detecting the signature of local adaptation associated with climatic variation is not an evidence of future adaptation to new climatic conditions, but it provides important information about standing genetic variation found among populations (Jump and Penuelas 2005; Valladares et al. 2014). This information can be used to build scenarios of species adaptation to climate change and to support decision-making in conservation management actions, such as ecological restoration (Gibson et al. 2016; Ramalho et al. 2017).

The ability of a species to adapt presupposes a match between the life stage at which organisms can express the phenotypic trait allowing for an adaptive response and the actual life stage at which the organism is submitted to an environmental demand. For instance, at low elevation,

mature life stages such as reproductive plants can be particularly exposed to severe summer conditions, whereas at high elevation early life stages such as seedlings would also be submitted to a strong environmental demand due to harsh spring conditions (*e.g.* cold, late and frequent frost, Korner 2003). Currently, how adaptation varies across the entire life cycle is still poorly understood. Early-life stages are often expected to contribute strongly to local adaptation (Donohue 2014; Postma and Agren 2016) but a meta-analysis of empirical studies did not allow the identification of a critical stage that would consistently contribute to local adaptation across species (Gibson et al. 2016). Instead, signatures of local adaptation have been found at any life stage, even though most studies focused on non-reproductive stages (Gibson et al. 2016).

Natural selection on early-life stages can influence the evolution of subsequent life stages, *i.e.* cascading effects (Donohue 2014). For instance, the timing of germination can influence the seasonal environment experienced by subsequent life stages, and the length of the growth period (Donohue 2002; Donohue et al. 2005). These cascading effects may also be transmitted across generations in the form of maternal effects

(Donohue 2014). Therefore, a reduced plant size at an adult plant stage could be due to a direct selection of adults, but also to an indirect selection of delayed germination at the seed life stage. These cascading effects are often complex, frequently invisible, and rarely considered in studies testing for local adaptation in the wild (Leimu and Fischer 2008; Hereford 2009; Gibson et al. 2016; Halbritter et al. 2018). Experiments testing for the effect of phenotypic traits on fitness components at later stages while mitigating the influence of previous stages are therefore likely to provide critical information about adaptive mechanisms along the life cycle of plants.

Studies comparing populations at different elevations in mountains are particularly popular for studying climate-related adaptation (Leimu and Fischer 2008; Hereford 2009; Halbritter et al. 2018). They provide strongly contrasting environments in close proximity and keep to a minimum the effects of confounding factors, such as photoperiod and geology or biogeographic history. Recently, Halbritter et al. (2018) combined the information from studies of multiple plant species along elevation gradients. They found significant evidence for adaptation to different elevations in terms of biomass, with a clear trend towards smaller adult plants at higher elevation. They also found that morphological, functional, and phenological trait differentiation along an elevation gradient is common in plants, but difficult to link to plant fitness afterwards. For instance, a common hypothesis in the literature is that the smaller size of plants at high elevation provides several advantages, including warmer microclimatic conditions close to the ground and protection from the wind (Korner 2003). Alternatively, smaller size at higher elevation could also reflect an overall lower performance of populations reaching their ecological range edge at high elevation (Korner 2003; Sexton et al. 2009). Therefore, experiments testing for the effect of phenotypic traits on fitness components along elevation gradients have the potential to shed light on the adaptive significance of phenotypic differentiation.

Here, we studied the direct contribution of adult life stages to the adaptation to elevation of populations of two subspecies of snapdragon plants (*Antirrhinum majus* L., Plantaginaceae). We used a parallel approach as described by Kawecki and Ebert (2004); the focus is on a specific ecological factor defining habitat types – here elevation. Several replicated populations (seven populations of yellow-flowered *A. m. striatum* and eight populations of magenta-flowered *A. m. pseudomajus*) were sampled across their similar altitudinal range and compared, in terms of fitness proxies

(flowering, number of flowers and survival), and phenotypic traits (functional, morphological and developmental traits) in low- vs high-elevation common gardens. We also evaluated the effect of phenotypic traits on fitness proxies in relation to elevation to identify traits potentially linked to fitness in these systems. To mitigate the influence of prior life stages on adult plant responses, seeds and seedlings were grown in homogenized controlled conditions before their transplant in common gardens. *A. majus* populations are a useful system to explore the potential for differential adaptive responses between closely related subspecies. Prior results of a common garden Q_{ST} - F_{ST} approach conducted on adult plants on these two subspecies of snapdragon plants suggested a differential adaptive response to elevation in adult life stages (Marin et al. 2020).

2. Material and methods

2.1. Study system

Antirrhinum majus L. (Plantaginaceae) is a hermaphroditic, self-incompatible, short-lived perennial species, characterized by a patchy distribution in southern Europe centred over the Pyrenees Mountains (Khimoun et al. 2013). This species occurs from sea level to an altitude of 1900 m (Andalo et al. 2010), on limestone or siliceous substrates and in habitats with contrasted moisture regimes (rainfall 500–1000 mm per year). *A. majus* thrives in disturbed habitats and is especially common along roadside and railway embankments (Khimoun et al. 2013).

2.2. Subspecies of *A. majus*

The two interfertile subspecies of *A. majus*, *A. m. pseudomajus* and *A. m. striatum* produce magenta and yellow zygomorphic flowers, respectively (Andalo et al. 2010). For putative neutral microsatellite loci, they show ca. 1% genetic differentiation (estimated via F_{ST}), which is one order of magnitude lower than the ca. 10% differentiation found among populations within subspecies (Pujol et al. 2017). There is evidence for gene exchange between subspecies in multiple populations across contact zones (Khimoun et al. 2011). Genome scans across a particular contact zone in the Pyrenees revealed little to negligible differentiation between the two subspecies, except for loci underlying flower color differences, which were characterized by high differentiation (Tavares et al. 2018; Whibley et al. 2006). Frequency-dependent selection exerted by pollinators on the basis of flower colour is acknowledged to maintain the two subspecies as separate entities (Tastard et al. 2003). The different

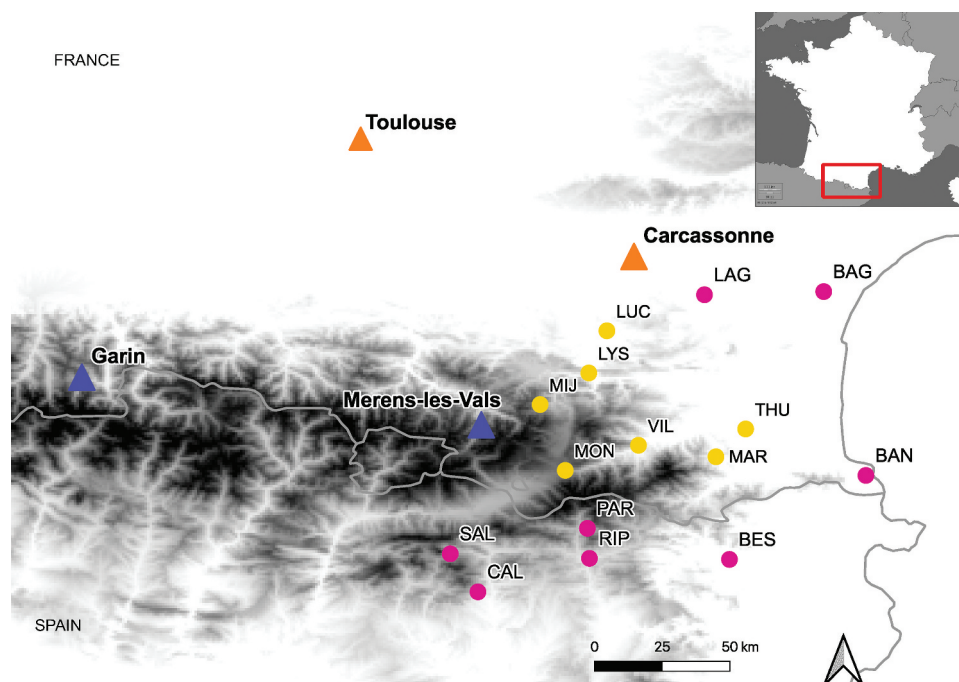


Figure 1. Map of *A. majus* populations that were sampled across the geographic range of the species in Southern France. Pink dots represent *A. m. pseudomajus* populations, yellow dots represent *A. m. striatum* populations. Blue triangles represent high-elevation gardens, orange triangles represent low-elevation gardens.

geographic distributions of *A. m. pseudomajus* and *A. m. striatum* are not explained by habitat differences, as illustrated by the substantial overlap of environmental conditions between the two species (Khimoun et al. 2013).

2.3. Collection sites and plant material

Fifteen wild populations of *A. majus* distributed across its native geographic range (between north-eastern Spain and south-western France) were sampled in 2011. For each subspecies, we sampled populations from low and high elevation habitats in many locations across the species geographic range but avoided populations on the range margins where evolutionary adaptive mechanisms might differ (Figure 1). The studied populations covered most of the altitudinal range of the species (0 m to 1600 m, see Table S1 in Supplementary Information).

They were sampled along elevation gradients where they are confronted with contrasted environmental conditions (Marin et al. 2020). These conditions ranged from 14.8°C and 52 mm (BAN, 61 m above sea level) to 6.1°C and 94 mm (MON, 1564 m above sea level), based on fifty-year averages (1950–2000) of mean annual temperature and average rainfall extracted from the WorldClim database (resolution 1 km², www.worldclim.org, Hijmans et al. 2005). Two elevation layers of climate conditions have been described where conditions are consistent within each layer and differ between layers (Marin et al. 2020): between 0 and 750 m (hereafter referred to as

“low-elevation”) and between 750 and 1800 m (hereafter referred to as “high-elevation”). In total, we used eight populations of *A. m. pseudomajus* and seven populations of *A. m. striatum*.

2.4. Experimental design: mitigating maternal effects and early life stage influence

Seed families used to produce the plants grown in this experiment were not sampled directly in the wild but produced by the last of two successive generations of parental plants that were germinated and grown in a common garden environment (Figure S1). Only the first parental generation of plants was germinated from seeds collected from field populations. These two generations of plants regenerated before our experiment are expected to have reduced maternal environmental effects that could have otherwise biased the trait values recorded during the experiments presented here. Seeds from 13 to 15 families from each of the 15 study populations were grown in May 2016 in a growth room at the Toulouse Plant-Microbe Phenotyping facility (TPMP, INRA Toulouse, France, Table S1). At the stage of the two cotyledons, 12 plants by family were moved for 3 weeks to a glasshouse at the TPMP facility pending their transfer to different sites. These plants grew for 3 weeks under controlled climatic conditions (temperature between 19 and 24°C, humidity range from 53% to 63%) and natural light (without supplementary lighting). Since natural selection on early-life stages can influence the evolution of subsequent life stages (cascading effects, Donohue et al.

2014), we chose to germinate and grow seedlings under controlled conditions to homogenize the age of seeds and seedlings and the resources available to them so as to diminish the influence of prior life stage on adult plant responses.

2.5. Common garden design

In July 2016, we transplanted 2403 juveniles in four common gardens located on four sites (Figure 1, ca. 600 plants in each garden). Two gardens were located at low elevation, in Toulouse (elevation 152 m) and Carcassonne (elevation 110 m), France. The other two were located at high elevation in the Pyrenees, in Garin (elevation 1126 m) and Merens-Les-Vals (1100 m), France. Thus, for each elevation (high vs low), there were two gardens (*i.e.* replicates). These four sites were chosen because their climatic conditions were representative of the average climatic conditions experienced by sampled populations from the lowest half and highest half of their altitudinal range, respectively. The gardens at higher elevation received more rainfall and had a less severe summer drought than the low-elevation gardens (see supplementary information, Figure S2). Over the period of the experiment, the average temperature measured on the experimental site (one record every hour during the whole experiment) was 25.9 °C in Carcassonne, 23 °C in Toulouse, 18.15 °C in Garin, and 17.4 °C in Merens-les-vals. In every garden, the 15 study populations (and families within populations) were the same, so that the four gardens were composed by a similar gene pool. In every garden, every seed family was represented by three individuals. In each garden, ca. 600 plants were arranged in a randomized block design (17 plastic containers, 600×400×120 mm) with each container containing 34 or 35 plants that were randomly allocated to the different containers. The bottom of each container was covered with an irrigation mat (400 g·m⁻²) that regulated and homogenized the moisture content of the compost. Plants were supplied with water in case of prolonged drought.

2.6. Phenotypic data

We investigated three fitness-related traits that were used as fitness proxies: flowering (yes/no), the number of flowers of flowering plants and survival (yes/no). Flowering was evaluated by recording whether plants had flowered or not during the experiment. Plants were left in the common gardens for 5 months to avoid a bias towards early flowering plants. The number of flowers referred exclusively to the fecundity of flowering plants (N = 678). Using flowering as a fitness variable obviously limits our measurement of fitness to a partial measurement of female potential fitness. Survival was evaluated by recording whether plants

were still alive, or not, at the end of the experiment. Flowering and survival rates were estimated for each population within each garden as the ratios between the number of reproductive plants and the number of plants still alive, respectively, on the initial number of plants.

We investigated five vegetative traits on each individual: a functional trait (specific leaf area, SLA), three morphological traits related to plant growth (vegetative height that is the height of the last vegetative node on the stem, basal diameter of the main stem, and number of nodes on the main stem), and a developmental trait (average internode length). The internode length is the ratio of the number of nodes to the plant vegetative height and is therefore not independent of these two traits. The functional trait (SLA) was calculated as the ratio between the cumulated area of five mature but non-senescent fresh leaves by plants and their oven-dried mass (Cornelissen et al. 2003; Pujol et al. 2008; Perez-Harguindeguy et al. 2016). Leaf area was measured by using the R package Momocs v. 1.2.9 (Bonhomme et al. 2014).

We also investigated three reproductive traits: a phenological trait (time of flowering since transplantation) and two morphological traits. These morphological traits were reproductive height (total height of the plant including reproductive part) and number of stems harbouring flowers on the plant. Not all the plants flowered, which resulted in fewer plants measured for reproductive traits (N = 678) and one fitness proxy (number of flowers for flowering plants) than plants for vegetative traits (N = 2403).

2.7. Statistical analysis

All statistical analyses were performed using the R.3.5.0 software (R Core Team 2014). All the generalized mixed-models were implemented in R via the lme4 package (Bates et al. 2015). Model averaging was performed using the package MuMIn (Barton 2009). Assumptions of models (*e.g.* heteroscedasticity, over or under dispersion) were checked.

2.7.1. Testing for a pattern of adaptation to elevation

The “local vs. foreign” elevation criterion (Kawecki and Ebert 2004) was chosen to analyse climate adaptation of fitness proxies and to compare phenotypic traits. Climate adaptation at different elevations is operating if populations originating from high-elevation habitats outperform populations from low-elevation habitats in high-elevation gardens, whereas populations originating from low-elevation habitats outperform populations from high-elevation habitats in low-elevation gardens. The reaction norms (*i.e.* phenotypic responses of similar genotypes between high and low elevation gardens) of populations originating from high- and

low-elevation habitats should be crossing in the presence of adaptation to elevation for measurements similarly related to fitness at high and low elevations.

We performed GLMMs (Generalized Linear Mixed Models) on fitness proxies (flowering, number of flowers and survival) and phenotypic traits, for each subspecies separately. The fixed effects included the elevation of gardens (as a discrete variable, “high” vs “low”), the elevation of origin of the populations (as a discrete variable, “high” vs “low”), and their interactions. The population and the site replication effects (*i.e.* Toulouse, Carcassonne, Garin and Merens-les-Vals) were included as random effects. Fitness proxies and traits were modelled differently depending on their distribution: (i) survival and flowering (0 vs 1) were modelled with a binomial model, (ii) the number of flowers, the number of nodes, the number of stems and the time to flowering were modelled with a Poisson model (with a log link function), and (iii) the remaining traits (SLA, vegetative height, basal diameter, internode length, reproductive height) were modelled with a Gaussian model. The link functions chosen satisfied the assumptions of the models.

2.7.2. Testing the effects of phenotypic traits on fitness-related traits between elevations

We tested whether (i) fitness proxies were correlated to phenotypic traits and whether (ii) these effects varied according to the elevation of origin and the elevation of the gardens. For the number of flowers, we calculated the relative fitness by dividing the number of flowers by their site mean value. We standardized trait values within each site by subtracting the site mean and dividing it by the standard deviation. Then, we used a $\log(x + 1)$ transformation and data were modelled following a Gaussian distribution. For survival and flowering, data were not standardised by the mean because they are binomial.

The effects of all phenotypic traits have not been tested on all the fitness proxies. For both survival and flowering, only three phenotypic traits (vegetative height, number of nodes and internode length) were included in these models. This is because other phenotypic traits were sometimes not measured on dead and non-flowering plants for technical reasons. The effect of phenotypic traits on survival has been tested exclusively on data from low-elevation gardens (reduced dataset, $N = 1338$) because all plants survived at high-elevation gardens. To test for a divergent effect of traits on fitness across treatments and origins, but to avoid three-ways interactions with traits (*i.e.* elevation of origin $\times$ elevation of the gardens $\times$ phenotypic trait), we combined elevation of origin and elevation of the gardens as a single variable called “elevation gardens/origin” and containing four levels “garden high/elevation high”, “garden low/ elevation low”, “garden high/elevation low”, “garden low/elevation high”.

We had no a priori hypothesis regarding which phenotypic traits might affect fitness proxies. Therefore, for each fitness proxy, we used an information-theoretic model averaging approach testing multiple traits, as outlined in Grueber et al. (2011). This approach does allow to test multiple hypotheses in the same analysis using the following four-step process: (i) we generated all possible sub-models from the set of predictor of interest (phenotypic traits in interaction with elevation of origin and gardens); (ii) we selected the best models given their AIC (Akaike Information Criterion, here we selected the 95% confidence set of models); (iii) we averaged the estimates of predictors among all selected models weighted by the Akaike weight of each model (Anderson and Burnham 2002; Symonds and Moussalli 2011); (iv) we calculated the 95% confidence intervals (CIs) around averages estimates to help interpret the significance of each variable (Turek and Fletcher 2012; Galipaud et al. 2014).

3. Results

3.1. No signal of climate adaptation on fitness component

3.1.1. Fitness proxies

Plant survival was exclusively affected by the elevation of gardens in both subspecies (Table 1 a). All plants survived in high-elevation gardens whereas less than half of the plants survived in low-elevation gardens (Figure 2a and Figure 2d). The elevation of origin of the populations had no effect on the survival rate of plants (Table 1 a). No significant elevation of gardens $\times$ elevation of origin effect was found, suggesting that the “local elevation vs. foreign elevation” criterion (*i.e.*, “local is best”) was not satisfied for survival in both subspecies.

In contrast, a significant elevation of gardens $\times$ elevation of origin effect was found for flowering in *A. m. striatum* and the number of flowers in *A. m. pseudomajus* (Table 1 b and c). Yet, the “local elevation vs. foreign elevation” criterion was not satisfied in *A. m. striatum* and partially satisfied in *A. m. pseudomajus*. Indeed, in *A. m. striatum*, populations from high-elevation habitats had a lower flowering rate in high-elevation gardens than populations from low-elevation habitats, which suggests the maladaptation of native populations (Figure 2b). These differences disappeared in low-elevation gardens, where all populations had the same flowering rate (Figure 2b). In *A. m. pseudomajus*, populations originating from low-elevation habitats had a higher flowering rate and produced more flowers in low-elevation gardens than populations from high-elevation habitats, which suggests climate adaptation to low elevation (Figure 2e and Figure 2f). However, in high-elevation gardens, all populations of *A. m. pseudomajus* produced the same number of flowers (Figure 2f).

Table 1. Results from the generalized mixed models (GLMM) testing for climate adaptation on fitness proxies in both subspecies of *Antirrhinum majus*. Random factors in all models are the population effect and the site replication effect. When we re-run the models without the random effect at zero, results were similar. Marginal R^2 is part of the variance explained by fixed effects. Conditional R^2 is part of the variance explained by both fixed and random effects.

	<i>A. majus striatum</i>	<i>A. majus pseudomajus</i>
a) Survival (binomial)		
Sample size	$N = 1162$	$N = 1241$
Coefficient of determination	$R^2_m = 0.67, R^2_c = 0.67$	$R^2_m = 0.62, R^2_c = 0.62$
<i>Fixed effects</i>		
Estimates (CI 95%)	Estimates (CI 95%)	Estimates (CI 95%)
Intercept	4.7 (3.56; 5.83)	4.11 (3.23; 5)
Elevation of gardens (low)	-5.3 (-6.46; -4.14)	-4.52 (-5.43; -3.61)
Elevation of origin (low)	-0.58 (-2.08; 0.93)	0.52 (-0.92; 1.96)
Elev. origin x gardens (low x low)	0.47 (-1.07; 2.02)	-0.25 (-1.72; 1.23)
<i>Random effects</i>		
Variance (CI 95%)	Variance (CI 95%)	Variance (CI 95%)
Populations effect	0 (0; 0)	0 (0; 0)
Site replication effect	0 (0; 0)	0 (0; 0)
b) Flowering (binomial)		
Sample size	$N = 1162$	$N = 1241$
Coefficient of determination	$R^2_m = 0.03, R^2_c = 0.21$	$R^2_m = 0.12, R^2_c = 0.21$
<i>Fixed effects</i>		
Estimates (CI 95%)	Estimates (CI 95%)	Estimates (CI 95%)
Intercept	-1.58 (-2.65; -0.5)	-1.79 (-2.6; -0.98)
Elevation of gardens (low)	0.39 (-0.88; 1.67)	0.42 (-0.49; 1.33)
Elevation of origin (low)	0.95 (-0.03; 1.92)	1.44 (0.66; 2.22)
Elev. origin x gardens (low x low)	-0.74 (-1.29; -0.19)	-0.18 (-0.72; 0.35)
<i>Random effects</i>		
Variance (CI 95%)	Variance (CI 95%)	Variance (CI 95%)
Populations effect	0.35 (0.32; 0.38)	0.24 (0.21; 0.26)
Site replication effect	0.39 (0.35; 0.42)	0.17 (0.15; 0.19)
c) Number of flowers (poisson)		
<i>Fixed effects</i>		
Estimates (CI 95%)	Estimates (CI 95%)	Estimates (CI 95%)
Intercept	8.49 (5.91; 11.06)	11.55 (9.33; 13.77)
Elevation of gardens (low)	0.17 (-1.6; 1.95)	-1.81 (-4.3; 0.69)
Elevation of origin (low)	2.9 (-0.85; 6.66)	0.72 (-1.98; 3.43)
Elev. origin x gardens (low x low)	-0.74 (-3.2; 1.71)	2.67 (-0.14; 5.47)
<i>Random effects</i>		
Variance (CI 95%)	Variance (CI 95%)	Variance (CI 95%)
Populations effect	4.78 (4.53; 5.03)	1.48 (1.36; 1.6)
Site replication effect	0 (0; 0)	0.13 (0.09; 0.17)

Populations and site replication effects explained different part of variance according to the fitness proxy considered (Table 1). There were some variation in number of flowers across populations and replicated site, and in flowering rate across populations, but no variation in survival between populations or site of replication (Figure 2).

3.1.2. Phenotypic traits

None of the eight traits satisfied the “local elevation vs. foreign elevation” criterion (Figure 3 for *A. m. striatum* and Figure S4 for *A. m. pseudomajus*): populations from low-elevation habitats tend to outperform population originating from high elevation, in particular in high-elevation gardens. Indeed, plants originating from high-elevation populations tend to have a reduced size (vegetative) with fewer nodes and shorter internode length than those from low-elevation populations (Figure 3a, b, c and Figure S4 for *A. m. pseudomajus*).

GLMMs showed a significant elevation of origin x elevation of gardens effect on the vegetative height and the internode length in *A. m. striatum* and on the vegetative height and the number of nodes in *A. m. pseudomajus* (Tables S2:S4). However, the marginal R^2 of these models were around 0.02 and 0.06 (see Tables S2 and S3) suggesting that the part of variance explained by fixed effects was very low. No elevation of origin x elevation of gardens effect was found on the other traits (Tables S2:S4). A significant elevation of the garden effect was found on the time of flowering and reproductive height in both subspecies; plants in high-elevation gardens were taller (reproductive height, around 10 cm difference) and flowered later (around 10 days difference) than plants in low-elevation gardens. For these two traits, fixed effects explained a significant part of the variance (around 30%, see Table S3). We found no effect of the garden elevation on the other traits (Tables S2:S4).

3.2. Consistent association between phenotypic traits and fitness proxies along elevation

A large part of the variation in the number of flowers and flowering was explained by differences in phenotypic traits, but this was not the case for survival. The model averaging approach provided a best model with an $R^2 = 0.6$ for the number of flowers ($R^2 = 0.6$ for *A. m. striatum* and $R^2 = 0.56$ for *A. m. pseudomajus*, Table 2), around 0.4 for flowering ($R = 0.44$ for *A. m. striatum* and $R = 0.39$ for *A. m. pseudomajus*, Table S3), and 0.1 for survival ($R^2 = 0.07$ for *A. m. striatum* and $R^2 = 0.1$ for *A. m. pseudomajus*, Table S3). Therefore, we did not discuss the significance of phenotypic traits on survival because the global variance explained by the model was negligible.

The relative number of flowers was significantly affected by two traits in *A. m. striatum* (vegetative height, and reproductive height, CIs not overlapping zero, Table 2), and by two traits in *A. m. pseudomajus* (reproductive height, number of reproductive stems). Thus, for both subspecies, taller flowering plants (higher reproductive height) – when all the other traits were similar or set to zero – produced more flowers than shorter flowering plants (Table 2). In *A. m. striatum*, plants with lower vegetative heights also produced more flowers. In *A. m. pseudomajus*, plants with more reproductive stems also produced more flowers. Reproductive height showed the strongest effect on the relative number of flowers (Table 2). For the relative number of flowers, none of the interactions between phenotypic traits and the combined origin/garden elevation were significant (Table 2). In other words, all phenotypic traits showed consistent effects on fitness proxies in gardens and populations of different elevations. A similar result was found for the absolute number of flowers (i.e. not divided by site mean); the

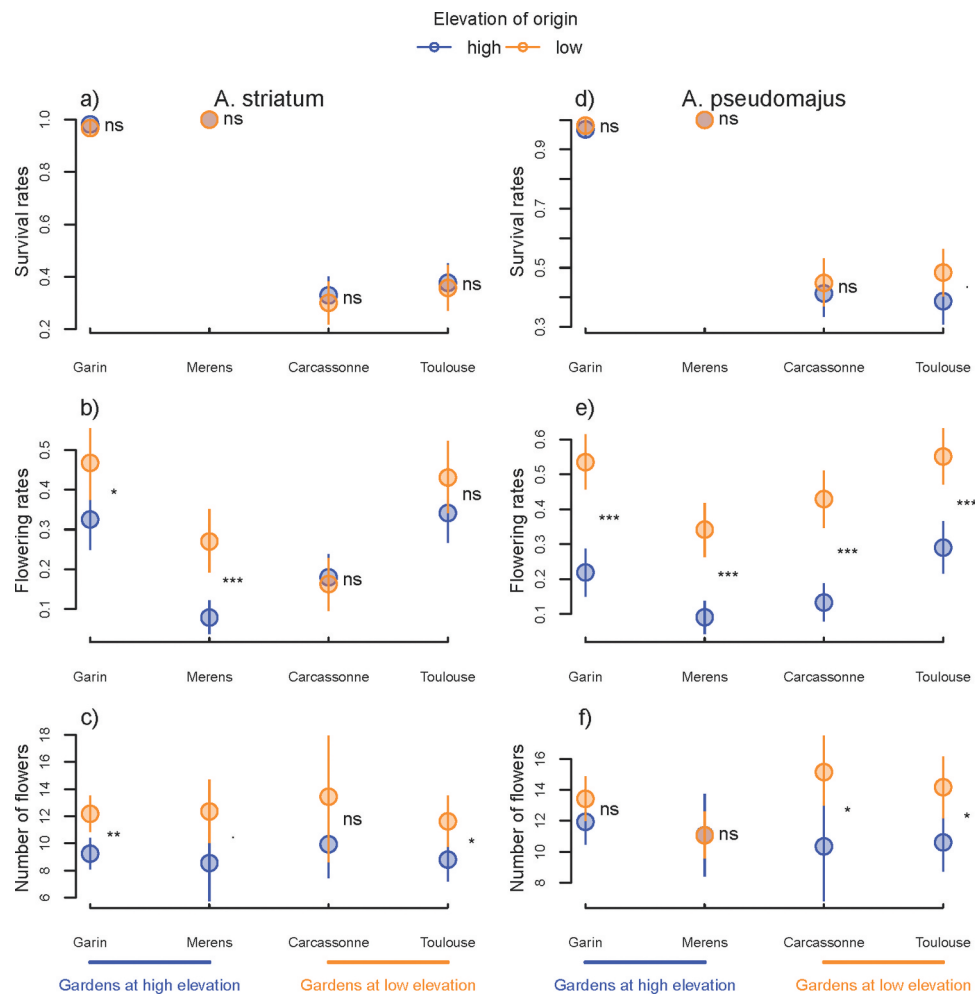


Figure 2. Fitness-related traits (mean values and 95% CI) of *Antirrhinum majus striatum* (a, b, c) and *A. m. pseudomajus* (d, e, f) in high and low-elevation gardens (mean values 95% CI). Significant differences for pairwise comparison (Wilcoxon tests) between populations originating from different elevations are indicated by asterisks. ***: p.value ≤ 0.001, **: 0.001 < p.value ≤ 0.01, *: 0.01 < p.value ≤ 0.05, .: 0.05 < p.value < 0.1, "ns": p.value ≥ 0.1.

direction of the phenotypic trait effect was systematically the same across gardens and populations despite significant interactions between phenotypic traits and the combined origin/garden elevation (Table S7).

Flowering rate was significantly affected by one trait in *A. m. pseudomajus* (vegetative height, CIs not overlapping zero) but was not affected by any trait in *A. m. striatum* (CIs overlapping zero, Table S3). In *A. m. pseudomajus*, taller plants before reproduction (vegetative height) tended to have a higher flowering rate than shorter plants. This pattern was consistent across populations of *A. m. pseudomajus* and gardens; none of the interactions between traits and the combined origin/garden elevations were significant (Table S3).

4. Discussion

4.1. No evidence of a contribution of adult life stages to adaptation to elevation

Common garden results showed no signal of adaptation to elevation in either *A. m. striatum* or *A. m. pseudomajus* adult plants. We found some genetic divergence between populations of high- and low-

elevation habitats in flowering, vegetative height and internode length in *A. m. striatum*, and in the number of flowers, vegetative height and number of nodes in *A. m. pseudomajus*. Yet the "local vs foreign" criterion did not hold for any of these fitness proxies and phenotypic traits. Populations of low-elevation habitats consistently outperformed others in the gardens, in contrast to what is predicted by theory. Thus, we found no pattern of climate adaptation to current conditions along elevation in adults (decoupled from early life stages). Additional studies are required to investigate whether there is a signal of climate adaptation at early-life stages, and whether this signal could propagate through adult life stages via selection on early-life stages. Most snapdragon plants behave as annuals and die after 1 year (around 70% to 90%; personal observation by the authors). A similar study conducted over multiple years would be ideal to bring information on plants living more than 1 year. Their cumulative phenotypic performance can only grow with age. Such a study might, however, be technically too demanding to be done.

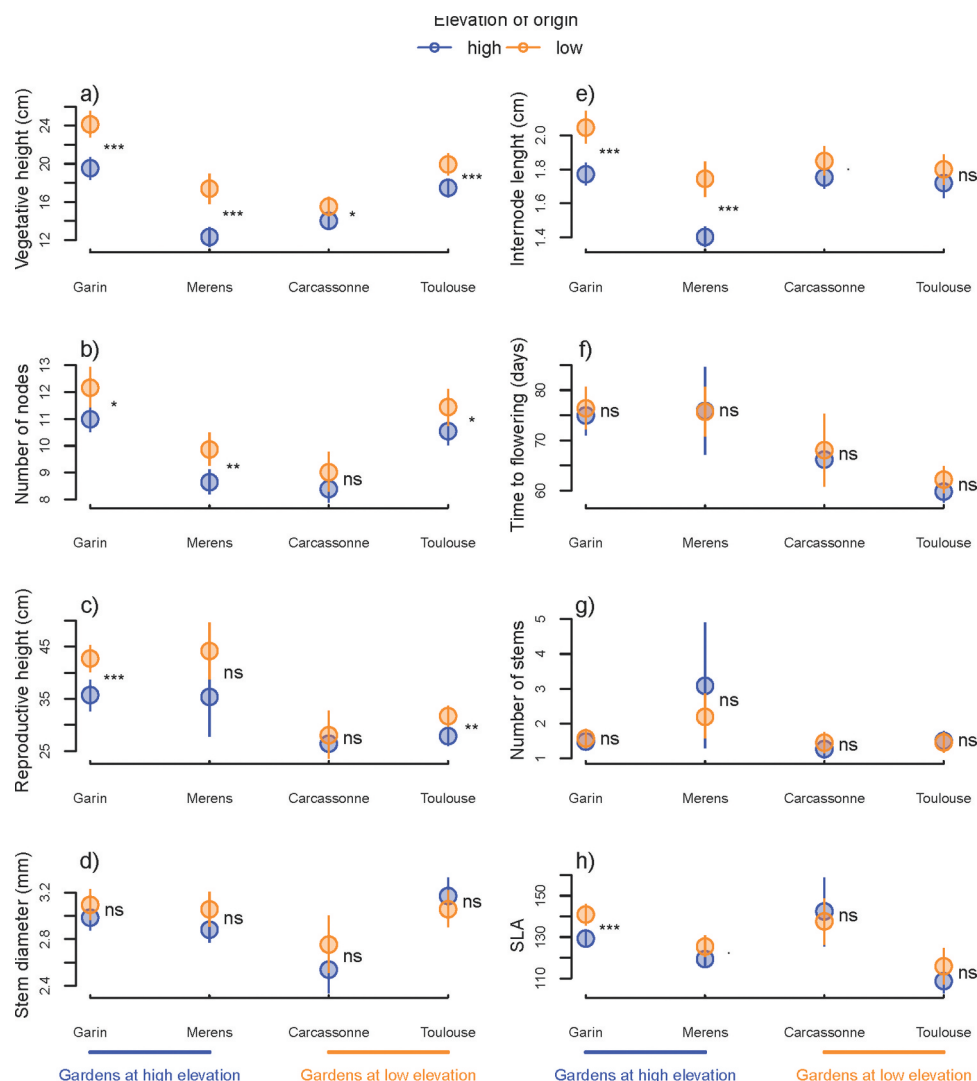


Figure 3. Phenotypic traits of *Antirrhinum majus striatum* in high and low-elevation gardens (mean values and 95% CI). Significant differences for pairwise comparison (Wilcoxon tests) are indicated by asterisks. ***: p.value ≤ 0.001, **: 0.001 < p.value ≤ 0.01, *: 0.01 < p.value ≤ 0.05, .: 0.05 < p.value < 0.1, "ns": p.value ≥ 0.1.

Our results differ from a previous indirect study on adult plants that used a Q_{ST} - F_{ST} approach in a unique common garden. Marin et al. (2020) brought indirect evidence supporting potential adaptive divergence in adult plants between *A. m. pseudomajus* and *A. m. striatum*. Using a Q_{ST} - F_{ST} approach, they showed that phenotypic differentiation along elevation was higher than what could be explained by neutral evolutionary divergence among populations of *A. m. striatum* for two biomass-related traits (number of nodes and number of branches). In our study, we found no evidence of adaptation to biomass-related traits (diameter, height, etc.) in both subspecies. Populations from higher elevations nevertheless systematically had a reduced vegetative size compared to populations from low elevation, which suggests a genetically based mechanism. The phenotypic differentiation found in adult plants in Marin et al. (2020) could be due to cascading effects from early-life stages, which were mitigating in our study. Thus, our study confirms that while Q_{ST} - F_{ST} potential signatures of past

adaptive evolution might be useful to identify potential candidate adaptive traits, as for biomass-related traits (number of nodes and number of branches) in *A. m. striatum*, reciprocal transplant experiments or multiple common garden experiments conducted while mitigating the influence of prior life stages are required to confirm the traits and/or the life stages involved in the differentiation of populations and their adaptive significance.

4.2. Effects of phenotypic traits on fitness proxies

Patterns of reduced plant size (either biomass or height) at high elevation are commonly found along elevation gradients (Halbritter et al. 2018). Our study provided no real support for any of the hypotheses (warmer microclimatic conditions and protection from the wind Korner 2003) linking reduced size to higher fitness in plants from high elevation. First, we found a significant genetic-based differentiation in vegetative height between plants originating from

Table 2. Estimates of model-averaging approach testing the effects of phenotypic traits on the relative number of flowers and the flowering rate in both subspecies of *Antirrhinum majus*. R^2 is part of the variance explained by the best model. Only significant interactions were shown here (complete table showed in appendix table S6). Estimates provided were back-transformed.

	<i>A. majus striatum</i> Estimates (CI 95%)	<i>A. majus pseudomajus</i> Estimates (CI 95%)
a) Relative number of flowers (gaussian)		
Coefficient of determination	$R^2 = 0.6$	$R^2 = 0.56$
Intercept	0.94 (0.84; 1.05)	0.95 (0.81; 1.09)
Vegetative height	-0.21 (-0.32; -0.09)	-0.09 (-0.21; 0.05)
Internode length	0.09 (-0.02; 0.22)	-0.02 (-0.1; 0.06)
Number of nodes	0.1 (-0.03; 0.24)	-0.02 (-0.12; 0.09)
SLA	0 (-0.04; 0.04)	0 (-0.03; 0.02)
Time to flowering	0.03 (-0.01; 0.07)	-0.01 (-0.04; 0.03)
Reproductive height	0.3 (0.21; 0.41)	0.26 (0.13; 0.4)
Number of reproductive stems	0 (-0.04; 0.05)	0.04 (0.01; 0.08)
Diameter of stems	0.03 (0; 0.05)	0.01 (-0.04; 0.07)
b) Flowering (binomial)		
Coefficient of determination	$R^2 = 0.44$	$R^2 = 0.39$
Intercept	-2.02 (-2.52; -1.52)	-2.97 (-3.73; -2.21)
Internode length	0.09 (-2.15; 2.33)	-0.14 (-1.33; 1.06)
Number of nodes	-0.35 (-3.02; 2.32)	-0.14 (-2.06; 0.81)
Vegetative height	2.97 (-0.03; 5.97)	2.79 (1.16; 4.42)
Elevation of gardens/origin (High/Low)	-0.34 (-1.11; 0.44)	1.93 (1.12; 2.73)
Elevation of gardens/origin (Low/High)	1.07 (0.47; 1.67)	1.54 (0.71; 2.37)
Elevation of gardens/origin (Low/Low)	0.64 (-0.02; 1.3)	3.16 (2.37; 3.96)

high and low elevation in both subspecies (with plants originating from high elevation being systematically smaller than plants originating from low elevation). However, we showed that the elevation (of origin and/or gardens) explained only a small part of the variance in plant height (around 2% and 6%). Therefore, a pattern of reduced plant size along elevation occurs in *A. majus*, but the major part of the variation in plant height still remains between sites, populations and individuals. Second, we showed that the effect of reduced plant size on fitness proxies did not change with the elevation of origin/gardens in both subspecies, suggesting no divergent selection between high and low elevation. In other words, reduced plant size did not provide a particular fitness advantage or disadvantage at high elevation compared to low elevation. Nevertheless, we found that plant height was associated with different fitness proxies for different subspecies; reduced (vegetative) plant size was associated with more flowers in *A. m. striatum*, and with a lower flowering rate in *A. m. pseudomajus*. Thus, while it is impossible to draw conclusions on the mechanisms underlying this size difference, we found that the ecological significance of reduced plant size differs between *A. majus* subspecies.

Phenotypic traits acting on fitness proxies varied across snapdragon subspecies. In *A. m. striatum*, taller flowering plants produced more flowers than shorter plants – when considering plants with similar trait values. This is coherent with the biology of snapdragon plants characterized by an indeterminate terminal inflorescence (*i.e.* the growing tip of the stem continues to grow and produce new flowers as it elongates); longer stems are more likely to produce more flowers. Therefore, it is not surprising that plants with shorter vegetative height also produced more flowers when

considering plants with a similar reproductive height. In *A. m. pseudomajus*, the reproductive height and the number of reproductive stems had a significant effect on the number of flowers. Therefore, the effect of other traits on the number of flowers is different in closely related species. This result suggests different potential targets for natural selection between subspecies.

4.3. Support for a scenario of adaptation to climate change

The range of climate conditions in the Pyrenees is already changing and set to change even more because of climate change (Lopez-Moreno et al. 2008). Here, our results suggest that *A. majus* adult plants were not adapted to the current climate conditions at different elevations. Yet, we found some elevation-related genetic variation, which might be related to climatic differences between sites and population origins. A hypothesis is that climate warming is already disturbing the signal of adaptation to historical conditions. Such a phenomenon has already been observed in other plant species, *e.g.* *Boechera stricta*, (Anderson and Wadgymer 2020). In the Pyrenees, conditions at high elevation are becoming more similar to conditions at lower elevation (Urli et al. 2014) which could explain why populations from low-elevation habitats outperformed population of high-elevation habitats in all habitats. Therefore, an alternative scenario of adaptation that we did not evaluate here might grant *A. majus* with some potential to adapt to climate change: populations from low-elevation habitats might colonize gradually via migration a range of habitats at higher elevation. Our results also showed a decrease in performance under warmer environments; survival dropped dramatically in low elevation-gardens compared to high-elevation gardens in all populations for both subspecies. These

findings suggest that *A. majus* will be at risk under climate change unless its populations undergo adaptive evolutionary changes, or migrate to track suitable climate conditions.

5. Conclusion

Our study provides no evidence of a direct contribution of adult plant stages to climate adaptation at different elevations in either *A. m. striatum*, or *A. m. pseudomajus*. This finding contrasts with previous studies on adult life stages, which detected a signal of adaptation to elevation in *A. m. striatum*, but not in *A. m. pseudomajus*. Our study calls for studying variations in climate adaptation along the entire life cycle of plants while mitigating the influence of prior life stages. This would help to reach a better understanding of the adaptive mechanisms of contrasted climates and thus help better predict the response of species in a warming climate (Valladares et al. 2014). We also found a genetically based pattern of reduced vegetative size in snapdragon plant populations originating from high elevation in both *A. m. striatum* and *A. m. pseudomajus*. Although such a pattern is generally described as adaptive, our results reveal a similar effect of vegetative size on fitness proxies in both low- and high-elevation gardens. Finally, the effect of phenotypic traits on fitness proxies varied between closely related subspecies, suggesting that the targets of natural selection might differ between subspecies of snapdragon plants.

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Disclosure statement

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BP designed the research program. SM, JA, ML, GO, TPK, VB, EC and BP carried out the experiments; AG, PM, PaMa performed statistical analyses, AG and BP wrote the manuscript.

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11. Appendices

11.1. Supplementary informations

Environmental conditions in planting sites

11.2. Supplementary figures

Figure S1. Experimental design. Figure S2. Ombrothermic diagram for each common garden. Graphs a) and b) refer to low elevation gardens, c) and d) to high elevation gardens. Figure S3. Phenotypic traits of *Antirrhinum majus pseudomajus* in high and low-elevation gardens. Figure S4. Effects of vegetative height on the number of flowers displayed by elevation of origin/gardens in a) *Antirrhinum majus striatum*, and b) *A. m. pseudomajus*.

11.3. Supplementary tables

Table S1. Description of *Antirrhinum majus* populations grown in the two common garden experiments.

Table S2. Results from the generalized mixed models (GLMM) testing for local adaptation on two phenotypic traits (internode length and number of nodes) in both subspecies of *Antirrhinum majus*.

Table S3. Results from the generalised mixed models (GLMM) testing for local adaptation on phenotypic traits in both subspecies of *Antirrhinum majus*.

Table S4. Results from the generalised mixed models (GLMM) testing for local adaptation on all phenotypic traits in both subspecies of *Antirrhinum majus*.

Table S5. Results from the generalised mixed models (GLMM) testing the effect of phenotypic traits on the survival and the probability of flowering in both subspecies of *Antirrhinum majus*. Results of all interactions are shown.

Table S6. Results from the generalised mixed models (GLMM) testing the effect of standardized-phenotypic traits on the relative number of flowers in both subspecies of *Antirrhinum majus*. Random factors for all models are (1-population). Results of all interactions are shown.

Table S7. Results from the generalised mixed models (GLMM) testing for local adaptation on the number of flowers in both subspecies of *Antirrhinum majus*. Results of all interactions are shown.