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Genetic variation underlies the plastic response to shade of snapdragon plants (*Antirrhinum majus* L.)

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ABSTRACT

A classic example of phenotypic plasticity in plants is the set of traits that change in response to shade. There is widespread evidence that plants in low light conditions often avoid shade by growing taller or by increasing their photosynthetic efficiency, i.e. the shade avoidance syndrome. Whether this plasticity might evolve in response to natural selection depends upon the presence of its standing genetic variation in wild populations. There is limited evidence for heritable standing variation in the plastic response of plants to shade. In this study, we used an experimental common garden approach to investigate this plastic response in snapdragon plants (*Antirrhinum majus* L.) originating from four natural populations from the Mediterranean region. Our results showed that individual plants reacted strongly to the presence of shade by growing longer shoots, longer internodes, and increasing their specific leaf area in these four populations. Our results also revealed genetic variation for the plastic response within these populations, as well as little genetic constraints to its evolution. Our findings imply that natural populations of *A. majus* harbour standing genetic variation for phenotypic plasticity in response to shade, providing them the potential to evolve in response to selection.

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Phenotypic plasticity; shade avoidance syndrome; quantitative genetics; stem elongation; specific leaf area

Introduction

Environmental heterogeneity is the norm rather than the exception in nature. As a consequence, organisms often experience variation in their environment across space and time. In this case, theory predicts that phenotypic plasticity (the capacity of one genotype to express different trait values in different environments) is likely to evolve (Via and Lande 1985; Schei ner 1993, 2013; Gavrilets and Scheiner 1993). The capacity of a population to evolve different levels of phenotypic plasticity is conditioned, however, by the presence of genetic variation for the plastic response. There is a large body of evidence showing a genetic basis to phenotypic plasticity for many traits (Beldade et al. 2011). A variety of approaches can be used to test for this genetic component (Pigliucci 2005). One classical method involves testing populations for gene-by-environment (GxE) interactions, by investigating how identical or related individuals sharing genes express different phenotypes in responses to a change in their environmental conditions.

The response of plants to a given light intensity and spectrum is a classic example of phenotypic plasticity (e.g. Smith 1982; Schmitt and Wulff 1993; Schmitt et al. 1995, 1999). In many species, shade triggers a suite of developmental, physiological, and phenological trait modifications. These modifications can include increased shoot (often through internode elongation), petiole or hypocotyl growth (Smith 1982; Ballaré et al. 1991; Ballaré 1999; Pierik et al. 2004; Pujol et al. 2005; Bell and Galloway 20 07; Franklin 2008), modification of leaf position (Pierik et al. 2003), increased apical dominance (Smith 1982; Smith and Whitelam 1997), reduction of branching or tillering (Smith 1982; Schmitt and Wulff 1993), earlier flowering (Smith 1982; Schmitt and Wulff 1993), and a greater leaf area to biomass ratio (increase in specific leaf area; Lewandowska and Jarvis 1977; Dong 19 95; Evans and Poorter 2001). Collectively, these trait mod ifications characterise the shade avoidance syndrome. Physiological and developmental mechanisms underlying this syndrome are well described (e.g. Ballaré et al. 1987, 1990; Ballaré and Pierik 2017), and involve the act ion of photoreceptors and phytohormones.

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The shade avoidance syndrome in plants is widely acknowledged as ecologically significant. This is because it is a functional mechanism that increases the ability of plants to reach or exploit light in the presence of competition. Stem elongation, for example, can result in longer shoots with the top leaves reaching above the canopy of competitors. The presence of this functional response in natural populations implies that past evolution has shaped the plant plasticity to light conditions. However, contemporary evolution requires both actual selection pressures and heritable variation. In terms of selection, the shade avoidance syndrome is often considered to be adaptive, and several studies have demonstrated a fitness advantage associated with its expression under neighbour shade (van Kleunen and Fischer 2001; Schmitt et al. 2003; Bell and Galloway 2007). In terms of heritable variation, knowledge is growing about the molecular mec hanisms underlying the plastic response of plants to shade in mutant and inbred lines. Most data on the heritable genetic variation for the magnitude of this plastic response is based on inbred lines and variation between specific mutants (e.g. Schmitt et al. 1995; Dono hue et al. 2000; Botto and Smith 2002; Huber et al. 2004). In contrast, knowledge regarding the presence of standing genetic variation for this plastic response is scarce in natural populations. Two notable exceptions are the stu dy of Bell and Galloway (2007) on *Geranium carolinianum* L., and a series of studies from Donohue and collea gues in field-derived inbred lines of *Impatiensis capensis* Meerb (e.g. Donohue and Schmitt 1999; Donohue et al. 2000; Huber et al. 2004). The presence of standing heritable variation for the expression of the plant response to shade at the scale of natural populations is the specific knowledge gap addressed by this study.

In this article, we investigated the plastic response to a modification of its environment induced by shade in individuals of four natural populations of *Antirrhinum majus* L. in a common garden experiment. Our aim was to evaluate the evolutionary potential of their plastic response to shade. *A. majus* grows in Mediterranean garrigues (dry scrubland comprised of a mixture of shrub and herb species) and Pre-Pyrenean mountain habitats, where there is variation in plant density and competition for light at both spatial and temporal scales. We therefore expected natural *A. majus* populations to exhibit a suite of plastic responses that are typical of the shade avoidance syndrome. We tested for a genetic component of plasticity variation by evaluating whether related individuals express a more similar plastic response than non-related individuals. We used a quantitative genetic approach to test for 1) the presence of a plastic response to shade in these four natural populations, and 2) genetic variation for this plastic response in these populations, and for each trait within the light and shade treatments separately, which would provide evidence for its evolutionary potential.

Material & methods

Study species

Antirrhinum majus L. is an hermaphroditic, self-incompatible (Andalo et al. 2010), short-lived perennial species, from the Plantaginaceae family. It has a patchy distribution in the eastern half of the Pre-Pyrenees and in the Mediterranean region, from Barcelona (Spain) to Montpellier (France) (Khimoun et al. 2013) at altitudes ranging from 0 to 1900 meters above sea level (Andalo et al. 2010). *Antirrhinum majus* is a species that colonises open habitats, typically limestone or siliceous substrates, and is adept at colonising screes, road banks and stone walls. *Antirrhinum majus* is characterised by a wide variation of floral morphology and colour and is easy to grow, with cultivated varieties being widely used in horticulture. Two subspecies have been described; *A. m. pseu domajus* and *A. m. striatum*, which are respectively characterized by magenta and yellow flowers. In this study, we studied *A. m. pseudomajus* populations. It has a diploid genome (2n = 16) and has become an important model in population genetics, speciation studies, and floral development research (Schwarz-Sommer et al. 2003).

Seed collection in wild populations

The plants used in this experiment originate from four natural populations of *A. m. pseudomajus* (Khimoun et al. 2011): Bages (BAG), Banyuls-sur-mer (BAN), Lagrasse (LAG), and Besalù (BES), which are located in Southern France and North Eastern Spain (Supplementary file 1). The general habitat of these four populations is typical of Mediterranean garrigues. In this dry environment, plants grow in a large variety of microhabitats, including open and shaded areas. The four populations used in this study were chosen on the basis of their high environmental heterogeneity in vegetation cover, resulting in different light conditions within a population. This choice was motivated by the expectation that shade is a stable cue for the presence of vegetation cover in these habitats and that plasticity is more likely to occur in predictably heterogeneous environments (Via and Lande 1985). Additionally, these four populations are significantly genetically differentiated at neutral markers (F_{ST} ranging from 0.08 to 0.12, Pujol et al. 2017) and thus can be considered as population replicates within the species level. In each population, we collected fruits that contained hundreds of seeds on 30 to 50 mature distant individuals in October 2011.

First generation of parents in a common environment

In order to reduce the influence of parental environmental effects, we grew a first generation of plants from

the seeds collected in the wild in a common environment during spring 2012 in the greenhouse at the CNRS experimental station in Moulis (Station d'Ecologie Théorique et Expérimentale, Moulis, France). We performed within-population crosses during spring and summer 2012 between maternal plants (BAG: 24 mothers, BAN: 25 mothers, BES: 23 mothers, LAG: 15 mothers) and different paternal plants (three to five paternal plants, sometimes including a plant that was used as a maternal plant in other crosses, were used to pollinate different flowers on a given maternal plant). This design produced three to five groups of full sibs (corresponding to three to five different fruits) pollinated by different paternal plants per maternal plant. At the global level, the fruits produced included a large amount of half sibs (groups of 25 to ca.100 plants shared either a father or a mother in the experimental plant po pulation), which optimises the relatedness variance for quantitative genetic analyses. Mature seeds were collected during autumn 2012 and were stored in paper bags at room temperature. Subsequently, we used the seeds produced in this common environment to perform a further common garden experiment where we manipulated shade levels.

Common garden experiment

In June 2014, we sowed seeds from 14 to 16 full sib families per population (Supplementary file 1) in peat-based universal compost in 8*8*7 cm pots. We sowed three seeds per pot. As soon as a seed germinated, seedlings that were found in excess in a pot (more than one) were transplanted into another pot. Around 900 seeds were sown and 336 seeds germinated and survived. These pots were spatially randomized in the EDB laboratory experimental garden at the Ecole Nationale Supéri eure de Formation de l'Enseignement Agricole (ENSF EA, Toulouse-Auzeville). The bases of the pots rested on an irrigation mat (400 g/m2) to regulate moisture content. A layer of soil of a few centimetres covered the base of the pots and the irrigation mat. To limit neighbour interference, pots were separated by at least 20 cm. On the 336 plants that grew, 88 individuals flowered. From June until September, approximately half the plants (n = 169) were exposed to natural light. The other half (n = 167) was exposed to a shade treatment. We randomly assigned half of the plants within each family to each treatment prior to the experiment. Shade was created for each plant individually, using vertical tunnels (height = 40 cm) that filtered the light that could reach the plant. These tunnels were made of a metallic mesh shaped into a tube, which was covered in green shading cloth, with an open top to allow for biotic interactions. No herbivory was recorded, probably as a result of the experimental garden isolated location and setting. Whether pollinators also had limited access to the plants is irrelevant to this study because we did not quantify plant reproductive success. We did not separate the direct effect of shade on plants from its potential indirect effects: reduced temperature, increased humidity and reduced mechanical disturbance caused by wind. All plants were watered similarly, with no nutrient addition, only when the lack of natural rain made it necessary to provide water to ensure the survival of plants in small pots. Average monthly temperatures ranged from 20.6°C to 21.5°C, and cumulative monthly rainfall ranged from 28.3 to 73.4 mm.

The shade treatment modified the light spectrum that the plants received (Supplementary file 2). In particular, it reduced the levels of Photosynthetically Active Radiation (PAR). PAR measurements were tak en once on a sunny day with a spectrometer (model AVASPEC-ULS 2048-USB2, Avantes, Apeldoorn, the Netherlands), from 8 am to 12 am, with one measurement per minute. The average PAR in the light treatment was 390.6 $\mu mol.m^{-}2.s^{-1}$, and its average in the shade treatment was 107.2 $\mu mol.m^{-}2.s^{-1}$. The average Red to Far Red ratio (R/FR) was reduced from 2.4 in the light to 1.7 in the shade, as expected from the effect of a green shading cloth.

Trait measurements

In order to quantify the response of plants to shade in the different populations, we took measures of plant height, internode length, and specific leaf area (SLA). We measured the vegetative height of every plant 40 days after germination, measured as distance (in centimetres) between the ground level and the last node at the top of the plant, or the last node below the first inflorescence if an individual flowered. We counted the number of nodes on this part of the stem, and divided the height by the number of nodes to obtain the mean internode length of 40-day-old plants (*Internode 40 days*, available for 327 individuals beca use 9 plants did not reach 40 days).

A similar measure was recorded at the date of first flowering (*Internode first flower*). However, flowering inhibition in the shade treatment resulted in very unbalanced data for this trait (N = 67 in the light and N = 21 in the shade, half of the latter belonging to the LAG population). We therefore chose not to perform analysis on this measurement other than testing for its correlation to the other measurements of internode length (see below). In order to obtain a measure of internode length for every individual, we measured the average internode length of the first six nodes of the stem starting from the base of the plant (*Internode six nodes*), on both flowering and non-flowering plants at the end of the experiment (N = 336).

For every plant, we estimated the specific leaf area (*SLA*), which is the ratio of the leaf area to its dry mass. For this, we sampled five mature non-senescent leaves on each plant. These leaves were stored in water and in

the dark during 6 h to 8 h before being scanned. They were then kept in silica gel until drying in the oven at 65°C for three days before weighing. SLA was derived by taking the ratio of the area of leaves to the mass of the dried leaves ($cm2.g^{-1}$).

Finally, at the end of the experiment, we estimated the total height of each plant, from the ground level to the top (*Height*, which includes the height of the inflorescence in individuals that flowered), and recorded whether each plant had flowered (*Flowering*).

Statistical analysis

Trait correlations

The first statistical analysis that we ran estimated the correlation between our three measurements of internode length (*Internode 40 days, Internode first flower* and *Internode six nodes*), by using a Pearson correlation test, to evaluate whether these traits were providing similar information. As these measures were well correlated, we only present analysis involving *Interno de six nodes*, for which we had most data. We used the statistical program R (R Core Team 2016) to estimate Pearson correlations.

Model 1: quantitative genetic linear mixed model to estimate families-level reaction norms

The second statistical analysis that we ran was a quantitative genetic linear mixed model. We used it to estimate the effect of the population of origin (*Population*) and the shade treatment (*Shade*) on *Height, SLA*, and *Internode six nodes* and the genetic variation of the plastic response. Genetic variation was estimated by the among-family variation of slopes between the two treatments (i.e. magnitude and direction of the change in trait values). We approximated the genetic variance underlying the plastic response by using the family effect which includes various degrees of genetic relatedness (e.g. Scheiner and Lyman 1989). This model was based on the availability of measurements for several members from the same maternal family in each treatment, which allowed us to estimate the between-family variance of the slope of the reaction norm between the light and the shade environments. This model included *Shade* and *Population* as fixed factors, as well as their interactions. The random part of the model included the interaction *Mother: Shade*, which allowed us to test whether individuals from different families had different reaction norms, thereby testing for the genetic basis of trait reaction norms in the broad sense. This model provided estimates for the between-family trait variance (intercept) in the light treatment. The variance term for the intercept was set for the light condition as the reference level but we also present the between-family trait variance for the intercept in the shade treatment. This model also provided estimates for the between-family variance of the slope of the reaction norm between light and shade as well as the covariance between the intercept value measured in the light treatment and the slope of the reaction norm. We allowed for, estimated and present different residual variances for light and shade treatments. For all these estimates, we present the posterior predicted 95% credible intervals.

Model 2: a second quantitative genetic linear mixed model

We ran two slightly different models that addressed slightly different questions. This second model was also a quantitative genetic linear mixed model. This approach is commonly used to decompose environmental and genetic effects (Charmantier et al. 2008; Pujol and Galaud 2013). We used it to consider the phenotypes expressed in the different environments as different traits, which allowed us to estimate the genetic covariance and calculate the genetic correlation between the two states (following the approach by Via and Lande 1985). This model thereby evaluates the independence of trait genetic variation between light and shade. The second model is a quantitative genetic linear mixed model, often referred to in the literature as the "Animal Model", with *Animal* referring to the additive genetic components estimated by the model. This model fully accounts for the different degrees of relatedness that can be identified in a population. In our dataset, the different degrees of relatedness between individuals were restricted to full sibs and half sibs, the only ones available to us from our crossing design. The added value of this approach compared to a classical linear mixed model is that it uses the relationship between individuals separated by different degrees of relatedness to estimate breeding values and thus the additive genetic component of the trait (the *Animal* random factor in this model). One must note that although this model produced estimates of the additive genetic component of phenotypic variance in each environment, this genetic effect was likely confounding additive, dominant and epistatic genetic effects in the absence of parent-offspring and double first cousin phenotypic data, thereby estimating the broad sense rather than the narrow sense genetic components of trait variance. This model included the same fixed effects as the first model: *Shade, Population* and their interaction. In order to estimate the additive genetic variance component in both treatments adequately, we excluded the possibility of a bias caused by the lack of independence of the estimates between the two treatments. To achieve this, we used an unstructured variance-covariance matrix between *Animal* (additive genetic

component) and *Shade* (environmental treatments). We then built a bivariate model where trait values obtained under shade and light could be analysed separately. We also used this model to estimate the genetic covariance between the two treatments. We derived genetic correlations between the trait value in the light environment and the trait value in the shade environment for each trait on the basis of these covariances (Falconer and Mackay 1996). This genetic correlation shows how the expression of the phenotype is affected across the two environments. Finally, a heterogeneous residual variance was included for each shade treatment, allowing different residual variances in the light and shade, but no covariance between the residuals in the two treatments. For all these estimates, we present the posterior predicted 95% credible intervals.

Common features of the two quantitative genetic linear mixed models

The two models were fitted in a Bayesian framework using MCMCglmm (Hadfield 2016) in the statistical program R (R Core Team 2016). Default prior assignments followed an inverse-wishart distribution (V = diag(2) and nu = 3 for the *Animal:Shade* (with animal being the default name for the additive genetic component in the MCMCglmm package) and the *Mother:Shade* effects, and V = diag(2) and nu = 0.002 for the residuals). We used a burn-in time of 900,000–4,800,000 iterations – thi
nning of 3000. These parameters were chosen because they allowed us to sample the posterior distribution 1300 times (except for *Internode six nodes*, where we used 1,500,000 burning iterations – 8,000,000 iterations – 5000 thinning to reduce autocorrelation). We used posterior distributions and model autocorrelation to assess the quality of model runs. Finally, we ran identical models with identical priors five times and used the Gelman–Rubin convergence criterion to assess run quality. This step yielded identical results, demonstrating high reliability between runs. 95% Credible Intervals (CIs) were derived from the runs for all parameters. The P values derived from Chi-squared tests were used for fixed effects. Random effects were considered significant if their 95% credible intervals did not overlap 0. Another approach that is often used to interpret whether random effects (family effects in model 1 and genetic effects in model 2) are meaningful is to compare a model with the random effect of interest and a simpler model excluding this factor by using information criterions (e.g. parametric linear models coefficient of determination R2 and Akaike Infor
mation Criterion for maximum likelihood estimates, Deviance Information Criterion for Bayesian linear models). An information criterion showing that more information is explained by the more complex model reflects a better fit of the data by the model including a meaning
ful random effect. However, this approach conducted by comparing DIC between Bayesian GLMMs may suffer methodological caveats (MacKenzie et al. 2018). We therefore chose to use CIs as explained above because this approach is highly reliable and gives all the necessary information about the parameter estimate. For example, a large but positive CI around a large parameter estimate value does confirm that the parameter estimate can be considered positive but also means that its value is imprecise and should not be considered to be high. Such information is relevant to evaluate whether the phenotypic variation of populations sampled in the wild has a genetic basis in an experimental setting. We did not compute the heritability estimates for reaction norms based on our experimental sibship data recorded in artificially modified environments because it would not provide meaningful quantitative information about the evolutionary potential of wild populations.

Results

Trait correlations

The three measures of internode length were strongly positively correlated (*Internode six nodes* and *Internode 40 days*: $\rho = 0.60$, $p < 10^{-15}$, *Internode six nodes* and *Internode first flower*: $\rho = 0.73$, $p < 10^{-15}$, *Internode 40 days* and *Internode first flower*: $\rho = 0.57$, $p < 10^{-7}$). We chose to focus on only one of these measurements, the *Internode six nodes* for the following analyses because this is the measure for which we had the most data.

Trait means

Table 1 presents trait values in the light and shade treaments. Overall, mean *Height* was 29.4 cm (sd = 16.2 cm) and was 23% to 42% less in the light than in the shade depending on the population (Figure 1). Overall, mean internode length (*Internode six nodes*) was 2.6 cm (sd = 1.1 cm) and was, depending on the population, 42% to 47% shorter in the light than in the shade (Figure 2). Overall, average *SLA* was 285.8 $cm2.g^{-1}$ (sd = 158.1 $cm2.g^{-1}$) and was 63% smaller in the light than in the shade (Figure 3). The probability of *Flowering* was 53% to 85% lower in the shade than in the light. We did not conduct an in-depth statistical analysis of this trait because too few plants flowered.

Model 1: quantitative genetic linear mixed model estimating families-level reaction norms

The effect of *Shade* on *Height* was significant (Table 2, *Light*: 20.9 cm (CI: 17.3 – 23.6); *Shade*: 31.2 cm (CI: 26.1

Table 1. Phenotypic trait values in the light and in the shade, by population. Population means (sd) for the traits in all four populations, in both the light and shade environments.

Trait	Population	Light	Shade
Flowering (probability)	BAG	0.4 (0.3)	0.1 (0.1)
	BAN	0.3 (0.3)	0 (0.1)
	BES	0.4 (0.3)	0.1 (0.2)
	LAG	0.4 (0.3)	0.2 (0.3)
Height (cm)	BAG	22.8 (9.3)	31.3 (8.8)
	BAN	25.9 (11.6)	36.5 (11.3)
	BES	24.7 (8.4)	32.1 (9.8)
	LAG	21.7 (8.6)	37.6 (12.2)
Internode six nodes (cm)	BAG	1.7 (0.5)	3.2 (0.8)
	BAN	2.2 (0.5)	4.1 (0.7)
	BES	1.9 (0.3)	3.2 (0.7)
	LAG	1.7 (0.3)	3.2 (0.5)
SLA ($cm2.g^{1}$)	BAG	151 (18.2)	411.9 (98.4)
	BAN	156.1 (24)	431.7 (98.7)
	BES	167.9 (14.5)	449.5 (88.6)
	LAG	145.6 (15.9)	397.3 (60.6)

– 35.8)). No differences in mean *Height* were detected among the four populations (Table 2, Supplementary file 3). There was no interaction between *Population* and the *Shade* treatment, as shown by null parameters for the interaction term (Supplementary file 4). There was significant variance between families for the intercept, i.e. mean trait values differed among families in the light treatment (V_{Light} = 0.14 (CI: 0.09 – 0.27), V_{Shade} = 0.25

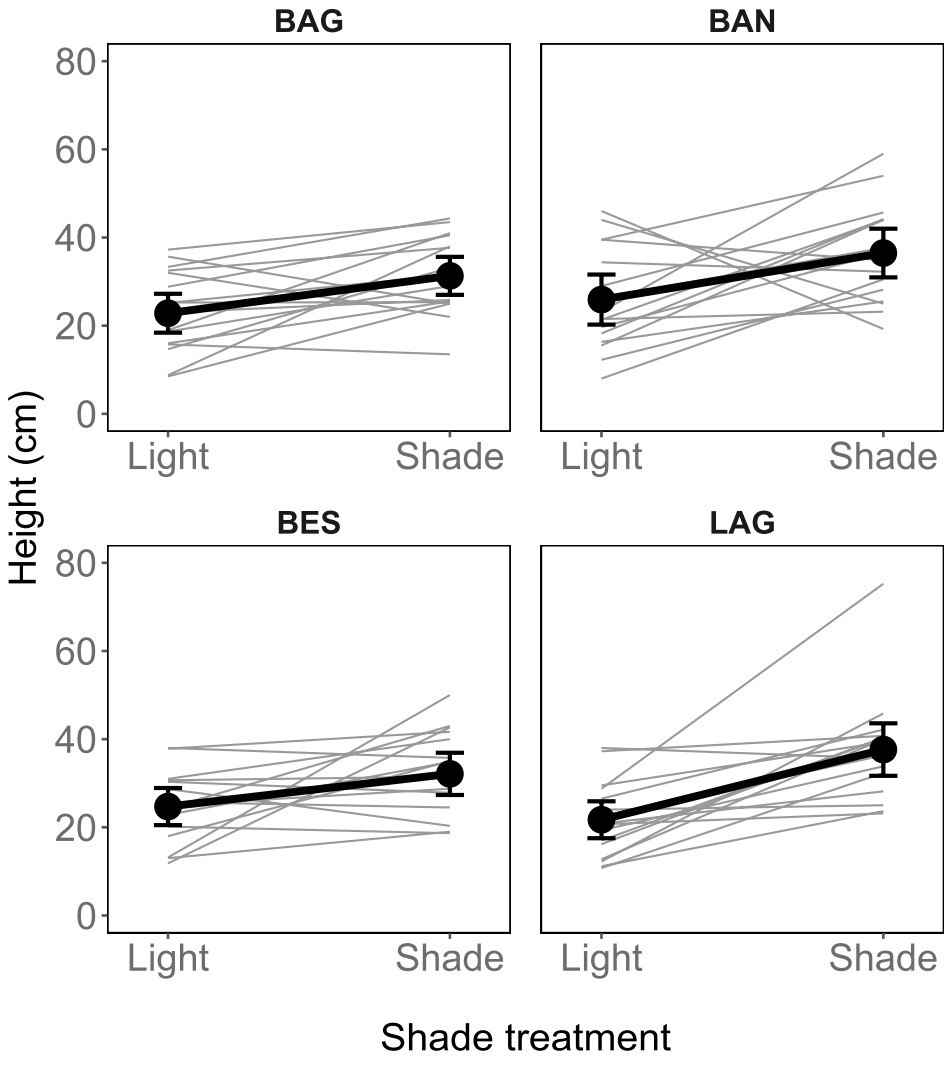

Figure 1. Longer stems in the shade than in the light. Average responses and family responses are presented for the four populations BAG, BAN, BES, and LAG. Grey: family means. Black dots and lines: population means based on family means. Error bars represent 95% credible intervals. These estimates are raw data summary statistics.

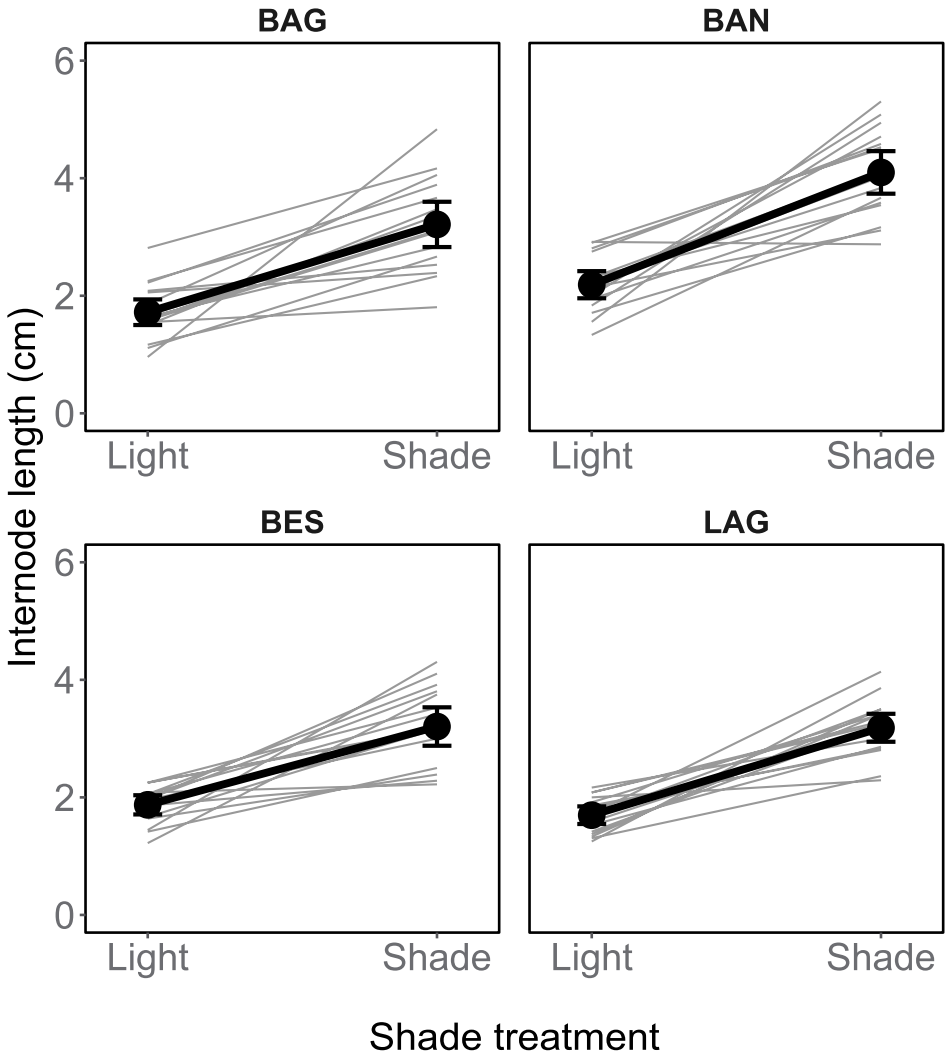

Figure 2. Longer internode length in the shade than in the light. Average responses and family responses are presented for the four populations BAG, BAN, BES, and LAG. Grey: family means. Black dots and lines: population means based on family means. Error bars represent 95% credible intervals. These estimates are raw data summary statistics.

(CI: 0.15 – 0.37), Table 3). Families also significantly differed in their response to the shade treatment: there was significant variance between families for the slope of the reaction norm (V_{slope} = 0.17 (CI: 0.1 – 0.31), Table 3). The covariance between the intercept (in the light) and the slope of the reaction norm was not different from zero (cov = 0.02 (CI: −0.11 – 0.14), Table 3).

The effect of *Shade* on *Internode six nodes* was significant (Table 2, *Light*: 1.9 cm (CI: 1.8 – 2); *Shade*: 3.4 cm (CI: 3.2 – 3.6)). There were significant differences in ave

Table 2. Significance tests for the *Population* and the *Shade* treatment. Chi-square tests are given for the fixed effects of the two models and the three traits of interest (*Height, Internode six nodes*, and *SLA*).

Trait	model	D.I.C.	Treatment	Chi2	D.f.	P.
Log Height	Model 1	604.2	*Population*	1.5	6	0.96
			Shade	22.7	4	**0.00**
	Model 2	282.8	*Population*	1.3	6	0.97
			Shade	15.3	4	**0.00**
Internode 6 nodes	Model 1	754.6	*Population*	15.8	6	**0.01**
			Shade	165.7	4	**< 10-3**
	Model 2	475.8	*Population*	15.7	6	**0.02**
			Shade	114.0	4	**< 10-3**
Log SLA	Model 1	−55.2	*Population*	1.9	6	0.93
			Shade	314.9	4	**< 10-3**
	Model 2	−898.2	*Population*	4.9	6	0.55
			Shade	304.0	4	**< 10-3**

D.f: degrees of freedom; P: P-value.

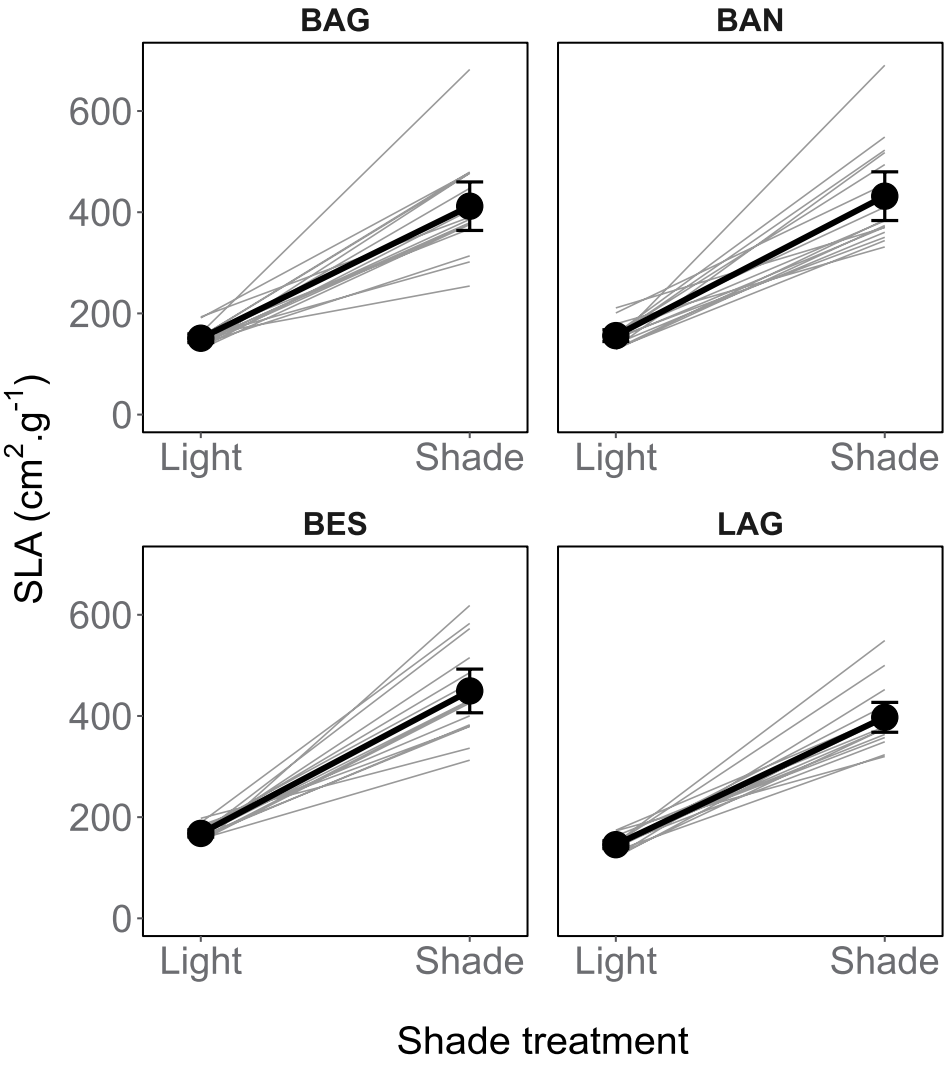

Figure 3. Higher specific leaf area in the shade than in the light. Average responses and family responses are presented for the four populations BAG, BAN, BES, and LAG. Grey: family means. Black dots and lines: population means based on family means. Error bars represent 95% credible intervals. These estimates are raw data summary statistics.

rage internode length among populations (Table 2, Supplementary file 3), but no interaction between *Population* and *Shade* (Supplementary file 4). There was significant variance between families for the intercept (V_{Light} = 0.18 (CI: 0.10–0.27), V_{Shade} = 0.4 (CI: 0.23 – 0.65), Table 3). There was significant variance between families for the slope of the reaction norm (V_{slope} = 0.31 (CI: 0.16 – 0.56), Table 3). The covariance between the intercept and the slope of the reaction norm was not different from zero (cov = – 0.04 (CI: – 0.14 – 0.02), Table 3).

The effect of *Shade* on *SLA* was significant (Table 2, *Light*: 153.4 m2.g^{-1} (CI: 139.5–165.6); *Shade*: 412.5 cm2. g^{-1} (CI: 357.6–459.4)). No differences in average *SLA* were detected among the four populations (Table 2, Supplementary file 3). There was no interaction between *Population* and the *Shade* treatment (Supplementary file 4). There was significant variance between families for the intercept (V_{Light} = 0.08 (CI: 0.05 – 0.12), V_{Shade} = 0.14 (CI: 0.1 – 0.24), Table 3) and for the slope of the reaction norm (V_{slope} = 0.1 (CI: 0.07 – 0.17), Table 3). The covariance between the intercept and the slope was not different from zero (cov = –0.01 (CI: – 0.04 – 0.02), Table 3).

In summary, all studied populations of *A. majus* exhibited a strong response to shade for all the three traits. There was no evidence of an interaction between *Population* and *Shade* in any of the traits (Supplementary file 3).

Model 2: a second quantitative genetic linear mixed model

The effect of *Shade* on *Height* was significant (Table 2, *Light*: 20.6 cm (CI: 17.3 – 23.8); *Shade*: 30.2 cm (CI: 26.6 – 36)). No differences in mean *Height* were detec ted among the four populations (Table 2, Supplement ary file 3). There was additive genetic variance in both

Table 3. Model 1. Family variation in the intercept and the slope of the reaction norm. Parameter estimates for the random effects estimated by Model 1 are given by the posterior mode estimates and their 95% credible intervals (C.I.). In this table, we present the family variance for the intercepts of the light and shade treatments (Variance Light of Shade intercept), the family variance for the slope of the reaction norm (Variance slope reaction norm), the covariance between the intercept of the light treatment and the slope of the reaction norm (Covariance Light-slope), and the residual variances in the two treatments (Residual variance Light and Shade) for the three traits of interest (*Height, Internode six nodes*, and *SLA*). Height and SLA were log transformed to meet the assumptions of the linear model. Values that were considered significant after examination of their credible intervals are presented in bold.

Trait	Term	Posterior mode	Lower 95% C.I	Upper 95% C.I.
Log Height	*Variance Light intercept*	**0.14**	0.09	0.27
	Variance slope reaction norm	**0.17**	0.10	0.31
	Variance Shade intercept	**0.25**	0.15	0.37
	Covariance Light-slope	−0.04	−0.14	0.02
	Residual variance Light	**0.35**	0.30	0.47
	Residual variance Shade	**0.21**	0.17	0.28
Internode 6 nodes	*Variance Light intercept*	**0.18**	0.10	0.27
	Variance slope reaction norm	**0.31**	0.16	0.56
	Variance Shade intercept	**0.4**	0.23	0.65
	Covariance Light-slope	−0.03	−0.14	0.05
	Residual variance Light	**0.32**	0.25	0.41
	Residual variance Shade	**0.70**	0.51	0.84
Log SLA	*Variance Light intercept*	**0.08**	0.05	0.12
	Variance slope reaction norm	**0.10**	0.07	0.17
	Variance Shade intercept	**0.14**	0.10	0.24
	Covariance Light-slope	−0.01	−0.04	0.02
	Residual variance Light	**0.03**	0.02	0.04
	Residual variance Shade	**0.05**	0.04	0.07

Table 4. Model 2. Genetic additive variance (*Va*) and covariances between light and shade. Parameter estimates for the random effects estimated by Model 2 are given by the posterior mode estimates and their 95% credible intervals (C.I.) for the three traits of interest (*Height, Internode six nodes*, and *SLA*). The last two traits were log transformed to meet the assumptions of the linear model. Values that were considered significant after examination of their CI are presented in bold.

Trait	Term	Posterior mode	Lower 95% C.I.	Upper 95% C.I.
Log Height	*Va Light*	**0.24**	0.12	0.50
	Va Shade	**0.28**	0.15	0.43
	Genetic covariance Light-Shade	0.02	−0.11	0.14
	Residual variance Light	**0.22**	0.07	0.35
	Residual variance Shade	0.00	0.00	0.16
Internode 6 nodes	*Va Light*	**0.26**	0.14	0.54
	Va Shade	**0.44**	0.24	1.17
	Genetic covariance Light-Shade	−0.02	−0.23	0.24
	Residual variance Light	0.15	0.00	0.24
	Residual variance Shade	0.00	0.00	0.61
Log SLA	*Va Light*	**0.06**	0.05	0.08
	Va Shade	**0.11**	0.08	0.13
	Genetic covariance Light-Shade	0.00	−0.02	0.02
	Residual variance Light	0.00	0.00	0.01
	Residual variance Shade	0.00	0.00	0.01

light (V_{Light} = 0.24 (CI: 0.12 – 0.54), Table 4) and shade (V_{Shade} = 0.28 (CI: 0.15 – 0.43), Table 4). The genetic covariance of the trait in the *Light* and *Shade* treatment was null, and with large credible intervals (Table 4).

The effect of *Shade* on *Internode six nodes* was significant (Table 2, *Light*: 1.9 cm (CI: 1.7 – 2.1); *Shade*: 3.4 cm (CI: 3.2 – 3.7)). There were significant differences in average internode length among populations (Table 2, Supplementary file S3). There was additive genetic variance in both light (V_{Light} = 0.26 (CI: 0.14 – 0.54), Table 4) and shade (V_{Shade} = 0.44 (CI: 0.24 – 1.17), Table 4), but no genetic covariance of the trait between the *Light* and *Shade* treatment (Table 4).

The effect of *Shade* on *SLA* was significant (Table 2, *Light*: 151.1 cm2.g^{-1} (CI: 141 – 161.3); *Shade*: 340.3 cm2.g^{-1} (CI: 373.6 – 445.6)). No differences in average *SLA* were detected among the four populations (Table 2, Supplementary file 3). There was additive genetic variance in both light (V_{Light} = 0.06 (CI: 0.05 – 0.08), Table 4) and shade (V_{Shade} = 0.11 (CI: 0.08 – 0.13), Table 4), but no genetic covariance of the trait between the *Light* and *Shade* treatment (Table 4).

In summary, all studied populations reacted strongly to shade, Model 2 estimated null (and small) covariances between the *Light* and *Shade* treatment, which means that individuals with high trait values in the light did not consistently have high (or low) trait values in the shade

environment. Consequently, the very weak genetic correlations derived from these covariances had very large credible intervals (*Log Height*: 0.16 (CI: -0.32 – 0.49); *Internode six nodes*: 0.10 (CI: -0.44 – 0.45); *Log Log SLA*: –0.02 (CI: -0.25 – 0.21)).

Discussion

Our results showed that *A. majus* plants have a large phenotypic plastic response to shade and its potential indirect effects on humidity, temperature and mechanical disturbance. When exposed to shade, *A. majus* plants increased in height, internode length, and SLA, typical of the shade avoidance syndrome (Smith and Whitelam 1997). In a situation where light may become, or is already, scarce, stem elongation through increased distance between nodes and/or stem height is expected to allow plants that are not shade tolerant to outgrow competitors. An increase in SLA in the shade, while technically not a shade avoidance strategy, is a functional response that has often been observed (Lewandowska and Jarvis 1977; Dong 1995; Evans and Poorter 2001; van Kleunen et al. 2011; Liu et al. 2016), and is expected to improve the physiological performance of plants in a light-limited environment. However, caution must be taken when interpreting SLA because its increase might also reflect a failure of plants to maintain adequate mass balance in tissues, possibly as a consequence of resource limitation (e.g. light restriction, given the large reductions in PAR experienced by plants in the Shade treatment).

Similar plastic responses have been observed in other studies involving *A. majus* or other *Antirrhinum* cultivars. In *A. majus* cultivars, Munir et al. (2004) found increased total height and reduced branching at lower light intensity and Khattak and Pearson (2005) found higher internode length in reaction to light filtering. In addition, Cremer et al. (1998) observed shorter flowering time under low R/FR ratio in an inbred *A. majus* line (Sippe50). Our results demonstrate the existence of a plastic response to shade of *A. majus* plants from wild populations and suggest the possibility of a shade avoidance syndrome in *A. majus*. The R/FR levels are relatively high in our experiment. They are usually lower under canopy shade. The high R/FR levels in the present experiment were probably too high to induce the full suite of responses to canopy shading and limited plants to mainly express response to reduced PAR, temperature, mechanical disturbance and increased humidity. One reason for the high R/FR levels in the shaded plants may have been the use of green shade cloth, which might not have significantly differed from black shade cloth. In order to simulate canopy shade r-absorbing filters have to be used, where the spectral composition is changed after the light passes through the filter.

Furthermore, we did not observe different levels of plasticity in the four populations. These populations are characterized by high habitat heterogeneity. The low population differentiation might indicate that there has been little variation in selection pressures among populations. Caution must nevertheless be taken when extrapolating our results to the natural environment. Our results do not necessarily mean that plants from these populations will express the same responses when growing in wild conditions. Complex environments may constrain the expression of plasticity, particularly if some phenotypes are more costly than others in a particular set of conditions (Donohue et al. 2000; Huber et al. 2004). The plastic response to shade is an evolutionary labile trait that can be lost quickly during evolutionary transitions, even at the within-species scale, such as between wild populations and cultivars (Pujol et al. 2005). However, the combined results from past studies of *A. majus* cultivars and this study support the hypothesis that plasticity is widespread among *A. majus* populations, whether it evolved post-speciation or was conserved from ancestral species.

For such a set of plastic responses to evolve, or to be conserved, individuals need to have access to reliable cues that describe the current and/or future light environment. Plants usually rely on a reduction of the ratio of red to far-red radiations (R/FR ratio) as a cue indicating the presence of competitors for light: plant responses under reduced R/FR ratios are similar to those observed in dense patches where there is competition for light (Smith 1982; Ballaré et al. 1987, 1990). The reduction of total light intensity, and in particular blue light, also induces a response to shade in some species (Ballaré 1999; Pierik et al. 2004; Franklin 2008, 2016; Ballaré and Pierik 2017). Previous studies on *A. majus* inbred lines and cultivars showed that the reduction of both the R/FR ratio and the amount of blue radiation are likely to be involved in *A. majus* response to shade (Cremer et al. 1998; Khattak and Pearson 2005). However, no study had evaluated this plastic response in *A. majus* plants originating from natural populations. In our experiment, we did not attempt to test these two cues in isolation. Instead, we evaluated the effect of a controlled light environment simulating competition for light but excluding other effects of high density (e.g., plant hormones modification, nutrient or water competition), and found that *A. majus* responded strongly to this controlled light environment, suggesting that either reduction of light intensity, R/RF ratio or both act as cues in this species.

We performed a study of the population variation of plant responses to shade, both between populations and between families, for several morphological and functional traits. Our results showed that families within populations varied in their phenotypic plasticity induced by shade, thereby supporting the presence of a genetic basis for this variation in plasticity. Family effects are often used to evaluate the genetic component of trait variation because members of the same family are genetically related (Lynch and Walsh 1998). This heritable variation in plasticity must be interpreted in the broad

sense because it encompasses additive genetic, dominance, maternal, and epistatic effects. While growing the parental generation in a common environment reduces the possible environmental component of maternal effects, it does not entirely eliminate it, and we cannot distinguish between all components of variance with our design.

Genotype by environment interactions are often found in studies of phenotypic plasticity (Pigliucci 2005). However, only a few cases of GxE interactions were documented for the plastic response of plants to shade or competition. For example, Donohue and Schmitt (1999) found a G × E interaction in the plastic response to plant density in natural settings, using inbred lines of *Impatiens capensis* Meerb. Botto and Smith (2002) found a G × E interaction for the plastic response of *Arabidopsis thaliana* (L.) Heynh. accessions to R/FR ratio modifications. However, for the shade avoidance response, most known G × E interactions correspond to comparisons between a plant wild type and mutant lines (Skalova and Krahulec 1992; Andersson and Shaw 1994; Schmitt et al. 1995, 2003), or between populations (Pigliucci et al. 1995; Van Hinsberg 1997), and habitats (Humphrey et al. 2018). Results from past studies did not necessarily support family variation in the plastic response to shade, i.e. variation in the slope of the reaction norm in plants from natural populations (e.g. Andersson and Widén 1994). As this genetic variation in the plastic response is the basis for the evolution of different degrees of plasticity, our results imply that *A. majus* populations have the potential to evolve under selection for higher or lower plasticity when grown under controlled conditions.

Whether the population evolutionary potential of a trait provided by its genetic variation can be realised in wild populations depends obviously on the presence of selection pressures that could not be quantified in this experimental setting. Under natural selection pressures, other biological mechanisms might however be acting as potential constraints to the response to selection (Pujol et al. 2018). In terms of trait reaction norms, pleiotropy between the differential expression of a trait in different environments might constrain the evolution of plasticity (Via and Lande 1985; van Kleunen and Fischer 2005). We therefore investigated trait genetic covariance between the two environments in each of our three traits and found no genetic covariances between trait values measured in the light and in the shade treatment, in all traits. Furthermore, the family means in the light treatment did not strongly co-vary with the slope of the reaction norm, which suggests no genetic correlations between the trait value in the light treatment and the plastic response. Collectively, these results suggest few genetic constraints on the differential expression of the trait in the light and in the shade, with genes involved in the mean trait response in one environment likely independent from the genes acting on the plastic response. This might be because genes expressed in the light environment are mostly different from the genes expressed in the shade environment. The absence of genetic covariance allowing families to evolve independently in a given environment might be the underlying mechanisms shaping the between-family variation in the *A. majus* plastic response to shade. A limitation of our approach is that the lack of genetic covariance between trait expression in light and shade may be masked by uncontrolled-for covariates that vary between Families (e.g. growth rate, growth rate under resource limitation). In contrast, other studies that estimated the genetic covariance or correlations between traits under light and shade conditions found a much higher correlation between the mean of the trait and the magnitude of the plastic response (Donohue and Schmitt 1999; Donohue et al. 2000). Whether the surprising lack of genetic covariance between environments that we detected questions the value of analysing plasticity as a trait in itself through the analysis of its family variation as a proxy for its genetic variation is a general hypothesis that would deserve further investigation. The relevance of our findings is obviously limited to their experimental background and it would be useful to evaluate whether our conclusions hold in the more stringent natural settings of the four natural populations that we studied. Nevertheless, our current findings suggest that genetic correlations between the differential expression of shade responsive traits in light and shade do not constrain the reaction norm to shade in these *A. majus* populations.

Conclusion & perspectives

Our experimental findings imply that *A. majus* plants can react to shade, its indirect effects on temperature, humidity and mechanical disturbance, and might potentially play a role in the competition for light in natural populations. This plasticity gives them the potential to respond to the temporal and spatial variation of density. Our results support the presence of genetic variation for the traits under both light and shade environments, between-family variation for the slope of the reaction norm, with no evidence for genetic correlations that could constrain the evolution of plasticity. Collectively, our findings suggest a plausible shade avoidance syndrome in *A. majus* but more investigations are needed to validate that specific aspect. To this aim, it is indeed necessary to take into account the allometric relationship between the plasticity of morphological traits and the plant overall growth under shade, and whether this relationship leads to fitness benefit in the presence of heterogeneous vegetation cover. Our findings strongly support the presence of heritable variation for the plastic response to shade in *A. majus* populations with the possibility for selection to act independently on trait values under shade and under light. This heritable variation, which represents the potential

for this plastic response to evolve in response to selection, might be widespread in *A. majus* populations.

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Author contributions

BP conceived the study. SM, JA and CB ran the experiment. SM, JA, CB and VB recorded the data. MM, JA and LG ran the analyses. MM and BP wrote the paper. All authors commented the manuscript.

Data archiving

Phenotypic data and pedigree data used in this article are available on Zenodo Open Aire repository, DOI: 10.5281/zenodo.3963754

Disclosure statement

The authors declare no conflict of interest.

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