Plasticity to simulated shade is associated with altitude in structured populations of *Arabidopsis thaliana*

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ABSTRACT

Plants compete for photosynthesis light and induce a shade avoidance syndrome (SAS) that confers an important advantage in asymmetric competition for light at high canopy densities. Shade plasticity was studied in a greenhouse experiment cultivating *Arabidopsis thaliana* plants from 15 populations spread across an altitudinal gradient in the Northeast area of Spain that contain a high genetic variation into a reduced geographical range. Plants were exposed to sunlight or simulated shade to identify the range of shade plasticity. Fourteen vegetative, flowering and reproductive traits were measured along to the life cycle. Shade plasticity in flowering time and dry mass was significantly associated with the altitude of population origin. Plants from coastal populations showed higher shade plasticity indexes than those from mountains. The altitudinal variation in flowering leaf plasticity adjusted negatively with average and minimum temperatures, while dry mass plasticity was better explained by negative regressions with the average, maximum and minimum temperatures, and by a positive regression with average precipitation of the population origin. The lack of an altitudinal gradient for the widest number of traits suggests that shade light could be a driver explaining the distribution pattern of individuals in smaller geographical scales than those explored here.

Key words: Light, phytochromes, phenotypic plasticity, shade avoidance syndrome, *Arabidopsis thaliana*, altitudinal gradient, structured populations, local adaptation.

A brief summary statement (3-4 sentences maximum) highlighting the importance of the work.

Low red/far-red (R/FR) ratio of the light environment provides an early and unambiguous warning of the presence of competing vegetation. This paper focuses on the study of the shade avoidance syndrome in structured populations of *Arabidopsis thaliana* originated in an altitudinal gradient of the Northeastern of Spain. Plasticity to shade of two important fitness traits, like flowering and aerial dry biomass, were associated with the altitudinal gradient of population origin being plants collected in the coast more plastic than those from the mountains. The clinal variation of the shade plasticity index was negatively associated with temperature for both traits and positively with precipitation for dry biomass, suggesting that these climatic parameters could be relevant for light adaptation in these populations.

INTRODUCTION

Plants need resources such as water, nutrients and light to grow. Under dense vegetation, light is a limiting resource and competition for light can strongly influence the success of a plant (Pierik and de Wit, 2013). Plants have evolved sophisticated mechanisms mediated by phytochromes that allow them to detect the early presence of neighboring plants and to initiate developmental adaptive developmental strategies that avoid shading before the canopy is closed (Ballaré et al., 1990). The most significant changes in the red/far-red (R/FR) ratios occur when daylight is reflected or transmitted by green vegetation. Absorption of red (R) and blue photons by chlorophyll and carotenoids results in a selective enrichment of far-red (FR) photons, reducing the R/FR ratios perceived by the plant tissues. As a result of changes in the light spectrum, plants display the shade avoidance syndrome (SAS), a set of physiological responses that increase vegetative structures like stems, petioles and hypocotyls, accelerates flowering, and reduce seed number and size (Casal, 2012).

In *Arabidopsis thaliana* and other species, phytochrome B (phyB) is the main phytochrome, and phyD and phyE contribute secondarily, mediating the SAS. In open environments, the Pr, the inactive form of the phytochromes located in the cell cytoplasm, migrates to the nucleus when it absorbs photons of R light and photo-transforms to Pfr. In the nucleus, the accumulated Pfr form interacts and degradates PIFs (Phytochrome Interacting Factors) through the proteosome leading to growth inhibition by the deactivation of gene expression (Lorrain et al. 2008). In opposition, the shade light converts Pfr to Pr form that no longer interacts with PIFs. These proteins will thus rapidly

re-accumulate promoting the expression of early shade genes such as *PIL1, ATHB2, HFR1,* and *PAR1* inducing cell elongation responses (Lorrain et al., 2008, Hornitschek et al., 2012). In addition, the full expression of SAS requires other photomorphogenic regulators like COP1 (McNellis et al., 1994, Pacín et al., 2013), SPA (Rolauffs et al., 2012) double B-Box proteins (Crocco et al., 2010; Gangappa et al., 2013) and bHLH/HLH transcription factors (Hao et al., 2012).

The hypothesis of adaptive plasticity predicts that the phenotype of shade avoidance induced by low R/FR ratios has a better fitness in dense canopies but is penalized at low densities (Schmitt et al., 1995). Because the light is a critical resource for plants, the SAS confers an important advantage in asymmetric competition for light at high densities. However in the absence of competition, allocation of resources to height at the expense of leaves, roots and branches may reduce growth and reproduction, and elongated stems may have a greater risk of mechanical damage (Casal and Smith, 1989; Schmitt and Wulff, 1993). Natural variation is a pre-requisite for the evolution of phenotypic plasticity (Via, 1985). At molecular level, nucleotide polymorphism at photoreceptor genes underlying natural variation in light responses (Aukerman et al., 1997; Balasubramanian et al., 2006; El-assal et al., 2001; Maloof et al., 2001). PhyB is the principal photoreceptor responsible for red light and shade avoidance responses, and is proposed to be the gene responsible for several QTL found when plants grow under shade (Borevitz et al., 2002; Botto et al., 2003; Botto and Coluccio, 2007; Kasulin et al., 2013). By the analysis of the phyB sequence in 33 *A. thaliana* accessions, Filiault et al. (2008) found 14 non synonymous polymorphisms with at least one of them responsible for the phenotypic variation

observed in seedlings exposed to red light. PIF4 polymorphisms are also supposed to be associated with internode length of inflorescence and reproductive timing and fitness under shade (Brock et al., 2010). Natural variation at *ELF3*, a gene involved in the circadian clock, was clearly associated with a function for shade avoidance in *A. thaliana*. In fact, a single amino acid change in the *ELF3* gene is responsible for the natural variation mediating cell elongation growth (Coluccio et al., 2011) and flowering time (Jiménez-Gómez et al., 2010) between two contrasting accessions originated in Bayreuth (Bay, Germany) and Shahdara (Sha, Tajikistan). Genetic diversity and structure analysis in more than 6000 wild genotypes from different world regions, at global and regional scales, suggest several major events in *A. thaliana* demographic history in Europe (Nordborg et al., 2005; Platt et al., 2010). In particular, high diversity has been described in the Mediterranean Peninsulas compared to Central and Northern Europe (Beck et al., 2008; Picó et al., 2008). The largest diversity has been found in the Iberian Peninsula, whose strong geographic structure has prompted the hypothesis of multiple Iberian glacial refuges with differential contribution to the colonization of Europe (Picó et al., 2008). The structure of northeastern Iberian populations is important and contains huge genetic variation across an altitudinal gradient suggesting that they may be locally adapted (Montesinos-Navarro et al., 2009; Montesinos-Navarro et al., 2011; Wolfe and Tonsor, 2014). These *A. thaliana* populations grow in two contrasting climatic environments: maritime lowland coastal area characterized by cool temperatures and moderate rainfall in the winter, low rainfall and high maximum temperatures in spring and summer; and higher altitude in mountainous areas, with higher rainfalls and lower minimum

115 temperatures in winter and a prolonged cool and wet spring. Interestingly, Montesinos-
116 Navarro et al. (2011) found that the phenotypes of these populations are associated with
117 a climatic gradient defined by altitudinal clines. Working with northeastern Iberian
118 populations, Tonsor´s group showed that biomass, leaf number, flowering time, and seed
119 weight increase, whereas translocation of resources to the root, vegetative growth and
120 number of seeds decrease with the altitude of the genotype origin. These life strategies
121 favor the selection of individuals for rapid life cycle in Mediterranean regions near the sea
122 avoiding typical warm dry summer periods, and long life cycles in individuals growing in
123 the mountains that help to maximize growth, cold tolerance in the winter and late
124 flowering.
125 The eco-physiological basis of the shade plasticity variation remains obscure. Some studies
126 have found a significant correlation between light sensitivity (Maloof et al., 2001; Stenøien
127 et al., 2002) and shade elongation response (Kasulin et al., 2013) with the latitude of
128 accession location, suggesting that light phenotypic variation could be a result of genotype
129 adaptation to a latitudinal gradient. Stenoien et al. (2002), working with 10 Norwegian
130 populations of *A. thaliana* collected in a narrow geographic range, found a latitudinal cline
131 in response to light: the northern genotypes are more responsive than southern
132 populations to R or FR continuous light during seedling de-etiolation. Furthermore, the
133 hypocotyl elongation response to a FR pulse at the end of the day, a laboratory treatment
134 that simulates shade avoidance, was positively associated with the increase of latitude for
135 European accessions collected between 15 and 65° (Kasulin et., 2013). However, in other
136 studies, hypocotyl and flowering shade response correlations with latitude are missing

(Botto and Smith, 2002; Filiault and Maloof, 2012). The strong structure of northeastern Iberian populations, containing a wide range of genetic diversity within a narrow geographical range, is an ideal system for testing hypotheses associated with the SAS. To have a better understanding about the drivers of shade avoidance plasticity, we designed a greenhouse experiment using the northeastern Iberian populations of *A. thaliana*. We evaluated the range of variation of vegetative, flowering and reproductive traits in response to simulated shade to answer the following questions:

a) What is the range of phenotypic variation to R/FR ratios in structured populations?

b) Is the expression of shade plasticity traits associated with an altitudinal gradient?

c) If the previous question is yes, what are the climatic drivers explaining this variation?

MATERIALS AND METHODS

Genetic material

Sixty genotypes from 15 populations of *A. thaliana* originated from the Northeast area of Spain were used in this study. These populations were collected in different locations defined by an altitudinal gradient (Montesinos-Navarro et al., 2009). In this area of collection, the rainfall increases and high spring temperatures and minimum winter temperatures decrease with the altitude (Montesinos-Navarro et al., 2011).

Culture conditions and light treatments

Seeds were sown in transparent plastic boxes on an agar solution of 0.8%. The boxes were placed in darkness at 5 °C for one week to break dormancy. After that, the boxes with

seeds were placed in a chamber with continuous white light for another week to induce uniform germination and the development of seedlings with well-developed green cotyledons and radicle. Then, seedlings were vernalized for two weeks in a light chamber under non-inductive short-day conditions (8+16h light, dark) at 5 °C before transplanting. The seedlings were transplanted on 27th August of 2011 to 7 x 4 cm pots (height x diameter) with a substrate of vermiculite, perlite and peat in a ratio of 30:30:10. After that, the plants were grown with natural radiation and controlled temperature in a greenhouse at IFEVA, Faculty of Agronomy, University of Buenos Aires (34º35'S, 58º29'W), Buenos Aires, Argentina. The pots with plants were watered with Hoagland solution (20 milliliters of Hakaphos Compo Red solution in 5 liters of water).

After a week of transplanting, the plants were exposed to sunlight or simulated shade, a treatment that consisted in sunlight plus lateral FR light mimicking neighboring plants (Rondanini et al. 2014). The FR light was provided by two banks equipped with 9 incandescent reflector lamps of 40W each, and a red acetate filter with two filters of blue acrylic Paolini (1 x 0.25 m long x wide) of 2 mm thick placed in front of the plants. To avoid the increase of temperature by the lamps, transparent bottles with water were placed between lamps and filters along with two fans that allowed ventilation. Plant grown in simulated shade received R/FR ratios ranged between 0.07 and 0.12 (Suppl. Table 1). Two lines of plants were located in front of the Paolini filters. The pots with plants were rotated every week to randomize light differences into the treatment. The sunlight treatment consisted of a similar experimental design without the addition of FR light. The lateral R/FR ratio was 0.65 (Suppl. Table 1). The sunlight on the top of the plants was

similar between both light conditions: the average photosynthetically active radiation (PAR) was near to 500 µmol m^{-2} s^{-1} at noon for sunny days and the R/FR ratios ranged between 0.94 and 1.02 (Suppl. Table 1). During the experiment, the daily average temperature ranged between 20.5 °C and 30.4 °C (Suppl. Fig. 1). PAR and R/FR ratios were measured with a model Spectroradiometer SPECTROSENSE2 / 2 + Meter, Skye Instruments Ltd (UK). The temperature was measured with a digital maximum and minimum thermometer (Thermometer, Germany).

Traits and shade plasticity index

Fourteen vegetative, flowering and reproductive traits were measured during the experiment: length and width of leaf, petiole length, leaf angle, rosette diameter and height, flowering time as number of leaves or days at flowering, length and diameter of primary axis, number of basal axes, number of secondary axes on the primary inflorescence, seed weight (100 seeds) and above-ground dry mass. The leaf angle was taken in the first and second week after starting the light treatments with a goniometer consisting of a protractor and a weight to mark the normal. The angle formed between the normal and the tallest petiole leaf was estimated. Vegetative traits were measured every week during the 28 d after the beginning of light treatments. Above-ground dry mass was assessed at the end of the trial (23/12/11) by placing the harvested aerial parts (including flowering axes) in an oven at 80 °C for 72 hours and then dried material was weighed with a precision balance. Seed yield, as the total weight of seeds, was not included in the analysis because heat stress increased towards the end of the experiment

(Suppl. Fig. 1) producing higher flower mortality at the extreme of inflorescences in the late flowering individuals compared with the earlier flowering individuals.

A shade plasticity index for each individual and trait was estimated as the difference between sunlight and simulated shade relative to sunlight as follow:

Shade plasticity index = 1+ [(simulated shade – sunlight)/sunlight]

Shade plasticity indexes higher than 1 indicate that simulated shade increases the response, and values lower than 1 indicate that simulated shade reduces the response with respect to sunlight. Values close to 1 mean that the individuals have low shade plasticity in opposition to higher or lower indexes that mean the individuals display strong shade plasticity.

Experimental design and statistical analysis

The experimental design was a randomized-block factorial design of two factors: population (P) consisted in 15 populations distributed along an altitudinal gradient, and light (L) consisted in two light conditions: sunlight and simulated shade. For each light condition, 8 replicates for population were established and each population was represented by 4 genotypes. Data were statistically analyzed by two-factor ANOVA including P, L, P x L and B (block) factors. Paired comparisons by Bonferroni test were included when the P X L interaction factor was significant. Genetic correlations for vegetative and reproductive traits within and between light treatments were estimated. Univariate regression analyses were done to evaluate clinal population differentiation between shade plasticity indexes and altitude or climatic parameters.

225 Because the SAS includes several morphological and developmental traits rather than any
226 single factor, multivariate analysis was done. The suite of SAS traits were treated as a
227 group testing the effect of light of all the measured traits (F= 14.57, $P < 0.0001$). The
228 overall clinal population differentiation between shade plasticity indexes and altitude was
229 evaluated using a multivariate analysis of variance MANOVA of all the measured traits as
230 dependent variables and altitude of population origin as the independent variable (F=
231 1.96, $P < 0.0001$). In addition, a principal component analysis (PCA) was conducted to
232 represent the complexity of data matrix in two principal axes. All measured traits for each
233 individual were included in the analysis as dependent variables. For graphical
234 representation, population and light were introduced as classification factors. Statistical
235 analyses were performed using the statistical program Infostat
236 (http://www.infostat.com.ar/).
237

238 **RESULTS**

239 **Time-course responses to simulated shade for vegetative traits**

240 Six vegetative traits (length and width of lamina, petiole length and leaf angle, and
241 diameter and height of rosette) were measured during the four weeks after the beginning
242 of light treatments to study the effect of simulated shade on the time-course of vegetative
243 growth. To increase the robustness of the analysis, the average response was estimated
244 for the 15 populations in each light condition and date. The time-course growth was
245 affected by simulated shade in four traits: leaf angle, petiole length, and height and
246 diameter of rosette (Fig. 1). Simulated shade increased the erect position of the young

leaves and rosette height at the starting of the experiment (Fig. 1, first and second weeks). Furthermore, the shade light increased significantly the petiole length and rosette diameter during the first month of the experiment (Fig. 1). The length and the width of the leaves also increased systematically during the first month but no significant differences were found between light treatments (Fig. 1).

Reaction norms to simulated shade

The average expression of the six vegetative traits with the exception of leaf length differed significantly among populations and light treatments (Fig. 2, Suppl. Table 2, see population and light factors). Simulated shade altered the vegetative phenotype in different intensities increasing the petiole length and the rosette height, and reducing the leaf angle with respect to the normal. In addition, simulated shade reduced marginally the leaf width and increased the rosette diameter in most of the populations (Fig. 2, Suppl. Table 3). No significant effects were detected for the population by light interaction factor in any of the six vegetative traits (Fig. 2, Suppl. Table 2).

Flowering was affected by population and light factors. As expected, the simulated shade accelerated flowering. The light factor was more sensitive for the number of leaves than for the number of days at flowering (Fig. 3, Suppl. Table 2). The population by light interaction effect was not significant for leaves and days at flowering. After flowering, six reproductive traits were measured. Population and light effects were significant for the number of basal axes, length of the principal axis, above-ground dry mass and seed weight. The population by light interaction factor was significant for the length of principal

axis and seed weight (Fig. 3, Suppl. Table 2). In these traits, plants from the ARU population showed significantly longer axes under simulated shade compared with sunlight (45.6 vs. 31.4 cm, respectively), and the individuals from the VDM population produced significantly lighter seeds under sunlight compared with simulated shade (2.7 vs. 3.6 mg/100 seeds, respectively). These differences disappeared in other populations, suggesting different light sensitivities to the same light signal (Suppl. Table 3). Although the population factor was significant for the principal axis diameter and the number of secondary axes, the light factor did not affect significantly these reproductive traits (Fig. 3 and Suppl. Table 2).

Genetic correlations for traits within and between light treatments

Least-squares means of vegetative, flowering and reproductive traits were used to estimate Pearson´s product correlations and genetic variance-covariance matrices within each light environment. Independently of the light factor, stronger positive correlations were found among leaf length with other vegetative traits like as leaf width, petiole length and rosette diameter ; and also between flowering time (leaves or days) with leaf angle, number of secondary axes and above-ground dry mass (Table 1). Some positive correlations were only found in plants cultivated in simulated shade. For example, plants with higher above-ground dry mass produced wider flowering axes and heavier seeds (Table 1).

A lower number of negative correlations were also found. For both light conditions, the rosette height showed a negative correlation with flowering (days or leaves) and

inflorescence length (Table 1). Interestingly, the rosette height was negatively correlated with fitness traits such as dry biomass and seed weight, specifically in plants cultivated under simulated shade (Table 1).

Population origin is the principal driver of the phenotypic variation

Multivariate analysis was applied for all the measurements of individuals corresponding to 15 populations and 14 traits in sunlight and simulated shade. The principal component analysis (PCA) reduced the variability of the data in a lower dimensional space than the original space of variables. The first and the second dimensions of the PCA analysis (CP1 and CP2) explained 34 % and 19.6 % of the data variability (Fig. 4). The first axis ordered the cases following a pattern that was very similar to the geographical gradient of the population origin. The biplot representation allowed the identification of the variables (arrows in the graph) that determine the location of the cases in this gradient: with some exceptions, vegetative traits and coastal populations were grouped together on the left side of the first axis, and flowering and reproductive traits together with populations originating from the mountains were grouped on the other side. Light was the secondary factor explaining the observations principally on the second axis. On the upper side of the graph appeared the cases associated with plants cultivated in simulated shade while in the bottom were grouped the cases of plants exposed to sunlight with the exception of BOS population (see 8: shade). .

Shade plasticity for flowering and dry mass is associated with an altitudinal cline

313 The shade plasticity was estimated in six vegetative and eight flowering and reproductive
314 traits for each population as the average response of the individuals. To test whether the
315 shade plasticity is associated with the altitude of the place of population origin, regression
316 analyses were done for each trait. In those traits associated significantly with an altitudinal
317 pattern, climatic parameters were examined in order to explain this variation. Number of
318 leaves or days at flowering and above-ground dry mass plasticity indexes showed a clear
319 and significant regression with the altitude of origin (Fig. 5). The distribution of shade
320 plasticity indexes adjusted to a regression line that differed from the horizontal (Fig. 5, P =
321 0.003 for leaves at flowering, P = 0.037 for days at flowering and P= 0.001 for above-
322 ground dry mass). Individuals from coastal areas showed a higher plasticity to shade than
323 those plants from mountain locations that displayed null or reduced shade plasticity (Fig.
324 5). Shade plasticity indexes for other vegetative and reproductive traits were not
325 associated with the altitude of population origin (Suppl. Fig. 2 and 3). Furthermore,
326 flowering leaf plasticity index showed a significant regression with average and minimum
327 temperatures (Fig. 6), but not with maximum temperature neither average precipitation
328 of the place of population origin (Suppl. Fig. 4). In other words, individuals from coastal
329 areas that experience higher average and minimum temperatures showed higher
330 flowering shade plasticity than those individuals from mountains sites. The shade
331 plasticity of above-ground dry mass showed a negative regression with temperatures
332 (average, minimum and maximum) and a positive regression with the average
333 precipitation (Fig. 6), but not with the distribution of precipitations in autumn and spring
334 (Suppl. Fig. 4). It means that higher dry mass plasticity index was associated with plants

335 from coastal areas growing with higher temperatures and lower average precipitation
336 than those from mountainous areas. No significant regressions were found for the
337 flowering day plasticity index and the six climatic parameters evaluated (Suppl. Fig. 5).
338
339 **DISCUSSION**
340 Shade response in vegetative, floral and reproductive traits was studied in 15 populations
341 of *A. thaliana* spread across an altitudinal transect in the northeastern area of Spain. These
342 populations contain a high genetic diversity and are characterized by a strong population
343 structure (Montesinos-Navarro et al., 2011; Picó et al., 2008). Vegetative traits such as
344 petiole length, leaf width, diameter and height of rosette, and angle of insertion of the
345 leaf were significantly affected by changes in R/FR ratios. The simulated shade produced
346 plants with narrower lamina, larger petioles and rosette diameter, and also more erected
347 leaves at early developmental stages compared with those plants cultivated under
348 sunlight (Fig. 1 and 2). In other species close to *Arabidopsis*, like rapeseed plants, it has
349 also been observed that low R/FR ratios induce dramatic shade avoidance responses.
350 Shade signals increase the leaf length but not the leaf width, and produce elevated leaf
351 angles in early stages of the development of a spring rapessed hybrid (Rondanini et al.,
352 2014). It is well known that the low R/FR ratios of the reflected light provide early
353 warnings of the presence and proximity of neighboring plants, allowing the initiation of
354 development adaptive strategies to avoid shading before the canopy is closed (Ballaré et
355 al., 1990). Furthermore, a huge natural variation for shade avoidance responses was
356 documented in a representative panel of *Arabidopsis* accessions (Botto and Smith, 2002).

Interestingly, natural variation at the *ELF3*, a circadian clock gene, is responsible for the shade avoidance variation for hypocotyl length elongation, leaf angle movement (Coluccio et al., 2011) and flowering response (Jiménez-Gómez et al., 2010) between Bay and Sha accessions of *A. thaliana*. The altered shade elongation response and leaf movement in Sha accession was associated to a rare Alanine by Valine substitution that alters ELF3-Sha circadian rhythms of leaf movements and clock gene expression (Coluccio et al., 2011, Anwer et al., 2014).

Shade light accelerates flowering response in *A. thaliana* plants. Some studies show that the number of leaves at flowering is a more sensitive trait than bolting time (Fig. 3, Callaghan and Pigliucci, 2002, Botto and Coluccio, 2007). In *Arabidopsis*, low R/FR ratios accelerate flowering by enhancing the expression of FLOWERING LOCUS T (FT), the gene involved in the induction of flowering by long days (Halliday et al., 2003). Callaghan and Pigliucci (2002) found that flowering time was accelerated by shade under field conditions, but not when *Arabidopsis* plants where grown in a greenhouse with the presence of grass neighbors. However, working with a wide range of natural variation, flowering time in response to low R/FR ratios was accelerated either when plants were cultivated in a light chamber (Botto and Smith, 2002) or in a greenhouse (Botto and Coluccio, 2007).

Reproductive traits were also affected by shade. Low R/FR ratios produced taller inflorescences and reduced the number of flowering axes, plant biomass and seed weight (Fig. 3). The effect of light on the inflorescence length was dependent on the population. Interestingly, the ARB population produced longer axes under simulated shade (P X L

interaction, *P*= 0.0022) but these differences disappeared in other populations suggesting that shade sensitivity depends on the origin of the population. Although cell elongation is stimulated by shade, Brock et al. (2010) found that most of the accessions of *A.thaliana* cultivated in low density in a greenhouse developed taller inflorescences than those growing in crowded stands. The authors interpreted these odd results as a limitation of translocation resources from leaves to fruits. Furthermore, branching is inhibited by low R/FR ratios. Loss of phyB function leads to a reduced branching by a down-regulation of the expression of auxin genes (Reddy and Finlayson, 2014; Su et al., 2011). Molecular and pharmacological assays suggest that the active form of phyB suppresses auxin signaling to promote branching (Reddy and Finlayson, 2014). Furthermore, in the present study, the simulated shade reduced above-ground dry mass and seed weight (Fig. 3), and these results are in accordance with previous evidence demonstrating that environments with resource limitation, such as low R/FR ratios, reduce plant growth and productivity (Sultan, 2000).

It is well known that climatic variables have important consequences for the geographical distribution of individuals and species. Shade plasticity indexes of flowering time and above-ground dry mass were significantly associated with the altitude of collection place (Fig. 5). However, other plasticity indexes of vegetative and reproductive parameters did not show clinal variation associated with the altitude (Suppl. Fig. 2 and 3). Interestingly plants from coastal populations showed higher plasticity to shade than mountainous populations, suggesting different light sensitivities according with the population origin. In fact, low R/FR ratios accelerated flowering and reduced the plant biomass more

401 dramatically in coastal populations than in mountainous populations. Furthermore, some
402 climatic parameters were significantly associated with shade plasticity indexes of some
403 traits. For example, the flowering leaf index showed a significant correlation with the
404 average temperature and the minimum temperature of population origin, while the shade
405 plasticity index for dry mass was better explained by the pattern of variation in
406 temperatures and the average precipitation (Fig. 6). Northeastern Iberian populations of
407 *A.thaliana* show strong demographic and genetic patterns defined by the altitude of
408 origin, with mountain populations less genetically diverse than coastal populations
409 (Montesinos-Novarro et al., 2009; Picó, 2012). The drivers of this altitudinal cline are
410 associated with colder winter temperatures and wetter and longer springs in mountain
411 areas (Montesinos-Navarro et al., 2009; Gomaa et al., 2011). Accordingly, the patterns of
412 evolutionary diversification in these structured populations can be influenced by the
413 plasticity to light. As predicted by the ecological theory, adaptation through natural
414 selection will not occur as readily for genetically distinct coastal populations because
415 individuals are more plastic and produce phenotypes more appropriate to different local
416 environments. Conversely, mountain ecotypes, in which individuals express limited
417 plasticity, would be predicted to show greater response to local selection regimes and
418 therefore greater genetic divergence (Sultan, 2000).
419 The altitudinal patterns in shade plasticity found for flowering leaf and biomass were
420 obtained from plants cultivated in optimal growth conditions. We should be cautious in
421 generalizing the conclusion of this work to other suboptimal environmental conditions. In
422 fact, the expression of the shade avoidance plasticity can be limited by

423 microenvironmental variation in water availability in seedlings from natural populations of
424 *Impatiens capensis* (Huber et al., 2004). The authors found that local seedling density was
425 a poor predictor of selection on shade-avoidance traits as a consequence of the
426 unpredictability of water availability, particularly in dry microsites that may affect the
427 costs and benefits of expressing shade avoidance (Huber et al., 2004).
428 The results of this work illustrate clearly that the shade plasticity for flowering and dry
429 biomass show clinal variation associated with altitude in structured populations of
430 *Arabidopsis* originating in the Northeast area of Spain. Ecotype differences in response to
431 shade signals were also documented for *Stellaria longipes* adapted to two ecological
432 environments. In concordance with the results showed here, the prairie ecotype responds
433 quickly to low R/FR ratios elongating their ramets as an adaptation to growth in dense
434 vegetation stands, in contrast to the alpine ecotype that displays dwarf phenotypes with
435 resistance to wind but unresponsive to shade signals allowing adaption to areas of sparse
436 vegetation where abiotic stresses predominate (Sasidharan et al., 2008). Furthermore, the
437 shade light, changing also across a micro-environmental context, may be a driver
438 explaining the distribution patterns of individuals in smaller geographical scales than those
439 explored here. New experimental approaches should be undertaken to test this
440 hypothesis. To evaluate this idea it is necessary to work with a bigger collection of
441 populations correctly described both in their geographical positions and types and
442 environments (woodland, scrubland, anthropic, prairie, etc.); as well as having detailed
443 descriptions of the environmental conditions of the collection site (radiation, light quality,
444 etc.). The atlas of ecological and climatic information along with genetic databases of each

individual and their corresponding phenotype may help to identify the underlying genes that express the enormous plasticity documented in the SAS. Deciphering the genetic and molecular basis of phenotypic plasticity is a challenge to understand how plants function, and it is essential to understand the evolutionary forces operating in the adaptation of species to a changing environment (Alonso-Blanco and Méndez-Vigo, 2014).

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Figure legends

Fig. 1: Time-course response to simulated shade for vegetative traits.

Response to R/FR ratios was calculated as the average of 15 Iberian populations at each date. The lines outside the box plot graphs indicate the minimum and maximum values for sunlight and simulated shade between 7 and 28 days after the starting of light treatments. Means were compared by Tukey test ($P < 0.05$) after analysis of One-Way ANOVA.

Fig. 2: Reaction norms to simulated shade for vegetative traits.

Each point represents the average response in the control and low R/FR treatments for each population. On the top of each graph is indicated the output of the ANOVA analysis for each independent variable (L= light, P= population) and the interaction between L x P. *, ** and *** indicate $P < 0.05$, 0.01 and 0.001, respectively. ns: not significant.

Fig. 3: Reaction norms to simulated shade for flowering and reproductive traits.

Each point represents the average response in the control and low R/FR treatments. On the top of each graph is indicated the output of the ANOVA analysis for each independent variable (L= light, P= population) and the interaction between L x P. *, ** and *** indicate $P < 0.05$, 0.01 and 0.001, respectively. ns: not significant.

Fig. 4: Multivariate analysis for all the traits from plants of 15 populations cultivated in sunlight and simulated shade.

623 Principal component analysis (PCA) was conducted to reduce the complexity of the data
624 matrix in two eigenvectors. All the measured traits for each individual were included in
625 the analysis as dependent variables and population and light as classification factors.
626 Numbers indicate populations as 1: PIN, 2: RAB, 3: SAL, 4: BAR, 5: HOR, 6: ARU, 7: COC, 8:
627 BOS, 9: MUR, 10: VDM, 11: ALE, 12: PAL, 13: BIS, 14: VIE, and 15: PAN. For additional
628 references on traits names see Table 1.
629
630 **Fig. 5: Clinal variation associated with altitude for flowering and above-ground mass**
631 **plasticity to simulated shade.**
632 Regression fitted between plasticity shade indexes for flowering and above-ground mass
633 and altitude of the population origin. Fitting regression lines are presented with R^2 and P
634 values indicating significant regression with respect to zero. Each point represents the
635 average plasticity response estimated as the difference between simulated shade and
636 sunlight relative to sunlight. Values closed to 1 mean that the population has null or low
637 plasticity in opposition to higher or lower indexes than 1 indicating that populations
638 display strong shade plasticity.
639
640 **Fig. 6: Climatic parameters associated with clinal variation for leaf flowering and above-**
641 **ground mass plasticity to simulated shade.**
642 Plasticity traits are represented as function of the climatic parameters associated to each
643 population. Fitting regression lines are presented with R^2 and P value indicating significant
644 regression with respect to zero. For other references see Fig. 5.

Suppl. Fig. 1: Average, minimum and maximum temperature during the experiment.

Daily temperatures (minimum, maximum and average) measured during the experiment into the greenhouse. The experiment started on 28th of August and finished on 23rd of December 2011.

Suppl. Fig. 2: Shade plasticity in vegetative traits regressed with altitude.

Plasticity traits are represented as function of the altitudinal gradients. Each point represents the average plasticity response estimated as the difference between simulated shade and sunlight relative to sunlight. Values closed to 1 mean that the population has null or low plasticity in opposition to higher or lower indexes than 1 indicating that populations display strong shade plasticity.

Suppl. Fig. 3: Shade plasticity for flowering and reproductive traits regressed with altitude.

Plasticity traits are represented as function of the altitudinal gradients. For other references see Suppl. Fig. 2.

Fig Suppl. 4: Climatic parameters not associated with clinal variation for leaf flowering and above-ground dry mass to simulated shade.

Plasticity traits are represented as function of the climatic parameters associated to each population.

Fig Suppl. 5: Climatic parameters not associated with clinal variation for flowering day to simulated shade.

Plasticity traits are represented as function of the climatic parameters associated to each population.

Table 1: Genetic correlation matrix of vegetative, flowering and reproductive traits from *A. thaliana* plants exposed to solar and low R/FR ratios. Sunlight (above the diagonal) and low R:FR conditions simulating foliar shade (below the diagonal). Significant correlations are indicated by *** (P<0.001), ** (P<0.01) and * (P<0.05). LL, leaf length; LW, leaf width; PL, petiole length; LA, leaf angle; RD, rosette diameter; RH, rosette height; FD, flowering in days; FL, flowering in leaves; PAL, principal axe length; PAD, principal axe diameter; SAN, secondary axe number; AN, basal axe number; DW, above dry weight; and SW, seed weight.

	LL	LW	PL	LA	RD	RH	FD	FL	PAL	PAD	SAN	AN	DW	SW
LL	----	0.55***	0.51***	-0.16	0.78***	0.4***	-0.23*	-0.08	-0.18	0.2*	-0.11	0.31**	0.42***	0.15
LW	0.69***	----	0.37***	-0.09	0.44***	0.16	0.001	0.03	-0.19	0.19*	-0.01	0.12	0.16	0.2
PL	0.64***	0.59***	----	-0.06	0.58***	0.05	0.1	0.14	-0.21*	0.12	0.009	0.31**	0.28**	0.07
LA	-0.08	-0.01	0.05	----	-0.13	-0.58***	0.34***	0.39***	0.1	-0.02	0.28***	-0.31***	0.03	0.13
RD	0.91***	0.71***	0.82***	-0.05	----	0.29**	-0.04	0.06	-0.15	0.15	0.004	0.22*	0.42***	0.1
RH	0.06	0.09	-0.16	-0.46***	-0.03	----	-0.57***	-0.48***	-0.2*	-0.04	-0.31***	0.27**	-0.01	-0.08
FD	-0.13	-0.11	0.06	0.38***	-0.06	-0.47***	----	0.9***	0.17	0.15	0.66***	-0.34**	0.34***	0.3*
FL	-0.02	-0.04	0.12	0.4***	0.04	-0.43***	0.92***	----	0.18	0.25**	0.67***	-0.26**	0.42***	0.35***
PAL	0.04	-0.1	0.14	0.17	0.11	-0.28**	0.16	0.21*	----	0.22*	0.25**	-0.11	0.21*	-0.08
PAD	0.13	0.13	0.3**	0.09	0.25**	-0.19	0.24*	0.3**	0.4***	----	0.31**	-0.06	0.018	0.03
SAN	-0.08	-0.01	0.13	0.09	0.01	-0.11	0.55***	0.54***	0.2*	0.46***	----	-0.23*	0.34***	0.26*
AN	0.2	0.13	0.28**	-0.02	0.25**	-0.04	-0.1	-0.1	0.04	0.02	-0.22*	----	0.28**	-0.15
DW	0.1	0.09	0.14	0.19	0.17	-0.35***	0.68***	0.68***	0.28**	0.34***	0.39***	0.19	----	0.18
SW	-0.17	-0.19	-0.16	0.44***	-0.15	-0.49***	0.35**	0.35**	0.29*	0.17	0.22	0.09	0.39***	----

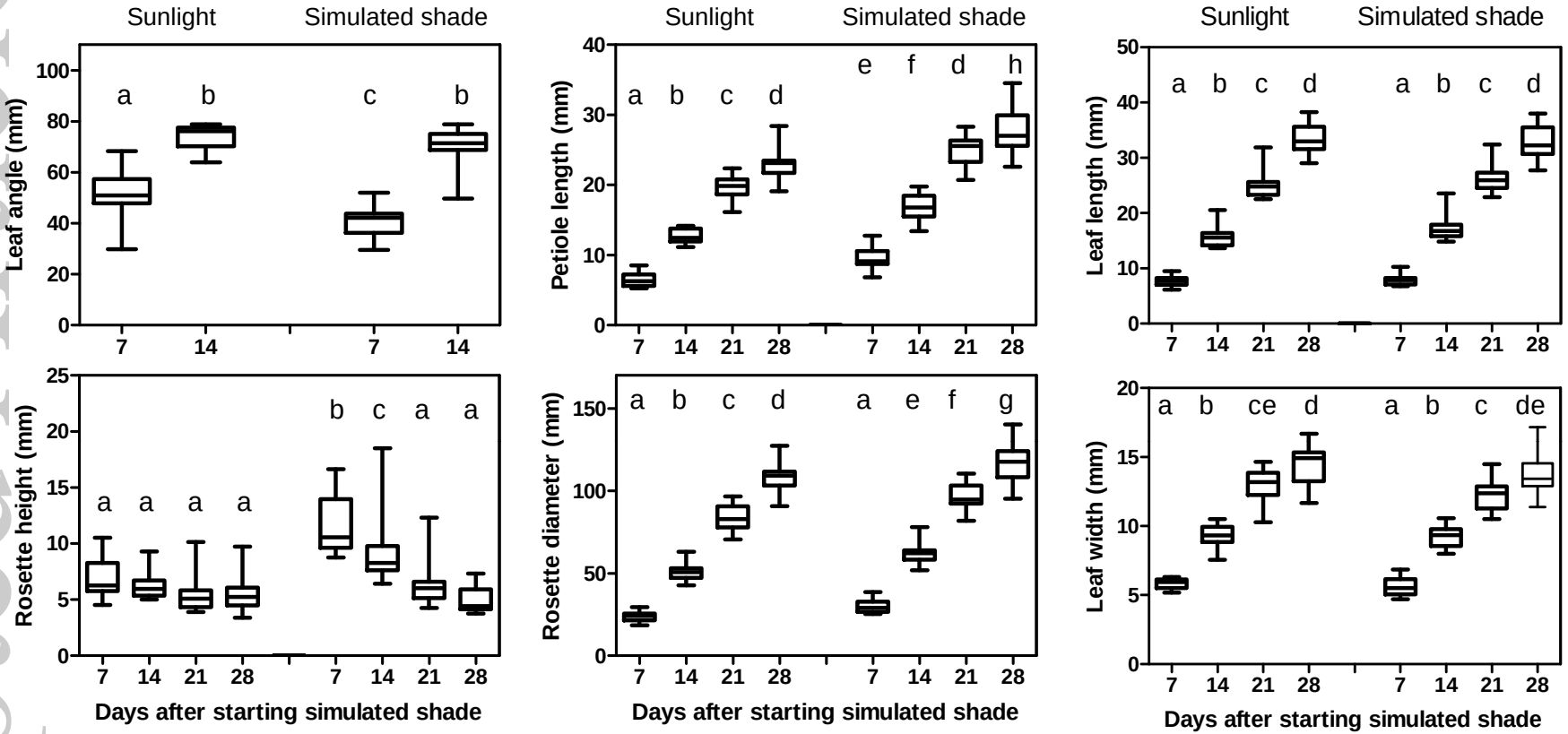


Fig. 1

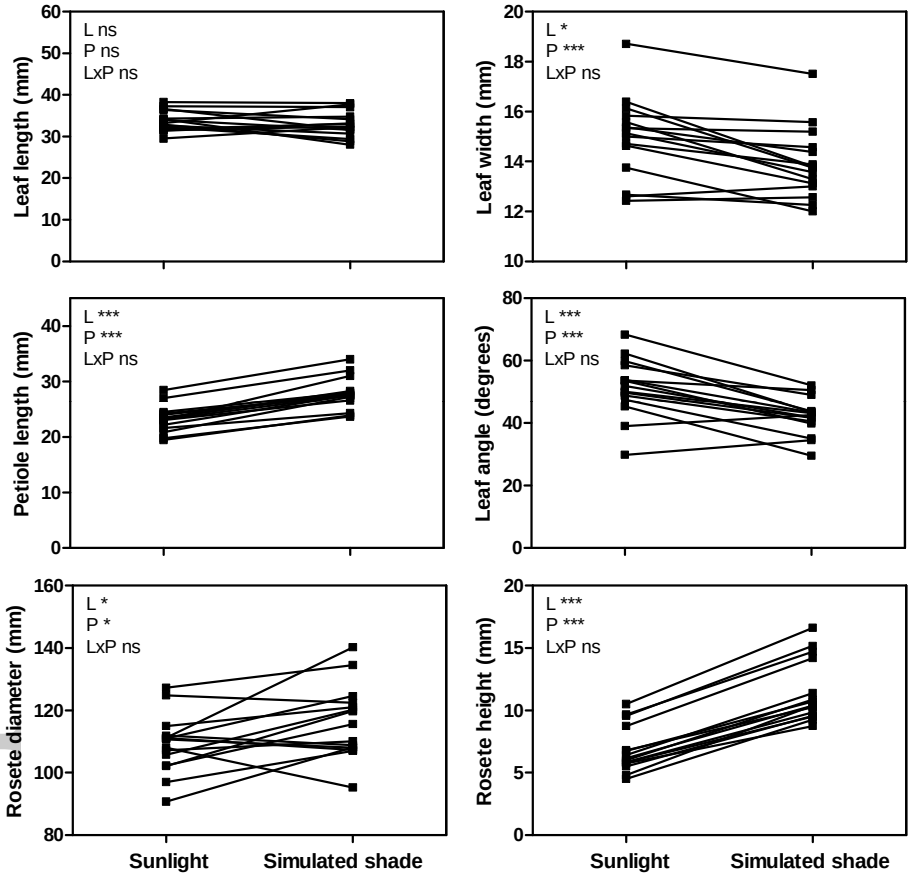
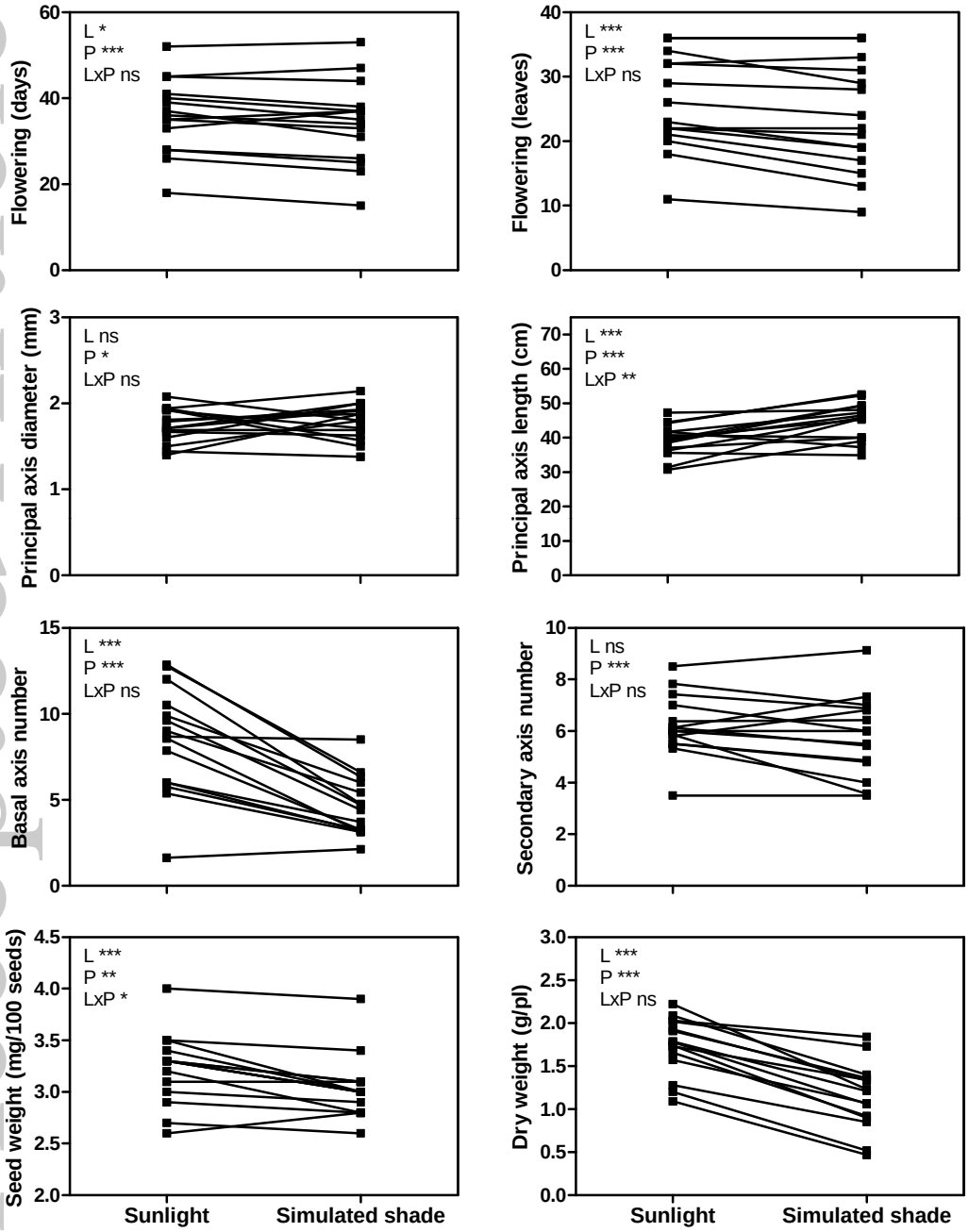


Fig. 2



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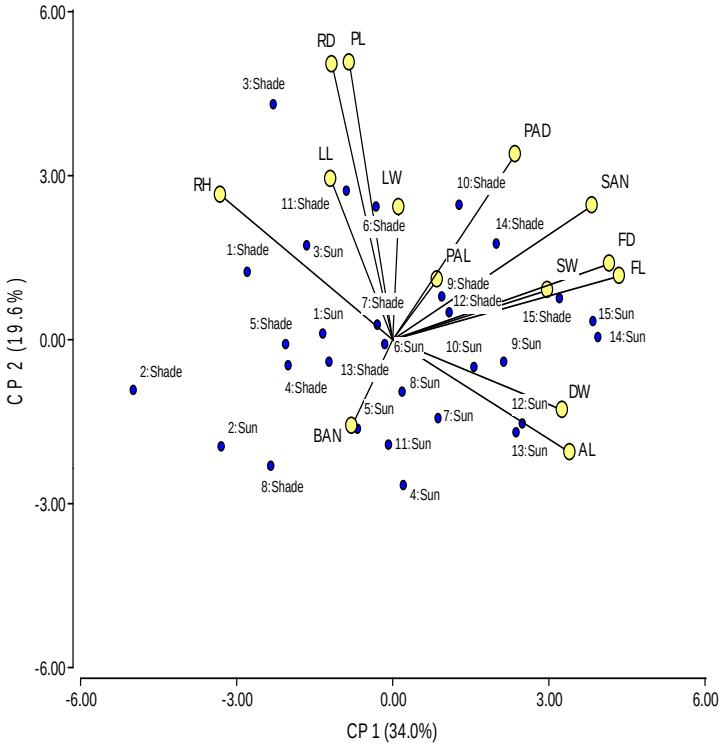

Fig. 4

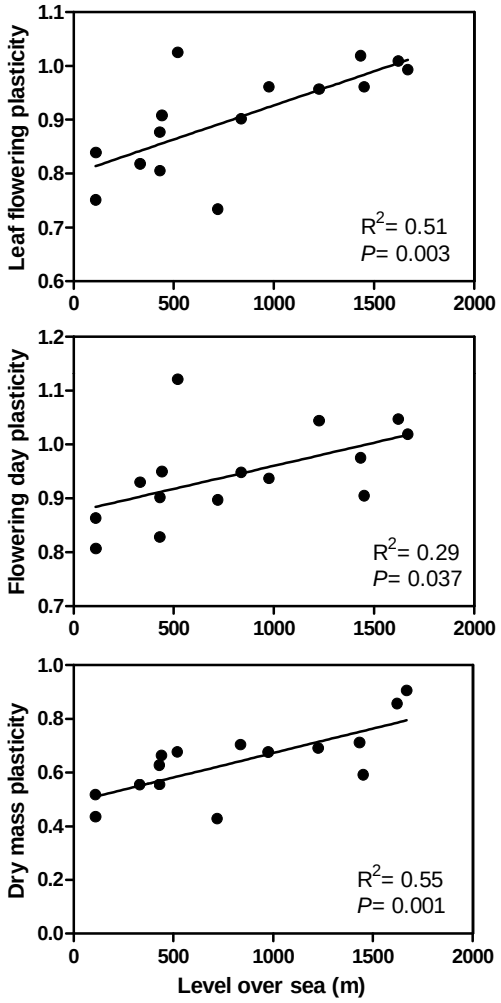

Fig. 5

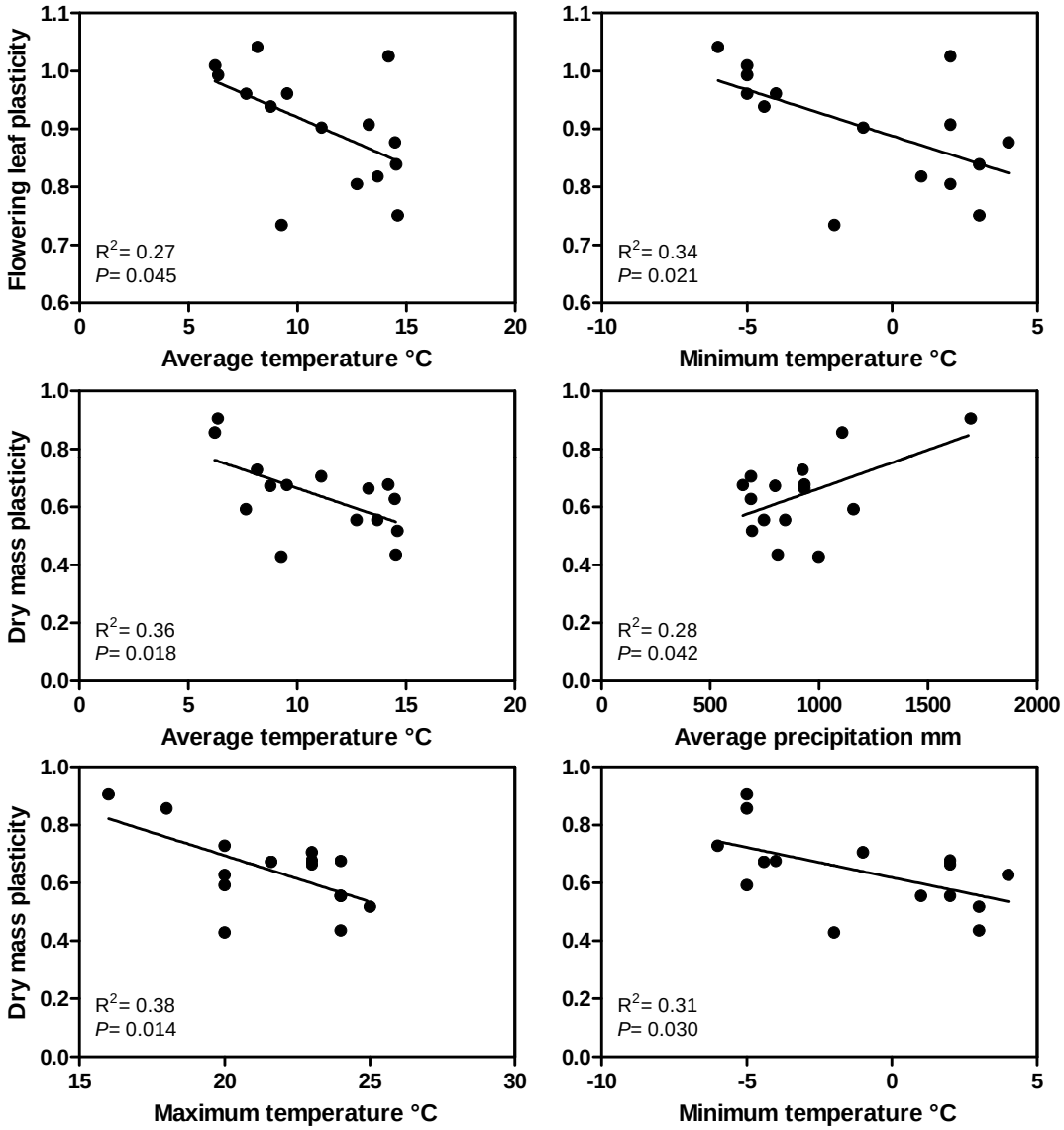

Fig. 6