Title: Water availability and population origin affect the expression of the tradeoff between reproduction and growth in *Plantago coronopus*

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Running title: The tradeoff between reproduction and growth is affected by population and water availability

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**Abstract**

Investment in reproduction and growth represent a classic tradeoff with implication for life history evolution. The local environment can play a major role in the magnitude and evolutionary consequences of such a tradeoff. Here we examined the investment in reproductive and vegetative tissue in 40 maternal half sib families from four different populations of the herb *Plantago coronopus* growing in either a dry or wet greenhouse environment. Plants originated from populations with an annual or a perennial life form, with annuals prevailing in drier habitats with greater seasonal variation in both temperature and precipitation. We found that water availability affected the expression of the tradeoff (both phenotypic and genetic) between reproduction and growth, being most accentuated under dry condition. However, populations responded very differently to water treatments. Plants from annual populations showed a similar response to drought condition with little variation among maternal families, suggesting a history of selection favoring genotypes with high allocation to reproduction when water availability is low. Plants from annual populations also expressed the highest level of plasticity. For the perennial populations, one showed a large variation among maternal families in resource allocation and expressed significant negative genetic correlations between reproductive and vegetative biomass under drought. The other perennial population showed less variation in response to treatment and had trait values similar to those of the annuals, although it was significantly less plastic. We stress the importance of considering intraspecific variation in response to environmental change such as drought, as conspecific plants exhibited very different abilities and strategies to respond to high versus low water availability even among geographically close populations.

**Key words:** annual, genetic correlation, life-history evolution, perennial, resource allocation, tradeoff, water availability.
Introduction

The environment plays a major selective role in shaping the life history strategies of plants (Stebbins, 1952). For instance, selfing may evolve in rapidly drying habitats (e.g. Ivey & Carr, 2012), and drought can select for a reduced lifespan (e.g. annual) and for early flowering thereby reducing evapotranspiration cost (e.g. Franke et al., 2006; Franks et al., 2007; McKay et al., 2003).

Theory predicts that an annual life history is favored over perennials in environments where the probability for a seed to become a flowering plant within one season, is greater than the probability for an adult to survive until the next flowering season (e.g. Bell, 1976; Charnov & Schaffer, 1973; de Jong & Klinkhamer, 2005). For an annual plant, lifetime fitness is determined by the number of seeds produced at the end of a single season. Consequently annuals are expected to attain their highest fitness when investing all resources in reproduction. For perennial plants, investment in reproduction in one flowering season must be balanced against investment in vegetative growth securing future survival and reproduction. Indeed, the allocation of resources in reproduction versus survival is a long recognized tradeoff affecting life-history evolution (Bulmer, 1994; Stearns, 1992).

In plants, vegetative and reproductive structures compete for the same resources and this represent a fundamental tradeoff. We therefore expect to find a negative correlation (both phenotypic and genetic) between these two traits (e.g. Agrawal et al., 2010). However, the magnitude and even sign of such a correlation can vary among environments. For instance, a phenotypic tradeoff can be obscured under very good growing conditions but revealed under stressful or resource poor conditions (Reznick, 1985; Van Noordwijk & de Jong, 1986). Plants, being sessile organisms, experience all extremes of their environment, and understanding how the environment and changes therein affect resource allocation requires knowledge on how variation in the environment affect phenotypic and genotypic tradeoffs. A genetic correlation between two traits can induce an
evolutionary constraint when the direction of the vector of selection operating on the traits is opposite to the direction of the genetic correlation (Etterson & Shaw, 2001; Roff, 1992). However only if the genetic correlation is -1 will evolution of larger values in both traits be prevented, and genetic correlations larger than -1 may slow down but not prevent a selective response for increased trait values (Via & Lande, 1985; Agrawal et al., 2010).

Evolution of resource allocation in response to environmental change will also be affected by the level of plasticity in the population, (i.e. phenotypic changes of identical genotypes across different environments). This is because plasticity may cause a weaker selection on a trait and thus a smaller genetic response to selection. It may be argued that high levels of plasticity impede evolutionary (genetic) response to selection. Nevertheless a plastic response may also be absolutely crucial for the maintenance of a local population subject to environmental change, allowing the immediate expression of a more optimal phenotype without a concomittant change in the underlying genotype (e.g. Chevin et al., 2010; Parmesan, 2006). Moreover, plasticity itself can be viewed as a trait upon which selection can operate (e.g. Via & Lande, 1985), and may entail fitness costs (DeWitt et al., 1998; Auld et al., 2010).

*Plantago coronopus* is an ideal species for studying how the local environment affects allocation to reproduction and growth, as it harbours both annual and perennial forms in separate populations. *P. coronopus* occurs in many different habitats from N Africa to N Europe, with annuals prevailing in drier habitats experiencing higher seasonal variation in temperature and precipitation (Braza et al., 2010; Villellas et al., in press). Plants from annual populations readily shift to a perennial life form under benign green house conditions, thus exhibiting high plasticity for this life-history trait. However, it is not known if perennial forms show a similar plastic response when grown in an "annual" habitat. Reports on current global changes predict both rising temperatures and a more frequent occurrence of drought events (IPCC, 2007). These are increasingly shifts towards
environmental conditions of the annual life forms. Therefore, one consequence of increased drought may be a reduction in life-span in perennial populations, with increased and/or earlier investment in reproduction over vegetative growth.

Here, we studied the variation in resource allocation to reproduction and growth in *P. coronopus* in plants from four native populations, two annual and two perennial. Populations were chosen to cover a large part of habitat variability of this species in the Mediterranean ecosystem. Because they were located in the same region they also experienced similar overall past selective forces related to climate events, but each one is adapted to particular local conditions. The study was conducted under controlled greenhouse conditions in order to estimate phenotypic and genetic correlations as well as cost of plasticity in plants grown under two different watering regimes (ample water and drought). The main purpose of the study was to examine if the expression of the tradeoff between allocation to vegetative and reproductive biomass varies with water availability and among populations of conspecifics exhibiting different life-histories. In short, we found evidence of a tradeoff between allocation to reproductive and vegetative biomass. The expression of the tradeoff was highest under drought, and depended on the origin of populations. Populations also varied with respect to levels of plasticity. We conclude that large within-species variation exists in both the ability and ways to respond to reduced water availability among geographically close populations.

**Materials and methods**

**Study species and study sites**

*Plantago coronopus* is a widespread species with native populations occurring from Morocco and the Iberian Peninsula to East Asia, and following the Atlantic coast to Northern Europe. In Spain both annual and perennial populations can be found across a relatively small geographic range, with annual life forms prevailing in drier habitats with lower minimum and higher maximum
temperatures than perennial forms (Braza et al., 2010). Flowers are outcrossing and wind pollinated, and mating system can be gynodioecious or hermaphroditic and varies highly among geographic regions. Populations in Spain consist almost of 100% hermaphrodites. In annual populations flowering begins in March, and plants usually die in June. Flowering occurs later in perennial populations (June-July), and most individuals do not flower in their first year (Braza et al., 2010).

Seeds were collected in 2009 from four large natural populations in south Spain; two annual and two perennial. The climate of the sampling region is typically Mediterranean, with summer drought (June-September), rainy winters, and high seasonal variability in amount and distribution of precipitation. The annual populations occur at sites that experience more extreme temperatures and lower precipitation than the perennial ones (Table 1). One of the perennial populations (4P) has a particularly wet microclimate because of its position on a mountain where fogs are frequent.

Given the mating system, and the fact that seeds from each maternal plant were obtained from different inflorescences, each individual seed family is expected to represent an outbred half-sib maternal family.

**Experimental set up**

Ten maternal plant families from each population were used, and eight seedlings represented each family. In early July, 2010, seeds from the four different populations of *Plantago coronopus* were sown in the greenhouse in seed trays (Pindstrup soil mixture no. 1) at Research Center Årslev, Denmark. Seeds from each maternal plant were sown in a separate seed tray. After sowing, seed trays were covered with an opaque acrylic plastic. The plastic was removed on day 3, while the acrylic was removed on day 10. On day 22, eight plants from each maternal plant were potted in...
individual pots (12 cm in diameter) all containing the same standard soil (Pindstrup sphagnum mix no. 2) with no extra fertilizer added. Care was taken to choose seedlings that all had the same size.

Four plants from each maternal family were subject to one of two treatments: wet or dry. In the wet treatment plants were watered every day, while the plants in the dry treatment only were watered every third day throughout the experiment. Pots in the dry treatment were observed to dry out in between watering, and leaves gradually lost their turgor until watering was applied again.

Within each treatment, the plants were randomized twice a week throughout the experiment, to avoid any position effect. All plants were harvested 45 days after the treatment had started, at the time where plants in the dry treatment started to show signs of wilting and just prior to fruit initiation. At harvest, inflorescences were cut off as close to the stem as possible. Leaf area (cm²) of each plant was estimated using a LI3100C Area Meter (LI-COR). The aboveground vegetative and reproductive tissue was placed in a dry cabinet (at 80 ºC) for 48 hours, and the dry weight was determined using a microgram precision balance. Although, in their natural populations, plants are known to differ in their flowering period, in the greenhouse all four populations produced their first inflorescences at the same time (July/August) – at the time where perennial populations in natural sites typically start their flowering. Onset of flowering was therefore not used as a variable in this study. In one population (3P), three of the ten maternal families did not produce any inflorescences in the wet treatment, in the dry treatment only one maternal family from this population failed to produce any inflorescences. All other maternal families produced inflorescences in both treatments.

Dry weight of inflorescences, dry weight of leaves and leaf area were used as response variables. In addition, we calculated an estimate of reproductive allocation (hereafter RA) as the biomass of reproductive tissue divided by the total biomass for each plant (cf Karlsson & Méndez, 2005).

**Statistical analysis**
We used general linear models to test for the effects of treatment, population, family (nested within populations), and their interactions on plant traits (biomass and RA). We specified treatment and population as fixed effects. Family was considered a random effect and estimation of associated variance component and test of significance were performed using REML. When a significant interaction between population and treatment was detected for a trait we used a Tukey’s HSD test to examine in which populations there was a different effect of treatment.

We also performed an ANCOVA on reproductive biomass, using vegetative biomass as covariate, to test if the tradeoff between allocation to reproduction and vegetative traits differed between treatments and populations.

Phenotypic correlations of the family means among vegetative and reproductive biomass were estimated using Pearson's correlation coefficients. The family mean correlations are sometimes used as a proxy for genetic correlation, but this approximation is severely biased when family size is small (Roff & Preziosi, 1994). We therefore also estimated genetic correlations between plant traits within each treatment (wet / dry) using the R package MCMCglmm (Hadfield, 2010). This is a Bayesian method that is less likely to underestimate standard errors (Hadfield, 2010). We used a multivariate normal model, fitting family as a random effect. As prior for both the within and between-family variance covariance matrix, we used a multivariate inverse gamma distribution ($V = \text{diag}(2)/3, \nu = 1.002$). Posterior distributions for the variance-covariance components were obtained using a Markov Chain Monte Carlo approach implemented in the R package MCMCglmm (Hadfield, 2010). One chain with 500,000 iterations was run, with the first 30,000 iterations discarded as burnin. The chain was thinned every 50 generations, yielding 9400 samples from posterior analysis. Genetic correlations were obtained from the estimates of additive variances and covariance of traits under the standard assumption that between half-sibs family variance (covariance) comprises additive effects (See Lynch & Walsh, 1998, ch 18 and ch 21). The posterior
distributions for the genetic correlations were roughly normal and we therefore use the posterior
mode as point estimate and the standard deviation of the posterior distribution as a summary of
statistical uncertainty around our point estimate of correlation (Gelman, 2004). We tested the
robustness of our analysis by using different priors (Inverse Gamma with slightly different
parameterization, flat prior) that yielded very close point estimates (results not shown).
We note that estimating additive genetic variance and covariance from the between family variance
of maternal half sibs relies on the assumption that genetic effects are not confounded with maternal
effects and our design does not allow to partition these. As most maternal effects in plants are
exerted via seed size and as effect of seed size on offspring wears off after a number of weeks
(Roach & Wulff, 1987; Weiner et al., 1997) it may be argued that for small-seeded plants (like
*Plantago*), maternal effects on adult offspring (i.e. not germination and initial seedling growth) are
expected to be minimal.

The plasticity of a trait was calculated by subtracting the family mean trait value in the dry
environment to the mean trait value of the same family in the wet environment (DeWitt, 1998;
DeWitt et al., 1998). Assuming a linear reaction norm, an estimate of plasticity was the absolute
difference between these trait values. This was repeated for all 40 families.

The relationship between plasticity of a trait and fitness was examined using multiple regression
where family mean and plasticity of the traits was regressed on relative fitness within the
environment. A cost of plasticity in a given environment would be indicated by a significant
negative partial regression coefficient between plasticity of the trait and relative fitness in that
environment (DeWitt et al., 1998). As relative fitness we used the relative biomass of inflorescence
weight. According to Primack (1979) most biomass of inflorescences in *Plantago* is devoted to seed
production, and biomass of inflorescences is therefore positively related to fitness - at least in the
dry environment associated with the annual life form. However, it is less obvious that a high
investment in reproduction in the first year of flowering is also correlated with high life-time fitness in a perennial site, as a high investment in reproduction may tradeoff with future survival. This study did not allow us to make good estimates of fitness in the wet treatment, and cost of plasticity was therefore only examined in the dry environment.

ANOVA, ANCOVA, Tukey’s HSD test, Pearson correlation coefficients, and multiple regression analysis were performed using the computer package JMP version 8.0 (SAS institute Inc.).

Results

Resource allocation is both population and environment dependent

All populations expressed the highest investment in reproductive biomass in the drought treatment (Table 2, 3, Fig. 1). The annuals’ increase in RA showed an absolute difference that was twice as high compared to that of the perennials, but the perennial population 3P showed the highest increase (from 0.08 – 0.19) although the absolute investment in reproductive tissue was much lower than in any of the other populations. We found a significant interaction between population and treatment for the vegetative traits and total biomass (Table 3). A Tukey’s test showed that for total biomass the interaction between population and treatments was due to plants from population 3P having a significantly higher total biomass in the dry treatment compared to the wet treatment (Tukey’s means difference (SE): 1.8 mg (0.4), \( P < 0.05 \)), whereas the other three populations did not show a significant difference in total biomass between treatments. For vegetative biomass and leaf area (two correlated traits), the Tukey test showed that the interaction between population and treatment was due to the two annual populations both having a significantly lower biomass of leaves and leaf area in the drought treatments compared to the wet treatment (Tukey’s means difference (SE) for leaf area and dry weight of leaves: 1A; 284.9 (41) and 1.54 (0.41), 2A; 287.9 (41), 1.45 (0.41) ), whereas the perennial populations did not differ in these traits between treatments.
A significant effect of family on inflorescence weight and RA (Table 3, Fig. 1) suggests the presence of genetic variation for these traits. Reaction norms (Fig. 1) show a similar response to treatment among families originating from both annual populations, whereas in particular population 3P showed larger variation among maternal families compared to the other study populations.

The ANCOVA on dry weight of inflorescences using the leaf weight as a covariate (Table 4) showed a significant interaction between populations and treatments suggesting that when accounting for variation in vegetative biomass the effect of treatment on reproductive biomass still varied among populations. More importantly, the significant interaction between the covariate (leaf biomass), population and treatment (Pop*Trt*Dw leaf, $P = 0.006$, Table 4, Fig. 2) shows that the expression of the fundamental tradeoff between allocation to vegetative and reproductive tissue varied both among populations and treatment. Analyzing each population separately showed no significant relationship between dry weight of inflorescences and dry weight of leaf among the maternal families in any of the study populations in the wet treatment, however in the dry treatment a significant negative relation was found in population 3P (slope (SE) = -0.64 (0.14), $t_9 = -4.64$, $P = 0.002$), and nearly so in 4P (slope (SE) = -0.55 (0.24), $t_9 = -2.27$, $P = 0.05$) and 2A (slope (SE) = -0.65 (0.33), $t_9 = -1.99$, $P = 0.08$). These negative regression coefficients were only detected in the drought treatment indicating that drought accentuates the expression of the tradeoff between vegetative and reproductive biomass in these populations. Note however, that for population 1A and 4P, the fitted regression line in the wet treatment is actually more negative than in the dry treatment (Fig. 2 compare solid versus dotted line). However, none of these regression lines were significantly different from zero (1A: slope (SE) = -0.33 (0.30), $t_9 = -1.14$, $P = 0.29$, 4P: slope (SE) = -0.68, $t_9 = -1.49$, $P = 0.17$).
Family mean and genetic correlations between traits

Family mean correlations between vegetative and reproductive biomass was overall negative but non-significant. With the exception of population 1A, the magnitude of the correlation was largest in the dry treatment (Table 5), as also suggested by the results from the ANCOVA. Some differences between populations and treatments are worth noticing: in the annual populations, we did not find significant negative correlations between biomass of inflorescences and leaves in any of the treatments. However, these correlations were significantly negative in the perennial population 3P and nearly so for the other perennial population 4P, but only in the drought treatment.

Estimates of genetic correlations (Table 5) were in the same direction as the family mean correlations. A significant negative genetic correlation biomass of inflorescences and leaves were detected for population 3P, but only in the drought treatment (Table 5). In the other populations, genetic correlations between reproductive and vegetative traits were often negative but not significant. This is not surprising given that very large sample sizes are needed to estimate precisely genetic correlations. Therefore we expect to have a low power to detect significant correlations (unless these are very strongly negative such as in 3P). However, the overall proportion of correlations with negative estimates is still quite telling: for both genetic and family mean correlations, 7 out of the 8 pair wise correlations estimated had negative point estimates (Table 5, binomial test assuming equal number of positive and negative correlations, $P < 0.035$).

Estimates of phenotypic plasticity and cost of plasticity

The level of plasticity for each of the four plant traits were estimated as the absolute difference in the family trait mean between dry and wet environment. In general, families from annual populations had a more similar mean value and a higher difference in trait mean between environments than perennial (Table 6). For leaf area and RA, estimates of plasticity were
significantly higher in the two annual populations compared to the perennial ones (test for difference between annual and perennial: $F_{1,36} = 16.44$, $P = 0.003$, and $F_{1,36} = 7.20$, $P = 0.01$, for leaf area and RA respectively). For dry weight of inflorescences, annual forms also showed higher values, and nearly significant differences in trait mean compared to perennials ($F_{1,36} = 3.98$, $P = 0.054$). For dry weight of leaves, no difference in plasticity was found between annual and perennial populations ($F_{1,36} = 0.02$, $P = 0.90$). Here, the perennial population 3P actually exhibited the largest estimate of plasticity while the other perennial population 4P had the lowest level of plasticity, and the two annual populations had levels of plasticity intermediate to the perennials (Table 6).

Multiple regression of the family mean of a trait within environment and the plasticity of the trait on the relative fitness within the dry environment did not show any significant effect of plasticity in dry weight of leaves (partial regression coefficient = -0.023, $t = 0.72$, $P = 0.47$), plasticity in leaf area (partial regression coefficient = 0.001, $t = 1.77$, $P = 0.10$), or plasticity in RA (partial regression coefficient = 0.11, $t = 0.53$, $P = 0.6$). Thus we did not detect any cost of plasticity for these traits.

**Discussion**

**Response in resource allocation to drought**

Plants from all study populations of *P. coronopus* increased their investment in reproductive biomass in the dry treatment, although in annual populations they showed a higher absolute increase under drought compared to plants from perennial populations. In a comparative study of 9 perennial and 6 annual *Plantago* species where reproductive allocation in natural sites was estimated, Primack (1979) found that annual species had a higher reproductive allocation than perennials. In this study, we examined the within-species variation in resource allocation and found that plants from annual populations showed a higher RA than one perennial population (3P), but not the other (4P). The
ANCOVA showed a phenotypic tradeoff between investment in reproductive and vegetative tissue, and this relation was generally more negative in the drought treatment. Genetic correlation estimates between reproductive and vegetative traits were also more negative in the drought environment. These results support previous findings (e.g. Stearns, 1992; Siemens et al., 2012) that the expression of a tradeoff becomes more pronounced under stressful conditions. As the expression of the phenotypic tradeoff between investment to reproduction and vegetative biomass was most pronounced in the drought treatment, we expect selection on resource allocation to be strongest under this condition. This may be one reason why maternal families from the annual populations all show very similar patterns of resource allocation in response to the water treatments (Fig. 1), suggesting that these annual populations have responded similarly to a selection history of repeated summer drought. The perennial populations originate from two very different types of habitat (mobile dunes with little vegetation (3P) and gaps in a foggy mountain forest with high plant competition (4P)). Perennial life form in this species is clearly adopted under very different environments subjecting plants to local selection histories. Unlike the annual populations, the two perennial populations behaved differently in their response to treatment. Population 4P showed little among-family variation relative to population 3P, and less plasticity relative to the two annual populations. Due to its expression of both a lower among family variation (suggesting a lower level of genetic variation for the response trait), and lower levels of plasticity relative to the other study population, it may actually represent the population with the least ability to respond to changes in water availability. The other perennial population 3P exhibited the largest variation among maternal families. In this population a significant negative genetic correlation between reproductive and vegetative investment was detected, but the correlation was larger than -1 suggesting that a response to selection for increased investment in both vegetative and reproductive tissue is possible. Moreover, the fact that the genetic correlation could at all be detected under the experimental
conditions show the presence of genetic variation for resource allocation within that population. In the perennial shrub *Artemisia ordosica*, growing in dunes, Li Li *et al.* (2011) found large variation in life-history traits among dunes in different fixation stages. They argue that this variation in growth and reproduction within a population was caused by the environmental differences across the dune habitat creating a mix of selection pressures on fecundity and survival (Li Li *et al.*, 2011). Similar reasoning may apply to explain the larger variation among maternal families in resource allocation in population 3P in the present study originating from a similar dune habitat.

In *P. lanceolata*, a close relative to *P. coronopus*, Shefferson & Roach (2010) studied the adaptive benefit of iteroparity. Plants flowered from one to six times, and fitness increased with number of reproductive years. Yet a large proportion of plants only reproduced once, despite having a post-reproductive lifetime, which could have allowed for another reproductive event. They argue that constraints from the environment on growth and size of plants may explain this observation. Indeed, the influence of plant size on flowering may be crucial for responding adaptively to environmental changes. Rapid growth and fast reproduction are considered a drought escape to allow completing the life cycle before drought kicks in. When a fast completion of the life cycle is advantageous, we expect selection for early flowering and hence a smaller threshold size for flower initiation. The minimum size required for flower induction, is a trait found to be both heritable and to vary among populations (e.g. Wesselingh *et al.*, 1993; de Jong & Klinkhamer, 2005; Wesselingh & De Jong 1995). If early flowering is favored to avoid drought stress later in the season (e.g. in habitats with annual populations in the present study), plants with a large threshold size may be at a disadvantage.

At the natural sites of our study populations, the average size of a flowering plant is largest in the perennial populations ((Braza *et al.*, 2010), Table 1 this study). Of course these size differences reported from natural sites are strongly influenced by perennial plants having had the opportunity to obtain a larger size over the course of possibly several years of growth. However, under the
greenhouse conditions in the present study, plants from the perennial populations 3P still achieved a
much larger vegetative size (dry weight of leaves, leaf area) than any of the other populations and
investment in reproduction was low relative to the other populations especially in the well-watered
treatment. This suggests that individuals from population 3P could have an inherent larger
minimum size of flower induction, but also that this can to some extend be environment-dependent,
as more inflorescences were produced in the drought treatment.

Magnitude and cost of plasticity

We found that the annual populations expressed higher levels of plasticity for investment in
reproductive traits than perennial populations. It has been predicted that greater levels of
environmental heterogeneity may select for higher levels of phenotypic plasticity (Alpert & Simms,
2002; Pigliucci, 2001). Differentiation in level of plasticity among populations has been
documented, whereby plants express higher levels of plasticity in populations found in dry habitats
and habitats experiencing greater annual variation in precipitation (Gianoli, 2004; Gianoli &
Gonzalez-Teuber, 2005; see however, Heschel et al., 2004). The fact that plants from both annual
sites in our study were also those expressing the highest plasticity suggests that the higher seasonal
variation in temperature and precipitation at those sites may also have selected for higher levels of
plasticity.

When a cost of plasticity is caused by a production cost, it is expected to be environment specific,
as e.g. stress may increase the cost due to resource limitation (Auld et al., 2010). It has been argued
that a cost of plasticity may be difficult to detect as selection may already have removed those
genotypes expressing a cost (DeWitt et al., 1998). Here, we examined the consequences of
plasticity in the drought treatment, the potentially most stressful of the two environments, and we did not find any evidence for a cost of plasticity.

To conclude, our study showed that populations originating from sites with different life histories varied in resource allocation in response to drought. Our results suggest that the ability for a species to respond and potentially adapt to environmental change is dependent on population origin and previous local selection history. This emphasizes the importance of considering within-species variation for responding to environmental changes rather than a fixed species perspective. If the same species, originating from the same small geographic region, can show different abilities and strategies to respond to changing water availability, then future projections of species distributions should take into account that responses to the ongoing climatic change are not as simple as usually predicted.

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References


Table 1. Location and characteristics of four Spanish study populations of *Plantago coronopus*. Data is obtained from Braza *et al.*, 2010, except average length of leaves, which was obtained by measuring the length of the longest leaf of flowering plants collected in the field (sample sizes: 110, 141, 148, and 226 individual plants for populations 1A, 2A, 3P and 4P respectively).

<table>
<thead>
<tr>
<th>Population</th>
<th>Lifespan</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Habitat</th>
<th>Mean biomass of adult plants (g) ± SD</th>
<th>Average length of leaves (mm) ± SD on adults plants</th>
<th>Min-max yearly temp. (°C)</th>
<th>Precipitation/year (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A. Trebujena (TRG)</td>
<td>Annual</td>
<td>36°53’55’’</td>
<td>6°17’33’’</td>
<td>Salt Marsh</td>
<td>0.097 ± 0.13</td>
<td>25.0 ± 15.0</td>
<td>12-24</td>
<td>578</td>
</tr>
<tr>
<td>2A. Aznalcázar (AZN)</td>
<td>Annual</td>
<td>37°15’51’’</td>
<td>6°13’44’’</td>
<td>Shrubland</td>
<td>0.064 ± 0.12</td>
<td>13.0 ± 9.3</td>
<td>11-25</td>
<td>558</td>
</tr>
<tr>
<td>3P. Camposoto (CA)</td>
<td>Perennial</td>
<td>36°25’35’’</td>
<td>6°13’43’’</td>
<td>Coastal dunes</td>
<td>NA</td>
<td>53.7 ± 14.9</td>
<td>14-22</td>
<td>622</td>
</tr>
<tr>
<td>4P. Bosque Niebla (BN)</td>
<td>Perennial</td>
<td>36°06’25’’</td>
<td>5°32’21’’</td>
<td>Foggy mountain</td>
<td>0.834 ± 0.69</td>
<td>54.1 ± 19.4</td>
<td>11-19</td>
<td>1045</td>
</tr>
</tbody>
</table>
Table 2. Means (SE) of plant traits in plants originating from four *Plantago coronopus* populations and growing in two different water treatments. LA: Leaf area, RA: reproductive allocation. For each population mean are given for N = 40 plants per treatment.

<table>
<thead>
<tr>
<th>Population</th>
<th>Dry Dw Infl (g)</th>
<th>Wet Dw Infl (g)</th>
<th>Dry Dw leaves (g)</th>
<th>Wet Dw leaves (g)</th>
<th>LA (cm²)</th>
<th>RA Dry</th>
<th>RA Wet</th>
<th>Total biomass (g) Dry</th>
<th>Total biomass (g) Wet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>4.10 (0.14)</td>
<td>2.97 (0.21)</td>
<td>2.20 (0.14)</td>
<td>3.73 (0.26)</td>
<td>305.49 (18.1)</td>
<td>0.66 (0.02)</td>
<td>0.45 (0.03)</td>
<td>6.29 (0.19)</td>
<td>6.71 (0.28)</td>
</tr>
<tr>
<td>2A</td>
<td>3.71 (0.17)</td>
<td>2.12 (0.18)</td>
<td>2.85 (0.18)</td>
<td>4.30 (0.33)</td>
<td>361.4 (20.3)</td>
<td>0.57 (0.02)</td>
<td>0.35 (0.03)</td>
<td>6.56 (0.19)</td>
<td>6.42 (0.34)</td>
</tr>
<tr>
<td>3P</td>
<td>1.32 (0.25)</td>
<td>0.43 (0.13)</td>
<td>5.92 (0.36)</td>
<td>5.00 (0.50)</td>
<td>540.96 (25.3)</td>
<td>0.19 (0.04)</td>
<td>0.08 (0.02)</td>
<td>7.23 (0.25)</td>
<td>5.44 (0.49)</td>
</tr>
<tr>
<td>4P</td>
<td>3.51 (0.12)</td>
<td>2.86 (0.22)</td>
<td>2.80 (0.17)</td>
<td>3.27 (0.21)</td>
<td>338.57 (18.1)</td>
<td>0.56 (0.02)</td>
<td>0.46 (0.03)</td>
<td>6.31 (0.17)</td>
<td>6.13 (0.25)</td>
</tr>
</tbody>
</table>
Table 3. Generalized linear model (REML method) examining the effects of treatment (Trt), population (Pop) and maternal family (Fam) on reproductive and vegetative traits in *Plantago coronopus*. LA: leaf area (cm²), and RA: reproductive allocation. For fixed effects the level of significance is given by F-statistics, and for random effects values correspond to the variance component estimate with standard error in brackets.

<table>
<thead>
<tr>
<th></th>
<th>Dw Infl.</th>
<th>Dw leaves</th>
<th>LA</th>
<th>RA</th>
<th>Total biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model $R^2$</td>
<td>0.70</td>
<td>0.51</td>
<td>0.47</td>
<td>0.74</td>
<td>0.31</td>
</tr>
<tr>
<td>Fixed effects</td>
<td>d.f.</td>
<td>$F$</td>
<td>$P$</td>
<td>$F$</td>
<td>$P$</td>
</tr>
<tr>
<td>Trt</td>
<td>1</td>
<td>61.44</td>
<td>&lt;0.0001</td>
<td>7.61</td>
<td>0.008</td>
</tr>
<tr>
<td>Pop</td>
<td>3</td>
<td>27.88</td>
<td>&lt;0.0001</td>
<td>14.41</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trt*Pop</td>
<td>1</td>
<td>2.17</td>
<td>0.09</td>
<td>6.22</td>
<td>0.0015</td>
</tr>
<tr>
<td>Random effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fam (Pop)</td>
<td>0.32</td>
<td>0.0016</td>
<td>0.42</td>
<td>0.08</td>
<td>2808.3</td>
</tr>
<tr>
<td></td>
<td>(0.13)</td>
<td>(0.25)</td>
<td>(2189.7)</td>
<td>(0.002)</td>
<td>(0.22)</td>
</tr>
<tr>
<td>Trt*Fam(Pop)</td>
<td>0.13</td>
<td>0.19</td>
<td>0.39</td>
<td>0.08</td>
<td>2839.8</td>
</tr>
<tr>
<td></td>
<td>(0.09)</td>
<td>(0.26)</td>
<td>(2459.9)</td>
<td>(0.0016)</td>
<td>(0.24)</td>
</tr>
</tbody>
</table>
Table 4. ANCOVA examining the effect of population (Pop), treatment (Trt) and the covariate dry weight of leaves (Dw leaves) on the dry weight of inflorescences in four populations of *Plantago coronopus*. Model $R^2 = 0.60$.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>SS</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pop</td>
<td>3</td>
<td>103.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trt</td>
<td>1</td>
<td>62.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dw leaves</td>
<td>1</td>
<td>35.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pop*Trt</td>
<td>3</td>
<td>11.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Pop*Dw leaves</td>
<td>3</td>
<td>0.9</td>
<td>0.84</td>
</tr>
<tr>
<td>Trt*Dw leaves</td>
<td>1</td>
<td>1.2</td>
<td>0.31</td>
</tr>
<tr>
<td>Pop<em>Trt</em>Dw leaves</td>
<td>3</td>
<td>14.4</td>
<td>0.006</td>
</tr>
<tr>
<td>Error</td>
<td>304</td>
<td>346.0</td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Estimates of the phenotypic family means correlation and the genetic correlation between reproductive and vegetative biomass in maternal plant families from four different *Plantago coronopus* populations and growing in either a dry (D) or wet (W) treatment. Standard deviation of the posterior distribution of the genetic correlations is given in parentheses. Significant correlations in bold, **: P<0.01, a: P<0.1

<table>
<thead>
<tr>
<th>Population</th>
<th>Treatment</th>
<th>Pearson family means correlation</th>
<th>Genetic correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dw infl.&lt;&gt;Dw leaves</td>
<td>Dw infl.&lt;&gt;Dw leaves</td>
</tr>
<tr>
<td>1A</td>
<td>D</td>
<td>0.06</td>
<td>0.07 (0.42)</td>
</tr>
<tr>
<td>1A</td>
<td>W</td>
<td>-0.37</td>
<td>-0.21 (0.48)</td>
</tr>
<tr>
<td>2A</td>
<td>D</td>
<td>-0.58</td>
<td>-0.30 (0.42)</td>
</tr>
<tr>
<td>2A</td>
<td>W</td>
<td>-0.23</td>
<td>-0.10 (0.52)</td>
</tr>
<tr>
<td>3P</td>
<td>D</td>
<td><strong>-0.85</strong></td>
<td><strong>-0.81 (0.22)</strong>**</td>
</tr>
<tr>
<td>3P</td>
<td>W</td>
<td>-0.13</td>
<td>-0.05 (0.47)</td>
</tr>
<tr>
<td>4P</td>
<td>D</td>
<td>-0.63a</td>
<td>-0.44 (0.36)</td>
</tr>
<tr>
<td>4P</td>
<td>W</td>
<td>-0.47</td>
<td>-0.22 (0.47)</td>
</tr>
</tbody>
</table>
Table 6. Plasticity (mean difference in trait value between environment) of plant traits in four populations of *Plantago coronopus* growing under two water treatment. Numbers in brackets indicate standard errors (SE).

<table>
<thead>
<tr>
<th>Population</th>
<th>Dw Inflorescences</th>
<th>Dw leaves</th>
<th>Leaf area (LA)</th>
<th>RA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>1.18 (0.23)</td>
<td>1.54 (0.25)</td>
<td>284.9 (30)</td>
<td>0.21 (0.03)</td>
</tr>
<tr>
<td>2A</td>
<td>1.58 (0.18)</td>
<td>1.45 (0.28)</td>
<td>287.7 (36)</td>
<td>0.21 (0.02)</td>
</tr>
<tr>
<td>3P</td>
<td>0.95 (0.27)</td>
<td>2.03 (0.48)</td>
<td>171 (29)</td>
<td>0.13 (0.04)</td>
</tr>
<tr>
<td>4P</td>
<td>0.92 (0.20)</td>
<td>0.88 (0.19)</td>
<td>158 (23)</td>
<td>0.14 (0.08)</td>
</tr>
</tbody>
</table>
Legends to figure

Figure 1. Reaction norms of maternal half sib families of *Plantago coronopus* plants from two
annual (1A and 2A) and two perennial (3P and 4P) populations. Each of the ten lines represents the
mean of plants from one maternal half sib family growing in a dry and a wet treatment (n = 4 plants
per family per treatment). Reaction norms are shown for the three plant traits: dry weight of
inflorescences (g), dry weight of leaves (g), and reproductive allocation (RA).

Figure 2. Linear relationship between investment in reproductive and vegetative biomass of
*Plantago coronopus* growing in either a dry (dotted line) or a well watered (solid line) environment
for plants from annual (1A and 2A) and perennial (3P and 4P) populations. Individual dots in each
graph depict a maternal family means, with closed circles representing dry and open triangles the
wet treatment.