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

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12 Running title: The tradeoff between reproduction and growth is affected by population and water
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1 **Abstract**

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

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

1 **Introduction**

2 The environment plays a major selective role in shaping the life history strategies of plants
3 (Stebbins, 1952). For instance, selfing may evolve in rapidly drying habitats (e.g. Ivey & Carr,
4 2012), and drought can select for a reduced lifespan (e.g. annual) and for early flowering thereby
5 reducing evapotranspiration cost (e.g. Franke *et al.*, 2006; Franks *et al.*, 2007; McKay *et al.*, 2003).
6 Theory predicts that an annual life history is favored over perennials in environments where the
7 probability for a seed to become a flowering plant within one season, is greater than the probability
8 for an adult to survive until the next flowering season (e.g. Bell, 1976; Charnov & Schaffer, 1973;
9 de Jong & Klinkhamer, 2005). For an annual plant, lifetime fitness is determined by the number of
10 seeds produced at the end of a single season. Consequently annuals are expected to attain their
11 highest fitness when investing all resources in reproduction. For perennial plants, investment in
12 reproduction in one flowering season must be balanced against investment in vegetative growth
13 securing future survival and reproduction. Indeed, the allocation of resources in reproduction versus
14 survival is a long recognized tradeoff affecting life-history evolution (Bulmer, 1994; Stearns, 1992).
15 In plants, vegetative and reproductive structures compete for the same resources and this represent a
16 fundamental tradeoff. We therefore expect to find a negative correlation (both phenotypic and
17 genetic) between these two traits (e.g. Agrawal *et al.*, 2010). However, the magnitude and even
18 sign of such a correlation can vary among environments. For instance, a phenotypic tradeoff can be
19 obscured under very good growing conditions but revealed under stressful or resource poor
20 conditions (Reznick, 1985; Van Noordwijk & de Jong, 1986). Plants, being sessile organisms,
21 experience all extremes of their environment, and understanding how the environment and changes
22 herein affect resource allocation requires knowledge on how variation in the environment affect
23 phenotypic and genotypic tradeoffs. A genetic correlation between two traits can induce an

1 evolutionary constraint when the direction of the vector of selection operating on the traits is
2 opposite to the direction of the genetic correlation (Etterson & Shaw, 2001; Roff, 1992). However
3 only if the genetic correlation is -1 will evolution of larger values in both traits be prevented, and
4 genetic correlations larger than -1 may slow down but not prevent a selective response for increased
5 trait values (Via & Lande, 1985; Agrawal *et al.*, 2010).

6 Evolution of resource allocation in response to environmental change will also be affected by the
7 level of plasticity in the population, (i.e. phenotypic changes of identical genotypes across different
8 environments). This is because plasticity may cause a weaker selection on a trait and thus a smaller
9 genetic response to selection. It may be argued that high levels of plasticity impede evolutionary
10 (genetic) response to selection. Nevertheless a plastic response may also be absolutely crucial for
11 the maintenance of a local population subject to environmental change, allowing the immediate
12 expression of a more optimal phenotype without a concomitant change in the underlying genotype
13 (e.g. Chevin *et al.*, 2010; Parmesan, 2006). Moreover, plasticity itself can be viewed as a trait upon
14 which selection can operate (e.g. Via & Lande, 1985), and may entail fitness costs (DeWitt *et al.*,
15 1998; Auld *et al.*, 2010).

16 *Plantago coronopus* is an ideal species for studying how the local environment affects allocation to
17 reproduction and growth, as it harbours both annual and perennial forms in separate populations. *P.*
18 *coronopus* occurs in many different habitats from N Africa to N Europe, with annuals prevailing in
19 drier habitats experiencing higher seasonal variation in temperature and precipitation (Braza *et al.*,
20 2010; Villellas *et al.*, *in press*). Plants from annual populations readily shift to a perennial life form
21 under benign green house conditions, thus exhibiting high plasticity for this life-history trait.
22 However, it is not known if perennial forms show a similar plastic response when grown in an
23 "annual" habitat. Reports on current global changes predict both rising temperatures and a more
24 frequent occurrence of drought events (IPCC, 2007). These are increasingly shifts towards

1 environmental conditions of the annual life forms. Therefore, one consequence of increased drought
2 may be a reduction in life-span in perennial populations, with increased and/or earlier investment in
3 reproduction over vegetative growth.

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

18 **Materials and methods**

19 **Study species and study sites**

20 *Plantago coronopus* is a widespread species with native populations occurring from Morocco and
21 the Iberian Peninsula to East Asia, and following the Atlantic coast to Northern Europe. In Spain
22 both annual and perennial populations can be found across a relatively small geographic range, with
23 annual life forms prevailing in drier habitats with lower minimum and higher maximum

1 temperatures than perennial forms (Braza *et al.*, 2010). Flowers are outcrossing and wind
2 pollinated, and mating system can be gynodioecious or hermaphroditic and varies highly among
3 geographic regions. Populations in Spain consist almost of 100 % hermaphrodites. In annual
4 populations flowering begins in March, and plants usually die in June. Flowering occurs later in
5 perennial populations (June-July), and most individuals do not flower in their first year (Braza *et*
6 *al.*, 2010).

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

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

16 **Experimental set up**

17 Ten maternal plant families from each population were used, and eight seedlings represented each
18 family. In early July, 2010, seeds from the four different populations of *Plantago coronopus* were
19 sown in the greenhouse in seed trays (Pindstrup soil mixture no. 1) at Research Center Årslev,
20 Denmark. Seeds from each maternal plant were sown in a separate seed tray. After sowing, seed
21 trays were covered with an opaque acrylic plastic. The plastic was removed on day 3, while the
22 acrylic was removed on day 10. On day 22, eight plants from each maternal plant were potted in

1 individual pots (12 cm in diameter) all containing the same standard soil (Pindstrup sphagnum mix
2 no. 2) with no extra fertilizer added. Care was taken to choose seedlings that all had the same size.
3 Four plants from each maternal family were subject to one of two treatments; wet or dry. In the wet
4 treatment plants were watered every day, while the plants in the dry treatment only were watered
5 every third day throughout the experiment. Pots in the dry treatment were observed to dry out in
6 between watering, and leaves gradually lost their turgor until watering was applied again.
7 Within each treatment, the plants were randomized twice a week throughout the experiment, to
8 avoid any position effect. All plants were harvested 45 days after the treatment had started, at the
9 time where plants in the dry treatment started to show signs of wilting and just prior to fruit
10 initiation. At harvest, inflorescences were cut off as close to the stem as possible. Leaf area (cm^2) of
11 each plant was estimated using a LI3100C Area Meter (LI-COR). The aboveground vegetative and
12 reproductive tissue was placed in a dry cabinet (at 80 °C) for 48 hours, and the dry weight was
13 determined using a microgram precision balance. Although, in their natural populations, plants are
14 known to differ in their flowering period, in the greenhouse all four populations produced their first
15 inflorescences at the same time (July/August) – at the time where perennial populations in natural
16 sites typically start their flowering. Onset of flowering was therefore not used as a variable in this
17 study. In one population (3P), three of the ten maternal families did not produce any inflorescences
18 in the wet treatment, in the dry treatment only one maternal family from this population failed to
19 produce any inflorescences. All other maternal families produced inflorescences in both treatments.
20 Dry weight of inflorescences, dry weight of leaves and leaf area were used as response variables. In
21 addition, we calculated an estimate of reproductive allocation (hereafter RA) as the biomass of
22 reproductive tissue divided by the total biomass for each plant (cf Karlsson & Méndez, 2005).

23 **Statistical analysis**

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

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

10 Phenotypic correlations of the family means among vegetative and reproductive biomass were
11 estimated using Pearson's correlation coefficients. The family mean correlations are sometimes used
12 as a proxy for genetic correlation, but this approximation is severely biased when family size is
13 small (Roff & Preziosi, 1994). We therefore also estimated genetic correlations between plant traits
14 within each treatment (wet / dry) using the R package MCMCglmm (Hadfield, 2010). This is a
15 Bayesian method that is less likely to underestimate standard errors (Hadfield, 2010). We used a
16 multivariate normal model, fitting family as a random effect. As prior for both the within and
17 between-family variance covariance matrix, we used a multivariate inverse gamma distribution (V
18 $= \text{diag}(2)/3$, $\nu = 1.002$). Posterior distributions for the variance-covariance components were
19 obtained using a Markov Chain Monte Carlo approach implemented in the R package MCMCglmm
20 (Hadfield, 2010). One chain with 500.000 iterations was run, with the first 30.000 iterations
21 discarded as burnin. The chain was thinned every 50 generations, yielding 9400 samples from
22 posterior analysis. Genetic correlations were obtained from the estimates of additive variances and
23 covariance of traits under the standard assumption that between half-sibs family variance
24 (covariance) comprises additive effects (See Lynch & Walsh, 1998, ch 18 and ch 21). The posterior

1 distributions for the genetic correlations were roughly normal and we therefore use the posterior
2 mode as point estimate and the standard deviation of the posterior distribution as a summary of
3 statistical uncertainty around our point estimate of correlation (Gelman, 2004). We tested the
4 robustness of our analysis by using different priors (Inverse Gamma with slightly different
5 parameterization, flat prior) that yielded very close point estimates (results not shown).
6 We note that estimating additive genetic variance and covariance from the between family variance
7 of maternal half sibs relies on the assumption that genetic effects are not confounded with maternal
8 effects and our design does not allow to partition these. As most maternal effects in plants are
9 exerted via seed size and as effect of seed size on offspring wears off after a number of weeks
10 (Roach & Wulff, 1987; Weiner *et al.*, 1997) it may be argued that for small-seeded plants (like
11 *Plantago*), maternal effects on adult offspring (i.e. not germination and initial seedling growth) are
12 expected to be minimal.

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

17 The relationship between plasticity of a trait and fitness was examined using multiple regression
18 where family mean and plasticity of the traits was regressed on relative fitness within the
19 environment. A cost of plasticity in a given environment would be indicated by a significant
20 negative partial regression coefficient between plasticity of the trait and relative fitness in that
21 environment (DeWitt *et al.*, 1998). As relative fitness we used the relative biomass of inflorescence
22 weight. According to Primack (1979) most biomass of inflorescences in *Plantago* is devoted to seed
23 production, and biomass of inflorescences is therefore positively related to fitness - at least in the
24 dry environment associated with the annual life form. However, it is less obvious that a high

1 investment in reproduction in the first year of flowering is also correlated with high life-time fitness
2 in a perennial site, as a high investment in reproduction may tradeoff with future survival. This
3 study did not allow us to make good estimates of fitness in the wet treatment, and cost of plasticity
4 was therefore only examined in the dry environment.

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

7 **Results**

8 **Resource allocation is both population and environment dependent**

9 All populations expressed the highest investment in reproductive biomass in the drought treatment
10 (Table 2, 3, Fig. 1). The annuals' increase in RA showed an absolute difference that was twice as
11 high compared to that of the perennials, but the perennial population 3P showed the highest increase
12 (from 0.08 – 0.19) although the absolute investment in reproductive tissue was much lower than in
13 any of the other populations. We found a significant interaction between population and treatment
14 for the vegetative traits and total biomass (Table 3). A Tukey's test showed that for total biomass
15 the interaction between population and treatments was due to plants from population 3P having a
16 significantly higher total biomass in the dry treatment compared to the wet treatment (Tukey's
17 means difference (SE): 1.8 mg (0.4), $P < 0.05$), whereas the other three populations did not show a
18 significant difference in total biomass between treatments. For vegetative biomass and leaf area
19 (two correlated traits), the Tukey test showed that the interaction between population and treatment
20 was due to the two annual populations both having a significantly lower biomass of leaves and leaf
21 area in the drought treatments compared to the wet treatment (Tukey's means difference (SE) for
22 leaf area and dry weight of leaves: 1A; 284.9 (41) and 1.54 (0.41), 2A; 287.9 (41), 1.45 (0.41)),
23 whereas the perennial populations did not differ in these traits between treatments.

1 A significant effect of family on inflorescence weight and RA (Table 3, Fig. 1) suggests the
2 presence of genetic variation for these traits. Reaction norms (Fig. 1) show a similar response to
3 treatment among families originating from both annual populations, whereas in particular
4 population 3P showed larger variation among maternal families compared to the other study
5 populations.

6 The ANCOVA on dry weight of inflorescences using the leaf weight as a covariate (Table 4)
7 showed a significant interaction between populations and treatments suggesting that when
8 accounting for variation in vegetative biomass the effect of treatment on reproductive biomass still
9 varied among populations. More importantly, the significant interaction between the covariate (leaf
10 biomass), population and treatment (Pop*Trt*Dw leaf, $P = 0.006$, Table 4, Fig. 2) shows that the
11 expression of the fundamental tradeoff between allocation to vegetative and reproductive tissue
12 varied both among populations and treatment. Analyzing each population separately showed no
13 significant relationship between dry weight of inflorescences and dry weight of leaf among the
14 maternal families in any of the study populations in the wet treatment, however in the dry treatment
15 a significant negative relation was found in population 3P (slope (SE) = $-0.64 (0.14)$, $t_9 = -4.64$, $P =$
16 0.002), and nearly so in 4P (slope (SE) = $-0.55 (0.24)$, $t_9 = -2.27$, $P = 0.05$) and 2A (slope (SE) = -
17 $0.65 (0.33)$, $t_9 = -1.99$, $P = 0.08$). These negative regression coefficients were only detected in the
18 drought treatment indicating that drought accentuates the expression of the tradeoff between
19 vegetative and reproductive biomass in these populations. Note however, that for population 1A and
20 4P, the fitted regression line in the wet treatment is actually more negative than in the dry treatment
21 (Fig. 2 compare solid versus dotted line). However, none of these regression lines were significantly
22 different from zero (1A: slope (SE) = $-0.33 (0.30)$, $t_9 = -1.14$, $P = 0.29$, 4P: slope (SE) = $-$
23 1.49 , $P = 0.17$)

24

1 **Family mean and genetic correlations between traits**

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

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

19 **Estimates of phenotypic plasticity and cost of plasticity**

20 The level of plasticity for each of the four plant traits were estimated as the absolute difference in
21 the family trait mean between dry and wet environment. In general, families from annual
22 populations had a more similar mean value and a higher difference in trait mean between
23 environments than perennial (Table 6). For leaf area and RA, estimates of plasticity were

1 significantly higher in the two annual populations compared to the perennial ones (test for
2 difference between annual and perennial: $F_{1,36} = 16.44$, $P = 0.003$, and $F_{1,36} = 7.20$, $P = 0.01$, for
3 leaf area and RA respectively). For dry weight of inflorescences, annual forms also showed higher
4 values, and nearly significant differences in trait mean compared to perennials ($F_{1,36} = 3.98$, $P =$
5 0.054). For dry weight of leaves, no difference in plasticity was found between annual and perennial
6 populations ($F_{1,36} = 0.02$, $P = 0.90$). Here, the perennial population 3P actually exhibited the largest
7 estimate of plasticity while the other perennial population 4P had the lowest level of plasticity, and
8 the two annual populations had levels of plasticity intermediate to the perennials (Table 6).

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

14

15 **Discussion**

16 **Response in resource allocation to drought**

17 Plants from all study populations of *P. coronopus* increased their investment in reproductive
18 biomass in the dry treatment, although in annual populations they showed a higher absolute increase
19 under drought compared to plants from perennial populations. In a comparative study of 9 perennial
20 and 6 annual *Plantago* species where reproductive allocation in natural sites was estimated, Primack
21 (1979) found that annual species had a higher reproductive allocation than perennials. In this study,
22 we examined the within-species variation in resource allocation and found that plants from annual
23 populations showed a higher RA than one perennial population (3P), but not the other (4P). The

1 ANCOVA showed a phenotypic tradeoff between investment in reproductive and vegetative tissue,
2 and this relation was generally more negative in the drought treatment. Genetic correlation
3 estimates between reproductive and vegetative traits were also more negative in the drought
4 environment. These results support previous findings (e.g. Stearns, 1992; Siemsen *et al.*, 2012) that
5 the expression of a tradeoff becomes more pronounced under stressful conditions. As the expression
6 of the phenotypic tradeoff between investment to reproduction and vegetative biomass was most
7 pronounced in the drought treatment, we expect selection on resource allocation to be strongest
8 under this condition. This may be one reason why maternal families from the annual populations all
9 show very similar patterns of resource allocation in response to the water treatments (Fig. 1),
10 suggesting that these annual populations have responded similarly to a selection history of repeated
11 summer drought. The perennial populations originate from two very different types of habitat
12 (mobile dunes with little vegetation (3P) and gaps in a foggy mountain forest with high plant
13 competition (4P)). Perennial life form in this species is clearly adopted under very different
14 environments subjecting plants to local selection histories. Unlike the annual populations, the two
15 perennial populations behaved differently in their response to treatment. Population 4P showed little
16 among-family variation relative to population 3P, and less plasticity relative to the two annual
17 populations. Due to its expression of both a lower among family variation (suggesting a lower level
18 of genetic variation for the response trait), and lower levels of plasticity relative to the other study
19 population, it may actually represent the population with the least ability to respond to changes in
20 water availability. The other perennial population 3P exhibited the largest variation among maternal
21 families. In this population a significant negative genetic correlation between reproductive and
22 vegetative investment was detected, but the correlation was larger than -1 suggesting that a response
23 to selection for increased investment in both vegetative and reproductive tissue is possible.
24 Moreover, the fact that the genetic correlation could at all be detected under the experimental

1 conditions show the presence of genetic variation for resource allocation within that population. In
2 the perennial shrub *Artemisia ordosica*, growing in dunes, Li Li *et al.* (2011) found large variation
3 in life-history traits among dunes in different fixation stages. They argue that this variation in
4 growth and reproduction within a population was caused by the environmental differences across
5 the dune habitat creating a mix of selection pressures on fecundity and survival (Li Li *et al.*, 2011).
6 Similar reasoning may apply to explain the larger variation among maternal families in resource
7 allocation in population 3P in the present study originating from a similar dune habitat.

8 In *P. lanceolata*, a close relative to *P. coronopus*, Shefferson & Roach (2010) studied the adaptive
9 benefit of iteroparity. Plants flowered from one to six times, and fitness increased with number of
10 reproductive years. Yet a large proportion of plants only reproduced once, despite having a post-
11 reproductive lifetime, which could have allowed for another reproductive event. They argue that
12 constraints from the environment on growth and size of plants may explain this observation. Indeed,
13 the influence of plant size on flowering may be crucial for responding adaptively to environmental
14 changes. Rapid growth and fast reproduction are considered a drought escape to allow completing
15 the life cycle before drought kicks in. When a fast completion of the life cycle is advantageous, we
16 expect selection for early flowering and hence a smaller threshold size for flower initiation. The
17 minimum size required for flower induction, is a trait found to be both heritable and to vary among
18 populations (e.g. Wesselingh *et al.*, 1993; de Jong & Klinkhamer, 2005; Wesselingh & De Jong
19 1995). If early flowering is favored to avoid drought stress later in the season (e.g. in habitats with
20 annual populations in the present study), plants with a large threshold size may be at a disadvantage.
21 At the natural sites of our study populations, the average size of a flowering plant is largest in the
22 perennial populations ((Braza *et al.*, 2010), Table 1 this study). Of course these size differences
23 reported from natural sites are strongly influenced by perennial plants having had the opportunity to
24 obtain a larger size over the course of possibly several years of growth. However, under the

1 greenhouse conditions in the present study, plants from the perennial populations 3P still achieved a
2 much larger vegetative size (dry weight of leaves, leaf area) than any of the other populations and
3 investment in reproduction was low relative to the other populations especially in the well-watered
4 treatment. This suggests that individuals from population 3P could have an inherent larger
5 minimum size of flower induction, but also that this can to some extent be environment-dependent,
6 as more inflorescences were produced in the drought treatment.

7

8 **Magnitude and cost of plasticity**

9 We found that the annual populations expressed higher levels of plasticity for investment in
10 reproductive traits than perennial populations. It has been predicted that greater levels of
11 environmental heterogeneity may select for higher levels of phenotypic plasticity (Alpert & Simms,
12 2002; Pigliucci, 2001). Differentiation in level of plasticity among populations has been
13 documented, whereby plants express higher levels of plasticity in populations found in dry habitats
14 and habitats experiencing greater annual variation in precipitation (Gianoli, 2004; Gianoli &
15 Gonzalez-Teuber, 2005; see however, Heschel *et al.*, 2004). The fact that plants from both annual
16 sites in our study were also those expressing the highest plasticity suggests that the higher seasonal
17 variation in temperature and precipitation at those sites may also have selected for higher levels of
18 plasticity.

19 When a cost of plasticity is caused by a production cost, it is expected to be environment specific,
20 as e.g. stress may increase the cost due to resource limitation (Auld *et al.*, 2010). It has been argued
21 that a cost of plasticity may be difficult to detect as selection may already have removed those
22 genotypes expressing a cost (DeWitt *et al.*, 1998). Here, we examined the consequences of

1 plasticity in the drought treatment, the potentially most stressful of the two environments, and we
2 did not find any evidence for a cost of plasticity.

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

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15 **Acknowledgments.**

16 The authors are grateful to R. Nielsen for her help in the greenhouse and to JG Sørensen, T
17 Bataillon, M. Mendéz, and R. Wesselingh for comments and suggestions to improve the
18 manuscript. MBG benefited from the support of two Spanish National projects (CGL2006-08507
19 and CGL2010-21642), BKE had support from a research project supported by the Villum
20 Foundation.

1 **References**

- 2 Agrawal, A. A., Conner, J. K., and Rasmann, S. 2010. Tradeoffs and negative correlations in
3 evolutionary ecology. In: *Evolution after Darwin: the first 150 years* (Bell, Eanes, Futuyma,
4 and Levinton, eds), pp. 243-268. Sinauer Associates, Sunderland, Massachusetts.
- 5 Alpert, P. & Simms, E. L. 2002. The relative advantages of plasticity and fixity in different
6 environments: when is it good for a plant to adjust? *Evol. Ecol.* **16**: 285-297.
- 7 Auld, J. R., Agrawal, A. A. & Relyea, R. A. 2010. Re-evaluating the costs and limits of adaptive
8 phenotypic plasticity. *Proc. R. Soc. B.* **277**: 503-11.
- 9 Bell, G. 1976. On breeding more than once. *Am. Nat.* **110**: 57-77.
- 10 Braza, R., Arroyo, J. & Garcia, M. B. 2010. Natural variation of fecundity components in a
11 widespread plant with dimorphic seeds. *Acta Oecol.* **36**: 471-476.
- 12 Bulmer, M. 1994. *Theoretical evolutionary ecology*. Sinauer Associates, Sunderland,
13 Massachusetts..
- 14 Charnov, E. L. & Schaffer, W. M. 1973. Life-history consequences of natural selection - Coles
15 result revisited. *Am. Nat.* **107**: 791-793.
- 16 Chevin, L. M., Lande, R. & Mace, G. M. 2010. Adaptation, plasticity, and extinction in a changing
17 environment: towards a predictive theory. *PLoS biology* **8**: e1000357.
- 18 de Jong, T. J. & Klinkhamer, P. G. L. 2005. *Evolutionary ecology of plant reproductive strategies*.
19 Cambridge University Press, Cambridge.
- 20 DeWitt, T. J. 1998. Costs and limits of phenotypic plasticity: Tests with predator-induced
21 morphology and life history in a freshwater snail. *J. Evol. Biol.* **11**: 465-480.
- 22 DeWitt, T. J., Sih, A. & Wilson, D. S. 1998. Costs and limits of phenotypic plasticity. *TREE* **13**: 77-
23 81.

- 1 Dorn, L. A. & Mitchell-Olds, T. 1991. Genetics of *Brassica campestris* .1. Genetic Constraints on
2 Evolution of Life-History Characters. *Evolution* **45**: 371-379.
- 3 Etterson, J. R. & Shaw, R. G. 2001. Constraint to adaptive evolution in response to global warming.
4 *Science* **294**: 151-154.
- 5 Falconer, D. S. & McKay, T. F. 1996. *Introduction to Quantitative Genetics. 4th edition*. Pearson
6 UK Longman group, London.
- 7 Franke, D. M., Ellis, A. G., Dharjwa, M., Freshwater, M., Fujikawa, M., Padron, A. & Weis, A. E.
8 2006. A steep cline in flowering time for *Brassica rapa* in Southern California: population-
9 level variation in the field and the greenhouse. *Int. J. Plant Sci.* **167**: 83-92.
- 10 Franks, S. J., Sim, S. & Weis, A. E. 2007. Rapid evolution of flowering time by an annual plant in
11 response to a climate fluctuation. *Proc. Natl. Acad. Sci. USA* **104**: 1278-1282.
- 12 Gelman, A. 2004. *Bayesian data analysis*, 2nd ed. Chapman & Hall/CRC, Boca Raton, Fla.
- 13 Gianoli, E. 2004. Plasticity of traits and correlations in two populations of *Convolvulus arvensis*
14 (Convolvulaceae) differing in environmental heterogeneity. *Int. J. Plant Sci.* **165**: 825-832.
- 15 Gianoli, E. & Gonzalez-Teuber, M. 2005. Environmental heterogeneity and population
16 differentiation in plasticity to drought in *Convolvulus chilensis* (Convolvulaceae). *Evol.*
17 *Ecol.* **19**: 603-613.
- 18 Hadfield, J. D. 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The
19 MCMCglmm R Package. *J. Stat. Software* **33**: 1-22.
- 20 Heschel, M. S., Sultan, S. E., Glover, S. & Sloan, D. 2004. Population differentiation and plastic
21 responses to drought stress in the generalist annual *Polygonum persicaria*. *Int. J. Plant Sci.*
22 **165**: 817-824.

- 1 IPCC (2007) Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III
2 to the Fourth Assessment. Report of the Intergovernmental Panel on Climate Change.
3 (Pachauri, R. K., Reisinger, A., eds.), pp. 104. IPCC, Geneva.
- 4 Ivey, C. T. & Carr, D. E. 2012. Tests for joint evolution of mating system and drought escape in
5 *Mimulus*. *Ann. Bot.* **109**: 583-598.
- 6 Karlsson, P. S. & Méndez, M. 2005. The resource economy of plant reproduction. In: Reproductive
7 allocation in plants (Reekie, E. G.; Bazzaz, F. A., eds.), pp. 1-49. Elsevier Academic Press,
8 Burlington, MA.
- 9 Li Li, S., Yu, F-H., Werger, M. J. A., Dong, M. & Zuidema, P. A. 2011. Habitat-specific
10 demography across dune fixation stages in a semiarid sandland: understanding the
11 expansion, stabilization and decline of a dominant shrub. *J. Ecol.* **99**: 610-620.
- 12 Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative traits*. Sinauer Associates Inc.
- 13 McKay, J. K., Richards, J. H. & Mitchell-Olds, T. 2003. Genetics of drought adaptation in
14 *Arabidopsis thaliana*: I Pleiotropy contributes to genetic correlations among ecological
15 traits. *Mol. Ecol.* **12**: 1137-1151.
- 16 Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Ann. Rev. Ecol.*
17 *Syst.* **37**: 637-669.
- 18 Pigliucci, M. 2001. *Phenotypic plasticity: Beyond nature and nurture*. Johns Hopkins University
19 Press, Baltimore.
- 20 Primack, R. B. 1979. Reproductive allocation in annual and perennial species of *Plantago*
21 (Plantaginaceae). *Am. Nat.* **114** 51-62.
- 22 Reznick, D. 1985. Costs of reproduction: an evaluation of the empirical evidence. *Oikos* **44**: 257-
23 267.
- 24 Roach, D. A. & Wulff, R. D. 1987. Maternal effects in plants. *Ann. Rev. Ecol. Syst.* **18**: 209-235.

- 1 Roff, D. A. 1992. *The evolution of life histories: theory and analysis*. Chapman and Hall, New
2 York.
- 3 Roff, D. A. & Preziosi, R. 1994. The estimation of the genetic correlation - the use of the Jackknife.
4 *Heredity* **73**: 544-548.
- 5 Shefferson, R. P. & Roach, D. A. 2010. Longitudinal analysis of *Plantago*: adaptive benefits of
6 iteroparity in a short-lived, herbaceous perennial. *Ecology* **91**: 441-447.
- 7 Stearns, S. 1992. *The evolution of life histories*. Oxford University Press, Oxford.
- 8 Stebbins, G. L. 1952. Aridity as a stimulus to plant evolution. *Am. Nat.* **86**: 33-44.
- 9 Van Noordwijk, A. J. & de Jong, G. 1986. Acquisition and allocation of resources: their influence
10 on variation in life history tactics. *Am. Nat.* **128**: 137-142.
- 11 Via, S. & Lande, R. 1985. Genotype-environments interaction and the evolution of phenotypic
12 plasticity. *Evolution* **39**: 505-522.
- 13 Villellas, J., J.M. Olesen, J. Ehrlén & García M.B. *In press*. Plant performance in central and
14 northern peripheral populations of the widespread *Plantago coronopus*. *Ecography*. doi:
15 10.1111/j.1600-0587.2012.07425.x
- 16 Weiner, J., Martinez, S., MullerScharer, H., Stoll, P. & Schmid, B. 1997. How important are
17 environmental maternal effects in plants? A study with *Centaurea maculosa*. *J. Ecol.* **85**:
18 133-142.
- 19 Wesselingh, R. A. & De Jong, T. J. 1995. Bidirectional selection on threshold size for flowering in
20 *Cynoglossum officinale* (hound's-tongue). *Heredity* **74**: 415-424.
- 21 Wesselingh, R. A., De Jong, T. J., Klinkhamer, P. G. L., van Dijk, M. J. and Schlatmann, E. G. M.
22 1993. Geographical variation in threshold size for flowering in *Cynoglossum officinale*. *Acta*
23 *Bot. Neerlandica* **42**: 81-91.

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

Population	Lifespan	Latitude	Longitude	Habitat	Mean biomass of adult plants (g) \pm SD	Average length of leaves (mm) \pm SD on adults plants	Min-max yearly temp. ($^{\circ}$ C)	Precipitation/ year (mm)
1A. Trebujena (TRG)	Annual	36 $^{\circ}$ 53'55'' N	6 $^{\circ}$ 17'33'' W	Salt Marsh	0.097 \pm 0.13	25.0 \pm 15.0	12-24	578
2A. Aznalcázar (AZN)	Annual	37 $^{\circ}$ 15'51'' N	6 $^{\circ}$ 13'44'' W	Shrubland	0.064 \pm 0.12	13.0 \pm 9.3	11-25	558
3P. Camposoto (CA)	Perennial	36 $^{\circ}$ 25'35'' N	6 $^{\circ}$ 13'43'' W	Coastal dunes	NA	53.7 \pm 14.9	14-22	622
4P. Bosque Niebla (BN)	Perennial	36 $^{\circ}$ 06'25'' N	5 $^{\circ}$ 32'21'' W	Foggy mountain	0.834 \pm 0.69	54.1 \pm 19.4	11-19	1045

4

1 Table 2. Means (SE) of plant traits in plants originating from four *Plantago coronopus* populations
 2 and growing in two different water treatments. LA: Leaf area, RA: reproductive allocation. For each
 3 population mean are given for N = 40 plants per treatment.

Population	Dw Infl (g)		Dw leaves (g)		LA (cm ²)		RA		Total biomass (g)	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
1A	4.10 (0.14)	2.97 (0.21)	2.20 (0.14)	3.73 (0.26)	305.49 (18.1)	590.35 (31.7)	0.66 (0.02)	0.45 (0.03)	6.29 (0.19)	6.71 (0.28)
2A	3.71 (0.17)	2.12 (0.18)	2.85 (0.18)	4.30 (0.33)	361.4 (20.3)	649.12 (44.87)	0.57 (0.02)	0.35 (0.03)	6.56 (0.19)	6.42 (0.34)
3P	1.32 (0.25)	0.43 (0.13)	5.92 (0.36)	5.00 (0.50)	540.96 (25.3)	563.59 (37.2)	0.19 (0.04)	0.08 (0.02)	7.23 (0.25)	5.44 (0.49)
4P	3.51 (0.12)	2.86 (0.22)	2.80 (0.17)	3.27 (0.21)	338.57 (18.1)	455.29 (27.0)	0.56 (0.02)	0.46 (0.03)	6.31 (0.17)	6.13 (0.25)

4

5

1 Table 3. Generalized linear model (REML method) examining the effects of treatment (Trt), population (Pop) and maternal family (Fam) on
 2 reproductive and vegetative traits in *Plantago coronopus*. LA: leaf area (cm²), and RA: reproductive allocation. For fixed effects the level of
 3 significance is given by F-statistics, and for random effects values correspond to the variance component estimate with standard error in brackets.

	Dw Infl.		Dw leaves		LA		RA		Total biomass		
Model R^2	0.70		0.51		0.47		0.74		0.31		
<u>Fixed effects</u>	d.f.	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Trt	1	61.44	<0.0001	7.61	0.008	62.87	<0.0001	78.33	<0.0001	3.83	0.06
Pop	3	27.88	<0.0001	14.41	<0.0001	5.81	0.0024	37.10	<0.0001	0.22	0.88
Trt*Pop	1	2.17	0.09	6.22	0.0015	8.50	0.0002	2.84	0.051	4.73	0.007
<u>Random effects</u>											
Fam (Pop)		0.32	0.0016	0.42	0.08	2808.3	0.18	0.007	0.001	0.34	0.12
		(0.13)		(0.25)		(2189.7)		(0.002)		(0.22)	
Trt*Fam(Pop)		0.13	0.19	0.39	0.08	2839.8	0.17	0.002	0.39	0.29	0.52
		(0.09)		(0.26)		(2459.9)		(0.0016)		(0.24)	

1 Table 4. ANCOVA examining the effect of population (Pop), treatment (Trt) and the covariate dry
 2 weight of leaves (Dw leaves) on the dry weight of inflorescences in four populations of *Plantago*
 3 *coronopus*. Model $R^2 = 0.60$.

Source	d.f.	SS	<i>P</i>
Pop	3	103.6	<0.0001
Trt	1	62.5	<0.0001
Dw leaves	1	35.6	<0.0001
Pop*Trt	3	11.1	0.02
Pop*Dw leaves	3	0.9	0.84
Trt*Dw leaves	1	1.2	0.31
Pop*Trt*Dw leaves	3	14.4	0.006
Error	304	346.0	

4

1 Table 5. Estimates of the phenotypic family means correlation and the genetic correlation between
 2 reproductive and vegetative biomass in maternal plant families from four different *Plantago*
 3 *coronopus* populations and growing in either a dry (D) or wet (W) treatment. Standard deviation of
 4 the posterior distribution of the genetic correlations is given in parentheses. Significant correlations
 5 in bold, **: P<0.01, a: P<0.1

Population	Treatment	Pearson family means correlation	Genetic correlation
		Dw infl.<>Dw leaves	Dw infl.<>Dw leaves
1A	D	0.06	0.07 (0.42)
1A	W	-0.37	-0.21 (0.48)
2A	D	-0.58	-0.30 (0.42)
2A	W	-0.23	-0.10 (0.52)
3P	D	-0.85**	-0.81 (0.22)**
3P	W	-0.13	-0.05 (0.47)
4P	D	-0.63a	-0.44 (0.36)
4P	W	-0.47	-0.22 (0.47)

6

1 Table 6. Plasticity (mean difference in trait value between environment) of plant traits in four
2 populations of *Plantago coronopus* growing under two water treatment. Numbers in brackets
3 indicate standard errors (SE).

Population	Dw Inflorescences	Dw leaves	Leaf area (LA)	RA
1A	1.18 (0.23)	1.54 (0.25)	284.9 (30)	0.21 (0.03)
2A	1.58 (0.18)	1.45 (0.28)	287.7 (36)	0.21 (0.02)
3P	0.95 (0.27)	2.03 (0.48)	171 (29)	0.13 (0.04)
4P	0.92 (0.20)	0.88 (0.19)	158 (23)	0.14 (0.08)

4

5

6

1 **Legends to figure**

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

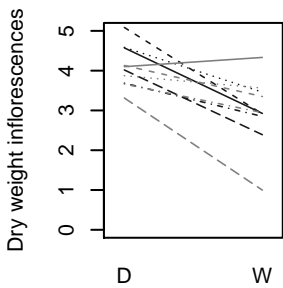
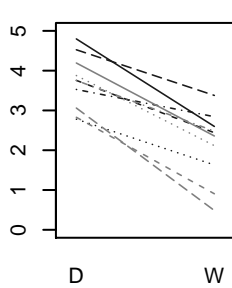
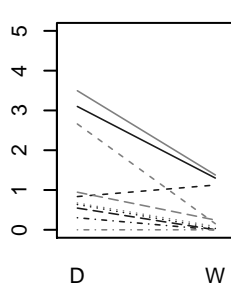
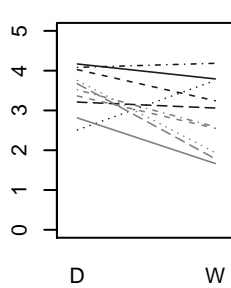
7

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

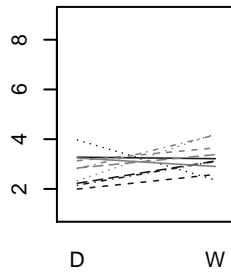
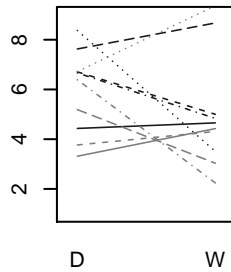
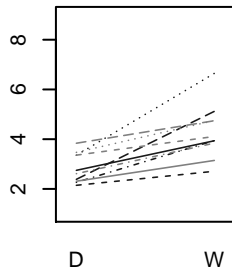
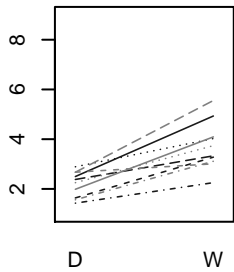
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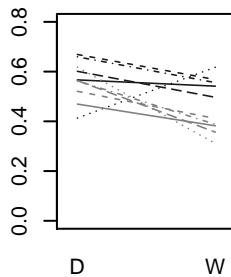
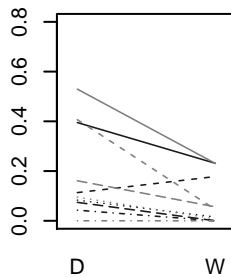
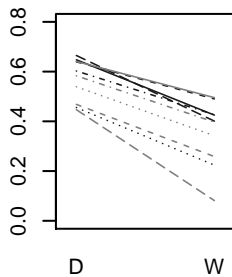
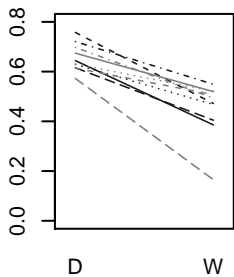
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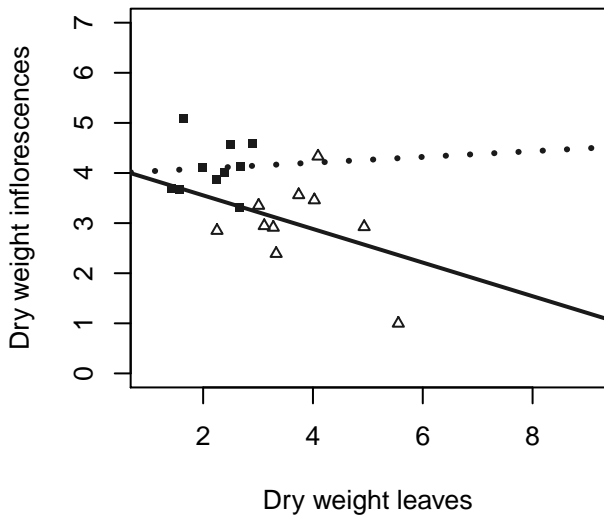
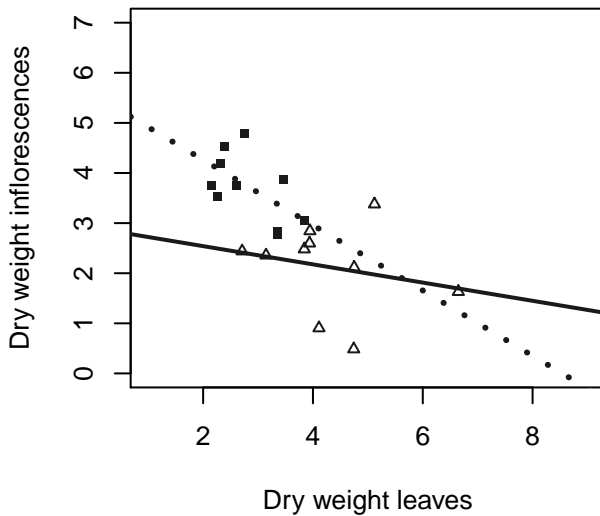
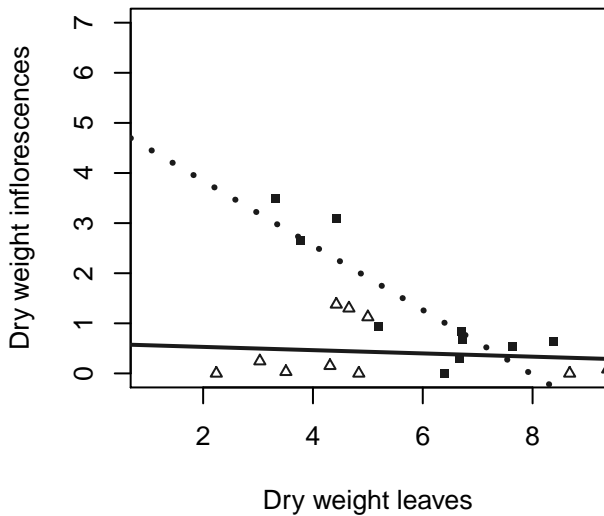
1A**2A****3P****4P**

Dry weight leaves



RA



1A**2A****3P****4P**