A tolerance-fecundity trade-off maintains intraspecific variation of seed traits in a widespread dimorphic herb

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Abstract

The coexistence of species with different seed sizes is a long-standing issue in community ecology, and a trade-off between fecundity and stress tolerance has been recently proposed to explain such co-occurrence in heterogeneous environments. Here we tested for the first time an intraspecific extension of this model: whether such trade-off also explains seed trait variation among populations of widespread plants under stress gradients. We collected seeds from 14 populations of Plantago coronopus along the Atlantic coast in North Africa and Europe. This herb presents seed dimorphism, producing large basal seeds with a mucilaginous coat that facilitates water absorption (more stress-tolerant), and small apical seeds without coat (less stress-tolerant). We analyzed variation among populations in number, size and mucilage production of basal and apical seeds, and searched for relationships with local environmental conditions. Populations under higher stress (higher temperature, lower precipitation and lower soil organic matter) showed fewer seeds per fruit, a higher predominance of basal relative to apical seeds, and larger basal seeds with thicker mucilaginous coats. These results strongly suggest that a trade-off between tolerance and fecundity at the fruit level underpins variation in seed traits among P. coronopus populations. In contrast, plant seed production per year and over the lifespan did not trade-off against tolerance traits, and seemed to be related with plant size and other life-cycle components. The tolerance-fecundity model may constitute a broader ecological framework to explain trait variation than the classical seed size-number compromise, although several fecundity levels should be considered to understand differences in seed characteristics among populations of widespread plants.
**Introduction**

Seed production and seed traits represent crucial components in plant fitness. Seed size, for example, is closely related to important ecological and demographic processes, such as dispersal, germination or seedling survival (Westoby *et al.* 1992; Chaplin III *et al.* 1993; Coomes & Grubb 2003). Seed production plays also a major role in individual fitness and population persistence (Lloyd 1987; Westoby *et al.* 2002), and a trade-off between the size and the number of seeds is expected (Smith & Fretwell 1974; Lloyd 1987). In addition, both seed size and total seed production might show a positive relationship with plant size (Primack 1987; Herrera 1991; Aarssen & Jordan 2001). The presence of mucilaginous seed coats in some plant species may also affect relevant seed-related processes, such as water stress tolerance, competition via allelopathy or adherence to soil particles (Harper & Benton 1966; Hasegawa *et al.* 1992; Lu *et al.* 2010). Many taxa present remarkable differences in seed characteristics among populations (*e.g.*, McWilliams *et al.* 1968; McKee & Richards 1996; Mendez 1997), and quantifying this intraspecific variation and determining its underlying causes may be important to understand why some plants are more successful than others in terms of colonization, or adaptation to new ecological or climatic scenarios (Buckley *et al.* 2003; Wright *et al.* 2006; Albert *et al.* 2010).

Environmental stress is a crucial factor in the ecology and evolution of plants (Grime 1977; Parsons 1991; Nevo 2001), and variation in stress levels may promote seed trait divergences among or within species. The hypothesis of the tolerance-fecundity trade-off (Muller-Landau 2010; see also Westoby *et al.* 2002), has been proposed to explain the coexistence of plant species with different seed sizes in environmentally heterogeneous communities. The underlying mechanism is related to a demographic process, *i.e.*, the differential probability of recruitment at the available
regeneration niches. In this process, high-stress regeneration sites would be eventually occupied by large-seeded species, thanks to their higher tolerance to environmental stress. Low-stress patches, in contrast, would be occupied by species of different seed sizes and tolerances, although small-seeded species would become dominant due to their higher seed production relative to large-seeded species. Because of its logic and simplicity, the mechanism underlying the tolerance-fecundity model could be rather general, and also explain variation in seed traits among populations of species occurring along environmental stress gradients. In this intraspecific extension of the model, populations in stressful environments would provide the seeds with additional resources at the cost of reducing seed number. In contrast, populations in less stressful conditions could afford to reduce resources investment per seed (and thus stress tolerance), in order to increase offspring number. These predictions rely on the assumption that available resources for seed production are constant across populations and do not covary with the traits involved in the trade-off. In addition, despite that seed size is the most frequently studied trait, other seed characteristics could be considered to evaluate stress tolerance (Muller-Landau 2010), such as some coat features or shape.

Widespread plants occurring along environmental gradients represent typical examples of high phenotypic variability (Joshi et al. 2001; Richards et al. 2005), and provide a good opportunity to analyze intraspecific variation in seed traits in relation to environmental conditions. *Plantago coronopus* is a common short-lived perennial herb present along a strong environmental gradient in the eastern Atlantic coast, and shows large differences among populations in fecundity (Braza et al. 2010, Villellas et al. 2012). Additionally, this taxon presents seed dimorphism (Dowling 1933; Schat 1981), whereby fruits produce both large basal seeds with a mucilaginous coat and small apical seeds without coat. For individuals emerging from basal seeds, plant performance
(germination, survival and fecundity) is positively correlated with original seed size (Koelewijn & Van Damme 2005). Moreover, basal seeds germinate better than apical ones, especially in dry years (Braza & García 2011), which likely results from higher provisions (Chapin III et al. 1993; Westoby et al. 2002; Coomes & Grubb 2003) and higher water absorption through their mucilaginous coat (Harper & Benton 1966; Schat 1981; Gutterman & Shem-Tov 1997).

In this study we analyze variation among populations of the widespread herb *P. coronopus* in a set of seed traits, and its relationship with environmental stress. Climate has a key role in plant performance at large scales (Woodward & Williams 1987), and the positive relationship between seed performance and seed size and mucilage in this species specifically suggests water and nutrient deficits as potential sources of stress. Consequently, we tested the effect of environmental stress on seed traits using: 1) water availability, estimated with precipitation (see also Harper & Benton 1966; Baker 1972; Wright & Westoby 1999) and with a more integrative metric of water deficit considering the balance between evapotranspiration and precipitation (Thornthwaite 1948); 2) temperature, which may reduce water availability (Baker 1972) or directly affect plant metabolic processes, as seeds require more energy to convert into seedling under warmer conditions (Lord et al. 1997; Murray et al. 2004); and 3) soil organic matter content, which can be used as an indicator of soil fertility (Reeves 1992), and which may be associated with soil water retention due to small particle sizes and high cation exchange capacity (Cobertera 1993).

To analyze variation in fecundity and seed traits in *P. coronopus*, we sampled 14 populations along the Atlantic Coast of North Africa and Europe, spanning a latitudinal gradient of 4000 km. Here, we first report variability among populations in the number, size and production of mucilage in basal and apical seeds. Given that *P. coronopus* is a
perennial plant, we consider fecundity at three levels: per individual over the lifespan, per individual per year, and per fruit. Second, we analyze if seed trait variation is associated with soil and climatic conditions, considering low water availability, high temperature and low organic matter content as representative of stressful conditions. Besides, we analyze if seed trait variation is affected by plant size. Third, we test whether a trade-off between fecundity (at the three levels) and stress tolerance promotes diversity in seed traits among populations of this dimorphic species. In that case, we would expect populations subject to higher stress to present: a) a higher predominance of basal (more stress-tolerant) relative to apical (less stress-tolerant) seeds, b) larger basal seeds with higher mucilage production, and c) a subsequent reduction in seed production due to trade-offs in resource allocation. To strengthen the analyses of trade-offs, we test the assumption that total resource investment in seeds is constant across populations and unrelated with seed traits.

**Materials and methods**

**Study species and populations**

*Plantago coronopus* L. subsp. *coronopus* (Plantaginaceae) is a widespread, short-lived perennial herb distributed along the Mediterranean basin, reaching North Europe through a narrow strip along the Atlantic coast (Fig. 1, Hultén & Fries 1986). The subspecies *coronopus* is present throughout most of the species’ range and differs from other less common subspecies in the morphology of the bracts (Chater & Cartier 1976). Our study was restricted to the common subspecies, and hereafter we will refer to it as *P. coronopus*. It presents high variability in morphological characters, and a life cycle that can be annual or perennial (Chater & Cartier 1976). Reproductive individuals have several spikes with wind-pollinated flowers, and present intermediate outcrossing rates,
with high variation among and within populations (Wolff et al. 1988). Fruits are capsules that produce two types of seeds (Dowling 1933; Schat 1981): up to four large basal seeds, and one or no small apical seed (Fig. 2). Basal seeds further differentiate from apical ones by the possession of a coat that becomes mucilaginous when moistened, which is virtually absent in the latter. *P. coronopus* is a colonizer plant occurring in many habitats, especially sand dunes, salt marshes, coastal prairies, and human-disturbed environments.

In this study we analyzed 14 perennial populations, spanning almost the entire latitudinal range of the species along the eastern Atlantic coast (Table 1; Fig. 1): two populations in Morocco (Tiznit, Cap Spartel), five in Spain (Ceuta, Tarifa, Camposoto, Corrubedo, Traba), one in NW France (Pen Bron), two in Denmark (Helnaes, Skallingen), two in Sweden (Glommen, Torekov) and two in Scotland (Aberdeen, Skye). All populations were located by the sea, although the species’ habitat in the seashore differed along the coast: populations in Tiznit, Cap Spartel and Ceuta were located in coastal cliffs; populations in Tarifa, Camposoto, Corrubedo, Traba and Pen Bron were situated in sand dunes; and populations in Helnaes, Skallingen, Glommen, Torekov, Aberdeen and Skye were located in coastal prairies.

**Environmental data**

To estimate soil fertility in populations, we collected 10 cm deep soil cores, and measured in the laboratory the percentage of soil organic matter content from the organic carbon (Heanes 1984). Meteorological data were obtained from several databases: the website http://www.allmetsat.com (MT); Direction Regional d’Hydraulique in Tetuan, Morocco (CS); Spanish National Meteorological Agency (CT, T and CA); MeteoGalicia (C and TB); MeteoFrance (F); Danish Meteorological Institute (DH and DS); Swedish Meteorological and Hydrological Institute (SG and
ST); and Met Office (EA and ES). We obtained mean monthly precipitation (mm), mean monthly maximum and minimum temperatures (°C) and mean annual temperature (°C), from 10-20 years within the last four decades (depending on the availability) from the nearest meteorological station to each population. We calculated total precipitation in the period of growing season where highest differences appeared among populations, i.e. from June to September (thereafter “summer precipitation”). We also calculated evapotranspiration (mm) with the equation (from Hargreaves 1985):

\[ ET = 0.0023 \cdot R_a \cdot TD^{0.5} \cdot (T_m + 17.8) \cdot d \]

where ET is monthly evapotranspiration, \( R_a \) is extraterrestrial radiation (calculated as a function of latitude and the month of the year, Allen et al. 1998), \( TD \) is the difference between mean monthly maximum and minimum temperatures, \( T_m \) is the average monthly temperature, and \( d \) is the number of days in each month. For each population, we summed ET from June to September to calculate summer ET, and then calculated a summer water stress index as the ratio between summer ET and summer precipitation.

**Seed collection and measurements**

We collected the spikes of 25 randomly chosen individuals in each population in the summers of 2007 or 2008. Fruits were dissected in the laboratory to measure a set of seed-related traits (Table 2). For five populations (MT, CS, CT, EA and ES) some variables were not calculated (Fig. 3). The number of basal and apical seeds per fruit was counted with magnifying glasses in 10 fruits per mother. The number of each type of seed per fruit and the total number of seeds per fruit (basal plus apical seeds; thereafter “fruit seed production”) was then averaged across individuals for each population. We also averaged across individuals the percentage of basal and apical seeds, and calculated the seed ratio from mean population values, dividing percentage of basal seeds by percentage of apical seeds (thereafter “seed ratio”).
Basal and apical seed mass was estimated for each population by weighting eight groups of 25 basal seeds and 25 apical seeds, respectively, from 12 individuals (seeds were weighted in groups due to their small size). The mucilaginous coat was measured with the aid of magnifying glasses in five basal seeds per plant, in 15 plants per population. Previously, we soaked the seeds for one hour in Petri dishes, until the mucilage became conspicuous (Fig. 2). We estimated the projected seed area and the total area that contained both the seed and the mucilaginous coat using the ellipse formula \( \text{area} = \pi \cdot a \cdot b; \) \( a \) and \( b \) correspond to the major and minor semiaxes), and we calculated the mucilage area by subtracting the seed area from the total area. For each population, we averaged across seeds the percentage areas of mucilage and seed, and then calculated from mean population percentages the ratio between mucilage and seed area (thereafter “mucilage ratio”). We used a relative measure of mucilage because we found with a linear regression (\( \text{lm} \) procedure, package \textit{stats}, R Core Development Team 2011) that it was positively correlated to seed area \( (t_{857} = 22.6, R^2 = 0.37, p < 0.001) \).

We estimated the total number of seeds per year (thereafter “annual seed production”) and the size of an average of 160 reproductive individuals per population and year, in annual censuses from 2007 to 2010. We recorded each year the number of leaves and inflorescences of individuals, and the length of an average leaf and an average inflorescence. Plant size was defined as number of leaves * length of an average leaf, and annual seed production was estimated as number of inflorescences * length of an average inflorescence * number of seeds per unit of inflorescence length (calculated with a regression equation for each population). We also calculated the total seed production over the lifespan (thereafter “lifetime seed production”) for those reproductive individuals that were monitored for their entire lives. For further details on the estimation of these parameters, see Villellas \textit{et al.} (2012). Annual seed production,
lifetime seed production and plant size were then averaged for each population across individuals and years.

Finally, we estimated for each population the total mass allocation to seeds per fruit, per plant per year, and per plant over the lifespan (thereafter “fruit seed mass”, “annual seed mass” and “lifetime seed mass”, respectively) from mean values of previous parameters: fruit seed mass = (number of basal seeds per fruit * basal seed mass) + (number of apical seeds per fruit * apical seed mass); annual seed mass = annual seed production * (fruit seed mass / fruit seed production); lifetime seed mass = lifetime seed production * (fruit seed mass / fruit seed production).

Analysis of seed trait variability, environmental factors and plant size

We analyzed among-population variability in seed traits with the coefficient of variation (CV) of population mean values. Since most of the traits were log-normally distributed, we also calculated the coefficient of variation for these distributions as

\[ CV_{ln} = \sqrt{e(s^2)} - 1 \]

where \( e \) is the base of the natural logarithm and \( s \) is the standard deviation of the natural-log transformed data (Koopmans et al. 1964).

The effects of environmental factors on seed trait variation were tested on those traits conferring stress tolerance to plants, i.e. seed mass and mucilage (see Introduction; thereafter “stress tolerance traits”). Considering the particular dimorphism of the species, in which basal seeds are larger than apical seeds and the latter lack the mucilaginous coat, we selected the following stress tolerance traits for analysis: seed ratio, basal seed mass and mucilage ratio (all of them log-transformed). We analyzed collinearity among environmental variables with an analysis of variance inflation factor (vif procedure, package car, R Core Development Team 2011), and we discarded summer water stress index from subsequent analyses because it showed high collinearity with summer precipitation (values much higher than 10; Kleinbaum et al.)
1988), and because the latter provided a better fit to our data. Thus, the environmental predictors were mean annual temperature, summer precipitation (log-transformed) and soil fertility (log-transformed). For each stress tolerance trait, we performed linear regressions with each of the three predictors, as well as multiple linear regressions with all possible combinations with two or three predictors (lm procedure, package stats, R Core Development Team 2011). To find out which model provided the best fit to our data we first compared AIC (Akaike’s information criterion) values from all regression analyses. Among the combinations of predictors with the lowest AIC values, we then checked with analyses of variance (anova procedure, package stats, R Core Development Team 2011) if the sequential addition of predictors significantly improved the previous simpler model. For these analyses, we used the populations for which we had data for all environmental predictors and stress tolerance traits (all except MT, CS and CT), so that AIC values were comparable.

We also analyzed whether plant size (log-transformed) was correlated with seed production traits and stress tolerance traits with linear regressions, although the effect of plant size on lifetime and annual seed production was instead analyzed with linear mixed models, including population and year as random factors (lme procedure, package nlme, R Core Development Team 2011).

**Analysis of the tolerance-fecundity trade-off**

To test for a trade-off between fecundity and stress tolerance, we used estimates of fecundity at three levels: lifetime seed production, annual seed production and fruit seed production. Firstly we performed simple linear regressions between each measure of seed production (response variables) and each stress tolerance trait (predictors), with log-transformed variables except for fruit seed production. Then we tested again the relationship between each seed production trait and each stress tolerance trait with...
multiple regressions, including plant size as a covariate to control for its possible
effects, and looking at the significance of the partial regression parameters of stress
tolerance traits. Finally, to check for the assumption of constant available resources for
seeds in the tolerance-fecundity trade-off, we performed Pearson’s correlation analyses
(cor procedure, package stats, R Core Development Team 2011) between lifetime,
annual and fruit seed mass on the one hand, and seed production traits and stress
tolerance traits on the other hand.

The tolerance-fecundity trade-off was tested for each fecundity level using three
stress tolerance traits, which may increase the probability of type I error. For all the
analyses, we thus performed at each fecundity level corrections on p values with the
false discovery rate method (Benjamini & Hochberg 1995), appropriate for analyses
with small sample sizes.

Results

Seed trait variability

Seed traits exhibited large differences in among-population variability (Table 2):
lifetime and annual seed mass, lifetime and annual seed production, and seed ratio,
showed the highest variability, whereas fruit seed mass was the least variable trait.
Apical seed mass was less variable across the study area than basal seed mass. Both
measures of variability among populations (CV and CV_{ln}) showed the same pattern
across traits.

Effects of environmental factors and plant size

There were notable differences among populations in temperature, summer
precipitation and soil fertility along the latitudinal gradient (Table 1). There was a
gradual decrease in temperature from south to north, and northern populations showed
in general higher precipitation, with a few exceptions in both climatic parameters. Southern populations in Spain and France showed lower soil fertility than most northern populations.

The separate effect of summer precipitation was more significant than that of temperature or soil fertility on seed ratio and mucilage ratio, whereas temperature showed the highest separate effect on basal seed mass (Table 3). In the case of seed ratio, the combination of summer precipitation and temperature showed the lowest AIC value, but the analysis of variance indicated that it did not explain differences among populations significantly better than summer precipitation alone. For basal seed mass, temperature and soil fertility together showed the lowest AIC value, and provided a better fit to the data than temperature alone, although with a marginal significance. In the case of mucilage ratio, the combination of the three predictors showed the lowest AIC value, but it did not improve a model with summer precipitation and soil fertility. However, the combination of summer precipitation and soil fertility explained differences in mucilage ratio better than summer precipitation alone, although with a marginal significance. Summer precipitation negatively affected seed ratio and mucilage ratio, but had no effect on basal seed mass. Temperature positively affected all stress tolerance traits, and the effect of soil fertility was always negative.

Plant size was significantly correlated with lifetime seed production ($F_{1,2618} = 858.4, R^2 = 0.34, p < 0.001$), annual seed production ($F_{1,5286} = 2317.3, R^2 = 0.21, p < 0.001$), fruit seed production ($F_{1,9} = 6.6, R^2 = 0.42, p = 0.030$), seed ratio ($F_{1,9} = 7.6, R^2 = 0.46, p = 0.022$) and mucilage ratio ($F_{1,9} = 14.9, R^2 = 0.62, p = 0.004$). In contrast, plant size showed no significant effect on lifetime seed mass ($F_{1,7} = 2.7, R^2 = 0.28, p = 0.144$), annual seed mass ($F_{1,9} = 2.3, R^2 = 0.21, p = 0.162$), fruit seed mass ($F_{1,9} = 2.0,$
$R^2 = 0.18, p = 0.188$), basal seed mass ($F_{1,9} = 0.5, R^2 = 0.05, p = 0.514$) and apical seed mass ($F_{1,9} = 0.1, R^2 = 0.02, p = 0.713$).

Tolerance-fecundity trade-off

Southern populations showed in general higher seed ratio, higher basal seed mass, and higher mucilage ratio than northern populations, whereas apical seed mass presented low variation along the latitudinal gradient (Fig. 3a-c). Southern populations had in general a lower fruit seed production than northern populations, but a higher lifetime and annual seed production (Fig. 3d).

Simple regression analyses showed that lifetime seed production was positively and significantly correlated with seed ratio, and marginally significantly correlated with basal seed mass and mucilage ratio, whereas in multiple regression analyses including plant size as a covariate, the partial correlations were not significant for any stress tolerance trait (Fig. 4a, b, c; Table 4). Annual seed production was positively correlated with seed ratio with a marginal significance, and not significantly correlated with basal seed mass and mucilage ratio, while none of their partial correlations were significant in regression analyses including plant size (Fig. 4d, e, f; Table 4). Fruit seed production was significantly and negatively correlated with seed ratio, basal seed mass, seed weight ratio and mucilage (Fig. 4g, h, i; Table 4); when accounting for plant size, the partial correlation was still significant and negative for basal seed mass, and marginally significant for seed ratio and mucilage ratio.

Lifetime seed mass was significantly correlated with lifetime seed production ($t_7 = 9.1, r = 0.96, p < 0.001$), seed ratio ($t_7 = 4.4, r = 0.86, p = 0.009$) and basal seed mass ($t_7 = 3.4, r = 0.79, p = 0.018$), and correlation with mucilage ratio was marginally significant ($t_7 = 2.3, r = 0.65, p = 0.056$). Annual seed mass was significantly correlated with lifetime seed production ($t_9 = 13.5, r = 0.98, p < 0.001$), seed ratio ($t_9 = 3.0, r =$
0.71, \( p = 0.023 \)) and basal seed mass (\( t_0 = 3.2, r = 0.73, p = 0.023 \)), although it showed no correlation with mucilage ratio (\( t_0 = 1.4, r = 0.44, p = 0.181 \)). Fruit seed mass showed no significant correlation with fruit seed production (\( t_{10} = -0.3, r = -0.09, p = 0.783 \)), seed ratio (\( t_{10} = 0.1, r = 0.01, p = 0.977 \)) or mucilage ratio (\( t_{10} = -0.4, r = -0.12, p = 0.977 \)), although it showed a marginally significant correlation with basal seed mass (\( t_{10} = 2.6, r = 0.64, p = 0.076 \)). Lifetime and annual seed mass decreased northwards, whereas fruit seed mass showed no clear latitudinal pattern (Fig. 3e).

**Discussion**

*Plantago coronopus* presents considerable variation along the Atlantic Coast in Europe and North Africa in a set of seed traits, *i.e.* the number and size of seeds, the proportion of basal and apical seeds and the production of mucilage. Similar levels of variability in seed traits have been found among populations of other widespread plants. For example, the CV for seed size similarly lies around 0.20-0.30 in the short-lived *Campanula Americana* (calculated from Kalisz & Wardle 1994) and the long-lived *Vaccinium stamineum* (Yakimowski & Eckert 2007), and the CV for annual reproductive output (number of seeds or fruits per plant per year) is also higher than 0.70 for those species. In this study, we tested whether the observed intraspecific variability was explained by a recent hypothesis proposed at the community level: the trade-off between stress tolerance and fecundity in heterogeneous environments (Muller-Landau 2010). Our results suggest indeed that a tolerance-fecundity trade-off underpins intraspecific variation in seed traits in *P. coronopus* at the fruit level, although lifetime and annual seed production appear to be shaped by different factors.

Stress tolerance traits (seed ratio, basal seed mass and mucilage ratio) were strongly correlated with climatic and soil conditions in *P. coronopus*. Seed ratio was
negatively affected by water availability, estimated with summer precipitation. Basal seed mass was enhanced by temperature, which may increase energy requirements of metabolic processes (Lord et al. 1997; Murray et al. 2004), and negatively affected by soil organic matter, which is associated with fertility (Reeves 1992), although both temperature and soil organic matter (Coberterera 1993) may have also indirectly affected seed mass through their effects on water availability. Finally, mucilage ratio was negatively affected by summer precipitation and soil organic matter, both associated with moisture, suggesting a role of mucilage in reducing water deficit. Overall, the three environmental parameters represent some kind of environmental stress (water and nutrient availability, and energy requirements), and significantly contribute to explain among-population differences in one or another stress tolerance trait. Our results support previous studies that found tolerance-related seed traits, most commonly seed size, positively correlated with higher temperatures (Baker 1972, Murray et al. 2004), lower precipitation or water availability in general (Baker 1972; Wright & Westoby 1999) and lower soil fertility (Lee & Fenner 1989; Parolin 2000). There is also abundant literature that relates seed size with seedling competitive ability (e.g. Tilman 1994, Geritz et al. 1999), but this factor does not help to explain seed trait variation in P. coronopus, because the populations exposed to highest competition, in northern coastal meadows, showed the smallest seeds.

Seed traits did not vary independently along the environmental gradient in P. coronopus. Fruit seed production showed the opposite trend than basal seed mass, which suggest the typical trade-off between seed size and seed number (Smith & Fretwell 1974; Lloyd 1988) at the fruit level. In addition, seed number seems to be also regulated by additional traits related with resource investment per seed, as indicated by the negative correlation between fruit seed production and seed ratio and mucilage ratio.
In contrast, lifetime and annual seed production showed positive or non-significant relationships with stress tolerance traits, suggesting there were no trade-offs at these higher levels. The finding that plant size was correlated with most of the traits measured in this study compelled us to analyze seed trait correlations accounting for plant size, and such analyses confirmed our previous interpretations. Indeed, fruit seed production still showed significant or marginally significant negative relationships with stress tolerance traits when considering the effect of plant size, whereas lifetime and annual seed production showed no significant correlations with any of the tolerance traits. Mendez (1997) also found in *Arum maculatum* a negative correlation between seed size and number only at the fruit level, although Devlin (1989) and Mehlman (1993) reported the trade-off at both levels in two perennial plants. The lack of consistency among studies confirms the importance of considering different levels when analyzing species’ seed production (Primack 1987; Herrera 1991).

The tolerance-fecundity model (Muller-Landau 2010) states that heterogeneous areas in terms of environmental stress provide different regeneration niches, allowing the maintenance of species of different seed sizes within communities, and assuming that seed size is related to stress tolerance. We believe that a similar mechanism underlies variability in seed traits among populations of *P. coronopus*, considering the large differences in climatic and soil conditions among locations and the corresponding variation in seed traits. Let’s consider the stress gradient that broadly coincides with the latitudinal gradient of the species, and along which the fecundity (at the fruit level) and the stress tolerance traits covary (Fig. 5). In this gradient, southern populations are subject to higher environmental stress (higher temperatures, lower summer precipitation and lower soil fertility) than northern populations, which limits their access to two essential resources for seed and seedling performance, *i.e.* water and nutrients. In
response to these conditions, plants produce basal seeds with more internal resources to
tolerate environmental stress (Chapin III et al. 1993; Westoby et al. 2002; Coomes &
Grubb 2003), and with higher amounts of mucilage to facilitate water absorption
reasons, southern plants also increase seed ratio, towards more basal relative to apical
seeds. Since total resources allocated to seeds by fruits are constant across populations,
the final outcome is a reduction in the total number of seeds per fruit. Conversely,
northern populations occur in less stressful conditions, and plants can thus reduce
investment in seed size and mucilage, allowing an increase in fruit seed production (Fig.
5). A decline in seed size with latitude seems to be indeed a common pattern within
widespread plant taxa (Moles & Westoby 2003). Moreover, *P. coronopus* adjusts the
coexistence of basal and apical seeds along the stress gradient, resulting in a higher
predominance of the more tolerant seed morph in populations under higher stress (in a
way equivalent to how big-seeded species would predominate over small-seeded species
in stressful sites within communities). Overall, our results strongly suggest that the
mechanism proposed by Muller-Landau (2010) for the maintenance of variation in seed
size among species can also explain the variability in seed traits among populations of
*P. coronopus*.

The tolerance-fecundity hypothesis may help to understand why many plants of
stressful and unpredictable environments produce different seed morphs with
contrasting tolerance attributes (Venable 1985; Imbert 2002). For example, Yao et al.
(2010) proposed a model for the widespread dimorphic *Chenopodium album*, in which
populations subject to the highest salinity stress produced higher proportions of the
tolerant seed morph by reducing total seed production. However, although the
mechanisms underlying the tolerance-fecundity trade-off seem particularly valid for
seed-heteromorphic species, this model might also apply to other taxa, constituting a broader framework than the frequently invoked compromise between seed size and number. Rather than focusing only on seed size, we would expect any additional investment on seed tolerance to stress, such as mucilage, to trade-off against fecundity, promoting thus variability among populations.

The tolerance fecundity model operates in *P. coronopus* at the fruit level but not at the individual level. The absence of trade-offs at this higher level, explained by the large variability in lifetime and annual seed mass and by the correlations between total resources and the variables involved in the trade-off, suggests that lifetime and annual seed production are shaped in *P. coronopus* by different factors than those regulating fruit seed production. Differences in seedling recruitment among populations may indeed explain why lifetime and annual seed production follow the opposite pattern than fruit seed production. Recruitment is significantly lower in southern than in northern locations (Villellas *et al.* 2012), and such failure ought to be compensated by an increase in total seed production for populations to persist. This is achieved to some extent through a larger size of individuals (Fig. 3f), as shown by a correlation between seed production at the individual level and plant size (see also Braza *et al.* 2010, Villellas *et al.* 2012). In contrast, the higher recruitment in northern populations, located in more humid and stable habitats, makes unnecessary a high investment in seed production. Thus, annual and lifetime seed production seem to be regulated to some extent by compensatory changes in life cycle components (fecundity vs. recruitment) among populations (Doak & Morris 2010, Villellas *et al.* 2012).

This study has shown that a gradient in environmental stress promotes variability among populations of the widespread *P. coronopus* in a set of seed traits, *i.e.* the size, the mucilage and the relative abundance of two seed morphs with contrasting tolerance.
attributes. Overall, our results support the hypothesis that a trade-off between fecundity and stress tolerance can underlie variation in seed traits not only among species, but also among populations of the same taxon. The tolerance-fecundity model may in fact constitute a broader ecological framework than the classical trade-off between seed size and number, at least for species occurring along gradients in environmental stress. However, our work shows also that fecundity can be regulated within plant species by different factors at the fruit and the whole individual level, to maximize the fitness of populations in each set of local conditions.

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References


McWilliams, E.L., Landers, R.Q., Mahlsted, J.P. (1968) Variation in seed weight and germination in populations of *Amaranthus retroflexus L.* *Ecology, 49*, 290-&.


Table 1 Location of *Plantago coronopus* populations in the study and mean values in environmental variables: annual temperature, summer precipitation ($P_S$), summer water stress index ($WSI_S$; see Materials and methods for details) and percentage of soil organic matter (SOM).

<table>
<thead>
<tr>
<th>Population</th>
<th>Location</th>
<th>Coordinates</th>
<th>Temperature (ºC)</th>
<th>$P_S$ (mm)</th>
<th>$WSI_S$</th>
<th>SOM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT</td>
<td>Tiznit, Morocco</td>
<td>29º45' N, 09º53' W</td>
<td>18.5</td>
<td>9</td>
<td>68.9</td>
<td>-</td>
</tr>
<tr>
<td>CS</td>
<td>Cap Spartel, Morocco</td>
<td>35º47' N, 05º55' W</td>
<td>17.7</td>
<td>63</td>
<td>10.4</td>
<td>-</td>
</tr>
<tr>
<td>CT</td>
<td>Ceuta, Spain</td>
<td>35º54' N, 05º21' W</td>
<td>16.1</td>
<td>44</td>
<td>13.9</td>
<td>-</td>
</tr>
<tr>
<td>T</td>
<td>Tarifa, Spain</td>
<td>36º02’ N, 05º38’ W</td>
<td>17.1</td>
<td>61</td>
<td>7.5</td>
<td>0.7</td>
</tr>
<tr>
<td>CA</td>
<td>Camposoto, Spain</td>
<td>36º25’ N, 06º13’ W</td>
<td>18.7</td>
<td>69</td>
<td>7.8</td>
<td>0.4</td>
</tr>
<tr>
<td>C</td>
<td>Corrubedo, Spain</td>
<td>42º33’ N, 09º01’ W</td>
<td>14.9</td>
<td>228</td>
<td>2.3</td>
<td>1.1</td>
</tr>
<tr>
<td>TB</td>
<td>Traba, Spain</td>
<td>43º11’ N, 09º03’ W</td>
<td>14.7</td>
<td>275</td>
<td>2.0</td>
<td>1.4</td>
</tr>
<tr>
<td>F</td>
<td>Pen Bron, France</td>
<td>47º18’ N, 02º30’ W</td>
<td>12.8</td>
<td>206</td>
<td>2.4</td>
<td>0.9</td>
</tr>
<tr>
<td>DH</td>
<td>Helsnaes, Denmark</td>
<td>55º08’ N, 09º59’ E</td>
<td>8.1</td>
<td>235</td>
<td>2.1</td>
<td>5.6</td>
</tr>
<tr>
<td>DS</td>
<td>Skallingen, Denmark</td>
<td>55º29’ N, 08º15’ E</td>
<td>9.1</td>
<td>355</td>
<td>1.3</td>
<td>17.9</td>
</tr>
<tr>
<td>SG</td>
<td>Glommen, Sweden</td>
<td>56º55’ N, 12º21’ E</td>
<td>8.0</td>
<td>377</td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>ST</td>
<td>Torekov, Sweden</td>
<td>56º23’ N, 12º38’ E</td>
<td>8.8</td>
<td>341</td>
<td>1.4</td>
<td>6.1</td>
</tr>
<tr>
<td>EA</td>
<td>Aberdeen, Scotland</td>
<td>57º20’ N, 01º55’ W</td>
<td>8.6</td>
<td>308</td>
<td>1.3</td>
<td>18.1</td>
</tr>
<tr>
<td>ES</td>
<td>Skye, Scotland</td>
<td>57º30’ N, 06º26’ W</td>
<td>9.1</td>
<td>585</td>
<td>0.7</td>
<td>17.7</td>
</tr>
</tbody>
</table>
Table 2 Description of seed traits measured in *Plantago coronopus*, and their variability among populations, estimated with the standard coefficient of variation (CV) of population mean values, and the coefficient of variation for log-normal distributions ($CV_{ln}$; see methods for details).

<table>
<thead>
<tr>
<th>Seed trait</th>
<th>Description</th>
<th>CV</th>
<th>$CV_{ln}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifetime seed production</td>
<td>Total number of seeds per plant over the lifespan</td>
<td>0.74</td>
<td>0.77</td>
</tr>
<tr>
<td>Annual seed production</td>
<td>Total number of seeds per plant per year</td>
<td>0.84</td>
<td>1.17</td>
</tr>
<tr>
<td>Fruit seed production</td>
<td>Total number of seeds per fruit (basal plus apical seeds)</td>
<td>0.24</td>
<td>0.25</td>
</tr>
<tr>
<td>Lifetime seed mass</td>
<td>Total mass of seeds per plant over the lifespan</td>
<td>1.12</td>
<td>1.20</td>
</tr>
<tr>
<td>Annual seed mass</td>
<td>Total mass of seeds per plant per year</td>
<td>1.05</td>
<td>1.64</td>
</tr>
<tr>
<td>Fruit seed mass</td>
<td>Total mass of seeds per fruit (basal plus apical seeds)</td>
<td>0.18</td>
<td>0.17</td>
</tr>
<tr>
<td>Seed ratio</td>
<td>Ratio between basal and apical seeds</td>
<td>0.74</td>
<td>0.57</td>
</tr>
<tr>
<td>Basal seed mass</td>
<td>Mass of basal seeds</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>Apical seed mass</td>
<td>Mass of apical seeds</td>
<td>0.23</td>
<td>0.27</td>
</tr>
<tr>
<td>Mucilage ratio</td>
<td>Amount of mucilage in basal seeds, relative to seed size</td>
<td>0.23</td>
<td>0.22</td>
</tr>
</tbody>
</table>
Table 3 Results from regression analyses between environmental predictors ($P_s$: summer precipitation; $T_e$: mean annual temperature; SOM: soil organic matter) and stress tolerance traits in *Plantago coronopus*. AIC values correspond to Akaike information criterion (only the three combinations of predictors with the lowest AIC values are shown). Model comparisons, performed with analyses of variance, show whether the sequential addition of predictors significantly improves the previous simpler model. The combination of predictors that constituted the best model for each stress tolerance trait is highlighted in bold. $F$ statistics are subindexed with corresponding degrees of freedom.

<table>
<thead>
<tr>
<th>Stress tolerance traits</th>
<th>Predictors</th>
<th>Effects of environmental gradient</th>
<th>Model comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$F$</td>
<td>$R^2$</td>
</tr>
<tr>
<td>Seed ratio</td>
<td>$P_s$</td>
<td>76.5</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>$P_s$ &amp; $T_e$</td>
<td>45.9</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>$P_s$ &amp; $T_e$ &amp; SOM</td>
<td>27.6</td>
<td>0.92</td>
</tr>
<tr>
<td>Basal seed mass</td>
<td>$T_e$</td>
<td>12.0</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>$T_e$ &amp; SOM</td>
<td>9.8</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>$T_e$ &amp; SOM &amp; $P_s$</td>
<td>5.9</td>
<td>0.72</td>
</tr>
<tr>
<td>Mucilage ratio</td>
<td>$P_s$</td>
<td>10.2</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>$P_s$ &amp; SOM</td>
<td>9.8</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>$P_s$ &amp; SOM &amp; $T_e$</td>
<td>7.5</td>
<td>0.76</td>
</tr>
</tbody>
</table>
Table 4 Tolerance-fecundity trade-off: regression analyses between fecundity traits (lifetime, annual and fruit seed production) and stress tolerance traits. In multiple regression analyses, plant size is included as a covariate and partial regression estimates ($\beta$) are shown. $F$ statistics are subindexed with corresponding degrees of freedom, and $p$ values are corrected by the false discovery rate method.

<table>
<thead>
<tr>
<th>Fecundity traits</th>
<th>Stress tolerance traits</th>
<th>Simple regression</th>
<th>Multiple regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$F$</td>
<td>$R^2$</td>
</tr>
<tr>
<td>Lifetime</td>
<td>Seed ratio</td>
<td>16.6,7</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>Basal seed mass</td>
<td>3.6,7</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Mucilage ratio</td>
<td>4.2,7</td>
<td>0.38</td>
</tr>
<tr>
<td>Annual</td>
<td>Seed ratio</td>
<td>6.4,9,7</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Basal seed mass</td>
<td>4.1,9,9</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Mucilage ratio</td>
<td>1.2,9,9</td>
<td>0.12</td>
</tr>
<tr>
<td>Fruit</td>
<td>Seed ratio</td>
<td>15.9,11,11</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Basal seed mass</td>
<td>20.4,10,10</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Mucilage ratio</td>
<td>24.7,10,10</td>
<td>0.71</td>
</tr>
</tbody>
</table>
Figures

Fig. 1 Location of populations of *Plantago coronopus* sampled in the study (black dots). The distribution range of the species, according to Hultén and Fries (1986), is highlighted in grey (including coastal outlines). See Table 1 for population acronyms.
Fig. 2 Seed dimorphism in *Plantago coronopus*. A basal (ba) and an apical (ap) seed after one hour soaked in water are shown. Basal seeds are larger than apical seeds, and possess a coat that becomes mucilaginous when moistened, as indicated by the arrow.
Fig. 3 Population averages (± S.E. in b, d and f) of seed traits in sampled populations of *Plantago coronopus*: a) seed ratio; b) basal seed mass (black) and apical seed mass (white); c) mucilage ratio, d) lifetime seed production (black), annual seed production (grey) and fruit seed production (white); e) lifetime seed mass (black), annual seed mass (grey) and fruit seed mass (white); and f) plant size. Populations are ranked from left to right by increasing latitude. Notice a logarithmic scale and a break in Y-axis in d) and e). For five populations (MT, CS, CT, EA and ES) some variables were not calculated.
Fig. 4 Relationship between lifetime, annual and fruit seed production on the one hand, and stress tolerance traits (seed ratio, basal seed mass and mucilage ratio) on the other hand, in *Plantago coronopus*. Continuous lines represent linear regressions between seed production traits and stress tolerance traits (left vertical axis), and dashed lines represent partial regressions between seed production traits and stress tolerance traits after controlling for plant size (right vertical axis). All variables were log-transformed except for fruit seed production. Notice small differences in scale among seed production traits and among stress tolerance traits. $R^2$ coefficients are accompanied by statistical significance: $\cdot p < 0.1$, $* p < 0.05$, $** p < 0.01$, $*** p < 0.001$; $p$ values were corrected by the false discovery rate method.
Fig. 5 Model showing a trade-off between fecundity (at the fruit level) and stress tolerance among populations of *Plantago coronopus* under a gradient in environmental stress. Diagram on the right represents differences in seed traits between the hypothetical extremes of the gradient (northern and southern populations). Basal seeds are surrounded by a mucilaginous coat (grey outline), which is absent in the smaller apical seed. Notice differences between fruits in size and mucilage production of basal seeds, and in the number of each seed morph.