

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

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12 **Short title:** A tolerance-fecundity trade-off at the intraspecific level

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15 *Plantago coronopus*, seed heteromorphism, seed number and weight, short-lived
16 perennial plant

26 **Abstract**

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

49

50

51 **Introduction**

52 Seed production and seed traits represent crucial components in plant fitness. Seed
53 size, for example, is closely related to important ecological and demographic processes,
54 such as dispersal, germination or seedling survival (Westoby *et al.* 1992; Chapin III *et*
55 *al.* 1993; Coomes & Grubb 2003). Seed production plays also a major role in individual
56 fitness and population persistence (Lloyd 1987; Westoby *et al.* 2002), and a trade-off
57 between the size and the number of seeds is expected (Smith & Fretwell 1974; Lloyd
58 1987). In addition, both seed size and total seed production might show a positive
59 relationship with plant size (Primack 1987; Herrera 1991; Aarssen & Jordan 2001). The
60 presence of mucilaginous seed coats in some plant species may also affect relevant
61 seed-related processes, such as water stress tolerance, competition via allelopathy or
62 adherence to soil particles (Harper & Benton 1966; Hasegawa *et al.* 1992; Lu *et al.*
63 2010). Many taxa present remarkable differences in seed characteristics among
64 populations (*e.g.*, McWilliams *et al.* 1968; McKee & Richards 1996; Mendez 1997),
65 and quantifying this intraspecific variation and determining its underlying causes may
66 be important to understand why some plants are more successful than others in terms of
67 colonization, or adaptation to new ecological or climatic scenarios (Buckley *et al.* 2003;
68 Wright *et al.* 2006; Albert *et al.* 2010).

69 Environmental stress is a crucial factor in the ecology and evolution of plants
70 (Grime 1977; Parsons 1991; Nevo 2001), and variation in stress levels may promote
71 seed trait divergences among or within species. The hypothesis of the tolerance-
72 fecundity trade-off (Muller-Landau 2010; see also Westoby *et al.* 2002), has been
73 proposed to explain the coexistence of plant species with different seed sizes in
74 environmentally heterogeneous communities. The underlying mechanism is related to a
75 demographic process, *i.e.*, the differential probability of recruitment at the available

76 regeneration niches. In this process, high-stress regeneration sites would be eventually
77 occupied by large-seeded species, thanks to their higher tolerance to environmental
78 stress. Low-stress patches, in contrast, would be occupied by species of different seed
79 sizes and tolerances, although small-seeded species would become dominant due to
80 their higher seed production relative to large-seeded species. Because of its logic and
81 simplicity, the mechanism underlying the tolerance-fecundity model could be rather
82 general, and also explain variation in seed traits among populations of species occurring
83 along environmental stress gradients. In this intraspecific extension of the model,
84 populations in stressful environments would provide the seeds with additional resources
85 at the cost of reducing seed number. In contrast, populations in less stressful conditions
86 could afford to reduce resources investment per seed (and thus stress tolerance), in order
87 to increase offspring number. These predictions rely on the assumption that available
88 resources for seed production are constant across populations and do not covary with
89 the traits involved in the trade-off. In addition, despite that seed size is the most
90 frequently studied trait, other seed characteristics could be considered to evaluate stress
91 tolerance (Muller-Landau 2010), such as some coat features or shape.

92 Widespread plants occurring along environmental gradients represent typical
93 examples of high phenotypic variability (Joshi *et al.* 2001; Richards *et al.* 2005), and
94 provide a good opportunity to analyze intraspecific variation in seed traits in relation to
95 environmental conditions. *Plantago coronopus* is a common short-lived perennial herb
96 present along a strong environmental gradient in the eastern Atlantic coast, and shows
97 large differences among populations in fecundity (Braza *et al.* 2010, Vilellas *et al.*
98 2012). Additionally, this taxon presents seed dimorphism (Dowling 1933; Schat 1981),
99 whereby fruits produce both large basal seeds with a mucilaginous coat and small apical
100 seeds without coat. For individuals emerging from basal seeds, plant performance

101 (germination, survival and fecundity) is positively correlated with original seed size
102 (Koelewijn & Van Damme 2005). Moreover, basal seeds germinate better than apical
103 ones, especially in dry years (Braza & García 2011), which likely results from higher
104 provisions (Chapin III *et al.* 1993; Westoby *et al.* 2002; Coomes & Grubb 2003) and
105 higher water absorption through their mucilaginous coat (Harper & Benton 1966; Schat
106 1981; Gutterman & Shem-Tov 1997).

107 In this study we analyze variation among populations of the widespread herb *P.*
108 *coronopus* in a set of seed traits, and its relationship with environmental stress. Climate
109 has a key role in plant performance at large scales (Woodward & Williams 1987), and
110 the positive relationship between seed performance and seed size and mucilage in this
111 species specifically suggests water and nutrient deficits as potential sources of stress.
112 Consequently, we tested the effect of environmental stress on seed traits using: 1) water
113 availability, estimated with precipitation (see also Harper & Benton 1966; Baker 1972;
114 Wright & Westoby 1999) and with a more integrative metric of water deficit
115 considering the balance between evapotranspiration and precipitation (Thornthwaite
116 1948); 2) temperature, which may reduce water availability (Baker 1972) or directly
117 affect plant metabolic processes, as seeds require more energy to convert into seedling
118 under warmer conditions (Lord *et al.* 1997; Murray *et al.* 2004); and 3) soil organic
119 matter content, which can be used as an indicator of soil fertility (Reeves 1992), and
120 which may be associated with soil water retention due to small particle sizes and high
121 cation exchange capacity (Cobertera 1993).

122 To analyze variation in fecundity and seed traits in *P. coronopus*, we sampled 14
123 populations along the Atlantic Coast of North Africa and Europe, spanning a latitudinal
124 gradient of 4000 km. Here, we first report variability among populations in the number,
125 size and production of mucilage in basal and apical seeds. Given that *P. coronopus* is a

126 perennial plant, we consider fecundity at three levels: per individual over the lifespan,
127 per individual per year, and per fruit. Second, we analyze if seed trait variation is
128 associated with soil and climatic conditions, considering low water availability, high
129 temperature and low organic matter content as representative of stressful conditions.
130 Besides, we analyze if seed trait variation is affected by plant size. Third, we test
131 whether a trade-off between fecundity (at the three levels) and stress tolerance promotes
132 diversity in seed traits among populations of this dimorphic species. In that case, we
133 would expect populations subject to higher stress to present: a) a higher predominance
134 of basal (more stress-tolerant) relative to apical (less stress-tolerant) seeds, b) larger
135 basal seeds with higher mucilage production, and c) a subsequent reduction in seed
136 production due to trade-offs in resource allocation. To strengthen the analyses of trade-
137 offs, we test the assumption that total resource investment in seeds is constant across
138 populations and unrelated with seed traits.

139

140 **Materials and methods**

141 **Study species and populations**

142 *Plantago coronopus* L. subsp. *coronopus* (Plantaginaceae) is a widespread, short-
143 lived perennial herb distributed along the Mediterranean basin, reaching North Europe
144 through a narrow strip along the Atlantic coast (Fig. 1, Hultén & Fries 1986). The
145 subspecies *coronopus* is present throughout most of the species' range and differs from
146 other less common subspecies in the morphology of the bracts (Chater & Cartier 1976).
147 Our study was restricted to the common subspecies, and hereafter we will refer to it as
148 *P. coronopus*. It presents high variability in morphological characters, and a life cycle
149 that can be annual or perennial (Chater & Cartier 1976). Reproductive individuals have
150 several spikes with wind-pollinated flowers, and present intermediate outcrossing rates,

151 with high variation among and within populations (Wolff *et al.* 1988). Fruits are
152 capsules that produce two types of seeds (Dowling 1933; Schat 1981): up to four large
153 basal seeds, and one or no small apical seed (Fig. 2). Basal seeds further differentiate
154 from apical ones by the possession of a coat that becomes mucilaginous when
155 moistened, which is virtually absent in the latter. *P. coronopus* is a colonizer plant
156 occurring in many habitats, especially sand dunes, salt marshes, coastal prairies, and
157 human-disturbed environments.

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

168 Environmental data

169 To estimate soil fertility in populations, we collected 10 cm deep soil cores, and
170 measured in the laboratory the percentage of soil organic matter content from the
171 organic carbon (Heanes 1984). Meteorological data were obtained from several
172 databases: the website <http://www.allmetsat.com> (MT); *Direction Regionale*
173 *d'Hydraulique* in Tetuan, Morocco (CS); Spanish National Meteorological Agency (CT,
174 T and CA); MeteoGalicia (C and TB); MeteoFrance (F); Danish Meteorological
175 Institute (DH and DS); Swedish Meteorological and Hydrological Institute (SG and

176 ST); and Met Office (EA and ES). We obtained mean monthly precipitation (mm),
177 mean monthly maximum and minimum temperatures (°C) and mean annual temperature
178 (°C), from 10-20 years within the last four decades (depending on the availability) from
179 the nearest meteorological station to each population. We calculated total precipitation
180 in the period of growing season where highest differences appeared among populations,
181 *i.e.* from June to September (thereafter “summer precipitation”). We also calculated
182 evapotranspiration (mm) with the equation (from Hargreaves 1985):

$$183 \quad ET = 0.0023 \cdot R_a \cdot TD^{0.5} \cdot (T_m + 17.8) \cdot d$$

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

190 Seed collection and measurements

191 We collected the spikes of 25 randomly chosen individuals in each population in
192 the summers of 2007 or 2008. Fruits were dissected in the laboratory to measure a set of
193 seed-related traits (Table 2). For five populations (MT, CS, CT, EA and ES) some
194 variables were not calculated (Fig. 3). The number of basal and apical seeds per fruit
195 was counted with magnifying glasses in 10 fruits per mother. The number of each type
196 of seed per fruit and the total number of seeds per fruit (basal plus apical seeds;
197 thereafter “fruit seed production”) was then averaged across individuals for each
198 population. We also averaged across individuals the percentage of basal and apical
199 seeds, and calculated the seed ratio from mean population values, dividing percentage of
200 basal seeds by percentage of apical seeds (thereafter “seed ratio”).

201 Basal and apical seed mass was estimated for each population by weighting eight
202 groups of 25 basal seeds and 25 apical seeds, respectively, from 12 individuals (seeds
203 were weighted in groups due to their small size). The mucilaginous coat was measured
204 with the aid of magnifying glasses in five basal seeds per plant, in 15 plants per
205 population. Previously, we soaked the seeds for one hour in Petri dishes, until the
206 mucilage became conspicuous (Fig. 2). We estimated the projected seed area and the
207 total area that contained both the seed and the mucilaginous coat using the ellipse
208 formula ($\text{area} = \pi \cdot a \cdot b$; a and b correspond to the major and minor semiaxes), and we
209 calculated the mucilage area by subtracting the seed area from the total area. For each
210 population, we averaged across seeds the percentage areas of mucilage and seed, and
211 then calculated from mean population percentages the ratio between mucilage and seed
212 area (thereafter “mucilage ratio”). We used a relative measure of mucilage because we
213 found with a linear regression (*lm* procedure, package *stats*, R Core Development Team
214 2011) that it was positively correlated to seed area ($t_{857} = 22.6$, $R^2 = 0.37$, $p < 0.001$).

215 We estimated the total number of seeds per year (thereafter “annual seed
216 production”) and the size of an average of 160 reproductive individuals per population
217 and year, in annual censuses from 2007 to 2010. We recorded each year the number of
218 leaves and inflorescences of individuals, and the length of an average leaf and an
219 average inflorescence. Plant size was defined as number of leaves * length of an
220 average leaf, and annual seed production was estimated as number of inflorescences *
221 length of an average inflorescence * number of seeds per unit of inflorescence length
222 (calculated with a regression equation for each population). We also calculated the total
223 seed production over the lifespan (thereafter “lifetime seed production”) for those
224 reproductive individuals that were monitored for their entire lives. For further details on
225 the estimation of these parameters, see Villellas *et al.* (2012). Annual seed production,

226 lifetime seed production and plant size were then averaged for each population across
227 individuals and years.

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

235 Analysis of seed trait variability, environmental factors and plant size

236 We analyzed among-population variability in seed traits with the coefficient of
237 variation (CV) of population mean values. Since most of the traits were log-normally
238 distributed, we also calculated the coefficient of variation for these distributions as
239 $CV_{\ln} = \sqrt{e^{(s^2)} - 1}$, where e is the base of the natural logarithm and s is the standard
240 deviation of the natural-log transformed data (Koopmans *et al.* 1964).

241 The effects of environmental factors on seed trait variation were tested on those
242 traits conferring stress tolerance to plants, *i.e.* seed mass and mucilage (see Introduction;
243 thereafter “stress tolerance traits”). Considering the particular dimorphism of the
244 species, in which basal seeds are larger than apical seeds and the latter lack the
245 mucilaginous coat, we selected the following stress tolerance traits for analysis: seed
246 ratio, basal seed mass and mucilage ratio (all of them log-transformed). We analyzed
247 collinearity among environmental variables with an analysis of variance inflation factor
248 (*vif* procedure, package *car*, R Core Development Team 2011), and we discarded
249 summer water stress index from subsequent analyses because it showed high
250 collinearity with summer precipitation (values much higher than 10; Kleinbaum *et al.*

251 1988), and because the latter provided a better fit to our data. Thus, the environmental
252 predictors were mean annual temperature, summer precipitation (log-transformed) and
253 soil fertility (log-transformed). For each stress tolerance trait, we performed linear
254 regressions with each of the three predictors, as well as multiple linear regressions with
255 all possible combinations with two or three predictors (*lm* procedure, package *stats*, R
256 Core Development Team 2011). To find out which model provided the best fit to our
257 data we first compared AIC (Akaike's information criterion) values from all regression
258 analyses. Among the combinations of predictors with the lowest AIC values, we then
259 checked with analyses of variance (*anova* procedure, package *stats*, R Core
260 Development Team 2011) if the sequential addition of predictors significantly improved
261 the previous simpler model. For these analyses, we used the populations for which we
262 had data for all environmental predictors and stress tolerance traits (all except MT, CS
263 and CT), so that AIC values were comparable.

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

269 Analysis of the tolerance-fecundity trade-off

270 To test for a trade-off between fecundity and stress tolerance, we used estimates of
271 fecundity at three levels: lifetime seed production, annual seed production and fruit seed
272 production. Firstly we performed simple linear regressions between each measure of
273 seed production (response variables) and each stress tolerance trait (predictors), with
274 log-transformed variables except for fruit seed production. Then we tested again the
275 relationship between each seed production trait and each stress tolerance trait with

276 multiple regressions, including plant size as a covariate to control for its possible
277 effects, and looking at the significance of the partial regression parameters of stress
278 tolerance traits. Finally, to check for the assumption of constant available resources for
279 seeds in the tolerance-fecundity trade-off, we performed Pearson's correlation analyses
280 (*cor* procedure, package *stats*, R Core Development Team 2011) between lifetime,
281 annual and fruit seed mass on the one hand, and seed production traits and stress
282 tolerance traits on the other hand.

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

288

289 **Results**

290 Seed trait variability

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

297 Effects of environmental factors and plant size

298 There were notable differences among populations in temperature, summer
299 precipitation and soil fertility along the latitudinal gradient (Table 1). There was a
300 gradual decrease in temperature from south to north, and northern populations showed

301 in general higher precipitation, with a few exceptions in both climatic parameters.
302 Southern populations in Spain and France showed lower soil fertility than most northern
303 populations.

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

319 Plant size was significantly correlated with lifetime seed production ($F_{1,2618} =$
320 858.4 , $R^2 = 0.34$, $p < 0.001$), annual seed production ($F_{1,5286} = 2317.3$, $R^2 = 0.21$, $p <$
321 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
322 $= 0.46$, $p = 0.022$) and mucilage ratio ($F_{1,9} = 14.9$, $R^2 = 0.62$, $p = 0.004$). In contrast,
323 plant size showed no significant effect on lifetime seed mass ($F_{1,7} = 2.7$, $R^2 = 0.28$, $p =$
324 0.144), annual seed mass ($F_{1,9} = 2.3$, $R^2 = 0.21$, $p = 0.162$), fruit seed mass ($F_{1,9} = 2.0$,

325 $R^2 = 0.18$, $p = 0.188$), basal seed mass ($F_{1,9} = 0.5$, $R^2 = 0.05$, $p = 0.514$) and apical seed
326 mass ($F_{1,9} = 0.1$, $R^2 = 0.02$, $p = 0.713$).

327 Tolerance-fecundity trade-off

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

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

345 Lifetime seed mass was significantly correlated with lifetime seed production ($t_7 =$
346 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
347 $= 3.4$, $r = 0.79$, $p = 0.018$), and correlation with mucilage ratio was marginally
348 significant ($t_7 = 2.3$, $r = 0.65$, $p = 0.056$). Annual seed mass was significantly correlated
349 with lifetime seed production ($t_9 = 13.5$, $r = 0.98$, $p < 0.001$), seed ratio ($t_9 = 3.0$, $r =$

350 0.71, $p = 0.023$) and basal seed mass ($t_9 = 3.2$, $r = 0.73$, $p = 0.023$), although it showed
351 no correlation with mucilage ratio ($t_9 = 1.4$, $r = 0.44$, $p = 0.181$). Fruit seed mass
352 showed no significant correlation with fruit seed production ($t_{10} = -0.3$, $r = -0.09$, $p =$
353 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
354 $= 0.977$), although it showed a marginally significant correlation with basal seed mass
355 ($t_{10} = 2.6$, $r = 0.64$, $p = 0.076$). Lifetime and annual seed mass decreased northwards,
356 whereas fruit seed mass showed no clear latitudinal pattern (Fig. 3e).

357

358 **Discussion**

359 *Plantago coronopus* presents considerable variation along the Atlantic Coast in
360 Europe and North Africa in a set of seed traits, *i.e.* the number and size of seeds, the
361 proportion of basal and apical seeds and the production of mucilage. Similar levels of
362 variability in seed traits have been found among populations of other widespread plants.
363 For example, the CV for seed size similarly lies around 0.20-0.30 in the short-lived
364 *Campanula Americana* (calculated from Kalisz & Wardle 1994) and the long-lived
365 *Vaccinium stamineum* (Yakimowski & Eckert 2007), and the CV for annual
366 reproductive output (number of seeds or fruits per plant per year) is also higher than
367 0.70 for those species. In this study, we tested whether the observed intraspecific
368 variability was explained by a recent hypothesis proposed at the community level: the
369 trade-off between stress tolerance and fecundity in heterogeneous environments
370 (Muller-Landau 2010). Our results suggest indeed that a tolerance-fecundity trade-off
371 underpins intraspecific variation in seed traits in *P. coronopus* at the fruit level,
372 although lifetime and annual seed production appear to be shaped by different factors.

373 Stress tolerance traits (seed ratio, basal seed mass and mucilage ratio) were
374 strongly correlated with climatic and soil conditions in *P. coronopus*. Seed ratio was

375 negatively affected by water availability, estimated with summer precipitation. Basal
376 seed mass was enhanced by temperature, which may increase energy requirements of
377 metabolic processes (Lord *et al.* 1997; Murray *et al.* 2004), and negatively affected by
378 soil organic matter, which is associated with fertility (Reeves 1992), although both
379 temperature and soil organic matter (Cobertera 1993) may have also indirectly affected
380 seed mass through their effects on water availability. Finally, mucilage ratio was
381 negatively affected by summer precipitation and soil organic matter, both associated
382 with moisture, suggesting a role of mucilage in reducing water deficit. Overall, the three
383 environmental parameters represent some kind of environmental stress (water and
384 nutrient availability, and energy requirements), and significantly contribute to explain
385 among-population differences in one or another stress tolerance trait. Our results
386 support previous studies that found tolerance-related seed traits, most commonly seed
387 size, positively correlated with higher temperatures (Baker 1972, Murray *et al.* 2004),
388 lower precipitation or water availability in general (Baker 1972; Wright & Westoby
389 1999) and lower soil fertility (Lee & Fenner 1989; Parolin 2000). There is also abundant
390 literature that relates seed size with seedling competitive ability (*e.g.* Tilman 1994,
391 Geritz *et al.* 1999), but this factor does not help to explain seed trait variation in *P.*
392 *coronopus*, because the populations exposed to highest competition, in northern coastal
393 meadows, showed the smallest seeds.

394 Seed traits did not vary independently along the environmental gradient in *P.*
395 *coronopus*. Fruit seed production showed the opposite trend than basal seed mass,
396 which suggest the typical trade-off between seed size and seed number (Smith &
397 Fretwell 1974; Lloyd 1988) at the fruit level. In addition, seed number seems to be also
398 regulated by additional traits related with resource investment per seed, as indicated by
399 the negative correlation between fruit seed production and seed ratio and mucilage ratio.

400 In contrast, lifetime and annual seed production showed positive or non-significant
401 relationships with stress tolerance traits, suggesting there were no trade-offs at these
402 higher levels. The finding that plant size was correlated with most of the traits measured
403 in this study compelled us to analyze seed trait correlations accounting for plant size,
404 and such analyses confirmed our previous interpretations. Indeed, fruit seed production
405 still showed significant or marginally significant negative relationships with stress
406 tolerance traits when considering the effect of plant size, whereas lifetime and annual
407 seed production showed no significant correlations with any of the tolerance traits.
408 Mendez (1997) also found in *Arum maculatum* a negative correlation between seed size
409 and number only at the fruit level, although Devlin (1989) and Mehlman (1993)
410 reported the trade-off at both levels in two perennial plants. The lack of consistency
411 among studies confirms the importance of considering different levels when analyzing
412 species' seed production (Primack 1987; Herrera 1991).

413 The tolerance-fecundity model (Muller-Landau 2010) states that heterogeneous
414 areas in terms of environmental stress provide different regeneration niches, allowing
415 the maintenance of species of different seed sizes within communities, and assuming
416 that seed size is related to stress tolerance. We believe that a similar mechanism
417 underlies variability in seed traits among populations of *P. coronopus*, considering the
418 large differences in climatic and soil conditions among locations and the corresponding
419 variation in seed traits. Let's consider the stress gradient that broadly coincides with the
420 latitudinal gradient of the species, and along which the fecundity (at the fruit level) and
421 the stress tolerance traits covary (Fig. 5). In this gradient, southern populations are
422 subject to higher environmental stress (higher temperatures, lower summer precipitation
423 and lower soil fertility) than northern populations, which limits their access to two
424 essential resources for seed and seedling performance, *i.e.* water and nutrients. In

425 response to these conditions, plants produce basal seeds with more internal resources to
426 tolerate environmental stress (Chapin III *et al.* 1993; Westoby *et al.* 2002; Coomes &
427 Grubb 2003), and with higher amounts of mucilage to facilitate water absorption
428 (Harper & Benton 1966; Schat 1981; Gutterman & Shem-Tov 1997). For identical
429 reasons, southern plants also increase seed ratio, towards more basal relative to apical
430 seeds. Since total resources allocated to seeds by fruits are constant across populations,
431 the final outcome is a reduction in the total number of seeds per fruit. Conversely,
432 northern populations occur in less stressful conditions, and plants can thus reduce
433 investment in seed size and mucilage, allowing an increase in fruit seed production (Fig.
434 5). A decline in seed size with latitude seems to be indeed a common pattern within
435 widespread plant taxa (Moles & Westoby 2003). Moreover, *P. coronopus* adjusts the
436 coexistence of basal and apical seeds along the stress gradient, resulting in a higher
437 predominance of the more tolerant seed morph in populations under higher stress (in a
438 way equivalent to how big-seeded species would predominate over small-seeded species
439 in stressful sites within communities). Overall, our results strongly suggest that the
440 mechanism proposed by Muller-Landau (2010) for the maintenance of variation in seed
441 size among species can also explain the variability in seed traits among populations of
442 *P. coronopus*.

443 The tolerance-fecundity hypothesis may help to understand why many plants of
444 stressful and unpredictable environments produce different seed morphs with
445 contrasting tolerance attributes (Venable 1985; Imbert 2002). For example, Yao *et al.*
446 (2010) proposed a model for the widespread dimorphic *Chenopodium album*, in which
447 populations subject to the highest salinity stress produced higher proportions of the
448 tolerant seed morph by reducing total seed production. However, although the
449 mechanisms underlying the tolerance-fecundity trade-off seem particularly valid for

450 seed-heteromorphic species, this model might also apply to other taxa, constituting a
451 broader framework than the frequently invoked compromise between seed size and
452 number. Rather than focusing only on seed size, we would expect any additional
453 investment on seed tolerance to stress, such as mucilage, to trade-off against fecundity,
454 promoting thus variability among populations.

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

472 This study has shown that a gradient in environmental stress promotes variability
473 among populations of the widespread *P. coronopus* in a set of seed traits, *i.e.* the size,
474 the mucilage and the relative abundance of two seed morphs with contrasting tolerance

475 attributes. Overall, our results support the hypothesis that a trade-off between fecundity
476 and stress tolerance can underlie variation in seed traits not only among species, but also
477 among populations of the same taxon. The tolerance-fecundity model may in fact
478 constitute a broader ecological framework than the classical trade-off between seed size
479 and number, at least for species occurring along gradients in environmental stress.
480 However, our work shows also that fecundity can be regulated within plant species by
481 different factors at the fruit and the whole individual level, to maximize the fitness of
482 populations in each set of local conditions.

483

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493

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Tables

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).

Population	Location	Coordinates	Temperature (°C)	P_s (mm)	WSI_s	SOM (%)
MT	Tiznit, Morocco	29°45' N, 09°53' W	18.5	9	68.9	-
CS	Cap Spartel, Morocco	35°47' N, 05°55' W	17.7	63	10.4	-
CT	Ceuta, Spain	35°54' N, 05°21' W	16.1	44	13.9	-
T	Tarifa, Spain	36°02' N, 05°38' W	17.1	61	7.5	0.7
CA	Camposoto, Spain	36°25' N, 06°13' W	18.7	69	7.8	0.4
C	Corrubedo, Spain	42°33' N, 09°01' W	14.9	228	2.3	1.1
TB	Traba, Spain	43°11' N, 09°03' W	14.7	275	2.0	1.4
F	Pen Bron, France	47°18' N, 02°30' W	12.8	206	2.4	0.9
DH	Helnaes, Denmark	55°08' N, 09°59' E	8.1	235	2.1	5.6
DS	Skallingen, Denmark	55°29' N, 08°15' E	9.1	355	1.3	17.9
SG	Glommen, Sweden	56°55' N, 12°21' E	8.0	377	1.2	0.8
ST	Torekov, Sweden	56°23' N, 12°38' E	8.8	341	1.4	6.1
EA	Aberdeen, Scotland	57°20' N, 01°55' W	8.6	308	1.3	18.1
ES	Skye, Scotland	57°30' N, 06°26' W	9.1	585	0.7	17.7

668 **Table 2** Description of seed traits measured in *Plantago coronopus*, and their
669 variability among populations, estimated with the standard coefficient of variation (CV)
670 of population mean values, and the coefficient of variation for log-normal distributions
671 (CV_{ln} ; see methods for details).

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Seed trait	Description	CV	CV_{ln}
Lifetime seed production	Total number of seeds per plant over the lifespan	0.74	0.77
Annual seed production	Total number of seeds per plant per year	0.84	1.17
Fruit seed production	Total number of seeds per fruit (basal plus apical seeds)	0.24	0.25
Lifetime seed mass	Total mass of seeds per plant over the lifespan	1.12	1.20
Annual seed mass	Total mass of seeds per plant per year	1.05	1.64
Fruit seed mass	Total mass of seeds per fruit (basal plus apical seeds)	0.18	0.17
Seed ratio	Ratio between basal and apical seeds	0.74	0.57
Basal seed mass	Mass of basal seeds	0.31	0.31
Apical seed mass	Mass of apical seeds	0.23	0.27
Mucilage ratio	Amount of mucilage in basal seeds, relative to seed size	0.23	0.22

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688 **Table 3** Results from regression analyses between environmental predictors (P_S :
689 summer precipitation; T_e : mean annual temperature; SOM: soil organic matter) and
690 stress tolerance traits in *Plantago coronopus*. AIC values correspond to Akaike
691 information criterion (only the three combinations of predictors with the lowest AIC
692 values are shown). Model comparisons, performed with analyses of variance, show
693 whether the sequential addition of predictors significantly improves the previous
694 simpler model. The combination of predictors that constituted the best model for each
695 stress tolerance trait is highlighted in bold). F statistics are subindexed with
696 corresponding degrees of freedom.
697

Stress tolerance traits	Predictors	Effects of environmental gradient				Model comparison	
		F	R^2	p	AIC	F	p
Seed ratio	P_S	76.5 _{1,9}	0.89	< 0.001	-19.3	-	-
	P_S & T_e	45.9 _{2,8}	0.92	< 0.001	-20.2	2.5 _{1,8}	0.152
	P_S & T_e & SOM	27.6 _{3,7}	0.92	< 0.001	-18.6	0.2 _{1,7}	0.664
Basal seed mass	T_e	12.0 _{1,9}	0.57	0.007	-18.0	-	-
	T_e & SOM	9.8 _{2,8}	0.71	0.007	-20.3	3.8 _{1,8}	0.086
	T_e & SOM & P_S	5.9 _{3,7}	0.72	0.024	-18.6	0.2 _{1,7}	0.674
Mucilage ratio	P_S	10.2 _{1,9}	0.53	0.011	-24.0	-	-
	P_S & SOM	9.8 _{2,8}	0.71	0.007	-27.4	5.0 _{1,8}	0.056
	P_S & SOM & T_e	7.5 _{3,7}	0.76	0.013	-27.5	1.5 _{1,7}	0.255

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707 **Table 4** Tolerance-fecundity trade-off: regression analyses between fecundity
708 traits (lifetime, annual and fruit seed production) and stress tolerance traits. In multiple
709 regression analyses, plant size is included as a covariate and partial regression estimates
710 (β) are shown. F statistics are subindexed with corresponding degrees of freedom, and p
711 values are corrected by the false discovery rate method.
712

Fecundity traits	Stress tolerance traits	Simple regression			Multiple regression					
		F	R^2	p	Stress tolerance trait			Plant size		
					β	t	p	β	t	p
Lifetime	Seed ratio	16.6 _{1,7}	0.70	0.014	0.90	2.7	0.104	0.08	0.2	0.865
	Basal seed mass	3.6 _{1,7}	0.34	0.099	0.97	1.7	0.210	0.72	1.7	0.423
	Mucilage ratio	4.2 _{1,7}	0.38	0.099	1.18	0.7	0.486	0.37	0.4	0.865
Annual	Seed ratio	6.4 _{1,9}	0.42	0.096	1.00	1.7	0.197	0.09	0.1	0.910
	Basal seed mass	4.1 _{1,9}	0.31	0.109	1.49	1.8	0.197	0.78	1.3	0.537
	Mucilage ratio	1.2 _{1,9}	0.12	0.298	-0.21	-0.1	0.927	1.1	1.0	0.537
Fruit	Seed ratio	15.9 _{1,11}	0.59	0.002	-1.97	-2.1	0.066	-1.00	-0.8	0.675
	Basal seed mass	20.4 _{1,10}	0.67	0.002	-4.10	-5.0	0.003	-2.16	-3.7	0.018
	Mucilage ratio	24.7 _{1,10}	0.71	0.002	-6.76	-2.5	0.059	-0.05	-0.4	0.971

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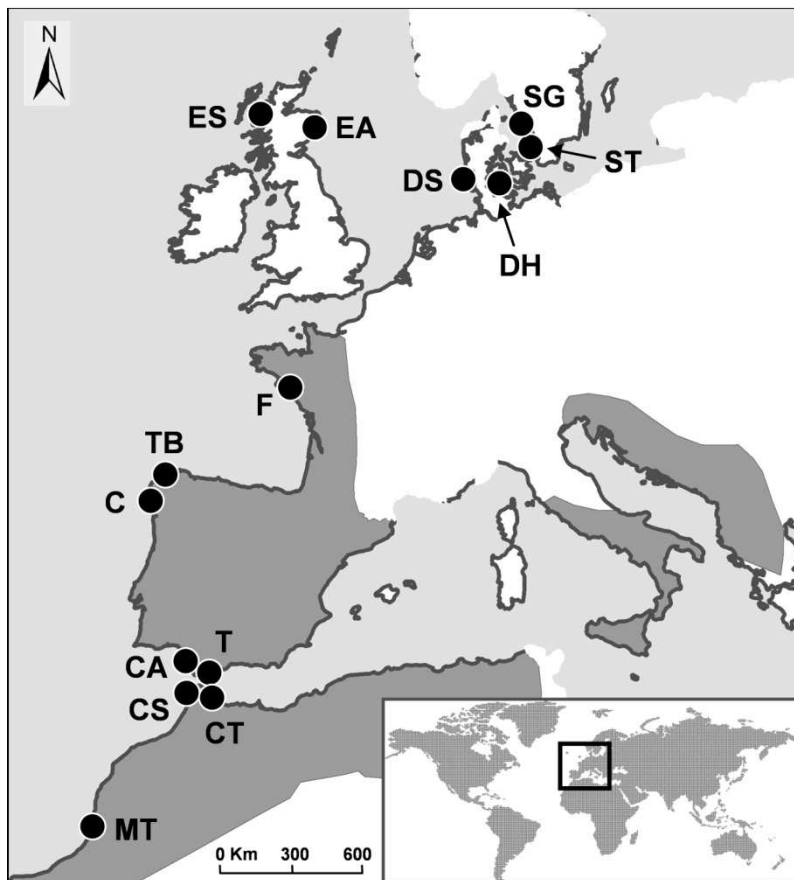
Figures

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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.

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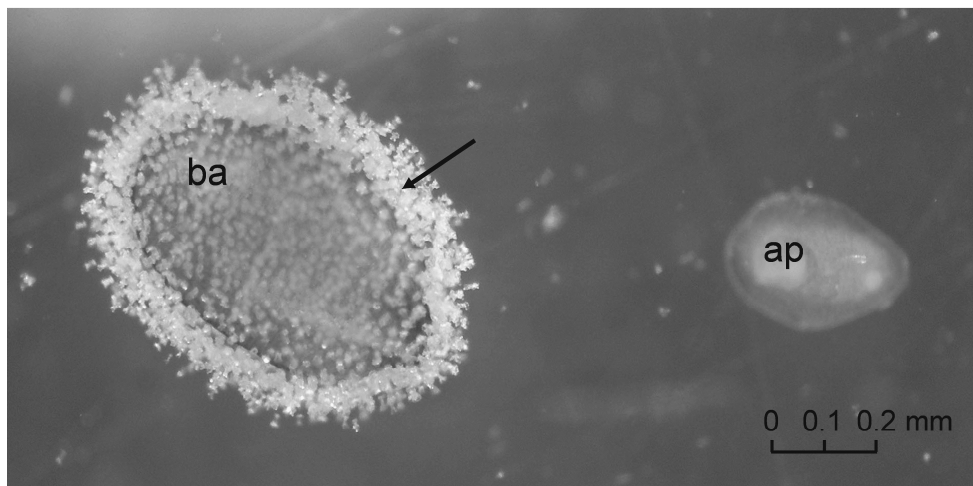
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740 **Fig. 2** Seed dimorphism in *Plantago coronopus*. A basal (ba) and an apical (ap)
741 seed after one hour soaked in water are shown. Basal seeds are larger than apical seeds,
742 and possess a coat that becomes mucilaginous when moistened, as indicated by the
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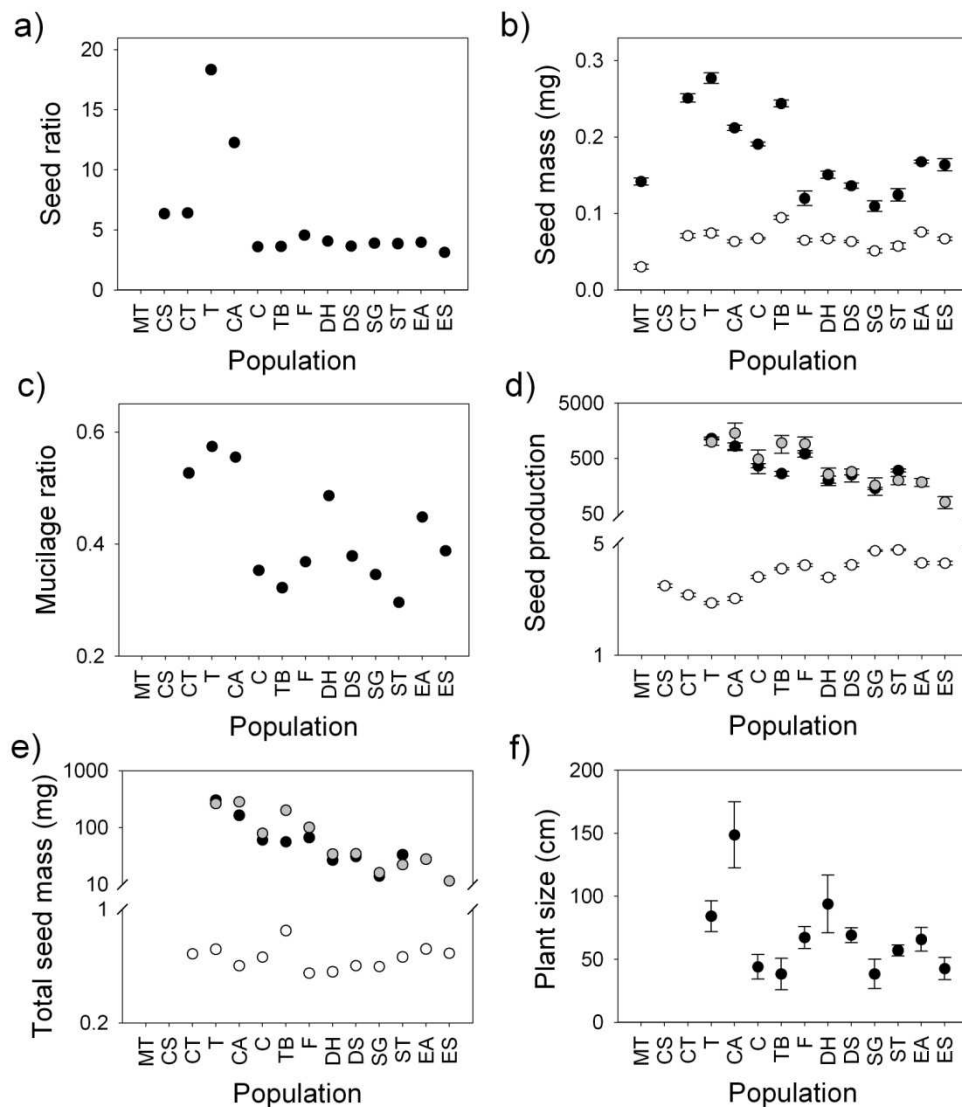
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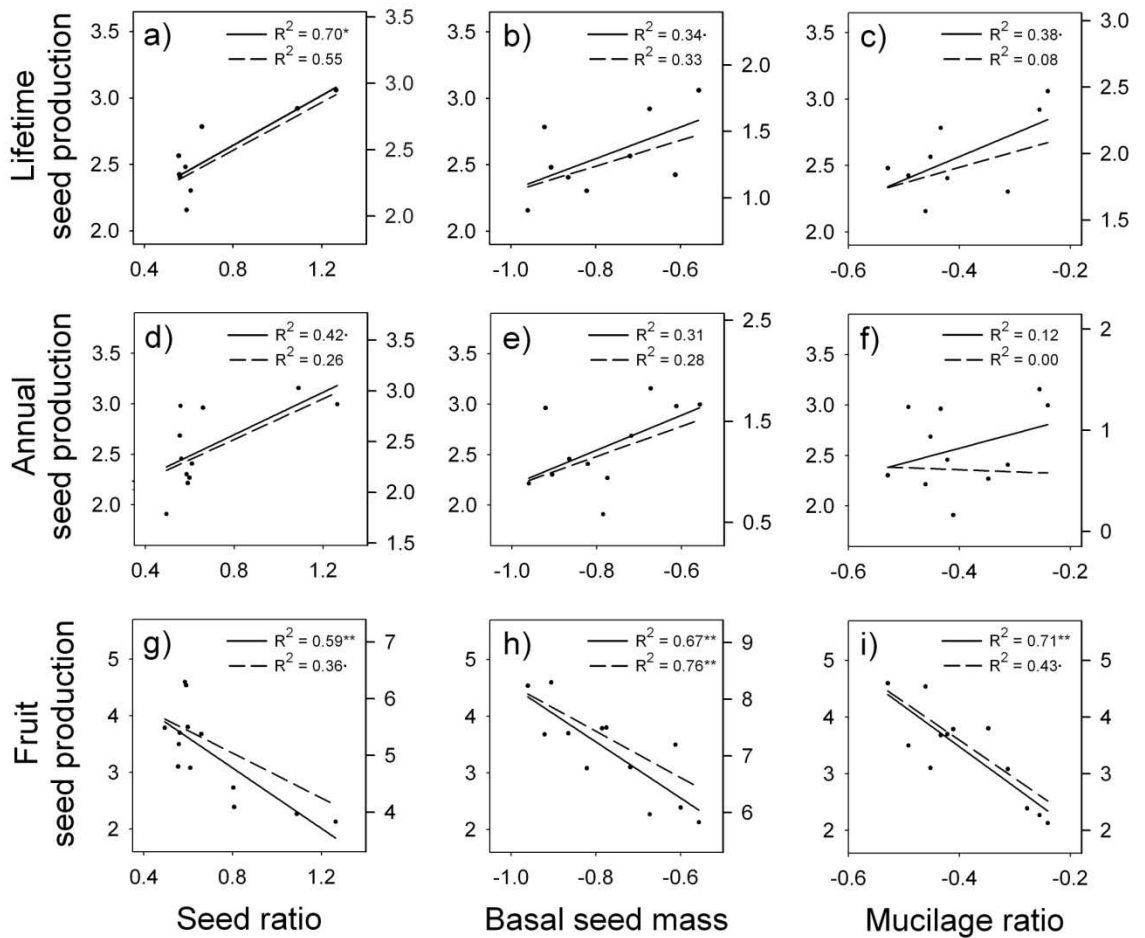
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758 **Fig. 3** Population averages (\pm S.E. in b, d and f) of seed traits in sampled
 759 populations of *Plantago coronopus*: a) seed ratio; b) basal seed mass (black) and apical
 760 seed mass (white); c) mucilage ratio, d) lifetime seed production (black), annual seed
 761 production (grey) and fruit seed production (white); e) lifetime seed mass (black),
 762 annual seed mass (grey) and fruit seed mass (white); and f) plant size. Populations are
 763 ranked from left to right by increasing latitude. Notice a logarithmic scale and a break in
 764 Y-axis in d) and e). For five populations (MT, CS, CT, EA and ES) some variables were
 765 not calculated.
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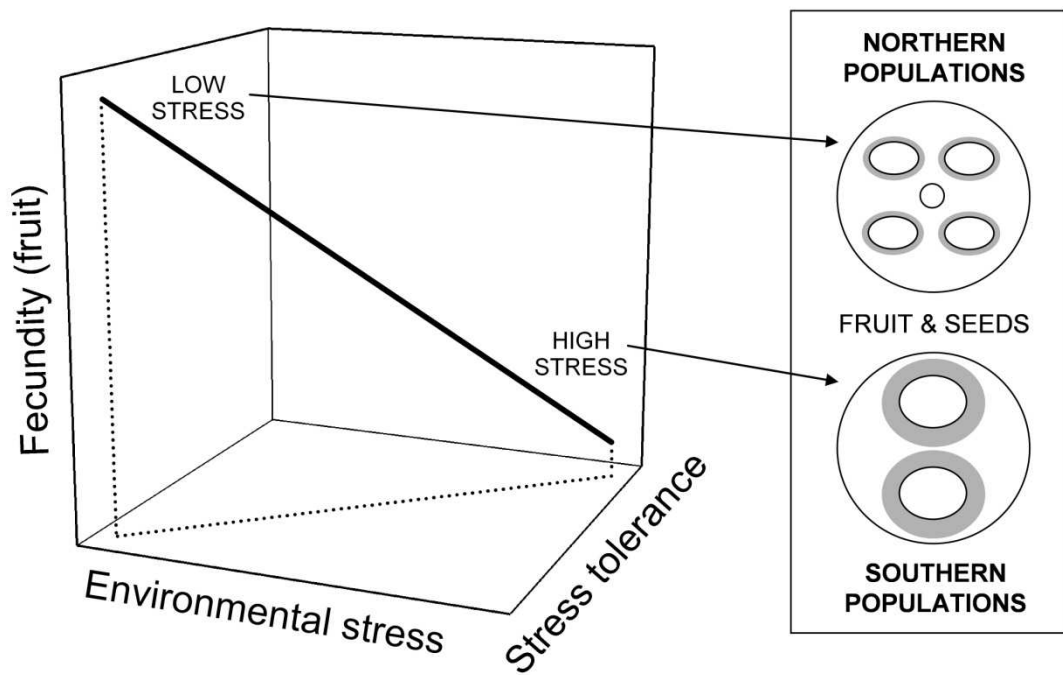
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768 **Fig. 4** Relationship between lifetime, annual and fruit seed production on the one
 769 hand, and stress tolerance traits (seed ratio, basal seed mass and mucilage ratio) on the
 770 other hand, in *Plantago coronopus*. Continuous lines represent linear regressions
 771 between seed production traits and stress tolerance traits (left vertical axis), and dashed
 772 lines represent partial regressions between seed production traits and stress tolerance
 773 traits after controlling for plant size (right vertical axis). All variables were log-
 774 transformed except for fruit seed production. Notice small differences in scale among
 775 seed production traits and among stress tolerance traits. R^2 coefficients are accompanied
 776 by statistical significance: $\cdot p < 0.1$, $* p < 0.05$, $** p < 0.01$, $*** p < 0.001$; p values
 777 were corrected by the false discovery rate method.



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779 **Fig. 5** Model showing a trade-off between fecundity (at the fruit level) and stress
780 tolerance among populations of *Plantago coronopus* under a gradient in environmental
781 stress. Diagram on the right represents differences in seed traits between the
782 hypothetical extremes of the gradient (northern and southern populations). Basal seeds
783 are surrounded by a mucilaginous coat (grey outline), which is absent in the smaller
784 apical seed. Notice differences between fruits in size and mucilage production of basal
785 seeds, and in the number of each seed morph.
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