

Does the spatial variation in selective pressures explain among-site differences in seed mass? A test with *Buxus balearica*

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Abstract Positive effects of seed size on germination and survival can be offset by a greater probability of predation or a poorer dispersal of larger seeds. We hypothesized that spatial variation in local selective pressures acting on seed mass may lead to differences in both optimal and observed seed mass among discrete populations. We first examined the variation in seed mass across a total of 14 populations of *Buxus balearica*; in six of such populations, we further examined the temporal variation by measuring seed mass during three consecutive years. Second, we evaluated seed mass effects on different recruitment phases (seed rain to seedling establishment) in three populations. Lastly, for these three populations, we estimated the resulting overall phenotypic selection on seed mass during recruitment and compared the observed and the predicted optimal seed masses. Most variation (c. 70%) in seed mass occurred among populations, and although we found inter-annual variation in seed mass, the differences in seed mass among populations were consistent over time. Conflicting selective pressures on seed mass appeared during recruitment, and their direction and strength varied among populations, depending on the relative local importance of seed predation vs. germination and establishment. Observed seed mass matched predicted optimal seed mass in two of the three examined populations, suggesting local adaptative responses to the spatial mosaic of selective pressures.

Keywords Balearic Islands · Iberian Peninsula · Local adaptation · Seed rain · Seed predation · Germination · Seedling establishment · Seed size · Seedling size · Phenotypic selection

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Introduction

The dependence of plant fitness on seed size has been extensively supported by empirical evidence in studies at the species level. Germination probability or germination rate (Baker et al. 1994; Greipsson and Davy 1995; Castro et al. 1999; Susko and Lovett-Doust 2000), seedling survival (Vaughton and Ramsey 1998; Eriksson 1999), seedling size (Stock et al. 1990; Vaughton and Ramsey 1998; Susko and Lovett-Doust 2000), and seedling competitive ability (Wulff 1986; Volis et al. 2002) have been shown to increase in larger seeds. Moreover, large seeds perform better than small ones under water and nutrient limitation (Stock et al. 1990; Eriksson 1999; Tungate et al. 2006). Nevertheless, conflicting selective pressures on seed mass may exist, as a large seed mass may represent a handicap in other phases of recruitment (Alcántara and Rey 2003; Gómez 2004). For instance, large seeds can be more prone to be preyed upon (Alexander et al. 2001) or to be dispersed to shorter distances from mother plants (Ganeshaiyah and Uma Shaanker 1991; Greene and Quesada 2005). Therefore, an accurate view of the relationship between seed mass and plant regeneration needs to consider the entire plant's life cycle.

To date, the relationship between seed mass and fitness has been estimated exclusively for one or a few recruitment phases (but see Alcántara and Rey 2003; Gómez 2004) and in a single population (e.g. Vander Wall 2003; Alcántara and Rey 2003; Gómez 2004; Martínez et al. 2007). However, the strength and sign of selective pressures on seeds mass in different recruitment phases may differ among populations depending on their ecological context, i.e., depending on the biotic and abiotic characteristics of each population, and on their relative importance on recruitment patterns. Such differences might lead to differences among populations in both optimal and observed seed mass, which may in turn be constrained by negative genetic correlations between seed mass and number (Smith and Fretwell 1974). Intraspecific and intrageneric studies at large spatial scales have mainly focused on seed mass variation across altitudinal and latitudinal ranges, in relation to gradients in length of the growing season or to variation in climatic conditions, and have led to conflicting hypotheses regarding the selective value of this trait (McWilliams et al. 1968; Baker 1972; Murray et al. 2004). A positive association between seed mass and environmental severity or unfavorable habitats has also been reported in some studies (e.g. Schimpf 1977; Willis and Hulme 2004; Yamada and Miyaura 2005).

For the occurrence of adaptive differentiation of seed mass among populations, two additional conditions must be met: (1) enough variability in seed mass at the appropriate level must exist, and (2) variation in seed mass must be heritable. While large intraspecific variation in seed mass is frequently reported, most of it has been examined within populations and has been found within individual plants (Michaels et al. 1988; Méndez 1997; Vaughton and Ramsey 1998). Such intra-individual variation has been associated with maternal effects (Roach and Wulff 1987; Galloway 2001) and/or with genetic differences among seed sires (Bañuelos and Obeso 2003), being sometimes interpreted in adaptive terms (Janzen 1977; Thompson 1984; Temme 1986). However, it has often been assumed to be related to physiological (Cavers and Steel 1984; Méndez 1997; Vaughton and Ramsey 1998) rather than to genetic effects. For some species, by contrast, most variation in seed mass occurs among individual plants (Castro et al. 1999; Willis and Hulme 2004; Halpern 2005) or among populations (Winn and Gross 1993; Greipsson and Davy 1995). A large amount of variance in seed mass among populations might indicate adaptive differentiation, provided that such differences have a genetic rather than environmental basis. Although the reasons behind these differences are

unknown in most cases, heritability of seed mass has been detected in several studies (Mazer 1987; Verhoeven et al. 2004).

In the present study, we examined the sources of spatial variation (at the scales of regions, populations, individuals and fruits) and temporal variation (three years) of seed mass in *Buxus balearica*, a shrub endemic to the Mediterranean Basin with a fragmented distribution (Benedí 1997). The species' recruitment is strongly limited (Lázaro et al. 2006a), and thus any factor affecting seed production and seedling establishment may have important implications for its population dynamics. We document here a prevalence of among-population variation in seed mass and quantify the effects of this trait on four recruitment phases (seed dispersal, seed predation, seed germination, and seedling survival) to assess its adaptive value in three populations. Finally, we go further evaluating whether among-population variation in seed mass could be explained by the existing variation in selective pressures on this trait. To our knowledge, this study is the first relating the variation in seed mass at a large spatial scale to the variation in selective pressures at different recruitment phases.

Material and methods

Study species

Buxus balearica Lam. (Buxaceae) is a shrub that can reach up to 6 m in height. It occurs in calcium rich soils, often on rocks, under subhumid conditions, between sea level and 1000 m (Benedí 1997). It is a mast seeding species, alternating years of high and almost nil seed production (Lázaro et al. 2006b), and shows ballistic seed dispersal, with fruits drying and dehiscing from June to July. Fruits usually contain six (sometimes four) ovules which develop into black seeds; these seeds bear a whitish elaiosome and are intensively removed mainly by ants in the Iberian Peninsula and by rodents in the Balearic Islands (Lázaro et al. 2006a). Neither ants nor rodents are effective seed dispersers of *B. balearica* (Lázaro et al. 2006a); thus, although secondary dispersal is possible, estimates of seed removal can actually be considered as estimates of seed predation. Seeds germinate in March, usually in low densities and under the parent plants; most seedlings die during the hot, dry summer, and there is no evidence of seed dormancy or the production of a seed bank in this species (Lázaro et al. 2006a). In general, remnant *B. balearica* populations show size structures biased towards old individuals, indicating low recruitment rates that are mainly caused by high post-dispersal seed predation, low germination, and high summer seedling mortality (Lázaro et al. 2006a).

Study areas

A total of 14 Spanish and Moroccan populations of *B. balearica* were included in the study (see Table 1 for details). Four of the Spanish sites are in the south of the Iberian Peninsula (the coast of Granada, Almirajara Mountains in Málaga and Gádor Mountains in Almería), whereas five are in the Balearic Islands (one in Cabrera Island and five in the Tramuntana Mountains, Mallorca Island; Table 1). The studied Moroccan populations are in the Rif Mountains (Jebha), Middle Atlas Mountains (Boulemane), and the North and South faces of Big Atlas Mountains (Cirque de Jaffar and Gorges du Todra, respectively). The predominant vegetation at all 14 sites consists of typical Mediterranean shrubs (see Lázaro 2005 for details).

Table 1 Location, geographic coordinates, elevation (m), distance to the nearest population (km), density (mean distance among individuals, m), and sampling type performed in each of the studied populations

Site	Coordinates	Region	Elevation	Distance	Density	Sampling type
Cerro Gordo	3°47' W 36°45' N	Iberian Peninsula	0	5	1.2	W
Frigiliana	3°53' W 36°46' N	Iberian Peninsula	350	5	3.1	W
Rágol I	2°42' W 36°58' N	Iberian Peninsula	440	70	2.1	W-SR-P-GG- FG
Rágol II	2°42' W 36°59' N	Iberian Peninsula	720	70	1.1	W
Cap Ventós	2°58' E 39°10' N	Balearic Islands	50	3.5		W
Andratx	2°24' E 39°31' N	Balearic Islands	5	10	1.0	W
Sant Vicenç	3°03' E 39°56' N	Balearic Islands	40	<1	1.1	W-P-GG -FG
Lluc	2°54' E 39°48' N	Balearic Islands	320	1.5	1.5	W-SR-P-GG-FG
Cúber	2°47' E 39°46' N	Balearic Islands	630	<1	2.7	W
Galatzó	2°29' E 39°38' N	Balearic Islands	900	1.5	3.5	W
Jebha	4°45' W 35°03' N	Morocco	150	<1	0.7	W
Boulemane	4°37' W 33°26' N	Morocco	1250	<1	1.8	W
Cirque de Jaffar	4°55' W 32°34' N	Morocco	900	<1	0.2	W
Gorges du Todra	5°35' W 31°36' N	Morocco	1500	<1	3.3	W

Sampling type: W, seed mass; SR, seed rain; P, seed predation; GG, germination in experimental garden; and FG, field experiment of germination and survival

Spatio-temporal variation in seed mass

In 2002, fruits of *B. balearica* were collected from all 14 populations. Additional samples were obtained in the summers of 2001 and 2003 in six Spanish populations (Rágol I, Rágol II, Sant Vicenç, Lluc, Cúber and Galatzó). A total of 20–35 fruits from each of 15–20 individuals were collected per population and year, except in Galatzó where fruits were obtained only from 7–10 shrubs due to the limited number of reproductive individuals in that population. The individuals were haphazardly chosen across the total area sampled in the study populations (varying from 0.1 to 0.3 km² depending on the size and characteristics of the populations). The masting behaviour of the species (Lázaro et al. 2006b) did not allow the collection of enough number of fruits of the same individuals all the years and therefore the sampling was conducted haphazardly each year. Fruits were collected when dried just before opening, all at the same stage of ripeness. They were weighed to the nearest mg, and subsequently dissected to count and weigh all their seeds (also to the nearest mg). We used these data to quantify the partitioning of variance of seed mass among regions, populations, individuals, and fruits (14 populations, 2002 data), as well as among-year variation in seed mass (six populations, 2001–2003). The years 2001 and 2003 were masting, whereas 2002 was a non-masting year.

Effect of seed mass on recruitment: from seed dispersal to seedling establishment

Seed rain

In the summer of 2003, we placed seed traps of 25 × 30 cm to quantify seed rain at two populations (Lluc and Rágol I). Traps were protected with a net against rodents and with

Tangle Foot to prevent ant entrance. Around each of 10 different individuals per population, we placed a total of 12 traps at three distances from the canopy (0—at the edge of the plant, 1 and 2 m) and at the four cardinal points. Seed rain was examined in plants haphazardly selected across the total area sampled in the study populations, separated from each other by at least 4 m. Slight differences in fruit mass were detected within individuals, due to their different seed number (results not shown). However, such differences were not due to the fruit position in the shrub that could affect the distance at which seeds of different sizes are dispersed. Traps were monitored weekly through the entire seed dispersal period (end of June, end of July). We recorded the number of seeds fallen in each trap, and weighed them in order to compare seed mass across distances and orientations.

Seed predation

We studied seed predation at three populations (Sant Vicenç, Lluc and Rágol I) during the seed dispersal period in the summer of 2003. We placed four traps made of green plastic mesh (1×1 mm) with 10 seeds each around the same 10 individuals per population used for the seed rain experiment, assigning four treatments to them: a factorial combination of two predator (control vs. vertebrate exclusion) and two microhabitat (open field vs. under canopy) treatments. The vertebrate exclusion treatment consisted of a 0.5×0.5 cm metal mesh covering the traps, allowing access to ants and other small invertebrates. To assess changes in seed mass due to selective predation, we obtained the total weight of the 10 seeds placed at each trap, and after 4 days we recorded the number of seeds remaining in each trap, and weighed them together again.

Germination and seedling survival

In the same three populations where seed predation experiments were performed, we estimated germination and seedling survival, both in the field and in an experimental garden. We carried out previous germination tests in an experimental garden outside our laboratory in autumn 2001. We weighed 100 seeds from a total of 10 haphazardly selected individuals across the area sampled in each population and sowed them (at 1–0.5 cm depth) on trays (100 pots/tray, 3 trays) filled with horticultural soil. We watered the trays weekly, and monitored germination every 4 days for about 9 months.

In autumn 2003, a total of 1000 individually weighed seeds (400 from Rágol I, 400 from Lluc, and 200 from Sant Vicenç) were sown at their sites of origin and individually marked using plastic tags. In order to include as much variability as possible, from the seeds collected for weight in 2003, we visually separated large and small fruits from each individual, and measured 20 seeds from each type of fruit (10 in the case of Sant Vicenç). We placed 10 grids per population under randomly chosen adults and 10 additional grids in the open field to assess whether the effect of seed mass on germination and survival varied between these two microhabitats. At Sant Vicenç, we were only able to use the 'under canopy' microhabitat due to the lower number of seeds available from this site. At each grid, of 30×30 cm size, we planted 20 seeds (two seeds per individual, one from a small fruit, and the other from a big one) uniformly distributed at 4 cm distances. Seed germination was recorded once in March–April of 2004, and seedling survival and size (length to the nearest mm) were also recorded once during the following autumn (September–October of 2004).

Phenotypic selection on seed mass

We estimated phenotypic selection on seed mass in three populations differing significantly in seed mass: Sant Vicenç, Lluc and Ràgol I. Following Gómez (2004), the estimation was obtained separately for each fitness component: (1) seed survival to predation, (2) seed germination (both in the experimental garden and under *Buxus* in the field, the least stressful microhabitat), and (3) seedling survival after the first summer under *Buxus*. For the first component, we used data on differences in mean seed mass per trap (only open traps used), before and after predation. We used the mean seed masses per trap before and after predation to define two curves, representing the seed mass distributions at the beginning and at the end of the experiment. By assuming normal distributions, we estimated the seed mass distributions from the mean seed masses per trap and variances of these means according to Sokal and Rohlf (1969, p. 179):

$$\sigma^2\bar{X} = \frac{\sigma^2}{n}$$

where $\sigma^2\bar{X}$ is the variance of the means in each population and n is the sample size on which means are based. Both curves (before and after predation) were multiplied by the sample size to obtain absolute frequencies. Finally, we used their ratio (after/before) to obtain the relationship between seed mass and survivorship to predation. For the two other fitness components, we used logistic regressions to estimate the probability of germination and seedling survival depending on seed mass (Gómez 2004).

The overall probability of a seed of a given mass to survive predation and become a one-year-old seedling was then obtained by multiplying the estimates of phenotypic selection acting on seed mass through each of the sequential fitness components (Gómez 2004). We used the curves estimated at each phase to calculate the overall phenotypic selection when their P -values <0.1 ; in the cases where P -values >0.1 , we used a constant representing the mean probability of survival. This overall estimate summarizes the reproductive output function for the life-cycle period studied here.

Statistical analysis

We analyzed the variation in seed mass among regions (Iberian Peninsula vs. Balearic Island vs. Morocco), populations, individuals, and fruits, by performing nested ANOVAs and a Variance Components Analysis with data gathered in 2002 from the 14 populations. To quantify the percentage of variance explained by annual differences, we performed another Variance Components Analysis with the six populations monitored during the three years. In these two analyses, all factors except region were considered as random factors, and the number of filled seeds per fruit was introduced as a covariate.

A General Linear Model was used to study seed mass variation among years, with individual plant nested within population and year. Both population and individual were considered as random factors whereas year as a fixed factor (as we specifically wanted to test for differences between mast and non-mast years). Both fruit mass and number of seeds per fruit were included as covariates. From all possible models including all these variables and their two- and three-order interactions, and any smaller subset of them, we selected that showing the lowest value of Akaike Information Criterion (AIC).

A separate mixed ANOVA was used to compare seed mass among the three populations (Sant Vicenç, Lluc and Ràgol I) in which the effect of seed mass on different

recruitment phases was experimentally studied. Here, we included population and year as fixed factors in order to test for differences among those specific populations and between the two study years. Again, individual was a random factor nested into population and year, and the number of filled seeds was introduced as covariate in this model.

The effects of seed mass on seed rain and seed predation were tested by means of repeated-measures analyses. In both cases, population was included as a fixed factor and individual plant as a random factor (subject). Distance and orientation (seed rain), and microhabitat and exclosure treatments (seed predation) were included as within-subject fixed factors, and seed mass as the dependent variable. The number of seeds collected per trap and the initial seed mass per trap were included as continuous covariates in the analyses of seed rain and seed predation, respectively. Germination in the field and in the experimental garden were analysed by means of logistic regressions, including population as a fixed factor and seed mass as a covariate. Survival in the field and the effect of microhabitat on germination and survival were assessed in separate analyses with data only from Lluc, due to the low number of seedlings available in the other populations. No seeds survived the beginning of the summer in the experimental garden.

The analyses including repeated measurements were conducted in SAS (v. 6.12, GLM procedure, SAS Institute Inc.), whereas all the others were performed in STATISTICA (v. 7). All reported results are based on Type III Sum of Squares, except those of the Variance Components Analysis, based on Type I Sum of Squares, due to unbalanced data. Multiple comparisons among levels of significant factors in ANOVAs were based on Tukey's *a posteriori* tests, whereas in mixed-model GLMs they were based on contrasts corrected with the sequential Bonferroni's test (Rice 1989). Means are accompanied by their standard deviations throughout the text, unless otherwise indicated.

Results

Seed mass variation

Spatial variation

Seed mass did not differ significantly among regions ($F_{2,10.2} = 0.03$, $P = 0.97$) but did vary among populations within regions ($F_{11,49.9} = 15.80$, $P < 0.0001$), among individuals within populations ($F_{52, 248.5} = 10.86$, $P < 0.0001$), and among fruits within individuals ($F_{136, 1154} = 3.17$, $P < 0.0001$) (Table 2). Contrary to the expected, the number of filled seeds per fruit was unrelated to seed mass ($F_{1,12.2} = 0.15$, $P = 0.71$). The largest variation in seed mass occurred among populations (69.1% of variance), followed by individuals (13.4%) and fruits (3.6%). By contrast, regions and the number of filled seeds within fruits showed percentages of variance close to zero. Such variation partitioning is much consistent among years (results not shown).

Temporal variation

Seeds were consistently lighter in the non-mast (2002) than in the two mast years (2001 and 2003) ($F_{2,17} = 10.22$; $P = 0.003$) and this was consistent across populations ($F_{9, 2941} = 1.64$; $P = 0.10$; Fig. 1); the Tukey's test showed no differences between 2001

Table 2 Mean seed mass (mg) and CV (SD/mean) per region and population

Region site	Site	Seed mass	
		Mean	CV
Iberian Peninsula	Cerro Gordo	9.49	0.15
	Frigiliana	9.26	0.14
	Rágol I	9.23	0.22
	Rágol II	8.56	0.17
	Total	9.18	0.21
Balearic Islands	Cap Ventós (Cabrera)	13.37	0.23
	Andratx (Mallorca)	7.50	0.20
	Sant Vicenç (Mallorca)	6.94	0.16
	Lluc (Mallorca)	7.19	0.32
	Cúber (Mallorca)	4.66	0.20
	Galatzó (Mallorca)	4.81	0.25
	Total	9.68	0.44
	Total Mallorca Island	6.62	0.31
Morocco	Jebha	18.38	0.13
	Boulemane	8.49	0.22
	Cirque de Jaffar	5.08	0.21
	Gorges du Todra	8.92	0.23
	Total	9.05	0.39

Data from 2002

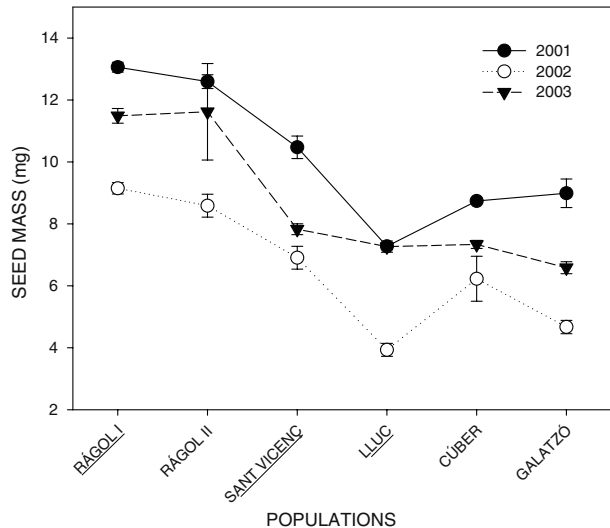
and 2003. Seed mass varied strongly among populations ($F_{5, 2941} = 13.55$; $P < 0.0001$; Fig. 1) and among individuals ($F_{149, 2941} = 6.63$; $P < 0.0001$). Mean seed mass and seed number tended to be negatively associated, but such relationship varied among populations and years (the three order interaction was significant: $F_{17, 2941} = 2.42$; $P = 0.001$). Such trade-off was generally clear and consistent in all populations in 2001, i.e., seed number and mean seed mass per fruit were negative related in all the populations that year. However, the trade-off was almost undetectable in 2002, when the negative relationship was found only at Rágol I. In 2003, seed mass and seed number were again negatively related at all the study populations except Rágol II and Lluc.

The Variance Components Analysis with data from the six populations monitored the three years showed again that the largest variation in seed mass occurred among populations (29.1% of variance), followed by individuals (3.6%), and finally by years (2.2%).

Effects of seed mass on different recruitment phases

The three study populations differed significantly in seed mass in the masting years ($F_{2, 65.7} = 70.81$, $P < 0.0001$). Both years, Rágol I showed the heaviest seeds (13.07 ± 2.86 mg, and 11.61 ± 3.17 mg, for 2001 and 2003 respectively), followed by Sant Vicenç (10.47 ± 2.41 mg, and 7.67 ± 2.47 mg) and Lluc (7.27 ± 2.24 mg, and 7.26 ± 2.38 mg). The interaction between population and year was, however, significant ($F_{2, 65.5} = 3.45$, $P = 0.038$), since the differences between both masting years were smaller at Lluc than at the other populations.

Fig. 1 Mean \pm SE seed mass in different years (2001–2003) and populations. Underlined names correspond to the populations where experiments were conducted in this study



Seed rain

Contrary to what might be expected, seeds remaining at the edge of the plant canopy were lighter than those dispersed at one or two meters from the mother plant at both populations (6.60 ± 1.06 vs. 7.40 ± 1.25 mg and 12.25 ± 1.78 vs. 12.38 ± 1.97 mg for Lluç and Rágol I respectively; distance: $F_{2, 215} = 6.92$, $P = 0.001$; distance \times population: $F_{2, 215} = 0.17$, $P = 0.846$). There was no effect of orientation ($F_{3, 215} = 1.17$, $P = 0.322$), although seeds facing east were heaviest at Lluç and lightest at Rágol I (orientation \times population: $F_{3, 215} = 2.69$, $P = 0.048$). A positive relationship between number of seeds collected and their weight was found in traps at Rágol I, but not at Lluç (interaction number of seeds \times population: $F_{2, 215} = 12.35$, $P < 0.001$). All other interactions were non-significant ($P \gg 0.05$).

Seed predation

Four days after setting up the experiment, all seeds from a large proportion of traps had disappeared (67% of dishes at Rágol I, 44% at Sant Vicenç, and 15% at Lluç). These traps could not be included in the following analyses of phenotypic selection due to the procedure used, since values before and after the experiment were required for each trap. On the other hand, no seeds were removed by predators during the experiment in 12% of the traps at Rágol I, 9% at Sant Vicenç, and 27% at Lluç. Our experience in the field leads us to assume that these traps were not found by predators since they quickly remove some seeds when they find a trap. None of these traps, which were excluded from further analyses on seed selection, differed in initial seed mass from the traps included in the analyses (all $P \gg 0.05$). The effect of predation on seed mass was inconsistent among populations (treatment: $F_{1, 54} = 0.59$, $P = 0.449$, and interaction treatment \times population: $F_{2, 54} = 6.68$, $P = 0.003$). Seeds escaping predation at Sant Vicenç and Lluç were lighter in open than in excluded traps, what suggests that rodents had selected larger seeds, whereas no differential removal occurred at Rágol I (Fig. 2). Moreover, the relationship

between initial and final seed mass varied among populations and treatments (interaction initial seed mass x treatment x population: $F_{6, 54} = 7.33$, $P < 0.0001$), indicating that the strength of selection on seed mass for each predation treatment also differed among populations (Fig. 2). Neither microhabitat nor its interaction with the other factors had significant effects on seed mass (all $P > 0.05$).

Germination and seedling survival

Germination in the experimental garden was 9% for seeds from LLuc, 23% for seeds from Sant Vicenç and 37% for seeds from Rágol I, although differences were not significant ($\chi^2 = 1.82$, $P = 0.402$). Mass positively affected total germination only for Rágol I seeds (interaction seed mass x population: $\chi^2 = 11.49$, $P = 0.009$).

In the field, the proportion of germinated seeds at LLuc was twice that at Sant Vicenç (14.6% vs. 7.5%, both for seeds under *Buxus*; $\chi^2 = 10.52$, $P = 0.001$) whilst none of the sown seeds germinated at Rágol I. Seed mass tended to be positively related to germination in both populations, although the relationship was only marginally significant ($\chi^2 = 2.75$,

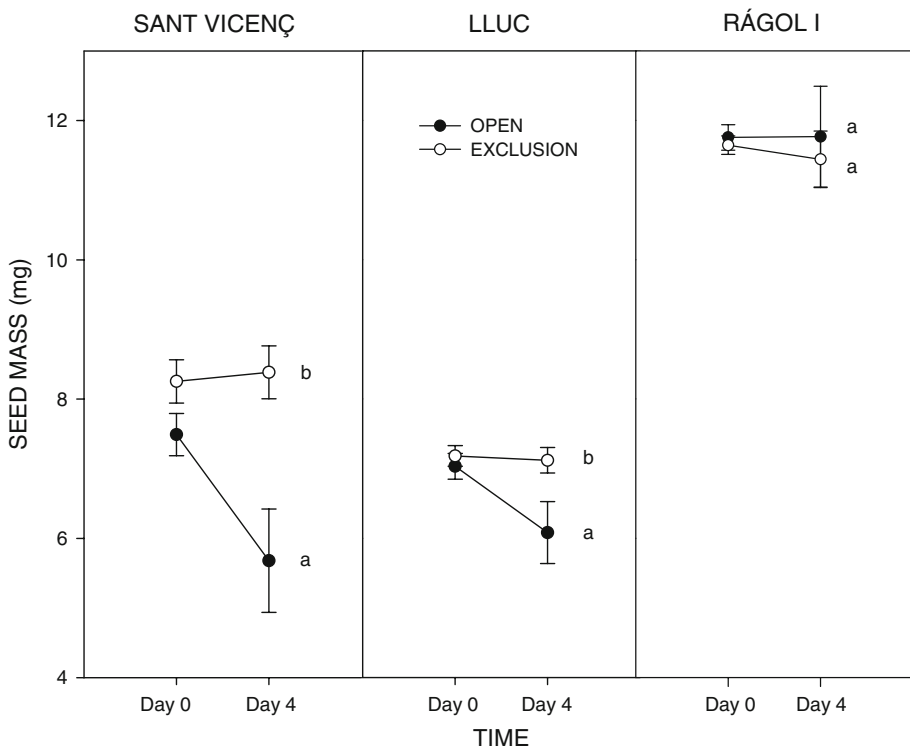


Fig. 2 Mean \pm SE seed mass at the beginning (Day 0) and at the end (Day 4) of the experiment of seed predation at Rágol I, Sant Vicenç, and LLuc. No significant differences between treatments (open vs. exclusion to vertebrate predation) existed at the beginning of the experiment. Mean seed mass sharing the same letter did not differ significantly within treatments at the end of the experiment. Microhabitats were previously pooled

$P = 0.097$). Seedling survival increased with seed mass at Lluc ($\chi^2 = 6.18$, $P = 0.013$) whereas the low germination and survivorship (15 seedlings of which only four survived) at Sant Vicenç precluded a rigorous test of such effect in that population.

When testing the effect of microhabitat at Lluc, we again found that seed mass did not alter the probability of germination under *Buxus* ($\chi^2 = 0.78$, $P = 0.378$; Fig. 3a) although it increased seedling survival (see above; Fig. 3b); however, seed mass did increase germination in the open field (interaction: $\chi^2 = 3.97$, $P = 0.046$; Fig. 3a). No seeds survived after the first summer in this microhabitat. Seed mass and one-year seedling size were not correlated ($r = 0.103$, $P = 0.715$, $n = 15$).

Phenotypic selection on seed mass

All phases were affected by seed mass in a different way and/or with different strength in the three populations. Figure 4 summarizes the results in the best possible conditions i.e. those that maximize seed survival: germination and survival under *Buxus*. In the case of Rágol I, for which we did not obtain data on germination in the field, the germination in the experimental garden is shown.

At Sant Vicenç, seed predation was greater for heavier seeds but seed mass had a slight positive effect on germination ($P < 0.10$) and an insignificant effect on seedling survival. Thus, the estimate of cumulative effects of seed mass results in stabilizing selection, with an optimal seed mass at around 6 mg. Predation was also greater for large seeds at Lluc, but this effect was weaker than at Sant Vicenç. The relationship between germination and seed mass was also similar to Sant Vicenç; however, the greater survival of heavier seeds in this population, make the balance much more favorable for larger seeds. The overall phenotypic selection at Lluc is therefore positive and directional, favoring large seeds. Finally, at Rágol I, predation affected all seed sizes at almost equal levels while germination was greater for larger seeds. Here, the estimate of cumulative effects of seed mass results in stabilizing selection with an optimal seed mass at approximately 13 mg, doubling the value obtained for Sant Vicenç.

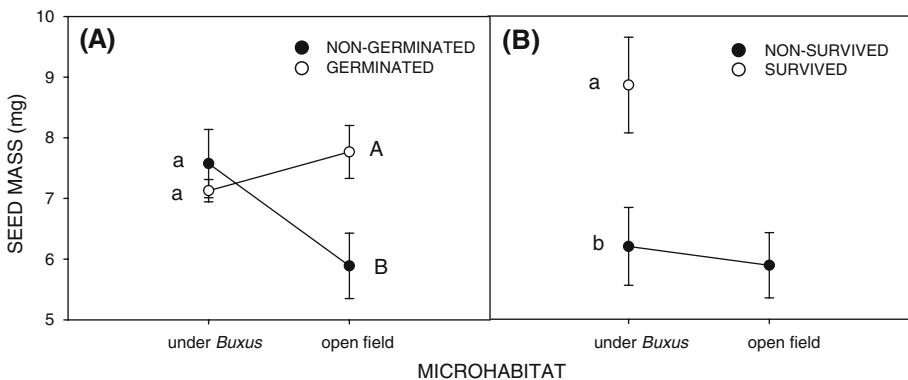


Fig. 3 Mean \pm SE mass of (a) germinated and (b) surviving seeds in different microhabitats (under *Buxus* and in open field) at Lluc. Mean seed mass with the same letter did not differ within microhabitats. No seeds survived in the open field

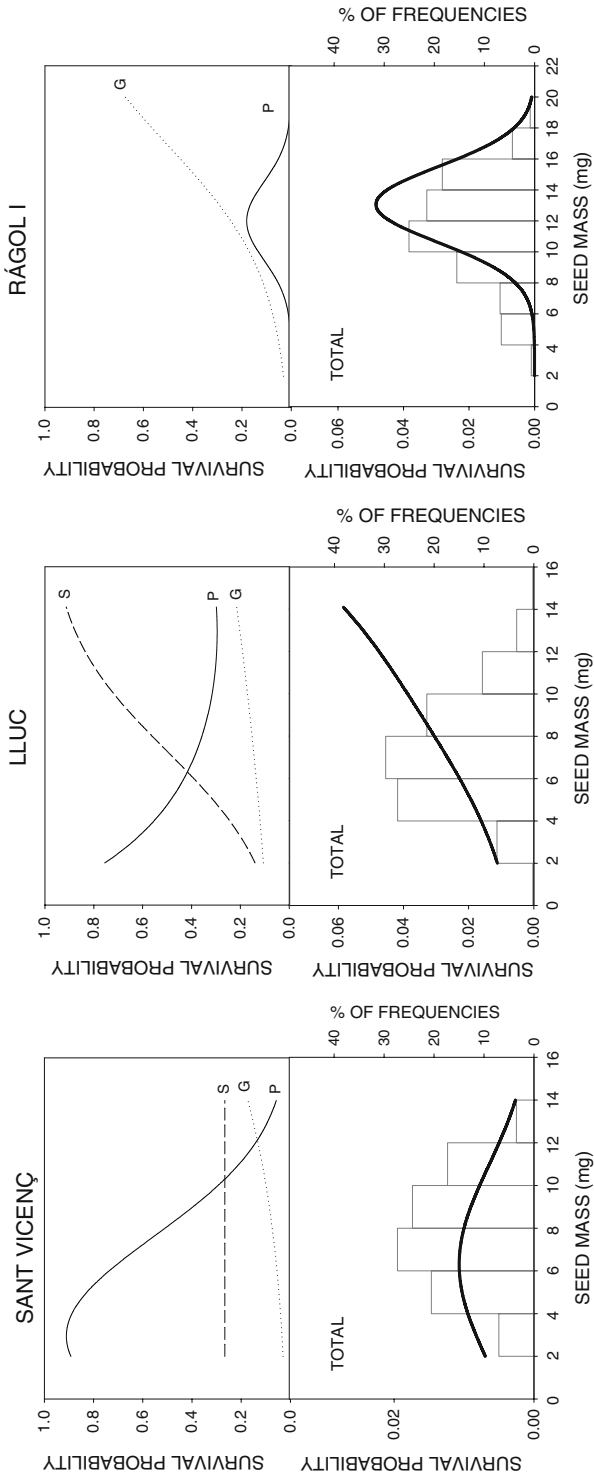


Fig. 4 Phenotypic selection on seed mass in different phases of recruitment and total phenotypic selection at three populations: Sant Vicenç, Lluç and Ràgol I. Only curves with test P -values < 0.1 were included; when P -values > 0.1 for an effect, a constant representing the mean probability of survival is shown. P: survival to predation; G: germination (under *Buxus*); in Ràgol I G shows germination in the experimental garden); S: seedling survival (under *Buxus*). Note that scale of axes differ in different graphs

Discussion

Spatio-temporal variation of seed mass

Buxus balearica showed a small fraction of variation in seed mass within individuals (c. 4%), moderate among individuals within populations (c. 13%), and a large fraction among populations (nearly 70%). This larger fraction of variation found among populations contrasts with other studies that have reported variation in seed mass predominantly within populations, either within individuals (Thompson 1984; Michaels et al. 1988; Hendrix and Sun 1989; Méndez 1997; Susko and Lovett-Doust 2000; Alexander et al. 2001), or among them (Castro et al. 1999; Willis and Hulme 2004; Halpern 2005). So far, only a few studies have compared seed mass at large spatial scales (but see Winn and Gross 1993; Greipsson and Davy 1995; Vargas et al. 2003). These studies have also shown strong differences among populations, which have been attributed either to genetic differentiation (e.g. Greipsson and Davy 1995 for *Leymus arenarius*) or to differences among environmental conditions (e.g. Winn and Gross 1993 for *Prunella vulgaris*).

Despite the great inter-annual variation in seed mass found in *B. balearica*, inter-population differences were generally consistent over time. Such temporal variation was particularly high between mast and non-mast years and was probably due to a response to resource levels. Plants are expected to alter seed number rather than seed mass if resource levels vary (Smith and Fretwell 1974); however, in *B. balearica*, both seed set (Lázaro et al. 2006b) and seed mass varied between mast and non-mast years (as found in other species, e.g. Vaughton and Ramsey 1998). Larger seed mass is a common phenotypic effect under circumstances of higher nutrient levels or water availability (Wulff and Bazzaz 1992), and variation among years has also been detected for Scots pine, with greater seed masses in years of higher rainfall (Castro et al. 1999).

The trade-off between seed mass and number predicted by life history theory (Smith and Fretwell 1974) has been reported in numerous studies, either at the plant (e.g. Vaughton and Ramsey 1998; Eriksson 1999) or fruit level (e.g. Baker et al. 1994; Eriksson 1999), although there seems to be also a number of exceptions (see review in Venable 1992). In *B. balearica*, such a trade-off at the fruit level was variable among years and populations. In masting years, the trade-off was generally found, whereas in the non-masting year (2002) the trade-off was detected only in the population with the largest fruit production (Ráfol I). Therefore, the existence of such a trade-off appeared to depend on resource availability: when resources were scarce, plants seemed to fail to adjust seed mass and number appropriately. A similar pattern has also been reported in studies examining seed mass changes over the flowering period (Cavers and Steel 1984; McGingley 1989).

In short, we found larger variation in seed mass among populations than within populations, this being consistent over time. In the next sections, we will discuss whether variation in local selective pressures on seed mass during recruitment can explain these among-site differences.

Effects of seed mass on the different phases of recruitment

Heavy seeds of wind-dispersed species are usually dispersed to shorter distances from mother plants than light seeds (Ganeshaiyah and Uma Shaanker 1991; Greene and Quesada 2005). We found the opposite trend for the ballistically dispersed seeds of *B. balearica*. It is possible that large seeds in ballistically dispersed species have structural benefits for

dispersing large distances. Unfortunately, there is no data available in the literature to test this hypothesis. An effect of compass orientation on seed mass was also observed, which may reflect within-individual variations due to external causes, such as exposure to sun. In spite of that, the effect of seed mass on distance achieved during dispersal was consistent across populations and orientations. This first potential advantage of large seeds dispersing longer distances could, however, be reduced as large seeds are usually more likely to be preyed upon than light seeds (e.g. Alexander et al. 2001; Celis-Diez and Bustamante 2005; but see Eriksson 1999). Interestingly, we found a higher predation on large seeds at Sant Vicenç and Lluc, but not at Ràgol I. These among-population differences in the effect of seed mass on survival to predation could be explained by differences in the main seed predators at each site. Parallel experimental work on *B. balearica* (Lázaro et al. 2006a) has shown that seed predation is consistently high across populations and years (over 80%), but that the main predators differ between the island and the mainland populations. While seeds are exclusively consumed by ants in the mainland, they are rapidly preyed upon by rodents in the island. Although the selective role of seed predators on seed size is still controversial (reviewed in Hulme and Benkman 2002), ants and rodents may select for different seed sizes (Rey et al. 2002), and in the range of size of *B. balearica*, rodents may select for larger seeds whereas ants may not. We believe that the relative importance of rodents and ants as predators is causing these spatial differences in selection for seed mass during the seed predation phase.

Total germination was rather low in *B. balearica*, both in the field and in the experimental garden. In the experimental garden, heavy seeds germinated more than light ones, but only for Ràgol I seeds, which we attribute to the largest seed mass and in turn the highest germinability in this population, allowing the adequate test of this relationship. Germination was only marginally influenced by seed mass in the most favourable conditions in the field (i.e. under *Buxus*); by contrast, germination increased with seed mass in the most stressful microhabitat (i.e. open field), consistently with what has been reported for other species (Vaughton and Ramsey 1998; Castro et al. 1999). Large seeds usually have a greater survivorship during establishment than small ones (e.g. Eriksson 1999), which is frequently attributed to a greater amount of reserves (Jurado et al. 1991; Vaughton and Ramsey 1998; but see Lacey et al. 1997). Our findings were also consistent with such pattern, although the effect was not significant in one of the populations, probably due to limited sample size. Lastly, larger seeds generate larger seedlings in many species (e.g., Stock et al. 1990; Vaughton and Ramsey 1998; Susko and Lovett-Doust 2000), but we did not find any relationship between seed mass and seedling size in *B. balearica* after 1 year of emergence. Early differences in seedling size are known to disappear over time in some species (Castro et al. 1999; Traveset et al. 2001; Rey et al. 2004), but can influence seedling survival during the first summer drought (Rey et al. 2004). Whether this is the case for *B. balearica* is unknown; however, seed mass in this species seems to affect more seedling establishment and the previous stages of recruitment than the subsequent ones.

Phenotypic selection on seed mass

Our results on phenotypic selection on seed mass in *B. balearica* are consistent with other studies that show opposing and conflicting selective pressures acting on seed mass. For instance, in *Betula monosperma*, Ganeshiah and Uma Shaanker (1991) showed selection on seed mass in opposite directions for dispersal efficiency and seedling establishment. In pines, larger seeds are less likely to disperse by wind, although secondary dispersal by

scatter-hoarding rodents may compensate the poor wind-dispersal (Vander Wall 2003). Conflicting selective pressures in seed size during and after dispersal have also been documented in *Olea europaea*, by using multiplicative components of fitness (Alcántara and Rey 2003). Large seeds of *Quercus ilex* are more prone to predation but germinate and produce seedlings that survive better than small ones (Gómez 2004). Lastly, Martínez et al. (2007) have recently reported that the advantage of large seeds of *Crataegus monogyna* during the dispersal stage is cancelled by an opposite selective pressure exerted by seed predators.

Besides finding conflicting selective pressures acting on seed mass in *B. balearica* during the recruitment phases, we also found that the direction and strength of such selective pressures vary among populations. In two of them, we detected significant positive phenotypic selection on seed mass during some phases of the life cycle whilst negative during others. Seed mass positively affected most fitness components related to seedling establishment, although such effect was not significant or equally strong in all populations. In general, heavier seeds had a higher probability of germinating and surviving as seedlings, but they suffered greater predation by rodents in the populations where these predators were present. The major consequences of such conflicting selective forces varying among populations were that the net phenotypic selection acting on seed mass was also population-dependent, and that the optimal seed masses differed among populations. Interestingly, the optimal seed masses derived from our estimates of net phenotypic selection match quite well the observed seed masses in two out of the three study populations (i.e. in Sant Vicenç and Ràgol I; Fig. 4). Two limitations related to the procedure used here may have influenced to some extent our estimates of phenotypic selection. First, the exclusion from the analyses of those traps in which seeds were completely removed could have overestimated the role of predation in the selection of seed masses. Nevertheless, the percentage of completely predated traps at Lluc was low, and at Ràgol I (the population where more traps were completely removed) predation did not have any important effect on the selection of seed masses. Therefore, we think that the exclusion of these traps may not have had a significant influence on the results in these two populations; however, we cannot discard a possible overestimation of the effect of seed predation at San Vicenç. Second, with the use of germination data from experimental garden for Ràgol I, we may have somehow underestimated the effect of seed mass on germination in the field in this locality, since this relationship is usually more pronounced in stressful conditions (Vaughton and Ramsey 1998; Castro et al. 1999). A slight displacement of the optimum towards larger seeds in both populations might be expected if such limitations in the procedure had the abovementioned effects. However, even in this case, the slightly larger optimum would still fit the observed masses in these populations, since we have used for the comparison the data from 2003 (the smallest masses for a masting year), but the observed masses varied from 13.07 to 11.61 at Ràgol I and from 10.47 to 7.67 at San Vicenç, depending on the year. Therefore, the results seem to indicate that the studied factors may be representative of the main selective forces influencing seed mass at these populations. On the contrary, the directional phenotypic selection favouring larger seeds found at Lluc does not match the observed seed masses that peak at low values at this population. A possible explanation for this mismatch could be that a trade-off between seed mass and number at the plant level could influence the optimal seed masses, since the maximization of the mother's fitness might impose selection towards more numerous but lighter seeds. However, we found no evidence of such a trade-off, neither when we separately analyzed populations and years (all $P \gg 0.05$; results not shown) nor when we analyzed the data from the seed rain experiment. Therefore, other factors (e.g., genetic or

environmental, such as resources limitation) may play a relevant role limiting seed size at that site.

Although the genetic differences among populations concerning seed mass and their heritability are unknown for *B. balearica*, opportunities for selection on this trait during recruitment obviously exist, and it is probable that they are the cause for the differences in seed mass among some populations. Alternatively, differences among populations could be due to differences in resources or water availability. We think, however, that this is an unlikely explanation for our results, since in years with lower resources the seeds are smaller (showing a pattern of lower resources, smaller seeds), whereas the populations of *B. balearica* with larger seeds are usually those having higher hydric stress and lower precipitation (Lázaro et al., 2006a; Lázaro and Méndez, 2007). On the other hand, we found no association between seed mass and altitude, latitude, or population characteristics related to fragmentation, distance to the nearest population and density (all $P \gg 0.05$; results not shown; see characteristics of the populations in Table 1 and seed masses in Table 2), contrary to what has been reported for other species (Ernst and Piccoli 1995; Susko and Lovett-Doust 2000; Sugiyama 2003). These findings lead us to believe that the differences in seed mass among some populations of *B. balearica* are likely the result of differences in phenotypic selection during recruitment.

To further deepen into the understanding on selection factors affecting plant traits, long-term studies that incorporate intra-population spatio-temporal variation are needed (Gómez 2004). We carried out the experiments on masting years. Seeds from non-masting years are smaller than those from mating years, as we have shown here. In addition, the availability of seeds and seedlings in the populations is much lower in non-masting years. This is mainly for two reasons. First, although the fraction of removed seeds in *B. balearica* does not depend upon seed production, neither in the island nor in the mainland populations (Lázaro et al. 2006b), the real number of seeds escaping predation may be lower in a non-masting year, since seed production is lower. Second, seedling emergence and seedling recruitment are greater after highly productive years (Lázaro et al. 2006b). Therefore, we need to consider that the lower production of seeds with lower seed masses in non-masting years may affect the patterns of phenotypic selection in each phase. For instance, the selection of seed masses during predation might be lower in non-masting years, as predators possibly expand their range of selected sizes when seeds are scarce. Moreover, the lower seed masses these years may also result into lower seed germinations. However, although the curves of phenotypic selection may be different in non-masting years (being probably more relaxed), masting years are those contributing the most to the reproductive success of the species and therefore our experiments may reflect the selection that is actually occurring on seed mass in this species. Anyway, the study of temporal variation in the selective pressures is certainly important for long-lived species, such as *B. balearica*, occurring in Mediterranean and semiarid environments, where survival at different phases is highly variable among years (e.g. Lázaro et al. 2006a). In addition, the study of local variations in the selective pressures related to the distance to mother plants may shed light on the role that the interactions among plants play on selection of seed mass.

Conclusions

In this study we document a prevalence of among-population variation in seed mass in *B. balearica* and demonstrate that the conflicting selective pressures appearing during recruitment varied in their direction and strength among populations, depending on the

relative local importance of seed predation vs. germination and establishment. Moreover, we show that observed seed mass matched predicted optimal seed mass (based on the estimates of phenotypic selection) in two out of the three populations, suggesting the existence of local adaptive responses to the spatial mosaic of selective pressures. Studies on genetic differences among populations concerning seed mass and their heritability in *B. balearica* would be desirable to corroborate such local adaptation.

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References

- Alcántara JM, Rey PJ (2003) Conflicting selection pressures on seed size: evolutionary ecology of fruit size and bird-dispersed tree, *Olea europaea*. *J Evol Biol* 16:1168–1176. doi:[10.1046/j.1420-9101.2003.00618.x](https://doi.org/10.1046/j.1420-9101.2003.00618.x)
- Alexander HM, Cummings CL, Kahn L, Snow AA (2001) Seed size variation and predation of seeds produced by wild and crop-wild flowers. *Am J Bot* 88:623–627. doi:[10.2307/2657061](https://doi.org/10.2307/2657061)
- Baker HG (1972) Seed weight in relation to environmental conditions in California. *Ecology* 53:997–1010. doi:[10.2307/1935413](https://doi.org/10.2307/1935413)
- Baker K, Richards AJ, Tremayne M (1994) Fitness constrains on the flower number, seed number, and seed size in the dimorphic species *Primula farinosa* L. and *Armeria maritima* (Miller) Willd. *New Phytol* 128(3):563–570. doi:[10.1111/j.1469-8137.1994.tb03002.x](https://doi.org/10.1111/j.1469-8137.1994.tb03002.x)
- Bañuelos MJ, Obeso JR (2003) Maternal provisioning, sibling rivalry and seed mass variability in the dioecious shrub *Rhamnus alpinus*. *Evol Ecol* 17:19–31. doi:[10.1023/A:1022430302689](https://doi.org/10.1023/A:1022430302689)
- Benedí C (1997) CVII. Buxaceae. In: Castroviejo S et al (eds) *Flora Ibérica*, vol VIII. Real Jardín Botánico, CSIC, pp 187–189
- Castro J, Gómez JM, García D, Zamora R, Hódar JA (1999) Seed predation and dispersal in relict Scots pine forests in southern Spain. *Plant Ecol* 145:115–123. doi:[10.1023/A:1009865703906](https://doi.org/10.1023/A:1009865703906)
- Cavers PB, Steel MG (1984) Patterns of change in seed weight over time on individual plants. *Am Nat* 124:324–335. doi:[10.1086/284276](https://doi.org/10.1086/284276)
- Celis-Diez JL, Bustamante RO (2005) Frequency dependent seed size selection on *Cryptocarya alba* (Mol.) Looser (Lauraceae): testing the effect of background. *Biol J Linn Soc* 84:137–142. doi:[10.1111/j.1095-8312.2005.00420.x](https://doi.org/10.1111/j.1095-8312.2005.00420.x)
- Eriksson O (1999) Seed size variation and its effects on germination and seedling performance in the clonal herb *Convallaria majalis*. *Acta Oecol* 20:61–66. doi:[10.1016/S1146-609X\(99\)80016-2](https://doi.org/10.1016/S1146-609X(99)80016-2)
- Ernst WHO, Piccoli F (1995) Fruit development and performance of *Shoenus nigricans* in coastal dune slacks of Europe: an extension of H.G. Baker’s seed mass-altitude to a seed mass-latitude relationship. *Acta Bot Neerl* 44(1):41–53
- Galloway LF (2001) The effect of maternal and paternal environments on seed characters in the herbaceous plant *Campanula americana* (Campanulaceae). *Am J Bot* 88:832–840. doi:[10.2307/2657035](https://doi.org/10.2307/2657035)
- Ganeshiah KN, Uma Shaanker R (1991) Seed size optimization in a wind dispersed tree *Butea monosperma*: a trade-off between seedling establishment and pod dispersal efficiency. *Oikos* 60:3–6. doi:[10.2307/3544984](https://doi.org/10.2307/3544984)
- Gómez JM (2004) Bigger is not always better: conflicting selective pressures on seed size in *Quercus ilex*. *Evolution* 58:71–80
- Greene DF, Quesada M (2005) Seed size, dispersal, and aerodynamic constrains within the Bombaceae. *Am J Bot* 92:998–1005. doi:[10.3732/ajb.92.6.998](https://doi.org/10.3732/ajb.92.6.998)
- Greipsson S, Davy A (1995) Seed mass and germination behaviour in populations of the dune-building grass *Leymus arenarius*. *Ann Bot (Lond)* 76:493–501. doi:[10.1006/anbo.1995.1125](https://doi.org/10.1006/anbo.1995.1125)
- Halpern SL (2005) Sources and consequences of seed size variation in *Lupinus perennis* (Fabaceae): adaptative and non-adaptative hypothesis. *Am J Bot* 92:205–213. doi:[10.3732/ajb.92.2.205](https://doi.org/10.3732/ajb.92.2.205)

- Hendrix SD, Sun I-F (1989) Inter- and intraspecific variation in seed mass in seven species of umbellifer. *New Phytol* 112:445–451. doi:[10.1111/j.1469-8137.1989.tb00336.x](https://doi.org/10.1111/j.1469-8137.1989.tb00336.x)
- Hulme PE, Benkman CW (2002) Granivory. In: Herrera CM, Pellmyr O (eds) *Plant-animal interactions: an evolutionary approach*. Blackwell Publishing, Oxford, pp 132–153
- Janzen DH (1977) Variation in seed size within a crop of a Costa Rican *Mucuna andreana* (Leguminosae). *Am J Bot* 64:347–349. doi:[10.2307/2441978](https://doi.org/10.2307/2441978)
- Jurado E, Westoby M, Nelson D (1991) Diaspore weight, dispersal, growth form, and perenniality of central Australian plants. *J Ecol* 79:811–830. doi:[10.2307/2260669](https://doi.org/10.2307/2260669)
- Lacey EP, Smith S, Case AL (1997) Parental effects on seed mass: seed coat but not embryo/endosperm effects. *Am J Bot* 84:1617–1620. doi:[10.2307/2446624](https://doi.org/10.2307/2446624)
- Lázaro A (2005) Aspectos ecológicos y filogeográficos del relicto mediterráneo *Buxus balearica* en poblaciones insulares y continentales. PhD thesis, University of the Balearic Islands
- Lázaro A, Méndez M (2007) Variation in sexual expression in the monoecious shrub *Buxus balearica* at different scales. *Plant Biol* 9:736–744. doi:[10.1055/s-2007-965249](https://doi.org/10.1055/s-2007-965249)
- Lázaro A, Traveset A, Castillo A (2006a) Spatial concordance at a regional scale in the regeneration process of a circum-Mediterranean relict (*Buxus balearica*): connecting seed dispersal to seedling establishment. *Ecography* 29:683–696. doi:[10.1111/j.2006.0906-7590.04667.x](https://doi.org/10.1111/j.2006.0906-7590.04667.x)
- Lázaro A, Traveset A, Méndez M (2006b) Masting in *Buxus balearica*: assessing fruiting patterns and processes at a large spatial scale. *Oikos* 115:229–240. doi:[10.1111/j.2006.0030-1299.14826.x](https://doi.org/10.1111/j.2006.0030-1299.14826.x)
- Martínez I, García D, Obeso JR (2007) Allometric allocation in fruit and seed packaging conditions: the conflict among selective pressures on seed size. *Ecol Evol* 21:517–533. doi:[10.1007/s10682-006-9132-x](https://doi.org/10.1007/s10682-006-9132-x)
- Mazer SJ (1987) The quantitative genetics of life history and fitness components in *Raphanus raphanistrum* L. (Brassicaceae): ecological and evolutionary consequences of seed-weight variation. *Am Nat* 130:891–914. doi:[10.1086/284754](https://doi.org/10.1086/284754)
- McGingley MA (1989) Within and among plant variation in seed mass and pappus size in *Tragopogon dubius*. *Can J Bot* 67:1298–1304. doi:[10.1139/b89-172](https://doi.org/10.1139/b89-172)
- McWilliams EL, Landers RQ, Mahlstedt JP (1968) Variation in seed weight in populations of *Amaranthus retroflexus* L. *Ecology* 49:290–296. doi:[10.2307/1934458](https://doi.org/10.2307/1934458)
- Méndez M (1997) Sources of variation in seed mass in *Arum italicum*. *Int J Plant Sci* 158:298–305. doi:[10.1086/297441](https://doi.org/10.1086/297441)
- Michaels HJ, Benner B, Hartgerink AP, Lee TD, Rice S, Willson MF et al (1988) Seed size variation: magnitude, distribution, and ecological correlates. *Evol Ecol* 2:157–166. doi:[10.1007/BF02067274](https://doi.org/10.1007/BF02067274)
- Murray BR, Brown AHD, Dickman CR, Crowther MS (2004) Geographical gradients in seed mass in relation to climate. *J Biogeogr* 31:379–388
- Rey PJ, Garrido JM, Alcántara JM, Ramírez JM, Aguilera A, García L et al (2002) Spatial variation in ant and rodent post-dispersal predation of vertebrate dispersed seeds. *Funct Ecol* 16:773–781. doi:[10.1046/j.1365-2435.2002.00680.x](https://doi.org/10.1046/j.1365-2435.2002.00680.x)
- Rey PJ, Alcántara JM, Valera F, Sánchez-Lafuente AM, Garrido JL, Ramirez JM et al (2004) Seedling establishment in *Olea europaea*: seed size and microhabitat affect growth and survival. *Ecoscience* 11:310–330
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution* 43:223–225. doi:[10.2307/2409177](https://doi.org/10.2307/2409177)
- Roach DA, Wulff RD (1987) Maternal effects in plants. *Annu Rev Ecol Syst* 18:209–235. doi:[10.1146/annurev.es.18.110187.001233](https://doi.org/10.1146/annurev.es.18.110187.001233)
- SAS Institute Inc (1997) SAS/STAT Software: changes and enhancements through release 6.12. SAS Institute, Cary
- Schimpf DJ (1977) Seed weight of *Amaranthus retroflexus* in relation to moisture and length of growing season. *Ecology* 58:450–453. doi:[10.2307/1935621](https://doi.org/10.2307/1935621)
- Smith CC, Fretwell SD (1974) The optimal balance between size and number of offspring. *Am Nat* 108:499–506. doi:[10.1086/282929](https://doi.org/10.1086/282929)
- Sokal RR, Rohlf FJ (1969) *Biometry. The principles and practice of statistics in biological research*. W.H. Freeman and Company, San Francisco
- Stock WD, Pate JS, Delfs J (1990) Influence of seed size and quality on seedling development under low nutrient conditions in five Australian and South African members of the Protaceae. *J Ecol* 78:1005–1020. doi:[10.2307/2260949](https://doi.org/10.2307/2260949)
- Sugiyama S (2003) Geographical distribution and phenotypic differentiation in populations of *Dactylis glomerata* L. in Japan. *Plant Ecol* 169:295–305. doi:[10.1023/A:1026081122985](https://doi.org/10.1023/A:1026081122985)
- Susko DJ, Lovett-Doust L (2000) Patterns of seed mass variation and their effects on seedling traits in *Alliaria petiolata* (Brassicaceae). *Am J Bot* 87:56–66. doi:[10.2307/2656685](https://doi.org/10.2307/2656685)

- Temme DH (1986) Seed size variability: a consequence of variable genetic quality among offspring? *Evolution* 40:414–417. doi:[10.2307/2408819](https://doi.org/10.2307/2408819)
- Thompson JN (1984) Variation among individual seed masses in *Lomatium grayi* (Umbelliferae) under controlled conditions: magnitude and partitioning of the variance. *Ecology* 65:626–631. doi:[10.2307/1941425](https://doi.org/10.2307/1941425)
- Traveset A, Riera N, Mas R (2001) The ecology of fruit-colour polymorphic species in *Myrtus communis* and differential effect of birds and mammals on seed germination and seedling growth. *J Ecol* 89:749–760. doi:[10.1046/j.0022-0477.2001.00585.x](https://doi.org/10.1046/j.0022-0477.2001.00585.x)
- Tungate KD, Burton MG, Susko DJ, Sermons SM, Ruffy TW (2006) Altered weed reproduction and maternal effects under low-nitrogen fertility. *Weed Sci* 54:847–853. doi:[10.1614/WS-05-145R.1](https://doi.org/10.1614/WS-05-145R.1)
- Vander Wall SB (2003) Effects of seed size of wind-dispersed pines (*Pinus*) on secondary seed dispersal and the caching behaviour of rodents. *Oikos* 100:25–34. doi:[10.1034/j.1600-0706.2003.11973.x](https://doi.org/10.1034/j.1600-0706.2003.11973.x)
- Vargas EM, Castro E, Macaya G, Rocha OJ (2003) Variation in fruit and seed size from 38 wild populations of *Phaseolus lunatus* (Fabaceae) from Central Valley, Costa Rica. *Rev Biol Trop* 51:707–724
- Vaughton G, Ramsey M (1998) Sources and consequences of seed mass variation in *Banksia marginata* (Proteaceae). *J Ecol* 86:563–573. doi:[10.1046/j.1365-2745.1998.00279.x](https://doi.org/10.1046/j.1365-2745.1998.00279.x)
- Venable DL (1992) Size-number trade-offs and the variation of seed size with plant resource status. *Am Nat* 10:287–304. doi:[10.1086/285413](https://doi.org/10.1086/285413)
- Verhoeven KJF, Biere A, Nevo E, van Damme JMM (2004) Differential selection on growth rate-related traits in wild barley, *Hordeum spontaneum*, in contrasting greenhouse nutrient environments. *J Evol Biol* 17:184–196. doi:[10.1046/j.1420-9101.2003.00636.x](https://doi.org/10.1046/j.1420-9101.2003.00636.x)
- Volis S, Mendlinger S, Ward D (2002) Differentiation in populations of *Hordeum spontaneum* along a gradient of environmental productivity and predictability: life history and local adaptation. *Biol J Lin Soc* 77:479–490. doi:[10.1046/j.1095-8312.2002.00120.x](https://doi.org/10.1046/j.1095-8312.2002.00120.x)
- Willis SG, Hulme PE (2004) Environmental severity and variation in the reproductive traits of *Impatiens glandulifera*. *Funct Ecol* 18:887–898. doi:[10.1111/j.0269-8463.2004.00907.x](https://doi.org/10.1111/j.0269-8463.2004.00907.x)
- Winn AA, Gross KL (1993) Latitudinal variation in seed weight and flower number in *Prunella vulgaris*. *Oecologia* 93:55–62
- Wulff RD (1986) Seed size variation in *Desmodium paniculatum*: III. Effects on reproductive yield and competitive ability. *J Ecol* 74:115–121. doi:[10.2307/2260352](https://doi.org/10.2307/2260352)
- Wulff RD, Bazzaz FA (1992) Effect of the parental nutrient regime on growth of the progeny in *Abutilon theophrasti* (Malvaceae). *Am J Bot* 79:1102–1107. doi:[10.2307/2445208](https://doi.org/10.2307/2445208)
- Yamada H, Miyaura T (2005) Geographic variation in nut size of *Castanopsis* species in Japan. *Ecol Res* 20:3–9. doi:[10.1007/s11284-004-0006-9](https://doi.org/10.1007/s11284-004-0006-9)