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Testing seed-size predictions in Mediterranean annual grasslands

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Abstract

On the basis of previous research, we predict that Mediterranean grasslands should show larger-seeded annuals in: (1) more-arid grasslands; (2) more-fertile soils; (3) less-grazed grasslands; and (4) grasslands with lower intensities of seed predation by ants. To test these predictions, we set 29 sampling units of 50 m × 50 m in a 1000 km^2 grassland area in Central Spain, and characterized them according to the former factors. We then recorded annual vegetation using ten quadrats of 20 cm × 20 cm in each sampling unit. Seed size at the community level was described using six variables: (1) mean seed mass; (2) standard deviation of seed mass; (3) weighted mean seed mass (by species frequencies); (4) proportion of small-seeded annuals; (5) proportion of medium-seeded annuals; and (6) proportion of large-seeded annuals. Most climate variables (mean annual temperature, length of the summer drought, water balance and mean annual precipitation) correlated with seed-size descriptors, showing that large-seeded annuals increase in warmer and more-arid communities. Mean seed size was modelled as a function of mean annual temperature and grazing pressure. According to this model, warmer and less-grazed communities tend to show a smaller mean seed size. These results confirm the importance of seed-size descriptors at the community level in Mediterranean grasslands, and the role of climate and grazing as major drivers in these communities. Conversely, hypotheses about soil fertility and seed predation by ants were not supported by our results.

Keywords: aridity, grazing, seed mass, seed predation, soil fertility

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Introduction

The development of predictive models for plant assemblages is one of the main goals of community ecologists, who often use species or community-level descriptors as response variables. In the case of Mediterranean grasslands, species composition is well correlated to environmental gradients such as climate, soils, landforms, microtopography, grazing pressure, successional stage and disturbance occurrence (Sterling *et al.*, 1983; Noy-Meir *et al.*, 1989; Figueroa and Davy, 1991; Peco and Espigares, 1994). The use of species does, however, have certain limitations, such as the difficulty of extrapolating predictions to different biogeographical regions. More synthetic indicators such as diversity, richness and productivity have also been modelled (de Pablo *et al.*, 1982; Seligman and Vankeulen, 1989; Montalvo *et al.*, 1993), and generally yield interpretations that have high transferability, although the underlying mechanisms are sometimes difficult to understand. More recently, researchers have focused on form and functional traits (Lavorel *et al.*, 1999; Sternberg *et al.*, 2000; Peco *et al.*, 2003, 2005; Diaz *et al.*, 2007; Acosta *et al.*, 2008), a more helpful approach to understanding ecological mechanisms that shape communities which is also suitable for predictions applied to different floras or regions. Studies on functional traits are based both on individual attributes of the species and synthetic values (e.g. mean or variance) obtained from groups of species (Ackerly *et al.*, 2002).

In this context, seed size (usually measured as seed mass) has been proposed as a key trait in plant strategies (Westoby, 1998) and its importance has also been highlighted for Mediterranean grasslands (Azcárate *et al.*, 2002; Peco *et al.*, 2003, 2009). Annual species are overwhelmingly predominant in this type of vegetation, making all processes that affect seed banks and seedling emergence and growth particularly relevant. Previous research has shown that seed size is affected by, or affects, persistence in the soil (Thompson *et al.*, 1993), herbivory (Osem *et al.*, 2006), seed predation (Azcárate and Peco, 2006),

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endozoochory (Pakeman *et al.*, 2002; Mouissie *et al.*, 2005), epizoochory (Römermann *et al.*, 2005), dyszoochory (Azcárate and Peco, 2007), germination (Kahmen and Poschlod, 2008a) and seedling growth and survival (Moles and Westoby, 2004). These processes are highly variable, and depend on both abiotic (climate, soil properties) and biotic factors (e.g. abundance and type of herbivores, seed predators and seed dispersers). Hence, a certain seed size implies costs or benefits for a given situation, and we can expect communities to differ in the range of seed sizes shown by the dominant annuals. For the particular case of Mediterranean annual grasslands, current knowledge leads to some predictions on the adaptive value of having either a large or a small seed for at least four factors: climate, soil fertility, grazing pressure and intensity of seed predation by ants.

In the first place, a more arid climate should favour large-seeded annuals. In general, large seeds show greater survival when emergence is affected by water stress (Leishman and Westoby, 1994; Veenendaal *et al.*, 1996; Westoby *et al.*, 1996; Kos and Poschlod, 2008). Although in Mediterranean grasslands there are no studies comparing seed sizes between areas with different climates, Peco *et al.* (2009) found an increase in the abundance of large-seeded species in years with dry autumns. This result suggests that Mediterranean species follow the general pattern, and large-seeded species survive better in situations of drought. It is therefore expected that Mediterranean grasslands affected by a drier climate show a greater proportion of large-seeded species in their floristic composition.

Secondly, larger-seeded annuals should be more abundant in more-fertile soils. This prediction is mainly based on the experimental study with Mediterranean species by Marañón and Grubb (1993). These authors concluded that smaller-seeded species should occupy soils with poorer nutrient supply and water retention capacity, as a consequence of their higher relative growth rates. This prediction is not universal, as shown by other contradictory results. For Australian species, Jurado and Westoby (1992) reported that seedlings of heavier-seeded species tend to survive longer than seedlings from lighter-seeded species when grown in the absence of mineral nutrients. Milberg and Lamont (1997) and Milberg *et al.* (1998) have proposed that large seeds might be an adaptation for enhancing establishment in nutrient-poor soils, although this hypothesis is by no means generally accepted. Pakeman *et al.* (2008), studying 12 sites across Europe, found no relationship between fertility and seed size, and Hanley *et al.* (2007) observed that the seed size of different floras responds in different ways to nutrient-deficient treatments, apparently confirming that the relationship between seed size and soil fertility is heavily dependent on floras or biomes.

Thirdly, small-seeded species should be promoted by increasing grazing pressure. Studies in different parts of the world have found that grazing is normally associated with an increase in small-seeded species in both seed banks and vegetation (McIntyre and Lavorel, 2001; Peco *et al.*, 2005; Osem *et al.*, 2006; Diaz *et al.*, 2007; Kahmen and Poschlod, 2008b). These findings are congruent with the effects of grazers, some of which directly involve seeds. For example, most studies analysing endozoochory report that more viable seeds are in grazer dung in the case of small seeds (Russi *et al.*, 1992; Pakeman *et al.*, 2002; Mouissie *et al.*, 2005). This has often been interpreted in terms of higher chewing and gut passage survival for small seeds, although Peco *et al.* (2006) and Bruun and Poschlod (2006) explain it as a consequence of the larger numbers of seeds produced by small-seeded plants. Other studies analysing epizoochory (seeds attached to animal skins) have also detected that light seeds are best retained (Römermann *et al.*, 2005; de Pablos and Peco, 2007), and hence are more capable of dispersal in grazed grasslands. Grazing also affects vegetation by promoting annual species, small-sized plants and certain growth forms (Noy-Meir *et al.*, 1989; Belsky, 1992; Diaz *et al.*, 2007). Some of these effects can also imply an increase in small-seeded species, since plant and seed size are often correlated (Aarssen and Jordan, 2001; Peco *et al.*, 2009) and a reduction of the abundance of the perennials relaxes competition at the moment of emergence (Jurjavcic *et al.*, 2002; Zeiter *et al.*, 2006), which probably benefits plants producing large numbers of small seeds.

Finally, seed predation by harvester ants should penalize large seeds, and hence indirectly favour small-seeded annuals. Previous research has shown that granivores often select certain seed sizes (Crist and MacMahon, 1992; Willott *et al.*, 2000a), and exclusion experiments have produced effects on vegetation that are attributable to seed-size selection by granivores (Davidson *et al.*, 1984). In Mediterranean grasslands, where *Messor* ants are the main seed predators, diet studies have revealed that ants select relatively large seeds, possibly because of their higher detectability (Detrain and Pasteels, 2000; Azcárate *et al.*, 2005). This fact could indirectly favour the seed banks of small-seeded species, as confirmed in at least one experimental study (Azcárate and Peco, 2006). However, the effect of harvester ants is not restricted to seed predation. Ants can also act as secondary dispersers (Wolff and Debussche, 1999; Retana *et al.*, 2004; Sánchez *et al.*, 2006), and middens and other nest-related structures are often favourable microsites for certain seedling types (Dean and Yeaton, 1992; Azcárate and Peco, 2007). In the case of Mediterranean grasslands, Azcárate and Peco (2007) found greater success of large-seeded species on *Messor barbarus* middens. This means that although seed predation by

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ants might affect the abundance of large-seeded species, their presence in the community may be guaranteed by their ability to colonize ant structures.

To date, no study has analysed these predictions together, considering the natural variability of Mediterranean annual grasslands at the regional scale. In this study, we test the validity of seed size as a predictable trait of Mediterranean grassland communities varying in climate, grazing pressure, intensity of seed predation by ants and soil fertility. We predict that communities should show larger-seeded annuals in (1) more-arid grasslands; (2) more-fertile soils; (3) less-grazed grasslands; and (4) grasslands with lower intensities of seed predation by ants.

Materials and methods

Study area and sampling units

The study was conducted at the foot of the Guadarrama Mountains (Madrid Region, Central Spain). We defined a study area of roughly 1000 km^2, bounded by the Manzanares River to the west, the Albalá and Guadalix rivers to the east, the 1500 m contour line to the north and the 650 m contour line and Madrid metropolitan area to the south. The selected area shows a continental Mediterranean climate, with a harsh summer drought and large thermal contrasts between winter and summer. Mean annual temperature and precipitation vary with altitude, ranging from 9°C to 14°C and 500–800 mm, respectively (Ninyerola *et al.*, 2005). The substrate is acidic, mainly composed of Carboniferous granite and Precambrian gneiss. On the southern border, igneous and metamorphic rock is covered by Miocenic sedimentary arkoses. Soils are shallow, and in general can be classified as dystric and eutric cambisols, although there are other types linked to particular landforms (Monturiol and Alcalá del Olmo, 1990). There are no biogeographical barriers in the study site, which is regarded as a floristic unit (Rivas-Martínez, 1987).

Most of the land has been used traditionally for sheep, goat and cattle grazing. As a result, the original Mediterranean forest has been transformed into a savanna-like landscape (*dehesa*) consisting of a grassland dominated by annuals with some scattered trees. *Quercus ilex* subsp. *ballota* is the predominant tree up to 1000–1200 m, *Q. pyrenaica* occurs at higher altitudes and *Fraxinus angustifolia* is frequent on most humid soils. Tree cover is usually lower than 50%, but it can be higher on abandoned land and game reserves. Agriculture is now almost non-existent, although in the past some fields were ploughed for barley and other cereals. Grazing pressure now varies considerably. Abandonment and grazing pressure reduction is sometimes explained by differences in productivity, but it is often simply a consequence of the distance from villages or roads.

Twenty-nine 50 m × 50 m sampling units were distributed in this study area. Although randomly selected, they had to meet four conditions: (1) annual-dominated grassland; (2) units in a continuous, homogeneous system of at least 1 ha; (3) valley floors and slopes with a $> 10\%$ gradient were avoided, in order to include only soils without excessive erosive or sedimentary processes; and (4) unchanged land use over at least the past 10 years. This latter condition was checked using owner interviews and aerial photographs.

Independent variables

Sampling units were characterized according to their climate, soil fertility, grazing pressure and intensity of seed predation by ants. Climate was described primarily by mean annual temperature (T) and mean annual precipitation (P). Autumn precipitation (Pa, precipitation recorded from September to November) was also included due to its influence on seedling emergence and survival. Two other aridity-related climate indices were also considered: annual water balance (WB) and length of the summer drought (D). WB was calculated as the difference between mean annual precipitation and annual potential evapotranspiration (Thornthwaite, 1948). D was calculated according to Walter–Gaussen ombrothermic diagrams (Gaussen, 1955): we plotted the diagram corresponding to each sampling unit and estimated the dates when temperature and precipitation curves crossed. We then calculated the length of the summer drought in days (Fig. 1). Climate variables were obtained from

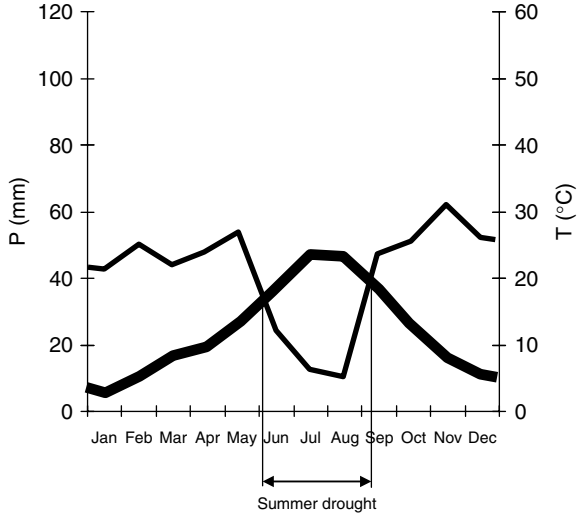


Figure 1. Walter–Gaussen diagram of one sampling unit. The diagram shows mean monthly precipitation, temperature and length of summer drought.

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Ninyerola *et al.* (2005) – a 200 m resolution digital climatic map of the Iberian Peninsula, based on meteorological stations with time series of at least 15 years.

Soil fertility was estimated using a bioassay. We used barley, a cereal commonly selected for bioassays in soils of the Mediterranean area (Moro *et al.*, 1997; Willott *et al.*, 2000b). In summer 2006, we collected ten soil cores from each sampling unit using a 4 cm width × 5 cm height cylindrical device. Sampling points were distributed at random, avoiding areas affected by trees. Each soil sample was then placed in a 4 cm wide × 5 cm tall pot, and sown with six barley seeds per pot. The pots were watered moderately in greenhouse conditions to maintain moisture. Only four seedlings were left in each pot after germination. Plants were cut 45 d after sowing, aerial biomass dry weight was measured after 48 h of oven drying at 75°C, using a 0.1 mg precision balance to measure all four plants in each pot together, and, finally, the average value of the ten soil samples collected in each sampling unit was defined for use as an index of soil fertility.

Grazing pressure was estimated from the presence of dung in the sampling units. In summer 2005, a grid of 2 m × 2 m cells was set in each plot to record presence/absence of dung in each cell. Tree-influenced areas were not taken into account. The proportion of cells containing dung of any grazer was adopted as a proxy for grazing pressure. Cattle were by far the most abundant grazers in the study area, with sheep, horse, rabbit and roe deer dung also occasionally found.

Intensity of ant seed predation was estimated using canary seed bait (*Phalaris canariensis*) in a 5-cm diameter Petri dish containing 3 g of seeds with a 4 mm steel mesh to exclude vertebrates and two 1 cm wide entrances to facilitate ant access. In August 2005, six baits were randomly distributed in each sampling unit, avoiding sites influenced by tree shade or litter. Baits were examined 48 h later, and we recorded the number discovered and depleted by ants. *Messor* harvester ants collect all seeds in a dish within a few minutes of its discovery. The likelihood of a bait being found was assumed to be a good index of the real seed harvesting intensity in the field. See Azcárate and Peco (2003) for a discussion of the suitability of this method for the study site.

Vegetation and seed-size descriptors

Vegetation was recorded in spring 2006. Ten 20 cm × 20 cm quadrats were set in each sampling unit, avoiding areas affected by tree shade or litter, and presence/absence data of all species was recorded. Annual species were characterized by their seed mass, used as a proxy for seed size. The term 'seed' is used here in a broad sense, following Bekker *et al.* (1998). In most cases, seed mass was obtained from Azcárate *et al.* (2002, 2005), Sánchez *et al.* (2002), and Azcárate and Peco (2006, 2007), all focusing on the same study area. For species not included in these studies, we followed the guidelines in Azcárate *et al.* (2002), which require the collection of at least 20 seeds from as many individuals as possible. For very small-seeded species, it is necessary to weigh seeds in groups, and hence it is necessary to collect a considerably higher amount of seeds. This procedure is extremely time-consuming for rare species, to the point of being virtually impossible in some cases. We therefore focused our efforts on the common species (defined as those present in more than four quadrats per sampling unit), completing the data set with as many rare species as possible.

We then divided the set of annuals into three seed-size classes: small-seeded species (< 0.1 mg), medium-seeded species and large-seeded species (> 1 mg). Thresholds between classes were defined after dividing the natural-log-transformed range of seed size into three equivalent segments. Finally, seed size at the community level was described using six variables: (1) mean seed mass; (2) standard deviation of the seed mass; (3) weighted mean seed mass (weighting by the species frequencies); (4) proportion of small-seeded annuals; (5) proportion of medium-seeded annuals; and (6) proportion of large-seeded annuals.

Statistical analysis

Prior to analysis, dependent variables were natural-log transformed for homoscedasticity and linearity in all parameters. The relationship between seed-size descriptors (6) and independent variables (8) was first analysed using Pearson's correlation indices, which were considered significant when $P < 0.05$. However, the probability of obtaining spurious significant results when analysing a multiple-correlation table (48 cells, in our case) is greater than the significance level (alpha inflation). Hitherto, there has been no consensus on a standardized approach to deal with this problem, and proposals range from the conservative sequential Bonferroni (Rice, 1989) to the use of no alpha-inflation correction procedure at all (Moran, 2003). In this paper, we report both the initial and the final post-correction significance following the false discovery rate (FDR) method. This procedure is based on the control of the proportion of erroneously rejected null hypotheses instead of the control of the family-wise error rate (FWER), first proposed by Benjamini and Hochberg (1995) and later by García (2003) for ecological studies.

We also estimated multiple regression models for seed-size descriptors, including the independent variables and their interactions. A backward elimination

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procedure was used to select independent variables. After the model estimations, normality in the distribution of residuals was examined graphically. All analyses were performed with Statistica version 6 (2002; StatSoft Inc., Tulsa, Oklahoma, USA).

Results

Sampling yielded 209 species, consisting of 155 annuals (74%) and 54 biennials or perennials. The mean proportion of annuals was 84.7% at the sampling unit scale (range 71.1–93.3%) and 87.4% at the quadrat scale (range 78.4–95.1%). Seed size was defined for 88 annuals (ranging from *Crassula tillaea*, 0.011 mg, to *Avena sterilis*, 9.045 mg), enough to characterize all the common annuals, and a mean of 92% of the annuals found per 20 cm × 20 cm quadrat. The impact of rare species without seed mass data in the estimation of the mean seed size at the community level was presumably low, as this value remained almost stable well before considering all the species for which we had available data.

Considering the initial P values, 15 significant correlations between seed-size descriptors and independent variables were found, involving climatic variables, grazing pressure and intensity of seed predation by ants. However, only eight correlation indices remained significant after the FDR correction (Fig. 2). Proportion of large-seeded annuals positively correlated with mean annual temperature ($r = 0.52$; $P = 0.0041$) and length of the summer drought ($r = 0.53$; $P = 0.0031$), and negatively correlated with mean annual precipitation ($r = -0.52$; $P = 0.0039$) and water balance ($r = -0.55$; $P = 0.0022$). Proportion of medium-seeded annuals showed the opposite correlations with the same climatic variables (mean annual temperature, $r = -0.61$; $P = 0.0005$; summer drought, $r = -0.61$; $P = 0.0005$; mean annual precipitation, $r = 0.55$; $P = 0.0019$; water balance $r = 0.60$; $P = 0.0006$). Seven correlation indices showed initial significant P values, but were rejected after the FDR correction: mean annual temperature versus mean seed size ($r = 0.42$; $P = 0.0252$); mean annual temperature versus standard deviation of the mean ($r = 0.38$; $P = 0.0397$); autumn precipitation versus proportion of medium-seeded annuals ($r = 0.46$; $P = 0.0127$); autumn precipitation versus proportion of large-seeded annuals ($r = -0.46$; $P = 0.0120$); length of the summer drought versus mean seed size ($r = 0.38$; $P = 0.0444$); grazing pressure versus weighted mean seed size ($r = -0.44$; $P = 0.0166$) and intensity of seed predation by ants versus standard deviation of the mean ($r = 0.38$; $P = 0.0426$). Soil fertility was the only independent variable showing no significant relationship with any seed-size descriptor.

Significant multiple regression models were obtained for five out of the six descriptors of community seed size (Table 1). No significant model could be obtained for the proportion of small-seeded annuals. Two seed-size descriptors (mean seed size and standard deviation of seed size) were dependent on two independent variables, mean annual temperature (positively) and grazing pressure (negatively). In both cases, temperature exerted more influence on the dependent variable. For the other seed-size descriptors, only one independent variable was accepted by the models.

Discussion

The results support two out of the four hypotheses tested in this study. The multiple regression models showed that mean seed size at the community level increased with mean annual temperature and decreased with grazing pressure. We also found several significant correlations between climate variables and seed-size descriptors, confirming that more-arid communities show a higher proportion of large-seeded species. Conversely, hypotheses concerning soil fertility and seed predation by ants were not supported by our results.

Correlations showed that large-seeded annuals increase in warmer and more arid grasslands, at the expense of the medium-seeded species. This effect is congruent with the increased abundance of large-seeded species in years with dry autumns found by Peco *et al.* (2009). Other authors have documented greater survival of large seeds when emergence is affected by water stress (Leishman and Westoby, 1994; Veenendaal *et al.*, 1996; Westoby *et al.*, 1996; Kos and Poschlod, 2008). Increases in the proportion of large-seeded species are at the expense of decreases in the proportion of medium-seeded species, but, interestingly, do not alter the proportion of small-seeded species. The independence between the proportion of small-seeded species and climate is perhaps a consequence of the higher amount of seeds produced by these species (Fenner and Thompson, 2005). A high seed production increases the likelihood of finding a 'safe site' in the regeneration phase, which makes it unlikely that these species would disappear from the community even when the general conditions are unfavourable.

Climatic indices (WB, D) showed higher correlations with seed-size descriptors than mean annual precipitation and autumn precipitation (in the latter case, correlations were rejected after FDR correction). However, mean annual temperature behaved similarly to climatic indices, suggesting that temperature is a better descriptor than rainfall. The literature contains few precedents supporting a major role of temperature

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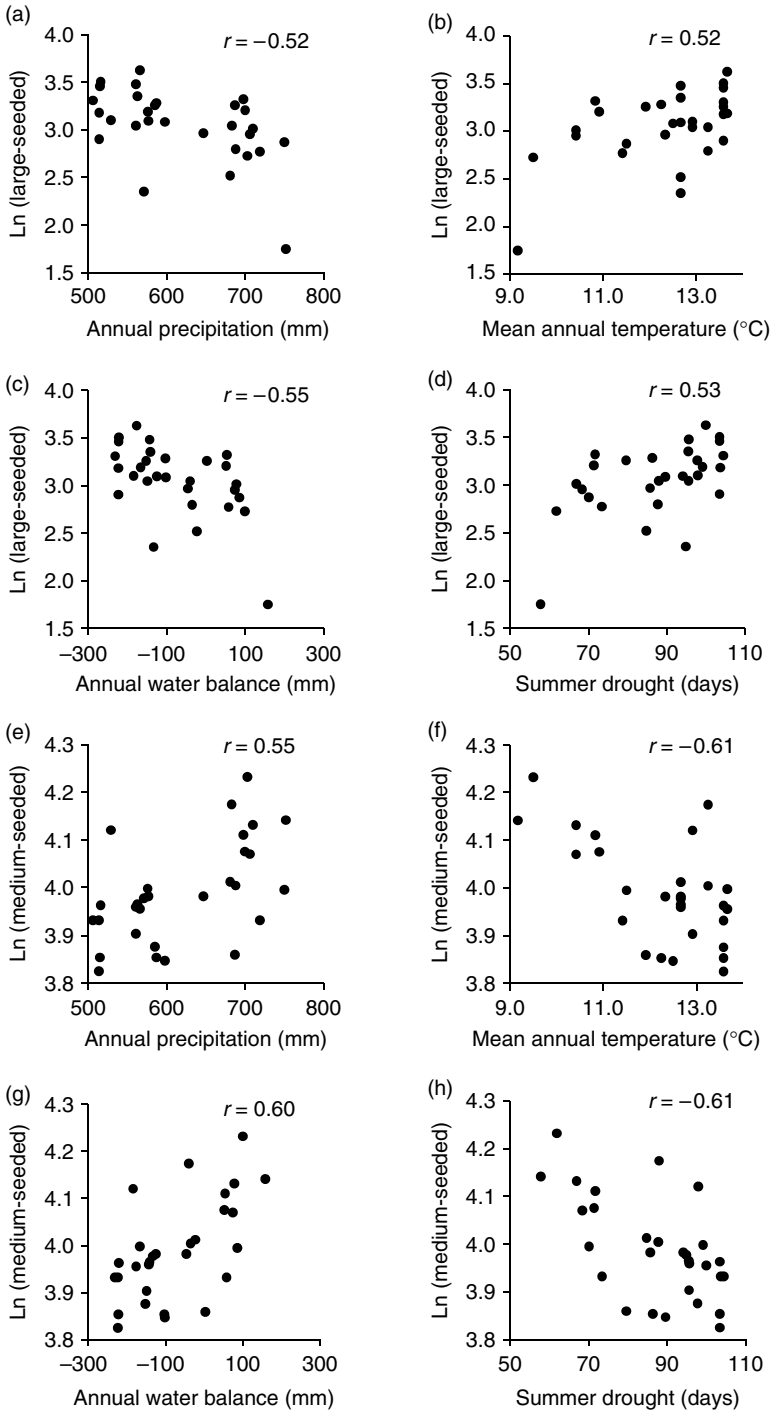


Figure 2. Significant relationships between climate descriptors and seed-size variables. Significance of the Pearson correlation indices were corrected using the false discovery rate (FDR) method ($P < 0.05$).

in determining seed size at the community level. For 12 sites across Europe, Pakeman *et al.* (2008) found that temperature was more correlated with seed mass than variables based on water availability and, in Australia, Murray *et al.* (2004) found that seed size was larger at low latitudes and inland areas, explained as a consequence of higher metabolic costs at high temperatures. One possible explanation for our results is that the rainfall gradient tested in this work (506–758 mm) was too narrow to exert more clear effects. Another possibility is that average values of precipitation are not the best indicators of aridity in Mediterranean systems. Variability is one of the main traits of Mediterranean climate, in the sense that rainfall is distributed irregularly both between and within years. Copious rainfall does not imply more

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Table 1. Stepwise multiple regression models of natural-log-transformed seed-size descriptors at the community level for the whole set of annuals. X_1, Mean annual temperature (°C); X_2, grazing pressure (proportion of 2 m × 2 m cells containing dung); X_3, summer drought (days)

Seed size descriptor (mg)	Equation	r^2	Analysis of variance
Mean	$Y = 0.15X_1 - 0.36X_2 - 2.10$	0.36	$F_{2,26} = 7.04$, $P < 0.0036$
Weighted mean	$Y = -0.40X_2 - 0.23$	0.19	$F_{1,27} = 6.50$, $P < 0.0068$
Standard deviation	$Y = 0.21X_1 - 0.53X_2 - 2.23$	0.35	$F_{2,26} = 6.90$, $P < 0.0039$
Small-seeded	–	–	–
Medium-seeded	$Y = -0.0046X_3 + 4.39$	0.37	$F_{1,27} = 15.64$, $P < 0.0004$
Large-seeded	$Y = 0.014X_3 + 1.79$	0.28	$F_{1,27} = 10.55$, $P < 0.0030$

water resources for plants, since excess water runs off after saturating the soil, while drought periods within the growing season can heavily influence vegetation (Peco *et al.*, 2009). The effects of these dry events are probably mediated by temperature, causing more harm in warmer locations. If this is the case, temperature could be a better proxy than precipitation for water shortage propensity or aridity, at least for the range of climate conditions included in our study.

We obtained a significant regression model for mean seed size as a function of mean annual temperature and grazing pressure, and also obtained a significant correlation index between grazing pressure and weighted mean seed mass, although it was rejected after the FDR correction. These results suggest that grazing tends to reduce seed size at the community level, but the effect is probably lower and subordinate to climate. Grazing can reduce community seed size, either favouring small seeds (e.g. endozoochory or epizoochory: Russi *et al.*, 1992; Pakeman *et al.*, 2002; Mouissie *et al.*, 2005; Peco *et al.*, 2005; de Pablos and Peco, 2007) or small plants (Noy-Meir *et al.*, 1989; Belsky, 1992; Diaz *et al.*, 2007), and hence indirectly promoting small seeds. Grazing also reduces competition levels by opening gaps and eliminating perennial species (Jurjavcic *et al.*, 2002; Zeiter *et al.*, 2006; Diaz *et al.*, 2007), which also benefits strategies aimed at the production of large numbers of small seeds. Standard deviation of seed mass is also dependent on mean annual temperature and grazing pressure. One possible interpretation is that, often, standard deviation scales with the mean, and hence similar relationships should be expected. In any case, the results also indicate that warmer and less-grazed communities contain a higher variability of seed sizes, which is consistent with the idea that changes in the community mainly affect large-seeded species.

Intensity of seed predation by ants only showed one significant correlation with seed-size descriptors (standard deviation); however, this was rejected after the FDR correction. The effect (if any) of ants on community seed size must be small, as otherwise this factor should be included in the models. It has been shown that large seeds are selected by ants in Mediterranean grasslands (Detrain and Pasteels, 2000; Azcárate *et al.*, 2005) and the effect of this process is visible in the seed banks (Azcárate and Peco, 2006). Furthermore, in this study we do not find any displacement of the community towards smaller seed sizes when ant granivory is intense. One possibility is that the influence of predation is overcompensated by a higher success of large seeds on ant middens (Azcárate and Peco, 2007). In any case, seed harvesting by ants is the only one of the four studied factors that could be driven by the vegetation rather than determining it. Some authors have shown a clear effect of structural factors such as vegetation density or biomass on ants (López *et al.*, 2000; Azcárate and Peco, 2004). Nevertheless, no data have been found to date to support a hypothetical dependence of *Messor* ants on vegetation composition.

Soil fertility does not predict seed size. One possible explanation is that soils of the 29 plots were too similar to exert different effects on vegetation, since we selected sites on similar geological substrata and landforms in order to avoid perennial-dominated grasslands. However, we recorded large differences in the barley biomass yielded by the soils from different plots (range: 0.191–0.381 g; $F_{28,261} = 7.57$; $P < 0.0001$), confirming that annual-dominated grasslands grow in a variety of soils, and that the sampling recorded a substantial part of this variety. The effect of soil fertility on seed size has been widely reported, but its sense is not consistent in the literature (Marañón and Grubb, 1993; Milberg and Lamont, 1997; Milberg *et al.*, 1998). Some studies have observed that different floras respond in different ways to nutrient-deficient treatments (Hanley *et al.*, 2007). In the Mediterranean, Marañón and Grubb (1993) suggested that species that tolerate soils with a poorer nutrient supply have smaller seeds and higher relative growth rate, but this experimental study was not accompanied by data showing that real communities followed this pattern. The simplest explanation for our results is that Mediterranean grasslands do not show a clear response to fertility, or that the effect is so small that

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more decisive factors such as climate and grazing make it irrelevant. Coinciding with our results, Pakeman *et al.* (2008) found no relationship between fertility and large-seeded plants when considering a large range of sites across Europe.

In conclusion, the results confirm our predictions for climate and grazing, perhaps the main factors driving Mediterranean grasslands, while soil fertility and seed predation intensity seem to be less relevant in terms of predicting community seed size. The use of seed size facilitates the interpretation of the patterns found for grazing and climate, and makes them testable in other regions with different floras. Future research on Mediterranean grasslands should include other grazing-dependent plant traits, apart from seed size (Diaz *et al.*, 2007) and combine spatial variability with interannual variability, since interannual climatic fluctuations, and not only average values, have been linked to seed size in these communities (Peco *et al.*, 2009).

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