



## Extended flowering in a Mediterranean shrub: Seasonal variability in seed quality and quantity

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### ABSTRACT

Fluctuating conditions throughout the year and changes in floral display may promote shifts in pollinator activity and predator pressure, influencing female reproductive output, especially for extended flowering species under seasonal climates. In this regard, flowering and fruit production were tracked in 2 different years in 2 populations of *Ononis tridentata* in Central Spain. Total fruit production was estimated, and fruits were harvested to obtain primary fruit investment, pollination success, predation incidence, seed production, seed weight and germination rates. *Ononis tridentata* combined spring mass flowering with a steady long flowering period across the summer and fall. The fewer flowers that are produced in fall were successfully pollinated, and produced fruits that were subject to minimal predation pressure relative to spring fruits. Moreover, fall fruits contained a higher number of heavier seeds and showed higher germination rates than those of spring seeds. Fall reproductive output represent 10% of annual viable seeds and thus may act as an important complement to the main spring reproductive investment. Extended flowering could be interpreted as a “bet-hedging strategy” for enduring Mediterranean unpredictable and changing environmental conditions.

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### Introduction

Most Mediterranean plant species present a single-peaked flowering period in spring (Bosch et al., 1997; Herrera, 1986; Petanidou et al., 1995). However, there are also Mediterranean species that flower for long periods (Picó and Retana, 2000; Wolfe and Burns, 2001). Extended flowering has been traditionally related to the maintenance of favourable and relatively uniform environmental conditions (Rathcke and Lacey, 1985). Under such circumstances, extended flowering seems to be a strategy that minimizes reproductive failure by increasing mating possibilities, improves control over a plant's relative investment in flowers and fruits, and avoids pre-dispersal seed predators (Bawa, 1983).

These predictions have been mainly tested on tropical species rather than species growing in seasonal climatic conditions. The number of plants with extended flowering in temperate regions, such as the Mediterranean Basin, has not been quantified, but appears to be far from negligible. Many of the most common and diversified Mediterranean shrub genera (i.e., *Erica*, *Cistus*, *Halimium*, *Helianthemum*, *Ulex*, *Cytisus*, *Lavandula*) represent examples of extended flowering patterns (Castroviejo, 1986–2011). Furthermore, there are also well-known Mediterranean species with

extended flowering, such as *Lobularia maritima* (Picó and Retana, 2000, 2003; Picó et al., 2002), *Ochradenus baccatus* (Hegazy et al., 2011; Wolfe and Burns, 2001). However, there remains uncertainty about the ecological role and evolutionary significance of this phenological strategy within a Mediterranean context.

Flowering period extension occurs at the population level by two mechanisms: (i) different plants, or groups of plants, flower with low synchrony during contrasting subperiods across the whole population flowering time (Tarayre et al., 2007), something that frequently occurs in relation to plant size (Bustamante and Búrquez, 2008); (ii) the same plants actively flower for a long period of time (Picó and Retana, 2000). Under the first scenario, assortative mating and reproductive isolation are more probable, because cross pollination is only possible between synchronously flowering plants (Fox, 2003; Hendry and Day, 2005). Under the second scenario, cross pollination is highly feasible as a great proportion of the plants continue flowering during the whole period.

Changes in flower density across the flowering period in combination with the seasonality of environmental conditions may profoundly affect plant interactions with both pollinators and seed predators (Brody, 1997; Elzinga et al., 2007; Grindeland et al., 2005; Pettersson, 1994). Under Mediterranean climate the density of both flowers and pollinators reach their highest values during spring, thus maximizing the probability of pollination (Thompson, 2005). But plants are also subject to pollen limitation and interference with pollen from other species (Knight et al., 2005). Moreover,

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periods with high flowering density may induce an increase in the rate of geitonogamous crosses (Mitchell et al., 2004; Miyake and Sakai, 2005). In addition, predator abundance reaches a maximum in spring too. Alternatively, flowering earlier or later may reduce the risk of predation (Lacey et al., 2003; Sánchez et al., 2008; Tarayre et al., 2007), but sub-optimal environmental conditions at these times may compromise pollination due to pollinator scarcity (Mahoro, 2002). Furthermore, fruit and seed production may also be lower, due to resource depletion or abiotic stress (Ausín et al., 2005; Giménez-Benavides et al., 2007). This constitutes a well-known evolutionary dilemma, whereby flowering in synchrony under mild spring conditions may favour pollination, but at the cost of a higher predation risk, whereas flowering during summer or fall may help plants escape predation, but at the cost of pollination failure.

The goal of our study is to evaluate whether fall reproductive output could represent a complement to that of spring, for extended flowering species in seasonal Mediterranean environments. We selected the plant *Ononis tridentata* L. (Fabaceae) as our model species, which is a long-flowering Mediterranean shrub that inhabits gypsum environments. A pilot study in our study site in central Spain (A.M. Sánchez, personal observation) has shown that flowering in this species peaks in June and continues for most of the plants until December, with a second but smaller fall flowering peak.

Based on this information, we hypothesize that fall fruit and seed production may positively contribute to total seed production of *O. tridentata*. In order to test this, we evaluated pollination success, predation incidence, the resulting female reproductive success and offspring quality, seed weight and seed germination rates, of this species along its extended reproductive period.

Evidences on the contribution of fall flowering to annual reproductive output could support the idea of extended flowering as “bet-hedging strategy” for enduring present and future Mediterranean variability on environmental conditions.

## Materials and methods

### Study species and study site

*Ononis tridentata* is a gypsophile shrub of up to 1.50 m in height that is widely distributed in gypsum areas in the Iberian Peninsula and NW Morocco. The flowering period of this shrub extends from May to December in central Spain, with a clear primary flowering peak in spring to early summer, and a secondary peak in fall. Flowers are grouped (1–3) in several axillary units along flowering branches, and look like a lax paniculated inflorescence (termed here as inflorescences). Pollination seems to be dependent on pollinator activity as bagged inflorescences do not produce any fruits (A.M. Sánchez, unpublished data). Fruits are 10–20 mm legumes containing 1–2 seeds.

The study was carried out in a gypsum semi-arid steppe near Morata de Tajuña, 40 km southeast of Madrid, central Spain (40°12'N, 3°25'W, 660 m a.s.l.). The area is covered by a mosaic of gypsum and calcicole vegetation, interspersed with olive groves. The vegetation is dominated by the perennial tussock *Stipa tenacissima* L. and gypsophile chamaephytes, such as *Helianthemum squamatum* (L.) Dum.Cours, *Lepidium subulatum* L., and *Centarurea hyssopifolia* Vahl. Most species in the community flower during the spring months, but some late summer/fall mass flowering shrubs are also present, such as *Gypsophila struthium* L. and *Launea pumila* (Cav.) Kuntze, in addition to some annuals, such as *Macrosyringium longiflorum* (Lam.) Rothm. and *Centaureum gypsicola* (Boiss. & Reut.) Druce, that continue flowering from late spring to early fall.

The climate is dry meso-Mediterranean, with a mean annual temperature of 14.2 °C, and an average annual rainfall of 438 mm

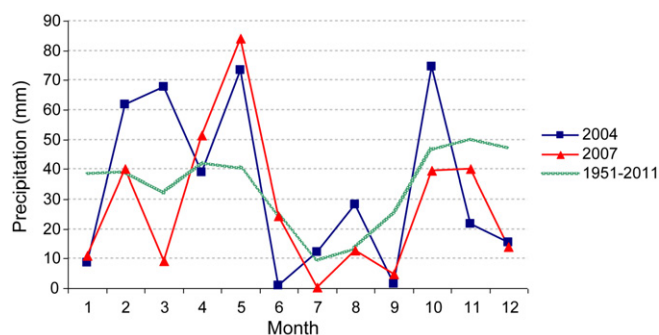


Fig. 1. Data on total precipitation per month for the two study years and mean values obtained from 1951–2011 data (Getafe thermopluviometric station).

(data from Arganda del Rey thermopluviometric station, 8 km distant from the study site,  $n=29$  years). The current study was conducted during 2 separate years: 2004 and 2007. Both years were relatively wet, but differed in the distribution of seasonal precipitation. Both years had relatively humid springs, but while the 2004 summer and fall were also relatively humid, the 2007 summer drought extended well into fall (Fig. 1).

### Data collection

Two *O. tridentata* populations that were located 1-km apart were selected. Thirty reproductive plants per population were randomly selected, tagged, and measured (i.e., plant height and 2 maximum crown diameters) before the onset of flowering. Plant volume, which served as a surrogate of plant size, was estimated as an ellipsoid ( $V=4/3\pi abc$ ). The distance from each individual to the three nearest *O. tridentata* reproductive neighbours was also recorded.

Plants were visited every 10 days during the 2004 blooming period (from late May to December). At each census, we counted the number of inflorescences with at least one open flower per plant. The total number of inflorescences with fruits was counted prior to legume dehiscence, to estimate the total number of spring and fall fruits. In addition, we obtained an estimate of spring and fall fruits per inflorescence, by counting the number of fruits in 12 inflorescences per plant (12 inflorescences  $\times$  30 plants  $\times$  2 populations = 720). Up to 50 mature legumes per plant were harvested after the spring flowering peak, while in fall each mature legume were harvested. Legumes were stored in paper bags under room conditions, until laboratory assays.

In 2007, the total number of flowers and fruits from each season were estimated for the same plants and, as in 2004, mature legumes were collected in spring and fall for analysis.

All collected legumes were dissected in the laboratory, and any sign of pre-dispersal predation, mainly caused by Hymenoptera and Lepidoptera larvae, was recorded. The number of non-fertilized ovules, mature seeds, and aborted seeds were recorded in legumes without signs of predation (up to 20 legumes per plant). Non fertilized ovules were easily identified by their small size, and aborted seeds were clearly smaller than mature ones and presented a rough, darker surface. In 2004, mature seeds were weighed to the nearest 0.0001 g ( $n=205$  seeds). Hard coated seeds were mechanically scarified by softly rubbing them with sandpaper. Once scarified, 97 spring and 140 fall seeds were placed in 11 and 14 Petri dishes, respectively, with moistened filter paper inside and 8–10 seeds per dish. All Petri dishes were placed in a germination chamber at 20 °C and 16 h light/8 h dark photoperiod. Germination was monitored every 2–3 days over 50 days.

The total number of ovules was used as an indicator of initial reproductive investment. Since the observed rate of passive autogamy was nil, pollination success was estimated by recording the

number of fertilized ovules (mature seeds + aborted seeds) per fruit, and by calculating the corresponding ovule set (percentage of fertilized ovules per fruit, Augspurger, 1981). The number of mature seeds and seed set (percentage of mature seeds per fruit) were respectively used as absolute and relative measures of reproductive success after predispersal seed predation. The number of aborted seeds was also recorded, and the abortion rate was calculated as the percentage of aborted seeds per fruit.

Among phenological predictors, we considered flowering length per plant as the number of days a plant is flowering, and flowering synchrony ( $S_i$ ; flowering overlap with any other plant in the studied population) was calculated as follows (Augspurger, 1983):

$$S_i = \frac{1}{n-1} \sum_{j \neq i} \frac{a_{ij}}{b_i}$$

where  $n$  is the total number of plants in each population,  $b_i$  is the number of days plant  $i$  is in flower, and  $a_{ij}$  is the number of days plant  $i$  and  $j$  overlap in their flowering periods. This index ranged between 0 and 1 (0 = no overlap; 1 = highest synchrony), and was calculated for each plant and flowering period.

To describe the relative importance of spring vs. fall flowering periods in terms of flower (Fw) and fruit (Fr) production, 2 seasonality indices were calculated for each plant as follows:

$$Fw(Fr) = \frac{Fs - Fa}{Fs + Fa}$$

where  $F_s$  is the number of inflorescences (or fruits) produced in spring, and  $F_a$  is the number of inflorescences (or fruits) produced for each individual in fall for Fw and Fr, respectively. These indices range between  $-1$  and  $+1$  ( $-1$  = 100% fall flower or fruit production;  $+1$  = 100% spring flower or fruit production).

### Data analysis

Spring and fall female reproductive output was modelled at two different levels: plant and fruit. We performed generalized linear models (GLMs) for the mean value per plant of several parameters that are indicative of female reproductive output at (1) the plant level: fruit production, parasitized fruit percentage, and seed production and (2) the fruit level: number of ovules, mature seeds, aborted seeds, seed set, abortion rate, and ovule set. All models include year as a repeated measures fixed factor ( $df=1$ ), season as a repeated measures fixed factor nested within a year ( $df=2$ ), and plant size as an additional fixed factor ( $df=1$ ). Population was also initially included in the models, but none of the response variables were significantly related to it, so it was removed from the models to be as parsimonious as possible.

GLMs were also performed to model which plant traits were related to the female reproductive output of each season. We used the same dependent variables at the plant and fruit level, and plant size (volume), neighbour distance (mean distance to the 3 nearest neighbours), flowering synchrony, and flowering length as fixed predictors. Data on flowering synchrony and flowering length were not collected in 2007 and so these models were performed only with 2004 data. A GLM was performed to test the effect of year (repeated measures fixed factor;  $df=1$ ), population (fixed factor;  $df=1$ ), and plant size (fixed factor;  $df=1$ ) on seasonality indices. Spearman correlations were performed between seasonality indices per plant calculated for both years to determine to what extent individual plants were consistent across time in their reproductive phenology. We also tested the existence of correlations for seasonality indices with total fruit and seed production to examine whether variation in phenology was directly related to the annual reproductive output.

We used a quasi-likelihood algorithm in all performed GLMs. The error distribution of the response variables was checked to

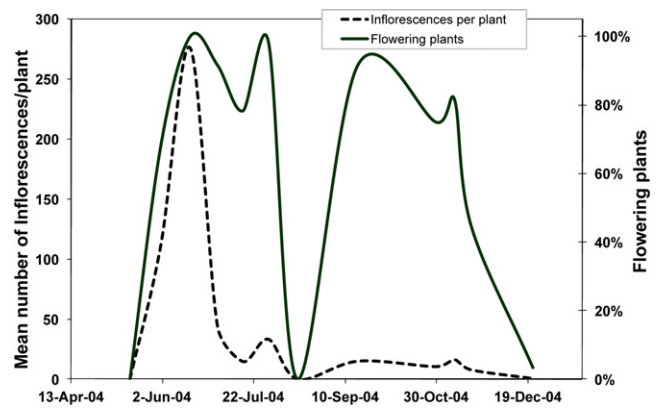


Fig. 2. Flowering pattern of *Ononis tridentata* plants studied in Morata de Tajuña (Madrid) in 2004.

select the appropriate link function. The maximum-likelihood method was used for parameter estimation and Wald  $\chi^2$ -tests were used to assess the significance of the fixed factors.

Seasonal variability in seed weight and percentage of seed germination was analysed using a Mann–Whitney  $U$  test.

Statistic computations were performed using SPSS 15.0 (SPSS Inc., 2006).

### Results

Mean  $\pm$  SD estimated plant volume was  $0.95 \pm 0.5 \text{ m}^3$  and mean distance to the three nearest *O. tridentata* reproductive neighbours was  $69.2 \pm 46.6 \text{ cm}$ .

All plants flowered across a long timespan, from late May to early December, except for three plants that did not bloom in the fall of 2004 and 2007, respectively. However, 2 and 18 plants failed to produce fruits in the fall of 2004 and 2007, respectively. Flowering intensity across the year was extremely variable, with a clear flowering peak in June, almost no flowering activity in August, and a lower flowering peak in September–October (Fig. 2). A total amount of 7416 (6862 spring + 554 fall) and 4638 (3938 spring + 700 fall) legumes were harvested and inspected in 2004 and 2007, respectively.

### Seasonal variability in female reproductive output

We found a significant effect of season for all female reproductive parameters at the plant level. Both total seed and fruit production were clearly greater and less variable in spring compared to fall periods (Fig. 3 and Table 1). Fall fruits had significantly lower predation pressure (5% of parasitized fruits) with less variability among plants, compared to fruits observed in spring (almost 60% of parasitized fruits). However, there was no variation in predation pressure between years (Fig. 3 and Table 1). Plant size was only directly related to fruit production per plant (Table 1).

Year and season significantly affected all variables observed at the fruit level. However, none of these variables were affected by plant size (Table 1). While all the variables showed quite stable spring values, fall values showed clear interannual fluctuations, in addition to higher interplant variability. Primary reproductive investment (ovules per fruit) exhibited a major decline in the fall of 2007 in comparison to the 2007 spring, and even fall 2004 values (Fig. 4 and Table 1). However, ovule set showed higher values in fall 2007 compared to those observed for both spring and fall 2004 fruits (Fig. 4 and Table 1). The number of aborted seeds and abortion rate decreased in the fall of 2004, but increased in the fall of 2007 in relation to respective spring values. Mature seed content per fruit and seed set was clearly higher in the fall of 2004

**Table 1**  
GLM models to explain variability of several parameters that are indicative of female reproductive output at both the plant and fruit level.

	Plant			Fruit					
	Fruit production (n = 238)	Parasitized fruits (%) (n = 213)	Seed production (n = 238)	Ovules (n = 97)	Mature seeds (n = 197)	Aborted seeds (n = 197)	Seed set (n = 197)	Abortion rate (n = 197)	Ovule set (n = 197)
Intercept	***	***	***	***	***	***	***	***	***
Year	***	ns	***	***	***	***	***	***	***
Season (year)	***	***	***	***	***	***	***	***	***
Plant size	**	ns	ns	ns	ns	ns	ns	ns	ns

Note: All models include the year as a repeated measures fixed factor (df = 1), season as a repeated measures fixed factor nested on year (df = 2), and plant size as a fixed factor (df = 1). Asterisks show the different degrees of significance of the Wald statistical test with the associated  $\chi^2$  value: \* < 0.5; \*\* < 0.01; \*\*\* < 0.001. n indicates number of plants for each target variable.

**Table 2**  
Mean values ( $\pm$ SD) of seed weight and seed germination (n = number of seeds and number of Petri dishes, respectively; 8–10 seeds per dish) and t and p values for the Mann–Whitney U test comparison between seasons.

	Spring	Fall	Mann–Whitney U test
Seed weight (mg)	6.9 $\pm$ 1.5 (n = 105)	7.8 $\pm$ 1.4 (n = 100)	U = 3237 p < 0.001
Germination (%)	61.3 $\pm$ 20.9 (n = 11)	91.4 $\pm$ 8.6 (n = 14)	U = 8 p < 0.001

**Table 3**  
Explicative variables significantly related to descriptors of plant reproductive output at both the plant and fruit level for the spring and fall period.

		Spring	Fall
Plant scale	Fruit production	–	Neighbour distance (**, –), flowering period length (*, +)
	Parasitized fruits (%)	–	Plant size (*, +), flowering length (*, +)
	Seed production	Plant size (*, +)	–
Fruit scale	Aborted seeds	Flowering synchrony (*, +)	–
	Mature seeds	–	Flowering synchrony (*, +)

Note: GLM model predictors comprise the following: plant size, neighbour distance, flowering synchrony, and flowering length. Asterisks show the different degrees of significance of the Wald statistical test with the associated  $\chi^2$  value: \* < 0.5; \*\* < 0.01; \*\*\* < 0.001. ( $\pm$ ) denote the sign for significant relations between explicative and dependent variables.

than in the spring of 2004. In 2007, the difference between the values of the two seasons decreased for both variables. Finally, fall seeds were significantly heavier and showed a higher percentage of germination than seeds from the spring flowering period (Table 2).

### Plant predictors related to female reproductive output

Female reproductive output in spring did not show any relationship with phenological parameters or with neighbour distance. Only seed production was positively related to plant size. However, fall reproduction was affected by some of the evaluated variables: fruit production decreased with distance to neighbours, and increased with the length of flowering. Longer flowering periods and larger plants were also related to a higher incidence of pre-dispersal predation (Table 3).

Primary reproductive investment and pollination success at the fruit scale were not affected by the studied predictors. However, aborted seeds in spring and mature seeds in fall were positively related to flowering synchrony (Table 3).

### Seasonality indices

Plant seasonality indices reflected the clearly higher intensity of the spring reproductive period, in terms of both the number of flowers and fruits, relative to the fall reproductive period (Table 4). Both indices significantly varied between years, but not in relation to plant size (Table 4). Values in 2007 were higher, indicating a higher importance of the spring flowering period, while values in 2004 were more variable among plants. Furthermore, seasonality indices based on fruits (Fr) varied between populations (Table 4).

Correlation between 2004 and 2007 seasonality indices was significant both for Fw (Rho = 0.44, p = 0.001) and Fr (Rho = 0.32,

p = 0.015). In just 2004, there was also a positive significant correlation for both seasonality indices (Fw and Fr) with respect to the total fruits per plant (Rho = 0.26, p = 0.044 and Rho = 0.42, p < 0.001, respectively). Neither Fw nor Fr showed any relationship with total seeds in any of the two study years (Table 5). Fruiting hierarchies were maintained between 2004 and 2007, as shown by the fact that the total fruits shed by each plant in both years were positively correlated (Rho = 0.37, p < 0.004). This was not the case for the total number of seeds (p > 0.05).

### Discussion

*Ononis tridentata* can produce viable seeds throughout its long flowering period. Seed production varied in quantity and quality, with spring seed production being more abundant, while fall seeds were heavier and performed better in germination trials. These results support our expectations, and revealed that pollinators, although probably a different ensemble for each season, maintained their activity and efficacy until the end of the flowering

**Table 4**  
GLM models to explain the variability of the seasonality indices based on flower (Fw) and fruit (Fr) numbers per plant.

	Fw (n = 118)		Fr (n = 118)	
	Wald $\chi^2$	p	Wald $\chi^2$	p
Intercept	492.98	0.000	21293.5	0.000
Year	32.05	0.000	38.96	0.000
Population	0.79	0.375	8.14	0.004
Year $\times$ population	1.81	0.178	3.66	0.056
Plant size	0.13	0.722	0.24	0.625

Note: Both models include year as a repeated measures fixed factor (df = 1), in addition to population (df = 1) and plant size (df = 1) as fixed factors.

**Table 5**

Spearman correlations between seasonality indices (Fw and Fr) and variables of plant reproductive output (total fruit and total seed production) observed in the two study years.

	Fw, 2004 (0.81 ± 0.20) §	Fr, 2004 (0.96 ± 0.05) §	Fruits, 2004	Seeds, 2004
Fw, 2007 (0.92 ± 0.10) §	0.44**	–	n.s.	n.s.
Fr, 2007 (0.99 ± 0.01) §	–	0.32*	n.s.	n.s.
Fruits, 2007	0.26*	0.42**	0.37**	–
Seeds, 2007	n.s.	n.s.	–	n.s.

Note: Asterisks show different levels of significance for Spearman's Rho: \* < 0.05; \*\* < 0.01. § Mean ± SD of Fw and Fr observed values.

period. In addition, fall fruits were subject to a major decline in predation risk.

The studied species combined spring mass flowering with a steady long flowering period across the summer and fall. All the plants in the observed populations had a similar flowering pattern, with minimal variation in the onset of flowering, duration, and seasonality indices. Plants exhibited a consistent flowering pattern across the two reproductive study periods. Specifically, the same plants showed lower seasonality indices for the two observed years. The seasonality indices indicated that flowering phenology was not related to plant size, as has been observed for other extended flowering species (Hegazy et al., 2011; Pettersson, 1994). Thus, the observed temporal consistency of plant flowering pattern indicates the existence of genotypic variability that may potentially be passed on to subsequent generations.

Moreover, plant size exerted almost any effect on female reproductive output. That was especially clear for variables at fruit level, pointing to the importance of processes at the population scale: flowering synchrony with other plants (Table 3) appeared to be more relevant than plant sizes. At plant level, fruit production was the only variable related to plant size all year round, while seed production was also higher in spring. That revealed a weaker effect than it was observed in other shrub species (Hegazy et al., 2011), probably due to the absence of a relation between plant size and flowering phenology.

A high percentage of plants were able to produce fall fruits (97% and 70% for 2004 and 2007, respectively). However, some data point to a possible fall resource limitation: (i) the low number of fall flowers observed; (ii) the fact that plants with a higher rate of resource-related intraspecific interference produced fewer fall, but not spring, fruits; (iii) the higher variability of most fall reproductive parameters at the fruit scale (Fig. 4). Environmental conditions in the Mediterranean region, mainly water availability, seem to modulate reproduction and are consequently critical during summer drought and later on during fall, till first rains appear. Thus, 2007 drier conditions could explain the observed reduction in the number of flowers and ovules per fruit, and the increase in aborted seeds and abortion rate during the 2007 fall season.

Fall resource limitation should be specifically tested, but what it is a fact at the light of our results is that, all the plants produced a significantly lower amount of flowers, fruits, and seeds in fall than in spring, but the seeds produced in fall were heavier and germinated better than spring seeds. These findings do not agree with previous observations in other extended flowering Mediterranean species. For instance, the ability for *Lobularia maritima* to germinate decreased throughout the course of the year (Picó and Retana, 2003), whereas the summer seeds of *Ochradenus baccatus* were smaller and less likely to germinate than winter seeds (Wolfe and Burns, 2001). In both cases, seasonal variation in seed fitness was explained as a consequence of progressive resource depletion that was associated with extended flowering. In our study, *O. tridentata* exhibited a large quantitative decline in seed production, but not in seed quality. That could be related to lower frequency of geitonogamous crosses, due to lower flower production in fall, which would promote an increase in fall seed fitness relative to spring seeds. Other studies have reported, how lower

flower density per plant is related to the number of flowers that a pollinator sequentially is visiting per plant, thus leading to a higher probability of pollen transfer between plants (Mitchell et al., 2004; Miyake and Sakai, 2005), and even, how flowering phenology may affect pollen flow distance and spatial genetic structure (Kitamoto et al., 2006). Pollinator activity, which was estimated by ovule set, was clearly affected by season, as ovule set values increased in the fruits produced during fall. It remains unclear whether these differences were produced by a change in pollinator identity, their abundance relative to flower number, or even their particular pollination behaviour. The observed higher values of ovule set from the fruits collected in fall may also be related to the post-pollination selection of spring fruits by seed predators. This indirect effect of predator selection might only be determined by recording fertilized ovules in predated fruits, which is not possible due to the act of predation itself, or by experimental predator exclusion (Herrera, 2000). In any case, it is of relevance that the extended duration of flowering is not limited by seasonal variability in pollinator availability (Rathcke and Lacey, 1985). In our study system, there must be sufficient fall pollinators to guarantee seed production. Moreover, as previously documented, fall seeds were heavier and also better at germinating, which are two well recognized fitness surrogates, in comparison to seeds produced in spring.

Pre-dispersal seed predation as well as pollinator activity both were clearly related to season. As observed in other Fabaceae shrubs that only flower during spring (Rabasa et al., 2005; Sánchez et al., 2008), an extremely high percentage of fruits in our study were predated. However, in the case of *O. tridentata*, fruits maturing from fall flowers did not experience such an intense predation pressure, as only a very low rate of these fruits were damaged by pre-dispersal seed predators. This seasonal shift in seed predator pressure clearly provides an adaptive advantage to the fall flowering period, as the effort made by plants, in allocating their resources towards fall flowers and fruits, was not threatened by seed predator activity. This pattern was especially significant for plants that produced fewer flowers and during a shorter period of time (Table 3). Variation in predation pressure across the reproductive period has been reported for a large number of species (Rathcke and Lacey, 1985), and is especially distinct in certain extended flowering species (Pettersson, 1994; Picó and Retana, 2000; Tarayre et al., 2007).

Absence of seasonality and the maintenance of favourable environmental conditions for long periods have been traditionally assumed to be one of the conditions associated with extended flowering (Bawa, 1983; Rathcke and Lacey, 1985). In contrast, the predictable Mediterranean shift from favourable to highly stressed periods is expected to promote an environmental restriction on flowering time, leading to a temporal adjustment of flowering to better environmental conditions. However, there are numerous exceptions to this reproductive behaviour. *Osyris quadripartita* (Herrera, 1988) or *Cneorum tricoccon* (Traveset, 1995) have a flowering pattern that is associated, not with the present Mediterranean climate, but with tropical conditions that existed in the region before the Pliocene. Recent studies have confirmed the basal position of *O. tridentata* within the *Ononis* genus (Turini et al., 2010), indicating that this species appeared during the early to mid Miocene, i.e., before the establishment of the Mediterranean

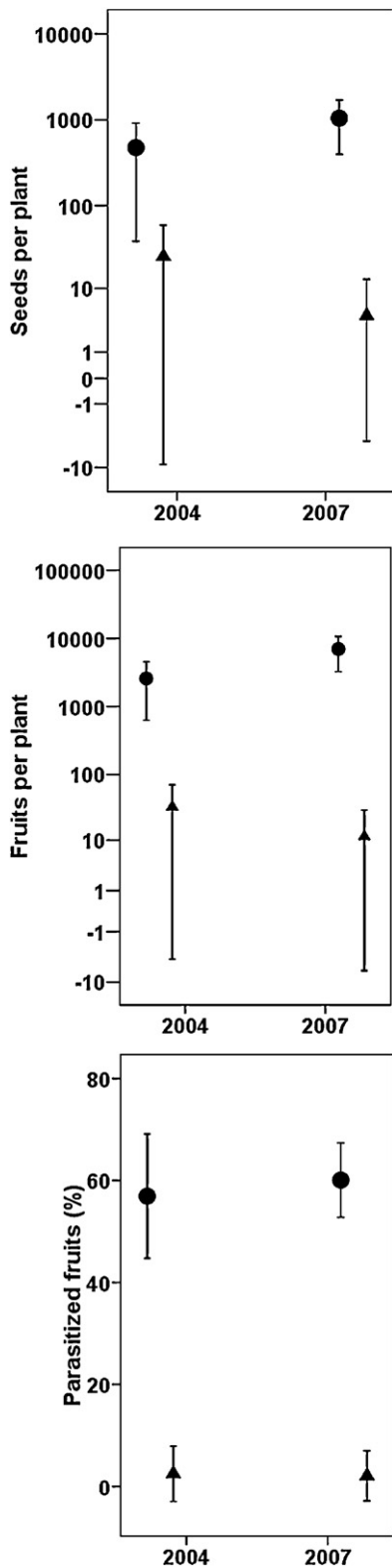


Fig. 3. Spring (●) and fall (▲) female reproductive output at the plant level (mean  $\pm$  S.D.). Fruits and seeds per plant are represented in log scale.

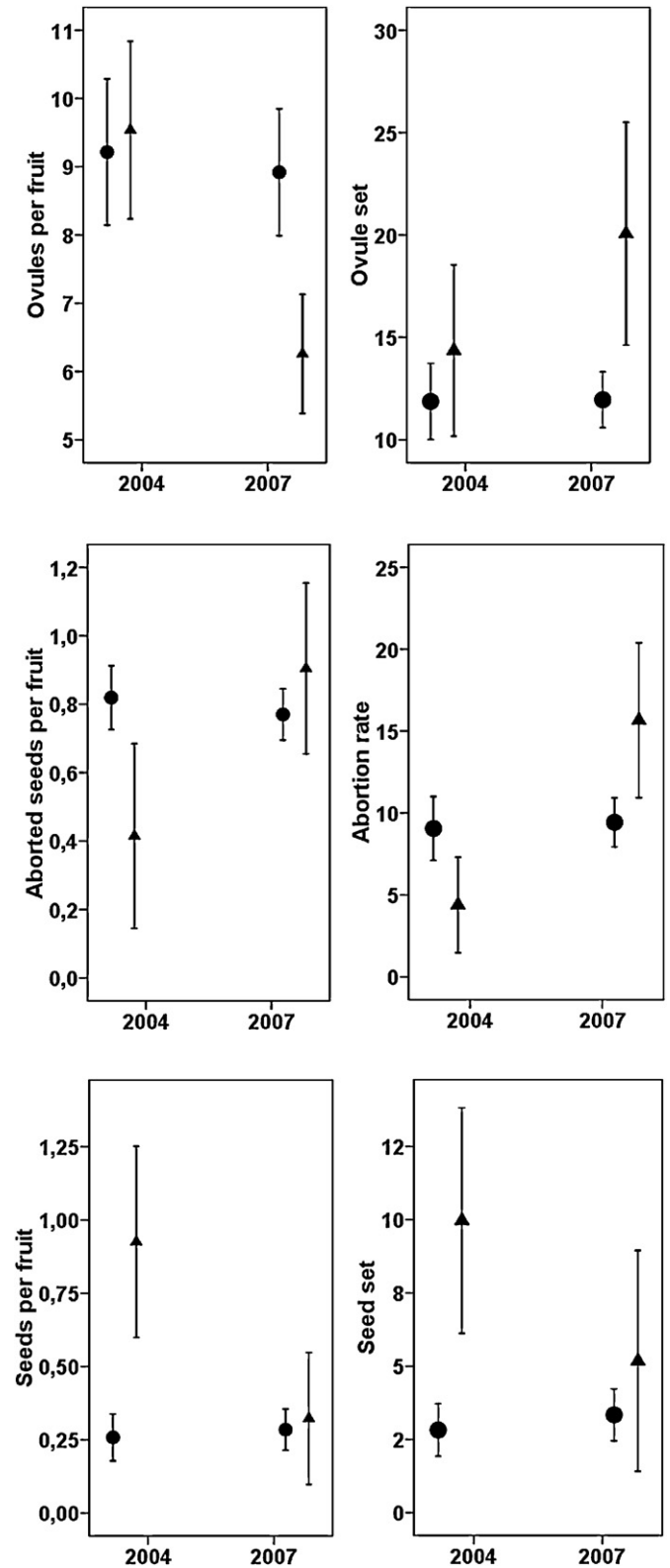


Fig. 4. Spring (●) and fall (▲) mean female reproductive output per plant at the fruit level (mean  $\pm$  SD).

climate. Our aim now is to figure out to what extent this reproductive biology confers any advantage to the investigated species in the present Mediterranean context.

It could be argued that plants producing a higher proportion of fall inflorescences, relative to spring inflorescences, and reaching a lower total fruit production, would not enjoy any advantage.

However, fall reproduction represents 10% of the annual viable seeds, and thus could be of relevance for the species population dynamics. The major decline in post-dispersal seed predation by granivorous *Messor* spp. harvester ants from late September onwards (Albert et al., 2005; Sánchez et al., 2006) and the larger

size and higher germination rate of fall seeds may favour this possibility (Metz et al., 2010).

Even if fall fruit production might not be of obvious relevance to the short-term population dynamics of *O. tridentata*, the adaptive potential of extended flowering periods should be considered in a broader temporal scale. It has been experimentally proved that variable and unpredictable environments promote the appearance of life-history strategies that allow individuals to spread the risk of reproductive failure in a larger temporal scale (Beaumont et al., 2009). The so called bet-hedging strategy (Slatkin, 1974) assumes the sacrifice of mean fitness levels to reduce variation in temporal fitness variation (or avoiding putting all your eggs in one basket) to maximize long-term fitness (Childs et al., 2010; Wilbur and Rudolf, 2006). Similarly as previously suggested for other extended flowering species (Picó and Retana, 2000; Tarayre et al., 2007), our study supports the idea that extended flowering may be adaptive, as it provides a species with the ability to persist in environments with changing selective pressures across time (Donohue, 2005). Under variable and unpredictable Mediterranean environmental conditions, “avoiding putting all the seeds in one season” confers plasticity in reproductive behaviour and may help to counteract environmental unpredictability and to prevail over the current processes of environmental change.

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