

SHORT COMMUNICATION

Effect of Pollination on Floral Longevity and Costs of Delaying Fertilization in the Out-crossing *Polygala vayredae* Costa (Polygalaceae)

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Received: 6 May 2008 Returned for revision: 8 July 2008 Accepted: 14 August 2008 Published electronically: 30 September 2008

• **Background and Aims** The effect of pollination on flower life span has been widely studied, but so far little attention has been paid to the reproductive consequences of delayed pollination in plants with long floral life spans. In the present study, *Polygala vayredae* was used to answer the following questions. (1) How does male and female success affect the floral longevity of individual flowers? (2) How does delaying fertilization affect the female fitness of this species?

• **Methods** Floral longevity was studied after experimental pollinations involving male and/or female accomplishment, bagging and open pollination. The reproductive costs of a delay in the moment of fertilization were evaluated through fruit set, seed–ovule ratio and seed weight, after pollination of flowers that had been bagged for 2–18 d.

• **Key Results** Senescence of the flowers of *P. vayredae* was activated by pollen reception on the stigmatic papillae, while pollen removal had no effect on floral longevity. Nonetheless, a minimum longevity of 8 d was detected, even after successful pollination and pollen dissemination. This period may be involved with the enhancement of male accrual rates, as the female accomplishment is generally achieved after the first visit. Floral life span of open-pollinated flowers was variable and negatively correlated with pollinator visitation rates. Delayed pollination had a major impact on the reproductive success of the plant, with fruit set, seed–ovule ratio and seed weight being significantly diminished with the increase of flower age at the moment of fertilization.

• **Conclusions** A strong relationship between pollination and floral longevity was observed. Flowers revealed the ability to extend or reduce their longevity, within some limits, in response to the abundance of efficient pollinators (i.e. reproductive fulfilment rates). Furthermore, with scarce or unpredictable pollinators, a long floral life span could maintain the opportunity for fertilization but would also have reproductive costs on production of offspring. Reduced female fitness late in the flower's life could shift the cost–benefit balance towards a shorter life span, partially counteracting the selection for longer floral life span potentially mediated by scarce pollination services.

Key words: Delayed pollination, endemic species, flower longevity, life span, pollen limitation, pollination, pollinator scarcity, *Polygala vayredae*, Polygalaceae, reproductive consequences, secondary pollen presentation.

INTRODUCTION

Floral longevity plays an important role in the reproductive ecology of plants. The length of time a flower remains open and functional influences the total number of pollinator visits and the size of floral displays, affecting the amount and quality of the pollen received and exported by the flower (Primack, 1985; Ashman and Schoen, 1996; Harder and Johnson, 2005) and, thus, its overall fitness (Rathcke, 2003). Flowers that present an extended longevity increase the opportunity for reproductive success through both pollen and ovules, but also require a high maintenance cost to sustain their functioning and attractiveness to pollinators (Ashman and Schoen, 1997).

Flowering plants exhibit a high diversity in floral life span, suggesting that floral longevity could represent a character adapted to the surrounding ecological conditions, where abiotic (e.g. temperature or water availability; Primack, 1985; Yasaka *et al.*, 1998) and biotic factors (e.g. pollinator visitation rates; Ashman and Schoen, 1994), as well as intrinsic features (e.g. breeding system;

Primack, 1985; Sato, 2002) could have an important effect in the selection of floral longevity. The effects of pollination on floral longevity variations have been evaluated in several plant species and, overall, the results have revealed a decrease in floral longevity with male and/or female accrual rates, i.e. with the accomplishment of flower function (e.g. Stead and Moore, 1979; Ishii and Sakai, 2000; Stpiczynska, 2003; Abdala-Roberts *et al.*, 2007). Pollen limitation due to scarcity of pollinators (insufficient pollen receipt) and/or poor quality of pollen received has been described as a common feature among animal-pollinated species, and may compromise seed production in many plant populations (e.g. Larson and Barrett, 2000; Ashman *et al.*, 2004; Knight *et al.*, 2005). If floral longevity can respond to pollen dissemination and/or pollen receipt, then it could have the necessary flexibility for optimizing the balance between reproductive output and resource efforts (Porat *et al.*, 1994), for example under unpredictable assemblages of pollinators. Scarce pollinators could thus be involved in the selection of a longer floral life span when floral maintenance costs are low (Ashman and Schoen, 1994). Nonetheless, to our knowledge, no study has experimentally evaluated the costs of a delay in the moment of

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pollination (due, for example, to scarce pollinators) to the reproductive outcome of the plant and its potential impacts on the selection of the floral longevity trait (but see Webb and Littleton, 1987).

Polygala vayredae (Polygalaceae) is a narrow endemic species from the oriental pre-Pyrenees, with long-lived papilionate flowers especially suited for entomophily. It flowers at the beginning of spring and, despite having long-lived flowers, different floral life spans were observed during the flowering season. Like other early flowering species (e.g. Schemske *et al.*, 1978; Navarro, 1998; Baker *et al.*, 2000; Griffin and Barrett, 2002), it is frequently subjected to pollinator unpredictability and pollen limitation, which detrimentally affected fruit and seed production (Castro, 2007; Castro *et al.*, 2008a). The objectives of the present work were, first, to evaluate how pollination affects floral longevity and, secondly, to assess the impacts of delayed fertilization, which frequently occurs due to pollinator scarcity, on the reproductive outcome of *P. vayredae*. Experiments involving different pollination treatments and xenogamous pollination of flowers with manipulated longevity were performed. By addressing these issues, this study constitutes the first report on floral longevity within the *Polygala* genus and contributes with further information on reproductive consequences resulting from the delay in the moment of fertilization beyond a mean floral longevity, a largely neglected topic in the literature.

MATERIALS AND METHODS

Plant and study area

Polygala vayredae Costa is an early flowering shrublet (April–May), occurring in a narrow area in Alta Garrotxa, Girona (Catalunya, Spain, UTM DG57 and DG58). Annually, new ramets are produced from a rootstock, leading to the formation of dense carpets of this plant. Protandrous flowers are developed in small axilar inflorescences of 1–3 units which, under natural conditions, are open for 8 ± 1.1 d (Castro *et al.*, 2008a). Pollen dehiscence occurs prior to anthesis, with most of the pollen grains being secondarily presented in a sterile branch of the stigma (secondary pollen presentation; Castro *et al.*, 2008b). The stigma receptivity reaches a maximum on day 3 and then decreases slightly, although always maintaining a large percentage of receptive flowers until senescence. Contrary to what has been described in other species of the genus (e.g. Lack and Kay, 1987; Weekley and Brothers, 2006), *P. vayredae* lacks an autonomous mechanism of self-pollination. This species is self-incompatible and relies strictly on pollination vectors to set fruits, with queens of the long-tongued bees *Bombus pascuorum* and *Anthophora* sp. being its main pollinators. Pollinator scarcity and unpredictability were observed during the period 2004–2007, frequently leading to pollen limitation and low fruit production (Castro, 2007; Castro *et al.*, 2008a).

The study was performed during the springs of 2006 and 2007 in the Coldecarrera population in the natural protected area of Alta Garrotxa (UTM DG57). This population

occurs at an altitude of 630 m, in mesophytic meadows (*Mesobromion*) under the *Buxo-Quercetum pubescentis* domain, with sparse cultivated *Pinus sylvestris*.

Effect of pollination on floral longevity

To determine the effect of pollination on floral longevity the following experiments were performed on randomly selected individual flowers during the spring of 2006: (a) bagged flowers with pollen removed from the pollen presenter (after successive recharging until no pollen remained to be presented; for details see Castro *et al.*, 2008b), i.e. male accomplishment; (b) bagged flowers hand-pollinated with xenogamous pollen, i.e. female accomplishment; (c) bagged flowers with pollen removed and hand-pollinated with xenogamous pollen, i.e. both male and female accomplishment; (d) bagged flowers without any treatment; and (e) open-pollinated flowers. In these experiments, the bagging process was conducted before anthesis to prevent natural pollination and the flowers were tagged with the day of flower opening. The pollen was removed immediately after anthesis (male accomplishment) and the pollinations were performed on the day 3 (female accomplishment), when the peak of stigmatic receptivity occurs (Castro *et al.*, 2008a). Flowers were monitored daily and floral longevity recorded.

Costs of delayed pollination for female fitness

The costs of delayed pollination on fruit and seed production and seed weight were evaluated during the spring of 2007. Previous experiments revealed that senescence of *P. vayredae* flowers is induced by pollination, allowing floral longevity to be manipulated experimentally (Ashman and Schoen, 1997). Hand pollinations were performed in several individual flowers at different times, in order to obtain flowers pollinated from day 2 to day 18, the maximum longevity observed. Day 1 was excluded as stigmatic receptivity was found to be <50% (Castro *et al.*, 2008a). Eight clusters of 1 m², with several reproductive ramets and bud flowers, were protected with a mosquito net to avoid natural pollination. Flowers were monitored daily and tagged with the day of anthesis until sets of flowers with delays of 2–18 d were obtained (2–4 flowers of the same age were tagged per cluster). Only ramets presenting one flower were selected. All flowers were hand-pollinated with a fresh pollen mixture collected from at least 10 distinct individuals. The mosquito net was maintained until flower senescence. Fruit and seed production were recorded when mature, and seeds were collected for determination of their weight. In the laboratory, seeds were dehydrated under natural conditions, maintained in a vacuum desiccator with silica gel for 24 h, and weighed in an analytical balance (0.01 mg precision).

Statistical analysis

Descriptive statistics were calculated for flower longevity and are presented as mean and standard deviation of the mean. Differences in floral longevity among treatments and

differences in fruit set, seed–ovule ratio and seed weight according to flower age were assessed. The effect of the pollination treatment on flower longevity was evaluated with a Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks using Dunn’s method for pairwise multiple comparison, including another treatment with the data on floral longevity of open-pollinated flowers obtained in the same population during 2005 (data from Castro *et al.*, 2008a). The effect of delayed pollination on the fruit set and seed–ovule ratio (categorical data) was analysed with a logistic regression model with a link function (logit) and approximated by a binomial distribution, while seed weight was analysed using a one-way ANOVA followed by a Tukey test (GLM procedure due to unbalanced data). The correlation between the moment of pollination and seed weight was investigated through the Pearson correlation coefficient.

RESULTS

Effect of pollination on floral longevity

Pollination treatments significantly affected the longevity of the flowers of *P. vayredae* ($H = 100.9$, $P < 0.001$; Fig. 1). Nonetheless, this effect was not similar after male and female achievement: while floral longevity was significantly reduced when female function was accomplished (regardless of male function), it did not vary when only the male function was involved (bagged flowers and flowers with accomplished male function showed similar longevity; Fig. 1). Under natural conditions, floral longevity varied significantly by year, with open-pollinated

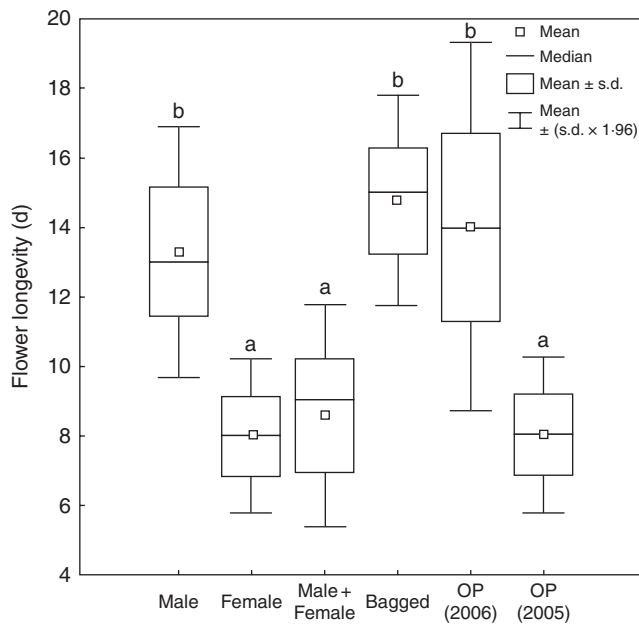


FIG. 1. Longevity of *Polygala vayredae* flowers after different pollination treatments: Male, bagged flowers with all the available pollen removed; Female, bagged flowers hand-pollinated with xenogamous pollen; Male + Female, bagged flowers with pollen removed and hand pollinated; Bagged, bagged unmanipulated flowers; OP, open-pollinated flowers from 2005 and 2006. Data from 2005 were obtained from Castro *et al.* (2008a). Different letters show significant differences at $P < 0.05$.

flowers presenting longer life spans during 2006 (despite being variable, the floral longevity was similar to that obtained in bagged flowers) than during 2005 (the floral longevity in this year was similar to longevities obtained after female accomplishment). Additionally, a minimum longevity of 8 ± 1.3 d appears to exist as flowers remain open even after early female fulfilment (i.e. when pollinations were performed during the first days of flowering).

Costs of delayed pollination for female fitness

The fruit set, seed–ovule ratio and seed weights obtained after pollination of flowers of different ages are presented in Fig. 2. Fruit set and seed–ovule ratio were higher when the pollinations were performed in the first days of the flower’s life span and significantly decreased with the increase in flower age ($\chi^2 = 81.7$, $P < 0.001$ and $\chi^2 = 113.8$, $P < 0.001$, for fruit set and seed–ovule ratio, respectively; Fig. 2A). Despite being highly variable, a similar result was obtained for the seed weight ($F = 4.90$, $P < 0.001$; Fig. 2B). There was a significant and negative relationship between the age at which the flower is pollinated and the seed weight (correlation coefficient = -0.488 , $P < 0.001$), i.e. an increase in flower age leads to a significant decrease in the seed weight (Fig. 2B).

DISCUSSION

Optimal floral longevity has been described as a trade-off between resource allocation to floral construction and floral maintenance (Ashman and Schoen, 1994, 1996; Schoen and Ashman, 1995). According to the model proposed by Ashman and Schoen (1994) and assuming a fixed resource availability, optimal floral longevities are determined by the interaction between the daily cost of flower maintenance in relation to the cost of producing a new flower (floral maintenance cost) and the rates of male and female fitness accruals with time (reproductive success). Thus, floral longevity could respond to pollen dissemination and/or pollen receipt, revealing the necessary flexibility for optimizing the balance between reproductive output and resource efforts (Porat *et al.*, 1994). In the present study, a strong effect of the reception of pollen over the stigmatic papillae on the longevity of *P. vayredae* flowers was observed. Pollinated flowers, regardless of having their pollen removed, live for shorter periods than bagged flowers or flowers where only the pollen was removed. On the other hand, the fulfilment of the male function did not affect floral longevity. Reception of pollen over the stigma revealed similar effects on floral senescence of several other species, regardless of pollen removal (e.g. Proctor and Harder, 1995; Ishii and Sakai, 2000; Luyt and Johnson, 2001; Martini *et al.*, 2003; Stpiczynska, 2003; Weber and Goodwillie, 2007). Experimental studies indicate that an increase in the production of endogenous ethylene is generally involved in corolla wilting and abscission (e.g. Nichols *et al.*, 1983), after a series of pollination-induced signals being generated within the floral tissues and transmitted through the style (e.g. Stead and Moore, 1979; Shibuya *et al.*, 2000).

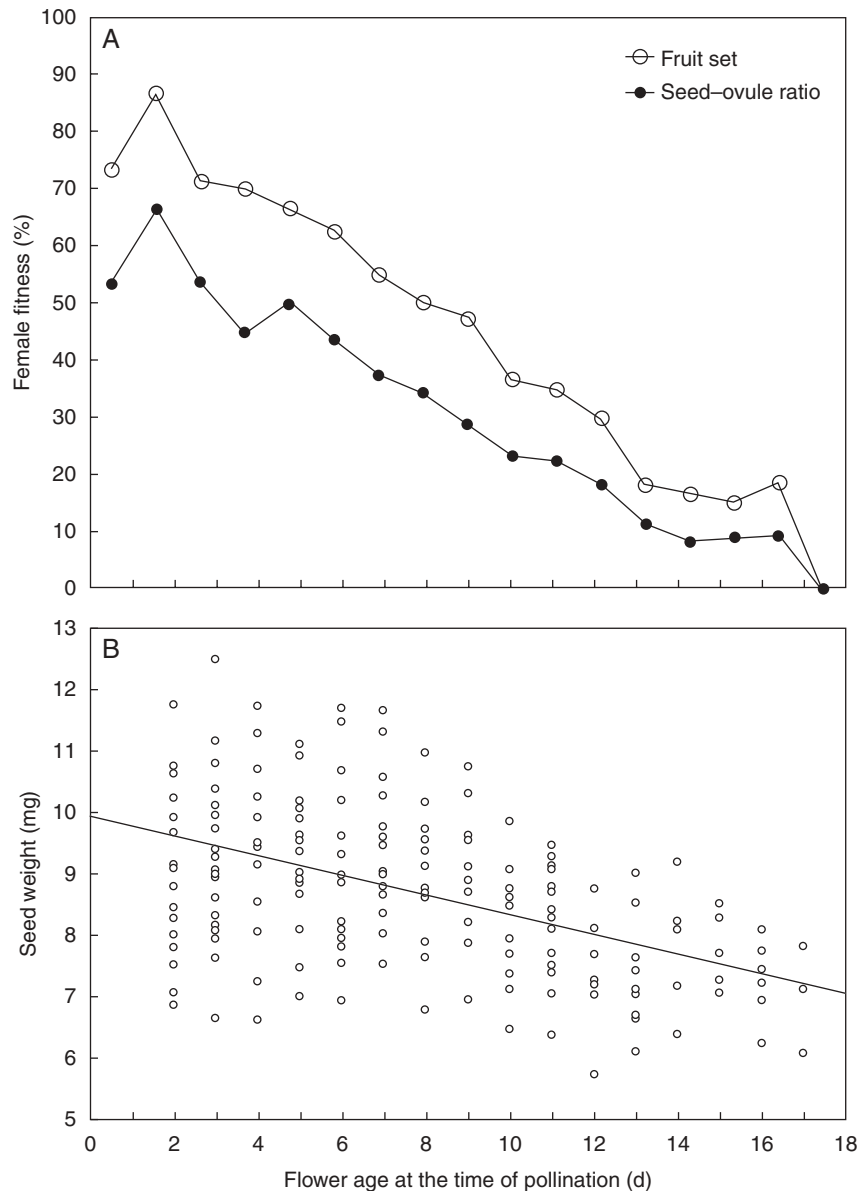


FIG. 2. Costs of delayed pollination for female fitness of *Polygala vayredae* flowers: (A) fruit set and seed-ovule ratio obtained after cross-pollination of flowers at different ages (from 2 to 18 d); (B) scatterplot of the seed weight as a function of the age at which flowers were pollinated (circles) and fitted linear model (line).

Furthermore, the amount of pollen received also appears to play a major role. In some species, the amount of ethylene produced by the flower has been positively correlated with the amount of pollen received in the stigma (e.g. Hill *et al.*, 1987; Stead, 1992).

However, while shortening of floral duration as a result of pollen reception is frequently observed (see references above, e.g. Proctor and Harder, 1995; Stpiczynska, 2003), an effective shortening of floral longevity after pollen removal is rarer (but see Devlin and Stephenson, 1984; Richardson and Stephenson, 1989; Sargent and Roitberg, 2000; Evanhoe and Galloway, 2002). Thus, assuming that flowers react to pollen reception but not to pollen removal, and considering the advantages of plasticity in floral longevity (i.e. reduction in maintenance costs), Ishii and

Sakai (2000) predicted that flowers will have a minimum longevity during which they do not abscise, even if most of their ovules are fertilized, so that male function can be enhanced. This was observed for *Erythronium japonicum* Decne., and similar patterns could also occur in other species which maintain their flowers for several days after being pollinated (Ishii and Sakai, 2000, and references therein). In *P. vayredae*, a minimum period of flower longevity also appears to be present as flowers remained open for about 8 d, despite female and male functions having been accomplished during the first days of a flower's life. Because this plant secondarily presents its pollen near the stigmatic papillae, the first pollinator's visit is vital for successful pollination (female accomplishment) (Brantjes, 1982; Castro, 2007). On the other hand, as pollen can still

be exported in subsequent visits, the minimum duration of floral longevity appears to be especially advantageous for a higher success of male fitness.

Because floral longevity is assumed to be a heritable trait, natural selection could play an important role in its optimization, with long-lived flowers being selected when fitness accrual rates and floral maintenance costs are low, and short-lived flowers being selected when fitness accrual rates and floral maintenance costs are high. Thus, plants could adapt to different levels of pollinator activity through evolving differences in floral longevity (Ashman and Schoen, 1994). In *P. vayredae*, the reliance on pollinators for seed production (Castro *et al.*, 2008a) and the low visitation rates of efficient pollinators (Castro, 2007) could be the most important factors leading to the long life span of its flowers. Furthermore, the floral duration appears to have some plasticity, as floral longevity varied significantly between years, with shorter longevity being observed during 2005 and longer longevity during 2006. These results were in accordance with the activity of effective pollinators and visitation rates reported for each year: the frequency of interactions between *P. vayredae* flowers and *B. pascuorum* queens, the main pollinator in Coldecarrera population, was clearly higher during 2005 (2.42, for 15 min following Herrera, 1989) than during 2006 (0.09) (Castro, 2007). Thus, it appears that, to some extent, the flowers presented variability in floral longevity as a response to the abundance of pollinators.

Many self-compatible species have mechanisms of delayed self-pollination as a means of ensuring reproduction when pollinators are scarce and/or unreliable (e.g. Kalisz and Vogler, 2003). On the other hand, self-incompatible species strictly rely on pollen vectors to transfer their male gametes to conspecific stigmas and achieve fertilization. As proposed above, plants could adapt to different levels of pollinator activity through evolving differences in floral longevity (Ashman and Schoen, 1994). Longer life spans could thus be achieved when pollination vectors are scarce and unreliable. Under these conditions, the plants will most probably experience a delay in the moment of pollination. However, little information is available on the reproductive consequences of a delayed pollination beyond the minimum or mean floral longevity (but see Webb and Littleton, 1987). Long life spans could have advantages, through maintaining the opportunity for pollen transfer, but could also have reproductive consequences, through the high energetic cost of flower maintenance (Ashman and Schoen, 1997). Factors, such as water balance (e.g. Nobel, 1977), nectar production (e.g. Southwick, 1984; Pyke, 1991; Ashman and Schoen, 1997), respiration rates (e.g. Bazzaz *et al.*, 1979; Werk and Ehleringer, 1983; Ashman and Schoen, 1994) and/or loss of gamete viability with the increase of flower age (e.g. Smith-Huerta and Vasek, 1984; Ashman and Schoen, 1997), have been described as the main direct costs involved in flower maintenance. The results of this study show a trade-off between the advantages of longer lived flowers and the disadvantages of delaying fertilization in *P. vayredae*. Fruit and seed production, as well as seed weight, declined significantly with an increase of flower

age at the moment of cross-pollination. Similar results were obtained in *Gentiana serotina* and *G. saxosa*. In these species, floral senescence was triggered by pollen reception, and the proportion of ovules that developed into seeds was negatively affected by the age of the stigma when it was pollinated (Webb and Littleton, 1987). In *P. vayredae*, a gradual decrease, unexpectedly even before the minimum longevity was achieved, was observed for fruit and seed production. This reduction in fruit and seed production could reflect a major energetic cost of maintaining flowers for a longer period, through increased resource allocation to floral maintenance (i.e. nectar production, respiration and transpiration) and/or through the loss of gamete viability with flower age, namely ovule viability, as peak receptivity occurs at day 3, slightly decreasing afterwards (Castro *et al.*, 2008a), similarly to what occurs with fruit and seed production. Reduced seed set or lower seed quality late in the flower's life could shift the cost-benefit balance towards a shorter life span, partially counteracting the selection for longer floral life span mediated by scarce pollination services. Thus, the reduction in female fitness over time may be an important factor in the evolution of floral longevity in this species. The equilibrium between reproductive outcomes and floral maintenance costs will determine the direction of floral longevity selection. As flowers are maintained for a minimum longevity of 8 d despite some loss in female fitness, other indirect outputs, besides male accrual rates, such as the contribution to the overall floral display of the plant, may also be involved. To understand better the selection of floral longevity, further studies should also evaluate experimentally the reproductive costs of delayed fertilization frequently occurring under scarce pollinator assemblages.

The present study constitutes the first report on floral longevity variation within the *Polygala* genus, revealing the role of stigmatic pollen reception on floral life span, as well as the ability to extend or reduce floral longevity, within some limits, in response to the abundance of efficient pollinators (i.e. reproductive fulfilment rates). Furthermore, the maintenance of the flowers for longer periods before cross-pollination negatively affected fruit and seed production as well as the seed weight, highlighting that a long floral life span could maintain the opportunity for fertilization but also have reproductive costs in offspring production.

ACKNOWLEDGEMENTS

The authors thank the Departamento de Medi Ambient of Generalitat de Catalunya, the Consorsis d'Alta Garrotxa and the Parc Natural de la Zona Volcànica de la Garrotxa for allowing this research and for all the support provided. They are also grateful to Dr João Loureiro for all his support and for critical reading of the manuscript. The Portuguese Foundation for Science and Technology (FCT) financed the work of S.C. (FCT/BD/10901/2002; FCT/BPD/41200/2007). The work was partially financed under grants PGIDT04PXIC31003PN from the Xunta de Galicia and CGL2006-13847-CO2-02 from the Spanish DGICYT to L.N.

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