

Breeding system and ecological traits of the critically endangered endemic plant *Limonium barceloi* (Gil and Llorens) (Plumbaginaceae)

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Abstract *Limonium barceloi* (Plumbaginaceae) is a narrow endemic seasonal halophytic plant, uniquely found in a highly degraded urban wetland in the Bay of Palma Mallorca, located in the northwest Mediterranean. It was awarded critically endangered status in 2004 and is the subject of a recovery plan administered by local government. Despite this, the last ten years have seen a dramatic decline in the population from ca. 3,000 individuals to just ca. 300; reasons for this decline are principally anthropogenic disturbance. Here we present the results of an investigation into some aspects of the reproductive biology of this species in the last remaining in situ population, in order to gain insight into its limited distribution and abundance, and to guide further development of management strategies. Findings indicate that although it provides important floral resources to a number of insect visitors, the plant is an autonomous apomictic that may also be functioning as an obligate asexual reproducer, and low genetic variation is suspected. Germinability is observed to be at ca. 70% and was positively correlated with maternal plant size characteristics. Two seed predators, the moth *Goniodoma limoniella* (Coleophoridae) and the ant *Messor bouvieri* (Myrmicinae), were discovered affecting both pre- and post-dispersal seeds. Rate of removal was high, although it is hypothesised that at this time species recruitment is not seed limited, but rather restricted by lack

of suitable microsites. The results of this study are used to make recommendations for the species recovery.

Keywords Apomixis · Balearic Islands · Plant conservation · Plant recovery plans · Reproductive biology · Sea lavender

Introduction

Recovery plans for endangered plants often require the creation of new, self-sustaining populations within their historic range and habitat (Knudsen 1987; Whitten 1990; Pavlik et al. 1993). Unfortunately, creating new self-sustaining populations that possess the genetic and ecological characteristics of natural populations remains a great challenge (Pavlik et al. 1993; Heywood and Iriondo 2003) and ought to begin with experiments to determine the ecological factors governing the growth of the founding population (Schemske et al. 1994). Amongst the key questions to be addressed are: what factors determine the viability of the population, which life stage is most critical for the viability of the population, and which management strategy offers the greatest chances for facilitating the survival of the population (Schemske et al. 1994; Heywood and Iriondo 2003)?

To provide adequate answers to these questions, a systematic collection of baseline data on the natural history of the species is needed (Schemske et al. 1994). A set of actions can then be established to minimise these factors, to reverse the declining trends and to fulfil the objectives of the recovery plan (Heywood and Iriondo 2003).

The Spanish Balearic Islands, part of the Tyrrhenian Islands (Sicily, Sardinia, Corsica, Balearic Islands) located in the Northwestern Mediterranean, is a “hotspot within a

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hotspot” for plant biodiversity (Medail and Quezel 1997) and currently 11 endangered plants are the subject of recovery plans. Ten of these species plans have as an objective the creation of new populations within the historic range (Conselleria de Medi Ambient 2007). One of the most emblematic of all the threatened Balearic plant species is the critically endangered endemic *Limonium barceloi* (Gil and Llorens). A member of the Plumbaginaceae, this sea lavender belongs to the family with the most endangered and rare species in Spain, where approximately 75% are considered endemic (Palacios and González-Candelas 1997).

L. barceloi, described for the first time in 1991 (Gil and Llorens 1991), is found uniquely in a small, highly degraded wetland area of 32 hectares known as Ses Fontanelles (39°32′05.92″N/2°43′41.60″E), located in the Municipal district of Palma de Mallorca. *L. barceloi* was included first in the Balearic Catalogue of threatened plants in 2001 (Sáez and Rosselló 2001) and later was incorporated into the IUCN Red List as critically endangered (Rosselló and i Sáez 2004). It is considered emblematic, as due to its presence in the remnant wetland, the last remaining habitat of its kind in the Bay of Palma (Amen-gual Pons and Ramis Crespi 2002), it is perceived to be a flagship species for the site.

Since it was first described, the plant’s distribution has decreased dramatically. Comparable with many other Mediterranean endemics with highly restricted ranges, the principal threats come from habitat destruction and fragmentation, as land is converted to accommodate tourist resort developments and the associated infrastructure (Blondel and Aronson 1999). A census conducted by the Sóller Botanical Garden in 2000 revealed approximately 3,000 plants in Ses Fontanelles divided into three subpopulations (Jardí Botànic de Sóller (JBS) 2001). In 2009, a second census revealed that just 301 individuals remain (Khan and Traveset 2009a). This dramatic reduction of 90% of the population in <10 years is the result of anthropogenic-based disturbances, namely construction activities on site during the building of an aquarium and later the accidental flooding of the site with fresh water (Khan and Traveset 2009a). The current in situ population of *L. barceloi* is divided between a subpopulation of 297 and another of 4, which for the purposes of this study are named hereafter *A* and *B*, respectively.

A recovery plan for this species was initiated in 2007 by local government. Amongst the objectives was the creation of a study site for propagation and research. Plants are grown from seed taken from the wild population, with the aim of reintroduction to the site so that the species might reoccupy its former distribution (Vicens and Bibiloni 2007).

Our goal in this study was thus to gather information on the factors that are limiting the reproductive success of *L. barceloi* in the only remaining in situ population at Ses Fontanelles. We examine the breeding system of the plant, not described so far, quantifying as well the frequency of floral visits by insects, seed production, germination success and levels of seed predation. Our specific questions were: (1) Do floral visitors contribute to seed set? (2) How viable are seeds produced by plants in the in situ population? (3) What factors govern seed germinability? (4) How detrimental are seed predators for species recruitment? Knowledge about the reproductive ecology and the factors that potentially impact upon recruitment in the species is essential for the successful management of existing populations of *L. barceloi* and the restoration of extirpated populations.

Materials and methods

Study species

L. barceloi shows a tetraploid chromosome number $2n = 36$. It was originally considered to be a hybrid formed by *L. gibertii* (Sennen) Sennen \times *L. boirae* L. Llorens & Tébar, although recent genetic analyses suggest that *L. cossonianum* Kuntze and *L. minutum* (Fourr.) Kuntze are implicated in its recent evolution (Rosselló 2008). Described as a perennial, multi-stem plant growing to a height of somewhere between 30 and 70 cm, the stems rise from a basal rosette of spatula shaped leaves (Gil and Llorens 1991). The stems themselves are leafless, but possess numerous branches of inflorescences. Flowers are lilac coloured, small (0.19–0.23 cm), held within a tubular calyx and clustered in racemes with five petals united at the base (Gil and Llorens 1991). Plants are hermaphrodite, and each flower holds one ovary; thus, one seed is produced per flower. Floration occurs between April and September and plants are self-incompatible due to the incompatibility of the pollen/stigma combination (Gil and Llorens 1991; Bibiloni 2000). *L. barceloi* also shows a high number of pollen morphological abnormalities (Bibiloni 2000) and is also thought to be apomictic, due to the genus’ propensity towards apomixis (Erben 1979). Seed production is high and completed 37 days after floration (Bibiloni 2000), with an average of 388.3 seeds produced per plant and in areas of high plant densities the seed bank has been seen to reach ca. 125,000/m² (Bibiloni 2000). *L. barceloi* is halophytic in nature and is found on the borders of halophytic plant communities where *Sarcocornia fruticosa* (L.) A.J. Scott and *Arthrocnemum macrostachyum* (Moris.) Moris & Delponte dominate (Khan and Traveset 2009b).

Floral visitors

Censuses were taken of insect floral visitors to both subpopulations *A* and *B*. Sections of plants constituting between 150 and 200 flowers were observed for 48 counts of 10 min, making a total of 8 h of observation on 22 days throughout the 2 months of principal floration, July and August in 2009 and 2010. Censuses were carried out between 10.00 h and 13.00 h, as previous studies show that from 13.30 h onwards there is a rapid reduction in the number of open flowers (Bibiloni 2000). Observations were based on species identification and number of flowers visited. Only those floral visitors touching reproductive parts of the flower were included in the study. Species were identified in the field and where this was not possible individuals were collected for taxonomic classification.

Pollination experiments

Given that the plant is self-incompatible and potentially apomictic, ten plants in the subpopulation *A* were chosen and subjected to pollen exclusion experiments to ascertain the level of apomixis occurring and if wind and/or pollinator agents also contribute towards seed set. Two treatments and a control were set up in each plant in June 2010. Before flower anthesis, flowering branches were bagged with white cloth of two different mesh sizes (1) that did not allow the passage of pollen or floral visitors (thus all seeds produced were apomictic), and (2) that permitted passage of pollen but not floral visitors (anemogamy plus apomixis, i.e. seeds produced were apomictic or the result of sexual reproduction from pollen transported by wind). The control branch was left unbagged (natural pollination), and thus all seeds produced in it came from apomixis or from sexual reproduction with pollen transported by insects or wind. Six weeks later, bags and controls were removed and the number of seeds produced counted.

Seeds extracted were placed in 85-mm petri dishes on top of a disc of filter paper and submerged in 8 ml of distilled water and placed in a germination chamber at 18°C with a 12 h photoperiod. These conditions were chosen as the optimum germination characteristics for the species based on earlier studies carried out by Bibiloni (2000). The number of seeds that germinated after 1 week was recorded. Germination was considered to have occurred on emergence of radicles.

Plant characteristics, seed production and germinability

To assess the relationship amongst plant characteristics, seed production and viability, mature inflorescences were collected on 5 August 2010 from 30 plants (not included in

the pollination exclusion experiment) from the subpopulation *A*. The subpopulation *B* was considered too fragile to permit seed removal. The seed material was held at 4°C until the experimental manipulation began.

Data were collected for each of the 30 plants from which inflorescences were collected. This included height, diameter, number of stems and number of flowers. Seeds were extracted for 20 of the samples and placed in petri dishes in optimum conditions for germination (Bibiloni 2000). Germination was considered to have occurred on emergence of the radicle.

When examining seeds from the different treatments, we noticed that a fraction of them were predated by insect (moth) larvae. We thus recorded the number of preyed seeds and seed set was based on actual seed count plus evidence of seed presence via moth predation holes.

Seed predation studies

Preliminary studies revealed the presence of two seed predators: a moth affecting pre-dispersal of seeds and an ant predated on pre- and post-dispersed seeds. Individuals were collected and sent for identification. We wanted to assess the impact of seed predation by moths and ants. For moths, we randomly chose 100 calyces from 20 of the samples taken from the plants (those used to establish seed set), and these were examined for evidence of seed predation by the moth. The presence of small holes at the base of the calyx was considered to be a sign of moth predation and a seed count was also taken for the same samples. Observations were also made of ant predation of *L. barceloi* seeds at the *A* and *B* populations. Both pre- and post-dispersal predation was observed. Four counts of 10 min were made on three different dates (25 September, 13 October, 27 October) to measure the rate of pre-dispersal seed removal by ants on plants from the *A* population. Seeds were included in the count when they were removed from the plant. Whether the seeds arrived at the nest or were lost along the way was not considered. On three dates in 2010, 12 August, 25 August and 9 September, post-dispersal seed predation was assessed. Four piles of 300 seeds were placed at random locations around the base of the plants in the subpopulation *A* on each of the three dates. After a period of 24 h, seeds were collected and recounted. Material discarded from ant nests was also collected from middens located adjacent to two of the entrance sites situated next to the subpopulation of *L. barceloi* on each of the following dates: 25 August 2009, 3 September 2009, 12 August 2010 and 18 November 2010. Three samples of 4 g were taken and divided into *L. barceloi* plant matter and other plant matter. The different sets of material were then weighed to the nearest 0.0001 mg with a Denver TARE balance.

Data analyses

Seed set in the two treatments and the control for the pollination experiments were compared by means of an ANOVA, after normalising the proportions with the angular transformation. Predation data were subjected to basic descriptive analysis and a general linear model was constructed to predict seed germinability, using number of flowers produced and number of predated seeds (bearing larval exit holes) as main effects. The R-system of statistical computing (R Development Core Team 2009) was employed for all analyses.

All means are given with their standard errors throughout the text if not otherwise indicated.

Results

Floral visitors

A total of 16 floral visitors were identified to species level; another 10 were classified to genus. Thus in total, 26 different morphospecies were recognised visiting the flowers of *L. barceloi* and interacting with reproductive parts of the flowers. Hymenoptera and Lepidoptera were the most frequent visitors (Table 1), and *Polistes gallicus*, *Ceylalictus variegatus* and *Andrena* spp were amongst the most numerous. *Apis mellifera* was ranked tenth. Mean number of flowers visited was 2.44 (± 0.12) per visit across all floral visitors, while the highest number of flowers visited by any one species per visit was observed in *Ceylalictus variegatus* (4.03 ± 0.21).

Pollination experiments

The exclusion experiments revealed that there was no significant difference in seed set amongst treatments ($F_{2,27} = 0.13$; $p = 0.88$). The level of apomixes is shown to be on average 23.41%, and seed set is not augmented by pollinator agents or wind. Germination success for all seeds across the treatments was 73.71% (± 4.5) and, again, no significant difference was observed in terms of germination success amongst treatments ($F_{2,23} = 0.02$; $p = 0.98$).

Plant characteristics, seed production and germinability

Measurements of the 30 plants sampled revealed a mean flower number of 1627.20 (± 115.00), mean height 59.23 cm (± 3.34), mean diameter 38.57 cm (± 2.40) and mean stem number 6 (± 0.37). These size variables are all positively correlated with number of flowers (height/number of flowers: adjusted $R^2 = 0.72$; $p < 0.01$ (Fig. 1), number of stems/number of flowers: adjusted $R^2 = 0.45$;

$p < 0.01$, number of stems/height: adjusted $R^2 = 0.26$; $p < 0.01$, number of stems/diameter: adjusted $R^2 = 0.23$; $p < 0.01$). As expected, the larger the plant was the greater the number of flowers; however, there was no significant correlation between plant size (specifically height) and number of seeds produced (adjusted $R^2 = -0.0517$; $p = 0.80$). Total germinability was observed to be at 70.3% (± 3.2) and a low but significant positive correlation exists between the height of plants and germinability ($R^2 = 0.21$; $p = 0.02$). Thus, plant size positively influences germination success (Fig. 2). One of the plants studied produced no seeds and was not included in the analyses. Table 2 gives all significant correlations found.

Seed predation studies

The seed predators were identified as the moth *Goniodoma limoniella* Stainton, 1884 (Coleophoridae) and the ant *Messor bouvieri* Bondroit 1918 (Myrmicinae). *G. limoniella* is a monophagous feeder specific to the *Limonium* genus, whereas *M. bouvieri* is a common polyphagous seed predator. *Goniodoma limoniella* lays eggs on open flowers, where the larvae will later feed on the newly formed seeds. When fully grown, the larvae bore a hole through the calyx and make their way down to a plant stem, where an incision is made and the stem entered. It is here that the larvae overwinters and emerges the following year in its imago form (Sammut 2008). Seed predation by *G. limoniella* varied amongst individuals, ranging from 1 to 78% of the seeds examined. No apparent correlation was seen between the number of emerging holes and plant height ($R^2 = 0.02$, $p = 0.46$), which suggests that moths are not preferentially attracted to larger plants. Substantial variance was observed in the data for moth seed predation (mean: 23.75; standard deviation 19.57) and the number of predated seeds [holes (n) per 100] was seen to have a negative influence on seed germination ($t = -1.77$, $p = 0.09$). However, we compared the linear model constructed to predict germinability using only the number of flowers ($t = 2.46$; $p = 0.03$) with a second model adding the predation variable by means of ANOVA; the second model was only marginally significantly better (model 1 vs. model 2, $F = 3.4485$; $p = 0.08$).

Pre-dispersal predation by the ant *M. bouvieri* revealed that there was a mean removal rate of 7.47 seeds min^{-1} (± 1.20) for the times sampled. Post-dispersal removal of seeds showed a mean of 104.17 (± 36.72) in 24 h, i.e. 8.68% of the seeds made available. Material collected from ant middens showed that 41.33% (± 5.61 ; mean of three replicas) of discarded material originated from *L. barceloi*. No intact seeds were found amongst the material. Of the midden material, 38.45% (± 4.87) was derived from *Avena barbata* whereas the remainder was of undetermined origin.

Table 1 Floral visitors to *Limonium barceloi* and visitation rates 2009-2010

Species/morphospecies	Family	Order	Number of census showing presence	Percentage of census showing presence	Mean number of flowers visited
<i>Andrena</i> spp. (Fabricius 1775)	Andrenidae	Hymenoptera	35	72.92	2.23 ± 0.21
<i>Aphaenogaster</i> spp. (Mayr 1853)	Formicidae	Hymenoptera	1	2.08	2.00 ± 0.00
<i>Apis mellifera</i> (Linnaeus 1758)	Apidae	Hymenoptera	13	27.08	2.85 ± 0.41
<i>Ceratina</i> spp. (Latreille 1802)	Apidae	Hymenoptera	22	45.83	3.65 ± 0.25
<i>Ceylalictus variegatus</i> (Olivier 1789)	Halictidae	Hymenoptera	31	64.58	4.03 ± 0.21
<i>Halictus</i> spp. (Latreille 1804)	Apidae	Hymenoptera	11	22.92	2.36 ± 0.28
<i>Lasioglossom</i> spp. (Curtis 1833)	Halictidae	Hymenoptera	20	41.67	2.55 ± 0.25
<i>Osmia</i> spp. (Panzer 1806)	Apidae	Hymenoptera	4	8.33	2.75 ± 0.48
<i>Polistes galicus</i> (Linnaeus 1767)	Vespidae	Hymenoptera	47	97.92	2.60 ± 0.17
<i>Prionyx</i> spp. (Vander Linden 1827)	Sphecidae	Hymenoptera	4	8.33	2.50 ± 0.65
<i>Rhodanthidium</i> spp. (Isensee 1927)	Apidae	Hymenoptera	1	2.08	200 ± 0.00
<i>Thyreus histronicus</i> (Illiger 1806)	Apidae	Hymenoptera	2	4.17	2.50 ± 0.50
<i>Ceriana</i> spp. (Rafinesque 1815)	Syrphidae	Diptera	15	31.25	2.80 ± 0.33
<i>Cheilisia</i> spp. (Meigen 1822)	Syrphidae	Diptera	1	2.08	3.00 ± 0.00
<i>Episyrphus</i> spp. (Matsumura & Adachi 1917) Syrphidae		Diptera	11	22.92	1.91 ± 0.21
<i>Melanostoma mellinum</i> (Linnaeus 1758)	Syrphidae	Diptera	13	27.08	2.54 ± 0.33
<i>Meliscaeva auricollis</i> (Meigen 1822)	Syrphidae	Diptera	11	22.92	1.91 ± 0.25
<i>Aricia cramera</i> (Eschscholtz 1821)	Lycaenidae	Lepidoptera	16	33.33	2.63 ± 0.27
<i>Colias croceus</i> (Fourcroy 1785)	Pieridae	Lepidoptera	4	8.33	0.82 ± 0.41
<i>Gonepteryx cleopatra</i> (Linnaeus 1767)	Pieridae	Lepidoptera	3	6.25	3.00 ± 0.58
<i>Gomodoma limoniella</i> (Stainton 1884)	Coleophoridae	Lepidoptera	15	31.25	2.07 ± 0.29
<i>Lysandra bellargus</i> (Rottemburg 1775)	Lycaenidae	Lepidoptera	6	12.50	2.50 ± 0.56
<i>Papilio machaon</i> (Linnaeus 1758)	Papilionidae	Lepidoptera	3	6.25	1.67 ± 0.33
<i>Pyronia cecilia</i> (Vallantin. 1894)	Nymphalidae	Lepidoptera	17	35.42	1.94 ± 0.22
<i>Vanessa cardui</i> (Linnaeus 1758)	Nymphalidae	Lepidoptera	3	6.25	2.67 ± 0.88
<i>Centrocons spmiger</i> (Fabricius 1803)	Coreidae	Hemiptera	1	2.08	2.00 ± 0.00

Discussion

The level of floral visitation observed in *L. barceloi* (26 morphospecies) indicates its importance as a resource for pollen and nectar to a diverse range of insects from different orders. However, insect floral visitation does not appear to contribute either to seed set or germinability success. As previously mentioned, the results from the pollination experiments indicate that seeds produced are the result of autonomous apomixis. No evidence was found that the apomictic seeds were any less viable than those produced in the anemogamous or natural pollination treatments, as no significant difference was observed in germination rates between treatments. Germination was seen at ca. 70% within the first week after setting the experiment, and this is ca. 16% higher than that previously reported for the species (Bibiloni 2001). Nonetheless, a fitness reduction of apomictic progeny might be expressed in later life stages (e.g. Kondrashov 1993; Chasnov 2000). To our knowledge, no previous studies have compared

progeny fitness for any species within the *Limonium* genus, but a higher fitness in sexually produced offspring compared to their apomictic counterparts has been reported for other species, such as *Ranunculus auricomus* (Izmailov 1996; Hörandl 2008) and *Boechera* (Voigt et al. 2007). However, the opposite has also been found, i.e. an increased fitness via the apomictic route compared to the sexual, in *Taraxacum officinale* (Van Dijk 2007) and *Antennaria parlini* (Michaels and Bazzaz 1986). Diverse results suggest that such assessments are species specific and that they more than likely depend on a combination of genetic and highly variable environmental factors.

The tetraploid nature of *L. barceloi* suggests diplospory as the probable mechanism for asexual production of seeds, as a strong correlation exists with tetraploidy and diplospory (Asker and Jerling 1992). Whitton et al. (2008) suggest that individuals exhibiting diplospory are also more likely to be obligate asexual reproducers, and while the existence of obligate apomictic species is controversial (Koltunow and Grossniklaus 2003; Tucker et al. 2003),

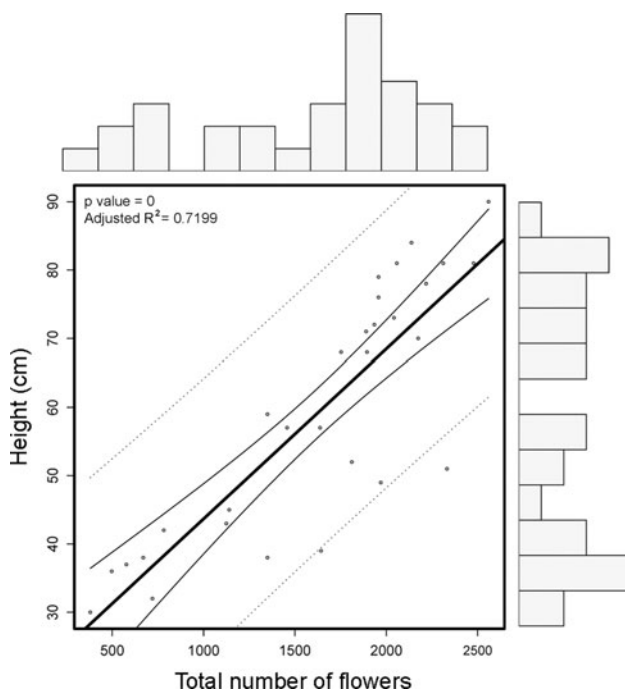


Fig. 1 Correlation between height of plants (cm) and number of flowers. Adjusted R^2 and P values have been included (*Black and dashed curves* indicate, respectively, the 95% confidence and prediction intervals of the regression. *Bars* in the margins represent the histograms of each variable compared.)

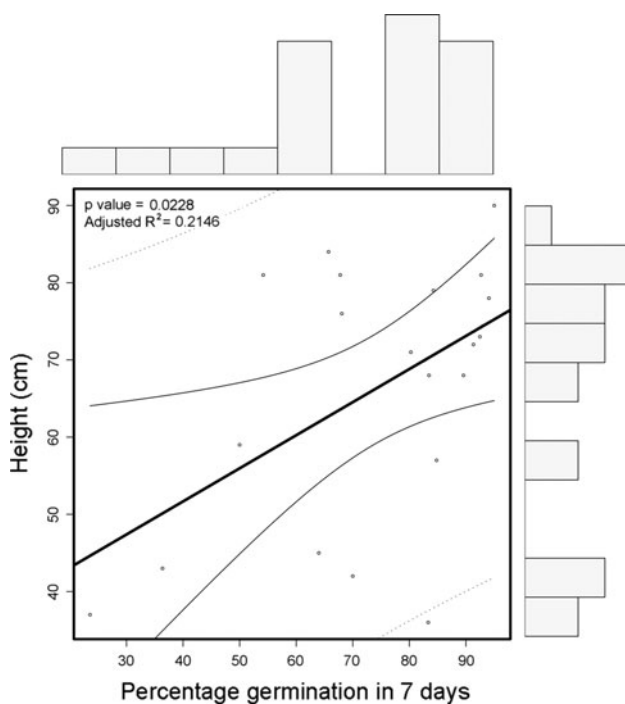


Fig. 2 Correlation between height of plants (cm) and percentage germination observed. Adjusted R^2 and P values have been included (*black and dashed curves* indicate, respectively, the 95% confidence and prediction intervals of the regression. *Bars* in margins represent the histograms of each variable compared.)

evidence suggests that *L. barceloi* may currently be functioning as obligate apomictic. High numbers of morphological anomalies are observed in the pollen (Bibiloni 2000). This is a characteristic of a high frequency apomixis-mediated reproduction (Maynard Smith 1978; Eckert 2002) and the presence of one of the study plants that produced flowers and no seeds could be an expression of the deleterious mutational load common in asexually reproducing plants as described by Navascués et al. (2010). Navascués et al. (2010) assert that this feature may develop under specific conditions such as self incompatibility, low population size and high clonal rate. Genetic testing would be required to confirm or refute this hypothesis and more research in this area is suggested.

Seed fitness was determined via germinability success. Interestingly, while large plant size does not appear to affect the quantity of seeds produced, it does influence the quality of seeds produced, as a correlation is found between plant size and germinability. Seed characteristics are usually determined by genotype and parental environment (Stanton 1984; Donohue and Schmitt 1998; Galloway 2001); however without genetic analysis, it cannot at this time be said whether seed germination performance has a genetic basis or is a consequence of the maternal plant environment. Authors have shown that a variety of factors, including drought stress, nutrient supply, increased CO_2 concentration and inter- and intra-specific competition for resources experienced by the maternal plant, can all have an effect on seed fitness (Alexander 1985; Roach and Wulff 1987; Fenner 1991; Paolini et al. 1999; Luzuriaga et al. 2006). It is thus likely that a larger plant will be able to compete more successfully for resources and thus produce fitter offspring.

Results of seed predation studies revealed two predators working at different phases in the plant's life cycle. Moths affect pre-dispersal and ants the pre- and post-dispersal. *Gonoidoma limoniella* (Coleophoridae) was first described for Spain in 1996 (Vives Moreno 1996). It is a pre-dispersal moth seed predator common to the *Limonium* genus. A high level of variation in predation rates, which can reach up to 78% of the seeds, was found, but these rates were not correlated to plant size. The genus *Messor* is an important group of granivorous ants in Mediterranean systems (Diaz 1994; Detrain and Tasse 2000; Azcárate and Peco 2007), and we found that *Messor bouvieri* (Myrmicinae) is an important predator of the *L. barceloi* seeds, indicated by both the removal rate (8.68% of seeds available in 24 h) and a high percentage of *L. barceloi* material found in middens (41.33%). Granivorous ants are commonly known to influence population dynamics of plant species through selective predation and dispersal (Janzen 1971; Andersen 1989; Brown and Human 1997; Retana et al. 2004); however, their effect on perennial plant

Table 2 Significant correlations between variables. (“Germinated” = % Germination in 7 days/Number of seeds)

Dependent–independent variables	Samples (<i>n</i>)	Adjusted R^2	<i>P</i> value	Dependent–independent variables	Samples (<i>n</i>)	Adjusted R^2	<i>P</i> value
Stem (<i>n</i>)–Diameter	20	0.3193	0.0056	Holes (<i>n</i> per 100)–Seeds (<i>n</i>)	20	0.2148	0.0228
Stem (<i>n</i>)–Height	20	0.7923	0	Holes (<i>n</i> per 100)–Germination in 7 days (<i>n</i>)	20	0.1853	0.0331
Stem (<i>n</i>)–Flowers (<i>n</i>)	20	0.7262	0	Holes (<i>n</i> per 100)–Seeds (<i>n</i> per 100)	20	0.3988	0.0017
Diameter–Stem (<i>n</i>)	20	0.3193	0.0056	Seeds (<i>n</i> per 100)–Seeds (<i>n</i>)	20	0.5155	2×10^{-4}
Diameter–Height	20	0.4116	0.0014	Seeds (<i>n</i> per 100)–Germination in 7 days (<i>n</i>)	20	0.2488	0.0146
Diameter–Flowers (<i>n</i>)	20	0.3453	0.0038	Seeds (<i>n</i> per 100)–Holes (<i>n</i> per 100)	20	0.3988	0.0017
Diameter–Germinations in 7 days (<i>n</i>)	20	0.2003	0.0274	Germinated–Diameter	20	0.3131	0.0061
Diameter–Germinated (<i>n</i>)	20	0.3131	0.0061	Germinated–Height	20	0.2146	0.0228
Height–Stem	20	0.7923	0	Germinated–Flowers (<i>n</i>)	20	0.203	0.0265
Height–Diameter	20	0.4116	0.0014	Germinated–Germination in 7 days (<i>n</i>)	20	0.2561	0.0133
Height–Flowers (<i>n</i>)	20	0.9375	0	Stem (<i>n</i>)–Diameter	30	0.2324	0.0041
Height–Germinated	20	0.2146	0.0228	Stem–Height	30	0.2616	0.0023
Flowers (<i>n</i>)–Stem	20	0.7262	0	Stem–Flowers (<i>n</i>)	30	0.449	0
Flowers (<i>n</i>)–Diameter	20	0.3453	0.0038	Diameter–Stem (<i>n</i>)	30	0.2324	0.0041
Flowers (<i>n</i>)–Height	20	0.9375	0	Diameter–Height	30	0.4091	1×10^{-4}
Seeds (<i>n</i>)–Germination in 7 days	20	0.7186	0	Diameter–Flowers (<i>n</i>)	30	0.3295	5×10^{-4}
Seeds (<i>n</i>)–Holes (<i>n</i> per 100)	20	0.2148	0.0228	Height–Stem (<i>n</i>)	30	0.2616	0.0023
Seeds (<i>n</i>)–Seeds (<i>n</i> per 100)	20	0.5155	2×10^{-4}	Height–Diameter	30	0.4091	1×10^{-4}
Germination in 7 days (<i>n</i>)–Diameter	20	0.2003	0.0274	Height–Flowers (<i>n</i>)	30	0.7199	0
Germination in 7 days (<i>n</i>)–Seeds (<i>n</i>)	20	0.7186	0	Flowers (<i>n</i>)–Stem (<i>n</i>)	30	0.449	0
Germination in 7 days (<i>n</i>)–Holes (<i>n</i> per 100)	20	0.1853	0.0331	Flowers (<i>n</i>)–Diameter	30	0.3295	5×10^{-4}
Germination in 7 days (<i>n</i>)–Seeds (<i>n</i> per 100)	20	0.2488	0.0146	Flowers (<i>n</i>)–Height	30	0.7199	0
Germination in 7 days (<i>n</i>)–Germinated	20	0.2561	0.0133				

populations is at present incompletely understood. Further, while our study examined rates of removal from plants and from controlled seed additions, no assessment was made of seed loss or abandonment en route to the nest; thus, we cannot discard the possibility that ants play a role in dispersal, favouring seedling establishment into new microsites.

In assessing the impact of seed predators, it would be wrong to assume that seed removal rates are equal to predation (Retana et al. 2004; Vander Wall et al. 2005; Martínez-Duro et al. 2010), and very few studies have looked at the effect of seed predators on recruitment rates in endangered, rare plants. Those that have been completed have found different results. Albert et al. (2005) showed that seed predation, although high, did not limit population growth for the rare *Erodium paularense* in central Spain,

while White and Robertson (2009) discovered the contrary for *Lepidium papilliferum*, a rare perennial herb in the US. It is thought probable that due to the high seed bank that has been reported for the species (Bibiloni 2000), recruitment in *L. barceloi* is not seed limited at this time, although more studies should be undertaken to assess the validity of this hypothesis.

Conclusions and recommendations for future conservation and management

Small populations are infamously prone to extinction from stochastic events (Goodman 1987), and at present the habitat of *L. barceloi* enjoys no official protected status and

is under constant threat of urban development. Therefore, a priori action should be the setting aside of subpopulation A as a micro reserve. Laguna et al. (2004) found that micro reserves were a useful tool offering effective protection for endangered plants in the Mediterranean context and the size of the subpopulation (ca. 300 plants) allows it to fit within the criteria defined by Frankel and Soulé (1981) and Lesica and Allendorf (1992), who suggest that a population of at least 50 individuals is required to avoid loss of genetic variation within plant populations in the short term.

Suitable habitat creation could also be initiated and plants translocated from the ex situ study sites to form new populations. We do not consider feasible the creation of subpopulations away from the plant's historic range, due to the lack of available salt marsh sites in Mallorca. The creation of new habitat and additional subpopulations within Ses Fontanelles is the only realistic hope for the species' continued survival and additional subpopulations could provide the potential to expand the genetic base of the species.

It should be recognised also that the timing of plantings is vital, as is the use of multiple sites. Further, it is suggested that large numbers of individuals are sown (Frankham et al. 2002) and that due to the vulnerability of seeds and seedlings (Primack and Drayton 1997) managers should consider transplanting young plants (Davy 2002). The implementation of extended monitoring procedures of at least 10 years is recommended to evaluate success based on long-term results (Godefroid et al. 2011).

In order to effectively create a suitable habitat, it is recommended that further studies into the ecological requirements of the species be undertaken; autecological studies are considered a critical pre-requisite to conservation science and action (Simberloff 1988; Brussard 1991; Schemske et al. 1994). Further, as it is seen that large plants produce fitter seeds, it is advised that a variety of soil substrates, soil saturation levels and salinity concentrations are experimented with in order to ascertain which provide for greater growth rates.

Finally, it is strongly recommended that a full genetic analysis of the variation in the species is undertaken; Godefroid et al. (2011) found that the rates of survival for reintroduced species were greater when an understanding of the level of genetic diversity is included in the project design.

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