



Key ecological determinants of the reproductive success and conservation strategy of *Delphinium pentagynum* subsp. *formenteranum* (*Ranunculaceae*), an endangered endemic species of the western Mediterranean Basin (Balearic Islands)

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Abstract

Delphinium pentagynum subsp. *formenteranum* is an endemic species restricted to the island of Formentera (Balearic Islands), where it is considered an endangered species. Ecological traits such as pollination and pollinator-plant interaction, and seed germination are key processes required for conservation efforts but remain poorly characterized. Floral traits were evaluated analysing total alkaloid content for vegetative plant, floral, nectar and pollen, and volatile emitted by flowers. Flowering phenology was assessed in the field in three different years (1998, 2008, 2018) and under cultivated conditions for *D. pentagynum* subsp. *formenteranum* and cohabiting species. Germination trials were conducted both at different constant and alternate temperatures, and seed lots conserved at room temperature were germinated across 7 years to assess seed viability with seed age. Micromorphological seed traits were assessed with scanning electron microscopy. Results show intraspecific flowering synchrony for *D. pentagynum* subsp. *formenteranum* with flowering occurring in May after the flowering peak of cohabiting species. Floral traits show lack of odor related volatiles and high alkaloid content in plant and flowers. Germination remains unaffected within a broad spectrum of temperatures and is only negatively affected at high temperatures (23°C). Seed viability maintains high germination rates for the first 2 years (~100%) and decreases after four-five years (40–60%) and six years (20%), being non-viable after seven years. Both phenological displacement and alkaloid content in flowers support pollination specificity, which is restricted almost exclusively to *Anthophora balearica*. Germination ecology is dependent mainly on water availability, but high seed longevity allows probable transient bank seed.

Keywords Flowering synchrony · Germination · Nectar · Pollination ecology

Introduction

Plant diversity of the Mediterranean islands has been strongly influenced by specific historical, biogeographical, and ecological conditions resulting in important biodiversity hotspots with high endemism (Médail 2017). However, due to several environmental threats many of these Mediterranean hotspots could face unpredictable consequences

(Médail 2017). For narrow endemics, these threats are mainly aggravated by their usually small population size, distribution area and capability to compete, which diminishes the possibilities for long term persistence (Laverne et al. 2004). Under these conditions, three main steps strongly influence plant life cycle and can be considered crucial, flowering, pollination, and germination (Chiang et al. 2009).

Floral phenology covers processes that span from flower opening to functionality and senescence (anthesis and post-anthesis till senescence) (Shivanna and Tandon 2014). It has been mainly linked and thought to be stimulated by photoperiod, temperature, or the plant developmental stage (Song et al. 2013). The specific conditions that trigger flowering in entomophilous species tend to be related in turn to periods of ecological relevance (Borchert et al. 2005). Extensive

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studies of flowering phenology show that flower display is intimately related to pollinators by synchronously blooming with pollinators cycles and/or arrival. These patterns are highly variable, spanning from specific plant-pollinator relations to non-specific ones during the peak pollination period (Waser and Ollerton 2006). Equally, plant-plant interactions have also been described, with various patterns at the community level. In this sense, two phenomena can be highlighted according to synchronous or asynchronous blooming: facilitation, where synchronous blooming among species can be explained as a mechanism to attract more pollinators due to higher conspicuousness (Thomson 1978; Rathcke 1983); and competitive exclusion, where asynchronous blooming, or allochory, entails that different species avoid competition for pollinators through different timing in flower phenology (Levin and Anderson 1970). The occurrence of synchronous or asynchronous patterns has implications in the different ecological relations, which can be especially meaningful in cross-pollinated species (Inouye and Morales 2002).

Interactions between plants and pollinators are often considered mutualistic, with pollinators facilitating the transfer of pollen to stigmas while seeking food rewards such as nectar and pollen. Through evolution, animal-pollinated plants have evolved a complex set of traits to increase the visibility and attractiveness of their flowers to pollinators. These include food rewards such as nectar and pollen abundance, the scent production, a showy appearance, mimicry, or deception (Willmer 2011; Ito et al. 2021). Thus, the visibility of flowers on the plant or in space is a first level of information, while traits such as colour arrangement and flower odour emission act as signals that announce to pollinators the location, quantity, and quality of floral rewards (Yan et al. 2016). The selection of the most efficient pollinators is a mechanism that plants have developed to increase pollination performance. One of the most common strategies to achieve greater attractiveness or deterrence is to modify the quantity of rewards or adapt their chemical quality (composition) (Willmer 2011; Ito et al. 2021).

Germination refers to the complex processes involved from seed imbibition to radicle protrusion (Baskin and Baskin 2014). It has been shown to be related to temperature, humidity, photoperiod, and stratification periods (Baskin and Baskin 2014). This set of conditions have been considered as environmental cues for favorable timing of plantlet emergence and development, selected as a result of long evolutionary processes (Hoyle et al. 2015). In certain species, germination is combined with dormancy, which allows further germination processes to take place in the following years favouring seed banks (Fenner and Thompson 2005). Likewise, non-dormant seeds can also maintain its viability for long periods of time to avoid germination under

non fitting conditions, favouring transient bank seed (Volis et al. 2004; Fenner and Thompson 2005). Besides physiological traits, germination and dormancy mechanisms can also couple with seed structures, which allow proper dispersal or are involved in speeding up specific processes needed for germination (Chambers and MacMahon 1994). In this sense, the various germination strategies and seed traits can be both considered of great importance for plant persistence, and important knowledge for proper ex situ and in situ conservation (Bacchetta et al. 2008).

Considering both flowering phenology and germination importance, the aim of this paper is to document the trends of both these processes in the endangered species *Delphinium pentagynum* Lam. subsp. *formenterianum* N. Torres, L. Sàez, J.A. Rosselló & C. Blanché (henceforth *D. formenterianum*), endemic to the island of Formentera (Balearic Islands, Spain), as well as the strategies for the establishment and persistence of its populations. For this purpose, we performed a study of:

1. The phenological traits of flowering in relation to the synchrony with the main entomophilous species of the plant community in which this species grows.
2. Pollination: pollinators and their relationship with production of floral scents and rewards.
3. The characteristics of germination ecology: (a) adaptive morphology of the seed; (b) temperature requirements; (c) evolution of germination and ability of seeds to form a long-lived seed bank in the soil.

Materials and methods

Location of study and species description

Plants under study and seeds were collected near Cala Saona (Cap de Barbaria, Formentera), coordinates: 38°41'18"N, 1°23'58"E, (UTM 31SCC68), altitude: (20–50 m). In this locality, reproductive individuals occupying xeric grasslands developed in clearing of thermo-Mediterranean *Pinus halepensis*-*Juniperus phoenicea* subsp. *turbinata* forest-brushes or calcicolous garrigues. Climate is Mediterranean, showing an average annual temperature of 18.1 °C (TMax = 29.7; Tmin = 9.1, and an annual rainfall of 350 mm, with the minimum during summer (Guijarro 1986).

D. formenterianum is a 40–80 cm high polycarpic perennial rhizomatous herb. In autumn, the rhizome produces a basal leaf rosette and, in May, a lax and few-branched flowering stem, with (3)5–7 blue-violet coloured and pubescent flowers of 17–19 mm in every terminal branch. Fruits are follicles that contain numerous small seeds covered with narrow scales. Under natural conditions the persistence of

populations depends on the partial survival of rhizomes and seed production. From a phylogenetic point of view, *D. formenterianum* is considered a (esquizo)endemism of the Formentera Island, vicariant taxon, adapted to dry insular conditions, and related with ibero-north African populations of *D. pentagynum*. Geographical isolation may have favored their individualization, determined by a response to dry conditions, with populations remaining moderately diverse (Torres et al. 2000; López-Pujol et al. 2003). Main differences that support taxonomical differentiation of *D. formenterianum* from *D. pentagynum* (and other subspecies of the *pentagynum* complex) rely on the smaller size both for vegetative (leaf and stomata size) and reproductive (narrower sepals and smaller corolla, spur, follicles and seeds) traits as described in Torres et al. (2000). Due to this critical population size and narrow distribution, their subpopulations are small and scattered over an area of about 12 km², this taxon has been listed as ‘critically endangered’ (CR) both in the Red List of Vascular Spanish Flora (Bañares et al. 2010), and in the Red Book of the Balearic Islands Vascular Flora (Sáez et al. 2017), being legally protected (Balearic Decree 75/2005).

Extracts preparation and analysis

For extractions of green tissues plants of 3 subpopulations were collected. For flower and pollen extractions, 10 plants of 3 subpopulations were used. Nectar was extracted from 10 plants and 10 flowers per plant using microvials, avoiding contact with the anthers. Phytochemicals were extracted from dried plant material and analysed using adapted procedures previously described (Gardner et al. 1999; Cook et al. 2013; Sadeq et al. 2021). Briefly, 500 mg for green parts, flowers and pollen were extracted in 15 ml of methanol for 6 h. The sample was mixed and then centrifuged. An aliquot of the extracted sample was diluted into 1:1 methanol/1% acetic acid for a total volume of 1 ml for analysis. Nectar samples were extracted using methods previously described (Gardner and Pfister 2009; Cook et al. 2013). In summary, a measured volume of nectar (10 µl) was extracted in 90 µl of 1:1 methanol/1% acetic acid. Reserpine (125 µg) was added as an internal reference after extraction.

Floral scent

Samples were collected from 3 plants per subpopulation. The volatiles emitted by flowers were obtained and analyzed using the headspace solid phase microextraction sampling technique (HS-SPME-GC-MS), an adapted protocol as proposed by Tomas et al. (2022). Briefly, volatile compounds were extracted from two flowers of each plant in a manual SPME holder with 10 mL glass vials

and Polydimethylsiloxane-Divinylbenzene (PDMS-DVB) fibres (Supelco Inc., Bellefonte, USA). GC-MS analyses were carried out on an Agilent 6980 GC - MDS 5975 inert XL (Agilent Technologies, EUA) using a Supelcowax 10 gas capillary column (60 m × 0.25 mm × 0.25 mm), and the total ion chromatograms and mass spectra were processed using GC-EM software, Turbomass version 5.1 (Perkin-Elmer, Inc.).

Floral visitor observations

Observations of floral visitors were made, on days with weather stability, by viewing recordings obtained with Bushnell HD cameras. The recordings were made between 06:00 h and 18:00, filming one inflorescence per plant, and two plants of three subpopulations, on 3 different days per location (216 h total). Insects that came into contact with flowers and had pollen grains deposited on their bodies were considered pollinators (Dafni 1992), based on field observation and pollen control in microscope specimens. The presence of pollen load in their bodies, was confirmed by pollen data from the published bibliography (Boi and Llorens 2007). In cultivated plants, observations were reduced to relative evaluations between visitors of the same plants.

Phenology and floral synchrony

To analyse the phenological processes, a first approach was conducted in 1997 with data from 11 perennial species cohabiting with *D. formenterianum* (*Crithmum maritimum* L., *Globularia alypum* L., *Helichrysum stoechas* (L.) Moench, *Limonium ebusitanum* (Font Quer) Font Quer, *Micromeria inodora* (Desf.) Benth., *Micromeria microphylla* (D’Urv.) Benth., *Micromeria nervosa* (Desf.) Benth., *Rosmarinus officinalis* L., *Teucrium capitatum* L. subsp. *majoricum* (Rouy) Nyman, *Thymbra capitata* (L.) Cav., and *Viola arborescens* L.) to assess flowering timing at the community scale.

Based on the obtained results, phenological data were recorded in the field in 1998, 2008 and 2018 for those species which displayed flowering overlap with *D. formenterianum*: *H. stoechas*, *T. capitatum* subsp. *majoricum* and *Th. capitata*. Additionally, flowering was assessed under controlled conditions in 2020 with plants cultivated in pots.

Phenological survey in the field was conducted by selecting 5 representative individuals of each species (10 in *D. formenterianum*), spaced at least 5 m from one another. In each of these plants a total of 25 randomly chosen floriferous branches were marked (5 per plant) and the number of opened flowers were counted weekly for each plant. Flowering under controlled conditions in 2020 were assessed by counting opened flowers every 5 days. Two different

branches per plant and 5 plants (pots) per species (total of 10 flowering branches per species) were followed. The interspecific synchrony index of *D. formenteranum* and of the species community were calculated (Augspurger 1983; Munguía-Rosas and Sosa 2010). Values varied from 0 (no overlap) to 1 (plant flowering overlaps completely with that of all other individuals).

Micromorphological seed traits

For scanning electron microscopy (SEM) investigation, seed samples were transferred from 70% ethanol to 100% ethanol, until dehydrated. After that, the samples were sputter coated with gold (sputter coater – Polaron) and examined under a scanning electron microscope – HITACHI 3400 N – with 10 kV acceleration voltages on low vacuum mode (Tomas et al. 2019).

Seed germination assays

Seeds were collected from representative plants of 3 sub-populations. Collection was carried out during June in 2010. Seeds were dried at room temperature (20–22 °C) in the laboratory by spreading them on trays from their collection in June to the following 1st September. At that moment, seeds were considered 0 months old and were stored within paper envelopes at room temperature until they were tested.

Germination trials were conducted by assigning 100 seeds into four 25-seed replicates. Each replicate was incubated in germination chambers (Selecta Hotcold UM germination chambers) on a double layer of filter paper moistened with distilled water in a 9-cm diameter Petri dish under dark conditions (Del Vecchio et al. 2012). Germinated seeds were counted with variable frequency until a maximum of 30 days, when ungerminated seeds were checked for presence of embryo as an approach of seed viability by the cut-test. Percentages of germination were computed based on viable seed counts and T50 was calculated (Del Vecchio et al. 2012).

Two germination experiments were conducted to assess the optimal temperature conditions, and to assess seed ageing resistance. In the first experiment germination tests were carried out at a constant temperature of 5, 10, 15 and 23°C, as well as at the following thermoperiods: 5/10, 5/15, 10/15 and 10/20 °C. In the second experiment seeds were

germinated at 15 °C under dark conditions, as these conditions ensured high germinability (according to the results from the first experiment), with seeds 1, 2, 4, 5, 6 and 7 years old.

Statistical analysis

Germination parameters were calculated using the package *Germinationmetrics* (Aravind et al. 2019) in R software (R Core Team 2013). Descriptive statistics and plots were carried out using the *Tidyverse* library (Wickham et al. 2019) and the *ggplot2* package (Wickham 2016). Cumulative germination in the different treatments was modelled considering the germination temperature and longevity as the experimental variables. For count response variables (day of the first germination, number of germinated seeds) and rate response variables (germination rate, final germination percentage) generalized linear models using the Poisson and quasibinomial families were used, respectively. In all cases, model selection was carried according to the Akaike selection Criterion using AICc (Bozdogan 1987). Model accuracy was evaluated using Q-Q plots and using McFadden Pseudo-R² when possible (Veall and Zimmermann 1996). Significant effects of the experimental variables were evaluated using ANOVA (Rutherford 2011), while differences among treatments were analysed using the Tukey Honest Significance Test (Abdi and Williams 2010).

Results

Chemistry and floral scent

Total alkaloid content in the green parts of the plants (leaves and stems), flowers, pollen loads, and nectar are presented in Table 1. Total alkaloid content showed maximum content in flower followed by vegetative structures. Significant content was also detected in flower reward structures such as pollen loads and nectar.

Only traces of 3-hexen-1-ol acetate and 3-hexen-1-ol were detected in the male phase of some flowers. Accordingly, we consider that *D. formenteranum* can be included in the odourless species group of the genus.

Flowers visitors

Table 2 shows the main visitors to the flowers of *D. formenteranum*. In both wild and cultivated populations, the importance of species of the genus *Anthophora* is evident. This is especially significant in the wild populations, where *A. balearica* is the key species for pollination. There is also a notable absence of visits by *Apis mellifera* which, although

Table 1 Total alkaloid content of vegetative (leaves + stem), flower, pollen load and nectar

Parameter	Total alkaloids
Plant (leaves+stems) (n = 3)	422 ± 37 µg/100mg
Flowers (n = 10)	705 ± 68 µg/100mg
Pollen loads (n = 10)	186 ± 22 µg/100mg
Nectar (n = 10)	7 ± 0.2 µg/mL

Table 2 Flower visitors for *Delphinium formenteranum* in wild and experimental populations. For wild population data is a mean $\pm$ SD of a total of 216 hours observations. For experimental crops, X is a measure of relative abundance

Pollinator	Site	Number of visited flowers (Total/plant)
<i>Anthophora balearica</i> (Friese, 1896)	Wild population	2.1 $\pm$ 1.9
<i>Anthophora</i> sp. (Latreille, 1803)	Experimental crop	XXX
<i>Xylocopa violacea</i> (Linnaeus, 1758)	Experimental crop	X
<i>Lasioglossum</i> sp. (Curtis, 1833)	Experimental crop	XX
<i>Macroglossum stellatarum</i> (Linnaeus 1758)	Experimental crop	X

it is a common species both in the areas of wild populations and in crops, only comes within a short distance of the flowers (up to 2–3 cm) and shies away from visiting them.

Phenology

The interspecific synchrony index is 0.18 ($\pm$ 0.05), which shows a low coincidence in the timing of flowering between the different species of the community. In contrast, the intra-specific synchrony of *D. formenteranum* plant population is very high at 0.91 ($\pm$ 0.02).

D. formenteranum displays its flowering peak between *Helichrysum stoechas* and *capitata* (Fig. 2A), lasting 22 ± 1.23 days. This flowering peak is only synchronous with the decrease of *Teucrium capitatum* flowering at both field and laboratory conditions, albeit with less intensity in the latter (Fig. 2B C). In 20 years of study, *D. formenteranum*, *H. stoechas*, *Th. capitata* and *T. capitatum* seem to have delayed their flowering for about 15 days on average from 1998 to 2018 (Fig. 2B).

Germination and seed traits

Seeds are subpyramidal with several lamellae covering their surface. The micromorphological analysis of these lamellae revealed a rugulose surface, papillate on the abaxial face (Fig. 1).

Incubation temperature had a significant effect in final germination for both constant (p-value < 0.01, $X^2 = 967.28$, df=3) and alternate temperatures (p-value < 0.01, $X^2 = 22.207$, df=3). The increase of temperatures has been generally associated with a decrease of germination for both conditions (Fig. 3). For constant temperatures, no substantial differences regarding final germination have been observed between 5, 10 and 15 °C, displaying germination rates near 100%, while incubation temperatures of 23 °C reduced germination to less than 20% (Fig. 3A).

For alternate temperatures, maximum germination rate was achieved at moderate temperature regimes and significant reduction occurred at the lowest and highest regimes (Fig. 3B).

Germination speed (T50) was also significantly affected by both constant (p-value = 0.004, $X^2 = 13.265$, df=3) and alternate temperature treatments (p-value < 0.01, $F = 186.4$, df=3). For conditions of constant temperature, an increasing germination delay was observed with increasing temperatures, while for alternate temperatures germination speed shows a similar pattern as germination rate, with maximum speed at medium temperatures (Fig. 4B). In the case of constant temperatures, first (p-value = 0.002, $X^2 = 15.065$, df=3) and last germination day (p-value = 0.004, $X^2 = 13.563$, df=3) followed similar patterns as germination speed, with a significant increase of days for germination with increasing temperature (Fig. 4C and D). Alternate temperature conditions also followed a similar pattern as germination speed for last day of germination (p-value = 0.002, $X^2 = 14.488$, df=3), but first day of germination was decreased steadily with increasing temperature regimes (p-value < 0.01, $X^2 = 20.087$, df=3) (Fig. 4C and D).

Regarding seed longevity, seed age was observed to impact germination (p-value < 0.01, $X^2 = 26.219$, df=4), significantly reducing it after 2 years, with relevant germination rates after four years and maintaining some germinability until the fifth year (Fig. 5).

Discussion

Floral traits and scent

Delphinium flowers have repeatedly been considered as a reference example of the “bee-flower syndrome” (Faegri and van der Pijl 1980), though other animals can also pollinate them effectively. For *D. formenteranum* scent results show plain absence of any odour production useful for pollinator attraction. Absence of scent has been previously suggested to be a common feature among bee-pollinated *Delphinium* species (pers. com. of Waser and Bosch in Johnson 2001). Yet, it has been challenged and attributed to unsuitable soil in at least one species of *Delphinium* (*D. elatum*), where enzymatic mechanisms to trigger floral scent still exist (Yang et al. 2009). Scent-related attractors have also been proposed to be related to nectar rather than other floral structures (Raguso 2004). However, since our results did not show chemical scent-related compounds at the flower level, it is unlikely that any flower structure (including nectar) could be responsible for any odour in the case of *D. formenteranum*. An alternative that seems plausible in

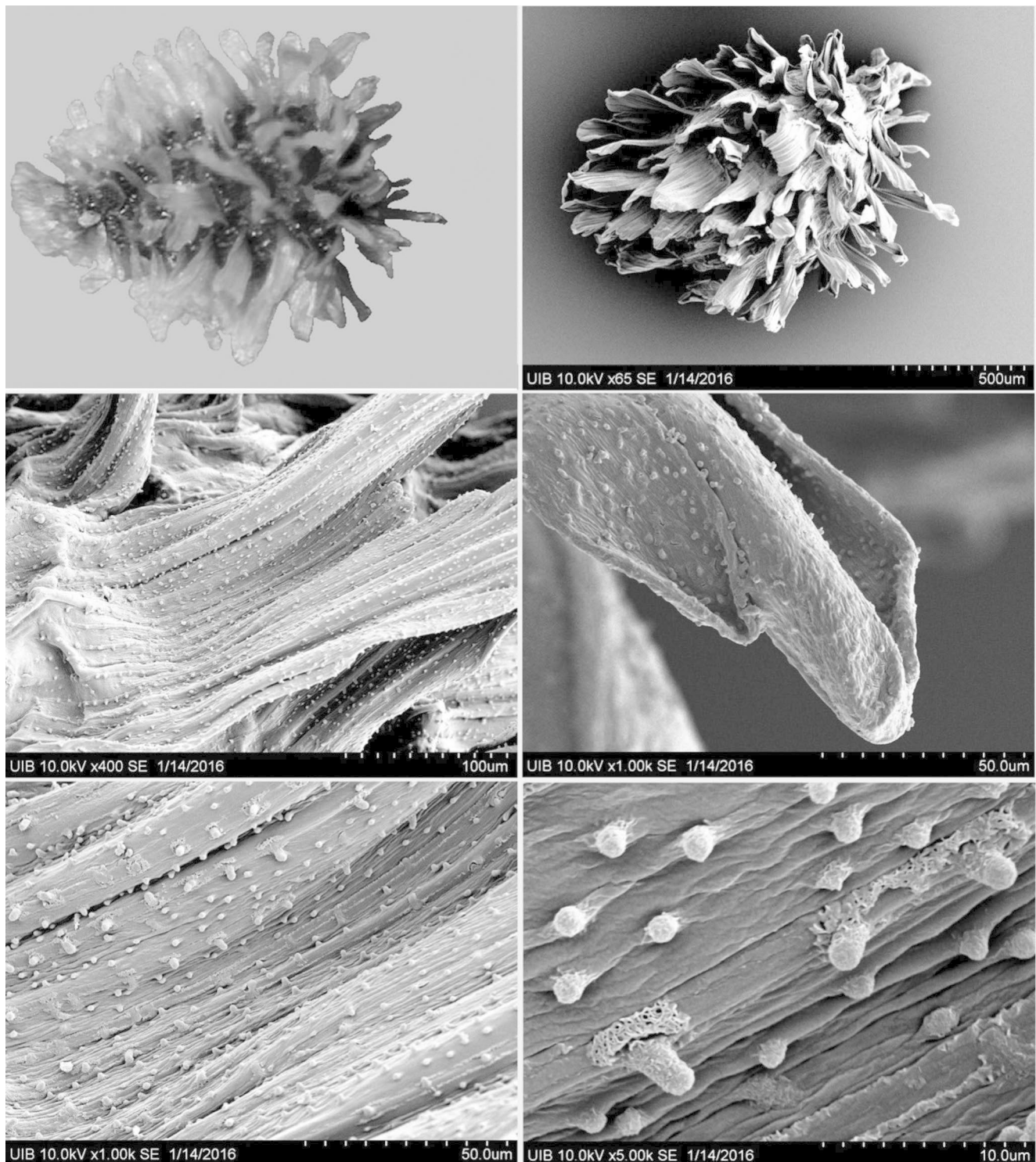


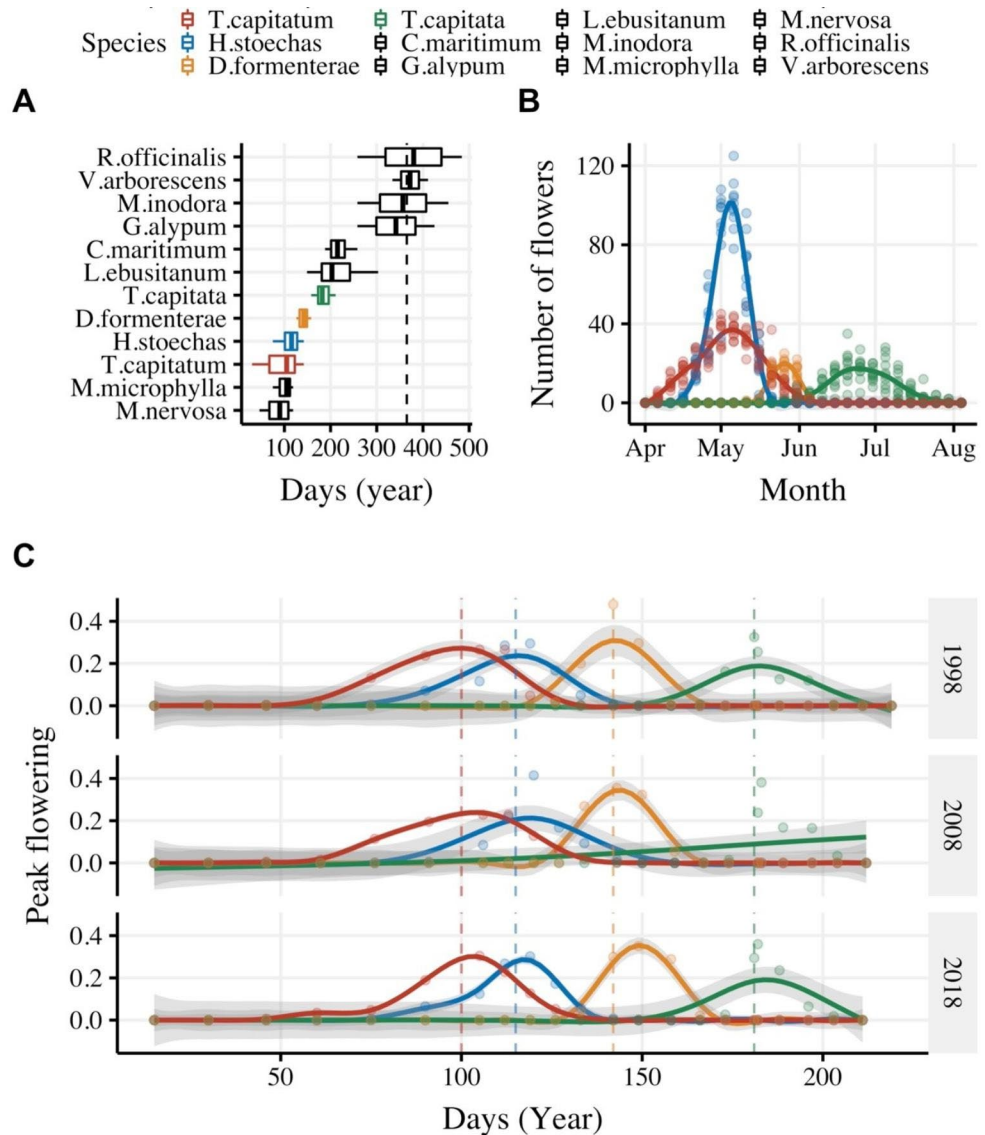
Fig. 1 Scanning electron microscopy images of *Delphinium formenteranum* seeds

Delphinium species can be related to nectar scent through yeast activity rather than per se production. Positive effects on male fitness and general fitness have been reported to occur in *Delphinium nuttallianum* and *D. barbeyi*, respectively, when nectar was contaminated by yeasts (Schaeffer

et al. 2014, 2015). These possibilities open the window for *D. formenteranum* to alternative explanations which require further investigation.

Regardless of alternative hypotheses on scent, nectar (as nutrition source), colour, disposition of flowers in the

Fig. 2 Phenological records of all the studied species along the year in field conditions (A), number of flowers of four overlapping species (*Delphinium formenteranum*, *Helichrysum stoechas*, *Thymra capitata* and *Teucrium capitatum*) from April to August of one year in laboratory conditions (B) and flowering peak of these species in a 20-year study in field conditions (C)



inflorescence and floral morphology can be considered responsible for pollinator attraction in *D. formenteranum*. Visual attraction has been extensively studied, pointing out its importance for pollinator orientation and flower tracking (Menzel et al. 1997; Miller et al. 2011). However, Reverte et al. (2016) indicated that colour itself is of less importance when pollinator targets are generalists, and Renoult et al. (2015) related generalist pollinators and pollination to colour conspicuousness. In the case of *D. formenteranum* both traits seem to play important roles. While colour does not seem to be attractive for all pollinators (i.e., *Apis mellifera*), conspicuousness based on higher flower size, and display investment compared to cohabiting species (e.g., arrangement in elongated inflorescences), allows better visibility. In this sense, it seems likely that *D. formenteranum* may relay much of its pollination and reproductive success on both flower conspicuousness and colour display.

Nectar and pollen composition may play a role in pollinator selection. It causes rejection in certain species, as it contains toxic secondary metabolites, such as diterpene alkaloids, (González and al. 1979; Díaz et al. 2004; Tiedeken et al. 2014; Stevenson 2020). Thus, through its nectar, *D. formenteranum* would achieve a certain specificity of pollination, allowing solitary generalist pollinator species present in the area, which can tolerate certain amounts of these toxic metabolites in the nectar, to act as priority or exclusive pollinators. In the same direction, due to low interspecific phenological synchrony, it is not feasible to use the mechanism of decreasing pollen toxicity by mixing pollen collections from several species (Rivest and Forrest 2020). Thus, the absence of reward visits by *A. mellifera*, either for nectar, which is often stolen in spurred-flower species, or for pollen could be explained by its toxicity, as it

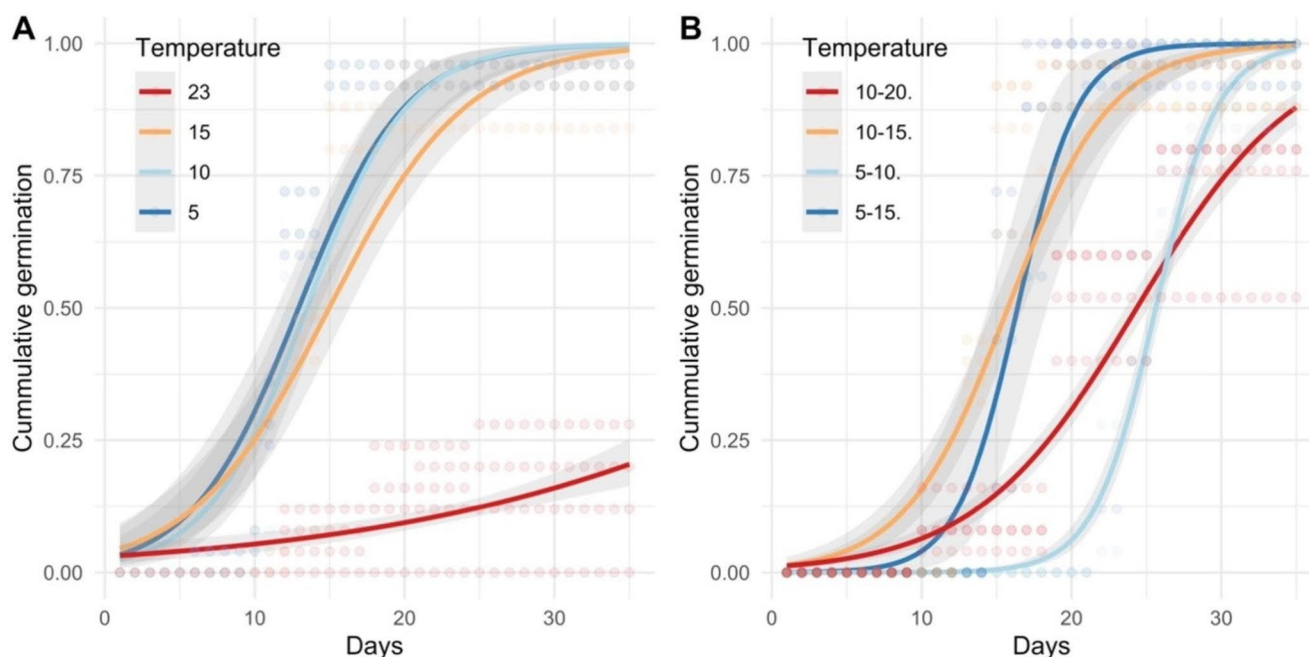


Fig. 3 Cumulative germination percentage of *Delphinium formenteranum* at different constant (A) and variable temperatures (B). N = 4–5

could not use it for feeding young or adults (Cook et al. 2013; Manson et al. 2013).

Phenology and pollinator competition

D. formenteranum lack of scent and high population variability are two major traits that entail the need of high visual attraction, which could be considerably diminished under high flowering crowdedness as stated in other species by Renoult et al. (2015). This competitive disadvantage seems to be resolved in *D. formenteranum* by coupling its floral phenology to avoid competition with other species, while allowing to exploit colour and conspicuousness. Both low phenological overlap with other species (flowering under low crowdedness) and high intraspecific synchrony appear to support this idea. Moreover, this phenological pattern seems to be constant across years, which could be related to possible photoperiodical stimulation, as is the case in other species (Borchert et al. 2005).

Interspecific competition has been previously indicated to happen in other *Delphinium* species (Ramírez-Rodríguez and Amich 2017; Orellana et al. 2008). However, references supporting any phenological adaptation to avoid or deal with this issue are scarce within the genera, with only one example given by the hummingbird pollinated *D. nuttallianum* (Waser 1978). Flowering displacement has been indicated to occur both to avoid the effect of heterospecific pollen (Morales and Traveset 2008) and pollinator dilution (Devaux and Lande 2009). Several studies have pointed out that the latter consequences of competition are especially

disadvantageous in xenogamic species with generalist pollinator (Buide et al. 2015 and included references). In the case of *D. pentagynum*, Bosch et al. (2001) noted low seed-set with spontaneous self-pollination (~6,1%), and moderate seed-set (~59,6%) with induced self-pollination, being considered as a facultative xenogamous species. Likewise, Blanché (2007) observed similar results for *D. formenteranum*. Regarding pollination, extensive studies in the Delphinieae tribe of the western Mediterranean have been conducted by Bosch et al. (1997). Specifically for the *Delphinium* genera, pollination process is known to be conducted by available fauna being mainly generalists of the Hymenoptera. These latter reproductive traits imply that *D. formenteranum* is highly dependent on cross-pollination, being this pollination process conducted by some generalist species. Considering previous observations and discussed characteristics on floral traits, it is highly plausible that flowering of *D. formenteranum* has evolved its flowering pattern to avoid competition with cohabiting species. However, the specific details behind phenological displacement by pollination competition (heterospecific pollen, pollen loss, pollinator dilution or a combination) remain to be investigated.

Germination and seed traits

The presence of lamellae on the seed surface has been related to the efficiency of seed dispersal, especially with anemochory (Blanché 1990), but may also be related to herpochory (with a hygroscopic movement that moves one seed away from the other). Due to the arid conditions where

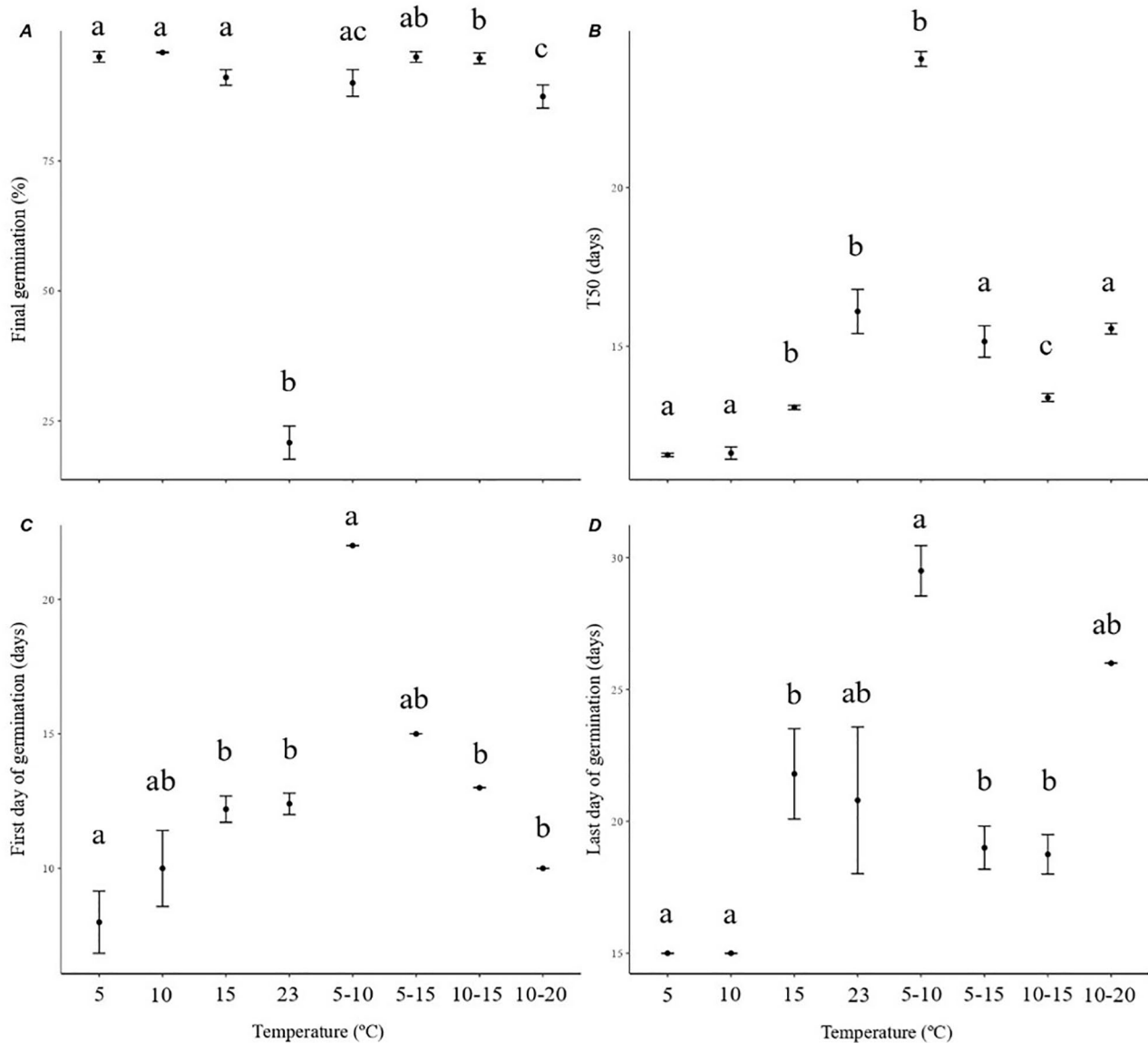


Fig. 4 Germination metrics in the different constant and variable temperature treatments for seeds of *Delphinium formenteranum*. (A) Final germination percentage, (B) T50, (C) First day of germination, (D)

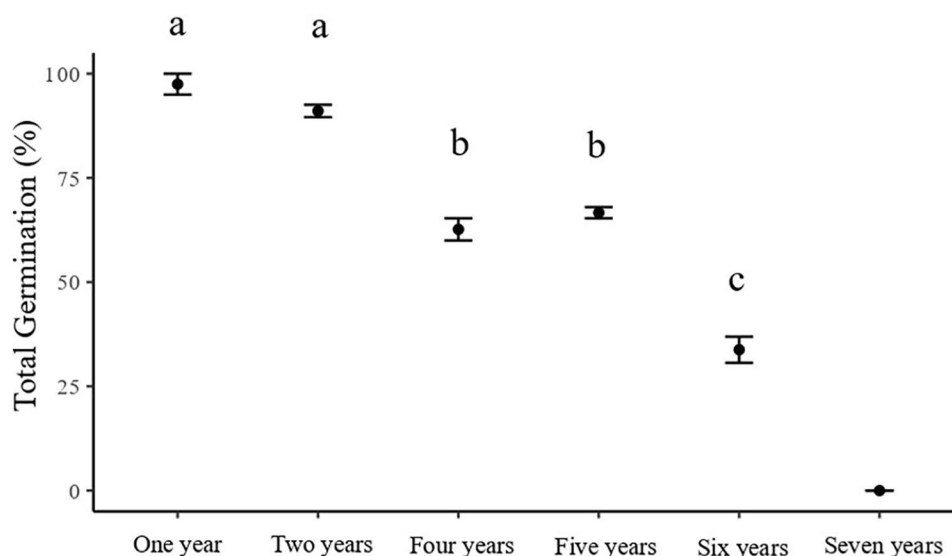
Last day of germination. Different letters indicate significant differences under Dunn test at alpha 0.05 (N = 4–5)

D. formenteranum inhabits, this seed traits can also be linked to a better imbibition capacity as stated for other species where rough surfaces allow better water impregnation to assure optimum moisture condition for faster germination (Cappelletti and Poldini 1984; Lijun et al. 2012; Zhang et al. 2019).

Germination results give further support to this idea, as fast imbibition and germination occur at a wide range of temperatures, with germination inhibition taking place only at high temperatures (23°C). Thus, these seeds can be considered physiologically as non-dormant or lacking primary dormancy, and that germination is constricted mainly

by water availability rather than temperature (Baskin and Baskin 2014). This same pattern has been described in several Mediterranean species, especially on taxa belonging to semiarid areas (Harel et al. 2011), species from arid environments like dune systems (Del Vecchio et al. 2012) and annuals (Sánchez et al. 2014). The latter authors tend to coincide that prompt germination at a wide range of temperatures with inhibition at extreme temperatures is a common strategy to take advantage of suitable conditions. In the specific case of *D. formenteranum*, germination optima also indicate that these conditions are met during late autumn, when rain and soil humidity are more regular and stable.

Fig. 5 Final germination percentages of seeds with different longevities (1–7 years) of *Delphinium formenteranum*. Different letters indicate significant differences under Dunn test at alpha 0.05 (N=5–8)



In this sense similar results have been previously described for other Mediterranean species (Del Vecchio et al. 2012) and have been attributed to both ensure germination under stable humid conditions and to permit plantlets to proper develop before summer harsh conditions.

Regarding seed longevity, *D. formenteranum* shows strong capability to maintain viable seeds for several years. It is a common trait described in many short lived or annuals species, where transient seed banks ensure survival by avoiding the risk of immediate germination under unpredictable conditions (Volis et al. 2004). Moreover, Mondoni et al. (2014) and references within, linked seed ageing resistance to higher temperature and heat stress during seed development, which can be applicable to *D. formenteranum* semiarid habitat. This adaptive germination response over the following years after seed production, can also be linked to higher genetic diversity, as it could promote crossing between individuals with different generational origin (López-Pujol et al. 2003 and included references). In this sense our results also suggest that the transient seed bank in *D. formenteranum*, in addition to securing plant development for further years, has the potential to be a useful mechanism to promote intraspecific diversity by favouring intergenerational crossing.

Conclusions

D. formenteranum displays several mechanisms both for flowering and seed and germination traits, which define its ecology and allow to promote moderate genetic diversity despite its restricted distribution. Phenological data show strong flowering synchrony while avoiding overlap with cohabiting species and the associated pollinator competition.

Pollinators seem to be restricted mainly to *Anthophora balearica*, with lack of visits or avoidance by other pollinators. Odourless flowers with relative high alkaloid content in both flower structure and rewarding substances appear to underlie pollination specificity. Regarding seeds, *D. formenteranum* displays high and fast germination at a broad range of (low) temperatures with micromorphological features that ensure rapid imbibition. This entails that this species has non-dormant seeds with germination mainly being related to water availability. However, high long-term viability indicates possible progressive germination across years, which in the natural localities probably ensures transient seed banks and inter-generational crossing.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author and Leonard Llorens on reasonable request.

Declarations

Conflicts of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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