Ecological and morphological seed traits of Polygala sardoa and P. sinisica: A comparative study on two endemic species of Sardinia

Efisio Mattana, Matthew I. Daws, Giuseppe Fenu, Gianluigi Bacchetta

Abstract

Polygala sardoa Chodat and P. sinisica Arrigoni (Polygalaceae) are two exclusive endemics to Sardinia and P. sinisica is affiliated under “Critically Endangered” in the IUCN Red Lists. In this work comparative studies on two populations of P. sardoa and in the only one of P. sinisica were carried out. In particular, seed output calculations and morphometric measurements on seed and elaiosome sizes were carried out. The effect of chipping, constant (15 °C) and alternating (25/10 °C) temperature regimes and light (8 h irradiance/day and dark) on germination were investigated. Seedling vigor assessments and a study on seed persistence in the soil were also carried out. P. sinisica had a higher seed output than P. sardoa and P. sinisica seeds were smaller than those of P. sardoa, with lower elaiosome area and elaiosome ratio values. Neither species had alternating temperature or light requirements for germination. P. sardoa achieved high germination percentages (>80%) at all the tested conditions. P. sinisica had a lower maximum germination (<60%), suggesting the presence of physiological dormancy, and took more than twice as long to reach the maximum germination percentage. Seedlings of P. sardoa were larger than those of P. sinisica, and neither species formed a persistent soil seed bank. These new data may help implement effective conservation measures for these two species and, more generally, for threatened endemic species of this genus.

Introduction

The genus Polygala Chodat (Polygalaceae) consists of ca. 725 species, has a subcosmopolitan distribution and presents a marked pattern of regional endemism and a significant representation in diversification centres, as defined by IUCN (Castro et al., 2008; Paiva, 1998). Four endemic species belonging to this genus have been inserted in the IUCN Red List (IUCN, 2009). In Sardinia, six Polygala species have been recognized (Conti et al., 2005) and two of them, P. sardoa Chodat and P. sinisica Arrigoni, are endemic to the Island. Taxonomically these two species are closely related and constitute a South Mediterranean complex together with P. preslii Sprengel (endemic to Sicily), P. venulosa Sibth. & Sm. (endemic to Greece) and P. aschersoniana Chodat (endemic to Cyrenaica) (Arrigoni, 1983). P. sardoa is comparatively common in rocky places in Southern and Central Sardinia (Arrigoni, 1983), mainly growing in areas characterized by the Mediterranean pluviseasonal oceanic bioclimate, with thermotypes ranging between the lower meso-Mediterranean and the lower supra-Mediterranean, and ombrotypes between the lower and the upper subhumid. In contrast, P. sinisica is only found in a single 1.6 ha site containing only ca. 70 individuals (Fenu and Bacchetta, 2008) along the coast in the Sinis peninsula (Central-Western Sardinia). This site experiences a Mediterranean pluviseasonal oceanic bioclimate, upper thermo-Mediterranean thermotype and upper dry ombrotype. This rare species is inserted in the IUCN Red Lists under “Critically Endangered” (Camarda, 2006).

A negative relationship has been reported between a narrow population size (or a low population density) and seed production, with the later being reduced in small, isolated populations because of both increased inbreeding and reduced number of compatible mates (Campbell and Husband, 2007; Vergeer et al., 2003). Under a Mediterranean climate, narrow endemics have been reported to be generally more stress-tolerant with a lower investment in pollen transfer and seed production than their widespread congeners, suggesting that local persistence is a key feature for the persistence of their populations (Lavergne et al., 2004). However, a high seed yield represents a successful trait for plant establishment: Mason et al. (2008) found that, at a global level, invasive species have a greater seed production than natives.

The presence of elaiosomes is associated with ant dispersal, or myrmecochory, and this seed appendage contains lipids and...
sometimes proteins and starch, which serve as a reward for ants (Lisci et al., 1996). Elaiosome size plays an important role in seed dispersal, as seed removal by ants is influenced by the ratio of elaiosome/seed size (Gunther and Lanza, 1989; Hughes and Westoby, 1992; Oostermeijer, 1989). In fact as seed size increases, elaiosome size must increase more than proportionally for seeds to remain equally attractive to ants (Edwards et al., 2006).

Seeds of species from the Polygalaceae have spatulate embryos (Martin, 1946) and are either non-dormant (ND) or physiologically dormant (PD) (Baskin and Baskin, 1998). However, germination ecology in species from the Polygalaceae has not been investigated in detail. Grime et al. (1981) found that a dry storage period at 5°C increased germination of P. vulgaris seeds compared with freshly collected seeds. Ferrara and Quinn (1987) found that after 4 months of dry storage, germination of P. paucifolia Willd. seeds increased with seed size. Liu et al. (2008) reported that some seed lots of Polygala species achieved high germination percentages (70–90%) only after pre-treatments like chipping with scalpel (P. vulgaris), followed by sterilization and imbibition (P. calcarea Schultz), while other species like P. comosa Schkuhr, P. mossii Exell and P. virgata Thunb., germinated quite well (50–87%) without any pre-treatments at both constant and alternating temperature regimes in the light. However, since the germination tests reported in Liu et al. (2008) were not designed in a factorial way, it is not possible to determine the key factors in stimulating germination of these species.

Among environmental factors, temperature is arguably the single most important factor governing the maximum germination percentage and rate of germination (Heydecker, 1977). Temperature also impacts on the success or failure of plant establishment (Kader and Jutzi, 2004) as well as seedling vigor (Hampton and Tekrony, 1995; Spears, 2002). Light and alternating temperature requirements for germination are related to seed mass (Jankowska-Blaszczyk and Daws, 2007; Milberg et al., 2000; Pearson et al., 2002, 2003; Probert, 1992) and seed mass also affects seedling survival at population level: larger seeds generally result in larger seedlings which often have a higher probability of survival (Daws et al., 2005) as a result of a greater ability to withstand either low levels of resources or various hazards (Leishman et al., 2000).

Seed size is also related to persistence in the soil. Soil seed banks can be classified as transient (TSB) or persistent (PSB) (Thompson and Grime, 1979) in accordance with the time that the seeds remain viable in the soil and persistent seeds are typically smaller and more rounded than seeds forming a transient seedbank (Cerabolini et al., 2003; Thompson et al., 1993). The ability to form a PSB is crucial to the survival of many rare or declining species (Eckstein et al., 2006; Keddy and Reznick, 1982; Quilichini and Debussche, 2000; Rowell et al., 1982) conferring a degree of resilience in the face of modern and intensive land use (Thompson et al., 1993). Within the genus Polygala, Cerabolini et al. (2003) identified two species able to form TSB, the temperate P. chamaebuxus L. and the Mediterranean P. nicaeensis W.D.J. Koch s.l.

In this work, comparative studies on the two closely related endemic Sardinian species, differing in their extent of distribution, were conducted on seed lots belonging to two populations of P. sardoa and the only population of P. sinisica. In particular, seed output, seed and elaiosome sizes and the effect of chipping, constant and alternating temperature regimes and light on germination were investigated. In addition, seedling vigor assessments as well as soil seed persistence analyses were carried out. The main aim of this study was to provide new data on seed biology, morphology and ecology of these previously unstudied species, in order to investigate their reproductive strategies and germination ecology. These data may help to implement conservation measures for these two species and, considering the marked pattern of regional endemism of this genus, for other threatened endemic Polygala species.

Materials and methods

Plant descriptions

Polygala sardoa Chodat is a perennial herb with stems not more than 15 cm in length, ascending from a wood stock, glabrous or slightly pubescent above. Leaves are all linear-lanceolate and bracts are slightly shorter. An inflorescence has 5–15 flowers on short stalks. Wings (7 mm × 3 mm) are glabrous, whitish with green veins, oblong-elliptical, acute, and slightly longer than the corolla tube. Petals are bluish or pink. The style is less than twice as long as stigma. The fruits bear hairy seeds with a short strophiophus. The flowering season lasts from early May to the first days of July (Arrigoni, 1983; McNeill, 1968).

Polygala sinisica Arrigoni is a perennial herb with stems 15–20(30) cm in length, erect or nearly erect. The tough, flexible stems are either hairless or slightly hairy. Lanceolate or linear, non-fleshy leaves alternate along the stem. Inflorescences have 15–20 flowers on short stalks, grouped in bunches at the ends of the stems. Three of the sepals are small and hairy; the two others resemble elliptical wings. The pink or bluish petals are 11–12 mm long. The fruits bear hairy seeds with a very short strophiophus. The plants flower in May and immediately afterwards produce fruits (Arrigoni, 1983; De Montmollin and Strahm, 2005).

Seed output assessment

A sample of 20 randomly chosen mature individuals for the two investigated populations of P. sardoa and 27 for the P. sinisica population (see below) were labelled and monitored twice at month during 2007. For each individual, the number of stems bearing flowers and fruit, as well as the number of ripe fruit for each stem were determined. The mean number of seeds per fruit was verified by checking 50 fruits from each population in the month when the maximum number of ripe fruits were detected and seed output was then calculated by multiplying the mean number of seeds/stem per the mean number of stems/individual.

Seed lot details

Seeds of the two species were collected from May to June 2007 at the time of natural ripening/dispersal from two populations of P. sardoa (Prados, Oliena–NU; 1210 m a.s.l.; lower supra-Mediterranean thermotype and upper subhumid ombrotrope; Sa Macchina Beccia, Iglesias–CI; 220 m a.s.l.; lower meso-Mediterranean thermotype and lower subhumid ombrotrope) and from the only population of P. sinisica (Sa Mesa Longa, San Vero Milis–OR; 25 m a.s.l.; upper thermo-Mediterranean thermotype and upper dry ombrotrope). Seeds were stored at the Sardinian Germplasm Bank (BG-SAR) where, once cleaned, by removal from the capsules, they were placed in the dry room at 15°C. Seed measurements and SEM image analysis

Images of the samples (150 seeds for P. sardoa and 339 for P. sinisica) were acquired using a flatbed scanner (Epson GT-15000), with a resolution of 200 dpi, on fresh seeds before they were stored in the dry room (15°C at the 15% of R.H.), to avoid any possible variation in dimension and shape. The acquisition method was performed with KS-400 V. 3.0 (Carl Zeiss, Vision, Oberkochen, Germany), according to Bacchetta et al. (2008). In order to take the measurements of the objects, a macro, formerly developed to identify diaspores of wild plants species (Bacchetta et al., 2008; Mattana et al., 2008), was applied both for the whole seeds and the elaiosomes. Elaiosome ratio (ER) was calculated as elaiosome area/whole seed area.

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SEM images on secondary electrons, acquired by “low vacuum” modality, were taken on seeds dried with silica-gel and mounted on metal stubs using double-stick tape and taken by a FEI ESEM QUANTA 200.

Germination tests

Three replicates of 10 seeds per treatment were sown, after removal of the elaiosome, on the surface of 1% agar water in 90 mm plastic Petri dishes and incubated at either 15°C or 25/10°C, in November 2007. The low number of seeds per replicate (10) was used because of limited seed availability, since *P. sinisica* has a very small population. For the same reason, no experiments, e.g. with a pre-chilling period, were conducted to investigate the presence of physiological dormancy.

In all treatments, seeds were exposed both to either irradiance for 8 h per day or constant darkness. In the alternating temperature regimes, the 8 h light period coincided with the elevated temperature period. Dark incubated seeds were scored in a dark room with a safe green light. To evaluate the effect of the safe green light on germination a test was carried out by sowing three extra replicates at 15°C in the dark, achieved by wrapping dishes in aluminium foil; seeds in this experiment were only scored once, at the end of the test, to avoid any exposure to irradiance (Baskin et al., 2006). Seeds were chipped using a scalpel, and to verify the effect of chipping on germination, an extra test was carried out under the same conditions, but with intact seeds for the seed lot with the larger amount of seed (Iglesias population). Germination counts for all experiments were made daily for 85 days and germinated seeds removed. Germination was defined as visible radicle protrusion. When no additional germination occurred for 2 weeks, the viability of any remaining seeds was checked by a cut-test and the final germination percentage calculated on the basis of the total number of filled seeds.

Seedling vigor

After visible germination, seedlings were incubated on 1% agar water under the same conditions as the germination test for a further 8 days. Subsequently, seedling dry mass was calculated by drying ten 8-day-old seedlings, for each germination condition, in an oven at 103°C for 17 h. Seedlings were then weighed to seven decimal places. To avoid any possible damage to the seedlings they were weighed with the seed coats in place.

Experimental seed burial

Experimental seed burials were carried out following a modification of the protocol in Arroyo et al. (2004). Sets of 5 replicates containing 10 seeds each were introduced into fine grain nylon mesh envelopes which were placed in plastic nets. The envelopes were filled with sieved local soil and buried so that the seed envelopes were at a depth of 5 cm at the original population locations. The burial sites were the four vertices and the centre of a square (1 m sides). Seed burials were performed at the time of natural seed dispersal. After 1 year the replicates were exhumed. Any remaining, intact, non-germinated seeds were sown immediately at 15°C in the darkness to check their viability and germination capacity.

Data analysis

One-way analysis of variance (ANOVA) was carried out on seed and elaiosome measures and ER values. As a result of multiple one-way comparisons, a Bonferroni correction was applied to the critical value of α for these comparisons (Sokal and Rohlf, 1995). Arcsine transformed germination percentages and seedling size values were also statistically analysed by one-way and two-way ANOVA; subsequently, *post hoc* Fisher least significant difference test (LSD) was conducted. The non-parametric Kruskal–Wallis test was carried out to test for differences in the time to final germination and the non-parametric Mann–Whitney *U*-test, for differences on the number of seeds/capsule between species and seeds/individual between each population, using MINITAB® release 11.21 (Minitab Inc.). As a result of multiple comparisons, a Bonferroni correction was applied to the critical value of α for the Mann–Whitney *U*-tests (Sokal and Rohlf, 1995).

Slopes generated from standard major axis (SMA) regressions were calculated using SMATR 2.0 (Falster et al., 2006). This method is more appropriate than least squares regression when the x-variable is measured with error and this approach has recently applied to comparing proportional changes in elaiosome and seed masses (in mg) in log–log analyses (Edwards et al., 2006). The SMA slopes were calculated as a measure of the proportional relationship between the elaiosome size \([f(x)]\) and the seed size \(x\), calculated as area in \([\text{mm}^2]\), and the ability to fit a common slope to the two species tested (Warton et al., 2006). To compare the relationship between the two species, shifts in elevation (residual axis scores, \(R\)) and along the common slope (fitted axis scores, \(F\)) were also analysed using the Wald statistic in SMATR 2.0 (Warton et al., 2006).

Results

Seed output

The vegetative season begins in April and ends in August for *P. sardoa*, while for *P. sinisica* starts in late January and ends in August. The fruiting season (Fig. 1) lasts from early March to late June/early July (ca. 135 days) for *P. sinisica*, showing a peak in June with 100% of the surveyed individuals bearing full, ripe capsules. The two *P. sardoa* populations showed a very similar trend with a fruiting period less than half as long as in *P. sinisica* (ca. 60 days), spreading from late May to June, when the peak was also detected, with the totality of individuals bearing ripe fruit (Fig. 1).

The mean number of capsules per stem in *P. sinisica* (18.6 ± 9.6) was more than three times greater than that in *P. sardoa*,

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Fig. 1. Fruit production annual trend for the three analysed populations in 2007. Field surveys were conducted twice at month. Percentages were calculated on a sample of 27 (*P. sinisica*) and 20 (for each *P. sardoa* population) randomly chosen mature individuals.
both in Oliena (5.8 ± 1.9) and Iglesias (5.6 ± 1.4) populations (Mann–Whitney U-test, p < 0.001; Fig. 2), with no significant differences between the two P. sardoa populations (Mann–Whitney U-test, p > 0.05). The mean number of capsules per individual was 161.1 ± 82.7 for P. sinisica and only 68.0 ± 21.6 and 44.8 ± 11.0 on P. sardoa for Oliena and Iglesias populations, respectively, with this difference being statistically significant (Mann–Whitney U-test, p < 0.001). While in P. sardoa the mean number of seeds per capsule was 2, this value was 1.8 ± 0.4 in P. sinisica, due to the presence of aborted seeds; this difference was statistically significant (Mann–Whitney U-test, p < 0.001). The mean number of capsules per individual was 290.0 ± 148.8 seeds per individual, significantly higher (Mann–Whitney U-test, p < 0.001) than those detected for P. sardoa (89.7 ± 20.0 and 136.0 ± 43.2 for Iglesias and Oliena populations, respectively, with no significant differences being detected between groups (Fig. 2).

**Seed sizes and elaiosome ratios**

The measurements taken on the whole seeds and elaiosomes are reported in Table 1. Seeds of the two species showed significantly different values (one-way ANOVA, p < 0.001). In particular, P. sinisica seeds with an average area of 4.3 ± 0.5 mm² are smaller compared with those of P. sardoa (average area 4.7 ± 0.6 mm²). Furthermore, the measurements taken on the elaiosomes detected an average area of 0.6 ± 0.2 and 0.8 ± 0.2 mm² for P. sinisica and P. sardoa, respectively. These differences were statistically significant (one-way ANOVA, p < 0.001).

SMA analyses, on seed and elaiosome areas, showed statistically significant results (Table 2). It was possible to identify a common slope between groups (SMA test statistic = 2.872; p > 0.05) both a significant elevation shift (F, SMA test statistic = 10.38; p < 0.001) and a shift along the common slope (R, SMA test statistic = 27.03; p < 0.001) between groups (Fig. 3).

The differences between the two species on the elaiosome and seed size resulted in a larger elaiosome ratio (ER) for P. sardoa compared with P. sinisica (average area 4.7 ± 0.6 mm²). Furthermore, the measurements taken on the elaiosomes detected an average area of 0.6 ± 0.2 and 0.8 ± 0.2 mm² for P. sinisica and P. sardoa, respectively. These differences were statistically significant (one-way ANOVA, p < 0.001).

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**Table 2**

Summary of analysis of covariation between elaiosome and seed size for P. sinisica and P. sardoa.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>R²</th>
<th>p</th>
<th>Slope Lower</th>
<th>Slope Upper</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. sinisica</td>
<td>339</td>
<td>0.04</td>
<td>&lt;0.001</td>
<td>0.463</td>
<td>0.417</td>
<td>0.515</td>
<td></td>
</tr>
<tr>
<td>P. sardoa</td>
<td>150</td>
<td>0.13</td>
<td>&lt;0.001</td>
<td>0.543</td>
<td>0.467</td>
<td>0.632</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>489</td>
<td>&lt;0.001</td>
<td>0.489</td>
<td>0.448</td>
<td>0.532</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 1**

Measurements taken on both the whole seeds and the elaiosomes of the two species. The features are explained in Mattana et al. (2008). Data are the mean of 339 seeds for P. sinisica and 150 for P. sardoa ±1 standard deviation (ns: p > 0.05 and *** p < 0.001, by one-way ANOVA). The critical p-value of 0.05 was corrected using a Bonferroni correction yielding a value of 0.0019.

**Fig. 2.** Seed output for the three investigated populations. Bars are the mean number of seeds per individuals, points the mean number of capsules per stem. Seed output values (expressed as the mean number of seeds per individuals) with different letters are significantly different at α = 0.001 (Mann–Whitney U-test, with Bonferroni correction). Data are the means of 27 (P. sinisica) and 20 (for each P. sardoa population) randomly chosen mature individuals (±1 standard deviation).

**Fig. 3.** Covariation between seed and elaiosome area on the two species, points indicate P. sinisica (n = 339) and triangles P. sardoa (n = 150) seeds. Test for common slope (SMA test statistic = 2.872; p > 0.05) both a significant elevation shift (F, SMA test statistic = 10.38; p < 0.001) and a shift along the common slope (R, SMA test statistic = 27.03; p < 0.001) between groups.

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The SEM images (Fig. 4) highlighted that the difference in size between the elaiosomes of the two species is due to a difference in morphology. In *P. sardoa* the two ventral arms of the elaiosome extend along the seeds, increasing the total surface while in *P. sinisica* the arms do not reach over the hilum region.

**Germination**

The preliminary test carried out on *P. sardoa* seeds from the Iglesias population, showed that chipping significantly increased germination (one-way ANOVA, *p* < 0.05); whereas seeds without any pre-treatments achieved 82.5 ± 13.6%, the chipped seeds reached final germination of 92.5 ± 7.5%. Consequently all the tests on the two species were carried out after chipping with a scalpel.

For both species there was no effect of temperature regime (constant vs. alternating) or the presence/absence of irradiance on germination percentages (two-way ANOVA, *p* > 0.05). In addition, whereas no statistical differences were found between the two populations of *P. sardoa*, *P. sinisica* seeds reached lower germination percentages at all the tested conditions (*p* < 0.001 by one-way ANOVA, followed by post hoc Fisher’s LSD test). The maximum germination percentage achieved for *P. sardoa* was 96.7 ± 5.8% (at 15 °C in the dark for the Iglesias population and in the light for the Oliena population), whereas for *P. sinisica* maximum germination was 42.7 ± 29.8% at 25/10 °C in the dark (Fig. 5).

The green safe light, under which scoring was carried out for the seeds sown in dark, did not affect germination levels. In fact, germination percentages achieved at 15 °C in the light (64.4 ± 24.5%), in the dark (scoring regularly under safe green light; 52.2 ± 33.1%) and in the dark (scored only at the end of the test; 71.1 ± 34.1%) were not significantly different (one-way ANOVA, *p* > 0.05).

*P. sinisica* seeds not only germinated to lower percentages but also more slowly than *P. sardoa* seeds (Kruskal–Wallis test, *p* < 0.05); *P. sardoa* reached the maximum observed germination percentage in 21.6 ± 8.2 days and *P. sinisica* took 45.0 ± 26.4 days. The curves in Fig. 6 show that at 25/10 °C in the dark *P. sinisica* needed 76 days to achieve the maximum germination percentage compared with 24 (Oliena population) and 33 days (Iglesias population) for *P. sardoa*.

**Seedling size**

Seedling dry mass was significantly different between the two species (one-way ANOVA *p* < 0.001). *P. sardoa* seedlings, with a dry

![Fig. 4. SEM images of the basal regions of *P. sardoa* (left) and *P. sinisica* (right) elaiosomes. Reference bar is 400 μm long.](image)

![Fig. 5. Final germination percentages at each temperature regime; values with the same letters are not significantly different at *p* > 0.05 (post hoc Fisher’s LSD test). Data are the means of 3 replicates (+1 standard deviation).](image)

![Fig. 6. Germination trends at 25/10 °C in the dark for the two populations of *P. sardoa* and for *P. sinisica*. Times to achieve the maximum germination percentages are shown. Points (*P. sardoa*) and triangles (*P. sinisica*) correspond to the actual data and solid lines indicate the fitted lines from the sigmoidal regressions. Data are the means of 6 replicate (+1 standard deviation).](image)
mass of 1.78 ± 0.50 mg, were heavier than those of *P. sinisica* (dry mass of 1.20 ± 0.29 mg).

**Seed persistence in the soil**

After 1 year of burial only a few *P. sinisica* seeds and no *P. sardoa* seeds were retrieved intact. In general, only empty seed coats were retrieved (92.0 ± 13.8% and 99.0 ± 3.2% of the seeds were retrieved empty for *P. sinisica* and *P. sardoa*, respectively), showing no statistical differences between species (one-way ANOVA, p > 0.05) and suggesting that the great majority of the seeds germinated during the year. All the retrieved intact *P. sinisica* seeds (4.0 ± 8.9%) germinated in the laboratory after incubation at 15 °C in the darkness and a further 4.0 ± 5.5% of seeds were not retrieved, being dead and decomposed in the soil before their exhumation.

**Discussion**

Significant differences were detected in all the analysed parameters between the two species. The two populations of *P. sardoa*, which differ from each others in terms of altitude and bioclimate, showed similar patterns of behavior. While no data on the vegetative season of both species is available in the literature, we were able to compare our data on the fruiting period with previous reports. The two species showed significant differences in seed outputs. The fruiting period, at least in 2007, was longer for *P. sinisica* than for both populations of *P. sardoa*, suggesting a higher investment in reproduction for this species. Our results confirm the data reported by Arrigoni (1983) for *P. sardoa* and broaden the periods previously reported for *P. sinisica* (Arrigoni, 1983; Camarda, 2006; De Montmollin and Strahm, 2005). The higher seed production detected in *P. sinisica* seems to be related to the higher number of capsules per stem, due to the larger size of the individuals of this species compared with those of *P. sardoa*, as reported in literature (Arrigoni, 1983; De Montmollin and Strahm, 2005) and also confirmed by our field observations, with stems of *P. sinisica* being longer than 30 cm. These data suggest that seed output is unlikely to constrain these species. Nevertheless this should be confirmed by planning a longer period of field monitoring. In fact, environmental factors such as light and water availability can affect fruit production, both directly because of their effects on resources available for fruit maturation, and indirectly because of their effects on flower production and on abundance of pollinators and seed predators (Agren et al., 2008). The two species showed differences in their external seed morphology, not previously described in a quantitative way (Arrigoni, 1983; Camarda, 2006; De Montmollin and Strahm, 2005): *P. sinisica* seeds were significantly smaller than those of *P. sardoa* and also elaiosome area and elaiosome ratio (ER) values were lower for *P. sinisica*. Seeds of both species seem to be adapted to the dispersal by ants (myrmecochory): the hairs in the seed coat facilitate seed handling and transport by ants that are attracted by the reward of the elaiosome. However, looking at the covariation of the seed and elaiosome areas and the lower ER values for *P. sinisica* it seems that these seeds could be less attractive for ants in terms of costs/benefits. The low values of the slopes of the covariation of the two species (<1; Fig. 3) indicate that elaiosome area increased more slowly than whole seed area. Therefore, in accordance with Edwards et al. (2006), the reward may be not compensatory with the cost of the efforts expended on their removal. However, to confirm this result more studies are needed to compare the obtained ER values with those of other species in this and other families. The difference in ER between the two species it is not only determined by the size, but also by different elaiosome shapes, as highlighted by the SEM images, with long arms of the elaiosomes on *P. sardoa* seeds that increase the total surface of this seed appendage. Therefore, this difference is not due to environmental factors like resources and nutrients availability, but to intrinsic species-specific effects. All these data suggest that the two species have different reproductive strategies. In particular, while *P. sinisica* produces a larger amount of smaller and unspecialized seeds per year, *P. sardoa* produces less, but larger seeds that are better adapted to ant dispersal.

As a result of the low numbers of available seeds it was not possible to carry out factorial seed germination experiments. Nonetheless, the germination experiments carried out in the laboratory indicated that chipping significantly increased the final germination percentages of *P. sardoa* seeds, but the high percentages achieved without pre-treatments excludes the possibility of physical dormancy (PY) sensu Baskin and Baskin (2004). Considering that the seeds of the two species have a very similar morphology, the lack of this class of dormancy may also be inferred for *P. sinisica* seeds. Neither species had an alternating temperature or light requirement for germination, suggesting that seedlings of both species are able to germinate in a range of environmental conditions (deeply or shallowly buried in the soil, in the sun/shade, etc.). Whereas *P. sardoa* seeds achieved high germination percentages at all the tested conditions, *P. sinisica* seeds germinated to significantly lower levels than its congener. In addition, *P. sinisica* seeds reached their maximum germination percentage more than twice as slowly as *P. sardoa* seeds (Fig. 6). These data (the differences on the final germination percentages in particular) suggest that, following the dormancy classification system (Baskin and Baskin, 1998, 2004; Nikolaeva, 1977), whereas *P. sardoa* seeds could be defined as non-dormant (ND), *P. sinisica* seeds may show physiological dormancy (PD). However, these data need to be confirmed by testing the effect of a pre-chilling period and several germination temperatures and conditions in a factorial way to identify the level and type sensu Baskin and Baskin (2004) of PD and to verify if the slow germination of these seeds was a function of the low quality of the seed lot, as suggested by the presence of aborted seeds.

The greater vulnerability of *P. sinisica* to extinction was also confirmed by the results of the seedling vigor measurements. Seedlings from larger seeds are typically more tolerant of a range of hazards (e.g. drought and herbivory; Daws et al., 2005; Leishman et al., 2000) suggesting that seedlings of *P. sardoa*, which were significantly larger than those of *P. sinisica*, will be both more robust and more likely to establish than those of *P. sinisica*.

The experimental seed burial data, in accordance with the results obtained by Cerabolini et al. (2003) on two other Polygala species, suggest that these species may not be able to form a PSB: although larger scale experiments would be needed to verify this. The vast majority of seeds of both species germinated in the soil before being exhumed. The seed shape of the two species is consistent with these results: persistent seeds tend to be small and compact, while short-lived seeds are normally larger and either flattened or elongated (Cerabolini et al., 2003; Fenner and Thompson, 2005; Funes et al., 1999; Thompson et al., 1993). The lack of a PSB represents a rarity trait that increases difficulties for long-term existence under threat for both species, but particularly for *P. sinisica*. It seems that the higher germination observed for *P. sinisica* seeds in the field than in the laboratory, confirms that seeds of this species may show a PD. Potentially, pre-chilling as well as dry after ripening may increase the final germination percentage in the field: this remains to be tested in the laboratory.

In conclusion, this study provides new data in terms of seed output, dispersal and germination, improving our knowledge on seed morphology and ecology of these two endemic species. Even if the achieved results need to be confirmed with further analysis (particularly monitoring of the populations and germination experiments to be carried out also under natural conditions in order to investigate the effect of temporary water availability), these data highlight...
the need of urgent in situ and ex situ conservation measures for the single population of *P. sinisica* for which a low seed viability and germination was detected. They may be helpful, as well, to activate similar conservation studies on threatened endemic species of this genus belonging to other territories.

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