

Reproductive biology of the narrow endemic *Anchusa littorea* Moris (Boraginaceae), an endangered coastal Mediterranean plant

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Abstract: Phenological and reproductive patterns are fundamental life-history components in the life cycle of plants. In this study, we analyzed phenological and reproductive data at the plant and population levels of the narrow endemic *Anchusa littorea* Moris, an endangered annual plant species growing in a few small patches at the Is Arenas dune system (SW Sardinia, Italy). Individuals from the main 2 patches were evaluated during a 4-year study period: plants were monitored on a monthly basis in each reproductive phenophase. To assess phenological variability within and among populations, an approach based on the Shannon–Wiener diversity index and the Morisita–Horn similarity index was applied. The seed output and the soil seed bank were also investigated in order to analyze the reproductive capacity of this plant species. *Anchusa littorea* showed a long flowering season and a high flowering synchrony between patches, which contrasted with a low within-patch synchrony. The seed production per plant was low and the species did not create a soil seed bank. Population size was characterized by considerable annual fluctuations: a significant migration occurred for *A. littorea* plants inside the dune system over the years. These results demonstrate the importance of the phenological pattern on the knowledge of the reproductive ecology of endangered coastal plant species and will contribute to orient management and conservation efforts.

Key words: Annual narrow endemic plant, phenology, sand ecosystem, Sardinia, seed output, soil seed bank

1. Introduction

Studies on reproductive biology of endangered plants are crucial for predicting their survival capacity and establishing the appropriate measures for their conservation (Evans et al., 2003). Plant reproductive success is determined by intrinsic characteristics of the reproductive system of a species, as well as by extrinsic abiotic and biotic factors (Abdala-Roberts et al., 2014).

Flowering and fruiting phenology may have an important influence on plant reproductive success in several ways (Augspurger, 1981; Elzinga et al., 2007). The timing of flowering can strongly influence the reproductive success at the individual level (plants that flower too early may not have adequate resources stored to mature fruits), at the population level (asynchronously flowering plants may not find mates), and at the species level (plants flowering at the “wrong” time might not be visited by pollinators, or may be disproportionately affected by seed predators (Molau, 1993; McIntosh, 2002)). Moreover, abiotic factors could influence the reproductive success because plants flowering early or late in the season may

be killed by adverse climatic factor before they can mature fruits (Molau, 1993; McIntosh, 2002).

In arid and semiarid habitats, like Mediterranean sandy coastal ecosystems, plant species are affected by the remarkable shifts that abiotic and biotic factors show throughout the year (Bosch et al., 1997; Fenu et al., 2013). Summer drought, the key characteristic of Mediterranean climate, appears to be the most limiting environmental factor for plant reproduction (Pettersson, 1994). Given the role that some of these factors may play as selective pressures on flowering times (Rathcke and Lacey, 1985; Pettersson, 1994), and considering that in the Mediterranean regions most plants flower during a few months and the blooming is concentrated in spring (Bosch et al., 1997), an extended flowering season in Mediterranean arid environments appears to be an exception to the rule (Picó and Retana, 2000).

Temperature is one of the most important environmental factors governing the timing of seed germination and seedling emergence and establishment (Fenner and Thompson, 2005) and represents an important driver of

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plant phenology (Rathcke and Lacey, 1985; Elzinga et al., 2007). More specifically, among the several environmental factors that affect flowering phenology, temperature is considered to be one of the major cues that initiate the onset of flowering (Blionis et al., 2001; Cornelius et al., 2013). Hence, studies of the relationship between temperature regimes and plant phenology are of great importance, in particular those regarding plants growing in arid ecosystems such as Mediterranean coastal dunes ones, where temperature represents an important limiting factor for plants (Maun, 2009). Furthermore, considering the documented effect of global warming in inducing advances in flowering (e.g., Cornelius et al., 2013), it is very important to study the relationships between small-scale temperature and flowering patterns, since knowledge about these events is scarce (Post et al., 2008). Climate change has given a new urgency to the need to understand how flowering phenology affects plant reproductive success and the ability of populations to adaptively adjust flowering time in response to changing conditions (Elzinga et al., 2007; Giménez-Benavides et al., 2011).

Phenological adjustments to deal with the Mediterranean summer drought have been previously studied (e.g., Fotelli, 2000). Highly seasonal climates stimulate high within-population phenological synchrony and the level of overlap between phenophases seems to be relevant for the adaptation of plants to the Mediterranean climate (Milla et al., 2010). Flowering synchrony among individuals is one of the main determinants of plant reproductive success, as strong flowering synchrony implies that each plant can exchange genes with most plants within the same population, thus increasing the genetic diversity (Augspurger, 1981; Ollerton and Lack, 1998). In fact, in exogamous species with asynchronous flowering phenology, those individuals that are highly asynchronous with respect to the population mode show remarkable reduction in reproductive fitness (Augspurger, 1981; Ollerton and Lack, 1998).

Information about the ecology of germination allows us to assess the potential persistence ability of seeds in the soil, which may be of special interest when studying threatened plant species (e.g., Cogoni et al., 2012). In coastal dunes ecosystems, the soil seed bank is characterized by a high spatial and temporal variability (Thompson, 1987), mainly due to the scarcity and irregular patterns of precipitation found in arid regions, which leads to the potential for spatial variability in processes important to the storage of germinable seeds (Pungraine and Lazaro, 2000; Cogoni et al., 2012).

As far as we know, few studies have explored the phenological and reproductive patterns of coastal plants in the Mediterranean Basin (e.g., Estiarte et al., 2011). In

particular, no studies are currently available on annual coastal plants and, to date, no exhaustive studies on phenology and reproductive biology have been carried out on *Anchusa littorea* Moris, an endangered, narrow endemic of Sardinia. In this study, we describe the most relevant phenological and reproductive traits of this annual plant; the specific aims rely on answering the following questions: (1) What is the phenological pattern and, more specifically, what is the flowering and fruiting phenology of the species, considering the small-scale temperatures trend? (2) What is the seed output for *A. littorea*? (3) Does the species create a soil seed bank? (4) How does flowering synchrony affect this coastal annual plant?

2. Materials and methods

2.1. Study species and area

Anchusa littorea (Boraginaceae) is an annual microphyte, with a small basal rosette (Figure 1). Each plant develops one or several cymes, with small tubular flowers distanced at the axil of cauline leaves, often also in the lower part of the stems just above ground level, on pedicels 2–3 mm long deflexed in fruit. Corolla, with tube 4 mm long and limb 4–5 mm, is light blue or white, rotate with rounded lobes; the small mericarps are light gray-brown small, 1.5–2 × 0.5–1 mm, with a lateral beak and a thin basal annulus, with finely tuberculate surface (Bacchetta et al., 2008).

Based on a recent study carried out on a part of the *A. littorea* population, the flowering period is approximately from March to May and the fruiting season from April to July (Fenu et al., 2013).

Anchusa littorea is currently restricted to the Is Arenas coastal dune system (SW Sardinia), where it grows in ephemeral plant communities occupying the open areas between *Juniperus macrocarpa* Sm. microforest (Bacchetta et al., 2008; Figure 2). This species grows exclusively within the “Is Arenas” penitentiary area, in a



Figure 1. *Anchusa littorea* Moris.



Figure 2. Habitat of *Anchusa littorea* in the Is Arenas coastal dune system.

few small and isolated patches within the dunal system, and in particular in 2 main patches, which are ca. 700 m distant from each other (Figure 3; Cogoni et al., 2013).

The Is Arenas dune system, mainly consisting of Holocene sandstones and aeolian sands forms (Annino et al., 2000), is one of the most important and well-preserved coastal ecosystems in Sardinia. Available climatic data from the nearest weather station (Montevecchio, ca. 12 km) indicate a typical Mediterranean annual pattern of temperatures (mean annual = 15.5 °C; T mean max = 19.4 °C; T mean min = 11.5 °C) and rainfall (mean annual: 737 mm) with a prolonged dry summer (>2 months).

2.2. Samplings

Over a period of 4 years (2009–2012) the *A. littorea* population was monitored monthly since access to the penitentiary area is allowed only once a month. More specifically, *A. littorea* was found in 2 main patches; the first one was discovered in 2005 and the second one in 2009 (called hereafter ANC1 and ANC2, respectively; Figure 3). Data collections were carried out from February to July, approximately the 10th day of each month, by monitoring 25 permanent plots of 1 × 1 m, randomly placed in the area where the plant was found; of these, 12 were placed in ANC1 and 13 in ANC2. In each plot, *A. littorea* plants were identified, tagged and marked with toothpicks, and reproductive traits (floral buds, anthesis flowers, immature and mature fruits) were recorded for each plant. Overall, a total of 1722 plants were monitored in the study period, varying from 278 (2009) to 583 (2011).

The average number of fruits per plant was determined as a ratio of the total number of fruits total number of plants monitored. In order to calculate seed output, each year in the peak season, 30 fruits were collected when ripe, outside plots, and seeds were extracted. The average number of seeds per fruit was multiplied by the average number of fruits per reproductive plant, in order to get a prediction of the mean reproductive capacity per plant.

To analyze the soil seed bank, a total of 43 superficial sand samples (ca. 500 g of sediment) were taken tri-monthly near the plots. The samples were transported to the laboratory and, once the sand had dried up (after ca. 30 days), they were sieved and seed extracts were analyzed with a stereoscope.

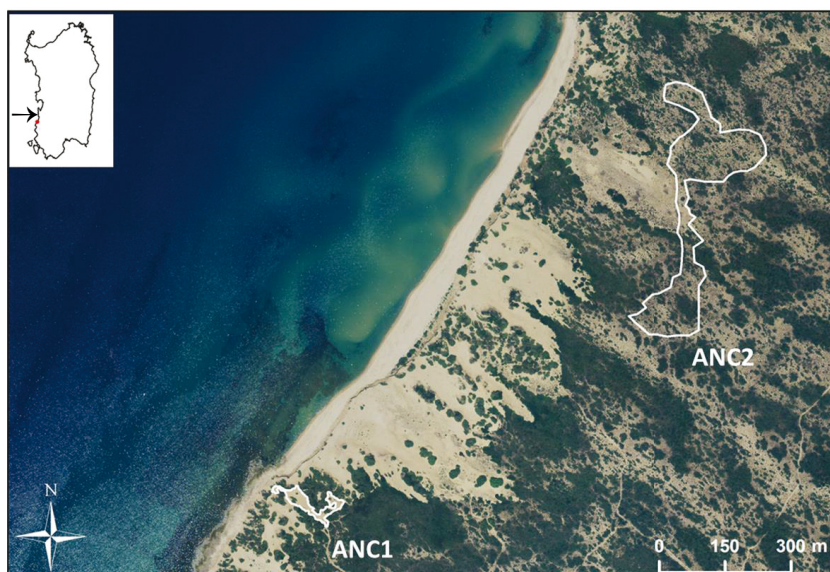


Figure 3. Distribution range of *Anchusa littorea* (Is Arenas, Arbus, SW Sardinia). The 2 main patches (ANC1 and ANC2), ca. 700 m distant from each other, occupying an area of ca. 3800 m² and 50,000 m², respectively. The area is located within the “Is Arenas” penitentiary and therefore access is restricted to authorized persons.

A temperature logger (TidbiT v2 Temp logger, Onset Computer Corporation, Cape Cod, MA, USA) was sown in order to record soil temperature at 90-min intervals during the phenological season (from January to July).

2.3. Statistical analysis

Phenological patterns and trends of the single phenophases were analyzed monthly to evaluate seasonal variations in flowering, fruiting, and fruit-set. Percentage values were calculated for each reproductive trait. The generalized linear model (GLM) was used to assess the *A. littorea* fruit-set in relation with patches and years, using the log link function and the Poisson error distribution. GLM analysis was performed using JMP 7.0 (SAS Institute, Cary, NC, USA).

In order to describe the phenological differences within patches and the similarity between patches, the diversity index (Shannon–Wiener diversity index) and the similarity index (Morisita–Horn similarity index) were used, respectively, following a modification of the protocol in Goulart et al. (2005). For each plant, a grade combination was attributed and different analyses were performed separately. Each observation was then categorized as a dichotomous dependent variable with 0 (absence of the characteristic) or 1 (presence of the characteristic). For each group of phenological phases, plants were characterized by the combination of assessed grades, e.g., a plant evaluated as 0-0-0 for phenological phases would show grade 0 for bud flower, grade 0 for anthesis flower, and grade 0 for withered flowers (the grades totaling 100%).

The Shannon–Wiener diversity index (H index) was used to estimate diversity of phenological behavior within patches (Goulart et al., 2005). Higher values for this index indicate higher phenological diversity, meaning lower phenological synchrony. According to Magurran (1988), the index is calculated following this formula:

$$H' = -\sum p_i \ln(p_i)$$

Frequencies of different phenological states (given by the grade combination) were used instead of frequencies of different species in a community. Accordingly, p_i was considered the proportion of individuals found in the *ith* combined grade, with $p_i = n_i/N$, n_i being the number of individuals showing the phenological status *i*, and *N* the total number of individuals in the population. Populations with greater numbers of phenological states show higher values of this index and less phenological synchrony (Goulart et al., 2005).

Finally, the similarity between ANC1 and ANC2 was evaluated monthly by the Morisita–Horn similarity index (MH index; Magurran, 1988); it was used to estimate synchrony between ANC1 and ANC2 (Goulart et al., 2005), with higher values indicating higher phenological synchrony:

$$MH = [2\sum n_{Ai}n_{Bi}]/(da + db)N_A N_B,$$

where $da = (\sum n_{Ai}^2)/N_A$, $db = (\sum n_{Bi}^2)/N_B$, N_A is the total number of individuals in ANC1, N_B the total number of individuals in ANC2, n_{Ai} is the number of individuals in the *ith* combined grade in A, and n_{Bi} is the number of individuals in the *ith* combined grade in B.

3. Results

3.1. Phenological pattern

The reproductive season of *A. littorea* starts either in late February or in early March and ends in late June, when all plants are dead (Figure 4). The flowering peak was observed mainly in April and subsequently in March, while the fruiting peak was recorded either in May or in April (Figure 4). The mean flowering duration was 87.4 ± 6.23 days, ranging from 77 to 93 days, while the mean fruiting duration was 94.40 ± 17.66 days, with a range from 77 to 124 days.

Mean percentage of flowered plants over the 4-year period, compared to the total number of monitored plants, was 52.7%, with lower values in 2011 and 2012 (45.4% and 34.7%, respectively) and higher in 2009 and 2010 (57.9% and 72.9%, respectively). Likewise, the mean percentage of fruited plants over the 4-year period was 63.3%, with lower values in 2011 and 2012 (59.0% and 34.9%, respectively) and higher in 2009 and 2010 (83.8% and 75.6%, respectively).

Flowering started when an average daily temperature of 7.5 °C was recorded (calculated considering a period of 10 days preceding the phenological monitoring), with values ranging from 5.9 and 9.4 °C. Flowering peak was recorded with a mean daily temperature of 15.7 °C, ranging from 11.6 and 18.6 °C, whereas the percentage of flowering plants strongly decreased when average temperatures exceeded 20 °C (Figure 4; Table 1). The fruiting season took place after a month from the start of the flowering period, and the fruiting peak was recorded in late April. Fruiting started when an average daily temperature of 13.5 °C was recorded (calculated considering a period of 10 days preceding the phenological monitoring), ranging from 8.1 and 18.6 °C. Fruiting peak was recorded with a mean daily temperature of 18.1 °C, ranging from 13.7 °C and 24.7 °C, whereas the percentage of fruiting plants strongly decreased when average temperatures exceeded 30 °C (Figure 4; Table 1).

The GLM analysis showed a significant effect of both the tested variables (patches and years) on the number of fruits per plant; the interaction between them also showed a significant effect on the fruit production per plant (Table 2). The mean number of fruits per plant varied according to the year and the patches (Figure 5); the highest number of fruits per plant was recorded in 2010 (3.85 ± 3.46 fruits per plant), while the lowest was found in 2012 (0.78 ± 1.12

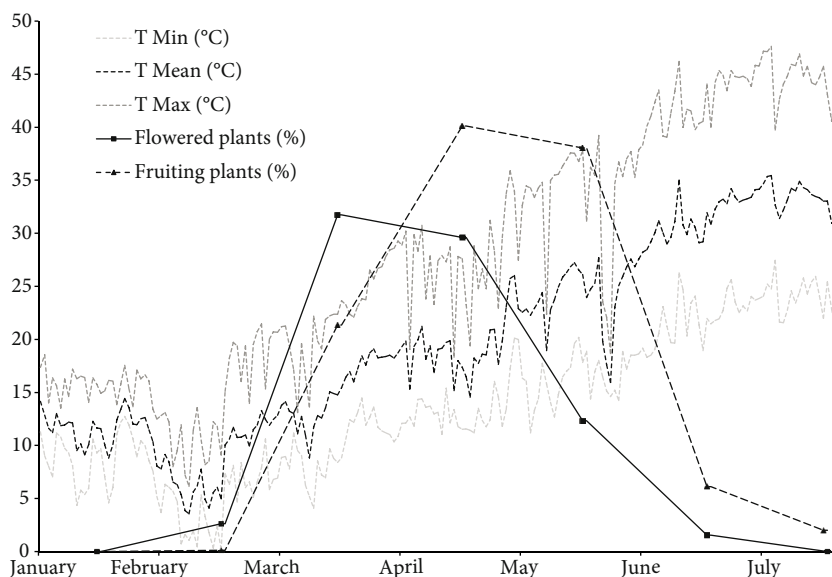


Figure 4. Flowering and fruiting patterns in relation to average monthly temperatures in *Anchusa littorea* over the 4 years of the study (2009–2012).

Table 1. Mean temperature values (°C) calculated considering the period of 10 days preceding the phenological monitoring in the *Anchusa littorea* population. Superscript letters indicate: “a” the start of flowering, “b” the peak of flowering, “c” the start of fruiting, and “d” the peak of fruiting.

	Years			
	2009	2010	2011	2012
February	7.15 ^a	7.5 ^a	9.40 ^a	5.92 ^a
March	11.60 ^{b,c}	15.67 ^c	8.10 ^c	12.42
April	13.75 ^d	16.40 ^b	16.05 ^{b,d}	18.66 ^{b,c}
May	20.70	17.45 ^d	16.00	24.76 ^d
June	22.90	19.30	18.35	30.56

Table 2. Generalized linear model (GLM) results examining the effects of patches and years on fruit output per plant. Observations = 738. Model: - LogLikelihood = 107.74; $\chi^2 = 215.48$; DF = 7; P-value < 0.0001.

	χ^2	DF	P-value
Year	55.16	3	<0.0001
Patches	36.24	1	<0.0001
Year × Patches	14.16	3	0.0027

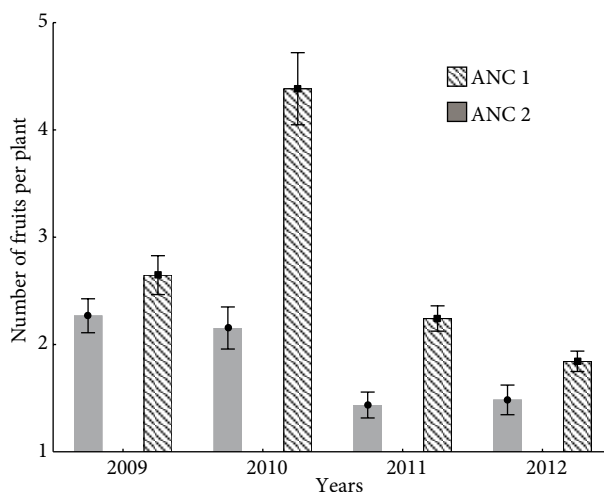


Figure 5. Mean number of fruits per plant (\pm standard error) over the 4 years of the study (2009–2012).

fruits per plant; Table 3). ANC1 generally showed lower fruit production per plant as compared to ANC2 (Figure 5; Table 3).

The mean number of seeds per fruit was 2.8 ± 0.62 , ranging from 2 to 4 seeds per fruit; however, the maximum value was rarely reached (8% of the fruits analyzed).

The analysis of the soil samples showed an extremely low number of seeds per sample (2.83×10^{-4} seeds per cm^3). Analysis with a stereoscope showed that all seeds retrieved from the sand sampled were empty.

3.2. Synchrony within and between patches

Plants from the same patches showed differences in the flowering dates, as reflected by the values of the Shannon–Wiener diversity index (Table 4). Throughout the study period, *A. littorea* showed an asynchrony among plants, due to a high degree of different phenophases overlapped (Shannon–Wiener diversity index value > 0.5). Conversely, low values were registered during the beginning and the end of the life cycle due to the existence of a single phenophase (Shannon–Wiener diversity index ≤ 0.5 ; Table 4). Patches exhibited the same monthly trend of diversity

as regards flowering (Shannon–Wiener diversity values), although plants in ANC2 had the highest flowering asynchrony value each month (Table 4).

High values of MH index between patches were measured, thus indicating a high level of synchrony (Table 5). MH index did not show a great monthly variation: values ranged from 0.83 (April 2010) to 1.00 (June 2011) and from 0.50 (April 2009) to 1.00 (February 2011) for flowering and fruiting seasons, respectively; April 2009 showed the lowest similarity values among years (Table 5).

4. Discussion

4.1. Flowering synchrony

A strong correspondence exists between resource availability and phenological patterns in almost all ecosystems that exhibit seasonal climate variation (Rathcke and Lacey, 1985). Sandy dune systems have limited resources available for plants, and only for a limited time during the growing season (Maun, 2009). In this type of temporally varying environments plants could have opportunistic and fixed phenological responses, which

Table 3. Reproductive traits in the *Anchusa littorea* population; the fruit and seed output for ANC1 and ANC2 over the study period were also reported.

	Year	No. of plants	No. of reproductive plants	Mean no. of fruits per reproductive plant (\pm SD)	Minimum and maximum no. of seeds per plant
POPULATION					
	2009	278	172	2.52 ± 1.74	2–12
	2010	377	164	3.85 ± 3.46	1–20
	2011	583	237	2.10 ± 1.57	1–10
	2012	484	165	0.78 ± 1.12	0–5
overall		1722	738		
ANC1					
	2009	72	56	2.27 ± 1.18	3–10
	2010	96	39	2.15 ± 1.22	3–9
	2011	139	39	1.43 ± 0.75	2–6
	2012	111	31	0.60 ± 1.12	0–5
overall		418	165		
ANC2					
	2009	206	116	2.65 ± 1.92	2–13
	2010	281	125	4.38 ± 3.75	2–23
	2011	444	198	2.24 ± 1.65	2–11
	2012	373	134	0.83 ± 1.18	0–6
overall		1304	573		

Table 4. Shannon–Wiener diversity index (H) mean values (\pm standard deviation) for reproductive phenology of *Anchusa littorea* plants within ANC1 and ANC2 patches, respectively (data from February 2009 to June 2012).

Month	Flowering		Fruiting	
	ANC 1	ANC 2	ANC 1	ANC 2
February	0.047 \pm 0.000	0.047 \pm 0.000		0.047 \pm 0.000
March	0.687 \pm 0.349	0.999 \pm 0.233	0.352 \pm 0.390	0.592 \pm 0.322
April	0.641 \pm 0.214	1.142 \pm 0.178	0.948 \pm 0.272	0.715 \pm 1.165
May	0.538 \pm 0.219	0.851 \pm 0.144	1.098 \pm 0.159	0.721 \pm 1.210
June	0.329 \pm 0.000	0.594 \pm 0.519	1.016 \pm 0.000	0.729 \pm 0.208

Table 5. Morisita–Horn similarity index (MH) between ANC1 and ANC2 patches of *Anchusa littorea* (data collected from February 2009 to June 2012).

Year	Month	MH Flowering	MH Fruiting
2009	February	-	-
	March	0.865	0.771
	April	0.951	0.507
	May	0.983	0.750
	June	-	-
2010	February		
	March	0.994	0.976
	April	0.832	0.962
	May	0.966	0.974
	June	-	-
2011	February	0.981	1.000
	March	0.950	0.939
	April	0.992	0.850
	May	0.976	0.940
	June	1.000	0.965
2012	February	0.981	1.000
	March	0.992	0.999
	April	0.980	0.972
	May	0.980	0.796
	June	-	-

represent contrasting strategies for optimizing fitness (Dyer et al., 2012). The ability to respond opportunistically at different phenological stages may be a metric for the long-term selective regime of the habitat (Novoplansky et al., 1994). In annual plants, development may be

constrained by the length of the growing season (Aronson et al., 1992): plants must initiate flowering with sufficient time to produce seed, but may initiate new tillers and buds if favorable conditions lead to the extension of the growing season (Dyer et al., 2012).

In this study, we showed the ability of *A. littorea* to exhibit opportunistic flowering responses to environmental variation in a Mediterranean coastal dune system. This plant is characterized by the ability to respond rapidly to changes in summer drought conditions; in fact, its flowering period starts at the beginning of its life cycle in order to avoid the subsequent reduction in soil moisture. In particular, the onset of flowering is closely related to a narrow range of mean daily temperatures, which is always recorded in February. Likewise, the peak of flowering is related to a narrow window of temperature range, independently from the beginning of flowering (which could occur both in March and in April), thus highlighting a strong correlation between temperature and flowering phases. This correlation is very important for *A. littorea*, an early flowering species, since it flowers within the window of favorable temperatures, therefore optimizing reproductive output (Cogoni et al., 2013). *Anchusa littorea* showed flowering and fruiting seasons overlapped and a long reproductive period lasting 4 to 5 months; this pattern may be considered an adaptive advantage in highly stochastic habitats, especially in those with unpredictable rainfall patterns (Braza and Garcia, 2011). Plant species with extended flowering seasons can be less seriously affected by this environmental unpredictability than species with concentrated flowering season (Picó and Retana, 2000). The extended blooming period can increase the individual's chance of having a large number of mates both as a pollen donor and as a recipient (Torres et al., 2002). In addition, the same phenological pattern has been observed for other plants and it has been suggested that it may be an adaptive response to attract pollinators that usually visit other species (Thompson, 1980; Torres et al., 2002).

Within each patch, plants of *A. littorea* flowered quite asynchronously. Tarayre et al. (2007) found that different plants, or groups of plants, flower with low synchrony across the whole population, flowering time causing reproductive isolation, because cross pollination is only possible between synchronously flowering plants (Hendry and Day, 2005). Conversely, a number of different interpretations have been given for flowering asynchrony; Ollerton and Lack (1992) hypothesized an evolutionary response to intraspecific competition for pollinators, an advantage of increasing mate availability, a variation in intensity and timing of seed predation and dispersal, or a selection on natural genetic variability and/or environmental heterogeneity. Other studies have also found that within population flowering asynchrony could be due to differential selection in different years depending upon environmental factors (e.g., Rathcke and Lacey, 1985). Since the first points cannot be confirmed as a consequence of the lack of studies on the specific

pollination system in *A. littorea*, the asynchrony among individuals may reflect species flexibility. Asynchrony could be considered an evolutionary adaptation to a long-term unfavorable and unpredictable environment, which increases the possibility of renewal of generations, especially for short-lived plants (Yu et al., 2009).

Conversely, a high reproductive synchrony was found between patches. Rathcke and Lacey (1985) suggested that there should be some advantage in partial synchrony of flowering between populations, as it promotes cross-pollination among distant individuals, thus enhancing genetic diversity, and it also helps to avoid competition for pollinators. The high degree of synchrony may also be related to attracting pollinators or it might simply be due to the fact that plants live in very homogeneous habitats as regards ecological conditions (Thompson, 1980). The latter could be an advantage for small populations, because strong synchrony implies that each plant can exchange genes with most plants within the same population, therefore increasing its genetic diversity (Martínez-Sánchez et al., 2011).

4.2. Seed production and soil seed bank

Annual plants show a wide yearly variability in population dynamics and reproductive traits: the number of individuals of a given year depends primarily on the seeds produced the previous years, and the percentage of individuals that become reproductive varies annually (Silvertown and Charlesworth, 2001; Cogoni et al., 2013). *Anchusa littorea*, in particular, shows a spatio-temporal variability in seedling emergence, survival, and reproduction (both in terms of survival and of fecundity), combined with an important relationship with the microhabitat availability (Cogoni et al., 2013). Therefore, the yearly number of fruits per plant (and consequently, the number of seeds per plant) also shows a high variability, with years in which low seed production is recorded (Silvertown and Charlesworth, 2001).

However, besides these general patterns at the population level, we found that the mean number of fruits per plant increased over time in ANC2 and decreased in ANC1. Patches of *A. littorea* with different size differed significantly in their seed production, with higher production per plant in the larger patch (ANC2) than in the smaller one (ANC1). These results are consistent with several previous studies that investigated the relationships between population size and reproductive success in rare and endangered plants (e.g., Fischer and Matthies, 1998; Fenu et al., 2011) and may be related to the patch size. Indeed, a negative relationship has been reported between narrow population size (or a low population density) and seed production due to several factors such as increased inbreeding, reduced number of compatible mates, and lack of pollinators leading to pollen limitation

(e.g., Fischer and Matthies, 1998; Campbell and Husband, 2007). Moreover, the low number of seed per fruit as compared to the number of ovules should be considered a result of the selective abortion of relatively low-quality offspring; this process can free resources for higher quality offspring and thereby increase the fitness of the maternal plant, as demonstrated for other species belonging to the family Boraginaceae (Melser and Klinkhamer, 2001). Accordingly, the mean number of seeds per fruit recorded in *A. littorea* was similar to that calculated for *A. crispera*, a psammophilous coastal endemic species of Sardinia and Corsica (Quilichini and Debussche, 2000).

The value of the soil seed bank detected in *A. littorea* is poor and all of the examined seeds were empty or dead: this result suggests that *A. littorea* is not able to create a persistent seed bank in the soil. This finding is discordant with previous studies indicating that annual plant species produce a soil seed bank in coastal Mediterranean semiarid ecosystems, and especially on sand dunes (Yu et al., 2008). However, the soil seed bank in arid ecosystems is characterized by a high spatial and temporal variability (Thompson, 1987) and most annual plants may exhibit ample variation in seed longevity in the soil, depending on the frequency of favorable conditions (Olano et al., 2012). Our results seem to confirm the same pattern found in *Dianthus morisianus* Vals., another narrow endemic plant growing in sandy coastal habitats that is unable to form a persistent soil seed bank (Cogoni et al., 2012). Furthermore, the congener *A. crispera*, a taxonomically related species that grows in similar sandy coastal habitats, was also reported to have a limited number of viable seeds in the soil (Quilichini and Debussche, 2000). Many biological, ecological, and stochastic factors can affect the formation of soil seed banks; for example, as suggested in previous studies for endemic and psammophilous plant species (Quilichini and Debussche, 2000; Fenu et al., 2013), human trampling could affect the depth of the seeds in the soil and the fate of the seed bank. A severe impact from human trampling on plant density and reproductive output in *A. littorea* populations has actually been reported: human disturbances, which modify the depth where most of the seeds become layered in the soil, could affect their germination success and consequently cause a reduction in seedling establishment (Fenu et al., 2013). Considering that a short-lived plant species in such an unpredictable environment is likely to depend on a long-lived seed bank for population persistence (Silvertown and Charlesworth, 2001), the lack of a persistent soil seed bank for *A. littorea* could thus be a critical factor. These threats and those already identified (Bacchetta et al., 2008; Fenu et al., 2013), the peculiar ecological requirements (Cogoni et al., 2013), the documented decline rate (in the past this species was distributed in several coastal systems), and the small population size make *A. littorea* prone to extinction,

and it is confirmed as Critically Endangered (Bacchetta et al., 2008).

The ANC2 discovery, in a site where this species was not found in previous years (Bacchetta et al., 2008), suggested that the *A. littorea* population might be adapted to large space shifts, inhabiting similar habitats: a significant migration could have occurred for this plant inside the dune system over the years. Likewise, our results could indicate a migration of the “population core” (in term of maximum seed productivity and population vitality) among patches: the “population core” has moved in the space from ANC1 to ANC2, suggesting that also the “population core” might move inside the dune system over the years.

The results of this study add further information to the conservation point of view: the *A. littorea* population is evidently susceptible to trampling damage, and, consequently, prohibiting and limiting human disturbances are essential measures. The recovery of *A. littorea* population needs investigations of other regeneration strategies in addition to protection, especially when regeneration from seeds is limited due to microhabitat conditions (Cogoni et al., 2013) and to the lack of a persistent soil seed bank. In addition, as the phenological behavior of the species is influenced by the Mediterranean climatic variability, more prolonged and detailed monitoring is needed in order to evaluate the conservation status of this narrow endemic plant.

In conclusion, this study provides new data in terms of phenological pattern and seed output, thus improving our knowledge on the reproductive ecology of this narrow endemic species growing in a selective habitat such as the Mediterranean coastal dunes. In addition, our results highlight the importance of the temperature regime in determining the phenological stages in *A. littorea*. Moreover, they also indicate that changes in this regime (as assumed by the scenarios on climate warming) could modify the biology and reproductive capacity of this coastal plant. Our findings are relevant because the Mediterranean flora presents a high number of endemic taxa that consist of a single population, such as the annual *A. littorea*. In particular, in Sardinia several narrow endemic plants that exhibit the same patterns [e.g., *Dianthus morisianus*, *Phleum sardoum* (Hack.) Hack.] are restricted to one or few coastal dune systems.

Further studies are needed to analyze this criticism and to explore several aspects related to the yearly dynamic variability, the selection imposed by environmental habitat parameters, and the adaptation of this species to its microhabitats, in order to implement appropriate conservation measures for this threatened endemic species. This approach may be helpful in assessing and

predicting phenological patterns in the genus *Anchusa* and in other plant growing on Mediterranean coastal dunes.

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