Climatic control of seed longevity of *Silene* during the post-zygotic phase: do seeds from warm, dry climates possess higher maturity and desiccation tolerance than seeds from cold, wet climates?

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5 **Climatic control of seed longevity of *Silene* during the post-zygotic phase. Do seeds from**
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7 **cold, wet climates?**

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15 **Abstract.** Seed longevity derived from artificial ageing experiments has been shown to be
16 related to the macroclimate at the sampling site and with individual seed traits. Nevertheless,
17 the ecological interpretation of artificial seed longevity remains to be clarified. In this article,
18 the ecological significance of seed longevity expressed by the p_{50} index was explored using 16
19 closely related populations of the genus *Silene* L. Seeds were subjected to an artificial ageing
20 treatment at 45°C, 60% relative humidity (RH) and regularly tested for germination. The
21 decline of viability to 50% (p_{50} index) was calculated using probit analysis. Its relationship with
22 known ecological predictors of seed longevity was assessed by multiple regression analysis.
23 Values of p_{50} ranged from 3.7 to 68.3 days. Seed lots which normally experience drought
24 during the post-zygotic phase in a dry, warm environment, were long-lived. Consistently,
25 precipitation during the driest months of the reproductive period was the strongest predictor of
26 p_{50} variability (partial regression, $R^2=0.424$). We could not find any relationship between *ex*
27 *situ* seed longevity and soil seed bank formation or seed size. Our results indicate that *ex situ*
28 seed longevity has an ecological significance related to long-term climatic differences at
29 population site. Increased longevity presumably reflects resistance to desiccation stress
30 attained by seeds through local adaptation to warm, dry climates. We cannot exclude that seed
31 tolerance to ageing could also represent phenotypic plasticity mediated by a drier maturation
32 environment. The vulnerability of seeds from wet, cool climates and the adaptive response of
33 seed longevity to different environments may have implications for *ex situ* conservation in the
34 face of climate warming.

Additional keywords: seed viability, climatic factors, desiccation stress.

Introduction

Seed longevity determines the storage potential of seed accessions held in *ex situ* collections such as germplasm banks and seed banks and, therefore plays a crucial role in securing the long-term survival of endangered species through *ex situ* conservation (Walters et al. 2005; Probert et al. 2009). Exposing seeds to a controlled ageing environment at 45°C, 60% relative humidity (RH) has been successfully employed to calculate an index of seed longevity expressed as the decline of viability to 50% (p_{50} index) for seeds preserved *ex situ* in seed banks (e.g. Ellis and Roberts 1980; Probert et al. 2009; Mondoni et al. 2011). Although the length of seed survival in *ex situ* collections declines predictably with increasing storage temperatures and higher seed moisture contents, seed longevity has been found to vary considerably among seed lots held in identical storage conditions (Walters et al. 2005). Such variation has been attributed to differences in taxonomy at family and/or genus level, macroclimate at the site of collection, or seed traits such as mass and oil content (Walters et al. 2005; Probert et al. 2009; Mondoni et al. 2011, 2014).

Although a measure of *ex situ* seed longevity obtained by controlled conditions in the laboratory may not explain the complex ageing response of seeds which occurs in nature, a number of studies have successfully used the p_{50} index to determine and interpret environmental-induced drivers of seed longevity (e.g. Probert et al. 2009; Mondoni et al. 2011, 2014; Bernareggi et al. 2015). We therefore assume that artificial ageing experiments in the laboratory can have an ecological significance. A screening of relevant literature reveals that ageing mechanisms tend to be ascribed to different and sometimes contrasting ecological causes. On one hand, Long et al. (2008) found evidence for a correlation between persistence in the soil under natural conditions and seed longevity estimated by laboratory experiments. Since individual seeds modulate their inclusion in the soil seed bank through germination and ageing (Kochanek et al. 2010), one can expect that seeds with higher resistance to ageing, thus long-lived in storage, would persist longer in the soil. On the other hand, in their extensive quantification of seed performance in storage, Walters et al. (2005) failed to detect a gene bank/soil seed bank correlation for seed longevity.

Following another hypothesis, *ex situ* seed longevity would reflect a selection pressure for seed resistance to ageing induced by local climatic factors, namely temperature and precipitation. A number of studies (e.g. Probert et al. 2009; Kochanek et al. 2010; Mondoni et al. 2011; Bernareggi et al. 2015) have stressed the importance of the climate of origin as the main driver

of variation in seed longevity, with seeds from wet, cool environments tending to be shorter-lived compared to those from warm, dry climates. Mondoni et al. (2014) observed that parental plants of *Silene vulgaris* growing in warmer environments might produce longer-lived seeds by promoting seed resistance to heat stress in the offspring. Furthermore, the fact that seeds experiencing drought during the period of maturation are characterized by longer life-spans (Kochanek et al. 2010) may suggest that desiccation tolerance determines a greater resistance to ageing in dry storage. The laboratory-derived index of seed longevity (p_{50} index) might therefore reflect tolerance to heat and/or desiccation stresses exercised by the local environment.

In the presented study, to clarify the ecological significance of seed resistance to ageing in long-term storage as expressed by the p_{50} index, seed accessions from con-generic species of the genus *Silene* L. were subjected to artificial ageing and the estimated p_{50} values were analysed using known ecological predictors of seed longevity. Specifically, we tested the hypotheses that the p_{50} index is related to (i) seed mass; (ii) an index of seed bank formation based upon all interacting factors known to affect seed persistence in the soil, such as dormancy breaking cues and germination traits (i.e. delayed germination, light requirement and reaction to fluctuating temperatures; Saatkamp et al. 2011); and (iii) long-term climatic conditions at population site (i.e. 50 years averages of temperature and precipitation).

Materials and Methods

Seed material

Seeds were selected from collections held for long-term conservation storage at the Millennium Seed Bank (Royal Botanic Gardens Kew, United Kingdom), according to seed availability, high initial viability (>80%) and to provide a broad climatic coverage (Fig. 1). Collections represent 11 species of the genus *Silene* L. (Caryophyllaceae), with four species including 2-3 populations from localities across Europe, the Near East and North America. Seeds were collected at the point of natural dispersal from fully ripened fruits between May and September and stored under international gene bank standard conditions (15% RH and -15°C; FAO/IPGRI 1994) at the Millennium Seed Bank for between 1 and 4 years (Table S1). Correlation analysis was performed to determine whether p_{50} is related to the time spent in storage prior to the commencement of the ageing experiment (B-E) or to the period between harvesting and banking (H-B). The relationship turned out to be statistically non-significant ($R^2 = 0.151$ and $R^2 = 0.004$, respectively; Table S1), indicating that the time spent between harvesting and the start of the experiment was unlikely to affect *ex situ* seed longevity of the studied species. For

each accession, mean seed mass was calculated by weighting five lots of 50 seeds (equilibrated to 15% RH, 15°C). For more detail on seed material see Table 1 and Table S1.

Artificial ageing and germination tests

Artificial ageing experiments were conducted between March and June 2016 at the Millennium Seed Bank, using a standard protocol (Davies and Probert 2004). For all accessions, eight samples of 90 seeds each were pre-equilibrated at 47% RH and 20°C for 2 weeks and then moved to the ageing environment of 60% RH at 45°C. The atmosphere of both environments was maintained within sealed 300 x 300 x 130 mm electrical enclosure boxes (Ensto UK Ltd, Southampton) by a non-saturated solution of LiCl. The RH value was checked every 2 weeks and re-equilibrated, if necessary (Hay et al. 2008). Samples were removed from the ageing environment after 0, 5, 10, 15, 20, 30, 50 and 70 days for germination tests. Seeds from each sample were uniformly sown on 1% agar in distilled water in three replicates of 30 seeds (Baskin and Baskin 1998) and placed in temperature ($\pm 1^\circ\text{C}$) and light (ca. $40 \mu\text{mol m}^{-2} \text{s}^{-1}$) controlled incubators at conditions (range and temperature regimes) previously found to be optimal for each accession (Table 1). Germination events were recorded weekly, with germination being defined as visible radicle protrusion of 3 mm (Ellis et al. 1985). At the conclusion of the experiment, germination percentages were corrected for viable seeds through cut-tests; empty seeds were excluded from the test.

Index of soil seed bank formation (SBI)

An index of soil seed bank formation (SBI) was based upon all interacting factors known to affect seed persistence in the soil, which include dormancy breaking cues and germination traits. The process of dormancy breaking in a winter soil seed bank can be simulated artificially by cold stratification (Godefroid et al. 2011). Seeds of all accessions were therefore cold-stratified at $-5/5^\circ\text{C}$ for 2 weeks. Cold stratification was tested under variables previously found to overcome dormancy with optimal results; i.e. fluctuating temperatures at a sufficient low mean temperature (ca. 0°C , as the 5°C range might cause precocious germination during the period of chilling). The duration of the test was determined using the ENSCONET Germination Database Report (<http://ensconet.maich.gr/Database.htm>), which indicates that for most tested accessions a two-week period under pre-treatment conditions was sufficient (Zani and Müller, unpublished data). The relative index of cold stratification requirement ΔG_{CS} was calculated as follows:

$$\Delta G_{CS} = [(G_{CS} - G_{opt}) / (G_{CS} + G_{opt})] * 100$$

where G_{CS} and G_{opt} are the number of germinated seeds at optimal temperature with and without applying cold stratification, respectively. An increase in germination rate induced by the pre-chilling treatment is signalled by positive values of ΔG_{CS} and likely indicates a winter dormancy syndrome (Mondoni et al. 2009).

Saatkamp et al. (2011) have shown that soil seed persistence can be related to delayed germination as quantified by T_{50} (days required to reach 50% of germination), light requirement (PGI, index of light requirement for germination; Koutsovoulou et al. 2014), and reaction to alternating temperatures (ΔG_{FT} , index of fluctuating temperatures requirement) (Table 2):

$$T_{50} = t_i + [(N/2 - n_i) * (t_i - t_j)] / (n_i - n_j)$$

in which N is the final number of emergence and n_i and n_j indicate the cumulative number of seeds germinated by adjacent counts at the i^{th} and j^{th} time, respectively, when $n_i < N/2 < n_j$;

$$PGI = 1 - (FGD/FGL)$$

where FGD and FGL are the percentages of germination in the dark and the light, respectively. The PGI results in values between 0 and 1 with a value of 0 corresponding to the germination percentage as equal in both light and darkness, while a value of 1 corresponds to germination only occurring in the light;

$$\Delta G_{FT} = [(G_{fluct} - G_{const}) / (G_{fluct} + G_{const})] * 100$$

where G_{fluct} and G_{const} are the number of seeds germinating at fluctuating and constant temperatures, respectively. Therefore, $\Delta G_{FT} < 0$ indicates that seeds germinate better under constant regime whereas $\Delta G_{FT} > 0$ occurs when fluctuating temperatures promote germination. In order to reduce SB traits (ΔG_{CS} , T_{50} , PGI, and ΔG_{FT}) to one meaningful index, principal component analysis (PCA) was performed on the z-transformed SB traits and the most explicative component (PC1) retained as index of seed bank formation (SBI). Correlations of single SB traits to PC1 were assessed by non-parametric Spearman rank correlation coefficients. Lastly, Walck et al. (2005) have stressed the importance of placing seed bank

formation in a plant life-cycle context, especially in the case of species with pronounced seasonal patterns as is the case with the present study. Thus, the germination pattern of each accession was used to locate the beginning and the end of soil bank formation at the seed dispersal peak and the germination event, respectively, in order to confirm the validity of SBI (Table 2).

Climatic data

Prevailing climatic conditions at the original sampling sites (temperature and precipitation) were extracted from WORLDCLIM dataset (download version 1.3 <http://www.worldclim.org/>; Hijmans et al. 2005) at a resolution of 2.5 minutes, based on data collected between 1950 and 2000. The period of plant activity was divided into three life cycle phases: (i) pre-zygotic, from the seedling to the flowering time; (ii) post-zygotic, including seed maturation up to dispersal; and (iii) post-dispersal, which may describe immediate germination (i.e. period of seedling establishment) or a quiescent period with no vegetative activity (i.e. overwintering). We derived the period of flowering for the studied species from Flora Europaea (Tutin 1980), Flora Iberica (Castroviejo et al. 1990), Flora of North America (Flora of North America Editorial Committee 1993) and the Red Data Book of the Republic of Bulgaria (Bulgarian Academy of Sciences 2011), whereas the time of seed dispersal was recorded on the field for each accession at the time of collection (Table 2). The time of germination and seedling establishment was estimated by Zani and Müller (unpublished data). According to the germination strategy adopted, the period of each phase varied with different populations (Table S1). Averages of temperature and precipitation were subsequently calculated for each accession by annual (= mean of all weekly mean values) and seasonal trends (= mean values for spring, summer, autumn and winter months, with seasons following the traditional solar timing of Europe and East Asia), and for each life cycle phase (Table 3). Next, in order to reduce the number of climatic explanatory factors and ensure their independence, principal component analyses (PCA) was performed on the z-transformed climatic variables and principal components (PCs) retained as climatic traits. PCA-axis scores were tested for correlation with single climatic variables by Spearman rank correlation coefficients (Table 4).

To assess whether long-term drought during the seed maturation period affected *ex situ* seed longevity, the heat-moisture index of the post-zygotic phase (PHM) was calculated for each seed origin (modified by Wang et al. 2006). The index PHM is given as $(P_{\text{temp}} + 10) / (P_{\text{pr}}/100)$, where P_{temp} and P_{pr} are the 50 years averages of temperature (°C) and precipitation (mm), respectively, using the post-zygotic months as time-scale. Drought was registered within the

seed maturation period with PHM>50 (moderate drought: 50<PHM<100; severe drought: 100<PHM<200; extreme drought: PHM>200) (Table 3). Although actual weather data from the seed ripening period were not available for each seed accession at the specific sampling location, WORLDCLIM data allow the assessment of long-term climatic differences among population sites, as is the goal of the present study.

Statistical analysis

A generalized linear model (GLM) with binomial error and probit link function was carried out on the data from the experiments (numbers of seeds germinating, numbers of seeds sown) using GenStat Release 11.1 to calculate the time for viability to fall to 50% (p_{50} index),

$$v = K_i - (p/\sigma)$$

where v is the viability (in normal equivalent deviates, NED) of the seed accession after p days in storage; K_i is the initial viability (NED) and σ is the time (days) for viability to fall by 1 NED (Ellis and Roberts 1980).

Relationship between seed longevity (p_{50}) and all potential explanatory factors (seed mass, index of drought of the post-zygotic phase PHM, index of seed bank formation SBI, and climatic traits as expressed by PCA-axis scores) was assessed by multiple regression analysis using backward elimination in Minitab version 13. The initial model was computed with all predictors included (full model); at each subsequent step the least significant variable was eliminated and the model re-fitted until all remaining variables were significant ($p<0.05$; reduced model) (Probert et al. 2009).

Results

For all seed lots, seed viability declined as the period of artificial ageing increased, with the exception of *S. caliacrae* (Fig. 2) which was identified as an outlier during multiple regression analyses (Cook's distance>1) and subsequently excluded from the dataset. Across the remaining accessions, we found a wide variation in seed viability ranging from 3.7d for *S. nutans* (ID9) to 68.3d for *S. reuteriana* (ID14) (Table 1). When comparing the time taken for viability to fall to 50% within different populations of the same species, only accessions belonging to *S. nutans* (ID9-10) and *S. otites* (ID11-13) showed intraspecific variability. The alpine population of *S. nutans* (3.7d; ID9) was shorter-lived than the population of Continental provenance (21.5d; ID10) and nearly six times less resistant to ageing, whereas the

Mediterranean accession of *S. otites* (ID12) was the longest-lived within the species (45.1d vs. 32.9d and 27.4d for ID11 and ID13, respectively; Table 1).

The principal component analysis (PCA) on soil seed bank (SB) traits extracted one factor (PC1=70.32%) which exhibited high and significant correlations with all SB traits and was therefore retained as index of soil seed bank formation (SBI) (Table 4). The ecological meaning of SBI was confirmed by the estimated periods of seed dispersal and germination for each accession, with SBI>0 indicating the formation of soil banks during winter or flooding seasons, and the absence of soil seed banks signalled by SBI<0 (Table 2). The PCA performed on climatic variables explained 82.9% of the variance (Table 4) and extracted three principal components. The first factor (PC1=45.1%) was positively correlated with all temperature variables at all time scales, thus representing the only trait for “temperatures”, whereas precipitation variables were described by the second (PC2=22.6%) and third (PC3=15.1%) factors. In particular, precipitation variables of the plant activity phases and summer correlated with PC2, describing “precipitation during the driest months of the reproductive period”, while PC3 explained the remaining precipitation variables (“annual and seasonal precipitation”) (Table 4).

The regression model constructed using all explanatory variables (seed mass, index of drought of the post-zygotic phase PHM, index of seed bank formation SBI, and climatic traits as expressed by PCA-axis scores) accounted for 84.6% of the variation in seed longevity ($R^2=0.846$, $F=13.782$, $p<0.001$). Backward elimination excluded seed mass and SBI as predictors of p_{50} and generated a reduced model ($R^2=0.863$, $F=23.998$, $p<0.001$) which included all climatic traits (PC1, PC2, PC3) and the index of drought (PHM). In the reduced model, partial regression analysis indicated a positive relationship between p_{50} and the explanatory variable for temperature as expressed by PC1, accounting for 27.5% of the variance (Fig. 3a). Negative and stronger relationships between p_{50} and the explanatory variables for precipitation were also observed. Annual and seasonal trends of precipitation explained 29.6% of the variation (partial regression, $R^2=0.296$; PC3; Fig. 3b), while a better fit was detected if only the mean rainfall level of the driest months during the reproductive period (PC2) was considered as predictor (partial regression, $R^2=0.424$; PC2; Fig. 3c). Seeds from drier and warmer environments tended therefore to survive for longer times in dry storage. This is consistent with the positive and strong relationship observed between p_{50} and the index of drought of the post-zygotic phase (PHM), which accounted for a high proportion of the variance in the reduced model (partial regression, $R^2=0.412$; PC1; Fig. 3d).

Discussion

Though the long-term survival of seeds in seed bank storage seems to be shaped by the local environment, the degree to which this trait can be ecologically determined is constrained by the species genetic background at the family and/or genus level (Walters et al. 2005). By selecting closely related populations of the same genus, we tried to control phylogenetic effects at the interspecific level and to evaluate the contribution of intraspecific variability. This approach of relating seed traits to the local environment at the species level was likewise adopted by Carta et al. (2016) to test whether germination preferences in *Romulea* seeds of phylogenetically related species reflected environmental conditions through local adaptation or phylogenetical constraints. The detection of meaningful differences in germination strategies associated with the local environment led the authors to argue for evolutionary adaptation mediated by phylogenetic relatedness. In the present study, intraspecific variation was found only among populations of widely differing climates for seed lots of *S. nutans* and *S. otites*, namely wet-cold (nearly three-fold in the alpine accession of *S. nutans*, ID9 vs. ID10) and dry-hot (nearly two-fold in the Mediterranean accession of *S. otites*, ID12 vs. ID13). Differences in seed longevity among sampled populations of *S. flos-cuculi* and *S. latifolia alba*, which occurred in relatively uniform habitats (i.e. marshland and temperate semi-dry grassland, respectively; Zani and Müller, unpublished data), failed to emerge. In agreement with Carta et al. (2006), we can hypothesize that variation of seed longevity under storage conditions reflects differential responses to the local climate attained by seeds through long-term adaptation rather than phylogenetic changes among our seed lots. However, the effect of the parental growth environment on seed ageing cannot be ruled out (Mondoni et al. 2014). Although we interpreted our results in terms of long-term climatic differences based on 50 years averages, WORLDCLIM predictions have been shown to strongly correlate with actual weather conditions during the seed ripening period (Fernández-Pascual et al. 2015). Therefore, we cannot exclude that climatically-related variation of longevity in our seed lots represents phenotypic plasticity mediated by the actual seed maturation environment. In this case, further investigations considering common garden experiments or reciprocal transplanting are required to disentangle plasticity from genetic effects.

In contrast to findings by Long et al. (2008), this study did not validate the hypothesis that seed life-span calculated by laboratory experiments is a reflection of seed persistence under natural conditions. In this regard, the case of alpine species is revealing. Seeds of alpine plants have been observed to be short-lived in storage (Mondoni et al. 2011), whereas they have a propensity to form long-term persistent soil seed banks in their natural environment

(Schwienbacher et al. 2010). The fact that seeds from cool, wet climates (i.e. alpine) have low resistance to laboratory-induced ageing at high temperature and partial hydration (45°C, 60% RH) despite showing high persistence in the natural environment may suggest that alpine climates do not exert on seeds much selection pressure for resistance to ageing since seeds under cool and fully hydrated conditions deteriorate slowly (Mondoni et al. 2014). Results of accelerated ageing experiments would thereby reflect climatic conditions at the population site rather than persistence in the soil seed bank. Accordingly, no significant relationship between the propensity to form soil banks and seed longevity in dry storage was revealed in our study, as the index of soil seed bank formation (SBI) was dropped during backward elimination along with ‘seed mass’ (Table 5). In agreement with our results, Probert et al. (2009) did not retain seed mass as a robust variable in the equation to predict seed life-span under storage conditions. Contrary to other authors (Thompson et al. 1993; Bekker et al. 1998; Arroyo et al. 2006) which found seed size to be related to soil longevity, no significant correlation between ‘seed mass’ and SBI was detected in our study (Table 5). Similarly, Saatkamp et al. (2011) excluded seed size as a predictor for persistence in the soil.

Climatic conditions experienced by the plants at the site of collection are likely be the main driver of viability for seeds in *ex situ* storage. The relationships observed between seed longevity and explanatory climatic factors support the notion of warm, dry climates originating long-lived seeds (e.g. Probert et al. 2009; Kochanek et al. 2010). For example, high temperatures and low levels of precipitation that characterize the Anatolian bioregion (with up to three months of water shortage in summer) were associated with the highest value for seed longevity (*S. reuteriana*, ID14; p_{50} =68.3d). At the other end of the spectrum, seeds of the alpine *S. nutans* (ID9; p_{50} =3.7d), which grows under the wettest and coolest environmental conditions analysed in our study, presents the lowest p_{50} value. Our results are consistent with Mondoni et al. (2011) who found that seed lots growing in alpine regions were considerably shorter-lived than lowland populations of the same taxa.

In their extensive study of the ecological predictors of *ex situ* longevity for seed bank-collected seeds, Probert et al. (2009) hypothesized that enhanced longevity evolved as an adaptation to hot, dry environments where seeds are required to survive under drought regime before successful seedling establishment. Kochanek et al. (2010) observed that dry conditions associated with warm temperatures during the seed development and ripening phase led to an increase in the viability of *Wahlenbergia tumidifructa* seeds. Similarly, Sinniah et al. (1998) found that the life-span of *Brassica campestris* seeds was enhanced if plants underwent a period of water shortage during seed development. This is consistent with our results that precipitation

during the period of plant activity was more critical than mean annual precipitation or precipitation outside of the life cycle phases (Table 5). Furthermore, the positive relationship of seed longevity with both, drought experienced during the post-zygotic phase (Fig. 3d) and temperature at population site (Fig. 3a) suggests that seed resistance to ageing can be related to the level of maturity normally attained by seeds in the local environment. Exposure to elevated temperatures combined with dry conditions during the post-zygotic phase promote seed ripening to full maturity which, in turn, determines higher initial viability and/or lower rates of germinability decline (Hay and Smith 2003; Probert et al. 2007; Bernareggi et al. 2015). Thus, seeds from hot, dry climates can attain a higher degree of maturity and potentially increase their longevity in storage. On the other hand, cooler climates with short growing seasons (i.e. alpine region) are characterized by a contracted phase of seed development that negatively affects seed ripening and thus longevity (Mondoni et al. 2011; Bernareggi et al. 2015).

Apart from enhancing the quality of seeds in term of initial viability, a higher degree of maturity can increase long-term survival in dry storage by promoting seed resistance to desiccation stress. The last phase of seed development on the maternal plant coincides approximatively with the acquisition of desiccation tolerance (Ellis et al. 1987; Welbaum and Bradford 1989), especially if seed maturity is attained under conditions of water-shortage which impose a further selective pressure for resisting dry conditions (e.g. Kochanek et al. 2010). This would explain why seed lots of *Silene* which normally experience drought during the maturation phase were longer-lived (Fig. 3d). Desiccation-tolerant seeds are better equipped to withstand the oxidative damage caused by abrupt changes in seed moisture content that inevitably take place during seed bank processing and accelerated ageing treatment (Donà et al. 2013). By hampering the activity of anti-oxidant molecules (inducing the formation of reactive oxygen species and subsequent DNA damage), dehydration and imbibition tend to impair the longevity of seeds lacking tolerance to desiccation stress (Rajjou and Debeaujon 2008; Waterworth et al. 2010; Ventura et al. 2012; Donà et al. 2013).

Optimal conditions for the increase in *ex situ* longevity (i.e. peak maturity and desiccation tolerance) are likely met when the post-zygotic phase of seeds is confined to the warmest and driest period of the year. The fact that our most critical precipitation variable retained ‘summer precipitation’ along with variable of the period of plant activity, thus describing rainfall during the driest months of the reproductive period (PC2, Table 5), suggests that our hypothesis is consistent. In a previous study (Zani and Müller, unpublished data), the germination strategy of seed lots from arid biogeographical regions (Anatolian, Macaronesian and Mediterranean

regions) were characterized by an autumnal emergence pattern with the maturation phase during the summer months (Table S1). Consistently, the highest p_{50} values in the current study are associated with seed lots from arid bioregions. On the other hand, seeds from wet, cool environments which have normally contracted growing seasons suffer from greater desiccation sensitivity due to a reduced period of maturation on the maternal plant located outside summer months, thereby resulting short-lived in storage (e.g. Daws et al. 2004).

Finally, *S. caliacrae* (ID1) was identified as an outlier and excluded from the multi-regression analysis. Apart from a p_{50} value (ID1; $p_{50}=203.8d$) almost three times higher than the highest value retained in the analysis (ID14; $p_{50}=68.3d$), the survival curve of *S. caliacrae* (Fig. 2) presents an anomalous increase of seed germinability from 0 (74%) to 10-30 (ca. 96%) days of ageing treatment, whereas seed viability should normally decline with increasing time of storage (Ellis and Roberts 1980). Zani and Müller (unpublished data) found that this species requires elevated temperatures ($>25^{\circ}C$) to germinate optimally and that climatic conditions at the site of collection determined the optimal range of temperature. It is possible that warm ageing conditions ($45^{\circ}C$) acted as an environmental cue for germination and after-ripened of *S. caliacrae* seeds, with nearly 100% of germinability during the first weeks. After 30 days of treatment, the seeds suffered the inevitable viability decline that takes place during artificial ageing (Ellis and Roberts 1980; Probert et al. 2009). Subjecting *S. caliacrae* seeds to a period of warm stratification prior to the commencement of the ageing experiment may help in fixing their irregular response to the treatment.

In this study, we clarified the ecological significance of *ex situ* seed longevity due to its important implications for the effective management of seed collections held in seed banks. We showed that climatic conditions likely affect *ex situ* longevity by selecting seed tolerance to desiccation stress through local adaptation to long-term conditions or phenotypic plasticity mediated by the seed maturation environment. The increased desiccation tolerance of seeds from warm, dry climates (reflected in a greater resistance to ageing conditions) might result from a higher degree of seed maturity attained under climatic conditions (high temperatures associated with drought regime) normally experienced during the post-zygotic phase in the local environment. The vulnerability of desiccation-sensitive seeds produced in wet, cool climates (i.e. alpine, Arctic and Boreal regions), which are predicted to be specifically targeted by climate warming, should be considered when selecting accessions for long-term conservation in germplasm banks (Mondoni et al. 2011, 2014; Bernareggi et al. 2015). In this light, transgenerational artificial ageing experiments might empirically test the plastic effect

exerted by climate warming on *ex situ* longevity of seeds from differing climates, exposed or not to seasonal drought.

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Fig. 1. Map of the collecting sites for the studied accessions. Black circles are followed by identification numbers (for ID no. abbreviations see Table 1).

Fig. 2. Survival curves fitted by probit analysis for all *Silene* accessions aged at 45°C, 60% RH.

Fig. 3. Relationships between p_{50} (*ex situ* seed longevity) and significant predictors derived by multiple regression analysis, **(a)** PC1 (temperature), **(b)** PC3 (annual and seasonal mean of precipitation), **(c)** PC2 (precipitation during the driest months of the reproductive period) and **(d)** PHM (heat-moisture index of the post-zygotic phase) at population site. Solid lines show the results of partial regression analysis.

Table 1. Details of accessions used in the study

Classification to species level, provenance (bioregion), time to 50% viability loss at 45°C, 60% RH ($p_{50} \pm \text{s.e.}$) and germination conditions used

ID no.	Species ^a	Bioregion ^b	$p_{50} \pm \text{s.e. (d)}$	Seed mass $\pm$ s.d. (mg)	Germination temperature (°C) ^c
1	<i>Silene caliacrae</i> Jordanov & Panov	Black Sea	203.8 ± 0.09	0.49 ± 0.004	25/15
2	<i>Silene donetzica</i> subsp. <i>sillingeri</i> (Hendrych) M. Sourkova	Pannonian	41.6 ± 0.16	0.44 ± 0.025	20
3	<i>Silene euxina</i> (Rupr.) Hand.-Mazz.	Black Sea	51.3 ± 0.10	0.50 ± 0.002	25/15
4	<i>Silene flos-cuculi</i> (L.) Clairv.	Continental	14.6 ± 0.12	0.13 ± 0.002	25/15
5		Atlantic	18.5 ± 0.13	0.13 ± 0.004	25/15
6	<i>Silene latifolia</i> subsp. <i>alba</i> (Miller) Greuter & Burdet	Anatolian	39.8 ± 0.09	1.31 ± 0.014	20
7		Anatolian	47.1 ± 0.13	1.48 ± 0.026	20
8		Pannonian	50.1 ± 0.10	0.64 ± 0.016	20
9	<i>Silene nutans</i> L.	Alpine	3.7 ± 0.13	0.34 ± 0.010	25/15
10		Continental	21.5 ± 0.08	0.47 ± 0.011	25/15
11	<i>Silene otites</i> subsp. <i>otites</i> (L.) Wibel	Continental	32.9 ± 0.16	0.21 ± 0.003	25/15
12		Mediterranean	45.1 ± 0.10	0.37 ± 0.005	25/15
13	<i>Silene otites</i> subsp. <i>hungarica</i> Wringley	Pannonian	27.4 ± 0.10	0.16 ± 0.006	25/15
14	<i>Silene reuteriana</i> Boiss. & Blanche	Anatolian	68.3 ± 0.18	0.30 ± 0.010	15
15	<i>Silene drummondii</i> Hook.	Boreal	40.6 ± 0.20	0.13 ± 0.002	25/15
16	<i>Silene parryi</i> (S. Watson) C.L. Hitchcock & Maguire	Boreal	32.4 ± 0.20	0.22 ± 0.002	25/15

^aPlant names follow the International Plant Name Index (IPNI: <http://www.ipni.org/>)

^bBiogeographical regions follow EEA reports on bioregions (<http://www.eea.europa.eu/>)

^cOptimal conditions for germination estimated for each accession (Zani and Müller, unpublished data)

Table 2. Traits and index of seed bank formation

Index of delayed germination (T_{50}) and relative germination rates for cold stratification pre-treatment (ΔG_{CS}), light requirement (PGI) and fluctuating temperatures (ΔG_{FT}) of the studied accessions. The index of soil seed bank formation (SBI) is calculated by PCA using SB traits (ΔG_{CS} , T_{50} , PGI, and ΔG_{FT} ; Table 4). The type of soil seed bank is estimated considering both, SBI and the germination and dispersal events

ID no.	Dispersal Peak ^a	Germination ^b	ΔG_{CS}	T_{50}	PGI	ΔG_{FT}	SBI	Type of soil seed bank
1	November	May	15.38	3.55	1.00	28.85	1.89	Flood-winter bank ^{c,d}
2	June-July	June-July	-1.16	1.31	0.01	0.00	-1.10	No ^e
3	October	June	9.54	2.08	0.00	1.82	0.20	Winter bank ^c
4	May	August	-5.88	3.93	0.49	15.38	1.10	Flood bank ^d
5	May	August	-2.86	5.72	0.29	30.43	1.89	Flood bank ^d
6	September	September	-12.05	3.44	0.19	0.00	-0.83	No ^e
7	September	September	-11.48	3.01	0.16	0.00	-0.94	No ^e
8	May	May	1.54	3.47	0.11	-1.75	-0.49	No ^e
9	November	July	11.80	3.86	0.30	1.31	0.51	Winter bank ^c
10	October	July	3.86	3.25	0.16	1.69	0.31	Winter bank ^c
11	May-June	May-June	0.00	1.58	0.00	0.00	-1.00	No ^e
12	September	September	-1.41	2.62	0.08	0.00	-0.53	No ^e
13	May-June	May-June	-1.41	1.89	0.00	0.00	-0.98	No ^e
14	September	October	0.59	4.19	0.85	-1.69	0.25	Delayed germination ^f
15	November	June	11.11	3.63	0.68	14.38	0.75	Winter bank ^c
16	November	June	9.09	3.76	0.60	13.09	0.62	Winter bank ^c

^aTime when ripe seeds were collected for study, or dispersal observed

^bMain period of germination estimated by Zani and Müller (unpublished data)

^cSeeds remain dormant during winter

^dSeeds of wetland species remain dormant during the flooding season

^eSeeds germinate immediately after dispersal

^fSeeds delay germination to spread the risk of emergence failure in mesic environments

Table 3. Climatic variables and drought index

Temperatures and precipitation are the 50 years averages (1950-2000) at annual (A = annual) and seasonal scales (W = winter; Sp = spring; Su = summer; Au = autumn), and of the plant life cycle phases (Pre = pre-zygotic; Post = post-zygotic; Dis = post-dispersal). Drought during the seed maturation period is given by PHM = heat-moisture index of the post-zygotic phase

ID no.	Temperature (°C)								Precipitation (mm)								Drought [HM index]	
	A	W	Sp	Su	Au	Pre	Post	Dis	A	W	Sp	Su	Au	Pre	Post	Dis	PHM	
1	12.3	3.3	10.5	21.3	14.0	19.1	20.3	11.6	42.5	44.3	38.3	39.3	48.0	40.7	38.5	51.5	312.5	Extreme
2	8.4	-2.1	8.8	17.9	9.0	13.9	8.8	17.8	49.8	29.7	48.0	75.7	45.7	39.8	48.0	78.5	39.1	NO
3	13.3	4.5	11.7	22.0	15.1	11.7	22.0	17.6	47.8	54.7	44.7	34.7	57.0	44.7	34.7	48.0	92.3	Moderate
4	13.9	6.3	12.4	22.0	14.8	20.0	10.4	18.4	102.1	107.0	87.7	78.0	135.7	102.0	88.5	85.0	23.0	NO
5	9.5	3.7	8.4	15.8	10.3	13.5	8.4	15.6	154.8	162.0	158.7	153.0	145.7	64.3	53.3	50.5	34.4	NO
6	7.2	-0.7	5.0	14.8	9.9	11.0	15.8	13.8	76.9	175.0	82.3	2.7	47.7	21.5	1.0	4.0	89.1	Moderate
7	7.2	-0.7	5.0	14.8	9.9	11.0	15.8	13.8	76.9	175.0	82.3	2.7	47.7	21.5	1.0	4.0	89.1	Moderate
8	10.1	0.0	10.4	19.7	10.5	10.4	19.6	15.4	46.7	37.0	42.7	63.7	43.3	42.7	61.5	49.3	48.1	NO
9	-1.4	-7.7	-3.5	5.6	0.1	5.6	2.3	-5.4	60.3	65.0	53.3	51.7	71.3	153.0	143.5	161.5	8.5	NO
10	5.8	-1.3	3.9	13.6	7.1	9.7	14.7	9.4	71.8	63.3	67.0	62.3	94.7	68.0	61.5	92.5	40.1	NO
11	9.9	0.3	10.0	19.3	10.2	15.0	7.6	16.6	53.0	38.7	50.0	76.3	47.0	49.8	43.0	71.0	40.8	NO
12	14.7	7.6	13.3	22.1	15.8	13.3	21.6	21.6	75.9	86.0	72.0	40.0	105.7	72.0	35.5	63.5	89.1	Moderate
13	9.3	-0.4	9.4	18.6	9.7	15.6	7.0	16.1	58.5	40.7	53.7	89.3	50.3	68.8	45.5	80.5	37.4	NO
14	14.6	7.0	13.0	21.6	16.7	13.0	21.1	21.3	92.3	221.3	94.7	1.0	52.0	94.7	1.0	3.0	158.0	Extreme
15	2.1	-13.4	2.8	15.9	3.2	15.9	7.5	-8.9	34.9	19.0	28.7	68.7	23.3	68.7	27.5	18.0	63.5	Moderate
16	0.7	-9.6	-0.3	11.0	2.0	11.0	5.2	-6.6	51.9	47.0	54.3	64.7	41.7	64.7	43.5	42.5	34.9	NO

Table 4. Results of Principal Component Analyses (PCA) for seed bank (SB) traits and climatic variables

Correlations of principal components (PCs) of PCA ordination with (a) SB traits (ΔG_{CS} , T_{50} , PGI, and ΔG_{FT}) and with (b) temperatures ($_{temp}$) and precipitation ($_{pr}$) at annual (A = annual) and seasonal scales (W = winter; Sp = spring; Su = summer; Au = autumn), and of the plant life cycle phases (Pre = pre-zygotic; Post = post-zygotic; Dis = post-dispersal). The most explicative component (PC1) of PCA for SB traits was retained as index of seed bank formation (SBI)

(a)	PC1	PC2	PC3	PC4	ΔG_{CS}	T_{50}	PGI	ΔG_{FT}											
Eigenvalue	2.813	0.673	0.293	0.221															
Variance	70.32	16.83	7.32	5.54															
ΔG_{CS}	0.810***				-														
T_{50}	0.735***				0.333	-													
PGI	0.868***				0.603	0.616	-												
ΔG_{FT}	0.711***				0.676	0.594	0.766	-											
(b)	PC1	PC2	PC3	PC4	A _{temp}	W _{temp}	Sp _{temp}	Su _{temp}	Au _{temp}	Pre _{temp}	Post _{temp}	Dis _{temp}	A _{pr}	W _{pr}	Sp _{pr}	Su _{pr}	Au _{pr}	Pre _{pr}	Post _{pr}
Eigenvalue	7.216	3.618	2.423	1.391															
Variance	45.10	22.61	15.14	8.69															
A _{temp}	0.965***	-0.156	0.189		-														
W _{temp}	0.849***	-0.050	0.437		0.929	-													
Sp _{temp}	0.976***	-0.118	0.079		0.978	0.851	-												
Su _{temp}	0.955***	-0.199	-0.096		0.931	0.732	0.967	-											
Au _{temp}	0.923***	-0.261	0.235		0.988	0.934	0.942	0.900	-										
Pre _{temp}	0.649**	0.085	-0.151		0.540	0.329	0.597	0.688	0.494	-									
Post _{temp}	0.611**	-0.567	0.051		0.711	0.660	0.636	0.653	0.770	0.083	-								
Dis _{temp}	0.839***	-0.157	0.319		0.900	0.910	0.876	0.753	0.889	0.309	0.590	-							
A _{pr}	0.139	0.182	0.958***		0.274	0.483	0.197	0.025	0.284	0.053	-0.024	0.366	-						
W _{pr}	0.038	-0.437	0.848***		0.281	0.451	0.155	0.039	0.379	-0.119	0.304	0.374	0.715	-					
Sp _{pr}	0.084	0.057	0.975***		0.243	0.454	0.163	-0.010	0.267	-0.012	0.005	0.360	0.987	0.769	-				
Su _{pr}	0.026	0.719***	0.121		-0.109	-0.106	-0.005	-0.071	-0.229	0.240	-0.562	-0.085	0.353	-0.357	0.290	-			
Au _{pr}	0.291	0.360	0.696***		0.312	0.417	0.238	0.096	0.283	0.140	-0.025	0.327	0.811	0.307	0.726	0.481	-		
Pre _{pr}	-0.233	0.673***	0.158		-0.250	-0.104	-0.279	-0.342	-0.277	-0.181	-0.410	-0.326	0.178	-0.015	0.081	0.177	0.357	-	
Post _{pr}	-0.231	0.894***	-0.076		-0.355	-0.197	-0.354	-0.426	-0.423	-0.222	-0.514	-0.328	0.011	-0.413	-0.098	0.565	0.381	0.731	-
Dis _{pr}	-0.133	0.879***	-0.155		-0.273	-0.111	-0.271	-0.361	-0.347	-0.211	-0.481	-0.183	-0.072	-0.482	-0.172	0.504	0.337	0.653	0.924

Levels of significance for the PCA results: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Significant values ($P < 0.05$) are given in bold in the correlation matrixes

Table 5. Results of the multiple regression analysis and correlation matrix of explanatory variables

Relationship between seed longevity (p_{50}), seed mass (mg), index of drought during the post-zygotic phase (PHM), index of seed bank formation (SBI), and climatic traits expressed as PC-axes scores, i.e. temperature (PC1), precipitation during the driest months of the reproductive period (PC2), and annual and seasonal precipitation (PC3)

Term	Coefficient	<i>t</i> -value	Seed mass	PHM	SBI	PC1	PC2	PC3
Full model								
Constant	28.818	3.172*						
Seed mass	-6.976	-0.805	-					
PHM	0.181	1.659	0.358	-				
SBI	-0.084	-0.026	-0.407	-0.208	-			
PC1	3.904	1.707	-0.180	0.310	-0.163	-		
PC2	-9.711	-1.990	-0.499	-0.424	0.426	0.018	-	
PC3	-3.936	-1.479	0.153	0.205	0.453	0.083	-0.019	-
Reduced model								
Constant	22.514	4.061***						
PHM	0.234	2.645***						
PC1	3.814	1.946*						
PC2	-4.752	-2.710***						
PC3	-6.259	-2.052**						

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

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