Large interspecific and intraspecific variation in seed longevity in storage

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Author contribution

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Acknowledgements

We thank Johannes Kasper, Lena Lerbs, Christina Mengel, Sarah Paschen, Nadine Pluquette, Annika Sommerfeld, and Helene Villhauer for technical assistance. This project was funded by a scholarship of Marburg University Research Academy (MARA) to LK.

Declaration of Interest statement

Authors have no conflicts of interest.

ABSTRACT

Premise: Conservation seed banks are an essential tool for plant ex-situ conservation. However, seeds stored for long periods of time slowly deteriorate and lose viability. While seed longevity is known to vary between species, the extent of variability within species and the factors driving it remain unclear.

Methods: We focused on both inter- and intraspecific variability in seed longevity and its determinants. We studied 41 common grassland species and 188 seed accessions from across Europe. We exposed the seeds to artificial ageing conditions (60% RH, 45°C) and estimated the seed longevity (p_{50}), defined as the time required for viability to decline to 50% of the initial viability. We then tested the relationships between seed longevity and both species- and origin-specific factors.

Results: Variability in seed longevity was only slightly greater between species than within species, with species identity explaining 51.9% of the total variation in seed longevity among seed accessions, leaving 48.1% of the variation within species. In interspecific species comparison, seed viability was predicted only by initial seed viability, but unrelated to species-specific seed mass or seed chemical composition. Within species, seed longevity increased with the initial viability of the seed accession and mean annual temperature at the accession origin, but was unrelated to seed mass or precipitation in the origin, and the residual variance was substantial.

Conclusion: Our findings highlight that seed longevity on the level of individual seed accessions remains challenging to predict.

KEYWORDS

artificial seed ageing, conservation seed bank, species comparison, intraspecific variability, seed longevity, seed storage, within-species variability

INTRODUCTION

Biodiversity conservation is one of the critical global challenges, and *ex situ* methods play an increasingly important role in this task (Cohen et al., 1991). One of the most common ex-situ methods for plant conservation are seed banks, dedicated facilities that store seeds of wild plant species, particularly the vulnerable ones (Peres, 2016). The stored seeds represent a snapshot of past populations and are valuable resources for species reintroductions or ecosystem restoration, and also for scientific research (Cochrane et al., 2007; Etterson et al., 2016; Wambugu et al., 2023). Thus, the ultimate task of conservation seed banks is to keep the seeds viable for decades or even centuries.

Seed survival in storage depends on the type of seeds and storage conditions. Some types of seeds, so-called recalcitrant seeds, do not survive desiccation, making long-term storage impossible. Other types of seeds, called orthodox, can survive desiccation, and they can be stored for a couple of months up to centuries, depending on the species (Hay & Probert, 2013). Conservation seed banks aim to maintain seed viability as long as possible by storing the seeds under low temperatures and humidity to slow down internal chemical processes such as lipid peroxidation and DNA damage (Nagel et al., 2015; Walters et al., 2005a). The seed longevity also depends on the quality and viability of the seeds (Hay & Probert, 2013; Niedzielski et al., 2009), and thus, guidelines for seed banking recommend storing accessions with high initial viability (ENSCONET, 2009; Millenium Seed Bank, 2022). Yet, even highly viable seeds stored under optimal conditions slowly deteriorate and eventually die. This mortality is of course faster when the seeds are stored in suboptimal conditions, for example when storing seeds for large-scale ecosystem restoration in ambient conditions at a farm (Merritt & Dixon, 2011). To optimise the long-term effectiveness of seed storage – be it for conservation, research or ecosystem restoration purposes – we need to understand which factors predict seed longevity in storage.

Studying seed longevity presents unique challenges, as it requires comparing old and fresh seeds of the same seed lot, which would need time travel. To circumvent this obstacle, researchers have developed artificial ageing protocols which simulate natural ageing by exposing the seeds to high temperatures and high relative humidity

(Delouche & Baskin, 2021). These conditions accelerate the same or at least similar chemical processes typical of natural ageing during long-term storage (Delouche & Baskin, 2021). Although this method may not perfectly replicate natural ageing, the longevity of seeds in artificial ageing correlates with the longevity of seeds stored in seed banks both across and within species (Ninoles et al., 2022; Probert et al., 2009). Nevertheless, artificial ageing is the best method we currently have to study seed longevity.

Seed survival in artificial ageing differs between species, and to some degree also between higher taxa like plant families, although large plant families harbour substantial variation in seed longevity (Probert et al., 2009; Walters et al., 2005a). Studies aiming to detect general patterns in seed longevity beyond evolutionary history yielded mixed results. Endospermic seeds survive shorter in storage than non-endospermic ones (Probert et al., 2009). The influence of seed weight is less clear; some studies suggest that seeds of small-seeded species survive longer (Satyanti et al., 2018), while others report no effect of seed weight (Davies et al., 2020; Merritt et al., 2014; Probert et al., 2009). Seed longevity is also affected by climatic conditions at seed collection sites: Seeds from hot or dry environments generally survive longer than those from cooler or wetter conditions (Merritt et al., 2014; Probert et al., 2009; Zani & Müller, 2017). Furthermore, seed longevity should hypothetically depend on the seed's chemical composition because high oil content should reduce seed longevity due to ongoing lipid peroxidation in storage (Narayana Murthy & Sun, 2000; Pritchard & Dickie, 2003), yet the results of empirical studies are inconsistent (Nagel et al., 2015; Probert et al., 2009; Walters et al., 2005a).

In contrast to between-species comparison, there is less comprehensive information on within-species variability on seed longevity in storage. Most data come from cultivated crops or model species (e.g. Bizouerne et al., 2023; Franks et al., 2018; Guzzon et al., 2021; Lee et al., 2019), with only a few studies addressing natural populations of wild species (Genna et al., 2020; Kochanek et al., 2009; Mondoni et al., 2011; White et al., 2023). These indicate that the variation in seed longevity within a species can be as substantial as the variation between species from the same ecosystem (Kochanek et al., 2009; Mondoni et al., 2011). The predictors of seed longevity on the level of populations, genotypes, or varieties appear to be similar to

those driving between-species differences: In general, lighter seeds of the same species typically survive longer than heavier seeds (Franks et al., 2019; Guzzon et al., 2018; Lee et al., 2019, but see Mira et al., (2019)). Consequently, seeds of genotypes or varieties with lighter seeds tend to survive longer in storage than accessions with heavier seeds, although this effect is inconsistent across studies (Guzzon et al., 2021; Schutte et al., 2008). The climate at the seeds' origin seems to be also a critical factor, with populations from warmer and dryer environments producing longer-lived seeds than populations from colder and more humid environments (Mondoni et al., 2011, 2014, but see Kochanek et al., (2009)). The differentiation in seed longevity among populations of the same species can be driven by two, mutually not exclusive mechanisms: genetic differentiation (Mondoni et al., 2014) or phenotypic plasticity, where parental growing conditions influence seed longevity (Kochanek et al., 2010; Mondoni et al., 2014; Sinniah et al., 1998). While the studies above provide valuable insights, we are still missing a comprehensive assessment of intraspecific variability in seed longevity across wild species, its magnitudes and predictors.

To fill this gap, we focused on more than 40 common grassland species represented by up to 11 accessions from across Europe. We exposed the seeds to artificial ageing, estimated their longevity, and analysed the data on two levels: Comparison across species and comparison across accessions within the species.

We expect that (1) seed longevity under artificial ageing conditions differs between species and accessions within species. The variation among species will be larger than the variation within species. (2) Across species, longevity will increase with seed quality (measured as initial viability) and decrease with seed weight. It will also be influenced by seed chemical composition, particularly oil content, and the plant family. (3) Within species, longevity will be higher in accessions with high initial viability, lighter seeds and coming from from warmer and drier environments.

MATERIAL AND METHODS

Study species and seed material

We focused on 41 species common in European grasslands, belonging to 11 families distributed across the phylogenetic tree of angiosperms (Table 1). They represent a wide variety of traits, some potentially associated with seed longevity, specifically seed weight, seed oil and protein content (Probert et al., 2009). All species have orthodox seeds, i.e. they survive desiccation and freezing during storage.

To test for within-species variability, most species were represented by multiple origins, further called accessions. The number of accessions per species was driven by seed availability and strongly varied between species. More than half of the species were represented by at least four origins, while for six species we managed to obtain only one accession (Table 1). In total, the experiment included 188 accessions.

The experiment required large amounts of seeds. Part of the seeds was obtained by direct collection in nature, or collecting at least 50 small plants in the wild and growing them in a cold greenhouse to produce seeds. However, most seeds come from on-farm propagation for restoration purposes. We are aware that on-farm propagation may cause unintended selection, which could alter seed traits (Conrady et al., 2023; Ensslin et al., 2018). In the seeds we used, this effect will be rather minor because most producers are relatively new in this business and the propagation does not take place for many generations (*personal communication*). Using farm-propagated seeds further has the advantage that the maternal plants grew under optimal conditions and the seeds were harvested when fully ripe. This reduces the variability in seed quality, a characteristic that has a massive effect on storage behaviour (Rahman & Ellis, 2019; Sano et al., 2016; Sinniah et al., 1998).

Artificial ageing

To artificially age the seeds, we exposed them to 45° C and 60 % relative humidity in a climate-controlled cabinet (Rumed ®, Rubarth Apparate GmbH, Laatzen, Germany). These conditions enhance similar chemical processes that are typical of

natural ageing during long-term storage and accelerate seed deterioration (Merritt et al., 2014; Probert et al., 2009).

We exposed the seeds to artificial ageing conditions for 0 (control group, fresh seeds), 1, 5, 9, 15, 20, 30, 40, 57 and 72 days (adjusted after Probert et al. (2009)). We put 3 replicates of 30 seeds of every accession and ageing duration into open 1.5 mL tubes in the ageing condition. The position of each accession in the ageing condition was randomised within the ageing duration. Before the ageing treatment, we manually scarified Fabaceae seeds with sandpaper to allow for moisture to be imbibed (Baskin, 2003). In total, our experiment contained 188 accessions, each with 10 ageing treatments and 3 replicates, that is 5,640 replicates containing 30 seeds each, that is 169,200 seeds.

Germination test

For germination tests, we used 6-well plates with a well-diameter of 39 mm (VWR International GmbH, Darmstadt, Germany, catalogue number: 734-2323), because they use space in the climate cabinet more efficiently than single petri dishes. For species with large seeds (*Bupleurum rotundifolium*, *Centaurea cyanus*, *Bromus hordeaceus*, *Arrhenatherum elatius*, *Lathyrus nissolia*, *Hordeum murinum*, *Hypochaeris radicata*, *Agrostemma githago*, *Poa annua*) we used petri dishes of 6 cm diameter to avoid overcrowding. We wrapped Petri dishes with seeds of *Bromus hordeaceus* in aluminium foil since light slightly inhibits germination (Andersson et al., 2002). After the intended ageing duration, we removed the seeds from the ageing conditions and transferred each replicate into one well or a petri dish laid out with two filter papers in a fully randomised design. We watered the seeds with a 0.025 % Gibberellic acid solution to break dormancy and closed the plates with transparent Parafilm® to avoid evaporation. Germination took place in a climate-controlled cabinet (Rumed®, Rubarth Apparate GmbH, Laatzen, Germany) with a 14 h daily photoperiod at 20° C and 10 h darkness at 10° C.

We checked for germination every seven days and added distilled water if necessary. When a radicle was longer than 2 mm, we considered a seed viable and removed it from the dish. For each replicate with viable seeds, we finished the trial when no

more seeds germinated for at least four weeks. If in a replicate no seeds germinated at all for ten weeks, we considered the seeds not viable. If samples were affected by mould, we added 1 ml of a 0.0835 % Difenoconazol solution.

Seed traits and climatic data

For each accession, we determined seed weight by weighing 2 replicates of 50 seeds and calculated the weight of a thousand seeds (further called seed weight for simplicity). We also obtained the mean annual temperature and precipitation for each accession location from WorldClim2 using the R package *raster* (Fick & Hijmans, 2017). In wild-collected seeds, we used the exact location of the seed collection. For farm-propagated seeds, we used the location of the farm, as the initial seeds used to establish the seed orchard was typically collected in the same region where the seed producer is located (Bucharova et al., 2019, Figure 1). The oil and protein content of the seeds was obtained from the Seed Information Database (SID) for each species (Society for Ecological Restoration et al., 2023).

Statistical analyses

First, we calculated seed longevity for each accession as the number of days in seed ageing needed to reduce the seed viability to 50 % of the initial viability. The germination rate was sometimes higher in the short-duration ageing treatments (one or five days) than in fresh seeds because short exposition to ageing treatment breaks dormancy in some species (Hay et al., 2021; Mira et al., 2019). We thus defined “initial viability” for each accession as the highest average germination rate of the 0, 1 or 5 days in the ageing treatment. We excluded accessions with poor initial viabilities (below 0.33) and then fitted seed-survival curves for each remaining accession using the *drc*-package for dose-response curves (Ritz et al., 2015). We selected two-parameter log-logistic models based on the AIC criteria. To estimate the longevity parameter p_{50}, we used the ED function of the *drc*-package. P_{50} indicates the time after which the germination rate decreases to half compared to the initial viability and we call p_{50} “seed longevity” in further text.

The ageing treatment took 72 days, and much longer seed longevity estimates are strong extrapolations and therefore unreliable. For the following analyses, we thus excluded 12 accessions with a p_{50}-value of more than 100 days. P_{50} estimates of most *Papaver rhoeas* accessions were higher than 100, so after excluding them the species' average longevity would have been underestimated. We therefore removed this species from further analyses.

Second, we compared the seed longevity between species. For this purpose, we averaged p_{50}-values across all the accessions of the same species. We then related the log-transformed average seed longevity as response variable to the species' mean initial viability, mean seed weight, and plant family as explanatory variables in a multiple linear regression model. As protein and oil contents were only available for 16 study species, we evaluated the effect of these in a separate multiple linear regression model, correcting for the initial viability.

Third, we analysed within-species variability in seed longevity. We included only species for which we had at least 4 accessions. We related accession-specific longevity (log-transformed p_{50}) as response variable to the species' identity (to account for it), the accession-specific initial viability, accession-specific seed weight, mean annual temperature and precipitation at the accession origin as explanatory variables in a multiple linear regression model. We scaled the seed weight within each species to standardise the variable across species. To understand the relative importance of the predictors for seed longevity across accessions, we fitted a multiple linear regression model containing only significant predictors of within-species variability, and determined the proportion of variance explained by each of them using the package *relaimpo* (Groemping & Lehrkamp, 2023).

All data analyses were performed using R version 2023.9.1.494 (R Development Core Team, 2024). We log-transformed the p_{50} as response variable to ensure that model assumptions were met. For all models, we verified that model assumptions were met by graphically evaluating the residuals (Zuur et al., 2010).

RESULTS

The estimated seed longevity, that is the time until half of the viable seeds have died (p_{50}), strongly varied among species (Figure 2). The average species seed longevity was shortest for *Bupleurum rotundifolium* (p_{50} = 5.39 days) and longest for *Achillea millefolium* (p_{50} = 62.88 days). The average species seed longevity significantly increased with mean initial viability but was unrelated to the species-specific seed weight (**Error! Reference source not found.**Table 2A, Figure 3), or the seed oil and protein content (Table 2B, Figure 3). We also did not detect significant differences between plant families (Table 2A, Figure 3).

The estimated seed longevity also strongly varied among accessions of the very same species. This variation was the highest in *Daucus carota*, in which the p_{50} varied more than 18-fold (Figure 2). In absolute values, the estimated seed longevity varied most in *Dianthus deltoides*, with 60.28 days difference between the longest and shortest-lived accession. After accounting for species identity, the accession-specific seed longevity increased with the initial viability of the given seed accession and mean annual temperature at the accession origin, but was unrelated to the annual precipitation and accession-specific seed weight (Table 3, Figure 4).

Species identity was the strongest predictor of accession-specific seed longevity, explaining 51.9% of the variation, followed by the accessions' initial viability (11.5%), and the mean annual temperature at the seed origin (2.3%). A total of 34.2% of the variance in seed longevity among accessions remained unexplained.

DISCUSSION

Effective conservation of seeds in ex-situ seed banks requires understanding of seed storage behaviour not only for individual species but also for accessions within the very same species. We found out that for multiple species common in European grasslands, species identity explained only 51.9% of the among-accessions variability in seed longevity. The residual variance was partly explained by initial seed viability and mean annual temperature at the accession origin, yet 34.2% of the among-accession variance remained unexplained. Across species, the seed longevity was predicted by initial seed viability only. Our findings suggest that seed longevity is hard to predict – both within and across species

Interspecific variability in seed longevity

In a comparison across species means, the only variable that predicted seed longevity in ageing was the initial seed viability, where species with more viable seeds survived longer in ageing. Lower initial viability might indicate that the seeds have already experienced some deterioration and have reached a point at the sigmoidal-shaped survival curve, where viability declines more rapidly, and p_{50} is lower (Hay et al., 2022). Such previous deterioration is naturally more likely in species with fast-deteriorating seeds. Alternatively, the seeds could have received suboptimal post-harvest treatment or have low quality from the point of collection, for example when the seeds were not fully ripe and consequently, had lower germination and shorter longevity in storage (Hay & Probert, 1995; Probert et al., 2007). Both previous deterioration and suboptimal post-harvest treatment are naturally more likely in species whose seeds are more vulnerable to ageing or environmental conditions during storage. Variability in initial viability might thus amplify the interspecific difference in seed longevity in artificial ageing. Our findings support current guidelines for conservation seed banks which recommend that conservation seed banks preferably select and store seeds with high initial viability (ENSCONET, 2009).

Seed longevity in artificial ageing was affected neither by seed weight nor by the chemical composition of the seeds. In general, seed weight seems to be an unreliable predictor of seed longevity in storage, as the effects reported in previous

literature strongly vary from negative, over no effect to, rarely, positive (Davies et al., 2020; Merritt et al., 2014; Pritchard & Dickie, 2003; Probert et al., 2009; Satyanti et al., 2018). Similarly, the chemical composition of the seeds did not predict seed longevity, which corresponds to previous empirical studies of wild species (Probert et al., 2009, Walters et al 2005). The expected effect of chemical composition on seed longevity in storage, especially oil content, is based on theoretical expectations and comparisons among oil crops, cereals, and legumes (Narayana Murthy & Sun, 2000; Pritchard & Dickie, 2003 Nagel et al., 2015), but the empirical evidence provided by others and us shows that it is likely not true for wild species.

Surprisingly, we did not detect significant differences between plant families, which contrasts with nearly all previous studies (Merritt et al., 2014; Mondoni et al., 2011; Probert et al., 2009; Walters et al., 2005b). This is likely caused by the low number of species per family (1-6), which resulted in a low power of the statistical tests. Nevertheless, we observed similar trends as previous studies: Fabaceae seeds were relatively long-lived, probably due to their physical dormancy (Merritt et al., 2014), Apiaceae were shorter-lived and there was large variability within Asteraceae (Merritt et al., 2014; Walters et al., 2005a).

Intraspecific variability in seed longevity

We detected substantial within-species variability in seed longevity in storage. Specifically, species identity explained only 51.9% of the variation among the accessions, leaving 48.1% to variation within species. Such high intraspecific variability is in line with previous works. On a continental scale, Kochanek et al. (2009) collected data on seed longevity in seven species represented by 2-8 populations, with populations within species largely aggregated in a specific biome. Here, the species identity explained 70% of the variability among accessions, leaving 30% to variation among accessions within species (own analysis of the data presented in Kochanek et al. (2009)). On a regional scale, Mondoni et al. (2011) examined seed longevity of six ecologically similar species that were each represented by two populations in contrasting environments, specifically high and low altitude. Here, species identity explained only 6% of variability, leaving astonishing 94% to variation within species (own analysis of the data presented in Mondoni et al.

(2011). In our study, we detected that species explained about half of the variability in seed longevity among accessions, yet this number might be underestimated because we excluded the species with the most long-lived seeds for methodical reasons (see methods). Nevertheless, the available evidence suggests that intraspecific variation in seed longevity is nearly as substantial as between-species variability, at least in species coming from the same type of habitat.

The intraspecific variation in seed longevity was predicted by initial seed viability and climate in seed origin, specifically mean annual temperature. These variables explained comparatively less variability in seed longevity than species identity, specifically 11.5% and 2.3%, respectively. The effect of initial seed viability is in line with the cross-species comparison, and is discussed above. The seed longevity of the accession increased with the mean annual temperature at the collection site, a pattern that was reported previously. Populations experiencing warmer climates are more long-lived than seeds from populations experiencing colder climates (Mondoni et al., 2011). Other studies attributed such a positive correlation between temperature and seed longevity mainly to the species identity, because they had species represented by single accessions (Merritt et al., 2014; Probert et al., 2009). We did not find any effect of mean annual precipitation on seed longevity, which contrasts with previous studies both within and across species (Merritt et al., 2014; Probert et al., 2009; White et al., 2023). This is likely because the mean annual precipitation was correlated with mean annual temperature (r=33), and as we used multivariate models, we were not able to tear the effect of both climate variables apart.

The intraspecific variability in seed longevity might be driven by two, mutually non-exclusive mechanisms – phenotypic plasticity, or genetic differentiation. For example, Mondoni et al. (2011) detected that seeds collected in populations in warmer environments survived longer in artificial ageing than conspecific seeds collected in cooler environments. A large proportion of this effect was caused by phenotypic plasticity, but the pattern was still significant, although weaker, in the second generation grown in a common environment, which indicates that the effect is partially heritable and thus genetic (Mondoni et al., 2014). Indeed, genes associated with seed longevity in storage were detected in some crops and model species (Bizouerne et al., 2023; Nagel et al., 2015; Raquid et al., 2021; Renard et al., 2020).

Our study worked with seeds that were collected in natural sites or produced in the region of origin; we thus cannot discriminate whether the observed effects are plastic or genetic.

CONCLUSION

Our results show that seed longevity in storage varies within species nearly as much as between species. Seed samples from different populations of the same species survive longer in storage if they have high initial seed viability and come from warmer climates, but there is substantial residual variability. This finding highlights that seed longevity remains challenging to predict. Conservation seed banks sometimes design their monitoring of seed viability based on species identity, with the assumption that accessions of the same species lose viability at the same rate when stored under the same conditions. Our data support existing concerns that this method might be suboptimal (Hay & Whitehouse, 2017).

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Figure 1: Origins of the 153 seed accessions used in the study and included in the analyses. Each point represents the location of a seed producer as a proxy for the origins of the individual seed accessions and thus, is the origin of multiple species. In three cases, the points represent the location of own seed collection.

Figure 2: (a) Estimated seed longevity (p_{50}) across 35 species and 153 accessions. Blue points represent species averages, green points accession estimates. (b) Proportion of explained variability in seed longevity by significant predictors and residuals (this analysis included only species represented by at least four accessions).

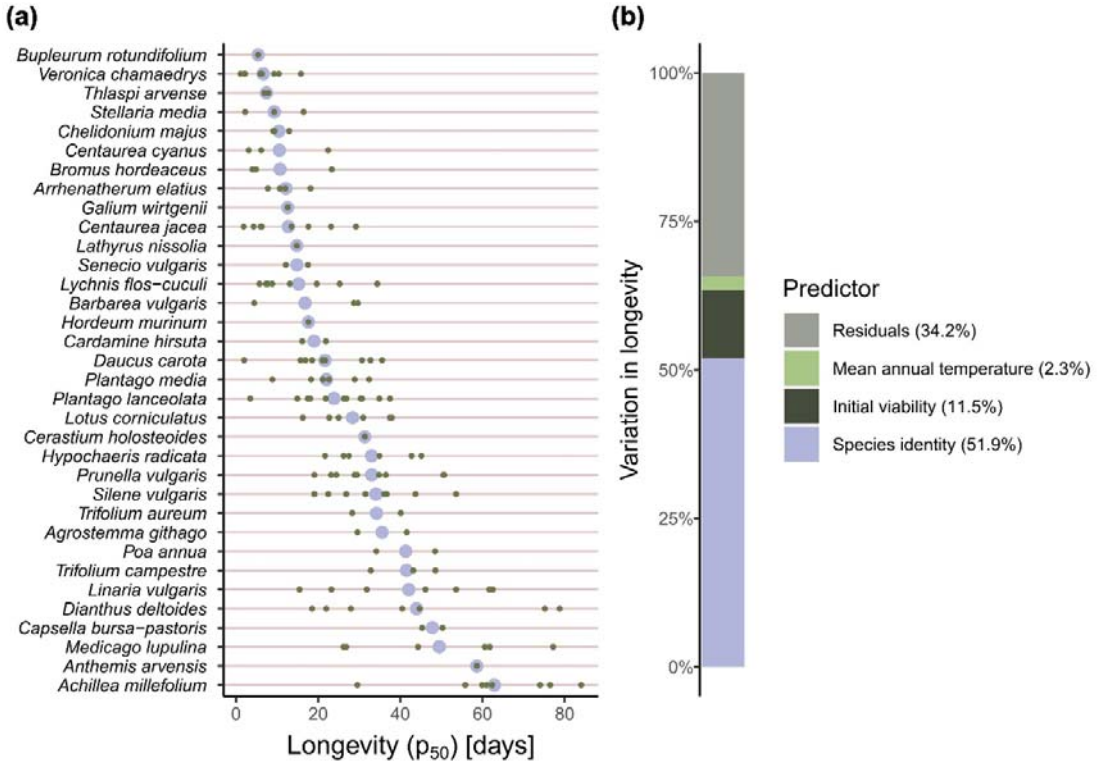

Figure 3: Relationship between average seed longevity (p_{50}) of each species and species-specific seed traits. Each point represents one species. (a) Initial seed viability, average across accessions of the same species; (b) seed mass, average across accessions of the same species; species-specific (c) protein and (d) oil content in the seeds; and (e) plant families. Dashed line indicates non-significant relationship. For full model results, see Table 2.

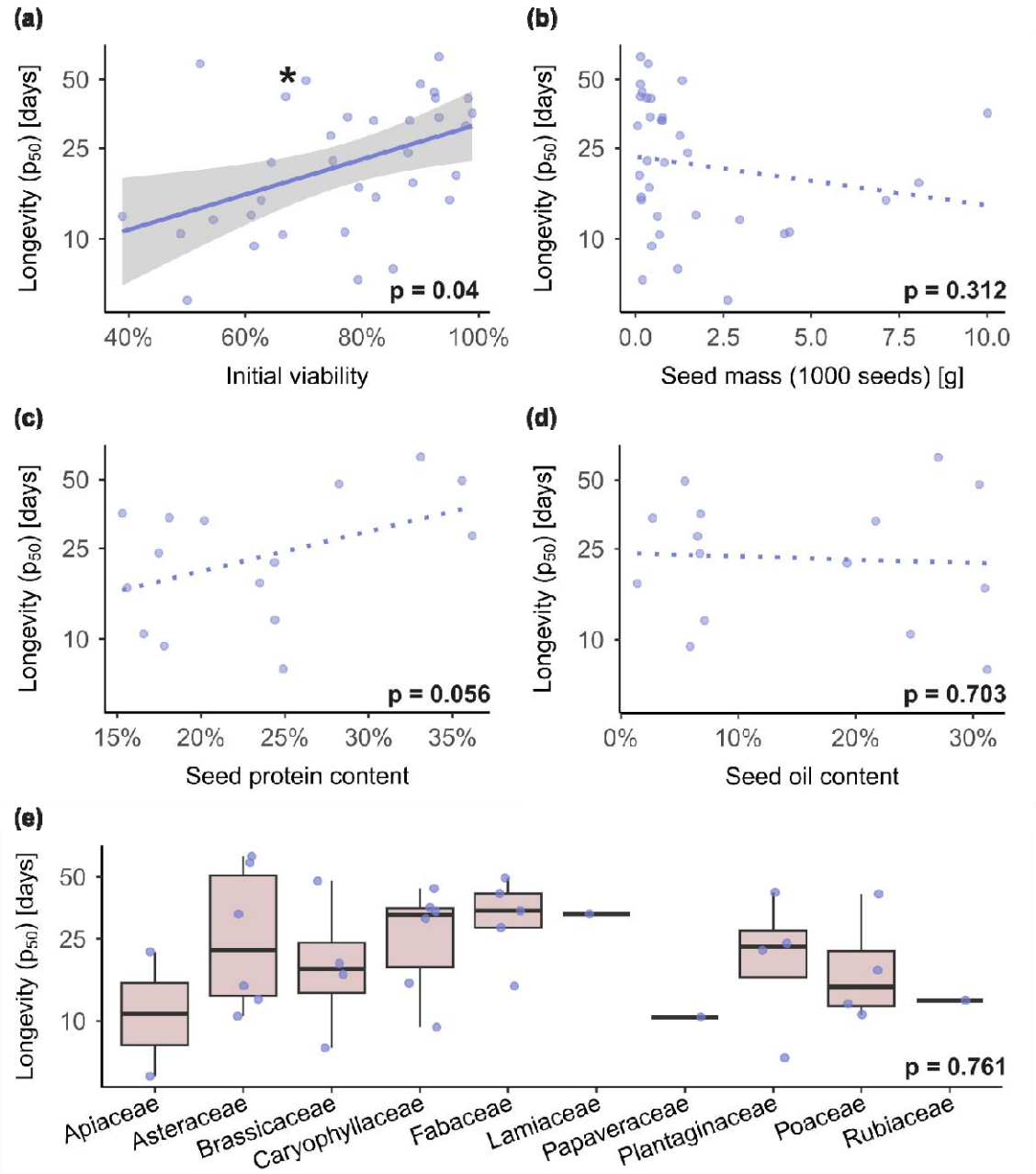

Figure 4: Relationship between seed longevity (p_{50}) of each accession, accession-specific seed traits, and environmental variables. Each point represents a seed accession. Seed longevity was adjusted for species identity. (a) Accession-specific initial seed viability; (b) accession-specific seed mass (scaled); (c) mean annual precipitation and (d) mean annual temperature at the origins of the accessions. For full model results see Table 3.

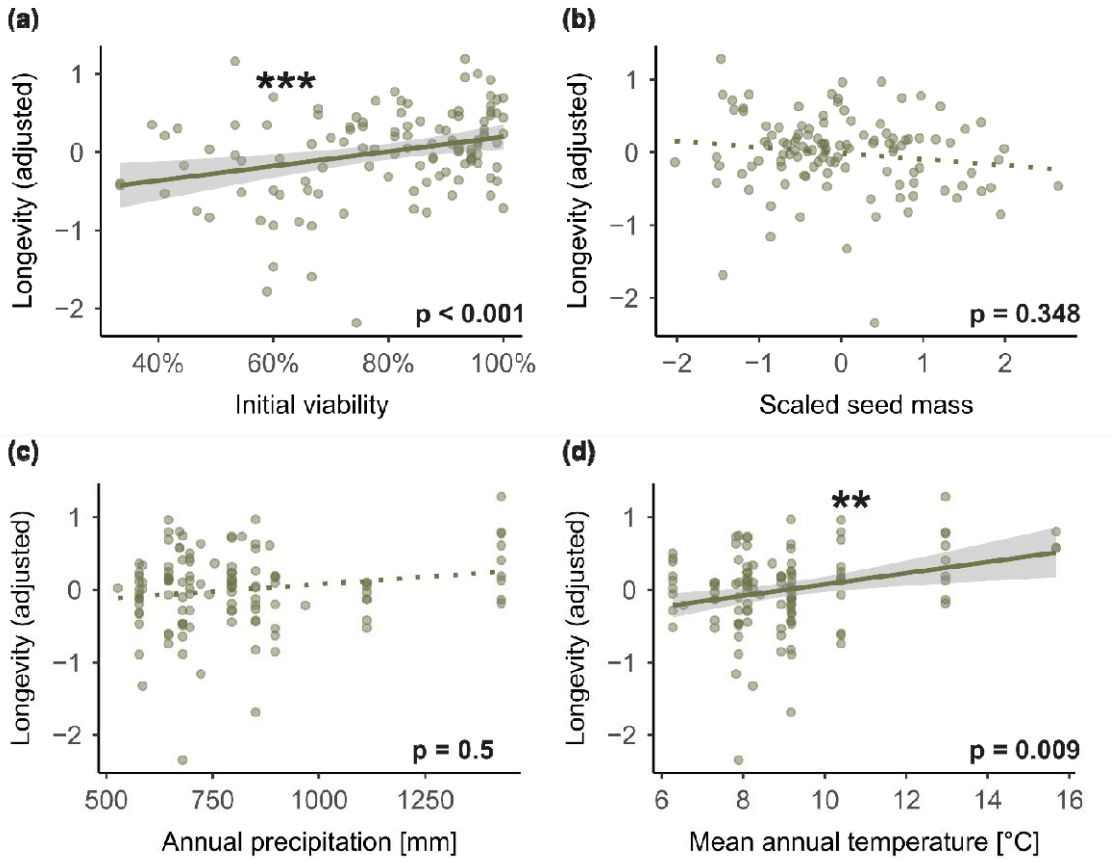

Table 1: Species included in the study. The number of accessions is the number of accessions used for the analyses and in brackets the initial number of accessions. Seed weight is the weight of 1000 seeds, averaged across all accessions of the same species. Oil and protein content are obtained from the Seed Information Database (Society for Ecological Restoration et al., 2023). Life cycle: A = annual, P = perennial, B = biennial. *: Information of conspecific

Species	**Number of accessions [initial number]**	**Seed weight [g]**	**Family**	**Life cycle**	**Oil content [%]**	**Protein content [%]**
Achillea millefolium	8 [10]	0.14	Asteraceae	P	27.03	33.1
Agrostemma githago	2 [2]	10.02	Caryophyllaceae	A	6.8	15.3
Ajuga reptans	0 [4]	1	Lamiaceae	P		
Anthemis arvensis	1 [1]	0.37	Asteraceae	A/B		
Arrhenatherum elatius	4 [5]	2.83	Poaceae	P	7.13	24.4
Barbarea vulgaris	4 [4]	0.39	Brassicaceae	A/B	31	15.6
Bromus hordeaceus	3 [3]	4.38	Poaceae	A		
Bupleurum rotundifolium	1 [1]	2.63	Apiaceae	A		
Capsella bursa-pastoris	2 [2]	0.15	Brassicaceae	A	30.53	28.23
Cardamine hirsuta	2 [2]	0.12	Brassicaceae	A		
Centaurea cyanus	3 [7]	3.94	Asteraceae	A	24.67	16.57
Centaurea jacea	8 [8]	1.72	Asteraceae	P		
Cerastium holosteoides	1 [1]	0.07	Caryophyllaceae	B/P		
Chelidonium majus	3 [5]	0.71	Papaveraceae	B		
Daucus carota	9 [9]	0.83	Apiaceae	A/B	19.26	24.38
Dianthus deltoides	7 [8]	0.2	Caryophyllaceae	P		
Galium wirtgenii	1 [1]	0.63	Rubiaceae	P		
Geranium pusillum	0 [1]	1	Geraniaceae	A		
Hordeum murinum	1 [1]	15.41	Poaceae	A	1.4	23.5
Hypochaeris radicata	6 [7]	0.74	Asteraceae	P		
Lamium album	0 [2]	1.57	Lamiaceae	P		
Lamium purpureum	0 [3]	0.69	Lamiaceae	A		
Lathyrus nissolia	1 [1]	7.13	Fabaceae	A		
Linaria vulgaris	7 [7]	0.15	Plantaceae	P		
Lotus corniculatus	6 [7]	1.33	Fabaceae	P	6.53	36.18
Lotus pedunculatus	0 [2]	0.52	Fabaceae	P		
Lychnis flos-cuculi	8 [8]	0.16	Caryophyllaceae	P		
Medicago lupulina	6 [6]	1.33	Fabaceae	A	5.45	35.57
Papaver rhoeas	0 [9]	0.1	Papaveraceae	A	43.47	24.53
Plantago lanceolata	11 [11]	1.49	Plantaginaceae	P	6.73	17.47

Plantago media	6 [6]	0.34	Plantaginaceae	P(/B/A)		
Poa annua	2 [2]	0.44	Poaceae	A/B		
Prunella vulgaris	9 [9]	0.77	Lamiaceae	P	21.7	20.2
Scandix pecten-veneris	0 [1]		Apiaceae	A		
Senecio vulgaris	2 [2]	0.17	Asteraceae	A		
Silene vulgaris	9 [9]	0.77	Caryophyllaceae	P	2.7	18.1
Stellaria media	3 [3]	0.46	Caryophyllaceae	A	5.9	17.8
Thlaspi arvense	2 [3]	1.18	Brassicaceae	A	31.2	24.9
Trifolium aureum	2 [2]	0.42	Fabaceae	A		
Trifolium campestre	3 [5]	0.31	Fabaceae	A/B		
Veronica chamaedrys	8 [8]	0.14	Plantaginaceae	P		

Table 2: **Interspecific** variability in seed longevity in storage, results of the linear model testing the effect of (A) initial viability, the family, the species-specific seed weight, and (B) and the protein and oil content on species-specific seed longevity. Analysis of variance with error type III. Significant effects ($p < 0.05$) are highlighted in bold.

	df	Resid.df	*F*	*p*	R^2
A		22			0.4
Initial viability	1		4.78	**0.04**	
Family	9		0.72	0.686	
Species-specific seed weight	1		1.07	0.312	
B		11			0.52
Initial viability	1		7.3	**0.021**	
Protein content	1		4.58	0.056	
Oil content	1		0.15	0.703	

Table 3: **Intraspecific** variability in seed longevity in storage, results of the linear model testing the effect of species identity, initial viability, accession-specific seed weight, mean annual temperature and mean annual precipitation on accession-specific seed longevity in storage. Analysis of variance with error type III. Significant effects (P < 0.05) are highlighted in bold.

	df	Resid.df	*F*	*p*	R^2
		94			0.67
Species	15		8.5	**< 0.001**	
Initial viability	1		25.6	**< 0.001**	
Accession-specific seed weight	1		0.89	0.348	
Mean annual temperature	1		7.09	**0.009**	
Annual precipitation	1		0.46	0.5	

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