

Research Paper

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Intra-specific variation in relative embryo length and germination of wild *Daucus carota* across climate gradients in North America and Europe

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Abstract

Although seed trait variations and their relationship to the ecological niche have been studied extensively at the species level, they do not necessarily reflect variations at the population level. In this study, we explored the intra-specific variation in relative embryo length, seed mass and germination speed in 40 populations of *Daucus carota* distributed across Europe and North America. By including information on local climate conditions, we aimed to examine the impact of the geographical origin on various seed functional traits and to detect potential local adaptation. No significant difference was observed in final seed germination for European and North American seeds incubated at 20°C, nor in seed viability. In European populations, relative embryo length significantly increased with increasing seed mass, but no such relation was found in North American populations. Larger relative embryo length at dispersal resulted in increased germination speed in both European and North American populations. Populations in drier areas typically had seeds with larger relative embryo lengths. Precipitation-related climate variables showed a negative relationship with relative embryo length, indicating a reduction in relative embryo length with increased precipitation. No clear relationship between climate and seed mass was observed. We can conclude that seed functional traits of *D. carota* are adapted to local climate conditions, as a clear gradient was observed in the relative embryo length of *D. carota*, which was associated with germination speed and climate. This gradient was less pronounced in North America, which can be explained by its relatively recent introduction to the continent.

Introduction

The reproductive success of plants relies on the alignment between seed characteristics with their environment (Baskin and Baskin, 2014; Igea et al., 2017; Soltani et al., 2021). Angiosperm seeds can, for example, germinate at widely different speeds, which are related to species' ecological strategy and habitat preference (e.g., Kadereit et al., 2017). How fast seeds can germinate is determined by seed morphology, physiology, gene regulation and environmental factors, with the process being irreversible but critical for species survival. Seed mass is another crucial trait in plant reproductive biology, influencing dispersal and population recruitment, with inter-specific variations related to species life form and habitat characteristics (Vandeloos et al., 2018; Kang et al., 2022). Seed mass indicates the amount of nutrient reserves the offspring are dispersed with (Moles, 2018; Kang et al., 2021), and plants face a trade-off between producing numerous small seeds for widespread distribution or a few large seeds for enhanced early seedling establishment (Leishman and Westoby, 1994; Moles et al., 2005). Although seed trait variations and their relation to the ecological niche have been studied extensively at the species level, they do not necessarily reflect variations at the population level (Saatkamp et al., 2019).

Although seed mass, like many seed morphological traits, is considered a stable trait at the species level (e.g., Harper, 1977), intra-specific variation in seed mass does exist and can range two- to six-fold (Hendrix and Sun, 1989). Inter-specific seed mass variation is mainly influenced by environmental and biotic factors (Moles et al., 2005), and the factors hypothesized to act on inter-specific trait variation may act on intra-specific variation as well (Fricke et al., 2019). Thermal and disturbance niche breadth also contribute to intra-specific seed mass variation, reflecting responses to temperature variations and disturbance levels (Wu et al., 2015; Fernández-Pascual et al., 2019). Kang et al. (2022) indicated that intra-specific seed mass variation in eastern Qinghai–Tibetan species is positively related to niche breadth, with significant direct effects of thermal niche and seed dispersal mode, emphasizing the

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simultaneous impact of intrinsic biotic attributes and extrinsic abiotic pressures on regional-scale intra-specific seed development. Ge et al. (2020), investigating seed mass and germination in eight *Allium* species and eight populations of *Allium chrysanthum*, found significant differences among both species and populations. The study highlighted the impact of elevation on seed mass and germination, with smaller seeds exhibiting enhanced germination, influenced by strong environmental effects, particularly at higher elevations.

Intra-specific variation in seed germination timing, often regulated by seed dormancy, is driven by climate in general and the climate seasonality in particular. Optimal primary seed dormancy is expected to be related to the length of the period following seed release, which is unfavourable for seedling establishment (Allen and Meyer, 1998; Wagmann et al., 2012) and varies among populations along a climate gradient. Sales et al. (2013) found that *Miconia albicans* seeds from grassland plants germinated slower and more asynchronous as compared to woodland plants in the Brazilian Cerrado. Allen and Meyer (2002), studying dormancy variation in *Bromus tectorum* populations, found that environmental conditions that lead to shortened seed ripening periods resulted in reduced seed dormancy. Moreover, there is evidence that intra-specific variation in germination characteristics coincides with variation in linked seed morphological traits. For example, Amimi et al. (2023) showed that variation in seed functional traits including morphological characteristics and physiological responses of *Quercus ilex* populations can be explained by climatic gradients. Notable differences in seed moisture content and germination rates associated with elevation and temperature variations were observed in *Q. ilex*.

While intra-specific variation in seed mass and dormancy have been relatively well studied, much less is known about intra-specific variation in other seed functional traits, such as the embryo-to-seed size ratio (hereafter referred to as relative embryo size). Relative embryo size is a proxy for the quantity of nutrient reserves stored in the embryo versus the endosperm. Relative embryo size has been shown to relate to seed mass, adult longevity, habitat shade and annual precipitation, supporting the tight relationship between embryo length and species ecology (Vandelook et al., 2012b, 2021). The relative embryo size in angiosperms is closely linked to physiological processes like seed germination, where species with larger embryos, for example, tend to germinate faster. As such, relative embryo length is a heritable and advantageous trait for early seedling establishment (Geber and Griffen, 2003; Verdú, 2006). Here, we will analyse intra-specific variation in the relative embryo size of *Daucus carota* L. and how it varies with the environment, perhaps mediated through other seed functional traits.

D. carota (wild carrot), the progenitor of the cultivated carrot, is a biennial herb native to Europe and Asia and introduced in North America by European settlers in the 17th century (Small, 1978; Iorizzo et al., 2013). It is now distributed throughout North America. Evidence provided by Magnussen and Hauser (2007) reveals hybridization between cultivated and wild *D. carota*, confirming the species' introduction from Europe as a weed. Mackowska et al. (2023), utilizing somatic hybridization techniques, emphasized the role of human-mediated hybridization in the weediness of *D. carota* in the United States. *D. carota* is particularly diverse in the Mediterranean region, with multiple subspecies and varieties (Spooner et al., 2014), while it is less diverse but still very common in more northern European regions. Like all other Apiaceae, *D. carota* seeds have an

underdeveloped embryo at dispersal and copious endosperm (Vandelook et al., 2012a).

Germination characteristics of *D. carota* have been shown to vary within a single plant and across populations. *D. carota* plants have a primary umbel and multiple secondary umbels. Germination percentages of *D. carota* seeds from primary umbels in early July are lower than those from secondary umbels ripening later (Gray, 1979). In addition, seeds from later primary umbels, though the same size, exhibited lower germination percentages than those from early primary umbels (but see Corbineau et al., 1995). Considerable variation in *D. carota* seed traits, linked to variation in reproductive timing, has been observed between populations along a latitudinal gradient in the United States (Lacey, 1988). Germination and dormancy characteristics of *D. carota* may also be mediated by seed size. Larger seeds of *D. carota* are, for example, less dormant than smaller ones (Jacobsohn and Globerson, 1980; Gross, 1984).

Detailed information on seed trait variation patterns within species on a continental scale is scarce, prompting questions about mean seed mass differences, relative embryo length, germination and bioclimate predictors contributing to variance within species. To improve our understanding of how seed trait variation at the population level is driven by climatic conditions, we analysed variation in seed mass and relative embryo length in *D. carota* along wide climatic gradients in the United States and Europe. More specifically, this study aims to reveal whether (i) seed germination percentage and rate relate to embryo size and/or seed mass, (ii) seed traits relate to local climate conditions; (iii) the same patterns are observed in its region of origin, Europe and in North America where it has become naturalized over the past 300 years.

Materials and methods

Seed samples

Seeds of 40 *D. carota* accessions (hereafter referred to as populations) were obtained from the USDA, Agricultural Research Service (Supplementary Table S1). Out of these 40 populations, 24 populations were originally sampled along a latitudinal gradient in western North America, while 16 populations had a European origin (Fig. 1). To better cover the climate range in Europe, two seed populations received from the IPK Gatersleben were added, as well as one population sampled in Rucava, Latvia (received from the National Botanic Garden of Latvia). All seeds had been dry stored for prolonged periods of time, which reduced the physiological dormancy present in the seeds. Since seeds were all stored for long periods in dry conditions, we assume that the dormancy that could be broken by dry storage was removed to a maximal extent. Any remaining dormancy, likely needed to be overcome by other treatments, such as cold stratification. Germination was always above 50% within 2 weeks after incubation, and it did not change significantly during storage (Supplementary Fig. S1). Information on seed accessions and collecting date of the original population is given in Supplementary Table S1. Some populations may have been regenerated in controlled conditions, but we have no further information on whether that was the case.

Germination tests

Germination tests were carried out in lab conditions in July at the Philipps University Marburg. For each population, three replicates

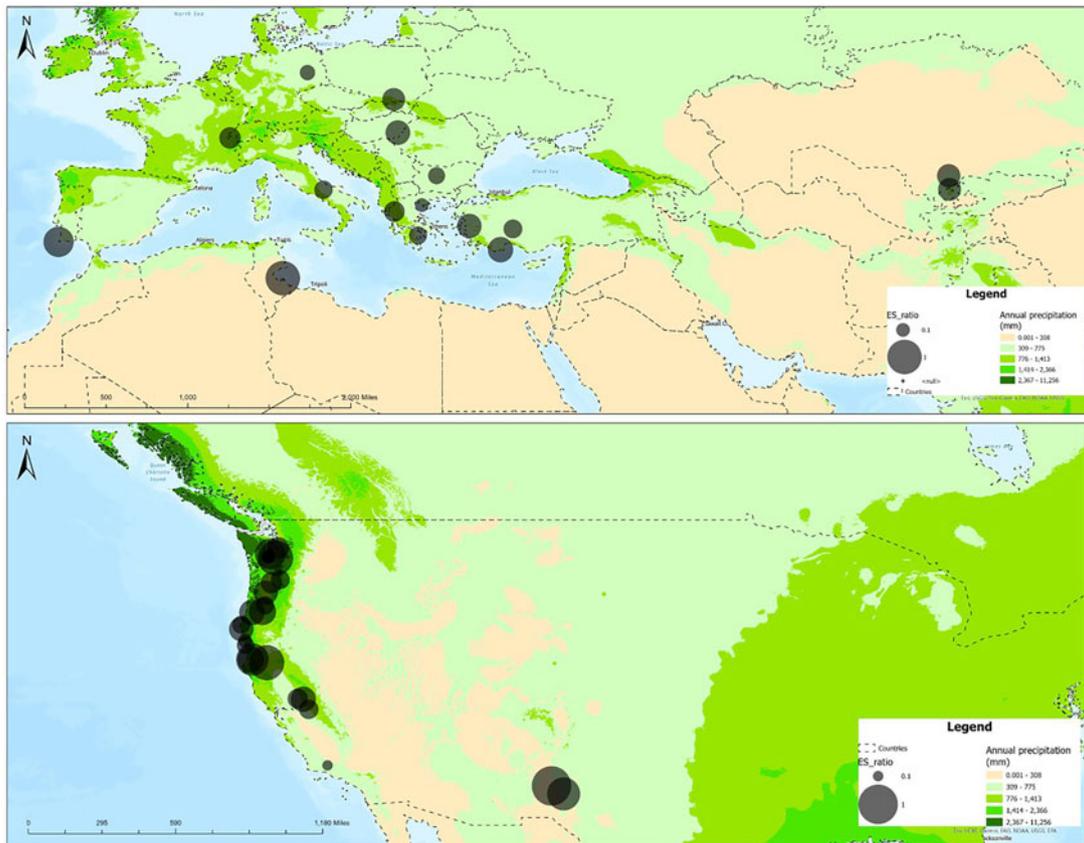


Figure 1. Geographic origin of 40 *D. carota* populations used in this study. The upper panel shows the locations of 16 European populations, including two from IPK Gatersleben and one from Rucava, Latvia. The lower panel shows the locations of 24 North American populations, sampled along a latitudinal gradient in western North America. The size of the dots reflects the mean length of the embryo-to-seed length ratio (E:S ratio) of the sampling sites. Data source: USDA, Agricultural Research Service (Supplementary Table S1).

of 25 seeds were placed on two layers of filter paper in 9 cm diameter Petri dishes and moistened with distilled water. All Petri dishes were placed in a temperature and light-controlled incubator (Binder GmbH) at a constant temperature of 20°C and a 12 h photoperiod (Philips TLD 80 fluorescent tubes with a photon flux density of $52 \mu\text{mol m}^{-2} \text{s}^{-1}$, 400–700 nm). The experiment ran for 15 days, during which germinated seeds were counted and removed every second day. Similarly, three replicates of 25 seeds from the North American populations were incubated at 10°C for 20 days. Seed germination of European populations was not tested at 10°C due to a lack of seeds. At the end of the experiment, a cut test was carried out on all non-germinated seeds to assess whether the seeds were dead or dormant. Seeds with an intact and white embryo and endosperm were considered viable. The final germination percentage was calculated, considering only intact seeds.

Calculation of germination speed

Germination speed was calculated using three indices, the inverse of Mean Germination Time ($1/\text{MGT}$), the inverse of time to 50% germination ($1/t_{50}$) using the Hill function (El-Kassaby et al., 2008; Joosen et al., 2010), and the inverse of time to actual 50% germination (R50) using Germin^{v3} (Soltani et al., 2015). MGT was calculated using the formula: $\text{MGT} = \sum n_i t_i / \sum n_i$, where t_i is the time from the start of the experiment and n_i the number of germinated seeds at the i th time (Ellis and Roberts, 1980). t_{50}

was extracted from a Hill function with formula $y = y_0 + (ax^b) / (c^b + x^b)$ fitted on the cumulative germination curve. Here, y is the cumulative germination percentage at time x (day), y_0 is the intercept on the y axis (≥ 0), a is the maximum cumulative germination percentage (≤ 100), b is controlling the shape and steepness of the curve and c is the time required for 50% of viable seeds to germinate (t_{50}). To calculate R50, Germin^{v3} was used that this tool is formulated using the Visual Basic (VB) Excel, and the corresponding codes can be found in Supplementary Table S2 and require the Macro VB section in Excel (<https://zenodo.org/records/10440963>). Germin^{v3} calculates the time to different percentiles of germination, including time to 50% of germination (t_{50}), through linear interpolation, subsequently determining the germination rate using its inverse.

Seed mass and embryo size measurements

Seeds mass was determined for each population by weighing all available seeds (usually about 100 seeds) on a precision balance. The total seed mass was divided by the number of weighted seeds to obtain an average value. All seed populations had been dry stored in the same lab conditions (40–60% relative humidity (RH)) for about 2 weeks prior to weighing.

Relative embryo length was expressed as the embryo-to-seed length ratio (E:S ratio). Embryo length and seed length were measured on seeds that were cut in half along the longitudinal axis. Ten seeds per population were placed in distilled water for 1

day to imbibe and were subsequently cut in half. Pictures of the cut seeds and embryos were taken using a Keyence digital microscope (VH-5000, Ver 1.5.1.1, Keyence Corporation). Embryo length and seed length along the longest axis were measured using the ImageJ software version 1.53t (Abramoff et al., 2004). Due to a lack of seeds, no embryo measurements were made for the two populations received from IPK Gatersleben and the population from Latvia.

Climate data

The geographical coordinates of the original sampling sites of all populations were given by the institutes that provided seed material. Using these coordinates, climatic data associated with the sampling or occurrence sites were estimated using the WorldClim database version 2.1 (<https://worldclim.org/>; downloaded on 7 Nov 2022) and accessed through DIVA-GIS version 7.5.0 (Hijmans et al., 2001). Altitude and climatic data incorporated in the study are listed in Supplementary Table S1.

Statistical analysis

Seed germination data were analysed using generalized linear mixed-effect models with a binomial error distribution and a logit link function in the package lme4 version 1.1-31 (Bates et al., 2015) in R version 4.2.1. (R Core Team, 2022). Populations were included as random effects in the models. Seed morphology and germination speed data were analysed using general linear models.

The relationships between predictors (coordinates and bioclimatic data) and the E:S ratio were investigated using general linear models, and the most important predictors were identified. Correlation coefficients and principal component analysis (PCA) were applied to explore the underlying structure of the dataset and identify the primary drivers of variation using SAS Institute Inc. (Cary, NC, USA, 2013). To visualize the PCA, the factoextra package in R was used (Kassambara and Mundt, 2020). The principal components (PCs) were interpreted to understand the correlations between the most important bioclimate variables (annual mean temperature, temperature seasonality, mean temperature of the wettest quarter, annual precipitation and precipitation seasonality) and seed morphology (seed mass and E:S ratio) or germination (germination percentage of viable seeds and dormancy and germination speed) data.

Stepwise analysis was used to identify the subset of significant predictors that best explained the variability in the E:S ratio using

SAS. The inclusion or exclusion of variables was iteratively evaluated by a stepwise backward selection procedure, optimizing the model's fit and preventing overfitting. Specifically, the stepwise selection process utilized the criteria of partial R^2 , F -value, and P -value for entry into and removal from the model. Also, $C(p)$ was calculated. ' $C(p)$ ' refers to the criterion used for the stepwise variable selection process. Specifically, it is often associated with the Change in Criteria (C) statistic, and ' p ' typically represents the probability associated with the change. Finally, the relationships between the most important predictor and embryo length, seed length and seed mass were investigated using the general linear model.

Results

Germination speed indices

The three germination speed indices applied here showed a similar trend but also some obvious differences (Fig. 2). In populations from Europe, R50 and $1/t_{50}$ showed no significant difference for the intercept (intercept = -0.009 ; $P = 0.89$), but there was a significant difference in slopes both in Europe ($P < 0.001$) and in North America at both 10 and 20°C ($P < 0.001$). However, $1/\text{MGT}$ was significantly different from both R50 and $1/t_{50}$ in European and North American populations tested at 10 or 20°C (Fig. 2). The differences between $1/\text{MGT}$ and R50 and between $1/\text{MGT}$ and $1/t_{50}$ were more obvious at 10°C for North American populations, at which R50 and $1/t_{50}$ showed a lower rate of germination compared to $1/\text{MGT}$. No significant difference in germination speed between European and North American populations was found for any of the indices ($1/t_{50}$: $P = 0.36$; R50: $P = 0.35$; $1/\text{MGT}$: $P = 0.86$).

Relationship between germination, seed mass and relative embryo size

No significant difference was observed in final seed germination percentage ($\beta = -0.41$; SE = 0.3; $P = 0.20$), nor seed viability ($\beta = -0.18$; SE = 0.3; $P = 0.60$), for European and North American seeds incubated at 20°C. Both European and North American populations germinated to about on average 80–85%, while seed viability was about 90% for both continents. Mass of European seeds was significantly higher as compared to those from North America ($\beta = 0.16$; SE = 0.04; $P < 0.001$). The range of average seed mass among the population was also considerably larger in Europe (0.7–3.3 mg) than in North America (0.7–1.6 mg).

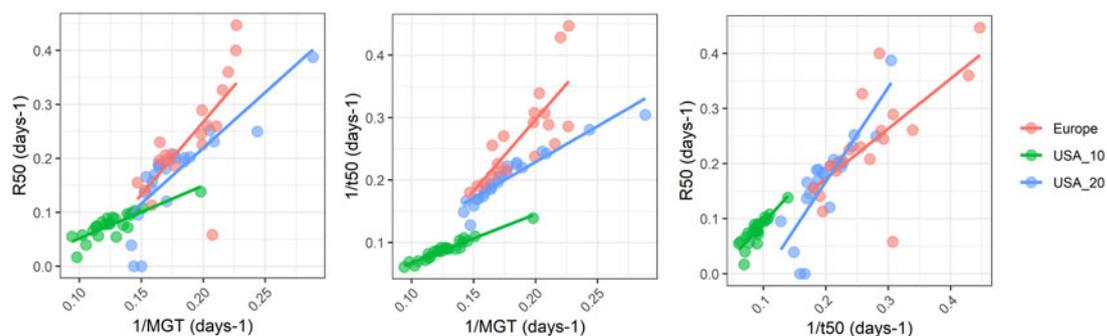


Figure 2. Relationship between three indices of germination speed in the European and North American population of *D. carota*. The germination of North American populations was tested at 10 and 20°C and European populations at 20°C only.

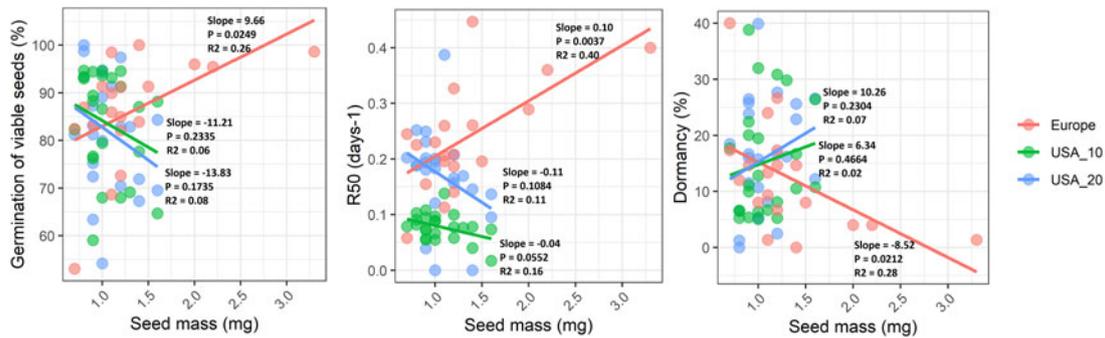


Figure 3. Relationships between seed mass and seed germination in European and North American *D. carota* populations. The germination of North American populations was tested at 10 and 20°C and European populations at 20°C.

A significant continent interaction ($t = -0.145$; $P < 0.001$) was observed for the relationship between seed mass and germination speed ($1/t_{50}$). While populations with heavier seeds germinated faster in Europe, they germinated slower in North America (Fig. 3). Germination percentage significantly increased ($P < 0.05$) with increasing seed mass in European populations. However, the germination percentage decreased with seed mass in North American populations incubated at 10 or 20°C, although this decrease was not statistically significant ($P = 0.23$ and $P = 0.17$, respectively). The speed of germination significantly increased ($P = 0.004$) with increasing seed mass in European populations, while no significant change was found in North American populations (neither at 10 nor at 20°C). The proportion of dormant seeds also significantly ($P = 0.02$) decreased with increasing seed mass in European populations, but no significant increase was observed in North American populations (at 10 and 20°C).

A marginally significant difference in the E:S ratio was observed between European and North American seeds ($\beta = -0.11$; $SE = 0.06$; $P = 0.08$). The E:S ratio in European populations (mean: 0.328; range: 0.173–0.659) was significantly higher than that in North American populations (mean: 0.249; range: 0.128–0.617). There was a significant interaction effect of continent on the relation between seed mass and the E:S ratio ($\beta = 0.17$; $SE = 0.05$; $P = 0.002$; Fig. 4). In European populations, relative embryo length significantly increased ($P = 0.003$) with increasing seed mass (Fig. 4), while in North American populations a

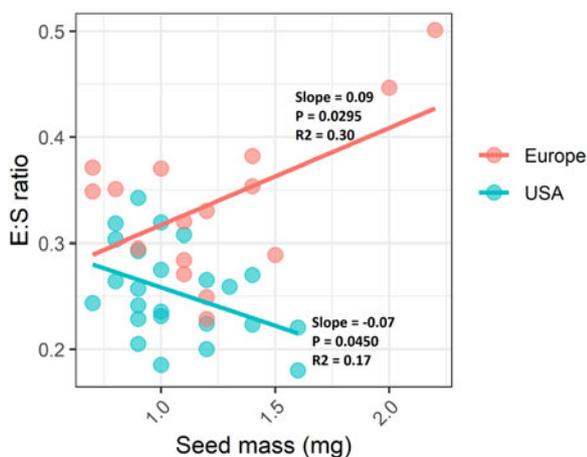


Figure 4. Relationships between seed mass and the E:S ratio in European and North American *D. carota* populations.

significant negative relationship was observed between seed mass and relative embryo length ($P = 0.05$).

No significant continent interaction was observed for the relationship between the E:S ratio and germination percentage ($P = 0.73$), germination speed ($P = 0.85$) and dormancy ($P = 0.79$) of seeds incubated at 20°C (Fig. 5). There was a significant positive relationship between the percentage of germination and the E:S ratio ($P = 0.08$) across all *D. carota* populations (Fig. 5). A significant positive relation between germination speed (R50) and the E:S ratio was also found across all populations ($P = < 0.001$). However, seed dormancy decreased with the E:S ratio, although the relationship was not significant ($P = 0.11$).

Relationship between relative embryo length and climate

The univariate relationships between 22 predictors (including bioclimate variables, altitude and geographical coordinates) and E:S ratios are shown in Table 1. Both geographical coordinates had a significant effect on the E:S ratio, but the longitude belonged to two different continents with negative values in North America and positive values in Europe, which led to a significant relationship. Therefore, we did not consider longitude in further analyses. The E:S ratio decreased significantly ($P = 0.049$) with increasing latitude (Table 1). Annual mean temperature, temperature seasonality, maximum temperature of the warmest month, mean temperature of the wettest quarter and mean temperature of the warmest quarter significantly increased with the E:S ratio (Table 1). An increase in annual precipitation, precipitation of the wettest month, precipitation of the wettest quarter, precipitation of the coldest quarter and isothermality resulted in a significant reduction in the E:S ratio.

In the multiple regression model constructed that best estimates the E:S ratio, only three climate variables were retained in the final model, including annual precipitation, mean temperature of the wettest quarter and annual mean temperature (Table 2). Annual precipitation significantly explained 70.6% of the changes in the E:S ratio ($P < 0.001$) and cumulative R^2 at the first step was 0.26 (Table 2). In the second step, the mean temperature of the wettest quarter was inserted into the model and explained 18.7% of the changes in the E:S ratio ($P = 0.06$), and cumulative R^2 increased to 0.33. Annual mean temperature explained 10.6% of changes in the E:S ratio and cumulative R^2 improved to 0.37, although it was not significant ($P = 0.14$).

Annual precipitation significantly decreased embryo length (slope = -0.00015 ; $P = 0.002$) and the E:S ratio (slope = -0.0001 ; $P < 0.001$) across *D. carota* European and North American

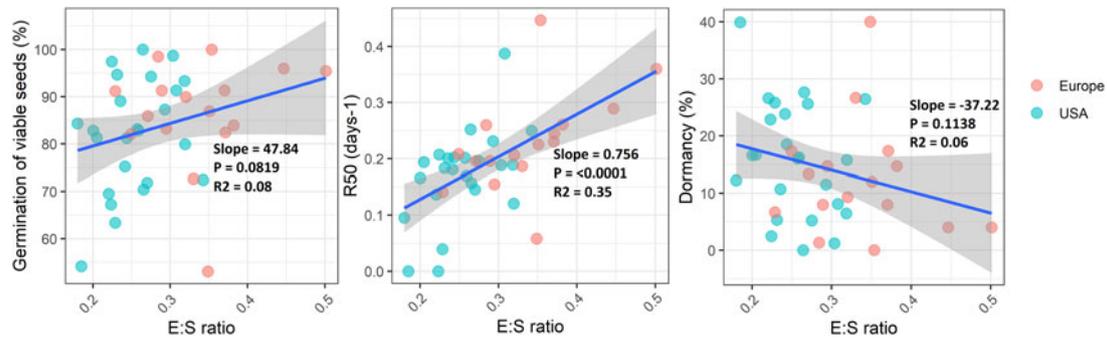


Figure 5. Relationships between the E:S ratio and seed germination characteristics for European and North American *D. carota* populations incubated at 20°C. The solid line shows the linear regression model, and the shaded area shows the 95% confidence interval.

populations, but it had no significant impact on either seed mass or seed length (Fig. 6). It showed that with a 1000-mm increase in precipitation, absolute embryo length decreases by about 0.15 mm.

The three first PC axes of the PCA explained 78% of the variation (Table 3; Fig. 7). The first PC explained 41% of the variation, and a positive correlation was observed between PC1 and the E:S ratio, germination percentage and rate, seed mass and temperature climate variables. There was a negative correlation between PC1 and the percentage of dormant seeds and the precipitation

climate variables (Fig. 7). The E:S ratio seems to align better with PC1, while PC2 is rather related to seed mass. European populations are predominantly situated on the right side of PC1, while North American populations are situated on the left side of PC1.

Discussion

Our results support the hypothesis that relative embryo length is positively related to germination speed at the population level.

Table 1 Results from the GLM of various predictors on the variation of intra-specific E:S ratio in 40 accessions of *D. carota* across North America and Europe

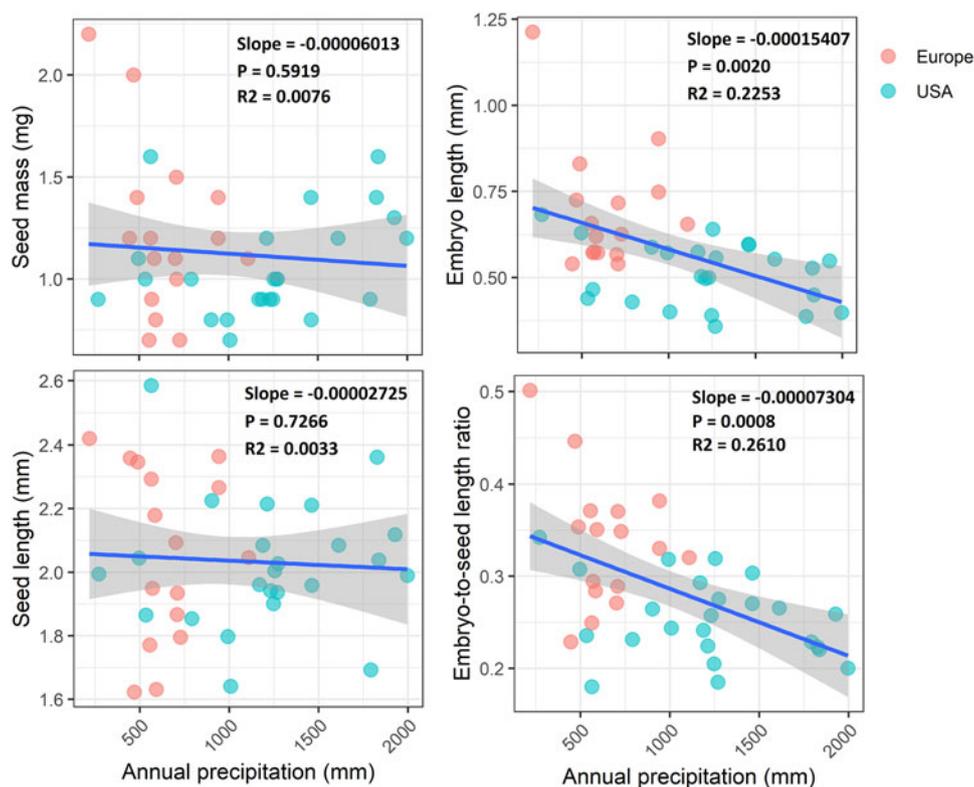
Predictors	Code	Unit	B	R ²	P
Altitude	Alt	M	0.00002	0.012	0.510
Geographical coordinates	Latitude	° NS	-0.00481	0.098	0.049*
Geographical coordinates	Longitude	° EW	0.00055	0.343	<0.001***
Annual mean temperature	Bio1	°C	0.00853	0.125	0.025*
Mean monthly temperature range	Bio2	°C	-0.00104	0.001	0.827
Isothermality	Bio3	°C	-0.00276	0.100	0.048*
Temperature seasonality	Bio4	°C	0.00012	0.100	0.046*
Maximum temperature of the warmest month	Bio5	°C	0.00551	0.115	0.032*
Minimum temperature of the coldest month	Bio6	°C	-0.00009	0.001	0.972
Temperature annual range	Bio7	°C	0.00280	0.060	0.129
Mean temperature of the wettest quarter	Bio8	°C	0.00692	0.250	0.001**
Mean temperature of the driest quarter	Bio9	°C	0.00049	0.003	0.762
Mean temperature of the warmest quarter	Bio10	°C	0.00950	0.225	0.002**
Mean temperature of the coldest quarter	Bio11	°C	0.00076	0.002	0.789
Annual precipitation	Bio12	mm	-0.00007	0.261	<0.001***
Precipitation of the wettest month	Bio13	mm	-0.00038	0.219	0.002**
Precipitation of the driest month	Bio14	mm	-0.00047	0.009	0.571
Precipitation seasonality	Bio15	mm	-0.00008	0.001	0.880
Precipitation of the wettest quarter	Bio16	mm	-0.00014	0.234	0.001**
Precipitation of the driest quarter	Bio17	mm	-0.00038	0.061	0.124
Precipitation of the warmest quarter	Bio18	mm	-0.00003	0.001	0.872
Precipitation of the coldest quarter	Bio19	mm	-0.00013	0.230	0.002**

Note: B is the regression slope and R² is the coefficient of determination.

*P < 0.05, **P < 0.01 and ***P < 0.001, respectively.

Table 2 Stepwise multiple regression analysis results for predictors influencing the E:S ratio in *D. carota* populations

Step	Predictor	Estimation	Contribution to E:S ratio variation (%)	Cumulative R^2	P-value
	Intercept	0.2202	–	–	0.001
1	Annual precipitation	–0.0001	70.61	0.26	0.001
2	Mean temperature of the wettest quarter	0.0045	18.67	0.33	0.059
3	Annual mean temperature	0.0051	10.62	0.37	0.143

**Figure 6.** Relationships between annual precipitation and seed morphology in European and North American *D. carota* populations. The solid line shows the linear regression model, and the shaded area shows the 95% confidence interval.

Moreover, relative embryo length is higher in drier (less annual precipitation) localities both in Europe and in North America. These results confirm the hypothesis that higher relative embryo length provides an adaptive advantage through the ability to germinate faster in dry regions. The distribution of variation in seed

mass across *D. carota* populations is harder to explain, as the relationships between seed mass on the one hand and seed characteristics and climate variables on the other are less equivocal as we will discuss below.

Seed mass effects: embryo and germination

Table 3 Eigenvalues of first three principal components and the percentage of variance explained by them for a PCA of six predictors (annual precipitation, precipitation seasonality, mean temperature of the driest quarter, annual mean temperature, temperature seasonality and mean temperature of the wettest quarter) and five seed morphology and germination traits (seed dormancy (%), seed mass (mg), E:S ratio, germination percentage of viable seeds (%) and germination speed (R50)) in 40 *D. carota* populations from Europe and North America

PCs	Eigenvalue	Percentage of variance	Cumulative (%)
1	4.46	40.51	40.51
2	2.64	24.04	64.55
3	1.51	13.68	78.23

The similar germination percentages and viabilities of European and North American *D. carota* populations at 20°C suggest a common germination process under optimal conditions. However, the relationship between seed mass and germination was different across continents. In Europe, larger seeds germinated faster and had fewer dormant seeds, which agreed with previous studies (Jacobsohn and Globerson, 1980; Gross, 1984). This confers an adaptive advantage in stressful and unpredictable environments (Moles et al., 2005; Vandeloek et al., 2018, 2021; Kang et al., 2021). Larger seeds also have more nutrient reserves and energy availability, increasing their environmental stress tolerance. The adaptive advantage of larger seeds in successful establishment and reduced dormancy aligned with established

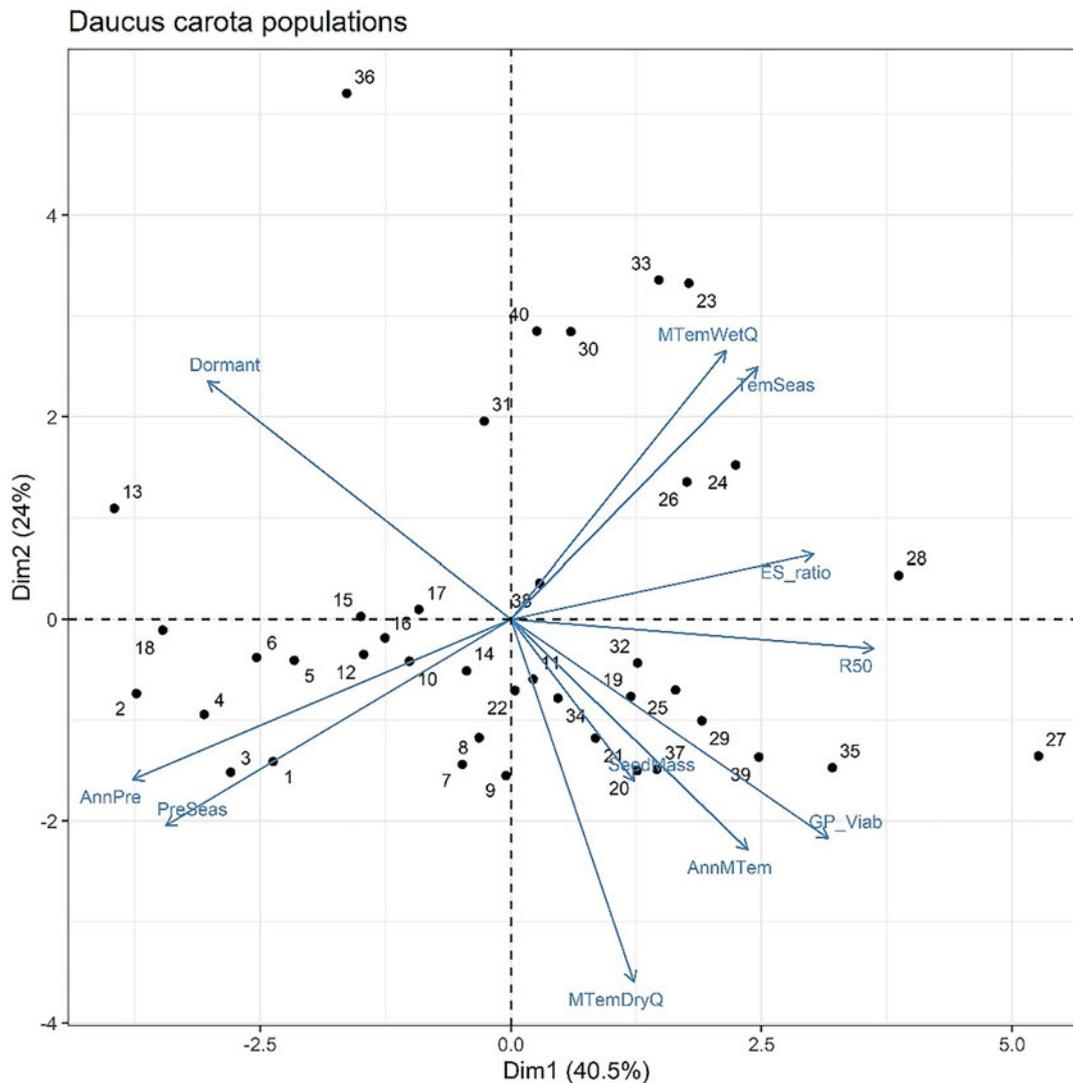


Figure 7. PCA of six predictors (annual precipitation, precipitation seasonality, mean temperature of the driest quarter, annual mean temperature, temperature seasonality and mean temperature of the wettest quarter) and five seed morphology and germination traits (seed dormancy (%), seed mass (mg), E:S ratio, germination percentage of viable seeds (%) and germination speed (R50)) in 40 *D. carota* populations from Europe (numbers 25–40) and North America (numbers 1–24).

ecological theories (Venable et al., 1998; Baskin and Baskin, 2014). Larger seeds also benefit from germinating faster, as they are exposed to higher risks of seed predation, because they can be detected more easily by seed predators, and they contain more nutrients (Dalling et al., 2011; Paulsen et al., 2014). In North America, however, larger seeds germinated slower, perhaps due to region-specific environmental factors. An equivocal relation between seed mass and germination speed has been observed in other studies (e.g., Milberg et al., 1996) and merits further study.

The relationship between seed mass and relative embryo length is complex and varies with species and context. In Apiaceae, a negative correlation was found between seed mass and relative embryo length at the species level (Vandelook et al., 2012a), while no relationship was found in Amaranthaceae (Vandelook et al., 2021). In our study, at the population level, seed mass and relative embryo length were related differently in European and North American populations. In Europe, larger

seeds had larger embryos and higher relative embryo lengths. In North America, larger seeds had smaller embryos, but no significant relationship was found with relative embryo length. These results imply that (i) the germination rate is not only determined by seed mass but also by environmental conditions and relative embryo length, and (ii) the relative embryo length does not always increase with seed mass. Larger embryos offer advantages in open and dry habitats or for species with short life cycles, providing an adaptive edge in unpredictable environments (Vivrette, 1995; Kadereit et al., 2017; Vandelook et al., 2021).

Relative embryo length and germination

The close relationship between relative embryo length ratio and germination has emerged as an important determinant shaping the reproductive success and ecological strategies of plant species (e.g., Vandelook et al., 2021). Relative embryo length and germination were related similarly in European and North American

populations unlike seed mass and germination. In both populations, germination percentage and rate increased, and seed dormancy decreased with increasing relative embryo length. Recent investigations showed that larger embryos germinated faster, which was a heritable and advantageous trait for early seedling establishment and provided a competitive advantage (e.g., Vandeloos et al., 2012a). In the present study on *D. carota* populations, we demonstrated that higher relative embryo length led to faster germination and lower seed dormancy. This pattern holds particular significance in seeds with underdeveloped embryos, such as *D. carota*, where relative embryo length affected germination more than seed mass (Fernández-Pascual et al., 2021). Intra-specific variation in relative embryo length within species and their implications for germination received limited research attention. Nonetheless, the subtle intricacies of intra-specific variation in embryo length provide valuable insights into germination processes within plant populations. Our study emphasized that within populations of the same species, the interplay between relative embryo length and germination responses was influenced by environmental factors, shaping the adaptive strategies of plants in diverse ecological contexts.

Relationship between seed mass, relative embryo length and local climate conditions

Climate variables are often poor predictors of ecological and evolutionary patterns of trait variation at the species level (Moles et al., 2005; Vandeloos et al., 2012a; but see Carta et al., 2022). Here we found for the first time that climate variables are good predictors for variation in relative embryo length between populations. Temperature-related variables, such as annual mean temperature, showed a positive relationship with relative embryo length, suggesting perhaps a connection between temperature and seed embryo development in mother plants. Conversely, precipitation-related variables, such as annual precipitation, showed a negative relationship with relative embryo length, indicating a decrease in relative embryo length in more humid regions. Overall rainfall increased and temperature decreased at higher latitudes, aligning with broader climatic trends. Vandeloos et al. (2012a), studying the relative embryo length of Apiaceae at the species level, also identified a negative and significant relationship between relative embryo length, rainfall, and latitude but a positive and non-significant relationship between relative embryo length and temperature. Hence, the exploration of seed trait variation within *D. carota* populations emphasizes the importance of considering intra-specific variations when unravelling the relationships between seed traits and environmental parameters. Past studies at the inter-specific level reported a negative correlation between latitude and seed size, corroborating the observed patterns (Moles et al., 2007). In contrast to relative embryo length, intra-specific variation in seed mass of *D. carota* was poorly predicted by local climate conditions, although a negative relationship between seed mass and latitude was also present in *D. carota* populations studied here (results not shown).

The response of relative embryo length to climate variation suggests potential adaptations to climatic conditions, emphasizing the adaptability of seed size and relative embryo size to various environmental cues. Annual precipitation significantly decreased with the relative embryo length of *D. carota* populations, but it had no significant impact on either seed mass or seed length. It seems that there is a trade-off in *D. carota*, balancing the

allocation of resources between two distinct reproductive strategies. The first strategy involves the investment in a nutrient-rich endosperm, a crucial component for potential seedling success, especially in environments with abundant precipitation and lower temperatures. This allocation ensures that seeds have the necessary resources to thrive in conditions where water availability is not a limiting factor. The second strategy involves directing resources towards the development of larger embryos, a strategic move aimed at enhancing seedling vigour. This strategy was advantageous in environments with lower precipitation and higher temperatures, where the size and strength of the developing embryo were more important than the endosperm richness. The trade-off highlights the plant's adaptive response to varying environmental conditions, showcasing a dynamic resource allocation strategy that matches the plant's reproductive needs in different ecological contexts.

However, intra-specific variation in relative embryo length may also be related to conditions experienced during seed maturation. Gray et al. (1984) described the development of endosperm and embryos in *D. carota* as a series of distinct phases. The endosperm first underwent a coenocytic phase, followed by rapid cell division and expansion, reaching its maximum volume and dry weight. Then, the endosperm volume slightly decreased as some cells near the embryo dissolved. The positive relationship between temperature and relative embryo length aligns with Gray et al. (1984), who observed that elevated temperatures during seed development favoured cell expansion, contributing to larger embryos. Conversely, the negative correlation between precipitation and relative embryo length suggests that increased precipitation could also affect seed components, perhaps influencing endosperm development, leading to a decrease in relative embryo length at dispersal. Increased precipitation might, for example, influence the dissolution of cells in the endosperm, as observed by Gray et al. (1984), potentially contributing to a decrease in relative embryo length. However, further studies are required to elucidate these patterns.

The differences that were observed between European and North American populations are potentially related to the longer local biogeographical history of European *D. carota* populations, as compared to the North American populations that were introduced on the continent about 300 years ago (Small, 1978; Iorizzo et al., 2013). *D. carota*, which originated in Europe and the Middle East, has a long evolutionary history on the continent and is very diverse, with multiple subspecies and varieties in the Mediterranean region (Spooner et al., 2014). Therefore, a much more developed local adaptation in Europe may explain the tighter relation between seed functional traits and climate in Europe as compared to North America. *D. carota* has been distributed across North America over the past 300 years, which is a very short period at the evolutionary scale. However, the distribution of *D. carota* over similar climate ranges in two different continents makes it an interesting case for studying micro-evolution at different temporary scales.

Methodological considerations

In literature, different metrics are being used to express germination speed and they tend to show subtle differences (Soltani et al., 2015), which was confirmed in this study. The existing methods to determine the seed germination rate can be grouped into three categories: (i) calculating an index by a formula, such as MGT, (ii) calculating time to 50% of maximum germination

by models, such as the Hill function, and (iii) calculating time to 50% germination, such as Germin^{v3}. In both traditional formulas, such as MGT, and in models designed to calculate the time to 50% of maximum germination (t_{50}), the time determined for mean germination, or 50% germination, depends on the maximum germination and the germination pattern during the test. Comparing different MGT and t_{50} values is not an appropriate method for comparing treatments. Similarly, when comparing the germination rate of seeds from different treatments, cultivars, habitats, populations or taxa, it is essential to evaluate them for a fixed percentage, such as 50% of the total population. Therefore, it is recommended to use alternative indices, like the inverse time to 50% germination (R50, or other rates, see Germin^{v3} in the Supplementary Files) to compare treatments accurately.

A downside of our study is the relatively unstandardized storage conditions before the onset of the germination experiments. In an ideal scenario, germination tests would have been performed with freshly harvested seeds, or seeds that had been stored in standardized conditions for the same period before the onset of the experiment. These shortcomings need to be considered when interpreting the results and limit the conclusions drawn. However, the high seed viability, even after prolonged periods of storage, and the consistent results we obtained do confirm the reliability of the conclusions drawn.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0960258524000230>.

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Author Contribution. F.V. conceptualized the research and assembled all data. A.V.V. carried out experiments. E.S. analysed the data. F.V. and E.S. wrote the manuscript. A.V.V. reviewed the manuscript.

References

- Abramoff MD, Magalhaes PJ and Ram SJ (2004) Image processing with ImageJ. *Biophotonics International* **11**, 36–42.
- Allen PS and Meyer SE (1998) Ecological aspects of seed dormancy loss. *Seed Science Research* **8**, 183–192.
- Allen PS and Meyer SE (2002) Ecology and ecological genetics of seed dormancy in downy brome. *Weed Science* **50**, 241–247.
- Amimi N, Ghouil H, Zitouna-Chebbi R, Joët T and Ammari Y (2023) Intraspecific variation of *Quercus ilex* L. seed morphophysiological traits in Tunisia reveals a trade-off between seed germination and shoot emergence rates along a thermal gradient. *Annals of Forest Science* **80**, 1–16.
- Baskin CC and Baskin JM (2014) *Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination*, 2nd Edn. San Diego, CA, USA: Academic Press/Elsevier.
- Bates D, Mächler M, Bolker B and Walker S (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**, 1–48.
- Carta A, Fernández-Pascual E, Gioria M, Müller JV, Rivière S, Rosbakh S, Saatkamp A, Vandelook F and Mattana E (2022) Climate shapes the seed germination niche of temperate flowering plants: a meta-analysis of European seed conservation data. *Annals of Botany* **129**, 775–786.
- Corbineau F, Picard MA, Bonnet A and Come D (1995) Effects of production factors on germination responses of carrot seeds to temperature and oxygen. *Seed Science Research* **5**, 129–135.
- Dalling JW, Davis AS, Schutte BJ and Elizabeth Arnold A (2011) Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. *Journal of Ecology* **99**, 89–95.
- El-Kassaby YA, Moss I, Kolotelo D and Stoehr M (2008) Seed germination: mathematical representation and parameters extraction. *Forest Science* **54**, 220–227.
- Ellis RH and Roberts EH (1980) Towards a rational basis for testing seed quality. In Hebblethwaite PD (ed.) *Seed Production*. London: Butterworths, pp. 605–635.
- Fernández-Pascual E, Mattana E and Pritchard HW (2019) Seeds of future past: climate change and the thermal memory of plant reproductive traits. *Biological Reviews* **94**, 439–456.
- Fernández-Pascual E, Carta A, Mondoni A, Cavieres LA, Rosbakh S, Venn S, Satyanti A, Guja L, Briceño VF, Vandelook F and Mattana E (2021) The seed germination spectrum of alpine plants: a global meta-analysis. *New phytologist* **229**, 3573–3586.
- Fricke EC, Tewksbury JJ and Rogers HS (2019) Linking intra-specific trait variation and plant function: seed size mediates performance tradeoffs within species. *Oikos* **128**, 1716–1725.
- Ge W, Bu H, Wang X, Martinez SA and Du G (2020) Inter- and intra-specific difference in the effect of elevation and seed mass on germinability of eight *Allium* species. *Global Ecology and Conservation* **22**, e01016.
- Geber MA and Griffen LR (2003) Inheritance and natural selection on functional traits. *International Journal of Plant Sciences* **164**, S21–S42.
- Gray D (1979) The germination response to temperature of carrot seeds from different umbels and times of harvest of the seed crop. *Seed Science and Technology* **7**, 169–178.
- Gray D, Ward JA and Steckel JR (1984) Endosperm and embryo development in *Daucus carota* L. *Journal of Experimental Botany* **35**, 459–465.
- Gross KL (1984) Effects of seed size and growth form on seedling establishment of six monocarpic perennial plants. *The Journal of Ecology* **72**, 369–387.
- Harper JL (1977) *Population Biology of Plants*. New York, NY: Academic Press, 892 p.
- Hendrix SD and Sun IF (1989) Inter- and intraspecific variation in seed mass in seven species of umbellifer. *New Phytologist* **112**, 445–451.
- Hijmans RJ, Guarino L, Cruz M and Rojas E (2001) Computer tools for spatial analysis of plant genetic resources data: 1. DIVA-GIS. *Plant Genetic Resources Newsletter* **127**, 15–19.
- Igea J, Miller EF, Papadopoulos AS and Tanentzap AJ (2017) Seed size and its rate of evolution correlate with species diversification across angiosperms. *PLoS Biology* **15**, e2002792.
- Iorizzo M, Senalik DA, Ellison SL, Grzebelus D, Cavagnaro PF, Allender C, Brunet J, Spooner DM, Van Deynze A and Simon PW (2013) Genetic structure and domestication of carrot (*Daucus carota* subsp. *sativus*) (Apiaceae). *American Journal of Botany* **100**, 930–938.
- Jacobsohn R and Globerson D (1980) *Daucus carota* (carrot) seed quality: I. Effects of seed size on germination, emergence and plant growth under subtropical conditions and II. The importance of the primary umbel in carrot-seed production. In Hebblethwaite PD (ed.) *Seed Production*. London and Boston: Butterworths, pp. 637–646.
- Joosen RV, Kodde J, Willems LA, Ligterink W, Van Der Plas LH and Hilhorst HW (2010) GERMINATOR: a software package for high-throughput scoring and curve fitting of Arabidopsis seed germination. *The Plant Journal* **62**, 148–159.
- Kaderit G, Newton RJ and Vandelook F (2017) Evolutionary ecology of fast seed germination — a case study in Amaranthaceae/Chenopodiaceae. *Perspectives in Plant Ecology, Evolution and Systematics* **29**, 1–11.
- Kang X, Zhou J, Du G and Qi W (2021) Multi-factor control of seed mass of species on the eastern part of the Qinghai-Tibetan Plateau: integration of environmental filters, local adaptation and correlated evolution. *Environmental and Experimental Botany* **187**, 104471.
- Kang X, Zhou J, Liu Y, Zhang S, Liu W, Bu H and Qi W (2022) Regional gradients in intraspecific seed mass variation are associated with species biotic attributes and niche breadth. *AoB Plants* **14**, plac013.
- Kassambara A and Mundt F (2020) R Package factoextra: Extract and Visualize the Results of Multivariate Data Analyses Version 1.0.7.
- Lacey EP (1988) Latitudinal variation in reproductive timing of a short-lived monocarp, *Daucus carota* (Apiaceae). *Ecology* **69**, 220–232.
- Leishman MR and Westoby M (1994) Hypotheses on seed size: tests using the semiarid flora of western New South Wales, Australia. *The American Naturalist* **143**, 890–906.

- Mackowska K, Stelmach-Wityk K and Grzebelus E (2023) Early selection of carrot somatic hybrids: a promising tool for species with high regenerative ability. *Plant Methods* **19**, 104.
- Magnussen LS and Hauser TP (2007) Hybrids between cultivated and wild carrots in natural populations in Denmark. *Heredity* **99**, 185–192.
- Milberg P, Andersson L, Elfverson C and Regner S (1996) Germination characteristics of seeds differing in mass. *Seed Science Research* **6**, 191–198.
- Moles AT (2018) Being John Harper: using evolutionary ideas to improve understanding of global patterns in plant traits. *Journal of Ecology* **106**, 1–18.
- Moles AT, Ackerly DD, Webb CO, Tweddle JC, Dickie JB, Pitman AJ and Westoby M (2005) Factors that shape seed mass evolution. *Proceedings of the National Academy of Sciences* **102**, 10540–10544.
- Moles AT, Ackerly DD, Tweddle JC, Dickie JB, Smith R, Leishman MR, Mayfield MM, Pitman A, Wood JT and Westoby M (2007) Global patterns in seed size. *Global Ecology and Biogeography* **16**, 109–116.
- Paulsen TR, Högstedt G, Thompson K, Vandvik V and Eliassen S (2014) Conditions favouring hard seedness as a dispersal and predator escape strategy. *Journal of Ecology* **102**, 1475–1484.
- R Core Team (2022) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Available at <https://www.R-project.org/>.
- Saatkamp A, Cochrane A, Commander L, Guja L, Jimenez-Alfaro B, Larson J, Nicotra A, Poschlod P, Silveira FAO, Cros A, Dalziel EL, Dickie J, Erickson TE, Fidelis A, Fuchs A, Golos PJ, Hope M, Lewandowski W, Merritt DJ, Miller BP, Miller R, Offord CA, Ooi MKJ, Satyanti A, Sommerville KD, Tangney R, Tomlinson S, Turner S and Walck JL (2019) A research agenda for seed-trait functional ecology. *New Phytologist* **221**, 1764–1775.
- Sales NM, Pérez-García F and Silveira FAO (2013) Consistent variation in seed germination across an environmental gradient in a Neotropical savanna. *South African Journal of Botany* **87**, 129–133.
- Small E (1978) A numerical taxonomic analysis of the *Daucus carota* complex. *Canadian Journal of Botany* **56**, 248–276.
- Soltani E, Ghaderi-Far F, Baskin CC and Baskin JM (2015) Problems with using mean germination time to calculate rate of seed germination. *Australian Journal of Botany* **63**, 631–635.
- Soltani E, Benakashani F, Baskin JM and Baskin CC (2021) Reproductive biology, ecological life history/demography and genetic diversity of the megagenus *Astragalus* (Fabaceae, Papilionoideae). *The Botanical Review* **87**, 55–106.
- Spoooner DM, Widrechner MP, Reitsma KR, Palmquist DE, Rouz S, Ghrabi-Gammar Z, Neffati M, Bouzbida B, Ouabbou H, El Koudrim M and Simon PW (2014) Reassessment of practical subspecies identifications of the USDA *Daucus carota* L. germplasm collection: morphological data. *Crop Science* **54**, 706–718.
- Vandelook F, Janssens SB and Probert RJ (2012a) Relative embryo length as an adaptation to habitat and life cycle in Apiaceae. *New Phytologist* **195**, 479–487.
- Vandelook F, Verdú M and Honnay O (2012b) The role of seed traits in determining the phylogenetic structure of temperate plant communities. *Annals of Botany* **110**, 629–636.
- Vandelook F, Janssens SB and Matthies D (2018) Ecological niche and phylogeny explain distribution of seed mass in the central European flora. *Oikos* **127**, 1410–1421.
- Vandelook F, Newton RJ, Bobon N, Bohley K and Kadereit G (2021) Evolution and ecology of seed internal morphology in relation to germination characteristics in Amaranthaceae. *Annals of Botany* **127**, 799–811.
- Venable DL, Dyreson E, Piñero D and Becerra JX (1998) Seed morphometrics and adaptive geographic differentiation. *Evolution* **52**, 344–354.
- Verdú M (2006) Tempo, mode and phylogenetic associations of relative embryo size evolution in angiosperms. *Journal of Evolutionary Biology* **19**, 625–634.
- Vivrette NJ (1995) Distribution and ecological significance of seed-embryo types in Mediterranean climates in California, Chile, and Australia. In Arroyo MKT, Zedler PH, and Fox MD (eds) *Ecology and Biogeography of Mediterranean Ecosystems in Chile, California and Australia*. New York, USA: Springer Verlag, pp. 274–288.
- Wagmann K, Hautekeete N C, Piquot Y, Meunier C, Schmitt S E and Van Dijk H (2012) Seed dormancy distribution: explanatory ecological factors. *Annals of Botany* **110**, 1205–1219.
- Wu GL, Shang ZH, Zhu YJ, Ding LM and Wang D (2015) Species abundance–seed-size patterns within a plant community affected by grazing disturbance. *Ecological Applications* **25**, 848–855.