

## Functional biogeography of the thermal thresholds for post-dispersal embryo growth in *Conopodium majus*

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**Original article: Functional biogeography of the thermal thresholds for post-dispersal embryo growth in *Conopodium majus***

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Running title: Functional biogeography of embryo growth

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## ABSTRACT

- **Background and Aims** Plant regeneration by seeds is driven by a set of physiological traits, many of which show functional intraspecific variation along biogeographic gradients. In many species, germination phenology depends on a germination delay imposed by the need for post-dispersal embryo growth (a.k.a. morphological dormancy). Such growth occurs as a function of environmental temperatures and shows base, optimum and ceiling temperatures (i.e. cardinal temperatures or thermal thresholds). However, the biogeographical variation in such thresholds has not been tested.
- **Methods** We used a thermal time approach and field experiments to assess intraspecific variation at the continental scale in the embryo growth thermal thresholds of the geophyte *Conopodium majus* (Apiaceae) across its distribution from the Iberian Peninsula to Scandinavia.
- **Key Results** Thermal thresholds for embryo growth varied across the latitudinal gradient, with the estimated optimum temperatures between 2.5 and 5.2 °C, ceiling temperatures between 12 and 20.5 °C and base temperatures between -6.6 and -2.7 °C. Germination in the field peaked in the months of January and February. The limiting factor for embryo growth was the ceiling temperature, which was negatively correlated with latitude and the bioclimatic environment of each population. In contrast, the optimal and base temperature were independent of local climate.

- **Conclusions** These results indicate that thermal thresholds for embryo growth are functional ecophysiological traits that drive seed germination phenology and seed responses to soil climatic environment. Therefore, post-dispersal embryo growth can be a key trait impacting climate change effects on phenology and species distributions.

## **KEYWORDS**

Cardinal temperatures for germination, Apiaceae, *Conopodium majus*, embryo:endosperm ratio, morphological dormancy, post-dispersal embryo growth, functional seed traits, plant regeneration, intraspecific variation

1

## 2 INTRODUCTION

3 The three aims of functional biogeography are to describe the distribution of  
4 functions along environmental gradients and across spatial scales; to use this  
5 information to explain the geographic distribution of organisms; and to predict  
6 their responses to environmental changes using trait-based predictive models  
7 (Violle et al., 2014). A relevant aspect of plant function that has been  
8 underutilized by biogeographical studies is the physiological thermal control of  
9 plant reproduction (Bykova et al., 2012), and especially seed germination  
10 (Rosbakh and Poschlod, 2015). The temperature to which imbibed seeds are  
11 exposed affects their germination rate (Fernández-Pascual et al., 2019). This  
12 phenomenon can be numerically described by the “cardinal germination  
13 temperatures”, i.e., the optimum temperature ( $T_o$ ), at which the germination rate  
14 is maximal and the base ( $T_b$ ) and ceiling ( $T_c$ ) temperatures that are,  
15 respectively, estimated to be the coldest and the warmest temperature at which  
16 the rate of germination tends to zero. The measurement of these temperatures  
17 for a given species enables prediction of its seed germination rate and  
18 germination success under different temperature scenarios (Orrù et al., 2012,  
19 Fernández-Pascual et al., 2015). Therefore, the cardinal temperatures can be  
20 key parameters to explain the contribution of regeneration thermal niches on  
21 species distributions and responses to climatic changes (Parmesan and Hanley,  
22 2015, Baskin and Baskin, 2022, Walck et al., 2011, Cochrane et al., 2015).

23 In many species, however, seed dormancy prevents germination even in the  
24 presence of suitable conditions, so that the exposure of seedlings to  
25 unfavourable environments is avoided and the timing of germination is matched

1 to favourable regeneration windows (Lamont and Pausas, 2023, Pausas et al.,  
2 2022). A particular case of seed germination delay occurs when the embryo, at  
3 the time of dispersal, is not completely developed and needs to grow to a critical  
4 size before germination can occur; a trait known as post-dispersal embryo  
5 growth or morphological dormancy (Baskin and Baskin, 2004, Vandelook et al.,  
6 2009b). Such embryo growth is a distinct ecophysiological process controlled by  
7 complex interactions between hormones and the seed tissues (Walker et al.,  
8 2021). As happens with germination, temperature is a major environmental  
9 driver of post dispersal embryo growth (Baskin et al., 2000, Phartyal et al.,  
10 2009, Vandelook et al., 2007, Vandelook et al., 2009a, Blandino et al., 2019,  
11 Vandelook and Van Assche, 2008), influencing the rate of development of the  
12 embryo, a mechanism that allows a precise timing of germination (Porceddu et  
13 al., 2017).

14 Within-species biogeographical variation in several germination parameters is  
15 well documented. For example, seeds from warmer sites have been shown to  
16 have lower minimum temperatures for germination at different geographical  
17 scales (Daws et al., 2004, Rosbakh and Poschlod, 2015). The requirements for  
18 cold stratification can also vary according to the local climate: populations from  
19 habitats with longer winters require a longer period of cold stratification  
20 compared with populations from milder habitats (Allen and Meyer, 1998,  
21 Fenner, 1991). However, much less research has been dedicated to traits  
22 related to post-dispersal embryo growth. Mondoni et al. (2008) compared post-  
23 dispersal embryo growth between mountain and lowland populations of the  
24 temperate woodland forb *Anemone nemorosa* and found that, although embryo  
25 size at dispersal was similar in all the populations, embryo growth at cool

temperatures was faster in the mountain population. This suggests a capacity of post-dispersal embryo growth to adapt to local conditions, either by local adaptation or phenotypic plasticity, analogous to that shown by other seed traits (Fernández-Pascual et al., 2013). However, Porceddu et al. (2017) found no variation in the thermal thresholds for embryo growth in two closely located populations of *Aquilegia barbaricina*. Further research is warranted, to measure the thermal thresholds for post-dispersal embryo growth across large biogeographical scales and investigate whether they vary in association with environmental gradients.

In this study, we assess the functional biogeography of post-dispersal embryo growth in the geophyte *Conopodium majus* (Apiaceae) across its latitudinal distribution, from the Iberian Peninsula to Scandinavia. To our knowledge, this is the first study to quantify the thermal thresholds that regulate embryo growth rate across the whole latitudinal distribution of a species. To do this, we develop a model of embryo growth as a function of temperature to describe its cardinal temperatures for embryo growth. It has been shown that Scottish populations of this species require post-dispersal embryo growth, and that embryo growth and germination occur optimally around 5 °C (Blandino et al., 2019). Such narrow thermal control in a species with a relatively wide latitudinal distribution could make the species' regeneration vulnerable to climate change (Walck et al., 2011), unless the species shows functional variation in its embryo growth thermal thresholds. Therefore, we hypothesized that (1) the thermal thresholds for embryo growth will show variation across populations sampled over the species' latitudinal gradient; and (2) the variation of thermal thresholds will be related to bioclimatic features along the latitudinal gradient. Specifically, we

1 predicted that thermal thresholds for embryo growth would be influenced by  
2 high maximum temperatures and drought at the lower latitudes, and by low  
3 minimum temperatures at the higher latitudes.

## 4 **MATERIALS AND METHODS**

### 5 ***Study species***

6 *Conopodium majus* is a geophyte with a European Atlantic distribution from  
7 Southern Spain to Central Norway (Tutin et al., 1968). *Conopodium majus* is a  
8 member of Apiaceae, a family that originated and diversified in the southern  
9 hemisphere, but that today is mostly distributed in the northern hemisphere  
10 (Calviño et al., 2016). As is common in Apiaceae (Walker et al., 2021, Baskin  
11 and Baskin, 2014, Vandeloos et al., 2012), seeds of *Conopodium majus*  
12 possess undeveloped linear embryos (Martin, 1946) and germinate when they  
13 extend to the full length of the endosperm (Blandino et al., 2019). For this  
14 reason, in this study, the relative embryo size (i.e. embryo length / endosperm  
15 length, hereafter referred to as “E:E ratio”) is used to describe embryo  
16 development, and germination is defined as the point at which E:E ratio is  $\geq 1$ .  
17 In *Conopodium majus*, germination has been observed to occur both at 0 and 5  
18 °C with a similar rate of embryo growth (Blandino et al., 2019). Such low  
19 temperature requirements are indicative of germination in winter. For a species  
20 adapted to woodlands as well as oligotrophic grasslands, the control of the  
21 germination process in this way can allow seedlings to establish before the  
22 development of a tree canopy or of competing vegetation.

### 23 ***Seed collection***



1 Mericarps of *Conopodium majus* were collected in the summer of 2016 from  
2 nine naturally occurring populations sampled across the western European  
3 latitudinal range of the species (Table 1). Since the seed cannot be separated  
4 from the fruit in this species, the dispersal unit will be hereafter referred to as  
5 the “seed”. A population was sampled only if it consisted of at least 200  
6 individual plants. Seeds were sampled from 50 plants within the population to  
7 secure a representative sample of the genetic variability of the population. At  
8 least 4000 seeds were collected from each population. All seeds were collected  
9 between July and August 2016 and the experiments started within three weeks  
10 of seed collection. Seeds were kept at below full hydration, dry exposed to the  
11 surrounding atmosphere, under ambient condition on a laboratory bench until  
12 the beginning of the tests.

### 13 ***Initial measurements***

14 Each collection was cleaned from debris and empty seeds were removed using  
15 a gravity seed separator machine. From each population, 10 seeds were  
16 selected randomly and allowed to rehydrate overnight at 20 °C and 100% RH.  
17 The seeds were then placed on 1% agar-water for 24 hours to become fully  
18 imbibed. Thereafter, seeds were prepared for vital staining with 1% aqueous  
19 solution of triphenyl tetrazolium chloride (TZ). A slice of seed coat was removed  
20 from the dorsal surface of each seed using a scalpel and seeds were incubated  
21 in TZ solution at 30 °C in the dark for 24 hours. Each seed was then cut  
22 longitudinally, and the embryo was extracted. Embryos and endosperms were  
23 photographed using a camera (Carl Zeiss AxioCam Colour) mounted on a Stemi  
24 SV 11 Microscope (Carl Zeiss, Welwin Garden City, Herts, UK) microscope and  
25 their lengths measured using the software Axiovision 3.1.2.1 (Carl Zeiss Vision

GmbH). The initial relative embryo length was measured only for the seeds that stained red with the TZ, i.e., indicating viability; unstained seeds / embryos were discarded. Relative embryo size was used because it describes the growth of the embryo regardless of the size of each seed. From each population 99 seeds were placed in a controlled humidity room at 15% RH and left to dry. The dry seed weight of 99 seeds for each population was measured using a precision scale.

### ***Embryo growth in controlled temperature conditions***

From each population and treatment, 16 subsamples of 15 seeds each were randomly taken and sown in separate, 8 cm diameter Petri dishes containing 1% agar-water substrate. Seeds were sown at -2.5 °C, 0 °C, 2.5 °C, 5 °C, 7.5 °C and 10 °C in incubators with a daily light regime of 12 hours. Every 14 days one subsample from each population and treatment was retrieved and the 15 seeds were placed for 24 hours in 1% TZ solution at 30°C in the dark, after a slice of the seed coat was removed. From this subsample, the embryo and endosperm length of 10 viable seeds was measured following further dissection of the seed. An E:E value of 1 was assigned to all germinated seeds. Seed measurement was stopped when the seeds ceased germinating. The experiment continued for 224 days, until all the 16 subsample assessments were concluded.

### ***Calculation of a thermal model for embryo growth***

The mean E:E ratio of 10 seeds for each population x temperature x time combination was calculated. All the temperatures for the same population had the same initial E:E ratio at time = 0, while the maximum value was fixed at 1, after which the seed was able to germinate. Since the data followed a sigmoidal growth distribution, except the treatments at -2.5 °C, a logistic model was fitted

to each population x temperature combination using the software OriginLab 9.0. The models of each population were bounded between the initial value of E:E for that population and 1. A linear model was fitted to the -2.5°C treatments. From the equation of the logistic and linear models, it was possible to calculate the time expressed in days (tr) at which each temperature x population combination would have reached the following deciles of relative embryo size: 0.3, 0.4, 0.5, 0.6 and 0.7. Deciles < 0.3 could not be calculated because they were less than the initial E:E. Deciles > 0.7 were not calculated to keep the symmetry of the analyses regarding deciles of the population. For each treatment, the embryo growth rate was calculated as 1/tr.

For each population and decile, embryo growth rate was plotted against temperature. Each dataset was visually divided into sub-optimal and supra-optimal ranges, using the point with the highest value of 1/ t as the dividing point. Linear regressions were fitted separately to the sub- and supra-optimal ranges. The intersection with the temperature axis of the sub-optimal and supra-optimal regression are, respectively, the base ( $T_b$ ) and the ceiling ( $T_c$ ) temperatures; these estimates are the temperatures below and above which the embryo growth rate is projected to be zero. The optimal temperature ( $T_o$ ), defined as the temperature at which the rate of embryo growth is estimated to be fastest, is the x-coordinate of the intersection point between sub-optimal and supra-optimal regressions. Then, for each population, the cardinal temperatures ( $T_b$ ,  $T_c$  and  $T_o$ ) were averaged across all the deciles calculated to define a mean value of the population (Ellis et al., 1986). The regression lines of each decile were recalculated and forced to pass through a common origin defined by the mean  $T_b$  (for the sub-optimal regressions) or the mean  $T_c$  (for the supra-optimal

regressions) (Hardegree, 2006). For the three southernmost populations, only the cardinal temperatures calculated for the relative embryo size of 0.4, 0.5 and 0.6 were used, because it was not possible to fit a supra-optimal regression to the 0.3 decile.

The slopes of these new linear regressions were then taken as a reciprocal to estimate the sub-optimal ( $\theta_b$ ) and supra-optimal ( $\theta_c$ ) thermal times for embryo growth.  $\theta$ , expressed in degree days ( $^{\circ}\text{Cd}$ ), indicates the cumulative thermal time units above  $T_b$  ( $\theta_b$ ) or below  $T_c$  ( $\theta_c$ ) that the seed must accumulate for the embryo to reach successive E:E deciles. For each population, the deciles were plotted against  $\theta_b$  and  $\theta_c$ , expressed both as their value and as the natural logarithm of the value, and linear regressions were fitted to the data. The regressions fitted to  $\theta$  and to  $\log(\theta)$  were compared in each case by their  $R^2$  (Hardegree, 2006). The regression models with the highest  $R^2$  were chosen to represent the rate of embryo growth as a function of thermal time for each population. The  $R^2$  of the models obtained fitting embryo growth and log-normal ( $\log ^{\circ}\text{Cd}$ ) were slightly higher than the  $R^2$  of the model obtained using normal distributed thermal times ( $^{\circ}\text{C}$ ). The only exception was constituted by the Spanish population of Central del Chorro (CHO), for which the best model fit was obtained using the non-transformed thermal time values, thus describing a linear increase of relative embryo size with accumulated heat.

### ***Embryo growth in natural conditions***

Embryo growth in the soil was recorded for three population representing the southern (CHO), middle (Wakehurst Place, UK, "WAK") and northern (Bergen, Norway, "BER") distribution of the species. The experiment was replicated in two locations where *Conopodium majus* naturally occurs: at Wakehurst Place,

England (site of collection of the “WAK” population); and in a meadow on the periphery of Bergen, Norway (close to the site of collection of the “BER” population). Sixteen subsamples of 20 seeds for each population and experimental site were mixed with 20 g of soil collected at the site and passed through a 3 mm sieve. Seeds and soil were placed in mesh net bags and buried at a depth of 5 cm. A datalogger that recorded soil temperature every 30 min was placed in each location (Tinytag View 2, Gemini Dataloggers Ltd., Chichester. UK and EasyLog USB-2, Lascar Electronics, in Norway). The seeds were buried in England on 1<sup>st</sup> September 2016 and in Norway on 14<sup>th</sup> September 2016. Every 14 days a bag for each population was retrieved and the soil washed. Seed bags buried in Norway were shipped to England for measurements within a week of retrieval. All the seeds retrieved were prepared for TZ staining and their embryo and endosperm lengths measured. It was easiest to measure the seeds when most of the seeds were not germinated. With an increasing number of germinated seeds and seedlings, the number of empty seed coats left in the soil bags made it difficult to distinguish between mouldy or germinated seeds. At this point, the experiment was terminated, representing nine measurements in Norway and thirteen in England.

### ***Validation of the thermal time model with field data***

To compare the embryo growth predicted by the thermal time model with embryo growth in natural conditions, embryo growth in the field sites was plotted against time. A logistic regression was fitted to these curves, and from the equations, the t to reach every decile of relative embryo growth was calculated. The units of thermal time required by each population to reach every t during the field experiment were calculated for both field locations using the

data recorded by the loggers. To account for every temperature fluctuation during the day, the thermal time was expressed in “°C 30 min” and the heat accumulated by the seed was calculated for every 30 min temperature record. The difference ( $\Delta T$ ) between each temperature record and the population  $T_o$  (averaged between deciles) was summed. When the temperature was higher than the mean  $T_c$  or lower than the mean  $T_b$  the heat accumulated was considered = 0 and the difference ( $\Delta T$ ) between each temperature record and the  $T_o$  was summed.

The time necessary in the field to accumulate enough heat to reach the thermal time necessary for each  $t$  was compared with the  $t$  estimated from the embryo growth data. The time (in days) needed to sum enough heat to reach the  $\theta T_b$  and  $\theta T_c$  calculated in the model, for each  $t$  decile ( $t_{\text{model}}$ ) in each population was compared with the time needed by each population to reach the same decile of relative embryo growth in the field ( $t_{\text{field}}$ ). These estimates were then graphically compared expressing the different  $t$  in function of E:E.

### ***Relationship between embryo growth and germination***

Germination was scored for each independent sample before measuring the relative embryo size and expressed as percentage of germinated seeds vs time. For each population, the germination data for the treatments at 2.5 and 5°C were fitted with the Boltzmann equation using the software OriginLab9. The other temperatures were not used because germination was too slow. For each population, from the fitted Boltzmann equation the day to reach 50% germination ( $t_{g50}$ ) was calculated. The  $t_{g50}$  was then used to calculate the corresponding E:E ratio at the same day using the logistic regression of the E:E data for the same treatment. For each population, the mean E:E ratio

corresponding to the  $t_{g50}$  for germination at the two temperatures used was displayed as the mean E:E ratio for 50% germination in that population. The mean between all the populations represented the mean for the species.

#### ***Relationship between environmental data and germination parameters***

The relationship between embryo development and seed germination parameters and geographical and bioclimatic data was explored for each population. A data matrix was built including latitude, altitude, mean annual temperature, precipitation of the driest month, mean maximum temperature of the hottest month and minimum mean temperature of the coldest month, seed dry mass, initial E:E ratio and cardinal temperatures for each population. Climatic data for the seed collection sites was extracted from WorldClim (Fick and Hijmans, 2017). Data was checked for autocorrelation using the Pearson correlation coefficient to exclude the variables with a strong autocorrelation. Finally, a PCA was run on the dataset, scaling the axis.

## **RESULTS**

#### ***Initial embryo length and seed mass***

The initial relative embryo size ranged from a mean value of 0.10 ( $\pm 0.03$  SD) for the population BER to a mean value of 0.19 ( $\pm 0.03$  SD) for the population CHO (Table 2). Mean seed dry mass ranged just under two-fold from 1.21 mg ( $\pm 0.38$  SD) in TRE to 2.03 mg ( $\pm 0.53$  SD) in FLE (Table 2).

#### ***Embryo growth in controlled temperature conditions***

The rate of embryo growth was strictly dependent on the temperature and the increase in embryo size could be appreciated already after 14 days of sowing for germination. For all the populations, the temperature treatments with the

highest rate of embryo growth were 2.5 and 5 °C. Clearly 0 °C was sub-optimal for embryo growth rate, and 7.5 and 10 °C were supra-optimal (Fig.1). The seeds survived cooling to -2.5 °C but the embryo did not grow at this temperature.

### ***Relationship between embryo growth and germination***

The first germination was scored after 84 days of imbibition in the four Spanish populations at temperatures of 0, 2.5 and 5 °C. The populations from WAK and BER first germinated after 112 days of imbibition. The last population to begin germinating was SCO, after 126 days of imbibition. Germination occurred when the embryo reached the same length of the endosperm ( $E:E = 1$ ) and a mean  $E:E = 1$  corresponded to 100% germination in the sample. The treatments that had the highest mean germination across all the populations after 32 weeks of imbibition were 2.5 °C, and 5 °C with, respectively, 97.7 and 98.4 % of seeds germinated at the last sampling. The lowest germination was observed at -2.5 and 10 °C. The population that reached, across all the treatments, the highest mean germination at week 32 (the end of the experiment), was TRE (80%  $\pm$  32 SD) while the lowest was achieved by SCO (59%,  $\pm$  42 SD). The time to reach 50 % germination ( $T_{g50}$ ), interpolated with the Boltzmann equation ranged between 111 (BAS) and 147 days (FLE) at 2.5 °C and between 116 (LEO) and 150 days (SCO) at 5 °C. The values of  $E:E$  corresponding to the estimated  $T_{50}$  in these two treatments were averaged between population and temperatures to describe a value of 0.89 ( $\pm$  0.02 SD) for the species.

### ***Cardinal temperatures for embryo growth***



Between populations,  $T_b$  estimates varied between -2.63 (SCO) and -6.65 °C (BER). In addition,  $T_o$  varied from 2.54 (LEO) and 5.23 °C (CHO). Finally,  $T_c$  was between 12.08 (BER) and 20.54 °C (TRE) (Table 2).

#### ***Embryo growth in natural conditions***

The minimum temperature recorded in Norway in winter was -2 °C in mid-November while the highest (18.5 °C) was recorded at the beginning of the experiment, on 15<sup>th</sup> September 2016. In England the minimum temperature recorded was 1.6 °C at the end of January and the maximum 17.0 °C, recorded on the same day as the Norwegian site, during an autumn heat wave. Embryo growth in natural condition was faster, for all the population tested, in the northern most location of Bergen (BER) where daily mean temperatures were lower than at Wakehurst (WAK), UK. However, in both sites the rate of embryo growth started to increase when the temperatures fell below 10 °C (Fig. 2). Even if the southern population (CHO) had the greater initial E:E ratio, its growth rate was not different from the other populations tested. Eventually, the three growth curves tended to converge when a mean E:E ratio approached 0.8 (Fig. 2). Germination in nature tended to peak in the months of January and February. Fitting a logistic regression to the curves permitted an estimation of the time, in days, to reach different deciles of relative embryo size.

#### ***Comparison of the model with field data***

The comparison of the thermal models against estimates of embryo growth in the field gave different results between the three populations but was consistent between experimental sites (Fig. 3). Estimates of time to reach successive deciles of E:E ratio were similar if calculated using the  $\theta T_b$  and  $\theta T_c$  of the WAK

population for both sites but higher than the  $Tr_g$  estimated from the logistic regression of embryo growth in the field. The BER population showed a rate of embryo growth that could be better predicted by the  $\theta T_c$  rather than by  $\theta T_b$  while both models diverged from the observed pattern of embryo growth in the southern population CHO.

### ***Environmental correlates of embryo growth traits***

A PCA ordered the populations according to their seed and germination parameters and to the climate of the collection site (Fig. 4). The first axis, that explained 50% of the variability in the data, separated the southern populations from the northern ones. The axis was described mostly by geographic and bioclimatic variables and the only seed traits that had a significant correlation with it were seed dry mass and the ceiling temperature for embryo growth ( $T_c$ ). In particular, there was a strong negative correlation between precipitation of the driest month and  $T_c$ . Mountain populations of *Conopodium majus* were in the southern portion of the distribution range of the species and were characterized by higher maximum temperatures and more severe drought stress. The seeds from these populations had a lower dry mass but a greater initial relative embryo size than the northern, lowland populations. The second axis explained 18.8 % of the variability in the data and had a significant correlation only with  $T_b$  and  $T_o$ . The two cardinal temperatures showed opposite trends, such that a higher optimum corresponded to a lower  $T_b$ . The two southern most populations, CHO and TRE, remained separated from the others: they came from the highest altitude and are exposed to the strongest heat and drought stress. The remaining populations can be separated in three

groups: SCO, BAS and LEO had the highest  $T_b$ , HER and FLE the biggest seeds and BER and WAK had the higher  $T_o$ .

### DISCUSSION

*Conopodium majus* shows considerable intraspecific variability in the thermal thresholds for post-dispersal embryo growth along its European distribution. Additionally, variability in post-dispersal embryo growth thresholds appears related to the climate of the collection sites, with warmer and drier sites correlating with a capacity for embryos to grow at warmer temperatures. These results indicate that there is functional variation in the embryo growth thermal thresholds, and therefore that these thresholds might be functional ecophysiological traits. To our knowledge, this is the first work showing biogeographical variation in the thermal thresholds for embryo growth. A previous study had found no variation in embryo thresholds between closely located populations of the Ranunculaceae species *Aquilegia barbaricina*, even if the same populations showed variation in the thermal thresholds for germination (Porceddu et al., 2017).

The ceiling temperature for embryo growth varied between 12 and 21 °C and had a strong negative correlation with latitude and precipitation. Populations from northern and wetter locations had lower ceilings; while the two southernmost populations (CHO and TRE) had a ceiling above 20 °C. Interestingly, the two southernmost populations (CHO and TRE) belong to the subspecies *Conopodium majus* subsp. *marizianum*, while the others belong to the typical subspecies *Conopodium majus* subsp. *majus* (the LEO population grows in the contact area between the two subspecies). *Conopodium majus* subsp. *marizianum* and all the other species in the genus are restricted to the

Iberian Peninsula and northern Africa, with the only exception of *Conopodium majus* subsp. *majus*, which is the only taxon to be widely distributed in western and northern Europe (Mateo and López Udias, 2003). This may suggest that the lower ceiling temperature for embryo growth in *Conopodium majus* subsp. *majus* played a key role in allowing the subsp. to colonize colder regions, a hypothesis that should be explored with broader comparative studies in the genus. From a functional point of view, the correlation between the ceiling temperature and precipitation reflects the general role of drought and frost as the major drivers of regeneration strategies (Jurado and Flores, 2005). A higher ceiling temperature in *Conopodium majus* subsp. *marizianum* would allow embryo growth during late summer and autumn, and therefore seedling emergence during winter, the season of lowest water stress in the Mediterranean mountains where the subspecies lives. Conversely, the lower ceiling temperature in *Conopodium majus* subsp. *majus* would retard embryo growth until winter, and lead to seedling emergence in early spring, a more favourable regeneration environment in cold-limited regions. Moreover, embryo growth (and the potential to germinate) under cold temperatures (close to 0°C) would enable the start of growth during winter and emergence around the time of snowmelt, a behaviour that has been described in sub-alpine meadows where *Conopodium majus* can be found (Fernández-Pascual et al., 2017, Shimono and Kudo, 2005). This could give the seedling an early start that can result in a competitive edge, making full use of a short growing season. In another frequent habitat of *Conopodium majus*, forest understoreys, early seedling emergence is a common trait in herbaceous species because it allows the young plant to start photosynthesis before the tree canopy closes.

The optimum temperature for embryo growth ranged between 2.5 and 5 °C (Table 2). This is a very low optimal temperature when compared to the optimal temperatures for germination found in other species: in the review by Dürr et al. (2015), the mean optimal temperature for germination across species was 27 °C, with the lowest value being 7 °C in the tree *Acer saccharum* (McCarragher et al., 2011). In the case of the Apiaceae crops *Apium graveolens* and *Daucus carota*, optimal embryo growth or germination occurred at 20-25 °C (Finch-Savage et al., 1998, Rowse and Finch-Savage, 2003, Walker et al., 2021), but it has been shown that crops tend to have higher thermal threshold for germination than wild species (Dürr et al., 2015). In the future, it would be interesting to explore whether *Conopodium majus* is an outlier, or embryo growth temperatures tend to be lower than the temperatures for seed germination across species. It is also worth noting that, in our case,  $T_o$  had a negative correlation with  $T_b$ , a phenomenon already observed for germination temperatures by Dürr et al. (2015). In other words, the populations with the higher  $T_b$  (BAS, LEO and SCO) also have the lower  $T_o$ , and therefore they have a narrower window of suboptimal conditions for embryo growth. In practical terms, these populations are at greater risk from climate change, which could produce a mismatch between their narrow germination niche and soil temperatures (Walck et al., 2011, Orrù et al., 2012, Maleki et al., 2024).

All the populations considered are estimated to have a negative base temperature for embryo growth, ranging from -6.7 °C in BER to -2.7 in BAS. Although it cannot be discarded that such low values are an artefact of the thermal time modelling approach, values of  $T_b$  lower than zero have been reported for some temperate trees, crops (mainly legumes) and wild plants but

are not common (Dürr et al., 2015). However, to our knowledge, no values as low as -6.7 °C have been reported previously, the lowest being a  $T_b$  of -3.9 °C for *Cryptantha minima* (Boraginaceae) (Wei et al., 2009) and -4.5 °C for *Krascheninnikovia lanata* (Amaranthaceae) (Wang et al., 2006). The germination of *Cryptantha minima* at negative temperatures was explained as an adaptation to take advantage of the water of the snowmelt in early spring and develop its annual cycle before the summer drought (Wang et al., 2006). In the case of *Conopodium majus*, that is a perennial, this strategy could, however, offer some advantage at the southern range of its distribution, where summer drought can be a recurrent issue, as already observed for Mediterranean subalpine species (Fernández-Pascual et al., 2017). *Krascheninnikovia lanata* seeds show a positive effect of seed size on the ability to germinate at sub-zero temperatures (Wang et al., 2006). The authors demonstrate that bigger seeds had a higher concentration of sugars (glucose, raffinose and sucrose) that probably lower the freezing point of the seed tissues. There are no reports on sub-zero germination in Apiaceae but an optimal temperature for embryo growth of 2 °C has been described for *Heracleum sphondylium* (Stokes, 1953) and it is not unlikely that this species, or others from the same family, could present equally low  $T_b$  for embryo growth. However, in practical terms, it is unlikely that such low temperatures do have a functional ecological role in embryo growth in the field: our field collected data and averaged climatic data from 1970-2000 for the collection sites show that such low mean temperatures are rare in the natural environment of *Conopodium majus*. Therefore, in the field it is likely that embryo growth is possible throughout the winter season and is limited only by the higher

1 temperatures (and the ceiling threshold) in autumn. In fact, results from the  
2 ordination analysis, showed that  $T_b$  is independent from climatic and geographic  
3 factors and is not even correlated to seed size or initial E:E ratio. Therefore, we  
4 can conclude that the limiting factor for embryo growth in *Conopodium majus* is  
5 the ceiling temperature and its interaction with warmer temperatures during the  
6 annual cycle.

7 Our thermal model outputs are supported by the results of the field sowings,  
8 although these should be taken with caution since we were not able to perform  
9 field sowing at the southernmost distribution of our experiment, in central Spain.

10 The responses of different populations to the warmer, drier sites in central  
11 Spain could provide useful data for predicting possible effects of a warming  
12 climate on seed regeneration in *Conopodium majus*. A most interesting follow  
13 up study would involve reciprocal transplants of populations across the  
14 latitudinal gradient of the species, to establish how much of the variation  
15 measured and modelled is genetic and how much is due to phenotypic plasticity  
16 (Franks et al., 2014). This would inform on the role for adaptation in any  
17 species' response to environmental change through seed regeneration.

## 18 **CONCLUSION**

19 Species responses to climate determine how plants cope with ongoing global  
20 change. Parmesan and Hanley (2015) identified three key issues for climate  
21 change research on plants: changing phenology, changing distributions and the  
22 role of plasticity and adaptation. Our study suggests that the thermal thresholds  
23 for post-dispersal embryo growth may have functional relevance for these three  
24 issues. The dependence of embryo growth on a relatively low ceiling  
25 temperature means that warmer winter temperatures could slow down post

dispersal embryo growth and potentially shift the emergence phenology from one season to another, as has been described in alpine systems in relation to another seed process, physiological dormancy release (Mondoni et al., 2012). Regarding distribution, we have found that two vicariant subspecies show diverging thermal thresholds for embryo growth. This supports the concept that seed ecophysiology can be a major driver of the distribution of taxa (Bykova et al., 2012), and of species migrations as a response to climate change (Walck et al., 2011, Baskin and Baskin, 2022). At the same time, our study also shows intraspecific variation in the embryo thermal thresholds, and therefore the potential for plasticity and/or adaptation to changes in the climatic environment (Cochrane et al., 2015, Nicotra et al., 2010, Franks et al., 2014).

To date, most studies on the responses of seeds to climate have focused on germination. To complete the picture, our study highlights the importance of post-dispersal embryo growth, showing intraspecific variation in this key ecophysiological trait across the latitudinal distribution of a species. Thus, embryo growth seems to be one of the many ways by which plants interact with changing soil temperatures (Amstutz et al., 2024). We make the case for more ecological, evolutionary and comparative studies on post-dispersal embryo growth, fully incorporating this trait into the research agenda on plant regeneration (Saatkamp et al., 2019) and plant responses to climate change (Parmesan and Hanley, 2015).

## **AKNOWLEDGEMENTS**

Álvaro Bueno Sánchez, Joseba Garmendia, Luis Carlón, Giles Laverack and Maria Marin helped with seed collection.

## **FUNDING**



This research received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n°607785.

#### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

#### AUTHOR CONTRIBUTIONS

**Cristina Blandino:** Conceptualization; Methodology; Investigation; Data Curation; Formal Analysis; Visualization; Writing – Original Draft Preparation; Writing – Review & Editing. **Brith Natlandsmyr:** Investigation; Writing – Review & Editing. **Sylvi M. Sandvik:** Investigation; Writing – Review & Editing. **Hugh W. Pritchard:** Funding acquisition; Conceptualization; Methodology; Writing – Review & Editing. **Eduardo Fernández-Pascual:** Conceptualization; Methodology; Investigation; Writing – Review & Editing.

#### DATA AVAILABILITY STATEMENT

Upon acceptance, all data will be deposited into Zenodo.

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## TABLES

**Table 1:** Provenance of seeds used in the experiments.

Population	Country	Location	Latitude	Longitude	Elevation (m a.s.l.)
HER	Norway	Herdla	60° 34' 29.784" N	4° 56' 53.627" E	37
BER	Norway	Bergen	60° 20' 7.35" N	5° 22' 17.79" E	97
FLE	Norway	Flekkeroya	58° 4' 5.34" N	7° 59' 53.56" E	19
SCO	UK	Dalreoch Farm	56° 44' 47.36" N	3° 32' 25.03" W	252
WAK	UK	Wakehurst Place	51° 04' 12.79" N	0° 05' 28.28" W	114
BAS	Spain	Ondarre	43° 01' 42.8" N	2° 03' 55.7" W	809
LEO	Spain	El Tendero	42° 54' 26.62" N	5° 49' 25.87" W	1426
CHO	Spain	Central del Chorro	40° 18' 26.17" N	5° 40' 09.39" W	1398
TRE	Spain	Tremedal	40° 22' 00.5" N	5° 37' 57.20" W	1555

**Table 2:** Initial E:E , seed dry mass and cardinal temperatures averaged between deciles (all as mean  $\pm$  SD) in seeds of all populations of *Conopodium majus* studied. To have a symmetric result around the middle value, when the lower deciles were excluded for being too close to the initial embryo size, the higher ones were excluded too.

Population	Initial E:E	Seed dry	T <sub>b</sub> (°C)	T <sub>o</sub> (°C)	T <sub>c</sub> (°C)	Deciles
------------	-------------	----------	---------------------	---------------------	---------------------	---------

	weight (mg)					used
HER	0.12 ±0.03	1.94 ±0.58	-4.01 ± 0.57	4.26 ± 0.80	12.90 ± 1.86	0.3 - 0.7
BER	0.10 ±0.03	1.80 ±0.55	-6.65 ± 0.62	4.58 ± 0.02	12.08 ± 1.32	0.3 - 0.7
FLE	0.17 ±0.04	2.03 ±0.53	-3.90 ± 0.14	4.50 ± 0.07	13.70 ± 0.71	0.3 - 0.7
SCO	0.13 ±0.02	1.63 ±0.54	-2.63 ± 0.38	2.80 ± 0.25	14.42 ± 2.47	0.3 - 0.7
WAK	0.10 ±0.02	1.75 ±0.42	-6.20 ± 0.89	4.59 ± 0.11	14.44 ± 1.72	0.3 - 0.7
BAS	0.15 ±0.02	1.43 ±0.59	-2.75 ± 0.10	2.69 ± 0.10	13.07 ± 0.93	0.3 - 0.7
LEO	0.12 ±0.03	1.66 ±0.52	-3.17 ± 0.06	2.54 ± 0.03	14.64 ± 2.23	0.4 - 0.6
CHO	0.19 ±0.03	1.24 ±0.38	-4.09 ± 0.59	5.23 ± 1.05	20.48 ± 9.09	0.4 - 0.6
TRE	0.11 ±0.02	1.21 ±0.38	-6.47 ± 0.41	4.86 ± 0.04	20.54 ± 7.25	0.4 - 0.6

1

2

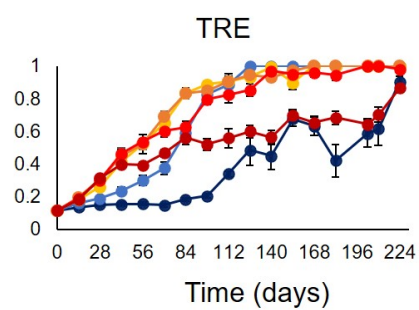
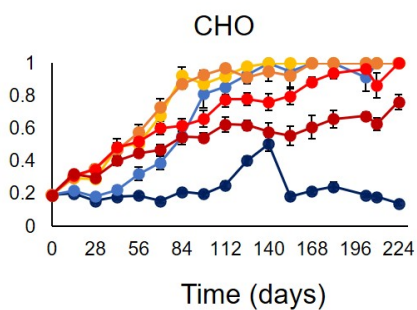
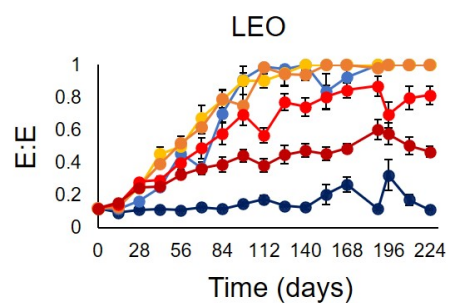
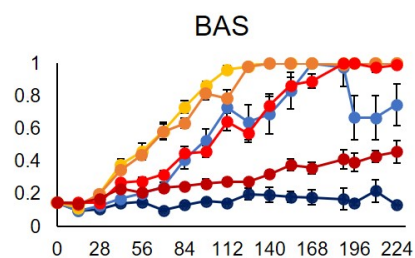
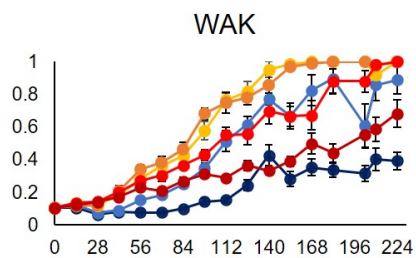
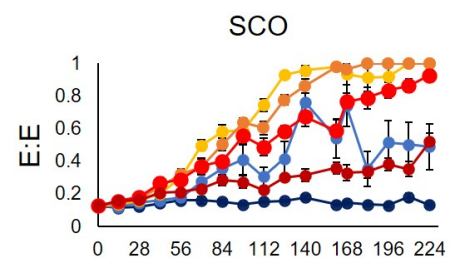
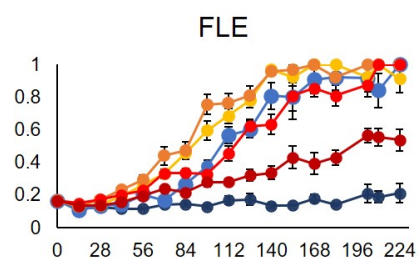
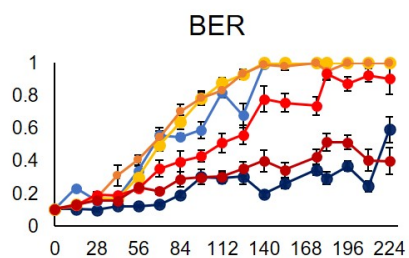
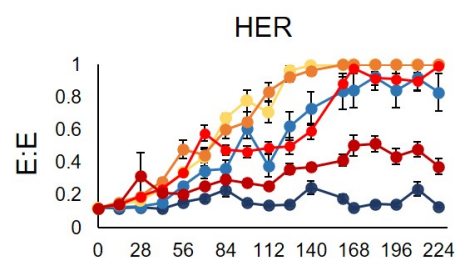
## FIGURES

**Fig. 1:** Patterns of embryo growth (E:E ratio) for all the seed populations of *Conopodium majus* and all temperatures tested. Each data point represents the mean of ten replicates ( $\pm$  SE).

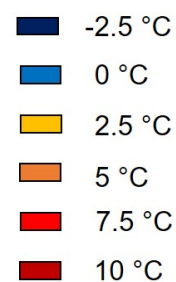
**Fig. 2:** Embryo growth in the field for buried seeds of *Conopodium majus*. Each data point represents the mean E:E ratio for 20 seeds of three representative populations: CHO for the southern edge of the distribution range, WAK for the middle and BER for the northern; soil temperature is also shown. The burial experiment was performed in England (on the collection site of the WAK population) and in Norway (on the collection site of BER population). The experiment started on 1<sup>st</sup> September 2016 in England and on 15<sup>th</sup> September 2016 in Norway. For each site, the experiment finished when all populations reached 100% radicle emergence (corresponding to E:E =1).

**Fig. 3:** Time (in days) required by each population of *Conopodium majus* seeds in each field location to reach different deciles of E:E ratio according to: 1) interpolation from the logistic regression of embryo growth in the field (grey line); 2)  $\theta T_b$  (blue line); and 3)  $\theta T_c$  (orange line) obtained from the model.

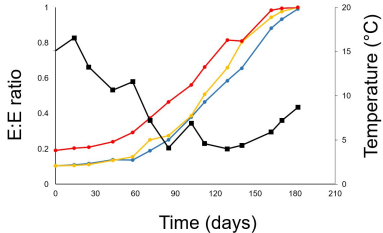
**Fig. 4:** Principal component analysis of seed traits (in red) and geographic and bioclimatic variables (in blue) across the latitudinal distribution of *Conopodium majus*.



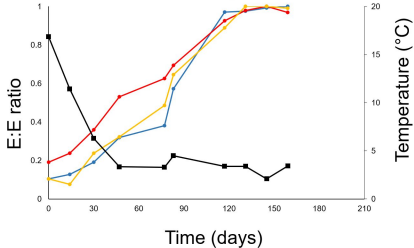
Legend



# England



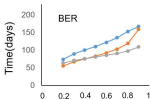
# Norway



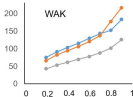
—●— BER —●— WAK —●— CHO —■— Mean temperature (°C)



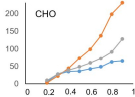
Norway



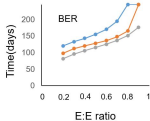
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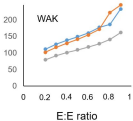
Norway



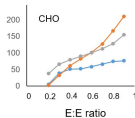
England



England



England

Predicted with  $\Theta_b$ Predicted with  $\Theta_c$ 

Inferred from logistic regression

