

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 some 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 ≥ 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 under ambient
11 condition on a laboratory bench until the beginning of the tests.

12 ***Initial measurements***

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

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

7 ***Embryo growth in controlled temperature conditions***

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

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

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

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 $1/t_r$ 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 (θ_b) and supra-optimal (θ_c) thermal times for embryo growth. θ , expressed in degree days ($^{\circ}\text{Cd}$), indicates the cumulative thermal time units above T_b (θ_b) or below T_c (θ_c) that the seed must accumulate for the embryo to reach successive E:E deciles. For each population, the deciles were plotted against θ_b and θ_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 θ 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 1st September 2016 and in Norway on 14th 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. 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_r to reach every decile of relative embryo growth was calculated. The units of thermal time required by each population to reach every t_r 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 (ΔT) 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 (ΔT) 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_r was compared with the t_r estimated from the embryo growth data. The time (in days) needed to sum enough heat to reach the θT_b and θT_c calculated in the model, for each t_r decile ($t_{r \text{ model}}$) in each population was compared with the time needed by each population to reach the same decile of relative embryo growth in the field ($t_{r \text{ field}}$). These estimates were then graphically compared expressing the different t_r 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 (± 0.03 SD) for the population BER to a mean value of 0.19 (± 0.03 SD) for the population CHO (Table 2). Mean seed dry mass ranged just under two-fold from 1.21 mg (± 0.38 SD) in TRE to 2.03 mg (± 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 (± 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 15th 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 θT_b and θT_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 shown a rate of embryo growth that could be better predicted by the θT_c rather than by θT_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 (Fig. 4) ordered the populations according to their seed and germination parameters and to the climate of the collection site. 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

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

14 The ceiling temperature for embryo growth varied between 12 and 21 °C and
15 had a strong negative correlation with latitude and precipitation. Populations
16 from northern and wetter locations had lower ceilings; while the two
17 southernmost populations (CHO and TRE) had a ceiling above 20 °C.
18 Interestingly, the two southernmost populations (CHO and TRE) belong to the
19 subspecies *Conopodium majus* subsp. *marizianum*, while the others belong to
20 the typical subspecies *Conopodium majus* subsp. *majus* (the LEO population
21 grows in the contact area between the two subspecies). *Conopodium majus*
22 subsp. *marizianum* and all the other species in the genus are restricted to the
23 Iberian Peninsula and northern Africa, with the only exception of *Conopodium*
24 *majus* subsp. *majus*, which is the only taxon to be widely distributed in western
25 and northern Europe (Mateo and López Udías, 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

1 *Krascheninnikovia lanata* (Amaranthaceae) (Wang et al., 2006). The
2 germination of *Cryptantha minima* at negative temperatures was explained as
3 an adaptation to take advantage of the water of the snowmelt in early spring
4 and develop its annual cycle before the summer drought (Wang et al., 2006). In
5 the case of *Conopodium majus*, that is a perennial, this strategy could,
6 however, offer some advantage at the southern range of its distribution, where
7 summer drought can be a recurrent issue, as already observed for
8 Mediterranean subalpine species (Fernández-Pascual et al., 2017).
9 *Krascheninnikovia lanata* seeds show a positive effect of seed size on the ability
10 to germinate at sub-zero temperatures (Wang et al., 2006). The authors
11 demonstrate that bigger seeds had a higher concentration of sugars (glucose,
12 raffinose and sucrose) that probably lower the freezing point of the seed
13 tissues. There are no reports on sub-zero germination in Apiaceae but an
14 optimal temperature for embryo growth of 2 °C has been described for
15 *Heracleum sphondylium* (Stokes, 1953) and it is not unlikely that this species, or
16 others from the same family, could present equally low T_b for embryo growth.
17 However, in practical terms, it is unlikely that such low temperatures do have a
18 functional ecological role in embryo growth in the field: our field collected data
19 and averaged climatic data from 1970-2000 for the collection sites show that
20 such low mean temperatures are rare in the natural environment of
21 *Conopodium majus*. Therefore, in the field it is likely that embryo growth is
22 possible throughout the winter season and is limited only by the higher
23 temperatures (and the ceiling threshold) in autumn. In fact, results from the
24 ordination analysis, showed that T_b is independent from climatic and geographic
25 factors and is not even correlated to seed size or initial E:E ratio. Therefore, we

1 can conclude that the limiting factor for embryo growth in *Conopodium majus* is
2 the ceiling temperature and its interaction with warmer temperatures during the
3 annual cycle.

4 Our thermal model outputs are supported by the results of the field sowings,
5 although these should be taken with caution since we were not able to perform
6 field sowing at the southernmost distribution of our experiment, in central Spain.
7 The responses of different populations to the warmer, drier sites in central
8 Spain could provide useful data for predicting possible effects of a warming
9 climate on seed regeneration in *Conopodium majus*. A most interesting follow
10 up study would involve reciprocal transplants of populations across the
11 latitudinal gradient of the species, to establish how much of the variation
12 measured and modelled is genetic and how much is due to phenotypic plasticity
13 (Franks et al., 2014). This would inform on the role for adaptation in any
14 species' response to environmental change through seed regeneration.

15 **CONCLUSION**

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

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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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17 TABLES

18 **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

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

Population	Initial E:E	Seed dry weight (mg)	T _b (°C)	T _o (°C)	T _c (°C)	Deciles used
HER	0.12 \pm 0.03	1.94 \pm 0.58	-4.01 \pm 0.57	4.26 \pm 0.80	12.90 \pm 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

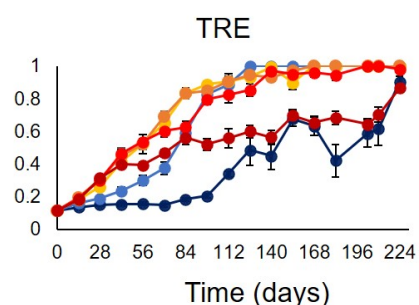
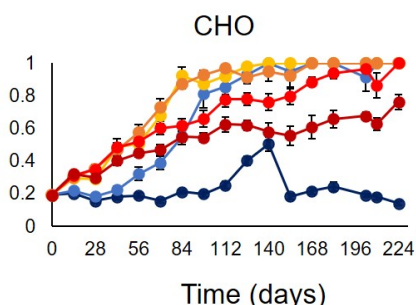
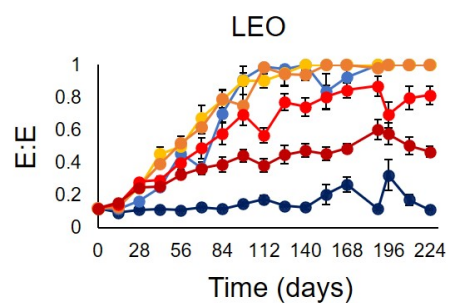
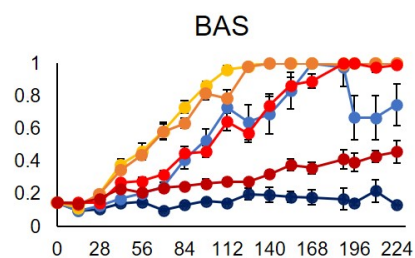
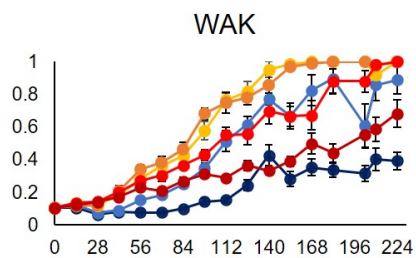
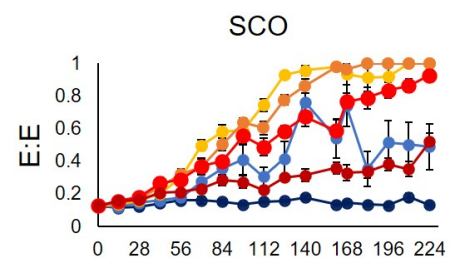
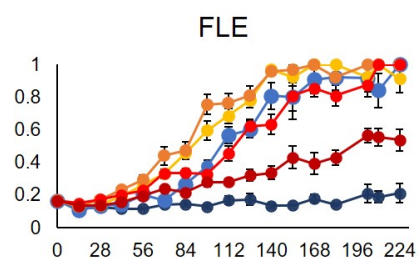
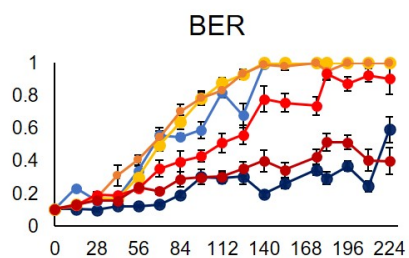
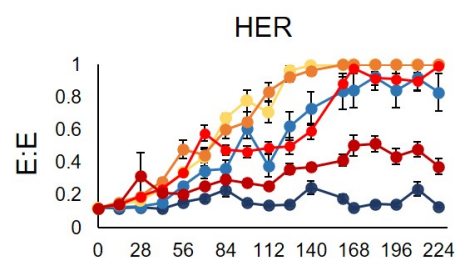
FIGURES

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).

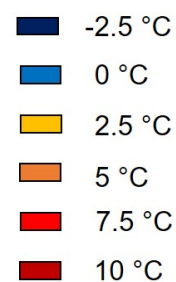
Fig. 2: Embryo growth in the field for buried seeds of *Conopodium majus*. Each data point represents the mean E:E ratio for 20 seeds of three representative populations: CHO for the southern edge of the distribution range, WAK for the middle and BER for the northern; soil temperature is also shown. 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 1st September 2016 in England and on 15th September 2016 in Norway . For each site, the experiment finished when all population 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) θT_b (blue line); and 3) θT_c (orange line) obtained from the model.

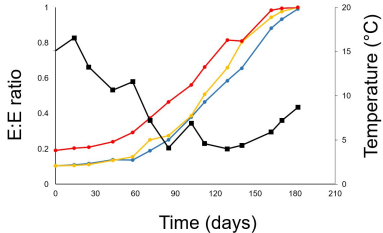
Fig. 4: Principal component analysis of seed traits (in red) and geographic and bioclimatic variables (in blue) across the latitudinal distribution of *Conopodium majus*.



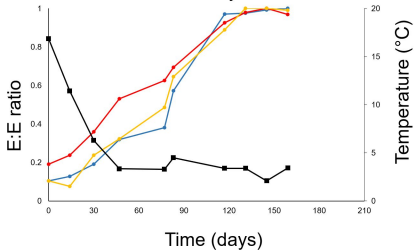
Legend



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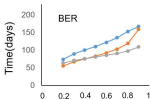


Norway

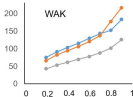


—●— BER —●— WAK —●— CHO —■— Mean temperature (°C)

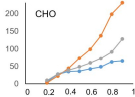
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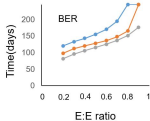
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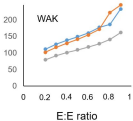
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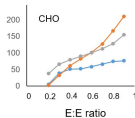
England



England



England

Predicted with Θ_b Predicted with Θ_c 

Inferred from logistic regression

