

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 appears not to have been tested at continental scales.
- **Methods** Here we have used a thermal time approach and field experiments to assess intraspecific variation in embryo growth thermal thresholds in the geophyte *Conopodium majus* (Apiaceae) across its distribution from the Iberian Peninsula to Scandinavia.
- **Key Results** Thermal thresholds 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 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 support that the thermal thresholds for embryo growth are a functional ecophysiological trait driving seed germination phenology and seed responses to the environment.

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. The
10 temperature to which imbibed seeds are exposed affects their germination rate
11 (Fernández-Pascual et al., 2019). This phenomenon can be numerically
12 described by the “cardinal germination temperatures”, i.e., the optimum
13 temperature (T_o), at which the germination rate is maximal and the base (T_b)
14 and ceiling (T_c) temperatures that are, respectively, estimated to be the coldest
15 and the warmest temperature at which the rate of germination tends to zero.
16 The measurement of these temperatures for a given species enables prediction
17 of its seed germination rate and germination success under different
18 temperatures (Orrù et al., 2012). Therefore, the cardinal temperatures are key
19 parameters to explain the contribution of regeneration thermal niches on
20 species distributions and responses to climatic changes (Maleki et al., 2024).

21 In many species however, seed dormancy prevents germination even in the
22 presence of suitable conditions, so that the exposure of seedlings to
23 unfavourable environments is avoided and the timing of germination is matched
24 to favourable regeneration windows (Lamont and Pausas, 2023, Pausas et al.,
25 2022). A particular case of seed germination delay occurs when the embryo, at

1 the time of dispersal, is not completely developed and needs to grow to a critical
2 size before germination can occur; a trait known as post-dispersal embryo
3 growth or morphological dormancy (Baskin and Baskin, 2004). As happens with
4 germination, temperature is a major environmental driver of post dispersal
5 embryo growth (Baskin et al., 2000, Phartyal et al., 2009, Vandelook et al.,
6 2007, Vandelook et al., 2009, Blandino et al., 2019, Vandelook, 2008),
7 influencing the rate of development of the embryo, a mechanism that allows a
8 precise timing of germination (Porceddu et al., 2017).

9 Biogeographical variation in several germination traits is well documented. For
10 example, seeds from warmer sites have been shown to have lower minimum
11 temperatures for germination at different geographical scales (Daws et al.,
12 2004, Rosbakh and Poschlod, 2015). The requirements for cold stratification
13 can also vary according to the local climate: populations from habitats with
14 longer winters require a longer period of cold stratification compared with
15 populations from milder habitats (Allen and Meyer, 1998). However, much less
16 research has been dedicated to traits related to post-dispersal embryo growth.
17 Mondoni et al. (2008) compared post-dispersal embryo growth between
18 mountain and lowland populations of the temperate woodland forb *Anemone*
19 *nemorosa* and found that, although embryo size at dispersal was similar in all
20 the populations, embryo growth at cool temperatures was faster in the mountain
21 population. This suggests a capacity of post-dispersal embryo growth to adapt
22 to local conditions, either by local adaptation or phenotypic plasticity, analogous
23 to that shown by other seed traits (Fernández-Pascual et al., 2013). Further
24 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 vulnerable to spatial shifts in its regeneration niche in a scenario of changing climate (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 predicted that thermal thresholds for embryo growth would be influenced by high maximum temperatures and drought at the lower latitudes, and by low minimum temperatures at the higher latitudes.

MATERIALS AND METHODS

Study species

Conopodium majus (Apiaceae) is a geophyte with a European Atlantic distribution from Southern Spain to Central Norway (Tutin et al., 1968). As is common in *Apiaceae*, seeds of *Conopodium majus* possess undeveloped linear embryos (Martin, 1946) and germinate when they extend to the full length of the endosperm (Blandino et al., 2019). For this reason, in this study, the relative embryo size (i.e. embryo length / endosperm length, hereafter referred to as “E:E ratio”) is used to describe embryo development, and germination is defined as the point at which E:E ratio is ≥ 1 . In *Conopodium majus*, germination has been observed to occur both at 0 and 5 °C with a similar rate of embryo growth (Blandino et al., 2019). Such low temperature requirements are indicative of germination in winter. For a species adapted to woodlands as well as oligotrophic grasslands, the control of the germination process in this way can allow seedlings to establish before the development of a tree canopy or of competing vegetation.

Seed collection

Mericarps of *Conopodium majus* were collected in the summer of 2016 from nine naturally occurring populations sampled across the western European latitudinal range of the species (Table 1). Since the seed cannot be separated from the fruit in this species, the dispersal unit will be referred hereafter to as the “seed”. A population was sampled only if it consisted of at least 200 individual plants. Seeds were sampled from 50 plants within the population to secure a representative sample of the genetic variability of the population. At least 4000 seeds were collected from each population. All seeds were collected between July and August 2016 and the experiments started within three weeks

from seed collection. Seeds were kept at below full hydration under ambient condition on a laboratory bench until the beginning of the tests.

Initial measurements

Each collection was cleaned from debris and empty seeds were removed using a gravity seed separator machine. From each population, 10 seeds were selected randomly and allowed to rehydrate overnight at 20 °C and 100% RH. The seeds were then placed on 1% agar-water for 24 hours to become fully imbibed. Thereafter, seeds were prepared for vital staining with 1% aqueous solution of triphenyl tetrazolium chloride (TZ). A slice of seed coat was removed from the dorsal surface of each seed using a scalpel and seeds were incubated in TZ solution at 30 °C in the dark for 24 hours. Each seed was then cut longitudinally, and the embryo was extracted. Embryos and endosperms were photographed using a camera (Carl Zeiss AxioCam Colour) mounted on a Stemi SV 11 Microscope (Carl Zeiss, Welwin Garden City, Herts, UK) microscope and 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 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 1% agar-water substrate. Seeds were sown at -2.5 °C, 0 °C, 2.5 °C, 5 °C, 7.5 °C
2 and 10 °C in incubators with a daily light regime of 12 hours . Every 14 days
3 one subsample from each population and treatment was retrieved and the 15
4 seeds were placed for 24 hours in 1% TZ solution at 30°C in the dark, after a
5 slice of the seed coat was removed. From this subsample, the embryo and
6 endosperm length of 10 viable seeds was measured following further dissection
7 of the seed. An E:E value of 1 was assigned to all germinated seeds. Seed
8 measurement was stopped when the seeds ceased germinating. The
9 experiment continued for 224 days, until all the 16 subsample assessments
10 were concluded.

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

12 The average E:E ratio of 10 seeds for each population x temperature x time
13 combination was calculated. All the temperatures for the same population had
14 the same initial E:E ratio at time = 0, while the maximum value was fixed at 1,
15 after which the seed was able to germinate. Since the data followed a sigmoidal
16 growth distribution, except the treatments at -2.5 °C, a logistic model was fitted
17 to each population x temperature combination using the software OriginLab 9.0.
18 The models of each population were bounded between the initial value of E:E
19 for that population and 1. A linear model was fitted to the -2.5°C treatments.
20 From the equation of the logistic and linear models, it was possible to calculate
21 the time expressed in days (tr) at which each temperature x population
22 combination would have reached the following deciles of relative embryo size:
23 0.3, 0.4, 0.5, 0.6 and 0.7. Deciles < 0.3 could not be calculated because they
24 were under the initial E:E. Deciles > 0.7 were not calculated to keep the

1 symmetry of the analyses regarding deciles of the population. For each
2 treatment, the embryo growth rate was calculated as $1/tr$.

3 For each population and decile, embryo growth rate was plotted against
4 temperature. Each dataset was visually divided into sub-optimal and supra-
5 optimal ranges, using the point with the highest value of $1/tr$ as the dividing
6 point. Linear regressions were fitted separately to the sub- and supra-optimal
7 ranges. The intersection with the temperature axis of the sub-optimal and
8 supra-optimal regression are, respectively, the base (T_b) and the ceiling (T_c)
9 temperatures; these estimates are the temperatures below and above which the
10 embryo growth rate is projected to be zero. The optimal temperature (T_o),
11 defined as the temperature at which the rate of embryo growth is estimated to
12 be fastest, is the x-coordinate of the intersection point between sub-optimal and
13 supra-optimal regressions. Then, for each population, the cardinal temperatures
14 (T_b , T_c and T_o) were averaged across all the deciles calculated to define an
15 average value of the population (ELLIS et al., 1986). The regression lines of
16 each decile were recalculated and forced to pass through a common origin
17 defined by the average T_b (for the sub-optimal regressions) or the average T_c
18 (for the supra-optimal regressions) (Hardegree, 2006). For the three
19 southernmost populations, only the cardinal temperatures calculated for the
20 relative embryo size of 0.4, 0.5 and 0.6 were used, because it was not possible
21 to fit a supra-optimal regression to the 0.3 decile.

22 The slopes of these new linear regressions were then taken as a reciprocal to
23 estimate the sub-optimal (θ_b) and supra-optimal (θ_c) thermal times for embryo
24 growth. θ , expressed in degree days ($^{\circ}Cd$), indicates the cumulative thermal
25 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 ($\log \text{ } ^\circ\text{Cd}$) 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

1 were buried in England on 1st September 2016 and in Norway on 14th
2 September 2016. Every 14 days a bag for each population was retrieved and
3 the soil washed. Seed bags buried in Norway were shipped to England for
4 measurements. All the seeds retrieved were prepared for TZ staining and their
5 embryo and endosperm lengths measured. It was easiest to measure the seeds
6 when most of the seeds were not germinated. With an increasing number of
7 germinated seeds and seedlings, the number of empty seed coats left in the soil
8 bags made it difficult to distinguish between mouldy or germinated seeds. At
9 this point, the experiment was terminated, representing nine measurements in
10 Norway and thirteen in England.

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

12 To compare the embryo growth predicted by the thermal time model with
13 embryo growth in natural conditions, embryo growth in the field sites was
14 plotted against time. A logistic regression was fitted to these curves, and from
15 the equations, the t_r to reach every decile of relative embryo growth was
16 calculated. The units of thermal time required by each population to reach every
17 t_r during the field experiment were calculated for both field locations using the
18 data recorded by the loggers. To account for every temperature fluctuation
19 during the day, the thermal time was expressed in “°C 30 min” and the heat
20 accumulated by the seed was calculated for every 30 min temperature record.
21 The difference (ΔT) between each temperature record and the population T_o
22 (averaged between deciles) was summed. When the temperature was higher
23 than the average T_c or lower than the average T_b the heat accumulated was
24 considered = 0 and the difference (ΔT) between each temperature record and
25 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 average E:E ratio corresponding to the t_{g50} for germination at the two temperatures used was displayed as the average E:E ratio for 50% germination in that population. The average between all the populations represented the average for the species.

Relationship between environmental data and germination traits

The relationship between embryo development and seed germination traits and geographical and bioclimatic data was explored for each population. A data matrix was built including latitude, altitude, average annual temperature, precipitation of the driest month, average maximum temperature of the hottest

month and minimum average 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 an average value of 0.10 (± 0.03 SD) for the population BER to an average value of 0.19 (± 0.03 SD) for the population CHO (Table 2). Average 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 imbibition. 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 an average $E:E = 1$ corresponded to 100% germination in the sample. The treatments that had the highest average 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 in the last sampling. The lowest germination was observed at -2.5 and 10 °C. The population that reached, across all the treatments, the highest average 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 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 where daily average temperatures were lower than at Wakehurst, 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 an average 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 traits 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

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 are functional ecophysiological traits.

1 The ceiling temperature for embryo growth varies between 12.1 and 20.5 °C
2 and has a strong negative correlation with latitude and precipitation. Species
3 from northern populations, that are less likely to experience long exposure to
4 high autumnal temperatures, have lower values; while the two southernmost
5 populations, CHO and TRE, stand out for high T_c above 20 °C. Water stress is
6 the main limiting factor for embryo development in these populations, that are
7 exposed also to a shorter winter and a Mediterranean continental climate. The
8 higher T_c can therefore be an adaptation to cope with higher daily fluctuations in
9 temperatures that can prevent the embryo from growing during warmer and
10 potentially drier days during late autumn or early spring. Moreover, embryo
11 growth (and the potential to germinate) under cold temperatures (close to 0°C)
12 will enable the start of growth during winter and emergence under the snow to
13 avoid drought, as has been suggested to be the case for other species able to
14 grow in sub-alpine Mediterranean and sub-Mediterranean mountains
15 (Fernández-Pascual et al., 2017).

16 The optimum temperature for embryo growth ranged between 2.5 and 5.2 °C
17 (Table 2) and had a negative correlation with T_b , a phenomenon already
18 observed for germination temperatures by Dürr et al. (2015). The second axis of
19 our PCA ordination analysis can therefore be interpreted as reflecting the width
20 of the suboptimal temperature range for embryo growth, i.e., the gap between
21 T_b and T_o . The populations with the higher T_b (BAS, LEO and SCO) also have
22 the lower T_o and therefore a narrower window of suboptimal conditions for
23 embryo growth. Therefore, these populations are at greater risk of exposure to
24 a reduced germination niche in the face of climate warming (Walck et al., 2011).

1 These are also some of the populations at the milder and central points of the
2 latitudinal gradient investigated, i.e. northern Spain and the British Isles.

3 All the populations considered are estimated to have a negative base
4 temperature for embryo growth, ranging from -6.7 °C in BER to -2.7 in BAS.

5 Although it cannot be discarded that such low values are an artefact of the
6 thermal time modelling approach, values of T_b lower than zero have been
7 reported for some temperate trees, crops (mainly legumes) and wild plants but
8 are not common (Dürr et al., 2015). However, to our knowledge, no estimated

9 values as low as -6.7 °C have been reported previously, the lowest being a T_b
10 of -3.9 °C for *Cryptantha minima* (Boraginaceae) (Wei et al., 2009) and -4.5 °C
11 for *Krascheninnikovia lanata* (Amaranthaceae) (Wang et al., 2006). The

12 germination of *Cryptantha minima* at negative temperatures was explained as
13 an adaptation to take advantage of the water of the snowmelt in early spring
14 and develop its annual cycle before the summer drought (Wang et al., 2006). In

15 the case of *Conopodium majus*, that is a perennial, this strategy could however
16 offer some advantage at the southern range of its distribution, where summer
17 drought can be a recurrent issue, as already observed for Mediterranean
18 subalpine species (Fernández-Pascual et al., 2017). *Krascheninnikovia lanata*

19 seeds show a positive effect of seed size on the ability to germinate at sub-zero
20 temperatures (Wang et al., 2006). The authors demonstrate that bigger seeds

21 had a higher concentration of sugars (glucose, raffinose and sucrose) that
22 probably lower the freezing point of the seed tissues. There are no reports on
23 sub-zero germination in Apiaceae but an optimal temperature for embryo
24 growth of 2 °C has already been described for *Heracleum sphondylium*
25 (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, 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 average 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 temperatures in autumn. In fact, results from the ordination analysis, showed that T_b is independent from climatic and geographic factors and is not even correlated to seed size or initial E:E ratio. Therefore, we can conclude that the limiting factor for embryo growth in *Conopodium majus* is the ceiling temperature and its interaction with warmer temperatures during the annual cycle.

CONCLUSION

Conopodium majus can be considered a model species for studying post-dispersal embryo growth due to its fine regulation of embryo growth by temperature and the coincidence between the temperature requirements for embryo growth and germination. To date only one study is known to have developed thermal models of embryo growth in a species of the Ranunculaceae family, *Aquilegia barbaricina* (Porceddu et al., 2017) and this work represents the first attempt to develop such a model on a species from Apiaceae. The thermal models developed in this study can be used to predict shifts in the species' temperature germination niche caused by different climate change scenarios. The dependence of embryo growth on a relatively low ceiling temperature means that warmer winter temperatures because of climate change could compromise post dispersal embryo growth and thus negatively

1 impact the regeneration of *Conopodium majus*. However, *Conopodium majus*
2 also shows potential for adaptation to the climatic environment along its
3 latitudinal distribution, as expressed by the breadth of the temperature
4 germination niche indicated by the cardinal temperatures of each population. In
5 fact, post-dispersal embryo growth in *Conopodium majus* shows an intraspecific
6 variability which is consistent with an ecological function in determining the
7 timing of seedling emergence and establishment. This highlights the potential of
8 embryo growth temperatures as a functional ecophysiological trait driving seed
9 germination phenology and seed responses to the environment.

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17 **CONFLICT OF INTEREST STATEMENT**

18 The authors declare no conflict of interest.

19 **AUTHOR CONTRIBUTIONS**

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

27 **DATA AVAILABILITY STATEMENT**

28 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 average \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 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 \pm 0.03 | 1.80 \pm 0.55 | -6.65 \pm 0.62 | 4.58 \pm 0.02 | 12.08 \pm 1.32 | 0.3 - 0.7 |
| FLE | 0.17 \pm 0.04 | 2.03 \pm 0.53 | -3.90 \pm 0.14 | 4.50 \pm 0.07 | 13.70 \pm 0.71 | 0.3 - 0.7 |
| SCO | 0.13 \pm 0.02 | 1.63 \pm 0.54 | -2.63 \pm 0.38 | 2.80 \pm 0.25 | 14.42 \pm 2.47 | 0.3 - 0.7 |
| WAK | 0.10 \pm 0.02 | 1.75 \pm 0.42 | -6.20 \pm 0.89 | 4.59 \pm 0.11 | 14.44 \pm 1.72 | 0.3 - 0.7 |
| BAS | 0.15 \pm 0.02 | 1.43 \pm 0.59 | -2.75 \pm 0.10 | 2.69 \pm 0.10 | 13.07 \pm 0.93 | 0.3 - 0.7 |
| LEO | 0.12 \pm 0.03 | 1.66 \pm 0.52 | -3.17 \pm 0.06 | 2.54 \pm 0.03 | 14.64 \pm 2.23 | 0.4 - 0.6 |
| CHO | 0.19 \pm 0.03 | 1.24 \pm 0.38 | -4.09 \pm 0.59 | 5.23 \pm 1.05 | 20.48 \pm 9.09 | 0.4 - 0.6 |
| TRE | 0.11 \pm 0.02 | 1.21 \pm 0.38 | -6.47 \pm 0.41 | 4.86 \pm 0.04 | 20.54 \pm 7.25 | 0.4 - 0.6 |

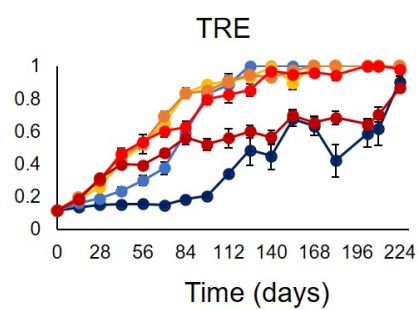
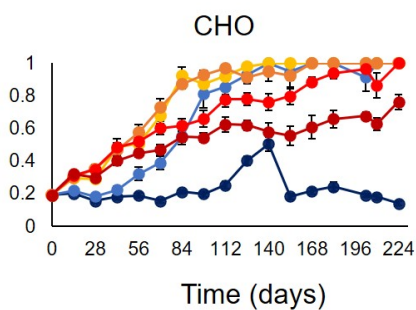
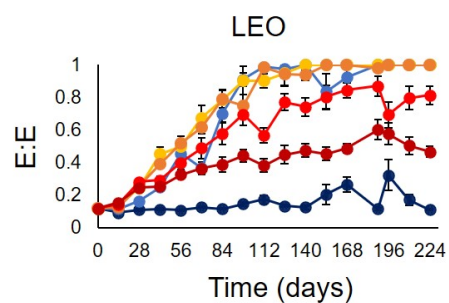
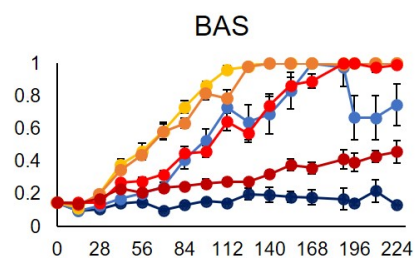
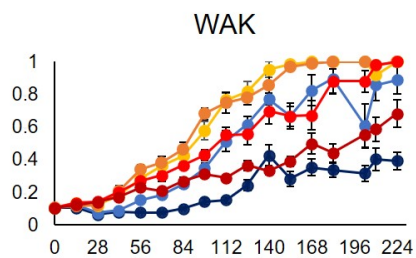
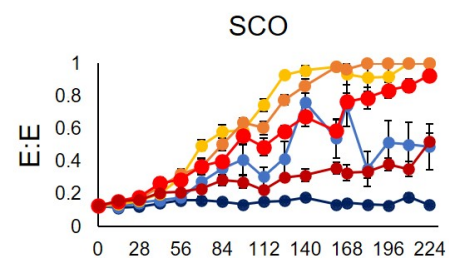
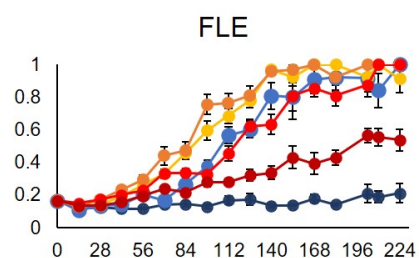
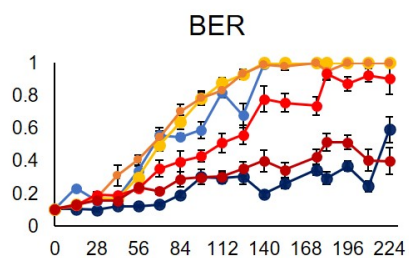
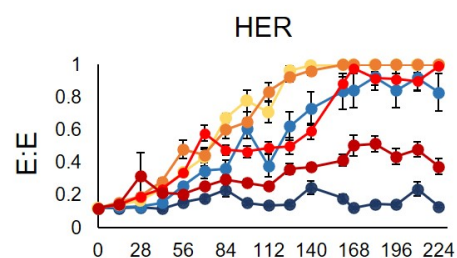
FIGURES

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 average of ten replicates (\pm SE).

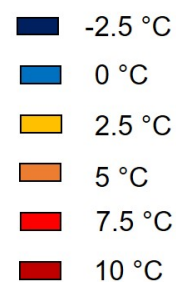
Fig. 2: Embryo growth in the field for buried seeds of *Conopodium majus*. Each data point represents the average E:E ratio for 20 seeds of three representative populations: CHO for the southern edge of the distribution range, WAK for the middle and BER for the northern; soil temperature is also shown. 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.

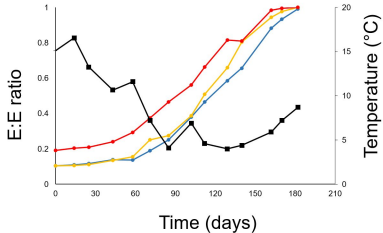
Fig. 4: Principal component analysis of seed traits (in red) and geographic and bioclimatic variables (in blue) across the latitudinal distribution of *Conopodium majus*.



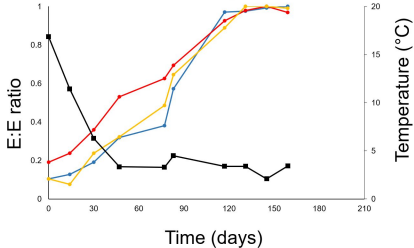
Legend



England

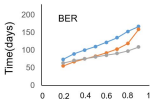


Norway

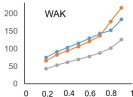


—●— BER
 —●— WAK
 —●— CHO
 —■— Mean temperature (°C)

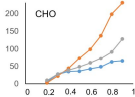
Norway



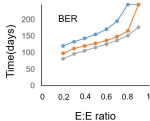
Norway



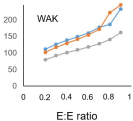
Norway



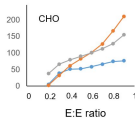
England



England



England

Predicted with Θ_b Predicted with Θ_c 

Inferred from logistic regression

