

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

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

3 Running title: Functional biogeography of embryo growth

4 **ABSTRACT**

- 5 1. Plant regeneration by seeds is driven by a set of physiological traits, many of which
6 show functional intraspecific variation along biogeographic gradients.
- 7 2. In many species, germination phenology depends on a germination delay imposed by
8 the need for post-dispersal embryo growth (a.k.a. morphological dormancy). Such
9 growth occurs as a function of environmental temperatures and shows base, optimum
10 and ceiling temperatures (i.e. cardinal temperatures or thermal thresholds). However,
11 the biogeographical variation in such thresholds appears not to have been tested at
12 continental scales.
- 13 3. Here we have used a thermal time approach and field experiments to assess
14 intraspecific variation in embryo growth thermal thresholds in the geophyte
15 *Conopodium majus* (Apiaceae) across its distribution from the Iberian Peninsula to
16 Scandinavia.
- 17 4. Thermal thresholds varied across the latitudinal gradient, with the estimated optimum
18 temperatures between 2.5 and 5.2 °C, ceiling temperatures between 12 and 20.5 °C
19 and base temperatures between -6.6 and -2.7 °C. Germination in the field peaked in
20 the months of January and February. The limiting factor for embryo growth was the
21 ceiling temperature, which was correlated with latitude and the bioclimatic environment
22 of each population. In contrast, the optimal and base temperature were independent
23 of local climate.

24 5. These results support that the thermal thresholds for embryo growth are a functional
25 ecophysiological trait driving seed germination phenology and seed responses to the
26 environment.

27 **KEYWORDS**

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

32 INTRODUCTION

33 The three aims of functional biogeography are to describe the distribution of functions along
34 environmental gradients and across spatial scales; to use this information to explain the
35 geographic distribution of organisms; and to predict their responses to environmental
36 changes using trait-based predictive models (Violle *et al.* 2014). A relevant aspect of plant
37 function that has been underutilized by biogeographical studies is the physiological thermal
38 control of plant reproduction (Bykova *et al.* 2012), and especially seed germination. The
39 temperature to which imbibed seeds are exposed affects their germination rate (Fernández-
40 Pascual, Mattana & Pritchard 2019). This phenomenon can be numerically described by the
41 “cardinal germination temperatures”, i.e., the optimum temperature (T_o), at which the
42 germination rate is maximal and the base (T_b) and ceiling (T_c) temperatures that are,
43 respectively, estimated to be the coldest and the warmest temperature at which the rate of
44 germination tends to zero. The measurement of these temperatures for a given species
45 enables prediction of its seed germination rate and germination success under different
46 temperatures (Orrù *et al.* 2012). Therefore, the cardinal temperatures are key parameters to
47 explain the contribution of regeneration thermal niches on species distributions and
48 responses to climatic changes (Maleki *et al.*, 2024).

49 In many species however, seed dormancy prevents germination even in the presence of
50 suitable conditions, so that the exposure of seedlings to unfavourable environments is
51 avoided and the timing of germination is matched to favourable regeneration windows
52 (Pausas *et al.* 2022; Lamont & Pausas 2023). A particular case of seed germination delay
53 occurs when the embryo, at the time of dispersal, is not completely developed and needs to
54 grow to a critical size before germination can occur; a trait known as post-dispersal embryo
55 growth or morphological dormancy (Baskin & Baskin 2004). As happens with germination,
56 temperature is a major environmental driver of post dispersal embryo growth (Baskin *et al.*

2000; Vandeloos, Bolle & Van Assche 2007; Vandeloos 2008; Phartyal *et al.* 2009; Vandeloos, Bolle & Van Assche 2009; Blandino *et al.* 2019), influencing the rate of development of the embryo, a mechanism that allows a precise timing of germination (Porceddu *et al.* 2017).

Biogeographical variation in several germination traits is well documented. For example, seeds from warmer sites have been shown to have lower minimum temperatures for germination at different geographical scales (Daws *et al.* 2004; Rosbakh & Poschlod 2015). The requirements for cold stratification can also vary according to the local climate: populations from habitats with longer winters require a longer period of cold stratification compared with populations from milder habitats (Allen & Meyer 1998). However, much less research has been dedicated to traits related to post-dispersal embryo growth. Mondoni *et al.* (2008) compared post-dispersal embryo growth between mountain and lowland populations of the temperate woodland forb *Anemone nemorosa* and found that, although embryo 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). 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 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

130 Axiovision 3.1.2.1 (Carl Zeiss Vision GmbH). The initial relative embryo length was measured
131 only for the seeds that stained red with the TZ, i.e., indicating viability; unstained seeds /
132 embryos were discarded. Relative embryo size was used because it describes the growth of
133 the embryo regardless the size of each seed. From each population 99 seeds were placed in
134 a controlled humidity room at 15% RH and left to dry. The dry seed weight of 99 seeds for
135 each population was measured using a precision scale.

136 ***Embryo growth in controlled temperature conditions***

137 From each population and treatment, 16 subsamples of 15 seeds each were randomly taken
138 and sown in separate, 8 cm diameter Petri dishes containing 1% agar-water substrate. Seeds
139 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
140 regime of 12 hours . Every 14 days one subsample from each population and treatment was
141 retrieved and the 15 seeds were placed for 24 hours in 1% TZ solution at 30°C in the dark,
142 after a slice of the seed coat was removed. From this subsample, the embryo and endosperm
143 length of 10 viable seeds was measured following further dissection of the seed. An E:E value
144 of 1 was assigned to all germinated seeds. Seed measurement was stopped when the seeds
145 ceased germinating. The experiment continued for 224 days, until all the 16 subsample
146 assessments were concluded.

147 ***Replication statement***

148 *Note for reviewers: this table is mandatory for submission to Functional Ecology*

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Population	Seed/embryo	9 populations x 16 scoring times x 6 incubation

		temperatures x 10 seeds/embryos
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149

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

151 The average E:E ratio of 10 seeds for each population x temperature x time combination was
 152 calculated. All the temperatures for the same population had the same initial E:E ratio at time
 153 = 0, while the maximum value was fixed at 1, after which the seed was able to germinate.
 154 Since the data followed a sigmoidal growth distribution, except the treatments at -2.5 °C, a
 155 logistic model was fitted to each population x temperature combination using the software
 156 OriginLab 9.0. The models of each population were bounded between the initial value of E:E
 157 for that population and 1. A linear model was fitted to the -2.5°C treatments. From the
 158 equation of the logistic and linear models, it was possible to calculate the time expressed in
 159 days (tr) at which each temperature x population combination would have reached the
 160 following deciles of relative embryo size: 0.3, 0.4, 0.5, 0.6 and 0.7. Deciles < 0.3 could not be
 161 calculated because they were under the initial E:E. Deciles > 0.7 were not calculated to keep
 162 the symmetry of the analyses regarding deciles of the population. For each treatment, the
 163 embryo growth rate was calculated as 1/tr.

164 For each population and decile, embryo growth rate was plotted against temperature. Each
 165 dataset was visually divided into sub-optimal and supra-optimal ranges, using the point with
 166 the highest value of 1/tr as the dividing point. Liner regressions were fitted separately to the
 167 sub- and supra-optimal ranges. The intersection with the temperature axis of the sub-optimal
 168 and supra-optimal regression are, respectively, the base (T_b) and the ceiling (T_c)
 169 temperatures; these estimates are the temperatures below and above which the embryo
 170 growth rate is projected to be zero. The optimal temperature (T_o), defined as the temperature

171 at which the rate of embryo growth is estimated to be fastest, is the x-coordinate of the
172 intersection point between sub-optimal and supra-optimal regressions. Then, for each
173 population, the cardinal temperatures (T_b , T_c and T_o) were averaged across all the deciles
174 calculated to define an average value of the population (ELLIS *et al.* 1986). The regression
175 lines of each decile were recalculated and forced to pass through a common origin defined
176 by the average T_b (for the sub-optimal regressions) or the average T_c (for the supra-optimal
177 regressions) (Hardegree 2006). For the three southernmost populations, only the cardinal
178 temperatures calculated for the relative embryo size of 0.4, 0.5 and 0.6 were used, because
179 it was not possible to fit a supra-optimal regression to the 0.3 decile.

180 The slopes of these new linear regressions were then taken as a reciprocal to estimate the
181 sub-optimal (θ_b) and supra-optimal (θ_c) thermal times for embryo growth. θ , expressed in
182 degree days ($^{\circ}\text{Cd}$), indicates the cumulative thermal time units above T_b (θ_b) or below T_c (θ_c)
183 that the seed must accumulate for the embryo to reach successive E:E deciles. For each
184 population, the deciles were plotted against θ_b and θ_c , expressed both as their value and as
185 the natural logarithm of the value, and linear regressions were fitted to the data. The
186 regressions fitted to θ and to $\log(\theta)$ were compared in each case by their R^2 (Hardegree
187 2006). The regression models with the highest R^2 were chosen to represent the rate of
188 embryo growth as a function of thermal time for each population. The R^2 of the models
189 obtained fitting embryo growth and log-normal ($\log ^{\circ}\text{Cd}$) were slightly higher than the R^2 of
190 the model obtained using normal distributed thermal times ($^{\circ}\text{C}$). The only exception was
191 constituted by the Spanish population of Central del Chorro (CHO), for which the best model
192 fit was obtained using the non-transformed thermal time values, thus describing a linear
193 increase of relative embryo size with accumulated heat.

194 ***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

220 data recorded by the loggers. To account for every temperature fluctuation during the day,
221 the thermal time was expressed in “°C 30 min” and the heat accumulated by the seed was
222 calculated for every 30 min temperature record. The difference (ΔT) between each
223 temperature record and the population T_o (averaged between deciles) was summed. When
224 the temperature was higher than the average T_c or lower than the average T_b the heat
225 accumulated was considered = 0 and the difference (ΔT) between each temperature record
226 and the T_o was summed.

227 The time necessary in the field to accumulate enough heat to reach the thermal time
228 necessary for each t_r was compared with the t_r estimated from the embryo growth data. The
229 time (in days) needed to sum enough heat to reach the θT_b and θT_c calculated in the model,
230 for each t_r decile ($t_{r \text{ model}}$) in each population was compared with the time needed by each
231 population to reach the same decile of relative embryo growth in the field ($t_{r \text{ field}}$). These
232 estimates were then graphically compared expressing the different t_r in function of E:E.

233 ***Relationship between embryo growth and germination***

234 Germination was scored for each independent sample before measuring the relative embryo
235 size and expressed as percentage of germinated seeds vs time. For each population, the
236 germination data for the treatments at 2.5 and 5°C were fitted with the Boltzmann equation
237 using the software OriginLab9. The other temperatures were not used because germination
238 was too slow. For each population, from the fitted Boltzmann equation the day to reach 50%
239 germination (t_{g50}) was calculated. The t_{g50} was then used to calculate the corresponding E:E
240 ratio at the same day using the logistic regression of the E:E data for the same treatment.
241 For each population, the average E:E ratio corresponding to the t_{g50} for germination at the
242 two temperatures used was displayed as the average E:E ratio for 50% germination in that
243 population. The average between all the populations represented the average for the species.

244 ***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 & 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. The ceiling temperature for embryo growth varies between 12.1 and 20.5 °C and has a strong negative correlation with latitude and precipitation. Species from northern populations, that are less likely to experience long exposure to high autumnal temperatures, have lower values; while the two southernmost populations, CHO and TRE, stand out for high T_c above 20 °C. Water stress is the main limiting factor for embryo development in these populations, that are exposed also to a shorter winter and a Mediterranean continental climate. The higher T_c can therefore be an adaptation to cope with higher daily fluctuations in temperatures that can prevent the embryo from growing during warmer and potentially drier days during late

autumn or early spring. Moreover, embryo growth (and the potential to germinate) under cold temperatures (close to 0°C) will enable the start of growth during winter and emergence under the snow to avoid drought, as has been suggested to be the case for other species able to grow in sub-alpine Mediterranean and sub-Mediterranean mountains (Fernández-Pascual, Jiménez-Alfaro & Bueno 2017).

The optimum temperature for embryo growth ranged between 2.5 and 5.2 °C (Table 2) and had a negative correlation with T_b , a phenomenon already observed for germination temperatures by Dürr *et al.* (2015). The second axis of our PCA ordination analysis can therefore be interpreted as reflecting the width of the suboptimal temperature range for embryo growth, i.e., the gap between T_b and T_o . The populations with the higher T_b (BAS, LEO and SCO) also have the lower T_o and therefore a narrower window of suboptimal conditions for embryo growth. Therefore, these populations are at greater risk of exposure to a reduced germination niche in the face of climate warming (Walck *et al.* 2011). These are also some of the populations at the milder and central points of the latitudinal gradient investigated, i.e. northern Spain and the British Isles.

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 estimated 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, Bai & Henderson 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*

367 *al.* 2006). In the case of *Conopodium majus*, that is a perennial, this strategy could however
368 offer some advantage at the southern range of its distribution, where summer drought can be
369 a recurrent issue, as already observed for Mediterranean subalpine species (Fernández-
370 Pascual, Jiménez-Alfaro & Bueno 2017). *Krascheninnikovia lanata* seeds show a positive
371 effect of seed size on the ability to germinate at sub-zero temperatures (Wang *et al.* 2006).
372 The authors demonstrate that bigger seeds had a higher concentration of sugars (glucose,
373 raffinose and sucrose) that probably lower the freezing point of the seed tissues. There are
374 no reports on sub-zero germination in Apiaceae but an optimal temperature for embryo
375 growth of 2 °C has already been described for *Heracleum sphondylium* (STOKES 1953) and
376 it is not unlikely that this species, or others from the same family, could present equally low
377 T_b for embryo growth. However, it is unlikely that such low temperatures do have a functional
378 ecological role in embryo growth in the field: our field collected data and averaged climatic
379 data from 1970-2000 for the collection sites show that such low average temperatures are
380 rare in the natural environment of *Conopodium majus*. Therefore, in the field it is likely that
381 embryo growth is possible throughout the winter season and is limited only by the higher
382 temperatures in autumn. In fact, results from the ordination analysis, showed that T_b is
383 independent from climatic and geographic factors and is not even correlated to seed size or
384 initial E:E ratio. Therefore, we can conclude that the limiting factor for embryo growth in
385 *Conopodium majus* is the ceiling temperature and its interaction with warmer temperatures
386 during the annual cycle.

387 CONCLUSION

388 *Conopodium majus* can be considered a model species for studying post-dispersal embryo
389 growth due to its fine regulation of embryo growth by temperature and the coincidence
390 between the temperature requirements for embryo growth and germination. To date only one
391 study is known to have developed thermal models of embryo growth in a species of the

392 Ranunculaceae family, *Aquilegia barbaricina* (Porceddu *et al.* 2017) and this work represents
393 the first attempt to develop such a model on a species from Apiaceae. The thermal models
394 developed in this study can be used to predict shifts in the species' temperature germination
395 niche caused by different climate change scenarios. The dependence of embryo growth on
396 a relatively low ceiling temperature means that warmer winter temperatures because of
397 climate change could compromise post dispersal embryo growth and thus negatively impact
398 the regeneration of *Conopodium majus*. However, *Conopodium majus* also shows potential
399 for adaptation to the climatic environment along its latitudinal distribution, as expressed by
400 the breadth of the temperature germination niche indicated by the cardinal temperatures of
401 each population. In fact, post-dispersal embryo growth in *Conopodium majus* shows an
402 intraspecific variability which is consistent with an ecological function in determining the
403 timing of seedling emergence and establishment. This highlights the potential of embryo
404 growth temperatures as a functional ecophysiological trait driving seed germination
405 phenology and seed responses to the environment.

406

407 **TABLES**

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

409

Populatio n	Countr y	Location	Latitude	Longitude	Elevation (m a.s.l.)
			60° 34'29.784"	4° 56' 53.627"	
HER	Norway	Herdla	N	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 Central del	42° 54' 26,62" N	5° 49' 25,87" W	1426
CHO	Spain	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

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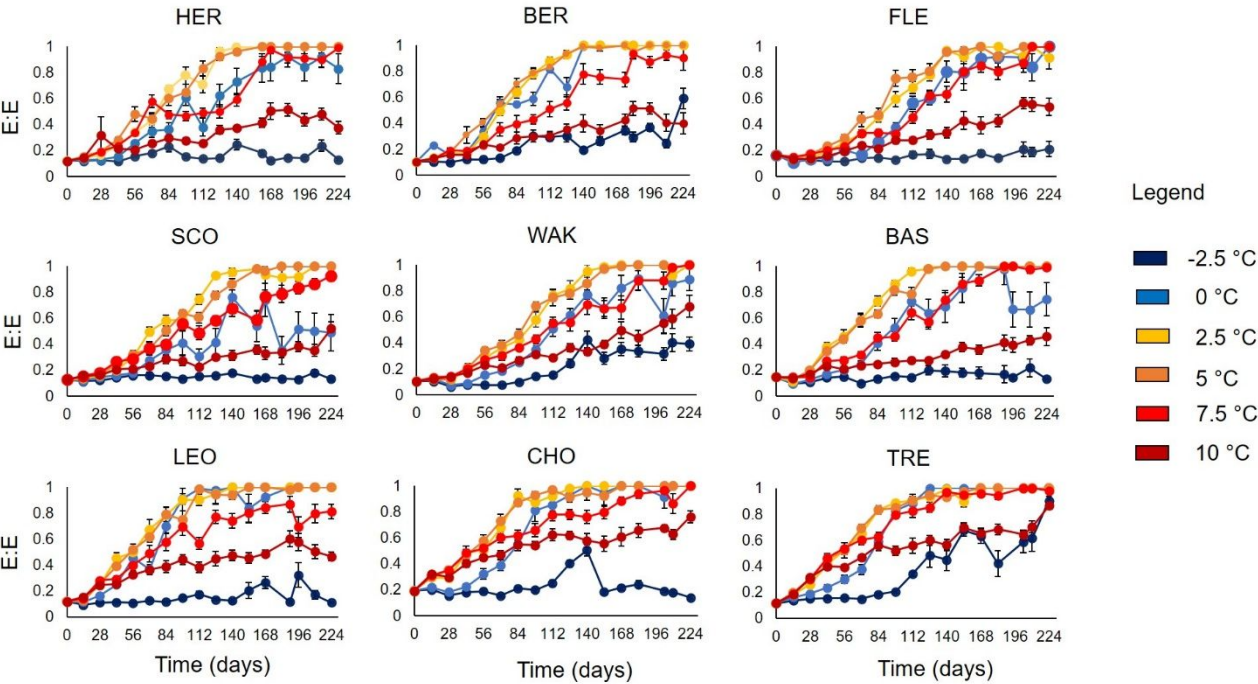
411 **Table 2:** Initial E:E , seed dry mass and cardinal temperatures averaged between deciles
412 (all as average \pm SD) in seeds of all populations of *Conopodium majus* studied. To have a
413 symmetric result around the middle value, when the lower deciles were excluded for being
414 too close to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

415

416

417 **FIGURES**



418

419 **Fig. 1:** Patterns of embryo growth (E:E ratio) for all the seed populations of *Conopodium*

420 *majus* and all temperatures tested. Each data point represents the average of ten replicates

421 (\pm SE).

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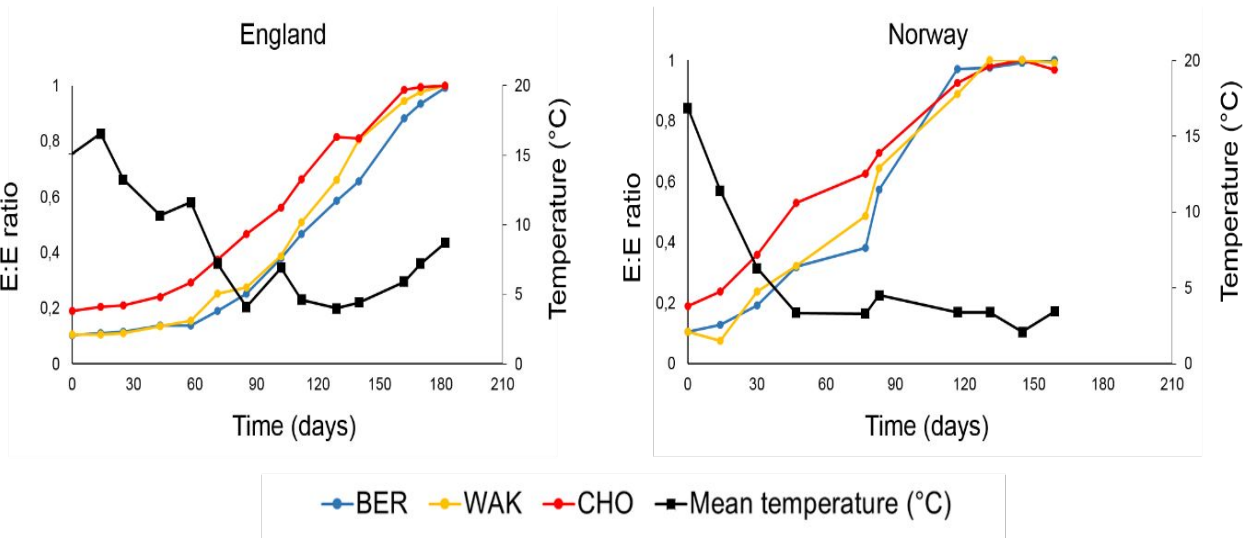


Fig. 2: Embryo growth in the field for buried seeds of *Conopodium majus*. Each data point represents the average E:E ratio for 20 seeds of three representative populations: CHO for the southern edge of the distribution range, WAK for the middle and BER for the northern; soil temperature is also shown.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

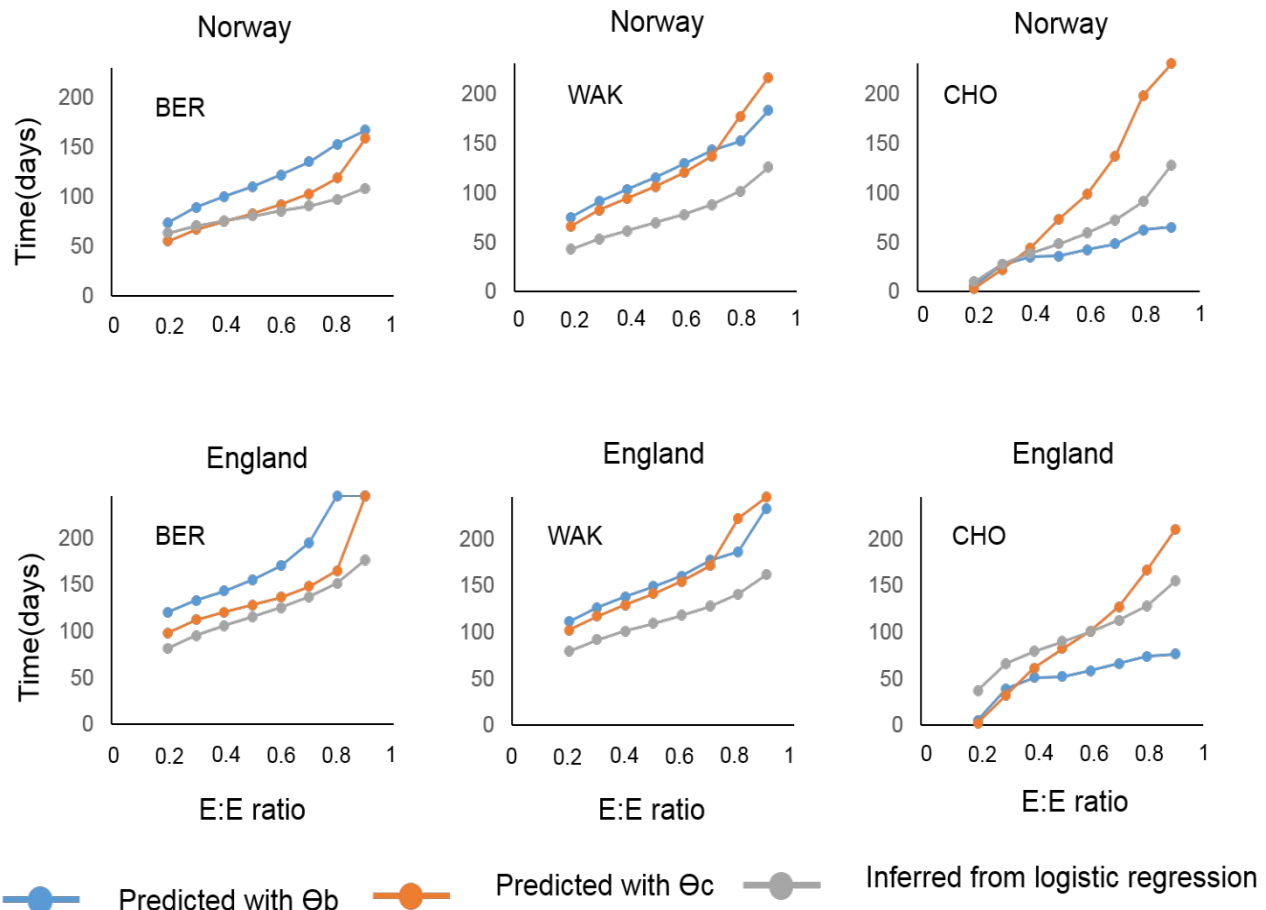


Fig. 3: Time (in days) required by each population of *Conopodium majus* seeds in each field location to reach different deciles of E:E ratio according to: 1) interpolation from the logistic regression of embryo growth in the field (grey line); 2) ΘT_b (blue line); and 3) ΘT_c (orange line) obtained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

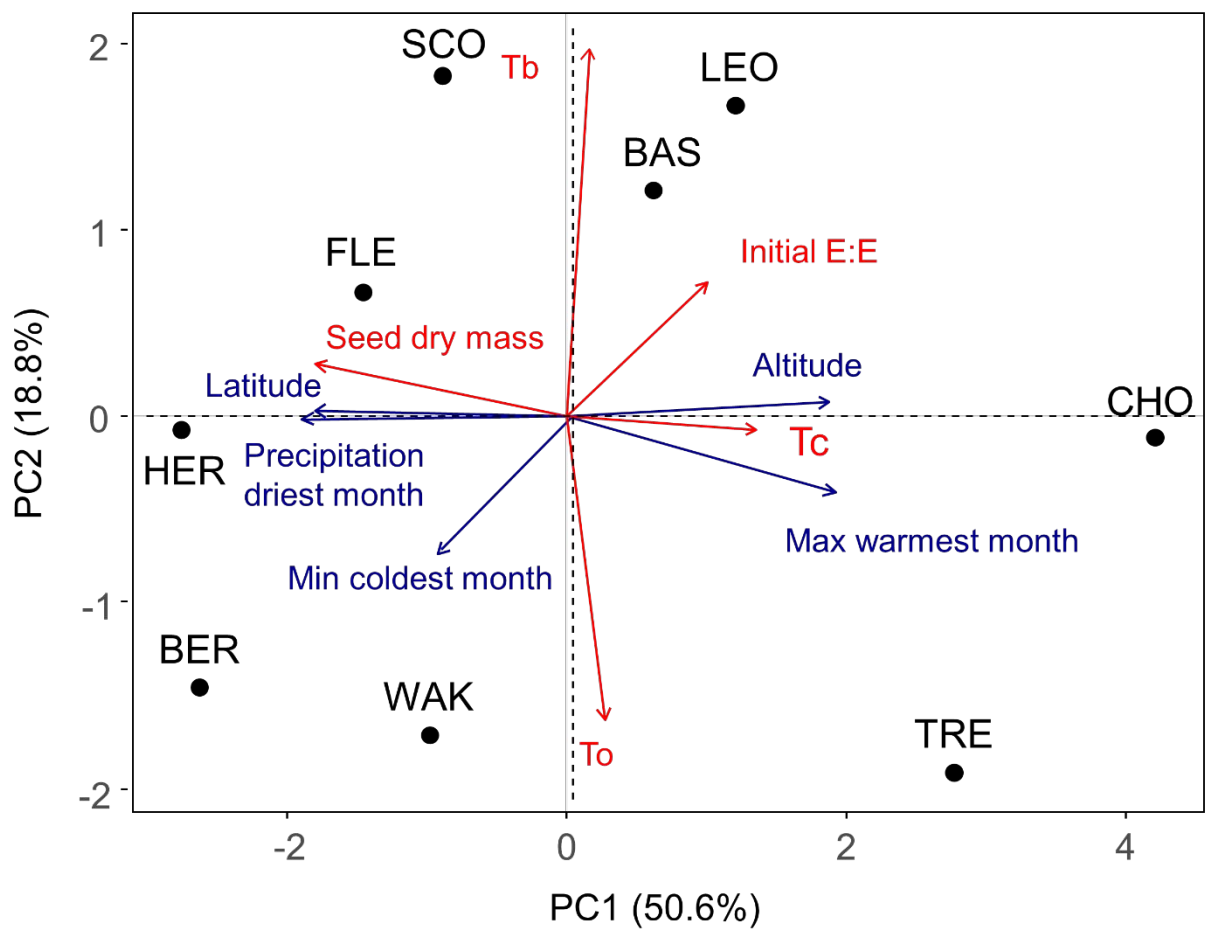


Fig. 4: Principal component analysis of seed traits (in red) and geographic and bioclimatic variables (in blue) across the latitudinal distribution of *Conopodium majus*.

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