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Winter temperature predicts prolonged diapause in pine processionary moth across its geographic range

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Prolonged diapause occurs in a number of insects and is interpreted as a way to evade adverse conditions. The winter pine processionary moths (*Thaumetopoea pityocampa* and *Th. wilkinsoni*) are important pest of pines and cedars in the Mediterranean region. They are typically univoltine, with larvae feeding across the winter, pupating in spring in the soil and emerging as adults in summer. Pupae may, however, enter a prolonged diapause with adults emerging one or more years later. We tested the effect of spatial variation in winter temperature on the incidence of prolonged diapause, using a total of 64 individual datasets related to insect cohorts over the period 1964-2015 for 36 sites in 7 countries, covering most of the geographic range of the species. We found high variation in prolonged diapause incidence over the species' range. Insect cohorts exposed to average winter temperatures lower than 0°C were associated with higher prolonged diapause incidence than cohorts exposed to intermediate temperatures. Prolonged diapause may represent a risk-spreading strategy although it is associated with high mortality because of a longer exposure to mortality factors, desiccation, and energy depletion. Climate change, and in particular the increase of winter temperature, may reduce the incidence of prolonged diapause at the colder sites whereas it may increase it at the warmer ones, with consequences on the population dynamics.

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Running head: Prolonged diapause ecology

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Abstract

Prolonged diapause occurs in a number of insects and is interpreted as a way to evade adverse conditions. The winter pine processionary moths (*Thaumetopoea pityocampa* and *Th. wilkinsoni*) are important pest of pines and cedars in the Mediterranean region. They are typically univoltine, with larvae feeding across the winter, pupating in spring in the soil and emerging as adults in summer. Pupae may, however, enter a prolonged diapause with adults emerging one or more years later. We tested the effect of spatial variation in winter temperature on the incidence of prolonged diapause, using a total of 64 individual datasets related to insect cohorts over the period 1964-2015 for 36 sites in 7 countries, covering most of the geographic range of the species. We found high variation in prolonged diapause incidence over the species' range. Insect cohorts exposed to average winter temperatures lower than 0°C were associated with higher prolonged diapause incidence than cohorts exposed to intermediate temperatures. Prolonged diapause may represent a risk-spreading strategy although it is associated with high mortality because of a longer exposure to mortality factors, desiccation, and energy depletion. Climate change, and in particular the increase of winter temperature, may reduce the incidence of prolonged diapause at the colder sites whereas it may increase it at the warmer ones, with consequences on the population dynamics.

Introduction

Although diapause is recognized as a common strategy among insects to overcome unfavourable periods, prolonged diapause spanning more than one year remains hardly studied (Danks, 1987; Soula and Menu, 2005). In general, diapause represents a break in development maximising the chance to survive predictably adverse conditions (Tauber et al., 1986). By contrast, the expression of prolonged diapause varies within a population or cohort and is generally considered as a way to spread the risk due to variably unfavourable conditions amongst years (Menu et al., 2000). If organisms can use a signal to predict an unfavourable condition, which affects fitness consequences, phenotypic plasticity evolves (Pigliucci, 2001). In contrast, if any unfavourable condition can't be measured or predicted using any signal, a genotype can increase the fitness of the organisms employing the tactic of "risk-spreading" (den Boer, 1968) or "bet hedging" (Slatkin, 1974) . In an uncertain fluctuating situation, bet hedging tactic wins over other tactics because it avoids worst-case scenarios (i.e., prolonged diapause in the pine processionary moth may avoid the extinction of an entire cohort in the event of an extremely cold winter) and reduces between-individual variance in fitness by increasing their geometric mean fitness in the long run (Seger and Brockmann, 1987; Starrfelt and Kokko, 2012). Prolonged diapause, however, can cause increased mortality (Sims, 1983), likely due to longer exposure to adverse factors and enemies, as well as reduced performance (Matsuo, 2006) related to extended energetic expenditure (Hahn and Denlinger, 2007).

If resource availability or some other essential environmental factors show a strong multiannual cyclic component, all individuals in a population may enter in prolonged diapause (Powell, 1974). Cohort splitting (a mixture of individuals with and without prolonged diapause) may occur in a relatively "fine grained" environment. Prolonged diapause can be induced either

density dependently or density independently. Whether current or parent generation is in control of diapause also determines the outcome of a diapause type. If the diapause is independent of population density and individuals are in control of the fate, chance of occurring prolonged diapause is limited. But, when parents control the fate of offspring in a density independent diapause occurrence, prolonged diapause becomes useful. In this case, mixture of offspring with and without prolonged diapause should be same for all the females in a population. The mixture should reflect highest fitness in the particular environment. If induction of diapause is density dependent and either individuals or parents are in control, prolonged diapause should occur. Females in this case may have different mixture of individuals with and without prolonged diapause (Hanski, 1988).

In Europe, Middle East, and Northern Africa, the winter pine processionary moth is represented by two sister species, *Thaumetopoea pityocampa* and *Th. wilkinsoni* (Lepidoptera, Notodontidae), which are pests of conifers and a threat to human and animal health because of urticating setae (Roques, 2015). Larvae feed during winter and generally adopt a univoltine life cycle, with the exception of Corsica where *Th. pityocampa* is strictly semivoltine (Geri, 1983). Temperature influences the duration of both larval and pupal stages (Démolin, 1969; Berardi et al., 2015; Robinet et al., 2015). Completing the development in silk tents, larvae leave the host tree in a typical head-to-tail procession in search of a pupation site in the soil. Burying 5–20 cm into the soil, they spin a cocoon and enter a phase of prepupal diapause, with the duration varying from 20 to 50 days (Salman et al., 2018). Breaking the prepupal diapause, they turn into pupae and enter pupal diapause, with the length varying from 1 month at high elevation/latitude to 5 months at low elevation/latitude. This variation in the length of pupal diapause occurs to match the local conditions for optimal development (Démolin, 1969). Moths generally emerge in

summer, but a variable fraction of the individuals may postpone their emergence and enter pupal prolonged diapause that may span as long as 9 years in *Th. wilkinsoni* (Halperin, 1990) and 8 years in *Th. pityocampa* (Salman et al., 2016).

The mechanisms of prolonged diapause induction and maintenance in the winter pine processionary moth are unknown. However, a phenology-based model suggests that prolonged diapause in the pine processionary moth is a developmental strategy to cope with adverse temperature conditions, at both ends of the scale (Démolin, 1969). Additionally, factors such as soil moisture (Torres-Muros et al., 2017), food quality, population density, and natural enemies (Geri, 1983) have been invoked to be linked to prolonged diapause, although without clear support. Battisti et al. (2005) have identified the temperature of the so-called 'cold period' (December-January-February) as key predictor for larval survival, and thus for the successful establishment of the insect in the expansion range associated with climatic change at both high elevation and latitude.

Here, to test the temperature hypothesis reported above (Démolin, 1969), we review all the available evidence of prolonged diapause across the range of *Th. pityocampa* and *Th. wilkinsoni*, keeping into account the genetic structure of the group (Kerdelhué et al., 2009; El Mokhefi et al., 2016) and excluding Corsica populations that are strictly semivoltine. We specifically test the effect of winter temperature as a driver of prolonged diapause in this species, and if prolonged diapause implies a cost in terms of mortality. We finally discuss the role of climate change on prolonged diapause and the potential effects on the population dynamics of this important pest.

Materials and methods

(a) Data collection

Published and unpublished data were retrieved from scientific databases and from institutional reports provided in the supplementary material (Supplemental file Dataset_S1). Available documents (initial n=42) were screened. Because we predicted winter temperature to be a mechanism causing PD, we only selected studies that included the following criteria: (i) the total number of individuals, (ii) insect collected using one or more of the five methods described below, (iii) the number of moths emerged in the year of collection and in the subsequent year(s), (iv) the number of dead larvae/pupae given for a specific year of sampling (namely a cohort), and (v) the average air temperature of the cold period (December-January-February) of the studied years. Information was obtained for seven countries from a total of 13 documents and 36 sites (Figure 1, Table 1, Supplemental file Dataset_S1). These sites belong to 6 out of 11 genetic subclades of the pine processionary moth described by Kerdelhué et al. (2009) and El Mokhefi et al. (2016) (Figure 1) and cover most of the climatic range of the species (Roques, 2015). The data span a latitude from 31.3° N to 47.8° N and longitude from -7.9° to 35.2° (Figure 1), and an elevation from 10 to 1910 m. For 11 sites, more than one year of observations was available, giving a total of 64 individual datasets on the occurrence of prolonged diapause.

For the year(s) of sampling, the temperature of each site was collected from the source document (n = 41) or from records of the nearest weather station (n = 23) (Supplemental file Dataset_S3). As average monthly temperature of the cold period (December- January-February) (Battisti et al., 2005; Roques, 2015) was available for all datasets, it was used for the analysis. For those sites with both average and minimum temperature, the correlation between the two

133 predictors was very strong (R^2=0.94), indicating that the choice of the metric did not affect the
134 results.

135 The mortality percentage was calculated as the number of individuals that did not
136 produce moths with respect to the initial number of individuals in the cohort. The prolonged
137 diapause percentage was calculated as the number of individuals that emerged over the year(s)
138 following the year of pupation, or that were still alive when the experiment was over one or more
139 years after pupation (emerged after prolonged diapause + living pupae at the last check), with
140 respect to the total number of emerged or living individuals (emerged univoltine + emerged after
141 prolonged diapause + living pupae at the last check).

142 Studies spanned an extended period (from 1964 to 2015) and geographic locations
143 (Figure 1, Table 1, Supplemental file Dataset_S1). Five major collection and rearing methods
144 were used. (i) Mature larvae in the pupation procession on the ground were followed in the field.
145 Pupation sites of the larvae were marked and flagged. When the individuals pupated, pupae were
146 dug out of the soil and taken to laboratory and kept in single vials for future observations. (ii)
147 Mature larvae in pupation procession were collected in the field and forced to pupate in pots.
148 After the emergence of the 1st year, leftover alive pupae were kept in vials for further observation
149 in the following year(s). (iii) Mature larvae were intercepted on the tree stems during their way
150 down to the ground with collar traps. Larvae were forced to pupate either in bags or pots, both
151 filled with soil, on site or at laboratory. After the pupation, the pupae were individually kept in
152 single vials and observations made. (iv) Tents full of mature larvae were collected in the field.
153 They were kept either in covered pots filled with soil or in cages with appropriate mesh netting,
154 individually or in group. After the moths emerged, pots/cages were checked, outcome recorded,
155 and observations continued with leftover alive pupae undergoing prolonged diapause. (v) Large

156 cages were constructed in the field and filled with tents of 4th or 5th instar larvae. Larvae were
157 provided with fresh pine branches and left to feed needles *ad libitum*. Upon feeding, larvae
158 pupated in the soil. Observations were made until emergence was not observed for three years.
159
160 *(b) Data analysis*
161 To test the effect of temperature on prolonged diapause incidence, we used a linear mixed-effect
162 model (Supplemental file Dataset_S2). The model included the linear, quadratic and cubic terms
163 of temperature as fixed effects and site nested within genetic subclade as random effects. We
164 omitted one data point using the Cook's distance (value >5). This very influential point was
165 related to the maximum observed mean temperature. The consideration of subclade and site as
166 random effects accounted for the biological and spatial dependence in the data. It is important to
167 stress that our model tested primarily the effect of spatial variation in winter temperature across
168 the species range, and only secondarily the inter-annual variation in temperature within the sites.
169 The analyses were run using the *nlme* package in *R* (Supplemental file Dataset_S3) (R Core
170 Team 2016). A model with the same random structure was used to test the association between
171 the incidence of prolonged diapause and pupal mortality.
172
173
174 **Results**
175 Prolonged diapause occurred in all genetic subclades of *Th. pityocampa* and *Th. wilkinsoni* for
176 which data were available (Figure 2a). All the 37 sites showed at least one year with occurrence
177 of prolonged diapause, and only 5 out of 65 individual datasets or cohorts did not show
178 occurrence of prolonged diapause. The incidence of prolonged diapause in the first year after

179 pupation varied greatly among sites and cohorts within sites. The mixed model indicated that
180 temperature affected the incidence of prolonged diapause in a non-linear fashion, i.e. linear
181 ($t_{1,25}$= -5.481, P<0.001), quadratic ($t_{1,25}$= 3.687, P=0.001) and cubic terms ($t_{1,25}$= -2.395,
182 P=0.025) were significant. Cold winters (temperature of December-January-February below
183 0°C) as well as warm winters (temperature above 10°C) resulted in high prolonged diapause
184 incidence, while prolonged diapause was less frequent at temperatures between 0 and 10°C,
185 irrespective of subclades (Figure 2a). However, the effect at higher temperatures was less
186 pronounced than that at low temperatures. Actually the 5 datasets with no occurrence of
187 prolonged diapause and almost all those with less than 25% of prolonged diapause were
188 observed at temperatures between 0 and 10°C (Figure 2a).
189 Pupal mortality was positively and significantly correlated to prolonged diapause
190 incidence (Figure 2b). Mortality varied greatly among sites and cohorts within sites. It reached
191 the highest values at both cold (lower than 0°C) and warm (higher than 10°C) winter sites,
192 indicating that cohorts with high incidence of prolonged diapause are more likely to die than
193 those with low incidence of prolonged diapause. In general, mortality rates lower than 25% were
194 associated with low incidence of prolonged diapause, especially in the subclade Rest of Europe.
195 The mortality factors were not systematically addressed in each study, so it was not possible to
196 analyse for these effects.
197
198
199 **Discussion**
200 Winter minimum temperature experienced by larvae was found to be a reliable predictor of the
201 prolonged diapause incidence in the processionary moth pupae, as prolonged diapause tends to

202 increase at both lower and upper ends of the temperature range, although a large variation is
203 observed throughout. The adaptive value of prolonged diapause in this species can be seen at
204 both ends of the temperature range, but for different reasons. At colder sites and years, winter is
205 directly challenging larval survival that depends essentially on suitable feeding conditions
206 (Battisti et al., 2005). Prolonged diapause in this case dilutes the risk of being exposed to
207 unfavourable conditions associated with cold weather among years (Danks, 1987). At warmer
208 sites and years, summer temperatures can impair the development of eggs and young larvae as
209 upper lethal thresholds can be reached (Halperin, 1990; Santos et al., 2011). The relationship
210 with prolonged diapause in pupae is in this case indirect, as winter temperature works as a proxy
211 of another thermal stressor that could become limiting later in the year when moths would
212 emerge and lay eggs. Thus, entering prolonged diapause at the southern edge of the range would
213 also allow risk spreading through time and contribute to persistence of populations (Hanski,
214 1988). Change of temperature within a few degrees may drastically change the survival rate of a
215 population. This is particularly a problematic for the pine processionary moth in the range edge
216 where temperature is limiting for their survival and spread beyond. In such conditions,
217 temperature may become more limiting any year. Therefore, a certain fraction of individuals
218 always enters prolonged diapause, even at a moderate temperature generally deemed harmless
219 for growth and survival. Had there been any fixed and certain cyclic component available with
220 which pine processionary moth could predict the extreme event of temperature, they would have
221 evolved a fixed life cycle using a fixed number of years with prolonged diapause (as might have
222 happened for the semivoltine population of pine processionary moth in Corsica, Geri 1983).
223 However, unavailability of such component leaves the option of stochastic phenotype switching
224 with frequency of fate depending on the temperature.

225 Diapause duration could not be tested in this study because most reports did not survey
226 the emergence of the moths for more than 2 years. As durations up to 8-9 years have been found
227 at a few sites (Halperin, 1990; Salman et al., 2016), it could be expected that the intensity of the
228 winter stress would be proportional to the duration of prolonged diapause, causing a longer
229 dilution of the risk in the most severe conditions. It could be also expected that high density of
230 larvae would result in higher incidence of prolonged diapause, as damaged trees may result
231 unpalatable for the larvae of the following generations (Battisti, 1988). This sort of density
232 dependent prolonged diapause would work with different stress mechanism than abiotic stresses,
233 e.g., temperature. Perhaps, the higher the density of larvae, the higher the unpalatability of
234 needles, the longer would be the diapause (Salman et al., 2016). Unfortunately, many studies on
235 prolonged diapause examined here did not report the insect density at the time of the sampling.
236 Other putative factors (such as disease, predation and parasitism) may respond to density
237 variation and thus prolonged diapause can be seen as an escape in time from mortality factors
238 (Hanski, 1988). Most parasitoids and predators of the pine processionary moth are univoltine and
239 cannot track the individuals entering prolonged diapause (Battisti et al., 2000).

240 Irrespective of the temperature conditions associated with prolonged diapause occurrence,
241 pupal mortality increased with prolonged diapause incidence likely because of a longer exposure
242 to chronic mortality factors (Sims, 1983), desiccation and depletion of the energy reserves (Hahn
243 and Denlinger, 2011). The higher mortality risks associated with prolonged diapause challenge
244 the hypothesis that prolonged diapause of processionary moth pupae represents a diversifying
245 risk-spreading strategy for the species. However, more attention should be given to climatically
246 non-typical years when benefits of such a strategy would be expected, ultimately securing the
247 long term persistence of populations (Menu et al., 2000). Prolonged diapause could also limit the

248 risks of local extinction after the generally high mortality observed at outbreak density (Salman
249 et al., 2016; Tamburini et al., 2013; Li et al., 2015). Survival, rather than reproduction, is more
250 important in a declining population, and is secured by entering prolonged diapause. However,
251 increased frequency of prolonged diapause helps declining population growth, or population
252 decrease itself causes increased frequency of prolonged diapause is not clear (Hanski, 1988). In
253 addition, the phenology of the pine processionary moth is severely constrained by climatic
254 factors (Robinet et al., 2015), and it could be that in certain years the required physiological
255 conditions to complete univoltine development are not met, thus leaving no alternative to
256 prolonged diapause.

257 Climate change may affect prolonged diapause in different ways. As winter is
258 experienced in two different life stages, the growing larva and the diapausing pupa, it is likely
259 that their respective vulnerability to stressful conditions differ. Larvae feeding on trees are
260 exposed to low, fluctuating temperatures, but are capable of selecting suitable microclimates and
261 have a silk tent regulating temperature (Battisti et al., 2005), while pupae in the soil are buffered
262 against temperature extremes, but passive. In addition, both stages have complex interactions
263 with precipitations and moisture, and related pathogenic agents (Torres-Muros et al., 2017).
264 Observations carried out between 1999 and 2016 in the coldest edge of the species' geographical
265 range indicated that prolonged diapause incidence markedly decreased as a consequence of
266 climate warming (Salman et al., 2016). Such changes in prolonged diapause, and related
267 mortality, have to be taken into consideration when modelling population dynamics and range
268 expansion, since prolonged diapause can alter the success of a population in a given area (Li et
269 al., 2015). For example, a reduction of prolonged diapause incidence would make predictions

easier because it will emphasize the role of density-dependent factors, which are important in this species (Tamburini et al., 2013; Toïgo et al., 2017).

Conclusion

Our study has shown winter temperature variation across most of the species range to be an important extrinsic factor for geographic variation in prolonged diapause incidence. Both low and high winter temperature are conducive to high frequency of prolonged diapause in the pupae, likely because different avoidance mechanisms (such as disease, intraspecific competition, predation, parasitism) of lower and upper lethal thresholds, respectively. A number of other factors have to be considered, however, for a better understanding of the physiological mechanism regulating prolonged diapause in the pine processionary moth. Namely, the population density and the nutritional condition of the larvae before pupation may reveal the role of prolonged diapause in the population dynamics of this important defoliating pest.

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375 **Captions**

376

377 Table 1:

378 Distribution of the 37 study sites among genetic clades and subclades of pine processionary

379 moths according to Kerdelhué et al. (2009) and El Mokhefi et al. (2016). The time period of

380 sampling and the pine host species are also given.

381

382 Figure 1:

383 Sites used for the analysis of prolonged diapause at the pupal stage, indicated with coloured dots

384 within each species and subclade of the pine processionary moth (a), and tree of genetic structure

385 based on Kerdelhué et al. (2009) and El Mokhefi et al. (2016) (b). Data were not available for the

386 subclades indicated in black.

387

388 Figure 2:

389 (a) Relationship between the daily mean temperature during the cold period of the larval

390 development (December, January, and February) and the percentage of prolonged diapause in the

391 cohorts of pupae originating from larvae experiencing those temperatures. The fitted line

392 represents predictions of a mixed model including subclade and site as random effects (Diapause

393 $= 80.39 - 28.69\ Temp + 3.96\ Temp^2 - 0.15\ Temp^3$, all terms $P<0.05$, $n=64$).

394 (b) Relationship between the percentage of individuals entering prolonged diapause and the

395 pupal mortality observed in the first year of prolonged diapause. The fitted line represents a

396 prediction of a mixed model including subclade and site as random effects (Mortality = 37.88 +

397 0.364 Diapause, $P<0.001$, $n=62$).

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Table 1 (on next page)

Summary list of the study sites

Distribution of the 37 study sites among genetic clades and subclades of pine processionary moths according to Kerdelhué et al. (2009) and El Mokhefi et al. (2016). The time period of sampling and the pine host species are also given.

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Clade	Subclade	No. of sites	Years	Host plant
Th. pityocampa	Rest of Europe	14	1971-2015	*Pinus brutia* *P. halepensis* *P. nigra* *P. pinaster*
Th. pityocampa	N/W Algeria, N Morocco	13	1988	*P. halepensis*
Th. pityocampa	S Algeria, S Morocco	3	1988-1995	*Cedrus atlantica* *P. halepensis*
Eastern North Africa	N/E Algeria, Tunisia	4	1983	*P. halepensis*
Th. wilkinsoni	Israel, Lebanon, E Turkey	3	1964-1970	*P. halepensis*

3
4

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Figure 1(on next page)

Location of the study sites in relation to the distribution of the pine processionary moth

Sites used for the analysis of prolonged diapause at the pupal stage, indicated with coloured dots within each species and subclade of the pine processionary moth (a), and tree of genetic structure based on Kerdelhué et al. (2009) and El Mokhefi et al. (2016) (b). Data were not available for the subclades indicated in black.

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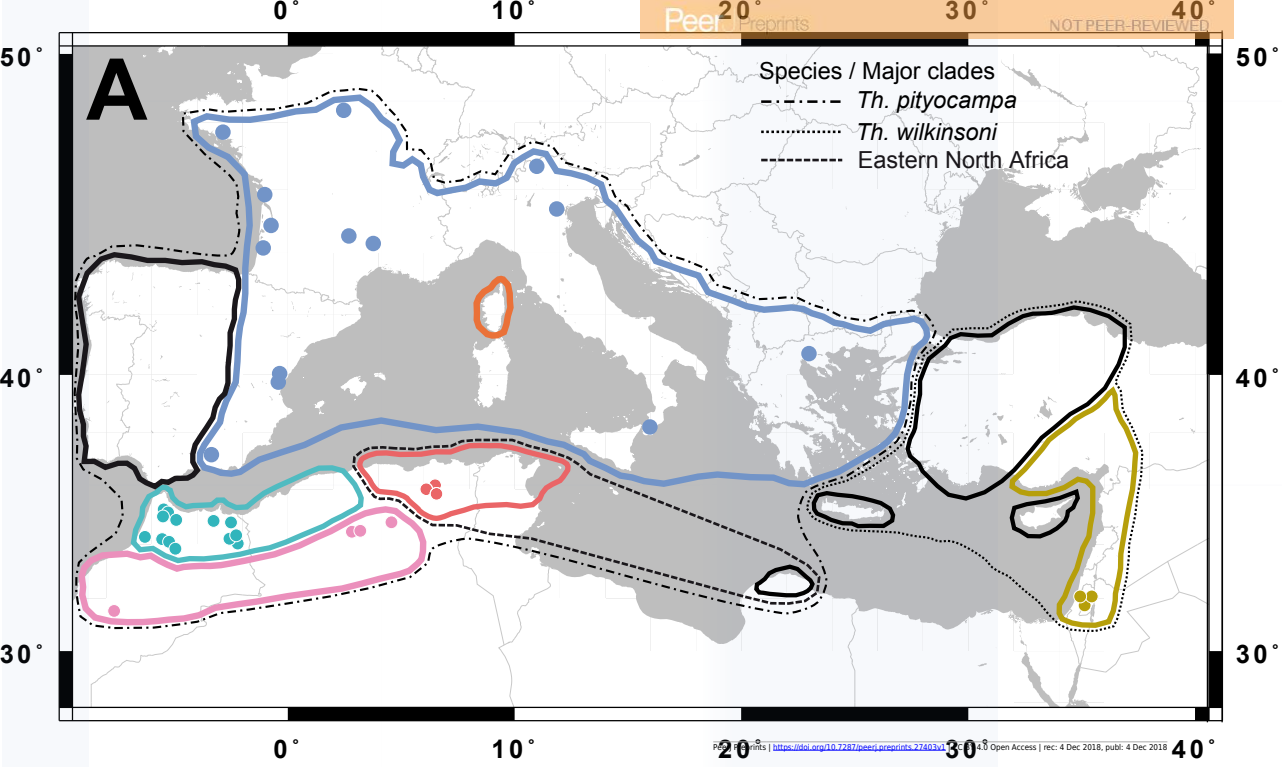

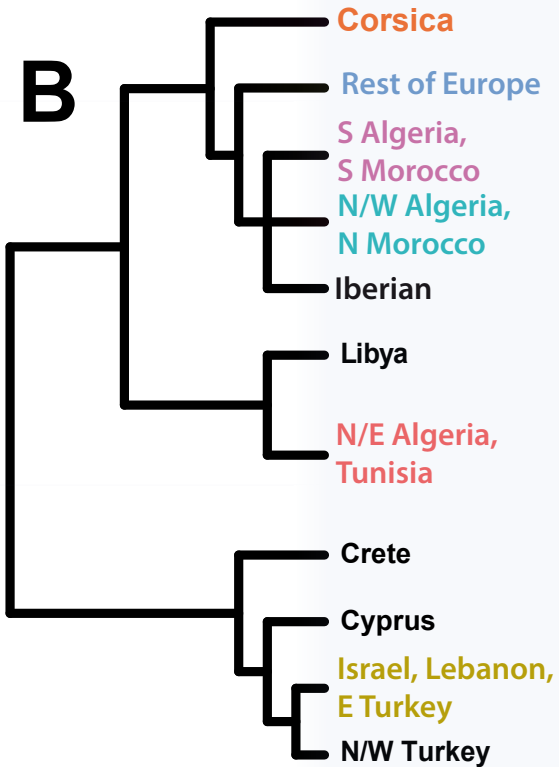

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Figure 2

Variation of prolonged diapause rate in relation to winter temperature and relationships between prolonged diapause and pupal mortality

(a) Relationship between the daily mean temperature during the cold period of the larval development (December, January, and February) and the percentage of prolonged diapause in the cohorts of pupae originating from larvae experiencing those temperatures. The fitted line represents predictions of a mixed model including subclade and site as random effects (Diapause = 67.02 - 12.50 Temp + 0.85 Temp^2, both terms P<0.001, R^2=0.32, n=65).

(b) Relationship between the percentage of individuals entering prolonged diapause and the pupal mortality observed in the first year of prolonged diapause. The fitted line represents a prediction of a mixed model including subclade and site as random effects (Mortality = 37.88 + 0.364 Diapause, P<0.001, R^2=0.18, n=65).