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Complex drivers of phenology in the pine processionary moth: lessons from the past

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Running title

Drivers of phenology in the pine processionary moth

ABSTRACT

1. Climate change affects the life cycle of many species. Yet, responses to yearly variation of weather can either help species track optimal conditions or be maladaptive.

2. We analysed phenological data of 46,479 pine processionary moths (*Thaumetopoea pityocampa*) during 15 years along an altitudinal gradient in Southern France. These larvae were sampled in situ and allowed to pupate in a common garden at lower elevation.

3. Individuals originating from higher elevation emerged earlier than those sampled at low elevation, which suggests local adaptation. Yearly variations in temperature also affected phenology. Warm springs caused an earlier adult emergence, while autumn temperatures had an opposite effect. Environmental cues could thus induce contradictory plastic responses.

4. Synchronization mechanisms were identified. Variability in the duration of the pupal phase is a key parameter to synchronize adult emergence in spite of different larval development rates that only marginally influenced emergence dynamics. Semivoltine individuals experiencing prolonged diapause were synchronized with univoltine individuals emerging the same year.

5. These data highlight some contradiction in the effect of spatial vs. temporal variations of temperature on adult emergence. This suggests that phenological responses to the current climate change cannot easily be anticipated by space-for-time substitution designs.

Keywords: temperature; elevation gradient; historical data; diapause; synchrony; *Thaumetopoea pityocampa*

INTRODUCTION

Phenology, the timing of life cycle events, is likely driven by a combination of short-term plastic and long-term evolutionary responses to environmental variations (Briscoe *et al.*, 2012; Robinet *et al.*, 2015). Widely distributed species encounter varying climatic conditions and ecological pressures, and local phenological adaptations optimize resource use and minimize risks encountered by each life stage (Abarca & Lill, 2019). It is critical that local adult emergence is synchronised to favour mate finding and increase reproductive success, in particular for univoltine species with short adult lifespans. The phenology of many species is also affected by yearly temperature variations, but the direction and strength of these responses can be very different between species. A recent study (Maurer *et al.*, 2018) using data from 215 moth species between 1895 and 2013 showed that species-specific phenological shifts induced by yearly variation ranged from a delay of 10.6 days to an advance of 10.3 days per degree Celsius. Determining if the relationship between temperature and phenology is closely matched over space and time would help develop our understanding of the evolution of phenology. It would also be useful to anticipate how species may respond to climate change, and determine if species are able to track the optimal conditions via plasticity (Roy *et al.*, 2015). Indeed, maladaptive shifts in phenology can expose sensitive life stages to detrimental environmental conditions such as frost or drought (Stewart *et al.*, 2020). Historical data are highly valuable and provide baseline phenological information useful in the context of climate change (Fenberg *et al.*, 2016).

The pine processionary moth (PPM) *Thaumetopoea pityocampa* (Denis & Schiff.) (Lepidoptera: Notodontidae) is distributed over a large part of the Western Mediterranean basin, from North Africa to Libya on the southern rim and from Portugal to Western Turkey in the northern part (Ipekdal *et al.*, 2020; Kerdelhué *et al.*, 2009). PPM is typically a univoltine winter-feeding insect with summer adult emergence. Adults emerge in early summer at colder high-elevation sites and in late summer at warmer sites. However, seasonal life history varies considerably both locally and regionally, and some proportion of the population can experience prolonged pupal diapause of one to several years (Salman *et al.*, 2019; Salman *et al.*, 2016). Such individuals are referred to as *semivoltine* individuals, as opposed to the *univoltine* ones which emerge as adult a few weeks after pupation. Adults mate immediately and die within 24 to 48h. Egg masses are laid on pine needles. After ca. a month of embryonic development, L1 larvae hatch and feed on the 1-year-old needles of their coniferous host. Larvae remain gregarious throughout their development across autumn and winter, and spin a typical white silken tent in which caterpillars shelter. The L5 larvae from a given nest leave the tree all together in late winter or early spring in a typical head-to-tail procession in search of an underground pupation site. Once buried, the larvae undergo a pre-pupal followed by a pupal phase, and experience an obligate diapause that can vary in duration, until univoltine adults emerge the following summer (Berardi *et al.*, 2015). The life cycle of the pine processionary moth is shown in Fig. 1. The PPM has been strongly affected by climate change and its northern and altitudinal expansions in Europe are well documented (Battisti *et al.*, 2005; Robinet *et al.*, 2014; Roques *et al.*, 2015).

- Figure 1 -

Spatial tuning of phenology is relatively well understood in this species. It can be linked to particular features of the life cycle coupled with life history traits (Huchon & Démolin, 1970; Robinet *et al.*, 2015). Briefly, the main thermal constraints influencing phenology are (*i*) the vulnerability of the first instars to high summer temperatures; (*ii*) the vulnerability of the second instars to early autumnal frosts and (*iii*) the vulnerability of late instars to extreme low winter temperatures (Robinet *et al.*, 2015). As a consequence, sexual reproduction and egg laying tend to occur earlier at the northern distribution edge and at high elevations (late June to mid July), which allows avoiding early frosts. On the contrary they occur later in the southern regions and at low elevations (August and September), thereby avoiding summer maximal temperatures. Consistently, the PPM does not occur in regions with both high summer heat and cold winters. Contrary to spatial variation, temporal variation in phenology, possibly due to yearly temperature variations, has been overlooked so far. Recent studies have proposed hypotheses about life cycle regulation in the PPM. The rate of larval development is highly dependent on temperature, as in any ectotherm. Consequently, there are strong local variations in the date of procession, i.e., the transition between the canopy-nesting larval stages and the underground prepupal and pupal stages. Several studies based either on field observations or laboratory rearing have shown that the duration of the underground stages tends to be shorter when processions occur later (and vice-versa), which helps synchronise adult emergence in spite of the high variations of the preceding life stages (Berardi *et al.*, 2015; Huchon & Démolin, 1970; Salman *et al.*, 2018). Yet, this phenomenon was not explicitly quantified and tested. It was also hypothesised that the existence of potential prolonged diapause could cause local variability in emergence patterns and dynamics (Salman *et al.*, 2016).

We recently obtained historical monitoring data concerning PPM phenology collected under the supervision of Guy Démolin, who devoted his career at INRA, France, to the study of the PPM and its life history traits. Our first author worked with G. Démolin and could retrieve well-organized data that were recently digitized. They correspond to a 15-years monitoring of phenology in 6 sites along an altitudinal gradient in Southern France, recorded between 1970 and 1984. We used these data to test some hypotheses regarding the drivers of phenological variations in the PPM. Yet, these data cannot directly be compared to monitoring designs using pheromone-baited traps, because the individuals remained in situ until the end of larval development and were then allowed to pupate and emergence in a common garden at lower altitude along the same gradient. In particular, we aimed at (*i*) testing if individuals that developed at high elevation still emerge earlier when pupation takes place in a common garden; (*ii*) determining if the emergence patterns of individuals (both males and females) from a given site are synchronized despite variations in procession dates; and (*iii*) determining if annual variation of the weather can affect emergence timing. If so, we asked whether this effect is consistent between sites and whether we can identify which weather parameters have the strongest impact. Lastly, we also explored the factors tuning the phenology of semivoltine individuals, and tested the hypotheses from the literature that the proportion of individuals entering prolonged diapause may be affected by population densities, winter temperatures (Salman *et al.*, 2019) or procession dates (Démolin, 1969).

METHODS

Study sites

Six sites were chosen across an altitudinal gradient along the D974 road in the Vaucluse Department between Malaucène and the Mont Ventoux Station (France), namely G445 (Portail St Jean, 445 m above sea level (a.s.l.), lat. 44.164 long. 5.141 dd), C671 (2ème Plateforme, 671 m a.s.l., lat. 44.159 long. 5.153 dd), B697 (Bramefam, 697 a.s.l., lat. 44.162 long. 5.158 dd), A688 (Le Camp, 688 m a.s.l., lat. 44.164 long. 5.155 dd), F781 (Fribouquet, 781 m a.s.l., lat. 44.168 long. 5.177 dd) and R923 (Les Ramayettes, 923 m a.s.l., lat. 44.169 long. 5.196 dd). The first 4 altitudinal steps were on the territory of the municipality of Malaucène while F781 and R923 were in the municipality of Beaumont-du-Ventoux. A map of the sites can be found in a companion data paper (Martin *et al.*, 2021).

Monitoring and sampling of processions

The same protocol was followed yearly from 1970 to 1984 by the team of the Laboratoire d'Ecologie du Mont Ventoux, wearing dedicated personal protective equipment because of the urticating nature of the larvae. At each study site, 10 Austrian pine trees (*Pinus nigra*) were selected and used during the whole study. Each tree was equipped with a wire and a net strapped on the trunk down to the ground to trap the caterpillars leaving the tree in procession at the end of larval development. When PPM density was very low, nests were sampled on other trees in the vicinity and seeded onto the selected pines. All the sites were visited daily from January 1st to May 31st each year. The caterpillars trapped at the base of each tree were counted daily, collected, and placed individually in glass tubes, with each tube given a unique identifier. When the number of trapped individuals was very high and outnumbered the laboratory capacity, the total number of trapped caterpillars was recorded and used as a proxy of population density in some of the analyses, and only a subset of the trapped caterpillars was actually sampled. They were brought to Malaucène (elevation 340 m a.s.l., lat. 44.192 long. 5.1403, dd) and reared in a laboratory under natural photoperiod at room temperature without heating or cooling (temperature not recorded). Caterpillars sampled from the same tree at the same date were identified as belonging to the same batch; note that a batch does not necessarily correspond to a single family.

Monitoring of adult emergence

A month after cocoon spinning, individuals were taken from the tubes and put into 25 x 15 x 8 cm plastic boxes filled with 4 cm of untreated sawdust. Individuals from the same batch were grouped in the same box, with 100 individuals maximum per box. The boxes were checked daily from the 1st of June to the 30th of September to record moth emergences (note that the sex of the emerged individuals was not recorded). In case all individuals did not emerge, the box was monitored again the following year to allow recording of the emergence date of the semivoltine individuals, until the fifth year.

Data sets and variables

Hereafter we will call "cohort" the individuals that hatched from eggs laid the same year at the same site. Year "N" refers to the year when procession occurred and caterpillars were sampled. For a given cohort, most individuals follow a univoltine cycle and emerge in the following summer, few weeks after the procession (Direct Emergence) while part of the individuals follow a semivoltine cycle and emerge after one or more year(s) of prolonged diapause as pupae (Prolonged Diapause N+1, N+2 etc.). The measured variables are shown in Fig. 1. For each individual, the main variables we used were the procession date (i.e., the date when it was sampled in the field), the emergence date (adult emergence observed in the lab), and the procession-to-emergence time lapse for the univoltine individuals emerging without prolonged diapause. This latter variable corresponded to the duration of the underground phase. It was calculated as the number of days between procession and adult emergence and hereafter named Proc2Em. For each site/year combination, we also calculated the proportion of semivoltine individuals (number of individuals emerged more than a year after the procession date / total number of emerged individual). To compare emergence dates across years and across cohorts, the dates of procession and of adult emergence were expressed as Julian days (January 1st = 1 to December 31st = 365 or 366 depending on leap years). For a given cohort (procession occurring in year N), we called $DirEm_N$ the variable corresponding to the dates of Direct Emergence of univoltine individuals of cohort N, and $ProlDiap_{N+1}$, $ProlDiap_{N+2}$ etc. the variables corresponding to the emergence date after 1, 2 (or more) years of prolonged diapause for semivoltine individuals, expressed as Julian days.

The phenological variability across sites and years was explored by calculating each year the median and mean emergence date (DirEm_N median, in Julian days) of univoltine adults.

Climatic data

The average monthly minimum and maximum temperatures (°C) over the period studied were retrieved from the historical weather data from worldclim v2.1 (https://www.worldclim.org/data/monthlywth.html) (Fick & Hijmans, 2017). Following Fick & Hijmans (2017), a monthly temperature was computed as the average of these minimum and maximum temperatures. Given the spatial resolution of these data (2.5 minutes, i.e. 0.04° or ~21 km^2) and the geographical proximity of the sampling sites and of the laboratory – all falling in three contiguous raster cells – we computed the mean value of these three tiles for each month and used it as an indicator of the local weather. The variation in weather due to elevation was simply represented by the elevation itself in the data analyses (see below).

Data analyses – statistical method

All analyses were performed using the R language for statistical computing (R Core Team, 2021). We used generalized linear mixed models (GLMMs) to analyze the data, a method appropriate to assess the simultaneous effects of various biotic/abiotic variables on ecological data (Bolker *et al.*, 2009). All models described below were fitted using the package 'lme4' (Bates *et al.*, 2015), with error distributions and link functions appropriate to the type of data modeled (see Table 1) and covariate scaling when required. Gaussian linear models (function "lm") were performed on all pairs of covariates and all yielded a correlation coefficient, $|r| < 0.7$, indicating limited collinearity issue (Dormann *et al.*, 2013). The validity of the model hypotheses with regard to the frequency of outliers, and to the distribution, dispersion and homogeneity of residuals, was checked with the package 'DHARMa' (Hartig, 2021) to ensure that model specifications were correct (see Appendices 1-3 in Supporting Information for details). Then the significance of fixed effects was assessed using deviance analyses (function 'Anova', package 'car', Fox & Weisberg, 2019) and non-significant fixed effects were removed one-by-one in decreasing significance order until all were significant (Faraway, 2016). The significant correlations between covariates and responses obtained from the models were retrieved with the packages 'ggeffects' (Lüdecke, 2018) and 'emmeans' (Lenth, 2021). Finally, the strengths of the correlations between the covariates and the response variable were assessed using part R^2 (Stoffel *et al.* 2021). For the whole model, we distinguished the marginal (fixed effects only) and conditional (fixed and random effects) R^2 (Nakagawa & Schielzeth, 2013). The different models and detailed statistical analyses are described below.

Testing the synchronization of adult emergence when procession dates vary: For univoltine individuals, we tested if individuals that pupated early tended to emerge early, or on the contrary if they spent more time as pupae, which would tend to synchronize emergences. In both cases we also accounted for the concomitant effect of weather. We first assessed the correlation between the procession-to-emergence time lapse of each moth (in number of days) and (*i*) the procession date of the larvae, (*ii*) the elevation of its collection site and (*iii*) the ten monthly temperatures from preceding September to current June, i.e., the temperatures experienced by the individuals during their larval development (Model 1). Random effects on the year of procession, on the study site and on the batch were introduced to account for data interdependence linked with our sampling design (Faraway, 2016). We then built a model with the same covariates but with the emergence date as the response variable (Model 2). Model features are summarized in Table 1. Because the structures of Model 1 and 2 are mathematically related (Emergence date = Procession date + Procession-to-emergence time lapse), we refer to “Model 1-2” for features shared by the two models.

Exploring the factors influencing the rates of prolonged diapause: We tested the hypotheses found in the literature that winter temperatures and population density may affect the proportions of semivoltine individuals. To do so, in a third model (Model 3) we assessed the correlation between the proportion of moths entering prolonged diapause in each batch and (*i*) winter temperature in the corresponding year (here, average of December, January, February and March), (*ii*) the procession date of the batch and (*iii*) population density in the corresponding site and year (using as proxy the total number of caterpillars trapped). Random effects on the year of procession and on the study site were introduced to account for data interdependence due to our sampling design.

Exploring the phenology of semivoltine individuals: Semivoltine individuals emerge after one or more year(s) of prolonged diapause. If their emergence pattern was influenced by the conditions they experienced as larvae, their phenology would be close to the phenology of individuals from the same cohort that emerged without prolonged diapause. We thus analysed the median emergence date of individuals experiencing a one-year prolonged diapause (ProlDiap_{N+1} median) as a function of the median emergence date of univoltine individuals from the same cohort (DirEm_{N} median). We expected that the slope of the regression would be 1 and the intercept 0 if the hypothesis was true (Model 4). The alternative hypothesis is that their emergence would be synchronized with that of the individuals emerging the same year (i.e., the following cohort), to optimize mating probability. To test this, we analysed ProlDiap_{N+1} median as a function of DirEm_{N+1} median (Model 5). In both models, the sampling site was used as a random factor to account for potential interdependence in the data due to the sampling design. The slope (resp. intercept) was compared to 1 (resp. 0) using a post-hoc t-test (function ‘summary.emmGrid’, package ‘emmeans’, Kenward-Roger method for the computation of degrees-of-freedom).

- Table 1 -

Graphics were made with the R packages ggplot2 (Wickham, 2016) and cowplot (Wilke, 2019).

Data availability

Data are available from the institutional INRAE data repository at data.inrae.fr (Martin *et al.*, 2020), http://dx.doi.org/10.15454/FGRKAY.

A detailed protocol, description of the available datasets and graphics showing the raw data (emergence patterns in each site and year as well as proportions of uni- and semivoltine individuals) can be found in a companion data paper (Martin *et al.*, 2021).

RESULTS

A total of 140,430 caterpillars were trapped during the 15 years of the whole study, and 46,479 were monitored in the laboratory (only a fraction of the trapped caterpillars was sampled when they were too numerous, see M&M). The numbers of monitored caterpillars leading to moth emergence per year varied between 329 in 1981 and 7,752 in 1978 because of substantial variations in population densities in the field. Number of monitored caterpillars leading to moth emergence per year and per site varied between 10 in B697 in 1980 and 2,319 in B697 in 1978, as shown in Fig. S4.1 (Appendix 4, Supporting Information). No larvae could be monitored in 14 cases, when nest densities were particularly low.

Adult emergence was highly variable across years and sites, even if individuals were all kept under identical conditions in a common garden after the procession. In most years, $DirEm_N$ median was earliest for the high elevation site (R923) and latest for the low elevation site (G445) (Fig. S4.2, Appendix 4, Supporting Information). On the other hand, yearly variation was very similar across sites. For example, emergences in 1976 were the earliest recorded for all sites, whereas they were latest for all sites in 1972, except for C671, which was marginally later in 1981 (Fig. S4.2).

Testing the synchronization of adult emergence when procession dates vary

The procession-to-emergence time lapse (Proc2Em) was negatively correlated with the procession date, elevation and the temperatures of previous December, February and June, but positively correlated with the temperature of previous October (Table 2). Proc2Em was 0.89 days shorter when procession date increased by 1 day and 2.71 days shorter for every 100 m rise in elevation (Fig. 2A&C, Table 2). Considering the effects of monthly temperatures, Proc2Em decreased by 4.56, 1.70 and 1.57 days for every 1°C rise in June, February and December temperatures, respectively (Fig. 2D, Table 2). Conversely, Proc2Em increased by 1.76 days for every 1°C rise in October temperature (Fig. 2D, Table 2). Proc2Em was therefore 13 days shorter in the highest (R923) than in the lowest site (G445) and 20 days shorter when June was the warmest (21 °C, average day temperature) than when it was the coldest (17 °C).

Consistently, emergence date was positively correlated with the procession date and with the temperature of previous October but negatively with the elevation and with the temperature of previous December, February and June (Table 2, Fig. 2B, 2C & 2D). Emergence occurred 0.10 days later when procession date increased by 1 day. All other regression slopes were exactly the same as those discussed above for Proc2Em. Consequently, the temporal dispersion of emergence dates was smaller than that of procession dates, i.e. emergences were more synchronized than processions (Fig. S4.3, Appendix 4, Supporting Information).

- Table 2 and Figure 2 -

Exploring the factors influencing the rates of prolonged diapause

The proportion of individuals entering prolonged diapause for each cohort can be found in the companion data paper (Martin *et al.*, 2021), in which detailed information and graphs are provided. The proportion of semivoltine individuals was highly variable between sites and years, and varied between 0 and 30%, except in F781 in 1978 when it exceptionally reached 64% (Fig. S5.1, Appendix 5). Model 3 shows that the proportion of moths entering prolonged diapause increased with procession date, population density, elevation and winter temperature (Table 2, Fig. 3). However, this model was very dependent on the extreme rate observed in site F781 in 1978. When the corresponding batches were removed, the proportion of moths entering prolonged diapause was no longer correlated with population density (Appendix 5). Overall, moths were therefore most likely to enter prolonged diapause if (*i*) they pupated late, (*ii*) the winter of the corresponding year was warm and (*iii*) they came from elevated sites. The correlation with moth density was less conclusive.

- Figure 3 –

Exploring the phenology of semivoltine individuals

ProlDiap$_{N+1}$ median was not correlated with DirEm$_N$ median (Table 2, Fig. 4A).

Contrastingly, ProlDiap$_{N+1}$ median was correlated with DirEm$_{N+1}$ median (Table 2, Fig. 4B).

The slope and intercept of this correlation were respectively 0.8 (± 0.1) and 37.1 (± 30.3) and did not differ significantly from 1 (t.ratio = -1.1, df = 20.4, P = 0.30) and 0 (t.ratio = 1.2, df = 20.5, P = 0.23), respectively. The relationship did not depart significantly from the expected “y = x” relationship, indicating no significant deviation from the phenology of the cohort with which the semivoltine individuals emerged.

- Figure 4 –

DISCUSSION

Understanding the drivers of phenology regulation in natural populations is a complex task. It requires long time series and detailed data to fully analyse the effects of space, climate and intrinsic individual parameters. In many studies, PPM phenology was monitored by trapping males in the field with pheromone-baited traps (see for instance Burban *et al.*, 2020; Salman *et al.*, 2016; Santos *et al.*, 2011). Such a monitoring design is relatively easy to handle but it does not allow to dissect the relationships between environmental factors and phenology, because much information is left unknown (exact location of the nest where the individual developed, voltinism status, procession date etc.). Here we had the opportunity to test several hypotheses concerning both spatial and temporal tuning of phenology. Historical data also allow to draw a baseline to which researchers will be able to add and compare data obtained in the current and future climatic conditions. Unfortunately, we could not address the question of male/female synchronization within sites, which should be explored in the future.

Relationships between PPM phenology and temperatures are complex

In a vast majority of cases, insect flight periods tend to occur later at higher elevations where temperature is lower, which is consistent with observations of climate-driven advances in phenological events over recent decades as climate warms (de Arce Crespo & Gutierrez, 2011; Roy *et al.*, 2015; Zografou *et al.*, 2020). However, here we observed that PPM adults emerged earlier at higher elevation, which is consistent with the documented variability of its life cycle across various environments. Indeed, Huchon & Démolin (1970) and Robinet and collaborators (2015) documented earlier adult emergence in the northern parts of the PPM distribution range and at high elevation. We showed that this counter-intuitive trend remains true when individuals are moved to a common-garden situation at the end of larval development. This could reveal either that these phenological differences are due to genetic differentiation between sites (i.e., local adaptations), or that they are driven by environmental cues experienced during the larval stages.

Interestingly, we also showed that monthly temperatures during development have a significant effect on adult emergence dates, and could explain yearly variations in phenology. In particular, higher June temperatures are associated to earlier adult emergence. This suggests that the diapause termination process and metamorphosis are accelerated when late spring temperatures are warmer, which is a classical plastic response in ectotherms (Zuo *et al.*, 2011). Yet, this plastic response is opposite to the geographical trends observed in this species, which tends to emerge later in warmer environments as shown in latitudinal or altitudinal gradients (Huchon & Démolin, 1970; Robinet *et al.*, 2015). A plausible hypothesis would be that some local adaptations are responsible for the maintenance of an altitudinal gradient in phenology in which individuals from higher elevation emerge earlier each year (spatial variation in climate-phenology interactions), while an inverse plastic response tends to shift emergence to earlier dates when spring temperatures are warmer (yearly variation in climate-phenology interactions). The question then arises whether this plastic response could be maladaptive in the pine processionary moth, because an earlier reproductive period in warmer years could lead to the young larvae facing the detrimental hot summer temperatures.

It would be interesting to test this hypothesis through modelling approaches and translocation experiments.

To make the picture more complex, we also found a positive relationship between the temperatures of the preceding month of October and adult emergence, suggesting that warm temperatures experienced in the early larval stages would delay emergence. This could correspond to a delayed effect of temperatures experienced in the beginning of the life cycle upon the ultimate stage. The mechanisms at play should now be explored, but we could hypothesise that such a correlation reflects a tuning of adaptive changes in phenology towards later reproduction in regions where autumns are warmer and where the risk of early frost are limited. Sparks and collaborators (2006), exploring the effects of climate on the phenology of 155 species of moths and butterflies in Southern England in the 19th century, concluded that "In general, species responded to increased temperature in the previous October by delayed appearance and to increased temperature in the current spring by advanced appearance". This pattern seems to hold true for the PPM, and we should now explore the possible mechanisms at play and the advantages of such an evolutionary strategy.

Variation in pupal diapause allows emergence synchronization

The life cycle of the PPM is generally univoltine, and it is well documented that the rate of larval development is highly variable. The consequence is that the timing of procession is also highly variable between sites and between years depending on environmental conditions (Berardi *et al.*, 2015; Robinet *et al.*, 2015). We identified that variations in the duration of the procession to emergence time lapse allowed a re-synchronization of adult emergence in each site. The slope of the regression (-0.89) shows that an individual ending its larval stage one day earlier will remain underground (as a prepupa and as a pupa) 0.89 days longer. It would be interesting in future studies to sex the emerged moth and to determine if both males and females follow the same pattern. The variability of the length of prepupal and pupal diapause thus appears as a key parameter allowing the synchronization of adult emergence at the population level, which was suggested but not quantified in previous studies (Berardi *et al.*, 2015; Salman *et al.*, 2018). Half of the synchronization effect may be due to prepupal diapause (Salman *et al.*, 2018), and our results suggest that pupal diapause flexibility would thus compensate the other half. Moreover, we suggest that phenology of larval development only marginally influences the dynamics of adult emergence. When local conditions such as increased temperature and high food quality speed up (or conversely, low temperature and/or poor food quality slow down) larval development, prepupal and pupal diapause lengths are modulated to compensate these effects and favour synchronization, thereby increasing reproductive success. Which external and internal drivers trigger this modulation and allow this synchronisation is still poorly understood, and dedicated studies should now be developed, for instance to test the effect of day length or other environmental cues on the dynamic of adult emergence. We also suggest that future projects should document the phenology of males and females separately, to bring information about emergence synchronisation between sexes.

Regulation of semivoltine individuals

Prolonged diapause corresponds to an extended period of pupal diapause over one to several years causing delayed emergence with a following cohort. Salman et al. (2016) suggested that these semivoltine individuals tend to emerge 3 to 4 weeks earlier than non-diapausing individuals emerging the same year. Yet, this pattern was described from comparisons of pheromone trapping data (thus including both non-diapausing and diapausing individuals without the possibility to separate them) to individuals sampled as larvae and kept in cages until emergence. Our results did not confirm this trend. On the contrary, the phenology of individuals emerging after one year of prolonged diapause fits the phenological patterns of the univoltine individuals emerging the same year, rather than those of their own cohort the previous year. This suggests that the semivoltine individuals react to environmental cues of their emerging year. Such a mechanism of regulation probably optimises the overlapping of emergence curves in a given year, and increases the chance of reproductive success for the individuals experiencing prolonged diapause. Data obtained from a similar monitoring protocol in different environments would be useful to determine if semivoltine individuals are usually synchronised with the emergence of the following cohort as we suggest, or if their emergence dynamics may vary among regions or habitats. Moreover, the individuals monitored in the present study were kept in a common garden after procession, and were thus exposed to similar environmental cues, which possibly tended to fade the site effects. Our study thus probably missed some of the parameters regulating prolonged diapause, even if it allowed to propose testable hypotheses.

Beyond phenology, we also questioned whether some environmental parameters could affect the proportion of individuals experiencing prolonged diapause in a given site. Consistent with the early hypothesis of Démolin (1969), we highlighted that a long larval development and thus a late procession date increased the probability of prolonged diapause. As pupation and metamorphosis take at least 6 weeks (Huchon & Démolin, 1970), prolonged diapause could be seen as a mechanism to avoid delayed emergence and de-synchronization with the rest of the cohort or with the optimal local timing of emergence when larval development is too long. We also found somehow contradictory results, as both higher elevation and warmer winters tend to favour semivoltinism. As suggested above for the complex relationship between temperature and emergence timing, this could be due to a decoupling between local adaptation at higher elevation favouring prolonged diapause and a plastic response to warmer winter conditions during some years. However, this observation contradicts the conclusions of

Salman and collaborators (2019) who relied on a dataset obtained at a large geographical scale. We hypothesize that they actually identified a site effect (i.e., corresponding to the local adaptation to high elevation we found here) rather than a direct effect of winter temperatures, as they did not rely on temporal series allowing to explore the effects of yearly weather conditions.

Predicting the effects of climate change in the PPM will be challenging

One of the main conclusions of the present work is that the effects of environmental factors on the phenological response of the PPM are complex and sometimes contradictory. In particular, we hypothesized that phenology regulation includes both local adaptations and plastic responses, with different tuning of spatial (early emergence at high elevations) and temporal variability (early emergence when springs are warmer and autumns are colder). These observations advocate for the design of complementary experiments along the same altitudinal gradient, using full common garden experiments as well as translocation designs, to dissect these opposing influences on the phenotype. We agree with Sparks and collaborators (2006) that these findings warn against expecting simple responses to climate warming, as differential warming trends in spring and autumn would have opposite consequences on species' phenology.

Moreover, altitudinal gradients are often used as space-for-time substitution designs that are supposed to help understanding how a species will react to climate change. Such studies suppose that investigating how phenotypic traits change along elevational gradients can contribute to the prediction of species phenological responses to future climate. This is because temperature varies strongly over a limited distance where photoperiod remains similar (de Arce Crespo & Gutierrez, 2011; Zografou *et al.*, 2020). However, the complex relationship between opposite geographical and temporal trends we identified in the present study suggests that using altitudinal gradients as space-for-time substitution devices would lead to erroneous conclusions in the PPM. A similar contradiction was reported for ring-width response to temperature variability in the Douglas-fir (Klesse *et al.*, 2020). When responses to spatial versus temporal temperature variation is opposite, one can suggest that spatial variation, caused by local adaptation and other slow processes, cannot be used to anticipate changes caused by rapid climate change (Klesse *et al.*, 2020). We support the conclusions of Zografou et al. (2020) and de Arce Crespo and Gutierrez (2011) who highlighted how caution is needed before extrapolating such results and predicting the effects of climate change on individual species' phenology.

Perspectives

The data we analysed was informative but did not fully allow to test how local genetic adaptation and plasticity interact, in particular because the first part of the life cycle occurred *in situ* while the post-larval stages occurred in a common garden at lower elevation, still in the same region. Several research perspectives could now be developed and built upon these results. First, as climate warming has been noticeable in the recent years in the study site (Appendix 6, Supporting Information), it would be interesting to add current observations using a similar protocol to determine how today's observations would fall when compared to historical data, and how phenological patterns have been modified by the on-going climate change. Second, as some studies have suggested that the response to environmental conditions may differ between habitats (Bell *et al.*, 2019), it would be valuable to determine whether the relationships between phenology and both spring and autumn temperatures correspond to a general trend in the PPM, or if it is true only under certain conditions (some habitats, or some ranges of temperatures). Third, building on the genomic resources recently developed for this species (Gschloessl *et al.*, 2018; Leblois *et al.*, 2018), we will test whether we can find signs of genomic adaptations along altitudinal gradients, which could explain the maintenance of the observed phenological patterns with earlier flight periods at higher elevation.

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The authors declare no conflict of interest.

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TABLES

Table 1: Details of the generalized linear mixed models used to analyze the procession-to-emergence time lapse (Model 1), the emergence date (Model 2), the proportion of individuals entering prolonged diapause (Model 3) and the phenology of semivoltine individuals (Models 4 & 5). In Models 1 & 2, “T_{09}, T_{10},… T_{06}” stand for “temperature of September, October,… June”. The temperatures of September, October, November and December are the ones of the previous year (N-1), i.e. those experienced by the young larvae. “#obs” stands for “total number of observations”.

Model 1-2 Phenology of univoltine individuals		
Response	Model 1	Procession-to-emergence time lapse
	Model 2	Emergence date
Fixed effects		Procession date Elevation T_{09}, T_{10}, T_{11}, T_{12}, T_{01}, T_{02}, T_{03}, T_{04}, T_{05}, T_{06}
Random effects		Site Year of procession Batch
Distribution (link)		Gaussian (identity)
#obs		41,847

Model 3 Proportion of individuals entering prolonged diapause	
Response	Proportion of moths entering prolonged diapause
Fixed effects	Procession date Winter temperature Elevation Population density
Random effects	Site Year of procession
Distribution (link)	Binomial (probit)*
#obs	1,025

Model 4-5 Phenology of semivoltine individuals		
Response		Median emergence date of individuals experiencing a one-year prolonged diapause
Fixed effects	Model 4	Median emergence date of univoltine individuals of the same cohort
	Model 5	Median emergence date of univoltine individuals of the following cohort
Random effects		Site Year of procession
Distribution (link)		Gaussian (identity)
#obs		55

* An observation-level random effect was used to correct overdispersion (Harrison, 2014).

Table 2 Values and significance of deviance analyses performed on the fixed effects of Models 1 to 5. “-” stands for “unsignificant fixed effect removed during model selection”. The quality of fit of the final models (i.e. once the unsignificant fixed effects were removed) is shown with the marginal (R^2_m, fixed effects only) and conditional (R^2_c, fixed and random effects) proportions of variance explained.

Fixed effects	χ^2	df	p	Regression equation	R^2_m	R^2_c
Model 1: Proc2Em of univoltine individuals					0.86	0.95
Procession date	13353.9	1	< 0.001	$y = -0.89 \cdot x + 188.91$		
Elevation	12.7	1	< 0.001	$y = -0.03 \cdot x + 111.48$		
T_{10}	5.7	1	0.017	$y = 1.76 \cdot x + 71.01$		
T_{12}	4.8	1	0.028	$y = -1.57 \cdot x + 98.36$		
T_{02}	5.3	1	0.022	$y = -1.70 \cdot x + 100.94$		
T_{06}	31.8	1	< 0.001	$y = -4.56 \cdot x + 176.93$		
T_{09}, T_{11}, T_{01}, T_{03}, T_{04}, T_{05}	-	-	-	-		
Model 2: Emergence date of univoltine individuals					0.57	0.84
Procession date	183.3	1	< 0.001	$y = 0.10 \cdot x + 188.91$		
Elevation	12.7	1	< 0.001	$y = -0.03 \cdot x + 219.04$		
T_{10}	5.7	1	0.017	$y = 1.76 \cdot x + 178.57$		
T_{12}	4.8	1	0.028	$y = -1.57 \cdot x + 205.92$		
T_{02}	5.3	1	0.022	$y = -1.70 \cdot x + 208.49$		
T_{06}	31.8	1	< 0.001	$y = -4.56 \cdot x + 284.48$		
T_{09}, T_{11}, T_{01}, T_{03}, T_{04}, T_{05}	-	-	-	-		
Model 3: Proportion of moths entering prolonged diapause					0.08	0.11
Procession date	55.4	1	< 0.001	$probit(y) = 0.0184 \cdot x - 5.9315$		
Population density*	29.2	1	< 0.001	$probit(y) = 0.0001 \cdot x - 2.4210$		
Winter temperature	10.1	1	0.001	$probit(y) = 0.2937 \cdot x - 4.6773$		
Elevation	6.6	1	0.010	$probit(y) = 0.0014 \cdot x - 3.8171$		
Model 4: ProlDiap$_{N+1}$ median					0.00	0.77
DirEm$_N$	-	-	-	-		
Model 5: ProlDiap$_{N+1}$ median					0.49	0.68
DirEm$_{N+1}$	40.4	1	< 0.001	$y = 0.84 \cdot x + 37.11$		

* Not significant when data from site F781 in 1978 were removed.

FIGURE LEGENDS

Fig. 1 Life cycle of the processionary moth showing the observed procession and emergence dates for two examples, and the corresponding values of procession-to-emergence time lapse (Proc2Em). The cycle shows that a proportion of individuals enter prolonged diapause and emerge one to several years after the univoltine individuals of the same cohort.

Fig. 2 Correlation between the procession-to-emergence time lapse (Proc2Em) and procession date (A), between emergence date and procession date (B), and between emergence date (or procession-to-emergence time lapse, Proc2Em, on the secondary axis) and elevation (C) or temperatures (D). As shown in Table 2, the regression slopes between the emergence date or Proc2Em and the altitude or temperatures are identical because Model 1 and 2 are mathematically related. The two responses are therefore displayed jointly in C and D. Raw data are displayed as squares (A & B) or as density traces ("violin plots", Hintze & Nelson, 1998) highlighting the distribution of the data (C & D). Colors show the number of moths for each (x, y) coordinate (A & B) and the regression lines from Model 1 (A) and Model 2 (B, C & D) are displayed in black. The procession and emergence dates have been translated to a "Month day" format for easier reading but the statistics were performed on dates expressed in Julian days. "pR^2" stands for "part R^2" and represents the part of variance explained by each covariate alone in Model 1 (A and green part on the right in C and D) and Model 2 (B and blue part on the left in C and D).

Fig. 3 Correlation between the proportion of individuals entering prolonged diapause and procession date (A), elevation (B) and winter temperature (C, average of December, January, February and March). Raw data are displayed as squares (A) or as density traces highlighting the distribution of the data (B & C). Colors show the number of moths for each (x, y) coordinate (A) and the regression lines from Model 3 are displayed with solid (model fitted with all data) or dashed (model fitted without site F781 in 1978, see text for details) lines. The procession date has been translated to a “Month day” format for easier reading but the statistics were performed on dates expressed in Julian days. “pR^2” stands for “part R^2” and represents the part of variance explained by each covariate alone in the model mentioned in subscript.

Fig. 4 Median emergence date of semivoltine individuals experiencing a one-year prolonged diapause as a function of the median emergence date of univoltine individuals of the same cohort (A) or of the following cohort (B). The dotted line shows the y = x diagonal expected if the phenological patterns are fully similar while the solid line depicts the correlations found in Model 5 (B, no significant correlation in A). If the correlation was significant, its slope and intercept were compared to the expected y = x diagonal and results are shown above graphs. The procession and emergence dates have been translated to a “Month day” format for easier reading but the statistics were performed on dates expressed in Julian days.

SUPPORTING INFORMATION

Supporting information contains the Appendices 1 to 6 cited in the text.

FIGURE 1

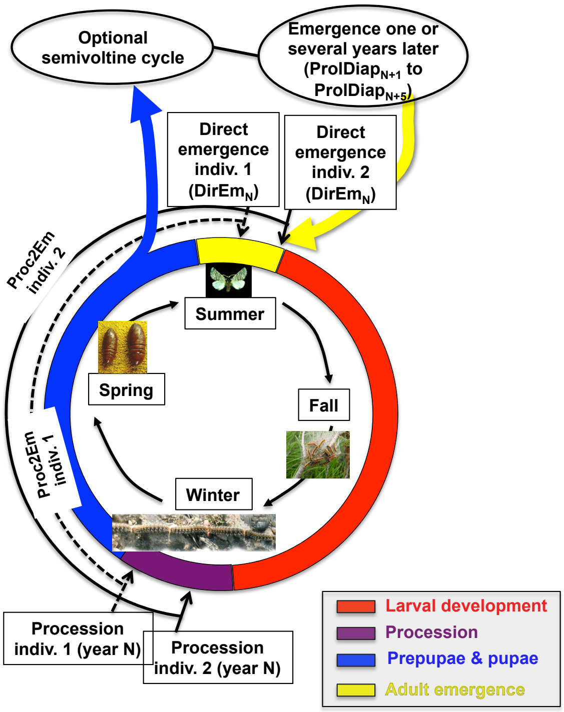

Optional semivoltine cycle
Emergence one or several years later (ProlDiap$_{N+1}$ to ProlDiap$_{N+5}$)
Direct emergence indiv. 1 (DirEm$_N$)
Direct emergence indiv. 2 (DirEm$_N$)
Proc2Em indiv. 2
Summer
Spring
Fall
Proc2Em indiv. 1
Winter
Procession indiv. 1 (year N)
Procession indiv. 2 (year N)
🟥 Larval development
🟪 Procession
🟦 Prepupae & pupae
🟨 Adult emergence

FIGURE 2

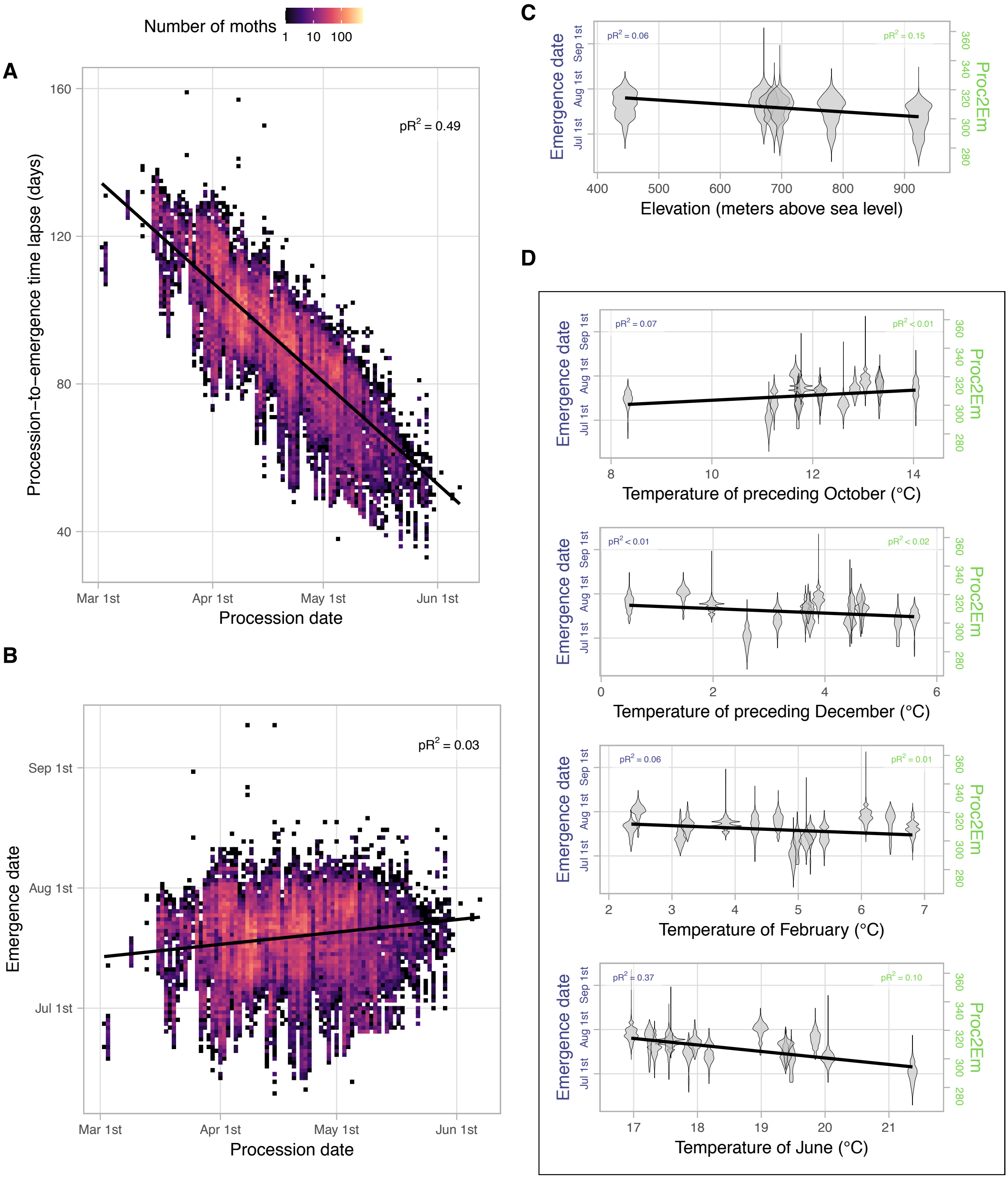

Number of moths
1
10
100
A
Procession−to−emergence time lapse (days)
160
120
80
40
pR^2 = 0.49
Mar 1st
Apr 1st
May 1st
Jun 1st
Procession date
B
Emergence date
Sep 1st
Aug 1st
Jul 1st
pR^2 = 0.03
Mar 1st
Apr 1st
May 1st
Jun 1st
Procession date
C
Emergence date
Jul 1st
Aug 1st
Sep 1st
pR^2 = 0.06
pR^2 = 0.15
Proc2Em
360
340
320
300
280
400
500
600
700
800
900
Elevation (meters above sea level)
D
Emergence date
pR^2 = 0.07
pR^2 < 0.01
Proc2Em
8
10
12
14
Temperature of preceding October (°C)
pR^2 < 0.01
pR^2 < 0.02
0
2
4
6
Temperature of preceding December (°C)
pR^2 = 0.06
pR^2 = 0.01
2
3
4
5
6
7
Temperature of February (°C)
pR^2 = 0.37
pR^2 = 0.10
17
18
19
20
21
Temperature of June (°C)

FIGURE 3

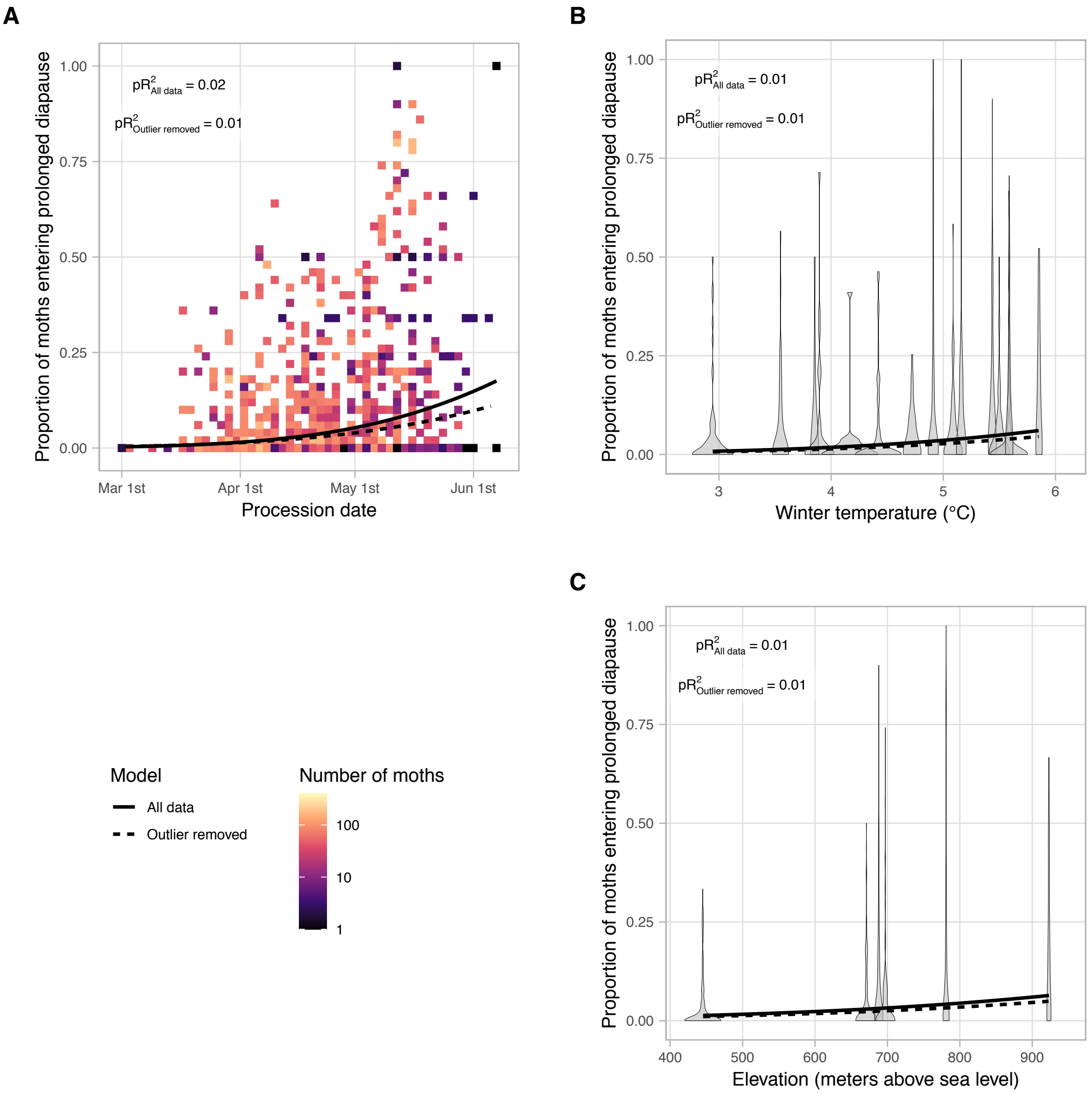
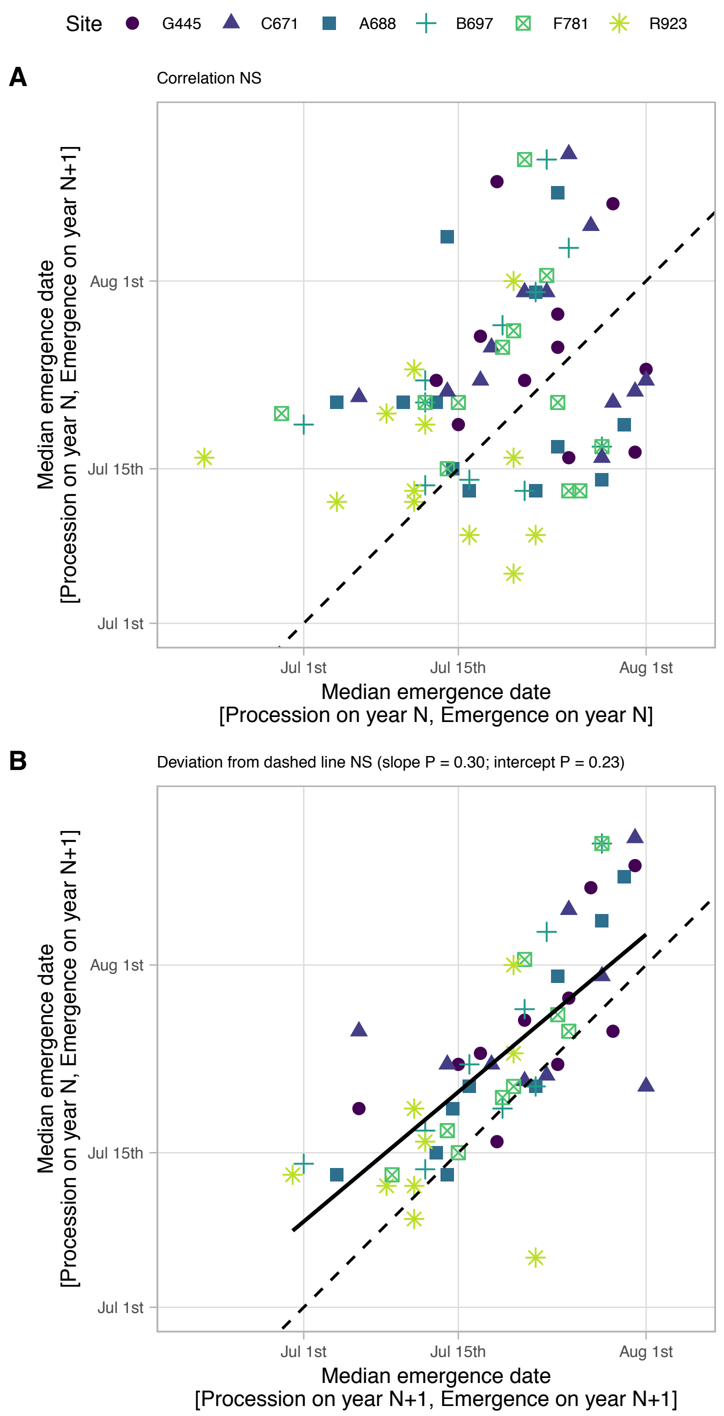

A
pR$^2_{\text{All data}}$ = 0.02
pR$^2_{\text{Outlier removed}}$ = 0.01
Proportion of moths entering prolonged diapause
1.00
0.75
0.50
0.25
0.00
Mar 1st
Apr 1st
May 1st
Jun 1st
Procession date
B
pR$^2_{\text{All data}}$ = 0.01
pR$^2_{\text{Outlier removed}}$ = 0.01
Proportion of moths entering prolonged diapause
1.00
0.75
0.50
0.25
0.00
3
4
5
6
Winter temperature (°C)
C
pR$^2_{\text{All data}}$ = 0.01
pR$^2_{\text{Outlier removed}}$ = 0.01
Proportion of moths entering prolonged diapause
1.00
0.75
0.50
0.25
0.00
400
500
600
700
800
900
Elevation (meters above sea level)
Model
All data
Outlier removed
Number of moths
100
10
1

FIGURE 4


Site
● G445
▲ C671
■ A688
+ B697
☒ F781
✳ R923
A
Correlation NS
Median emergence date [Procession on year N, Emergence on year N+1]
Aug 1st
Jul 15th
Jul 1st
Median emergence date [Procession on year N, Emergence on year N]
B
Deviation from dashed line NS (slope P = 0.30; intercept P = 0.23)
Median emergence date [Procession on year N, Emergence on year N+1]
Median emergence date [Procession on year N+1, Emergence on year N+1]