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

2

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22 **Running title**

23 Drivers of phenology in the pine processionary moth

24

25 **ABSTRACT**

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

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

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

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

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

43

44 **Keywords:** temperature; elevation gradient; historical data; diapause; synchrony;

45 *Thaumetopoea pityocampa*

46 **INTRODUCTION**

47 Phenology, the timing of life cycle events, is likely driven by a combination of short-term  
48 plastic and long-term evolutionary responses to environmental variations (Briscoe *et al.*,  
49 2012; Robinet *et al.*, 2015). Widely distributed species encounter varying climatic conditions  
50 and ecological pressures, and local phenological adaptations optimize resource use and  
51 minimize risks encountered by each life stage (Abarca & Lill, 2019). It is critical that local  
52 adult emergence is synchronised to favour mate finding and increase reproductive success, in  
53 particular for univoltine species with short adult lifespans. The phenology of many species is  
54 also affected by yearly temperature variations, but the direction and strength of these  
55 responses can be very different between species. A recent study (Maurer *et al.*, 2018) using  
56 data from 215 moth species between 1895 and 2013 showed that species-specific  
57 phenological shifts induced by yearly variation ranged from a delay of 10.6 days to an  
58 advance of 10.3 days per degree Celsius. Determining if the relationship between temperature  
59 and phenology is closely matched over space and time would help develop our understanding  
60 of the evolution of phenology. It would also be useful to anticipate how species may respond  
61 to climate change, and determine if species are able to track the optimal conditions via  
62 plasticity (Roy *et al.*, 2015). Indeed, maladaptive shifts in phenology can expose sensitive life  
63 stages to detrimental environmental conditions such as frost or drought (Stewart *et al.*, 2020).  
64 Historical data are highly valuable and provide baseline phenological information useful in  
65 the context of climate change (Fenberg *et al.*, 2016).

66

67 The pine processionary moth (PPM) *Thaumetopoea pityocampa* (Denis & Schiff.)  
68 (Lepidoptera: Notodontidae) is distributed over a large part of the Western Mediterranean  
69 basin, from North Africa to Libya on the southern rim and from Portugal to Western Turkey  
70 in the northern part (Ipekdal *et al.*, 2020; Kerdelhué *et al.*, 2009). PPM is typically a

71 univoltine winter-feeding insect with summer adult emergence. Adults emerge in early  
72 summer at colder high-elevation sites and in late summer at warmer sites. However, seasonal  
73 life history varies considerably both locally and regionally, and some proportion of the  
74 population can experience prolonged pupal diapause of one to several years (Salman *et al.*,  
75 2019; Salman *et al.*, 2016). Such individuals are referred to as *semivoltine* individuals, as  
76 opposed to the *univoltine* ones which emerge as adult a few weeks after pupation. Adults mate  
77 immediately and die within 24 to 48h. Egg masses are laid on pine needles. After ca. a month  
78 of embryonic development, L1 larvae hatch and feed on the 1-year-old needles of their  
79 coniferous host. Larvae remain gregarious throughout their development across autumn and  
80 winter, and spin a typical white silken tent in which caterpillars shelter. The L5 larvae from a  
81 given nest leave the tree all together in late winter or early spring in a typical head-to-tail  
82 procession in search of an underground pupation site. Once buried, the larvae undergo a pre-  
83 pupal followed by a pupal phase, and experience an obligate diapause that can vary in  
84 duration, until univoltine adults emerge the following summer (Berardi *et al.*, 2015). The life  
85 cycle of the pine processionary moth is shown in Fig. 1. The PPM has been strongly affected  
86 by climate change and its northern and altitudinal expansions in Europe are well documented  
87 (Battisti *et al.*, 2005; Robinet *et al.*, 2014; Roques *et al.*, 2015).

88 - Figure 1 -

89 Spatial tuning of phenology is relatively well understood in this species. It can be linked to  
90 particular features of the life cycle coupled with life history traits (Huchon & Démolin, 1970;  
91 Robinet *et al.*, 2015). Briefly, the main thermal constraints influencing phenology are (i) the  
92 vulnerability of the first instars to high summer temperatures; (ii) the vulnerability of the  
93 second instars to early autumnal frosts and (iii) the vulnerability of late instars to extreme low  
94 winter temperatures (Robinet *et al.*, 2015). As a consequence, sexual reproduction and egg  
95 laying tend to occur earlier at the northern distribution edge and at high elevations (late June

96 to mid July), which allows avoiding early frosts. On the contrary they occur later in the  
97 southern regions and at low elevations (August and September), thereby avoiding summer  
98 maximal temperatures. Consistently, the PPM does not occur in regions with both high  
99 summer heat and cold winters. Contrary to spatial variation, temporal variation in phenology,  
100 possibly due to yearly temperature variations, has been overlooked so far. Recent studies have  
101 proposed hypotheses about life cycle regulation in the PPM. The rate of larval development is  
102 highly dependent on temperature, as in any ectotherm. Consequently, there are strong local  
103 variations in the date of procession, i.e., the transition between the canopy-nesting larval  
104 stages and the underground prepupal and pupal stages. Several studies based either on field  
105 observations or laboratory rearing have shown that the duration of the underground stages  
106 tends to be shorter when processions occur later (and vice-versa), which helps synchronise  
107 adult emergence in spite of the high variations of the preceding life stages (Berardi *et al.*,  
108 2015; Huchon & Démolin, 1970; Salman *et al.*, 2018). Yet, this phenomenon was not  
109 explicitly quantified and tested. It was also hypothesised that the existence of potential  
110 prolonged diapause could cause local variability in emergence patterns and dynamics (Salman  
111 *et al.*, 2016).

112 We recently obtained historical monitoring data concerning PPM phenology collected under  
113 the supervision of Guy Démolin, who devoted his career at INRA, France, to the study of the  
114 PPM and its life history traits. Our first author worked with G. Démolin and could retrieve  
115 well-organized data that were recently digitized. They correspond to a 15-years monitoring of  
116 phenology in 6 sites along an altitudinal gradient in Southern France, recorded between 1970  
117 and 1984. We used these data to test some hypotheses regarding the drivers of phenological  
118 variations in the PPM. Yet, these data cannot directly be compared to monitoring designs  
119 using pheromone-baited traps, because the individuals remained in situ until the end of larval  
120 development and were then allowed to pupate and emergence in a common garden at lower

121 altitude along the same gradient. In particular, we aimed at (i) testing if individuals that  
122 developed at high elevation still emerge earlier when pupation takes place in a common  
123 garden; (ii) determining if the emergence patterns of individuals (both males and females)  
124 from a given site are synchronized despite variations in procession dates; and (iii) determining  
125 if annual variation of the weather can affect emergence timing. If so, we asked whether this  
126 effect is consistent between sites and whether we can identify which weather parameters have  
127 the strongest impact. Lastly, we also explored the factors tuning the phenology of semivoltine  
128 individuals, and tested the hypotheses from the literature that the proportion of individuals  
129 entering prolonged diapause may be affected by population densities, winter temperatures  
130 (Salman *et al.*, 2019) or procession dates (Démolin, 1969).

131

## 132 **METHODS**

### 133 *Study sites*

134 Six sites were chosen across an altitudinal gradient along the D974 road in the Vaucluse  
135 Department between Malaucène and the Mont Ventoux Station (France), namely G445  
136 (Portail St Jean, 445 m above sea level (a.s.l.), lat. 44.164 long. 5.141 dd ), C671 (2ème  
137 Plateforme, 671 m a.s.l., lat. 44.159 long. 5.153 dd), B697 (Bramefam, 697 a.s.l., lat. 44.162  
138 long. 5.158 dd), A688 (Le Camp, 688 m a.s.l., lat. 44.164 long. 5.155 dd), F781 (Fribouquet,  
139 781 m a.s.l., lat. 44.168 long. 5.177 dd) and R923 (Les Ramayettes, 923 m a.s.l., lat. 44.169  
140 long. 5.196 dd). The first 4 altitudinal steps were on the territory of the municipality of  
141 Malaucène while F781 and R923 were in the municipality of Beaumont-du-Ventoux. A map  
142 of the sites can be found in a companion data paper (Martin *et al.*, 2021).

143

### 144 *Monitoring and sampling of processions*

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

162

### 163 *Monitoring of adult emergence*

164 A month after cocoon spinning, individuals were taken from the tubes and put into 25 x 15 x  
165 8 cm plastic boxes filled with 4 cm of untreated sawdust. Individuals from the same batch  
166 were grouped in the same box, with 100 individuals maximum per box. The boxes were  
167 checked daily from the 1<sup>st</sup> of June to the 30<sup>th</sup> of September to record moth emergences (note  
168 that the sex of the emerged individuals was not recorded). In case all individuals did not



169 emerge, the box was monitored again the following year to allow recording of the emergence  
170 date of the semivoltine individuals, until the fifth year.

171

### 172 *Data sets and variables*

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

193 The phenological variability across sites and years was explored by calculating each year the  
194 median and mean emergence date (DirEm<sub>N</sub> median, in Julian days) of univoltine adults.

195

#### 196 *Climatic data*

197 The average monthly minimum and maximum temperatures (°C) over the period studied were  
198 retrieved from the historical weather data from worldclim v2.1

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

206

#### 207 *Data analyses – statistical method*

208 All analyses were performed using the R language for statistical computing (R Core Team,  
209 2021). We used generalized linear mixed models (GLMMs) to analyze the data, a method  
210 appropriate to assess the simultaneous effects of various biotic/abiotic variables on ecological  
211 data (Bolker *et al.*, 2009). All models described below were fitted using the package ‘lme4’  
212 (Bates *et al.*, 2015), with error distributions and link functions appropriate to the type of data  
213 modeled (see Table 1) and covariate scaling when required. Gaussian linear models (function  
214 “lm”) were performed on all pairs of covariates and all yielded a correlation coefficient,  
215  $|r| < 0.7$ , indicating limited collinearity issue (Dormann *et al.*, 2013). The validity of the  
216 model hypotheses with regard to the frequency of outliers, and to the distribution, dispersion  
217 and homogeneity of residuals, was checked with the package ‘DHARMA’ (Hartig, 2021) to

218 ensure that model specifications were correct (see Appendices 1-3 in Supporting Information  
219 for details). Then the significance of fixed effects was assessed using deviance analyses  
220 (function ‘Anova’, package ‘car’, Fox & Weisberg, 2019) and non-significant fixed effects  
221 were removed one-by-one in decreasing significance order until all were significant (Faraway,  
222 2016). The significant correlations between covariates and responses obtained from the  
223 models were retrieved with the packages ‘ggeffects’ (Lüdtke, 2018) and ‘emmeans’ (Lenth,  
224 2021). Finally, the strengths of the correlations between the covariates and the response  
225 variable were assessed using part  $R^2$  (Stoffel *et al.* 2021). For the whole model, we  
226 distinguished the marginal (fixed effects only) and conditional (fixed and random effects)  $R^2$   
227 (Nakagawa & Schielzeth, 2013). The different models and detailed statistical analyses are  
228 described below.

229

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

242 mathematically related (Emergence date = Procession date + Procession-to-emergence time  
243 lapse), we refer to “Model 1-2” for features shared by the two models.

244

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

254

255 *Exploring the phenology of semivoltine individuals:* Semivoltine individuals emerge after one  
256 or more year(s) of prolonged diapause. If their emergence pattern was influenced by the  
257 conditions they experienced as larvae, their phenology would be close to the phenology of  
258 individuals from the same cohort that emerged without prolonged diapause. We thus analysed  
259 the median emergence date of individuals experiencing a one-year prolonged diapause  
260 ( $\text{ProlDiap}_{N+1}$  median) as a function of the median emergence date of univoltine individuals  
261 from the same cohort ( $\text{DirEm}_N$  median). We expected that the slope of the regression would  
262 be 1 and the intercept 0 if the hypothesis was true (Model 4). The alternative hypothesis is  
263 that their emergence would be synchronized with that of the individuals emerging the same  
264 year (i.e., the following cohort), to optimize mating probability. To test this, we analysed  
265  $\text{ProlDiap}_{N+1}$  median as a function of  $\text{DirEm}_{N+1}$  median (Model 5). In both models, the  
266 sampling site was used as a random factor to account for potential interdependence in the data

267 due to the sampling design. The slope (resp. intercept) was compared to 1 (resp. 0) using a  
268 post-hoc t-test (function ‘summary.emmGrid’, package ‘emmeans’, Kenward-Roger method  
269 for the computation of degrees-of-freedom).

270

271 - Table 1 -

272 Graphics were made with the R packages ggplot2 (Wickham, 2016) and cowplot (Wilke,  
273 2019).

274

#### 275 *Data availability*

276 Data are available from the institutional INRAE data repository at data.inrae.fr (Martin *et al.*,  
277 2020), <http://dx.doi.org/10.15454/FGRKAY>.

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

281

## 282 **RESULTS**

283 A total of 140,430 caterpillars were trapped during the 15 years of the whole study, and  
284 46,479 were monitored in the laboratory (only a fraction of the trapped caterpillars was  
285 sampled when they were too numerous, see M&M). The numbers of monitored caterpillars  
286 leading to moth emergence per year varied between 329 in 1981 and 7,752 in 1978 because of  
287 substantial variations in population densities in the field. Number of monitored caterpillars  
288 leading to moth emergence per year and per site varied between 10 in B697 in 1980 and 2,319  
289 in B697 in 1978, as shown in Fig. S4.1 (Appendix 4, Supporting Information). No larvae  
290 could be monitored in 14 cases, when nest densities were particularly low.

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

298

### 299 *Testing the synchronization of adult emergence when procession dates vary*

300 The procession-to-emergence time lapse (Proc2Em) was negatively correlated with the  
301 procession date, elevation and the temperatures of previous December, February and June, but  
302 positively correlated with the temperature of previous October (Table 2). Proc2Em was 0.89  
303 days shorter when procession date increased by 1 day and 2.71 days shorter for every 100 m  
304 rise in elevation (Fig. 2A&C, Table 2). Considering the effects of monthly temperatures,  
305 Proc2Em decreased by 4.56, 1.70 and 1.57 days for every 1°C rise in June, February and  
306 December temperatures, respectively (Fig. 2D, Table 2). Conversely, Proc2Em increased by  
307 1.76 days for every 1°C rise in October temperature (Fig. 2D, Table 2). Proc2Em was  
308 therefore 13 days shorter in the highest (R923) than in the lowest site (G445) and 20 days  
309 shorter when June was the warmest (21 °C, average day temperature) than when it was the  
310 coldest (17 °C).

311 Consistently, emergence date was positively correlated with the procession date and with the  
312 temperature of previous October but negatively with the elevation and with the temperature of  
313 previous December, February and June (Table 2, Fig. 2B, 2C & 2D). Emergence occurred  
314 0.10 days later when procession date increased by 1 day. All other regression slopes were  
315 exactly the same as those discussed above for Proc2Em. Consequently, the temporal

316 dispersion of emergence dates was smaller than that of procession dates, i.e. emergences were  
317 more synchronized than processions (Fig. S4.3, Appendix 4, Supporting Information).

318

319 - Table 2 and Figure 2 -

320

321 *Exploring the factors influencing the rates of prolonged diapause*

322 The proportion of individuals entering prolonged diapause for each cohort can be found in the  
323 companion data paper (Martin *et al.*, 2021), in which detailed information and graphs are  
324 provided. The proportion of semivoltine individuals was highly variable between sites and  
325 years, and varied between 0 and 30%, except in F781 in 1978 when it exceptionally reached  
326 64% (Fig. S5.1, Appendix 5). Model 3 shows that the proportion of moths entering prolonged  
327 diapause increased with procession date, population density, elevation and winter temperature  
328 (Table 2, Fig. 3). However, this model was very dependent on the extreme rate observed in  
329 site F781 in 1978. When the corresponding batches were removed, the proportion of moths  
330 entering prolonged diapause was no longer correlated with population density (Appendix 5).  
331 Overall, moths were therefore most likely to enter prolonged diapause if (i) they pupated late,  
332 (ii) the winter of the corresponding year was warm and (iii) they came from elevated sites.  
333 The correlation with moth density was less conclusive.

334

335 - Figure 3 -

336

337 *Exploring the phenology of semivoltine individuals*

338 ProlDiap<sub>N+1</sub> median was not correlated with DirEm<sub>N</sub> median (Table 2, Fig. 4A).

339 Contrastingly, ProlDiap<sub>N+1</sub> median was correlated with DirEm<sub>N+1</sub> median (Table 2, Fig. 4B).

340 The slope and intercept of this correlation were respectively 0.8 ( $\pm$  0.1) and 37.1 ( $\pm$  30.3) and

341 did not differ significantly from 1 (t.ratio = -1.1, df = 20.4, P = 0.30) and 0 (t.ratio = 1.2, df =  
342 20.5, P = 0.23), respectively. The relationship did not depart significantly from the expected  
343 “y = x” relationship, indicating no significant deviation from the phenology of the cohort with  
344 which the semivoltine individuals emerged.

345

346 - Figure 4 –

347

348

## 349 **DISCUSSION**

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

362

### 363 *Relationships between PPM phenology and temperatures are complex*

364 In a vast majority of cases, insect flight periods tend to occur later at higher elevations where  
365 temperature is lower, which is consistent with observations of climate-driven advances in



366 phenological events over recent decades as climate warms (de Arce Crespo & Gutierrez,  
367 2011; Roy *et al.*, 2015; Zografou *et al.*, 2020). However, here we observed that PPM adults  
368 emerged earlier at higher elevation, which is consistent with the documented variability of its  
369 life cycle across various environments. Indeed, Huchon & Démolin (1970) and Robinet and  
370 collaborators (2015) documented earlier adult emergence in the northern parts of the PPM  
371 distribution range and at high elevation. We showed that this counter-intuitive trend remains  
372 true when individuals are moved to a common-garden situation at the end of larval  
373 development. This could reveal either that these phenological differences are due to genetic  
374 differentiation between sites (i.e., local adaptations), or that they are driven by environmental  
375 cues experienced during the larval stages.

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

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

393 To make the picture more complex, we also found a positive relationship between the  
394 temperatures of the preceding month of October and adult emergence, suggesting that warm  
395 temperatures experienced in the early larval stages would delay emergence. This could  
396 correspond to a delayed effect of temperatures experienced in the beginning of the life cycle  
397 upon the ultimate stage. The mechanisms at play should now be explored, but we could  
398 hypothesise that such a correlation reflects a tuning of adaptive changes in phenology towards  
399 later reproduction in regions where autumns are warmer and where the risk of early frost are  
400 limited. Sparks and collaborators (2006), exploring the effects of climate on the phenology of  
401 155 species of moths and butterflies in Southern England in the 19<sup>th</sup> century, concluded that  
402 "In general, species responded to increased temperature in the previous October by delayed  
403 appearance and to increased temperature in the current spring by advanced appearance". This  
404 pattern seems to hold true for the PPM, and we should now explore the possible mechanisms  
405 at play and the advantages of such an evolutionary strategy.

406

#### 407 *Variation in pupal diapause allows emergence synchronization*

408 The life cycle of the PPM is generally univoltine, and it is well documented that the rate of  
409 larval development is highly variable. The consequence is that the timing of procession is also  
410 highly variable between sites and between years depending on environmental conditions  
411 (Berardi *et al.*, 2015; Robinet *et al.*, 2015). We identified that variations in the duration of the  
412 procession to emergence time lapse allowed a re-synchronization of adult emergence in each  
413 site. The slope of the regression (-0.89) shows that an individual ending its larval stage one  
414 day earlier will remain underground (as a prepupa and as a pupa) 0.89 days longer. It would  
415 be interesting in future studies to sex the emerged moth and to determine if both males and

416 females follow the same pattern. The variability of the length of prepupal and pupal diapause  
417 thus appears as a key parameter allowing the synchronization of adult emergence at the  
418 population level, which was suggested but not quantified in previous studies (Berardi *et al.*,  
419 2015; Salman *et al.*, 2018). Half of the synchronization effect may be due to prepupal  
420 diapause (Salman *et al.*, 2018), and our results suggest that pupal diapause flexibility would  
421 thus compensate the other half. Moreover, we suggest that phenology of larval development  
422 only marginally influences the dynamics of adult emergence. When local conditions such as  
423 increased temperature and high food quality speed up (or conversely, low temperature and/or  
424 poor food quality slow down) larval development, prepupal and pupal diapause lengths are  
425 modulated to compensate these effects and favour synchronization, thereby increasing  
426 reproductive success. Which external and internal drivers trigger this modulation and allow  
427 this synchronisation is still poorly understood, and dedicated studies should now be  
428 developed, for instance to test the effect of day length or other environmental cues on the  
429 dynamic of adult emergence. We also suggest that future projects should document the  
430 phenology of males and females separately, to bring information about emergence  
431 synchronisation between sexes.

432

### 433 *Regulation of semivoltine individuals*

434 Prolonged diapause corresponds to an extended period of pupal diapause over one to several  
435 years causing delayed emergence with a following cohort. Salman *et al.* (2016) suggested that  
436 these semivoltine individuals tend to emerge 3 to 4 weeks earlier than non-diapausing  
437 individuals emerging the same year. Yet, this pattern was described from comparisons of  
438 pheromone trapping data (thus including both non-diapausing and diapausing individuals  
439 without the possibility to separate them) to individuals sampled as larvae and kept in cages  
440 until emergence. Our results did not confirm this trend. On the contrary, the phenology of

441 individuals emerging after one year of prolonged diapause fits the phenological patterns of the  
442 univoltine individuals emerging the same year, rather than those of their own cohort the  
443 previous year. This suggests that the semivoltine individuals react to environmental cues of  
444 their emerging year. Such a mechanism of regulation probably optimises the overlapping of  
445 emergence curves in a given year, and increases the chance of reproductive success for the  
446 individuals experiencing prolonged diapause. Data obtained from a similar monitoring  
447 protocol in different environments would be useful to determine if semivoltine individuals are  
448 usually synchronised with the emergence of the following cohort as we suggest, or if their  
449 emergence dynamics may vary among regions or habitats. Moreover, the individuals  
450 monitored in the present study were kept in a common garden after procession, and were thus  
451 exposed to similar environmental cues, which possibly tended to fade the site effects. Our  
452 study thus probably missed some of the parameters regulating prolonged diapause, even if it  
453 allowed to propose testable hypotheses.

454 Beyond phenology, we also questioned whether some environmental parameters could affect  
455 the proportion of individuals experiencing prolonged diapause in a given site. Consistent with  
456 the early hypothesis of Démolin (1969), we highlighted that a long larval development and  
457 thus a late procession date increased the probability of prolonged diapause. As pupation and  
458 metamorphosis take at least 6 weeks (Huchon & Démolin, 1970), prolonged diapause could  
459 be seen as a mechanism to avoid delayed emergence and de-synchronization with the rest of  
460 the cohort or with the optimal local timing of emergence when larval development is too long.

461 We also found somehow contradictory results, as both higher elevation and warmer winters  
462 tend to favour semivoltinism. As suggested above for the complex relationship between  
463 temperature and emergence timing, this could be due to a decoupling between local  
464 adaptation at higher elevation favouring prolonged diapause and a plastic response to warmer  
465 winter conditions during some years. However, this observation contradicts the conclusions of

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

471

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

473 One of the main conclusions of the present work is that the effects of environmental factors  
474 on the phenological response of the PPM are complex and sometimes contradictory. In  
475 particular, we hypothesized that phenology regulation includes both local adaptations and  
476 plastic responses, with different tuning of spatial (early emergence at high elevations) and  
477 temporal variability (early emergence when springs are warmer and autumns are colder).

478 These observations advocate for the design of complementary experiments along the same  
479 altitudinal gradient, using full common garden experiments as well as translocation designs,  
480 to dissect these opposing influences on the phenotype. We agree with Sparks and  
481 collaborators (2006) that these findings warn against expecting simple responses to climate  
482 warming, as differential warming trends in spring and autumn would have opposite  
483 consequences on species' phenology.

484 Moreover, altitudinal gradients are often used as space-for-time substitution designs that are  
485 supposed to help understanding how a species will react to climate change. Such studies  
486 suppose that investigating how phenotypic traits change along elevational gradients can  
487 contribute to the prediction of species phenological responses to future climate. This is  
488 because temperature varies strongly over a limited distance where photoperiod remains  
489 similar (de Arce Crespo & Gutierrez, 2011; Zografou *et al.*, 2020). However, the complex  
490 relationship between opposite geographical and temporal trends we identified in the present

491 study suggests that using altitudinal gradients as space-for-time substitution devices would  
492 lead to erroneous conclusions in the PPM. A similar contradiction was reported for ring-width  
493 response to temperature variability in the Douglas-fir (Klesse *et al.*, 2020). When responses to  
494 spatial versus temporal temperature variation is opposite, one can suggest that spatial  
495 variation, caused by local adaptation and other slow processes, cannot be used to anticipate  
496 changes caused by rapid climate change (Klesse *et al.*, 2020). We support the conclusions of  
497 Zografou *et al.* (2020) and de Arce Crespo and Gutierrez (2011) who highlighted how caution  
498 is needed before extrapolating such results and predicting the effects of climate change on  
499 individual species' phenology.

500  
501 *Perspectives*

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

516 of genomic adaptations along altitudinal gradients, which could explain the maintenance of  
517 the observed phenological patterns with earlier flight periods at higher elevation.

518

519

520

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527

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669

670

671

672 **TABLES**

673 **Table 1:** Details of the generalized linear mixed models used to analyze the procession-to-  
 674 emergence time lapse (Model 1), the emergence date (Model 2), the proportion of individuals  
 675 entering prolonged diapause (Model 3) and the phenology of semivoltine individuals (Models  
 676 4 & 5). In Models 1 & 2, “T<sub>09</sub>, T<sub>10</sub>,... T<sub>06</sub>” stand for “temperature of September, October,...  
 677 June”. The temperatures of September, October, November and December are the ones of the  
 678 previous year (N-1), i.e. those experienced by the young larvae. “#obs” stands for “total  
 679 number of observations”.

680

<b>Model 1-2 Phenology of univoltine individuals</b>	
Response	Model 1 Procession-to-emergence time lapse
	Model 2 Emergence date
Fixed effects	Procession date Elevation
	T <sub>09</sub> , T <sub>10</sub> , T <sub>11</sub> , T <sub>12</sub> , T <sub>01</sub> , T <sub>02</sub> , T <sub>03</sub> , T <sub>04</sub> , T <sub>05</sub> , T <sub>06</sub>
Random effects	Site
	Year of procession
	Batch
Distribution (link)	Gaussian (identity)
#obs	41,847
<b>Model 3 Proportion of individuals entering prolonged diapause</b>	
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
<b>Model 4-5 Phenology of semivoltine individuals</b>	
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

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

682

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

Fixed effects	$\chi^2$	df	p	Regression equation	$R^2_m$	$R^2_c$
<b>Model 1: Proc2Em of univoltine individuals</b>					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 <sub>10</sub>	5.7	1	0.017	$y = 1.76 \cdot x + 71.01$		
T <sub>12</sub>	4.8	1	0.028	$y = -1.57 \cdot x + 98.36$		
T <sub>02</sub>	5.3	1	0.022	$y = -1.70 \cdot x + 100.94$		
T <sub>06</sub>	31.8	1	< 0.001	$y = -4.56 \cdot x + 176.93$		
T <sub>09</sub> , T <sub>11</sub> , T <sub>01</sub> , T <sub>03</sub> , T <sub>04</sub> , T <sub>05</sub>	-	-	-	-		
<b>Model 2: Emergence date of univoltine individuals</b>					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 <sub>10</sub>	5.7	1	0.017	$y = 1.76 \cdot x + 178.57$		
T <sub>12</sub>	4.8	1	0.028	$y = -1.57 \cdot x + 205.92$		
T <sub>02</sub>	5.3	1	0.022	$y = -1.70 \cdot x + 208.49$		
T <sub>06</sub>	31.8	1	< 0.001	$y = -4.56 \cdot x + 284.48$		
T <sub>09</sub> , T <sub>11</sub> , T <sub>01</sub> , T <sub>03</sub> , T <sub>04</sub> , T <sub>05</sub>	-	-	-	-		
<b>Model 3: Proportion of moths entering prolonged diapause</b>					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	$probit(y) = 0.0014 \cdot x - 3.8171$		
<b>Model 4: ProlDiap<sub>N+1</sub> median</b>					0.00	0.77
DirEm <sub>N</sub>	-	-	-	-		
<b>Model 5: ProlDiap<sub>N+1</sub> median</b>					0.49	0.68
DirEm <sub>N+1</sub>	40.4	1	< 0.001	$y = 0.84 \cdot x + 37.11$		

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

689

690 **FIGURE LEGENDS**

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

695

696 **Fig. 2** Correlation between the procession-to-emergence time lapse (Proc2Em) and procession  
697 date (A), between emergence date and procession date (B), and between emergence date (or  
698 procession-to-emergence time lapse, Proc2Em, on the secondary axis) and elevation (C) or  
699 temperatures (D). As shown in Table 2, the regression slopes between the emergence date  
700 or Proc2Em and the altitude or temperatures are identical because Model 1 and 2 are  
701 mathematically related. The two responses are therefore displayed jointly in C and D. Raw  
702 data are displayed as squares (A & B) or as density traces (“violin plots”, Hintze & Nelson,  
703 1998) highlighting the distribution of the data (C & D). Colors show the number of moths for  
704 each (x, y) coordinate (A & B) and the regression lines from Model 1 (A) and Model 2 (B, C  
705 & D) are displayed in black. The procession and emergence dates have been translated to a  
706 “Month day” format for easier reading but the statistics were performed on dates expressed in  
707 Julian days. “pR<sup>2</sup>” stands for “part R<sup>2</sup>” and represents the part of variance explained by each  
708 covariate alone in Model 1 (A and green part on the right in C and D) and Model 2 (B and  
709 blue part on the left in C and D).

710

711 **Fig. 3** Correlation between the proportion of individuals entering prolonged diapause and  
712 procession date (A), elevation (B) and winter temperature (C, average of December, January,  
713 February and March). Raw data are displayed as squares (A) or as density traces highlighting  
714 the distribution of the data (B & C). Colors show the number of moths for each (x, y)

715 coordinate (A) and the regression lines from Model 3 are displayed with solid (model fitted  
716 with all data) or dashed (model fitted without site F781 in 1978, see text for details) lines. The  
717 procession date has been translated to a “Month day” format for easier reading but the  
718 statistics were performed on dates expressed in Julian days. “pR<sup>2</sup>” stands for “part R<sup>2</sup>” and  
719 represents the part of variance explained by each covariate alone in the model mentioned in  
720 subscript.

721

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

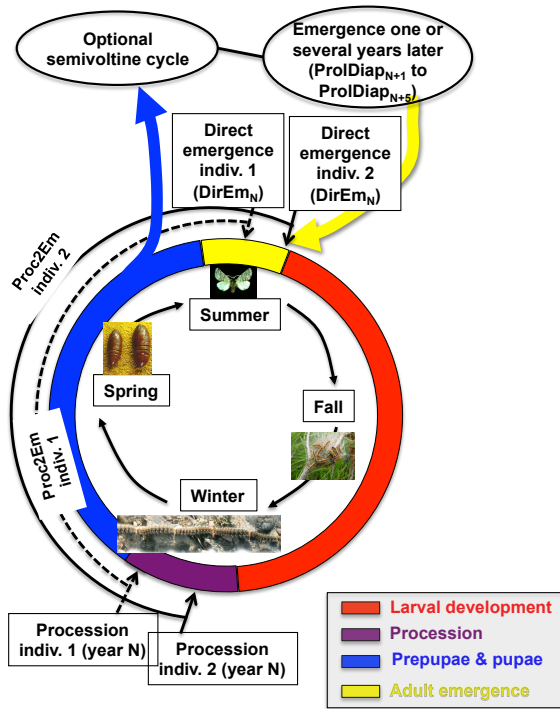
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#### 731 **SUPPORTING INFORMATION**

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

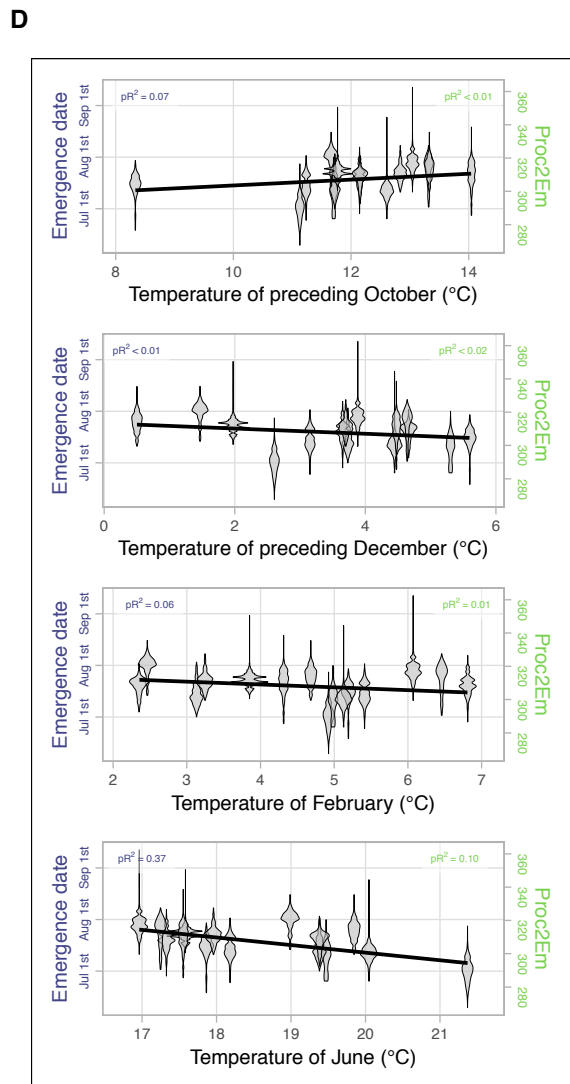
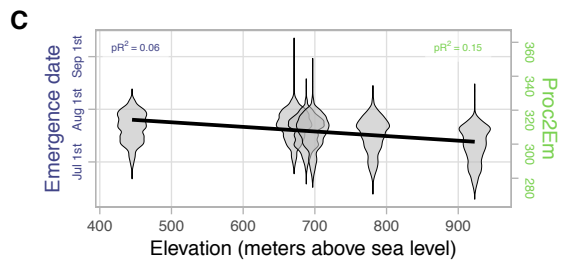
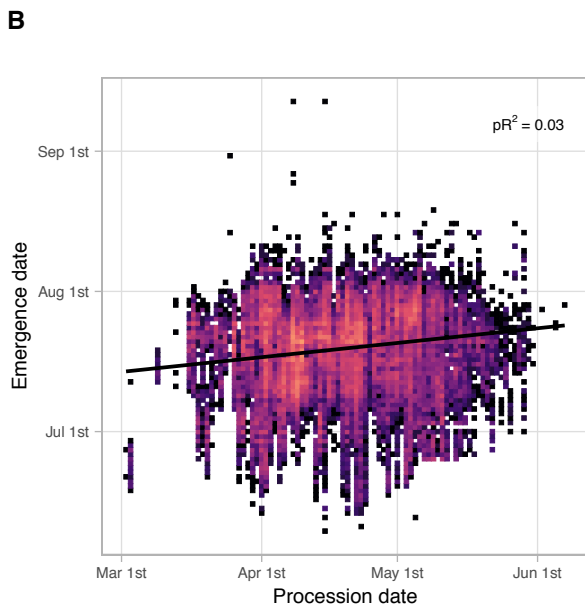
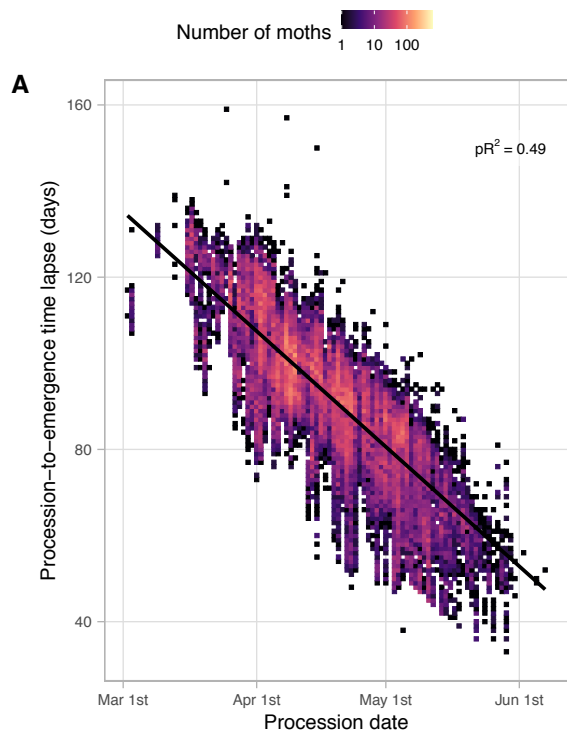
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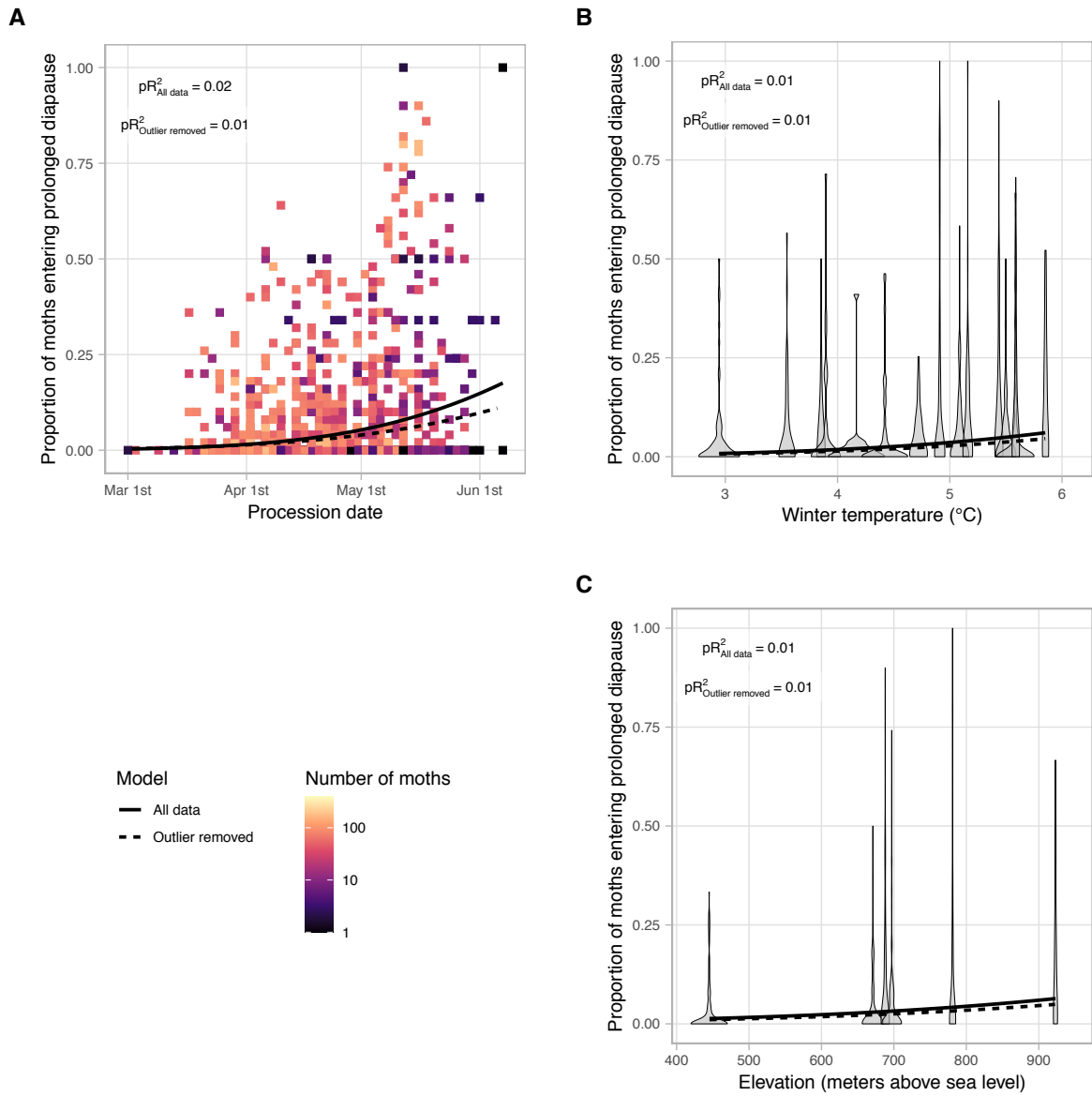
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