

1     **Effects of climate warming on the pine processionary moth at the**  
2     **southern edge of its range: a retrospective analysis on egg survival**  
3                                     **in Tunisia**

4             Asma BOUROUGAAOUI<sup>1,2,3</sup>, Christelle ROBINET<sup>3</sup>, Mohamed L. BEN JAMAA<sup>1</sup>, Mathieu LAPARIE<sup>3</sup>

6     <sup>1</sup> Université de Carthage, Institut National de Recherches en Génie Rural, Eaux et Forêts-Laboratoire  
7     de Gestion et de Valorisation des Ressources Forestières, Ariana, Tunisia

8     <sup>2</sup> Université de Carthage, Institut National Agronomique de Tunis, Tunis, Tunisia

9     <sup>3</sup> INRAE, URZF, 45075, Orléans, France

11    \* Corresponding author: **Mathieu Laparie**, [mathieu.laparie@inrae.fr](mailto:mathieu.laparie@inrae.fr)

18    Journal: PCI Ecology

19    Type of paper: Research Article

21    Number of figures: 6

22    Number of Tables: [13](#)

23    Supplementary Material: [119](#)

25    Running title: **Effects of climate warming on eggs of the pine processionary moth in Tunisia**

## 26 **Abstract**

27 In recent years, ectotherm species have largely been impacted by extreme climate events, essentially  
28 heatwaves. In Tunisia, the pine processionary moth (PPM), *Thaumetopoea pityocampa*, is a highly  
29 damaging pine defoliator, which typically lays eggs in summer. Its geographical range is expanding  
30 northwards in Europe while retracting from South Tunisia where summer temperatures can reach  
31 extremely high values. In this study, we aimed at exploring the effects of climate change on this  
32 species at its southern range edge. We investigated variations of fecundity and causes of egg  
33 mortality over time using historical and contemporary collections of egg masses from different  
34 Tunisian sites to seek relationships with regional climate change over three decades (1990-2019).  
35 Our results suggest negative effects of summer heat on egg survival, reflected in a decrease of  
36 hatching rate down to 0% in one site during a heatwave. Such a high hatching failure was found to  
37 result from both high egg sterility (our results did not allow distinguishing impeded mating success  
38 from failed egg maturation or early death of the embryo) and increased abortion of more developed  
39 embryos, but little effects of parasitism rate, thereby suggesting vulnerability to heat during  
40 embryonic development. We also observed decreasing female fecundity (*i.e.*, number of eggs laid per  
41 female) in regions where data were available both in the 1990s and the 2010s, which was associated  
42 with a decrease in parasitism rate, while the climatic variability increased. This study investigated  
43 direct hatching failure in nature that may be related to the magnitude of warming in summer.  
44 Previous studies have confirmed the thermal sensitivity of early instars of the PPM to temperatures  
45 observed in the present work, including one population from South Tunisia. However, further work is  
46 required to evaluate the relative importance of warming summers among populations because the  
47 risk of heat stress depends on the phenology of sensitive instars, and populations from the warmest  
48 areas may not necessarily be the most vulnerable to climate change if they already evolved  
49 phenological heat avoidance. In addition to heat-induced mortality, the ultimate fitness of individuals  
50 that survive challenging heat stresses during early developmental stages should also be explored to  
51 determine potential carry-over effects on subsequent life stages.

52

## 53 **Keywords**

54 Egg mass, heatwave, pine processionary moth, pinus, *Thaumetopoea pityocampa*, Tunisia

## 55 Introduction

56 During the period 1901-2010, land temperature has risen by 1.12 and 0.84°C in the Northern and  
57 Southern hemispheres, respectively (Jones et al., 2012). Climate change is predicted to increase not  
58 only mean temperatures but also temperature variability and, in turn, the magnitude and frequency  
59 of stochastic extreme thermal events (Allen et al., 2012). This is already being increasingly observed  
60 over most parts of the world (Allen et al., 2012; Coumou & Rahmstorf, 2012; Fischer & Schär, 2010),  
61 particularly northern Africa (Fontaine et al., 2013; Nangombe et al., 2019; Zittis et al., 2021). Mean  
62 temperature has risen by about 1.4°C since 1901 in Tunisia, with a remarkable average increase of  
63 +0.4°C per decade in the last 30 years, primarily observed during summer in southern regions where  
64 temperatures can exceed 40°C (Verner et al., 2013). Together with average warming, increasing  
65 thermal fluctuations and extreme events may impact all fitness components (e.g., phenology,  
66 morphology, behaviour, locomotor activity, and physiology) of organisms (Charmantier & Gienapp,  
67 2014; Chuine, 2010; Chuine et al., 2013; Gardner et al., 2011; Ghosh et al., 2013; Kingsolver et al.,  
68 2013; Liu et al., 1995; Pigliucci, 2001, 2005; Pincebourde et al., 2021; Pincebourde & Woods, 2020;  
69 Sheridan & Bickford, 2011; Thompson et al., 2013; Woods et al., 2015; Wu et al., 2019). Moreover,  
70 the concomitant and mutually interacting facets of climate change may ultimately translate into  
71 survival and in turn alter genetic frequencies, population density in given habitats, as well as  
72 persistence and distribution of many organisms (Root et al., 2003; Vasseur et al., 2014). In the  
73 twentieth century, a wide range of taxa ranging from invertebrates to mammals and from grasses to  
74 trees have shifted their ranges poleward, upslope or both (Crozier, 2004; Hickling et al., 2005; Karban  
75 & Strauss, 2004; Parmesan et al., 1999; Parmesan & Yohe, 2003; Root et al., 2003; Walther et al.,  
76 2002).

77 ~~During the period 1901-2010, land temperature has risen by 1.12 and 0.84°C in the Northern and~~  
78 ~~Southern hemispheres, respectively (Jones et al., 2012).~~ Climate warming (Parmesan & Yohe, 2003)  
79 ~~may induce heritable as well as plastic changes in a wide range of traits at multiple phenotypic levels~~  
80 ~~(Pigliucci, 2001, 2005), such as (i) the timing of life cycle events such as flowering, migration and egg~~  
81 ~~laying (Charmantier & Gienapp, 2014; Chuine, 2010; Chuine et al., 2013; Liu et al., 1995), (ii) the~~  
82 ~~behaviour such as locomotor activity or movements to seek suitable microclimates (Pincebourde et~~  
83 ~~al., 2021; Pincebourde & Woods, 2020; Woods et al., 2015), the morphology such as body size~~  
84 ~~(Gardner et al., 2011; Ghosh et al., 2013; Sheridan & Bickford, 2011; Wu et al., 2019), or (iii) the~~  
85 ~~physiology such as thermal acclimation or adaptation to thermal stressors. Those changes may~~  
86 ~~ultimately translate into survival and in turn alter genetic frequencies, population density in given~~  
87 ~~habitats, as well as distribution (Root et al., 2003).~~ In the twentieth century, a wide range of taxa  
88 ~~ranging from invertebrates to mammals and from grasses to trees have shifted their ranges~~

89 poleward, upslope or both (Crozier, 2004; Hickling et al., 2005; Karban & Strauss, 2004; Parmesan et  
90 al., 1999; Parmesan & Yohe, 2003; Root et al., 2003; Walther et al., 2002). Climate change is also  
91 expected to increase temperature variability and, in turn, increase the magnitude and frequency of  
92 stochastic extreme thermal events, which is already increasingly observed over most parts of the  
93 world (Allen et al., 2012; Coumou & Rahmstorf, 2012; Fischer & Schär, 2010), particularly northern  
94 Africa (Fontaine et al., 2013; Nangombe et al., 2019; Zittis et al., 2021). In Tunisia, the mean average  
95 temperature has risen by about 1.4°C since 1901, with a remarkable increase in the last 30 years with  
96 an average warming of 0.4°C per decade, and most substantial warming has been observed during  
97 summer in southern regions where average maximum temperatures can often exceed 40°C (Verner  
98 et al., 2013). Together with average warming, increasing thermal fluctuations and extreme events  
99 may impact organismal fitness (Kingsolver et al., 2013; Thompson et al., 2013), and the combination  
100 of these is expected to dramatically impact survival, distribution and life cycles of many organisms.

101 The pine processionary moth (hereafter referred to as PPM), *Thaumetopoea pityocampa* (Denis  
102 & Schiffermüller, 1776) (Lepidoptera, Notodontidae), is a highly damaging pest of pine forests across  
103 the circum-Mediterranean region (Carus, 2009; Démolin, 1969; Jacquet et al., 2013; Sbay & Zas,  
104 2018). The geographic range of the PPM extends from northern Africa to southern Europe, from the  
105 Atlantic coast to the western part of Turkey (EPPO, 2004; Roques, 2015). The PPM is a well-  
106 documented insect that has been acknowledged by the Intergovernmental Panel on Climate Change  
107 (IPCC) as one of the few species for which the causal relationship between climate warming and  
108 range expansion has been thoroughly proven (Battisti et al., 2005; Rosenzweig et al., 2007). The  
109 distribution range remained relatively steady until the late 1990s but then expanded towards higher  
110 latitudes and elevations in southern Europe. Indeed, warming winter temperatures have facilitated  
111 feeding in this winter-developing species and thus indirectly contributed to improving survival rate  
112 and growth rate in newly colonized areas (Battisti et al., 2005, Robinet et al., 2007).

113 Contrary to the beneficial effects of climate change demonstrated near the northern distribution  
114 edge of the PPM, adverse effects of climate change have been observed on the southern range edge  
115 (North Africa). Range retraction has been described in southern Tunisia and was found to result from  
116 increasing mortality rates of early life stages in a translocation experiment along a natural thermal  
117 gradient, which could be ascribed to local effects of climate warming (Bourougaaoui et al., 2021).  
118 Fecundity, hatching rate and predation at the egg stage (mostly from parasitoids) presumably play an  
119 important role in the PPM because this species is gregarious. Several studies have emphasized how  
120 larval performance depends on the realised group size, i.e. the number of neonates, and ultimately  
121 the survival of the whole colony until the end of larval growth (Clark & Faeth, 1997; Denno & Benrey,  
122 1997; Ronnås et al., 2010). Colony density has been suggested to influence feeding activity and

123 feeding efficiency of individual larvae, which is particularly critical in early stages when individuals  
124 have little desiccation and starvation resistances. The number of larvae was also shown to impact silk  
125 weaving activity to build and maintain the nests that shelter larvae during the day until their  
126 pupation in spring (Démolin, 1965; Martin, 2005). As a result, the number of surviving tents and the  
127 average proportion of living larvae per tent were positively correlated to colony size (Pérez-Contreras  
128 et al., 2003; Roques et al., 2015). Focusing on the main drivers of colony size in early development is  
129 therefore of key importance to understand distribution changes and responses to climate change.

130 In Tunisia, the life cycle of PPM is generally univoltine, however it can extend over two years at  
131 high altitudes due to prolonged diapause in a fraction of the pupae (Roques, 2015). Flight periods are  
132 poorly documented, nonetheless a study conducted by Démolin and Rive in 1968 in high and  
133 medium latitudes, revealed that most individuals fly in the second half of July at high elevations and  
134 August to September at mid elevations (Ben Jamâa & Jerraya, 1999; Démolin & Rive, 1968). Due to  
135 the short lifespan of adults, egg laying occurs immediately after adult flights, and eggs and neonate  
136 larvae are presumably the instars that are most likely exposed to acute heat during the whole life  
137 cycle. Understanding the effects of warming on female fecundity, egg survival and egg parasitoids is  
138 crucial to explore the overall effects of climate change of this species at its southern range edge  
139 where warming is known to be of great magnitude.

140 In this study, we explored how climate warming over the last three decades may have impacted  
141 egg survival and hatching rate in Tunisia. To address this question, we combined historical and  
142 contemporary collections of egg masses originating from different Tunisian localities in the 1990s  
143 (1992, 1993, and 1995) and in the 2010s (2010, 2014, 2017, 2018, and 2019). Egg phenotypes and  
144 survival rate were investigated with regard to regional climatic features and contrasts analyzed from  
145 30-year climatic data series across Tunisia. A cornerstone of this study is the identification of climate  
146 regions computed from multiple meteorological series, which allows comparing eggs from multiple  
147 sites within statistically consistent climates, instead of using arbitrary groups such as administrative  
148 regions. A grouping method was mandatory to analyze the long term data available on PPM eggs  
149 because exact sampling sites have changed over the years.

## 150 **Materials and Methods**

### 151 **1 Historical data (1992-2014) and egg sampling done for this study (2017-2019)**

152 A total of 755 egg masses from historical datasets and recent collections were analyzed in this  
153 study. Egg masses originated from 22 sites distributed across the PPM distribution in Tunisia (Table 1;  
154 Figure 1; Table SM1). Historical datasets on egg masses collected in 1992, 1993, 1995, 2010 and 2014

155 on Aleppo pine stands, *Pinus halepensis* Miller, were retrieved from institutional reports  
 156 (unpublished data, INRGREF). These datasets report the length of egg masses, the number of eggs  
 157 per egg mass, and the phenotype of individual eggs (parasitized, aborted, sterile, hatched). In  
 158 addition, we collected egg masses in various locations in 2017, 2018, and 2019. All these egg masses  
 159 were also collected on Aleppo pine stands, before hatching but as late as possible in each region to  
 160 ensure eggs were exposed to natural conditions, and then and kept at ambient temperature ( $25 \pm$   
 161  $2^\circ\text{C}$ ) at the INRGREF laboratory near Tunis where we followed a protocol similar to that used for  
 162 historical collections of egg masses. Egg masses were kept individually in test tubes capped with  
 163 cotton to allow ventilation. Egg hatching was checked daily. After a period of at least 40 days with no  
 164 additional hatching, the protective scales that cover PPM egg masses were removed to observe  
 165 individual eggs under a binocular magnifier and collect data similar to that available in historical  
 166 datasets: length of egg masses, number of eggs per egg mass (fecundity), and egg phenotype. First,  
 167 hatched eggs were distinguished from unhatched eggs based on the presence of the characteristic  
 168 large jagged exit hole from which the neonate left the egg, and an empty transparent shell. Then,  
 169 unhatched eggs were dissected to assess the cause of mortality (parasitized, *i.e.*, eggs with a small  
 170 parasitoid exit hole and/or containing a dead parasitoid and/or containing parasitoid meconium;  
 171 aborted, *i.e.*, dead embryo or dead PPM larva; and sterile, *i.e.*, undeveloped egg with dried-up yolk)  
 172 (Imbert, 2012). Parasitism rate was calculated taking into account both emerged parasitoids found in  
 173 the test tubes and dead ones found inside unhatched eggs.

174

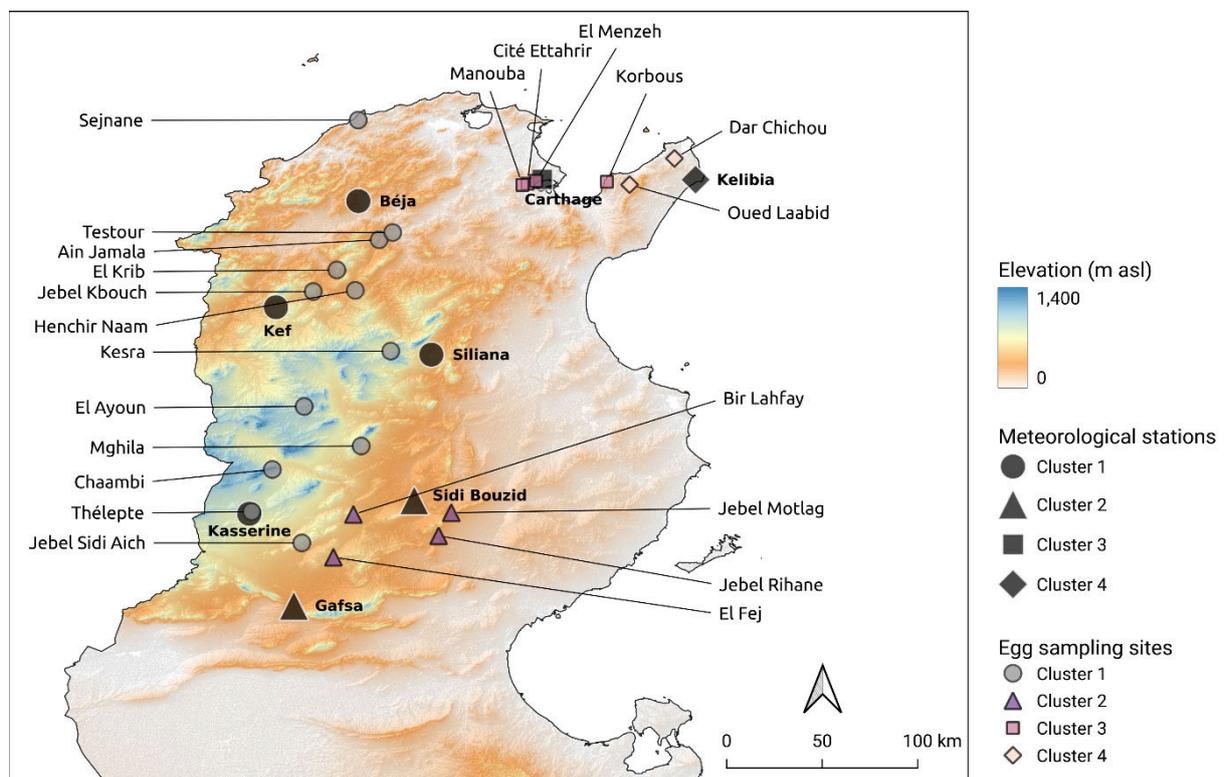
175 **Table 1** . Collection of PPM egg masses in Tunisia (see Table SM1 for coordinates of the sites).  
 176 Calculated climate clusters are indicated to represent the amount of data available per cluster.

Site	Year of collection	Number of egg masses	Cluster	Nearest meteorological station (within 100 km and 350 m in elevation); distance
Sejnane	1995	20	1	Béja; 51.0 km
Testour	2014	12	1	Béja; 26.3 km
Ain Jamala	2010	15	1	Béja; 26.5 km
El Krib	2010	18	1	Kef; 38.7 km
Henchir Naam	1992	53	1	Kef; 41.5 km
Jebel Kbouch	1993	56	1	Kef; 21.3 km
El Ayoun	1993	30	1	Kasserine; 73.2 km
Chaambi	1995	27	1	Kasserine; 30.3 km
	2014	11		
Thélepte	2017	43	1	Kasserine; 1.9 km
	2019	30		
Jebel Sidi Aich	2014	31	1	Kasserine; 32.7 km
Kesra	2010	18	1	Siliana; 20.8 km
Mghila	2014	51	1	Kasserine ; 71.6 km

Bir Lahfay	2014	12	2	Sidi Bouzid; 32.5 km
Jebel Motlag	2017	38	2	Sidi Bouzid; 20.6 km
	2018	29		
Jebel Rihane	2017	25	2	Sidi Bouzid; 25.7 km
El Fej	2017	18	2	Gafsa; 36.6 km
El Menzeh	1992	19	3	Carthage; 3.2 km
	1993	57		
	2014	10		
Cité Ettahrir	2014	21	3	Carthage; 7.9 km
Manouba	2010	15	3	Carthage; 10.5 km
Korbous	1992	30	3	Carthage; 32.5 km
	2010	15		
Dar Chichou	1995	20	4	Kelibia; 17.1 km
Oued Laabid	1995	31	4	Kelibia; 33.3 km

177

178



179

180 **Figure 1.** Location of egg sampling sites and meteorological stations, with associated calculated  
 181 climate cluster.

182

## 183 2 Climate data

184 We used series of daily temperatures recorded (by the Institut National de Météorologie, INM,

185 Tunis, Tunisia) in eight meteorological stations distributed within the PPM range in Tunisia (Fig. 1;

186 Table SM2). To fill missing data in INM time series, satellite measurement of daily temperatures were

187 also retrieved from the NASA Prediction of Worldwide Energy Resources website  
188 (<https://power.larc.nasa.gov/data-access-viewer/>) on the grid cells of 0.5 degree × 0.625 -degree (~  
189 50 km × 60 km) matching the location of INM weather stations (Table SM2). The similarity of both  
190 sources of data was evaluated using Pearson correlations tests for daily maximal and daily minimal  
191 temperatures in Tunis, where the data series from INM since 1990 was the most comprehensive.  
192 Daily maximal and minimal temperatures from both data sources were found to be strongly  
193 correlated (Pearson tests,  $r = 0.95$ ,  $p < 0.001$  and  $r = 0.94$ ,  $p < 0.001$ , respectively). The two types of  
194 datasets were therefore combined in case of missing data in other INM series to reconstruct  
195 uninterrupted series for the period 1990-2019 (Table SM2).

196 Each site of egg sampling was assigned to the nearest meteorological station (< 100 km in all  
197 cases) among those situated at an elevation within 350 meters of the egg site, an arbitrary threshold  
198 we chose to mitigate potential climatic differences along elevation gradients (Table 1, Figure SM3).

199 To better understand climatic features in each of the eight meteorological series (Table SM2), (i)  
200 the normal daily temperatures with seasonal contrasts over the period, as well as (ii) the overall  
201 trend since 1990, were calculated. For (i), we averaged 30 years of daily maxima (TX) and minima  
202 (TN) by day of the year, and calculated the likelihood for each day of temperatures below 0 or above  
203 32 and 40°C, which have been suggested by [Démolin](#) (~~Démolin~~, 1969) and Huchon & Démolin (1970)  
204 as pivotal thresholds for phenological strategies and survival in the PPM (see also discussion in  
205 Robinet et al. 2015). For (ii), daily TX and TN were averaged per year and represented along the 30  
206 years of data, together with the total number of days below 0 or above 32 and 40°C. Those per-  
207 station climate summaries are provided in SM4.

### 208 **3 Statistical analyses**

209 **Climate clusters.** The unbalanced egg sampling design throughout historical data and recent  
210 collections prevents allochronic comparisons of egg phenotypes within individual sampling sites.  
211 Therefore, we investigated climatic similarities and dissimilarities among meteorological series in  
212 order to identify regional climate clusters within which multiple meteorological series and associated  
213 egg sampling sites could be statistically grouped together. Climate-based grouping appeared more  
214 relevant and less arbitrary than using administrative regions because of the heterogeneous  
215 landscape and overall size of some regions. To do so, the monthly averages of TN and TX were  
216 calculated in each meteorological series over the period 1990-2019, resulting in a set of 24 variables

217 (2 × 12 months) and 30 values per series (30 years). A Principal Component Analysis (PCA) was used  
218 on the covariance matrix of those variables to project the 30 years of data from each of the eight  
219 meteorological locations and better visualize their intra- and inter-group variance on reduced  
220 dimensionality. The resulting multivariate object then fed a K-medoid clustering analysis using the  
221 PAM method (Partitioning Around Medoids, see Reynolds et al. 2006, Schubert and Rousseeuw 2019)  
222 to identify relevant climate clusters (listed in Table1). –The PCA could be performed on unscaled  
223 temperature variables since they were all measured in the same unit (covariance PCA), thereby  
224 giving most weight to summer months and to TX, due to ~~due to generally~~ higher temperature values,  
225 without neglecting other months and TN in the overall variance structure. As a consequence, the  
226 climate clusters identified using all four seasons are mostly influenced by the season eggs are  
227 exposed to (roughly June to September). Details of cluster assignation to individual points in each  
228 meteorological series are detailed in SM5. Monthly means of TN and TX of the medoid of each  
229 cluster, *i.e.*, the individual point that best represents its cluster due to low average dissimilarity to all  
230 other points, are represented in SM6.

231 ***Interannual fluctuation of maximal summer temperature within clusters.*** To explore regional  
232 warming trends to which eggs are subjected within clusters over 1990-2019, the monthly means of  
233 daily maximal temperatures from meteorological series within each cluster were calculated from  
234 June to September. A linear model was then built for each cluster and each month to plot regressions  
235 over time and determine the slope for each cluster. The adequacy of residuals to Normality was  
236 checked using QQ plots.

237 ***Egg phenotype comparisons.*** Egg phenotypes could not be compared allochronically in all clusters  
238 identified because the data set was unbalanced, with only two of four clusters grouping egg samples  
239 from both periods. Further analyses on eggs are therefore focused on those two clusters, but a  
240 complementary synchronic analysis is provided in SM7 to compare egg phenotypes across all clusters  
241 within the period(s) they have in common. Since egg phenotype variables did not meet assumptions  
242 of homoscedasticity and normality for parametric tests, ~~therefore~~ we used the non-parametric  
243 RANCOVA ~~test (RANCOVA)~~ proposed by Quade (1967) to compare eggs sampled between clusters (1  
244 and 3) and periods (1990s and 2010s) ~~within each of the four clusters in the 1990s or in the 2010s.~~  
245 First, the response variables (Fecundity, Hatching, Sterility, Abortion and Parasitism rates; Clutch  
246 length was discarded due to its high correlation and redundancy with Fecundity) and the covariate  
247 (monthly means of TX averaged from June to September per year per cluster) were ranked  
248 separately. Second, residuals from the respective linear regression of each ranked response variable  
249 on the ranked covariate were calculated. Third, the effects of grouping factor(s) on residuals were  
250 investigated for each response variable using ~~the~~ Quade's RANCOVA (factors Cluster, Period, and

251 their interaction). It was followed by pairwise t-tests and a Bonferroni correction when a significant  
252 interaction term was found.—Since the dataset is unbalanced, with both past and recent samples in  
253 only two of the four climate clusters, we conducted two separate Quade’s RANCOVA analyses:

254

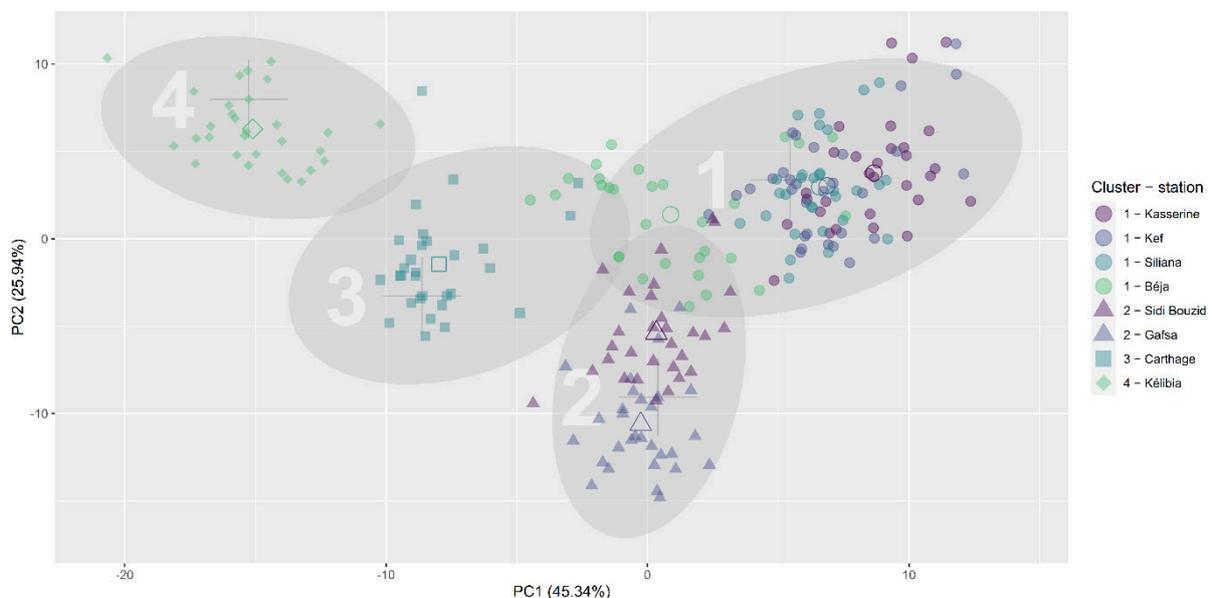
255 i. Analysis (1) was conducted on a subset of the data containing only clusters 1 and 3  
256 (where past and recent samples exist), in which we included both Cluster and Period  
257 factors as well as their interaction. When the interaction was significant, pairwise  
258 comparisons were performed using pairwise t-tests and Bonferroni correction.

259 ii. Analysis (2) synchronically compared clusters within each period subset, i.e., all clusters  
260 were considered but compared only to other clusters during the period(s) they shared. A  
261 Tukey post hoc procedure was used to investigate pairwise differences when a main  
262 effect was found.

## 263 Results

### 264 1 Climate clusters

265 The first plane (PC1 × PC2) of the PCA performed on climatic data from all eight meteorological  
266 series based on monthly averages of TN and TX each year ( $n = 8 \times 30 = 240$  data points) accounted  
267 for 71.28% of the total inertia (Fig. 2). PAM clustering on the PCA scores indicated four relevant  
268 groups with little overlapping (Fig. 2). Cluster 1 grouped Kef, Kasserine, Siliana and Béja together,  
269 cluster 2 grouped Sidi Bouzid and Gafsa together, while cluster 3 and cluster 4 corresponded to single  
270 meteorological series, Carthage and Kélibia, respectively. Depending on the meteorological series,  
271 between 76.67 and 100% of data points (years) were correctly assigned to their cluster (SM5).



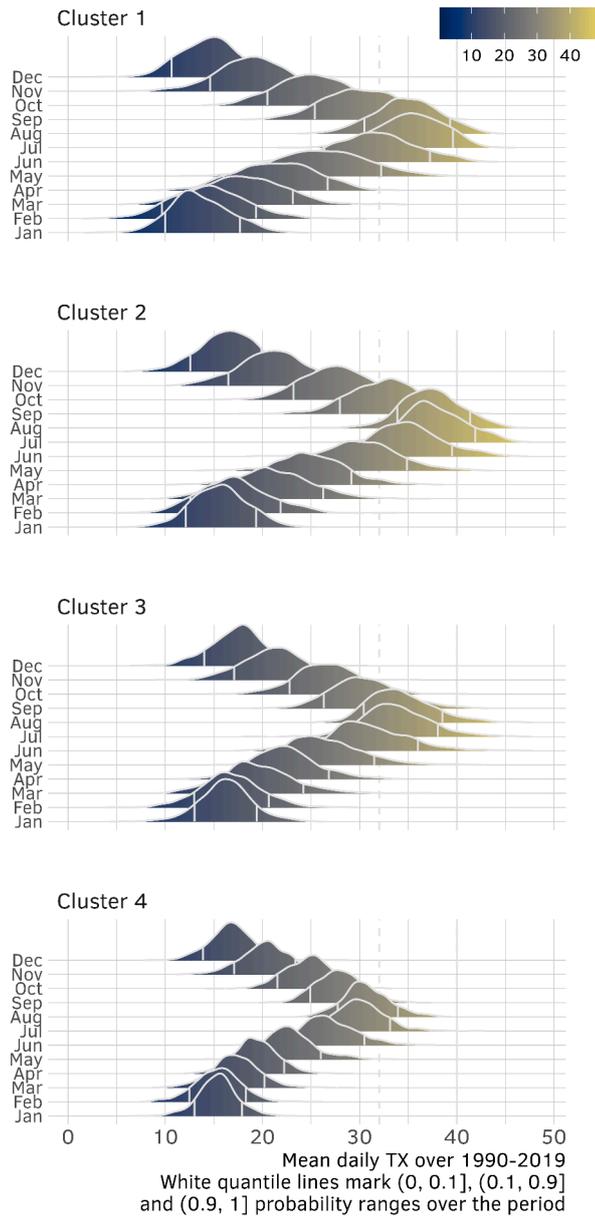
272

273 **Figure 2.** PCA scores for each year of data from the eight meteorological series (INM and NASA  
274 series, see table SM2) and 24 TN monthly average and TX monthly average variables. Each of the

275 eight series are identified with different symbol and colour combinations. PAM clustering results are  
276 overlaid on the PCA scores with 95% confidence ellipses and different symbols for different clusters.  
277 Open points correspond to the centroids of each meteorological series, while large thin crosses mark  
278 the medoid point of each cluster.

279

280 Per-cluster climate reconstructions averaged from daily means of TX over years (Fig. 3) indicated  
281 comparatively cold winters and hot summers with a high interseasonal variability in cluster 1,  
282 warmer winters and summers in cluster 2 with similar interseasonal variability, no extreme winters  
283 or summers and lower interseasonal variability in cluster 3, and the lowest interseasonal variability  
284 with comparatively mild summers in cluster 4. Within-month variability also appeared to be the  
285 highest over the last 30 years in clusters 1 and 2. The probability to overreach 40°C in summer was  
286 found to be the highest in cluster 2, while cluster 4 showed the lowest probability of overreaching  
287 32°C, with clusters 1 and 3 sitting in between those extremes. July and August are the warmest  
288 months in all clusters (Fig 3, Fig 4). TN monthly average within each medoid appeared to roughly  
289 reflect TX monthly average across each months of the year (SM6), indicating that similar trends can  
290 be inferred for per-cluster TN monthly average.



291 **Figure 3.** Ridge lines showing the distribution (kernel density) of daily means of TX over 1990-2019  
 292 for each cluster. The area of each distribution equals 1 and white vertical lines mark 0.1 and 0.9  
 293 quantiles.

294  
 295 Fluctuations of monthly averaged TX during summer over the last three decades (Fig. 4) in each  
 296 cluster indicated that September has not warmed significantly over time (no apparent slopes, non-  
 297 significant linear regressions). Contrariwise, July got significantly warmer over time, particularly in  
 298 cluster 2 and 3 (positive slopes, significant linear regressions). Cluster 2 appeared as the cluster with  
 299 the most striking summer warming, with positive increases of TX monthly average spanning not only  
 300 July, but also June (non-significant trend ( $p = 0.11$ ), similar to cluster 3) and August (significant  
 301 increase). Contrariwise, summers in clusters 1 and 4 appeared as the least subject to warming since  
 302 1990.



304

305

Figure 4. Fluctuations over time of monthly maximal temperature within each cluster, from June to September. The upper and lower lines of each ribbon correspond to 95% confidence intervals.

306

307

308

## 2 Egg comparisons

309

310

311

312

313

314

315

316

317

318

319

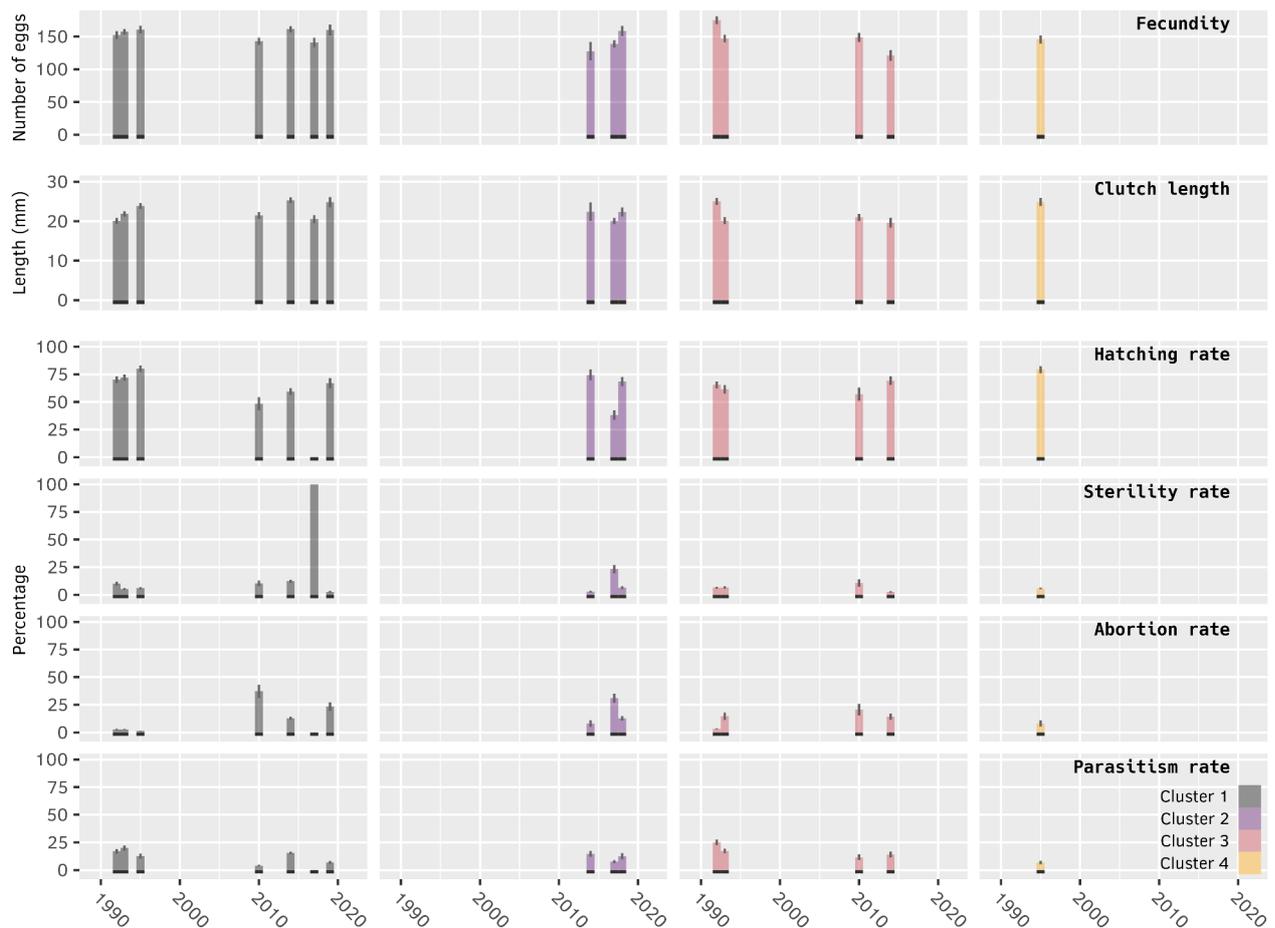
320

321

322

323

**Clutch size.** The length of egg masses was measured in case the distance between eggs of a clutch would differ among areas or periods, but this variable was found to be highly positively correlated to fecundity (Spearman correlation test,  $r_s = 0.72$ ,  $p < 0.001$ ), thereby leaving little room for variations in the fecundity/length ratio. Therefore, we focused further analyses on fecundity only, which ranged from  $121 \pm 8.2$  SE to  $174.6 \pm 5.9$  SE among clusters and periods (Figure 5). [The Quade's RANCOVA Analysis \(1\)](#) conducted on clusters where both past and present samples have been collected (clusters 1 and 3) showed no significant difference in fecundity between those two clusters (Quade's RANCOVA,  $F_{df} = 2.88_{1,}$ ,  $p = 0.09$ ) or periods ( $F_{df} = 3.01_{1,}$ ,  $p = 0.083$ ). However, a significant crossover interaction suggested non-parallel trends over time between them (Quade's RANCOVA,  $F_{df} = 6.42_{1,}$ ,  $p = 0.012$ ). Indeed, cluster 3 was the only cluster where fecundity changed over time (pairwise t-test,  $t_{df} = 3.1_{1,}$ ,  $p = 0.002$  adjusted by Bonferroni correction), with an average decrease of 16%. [Analysis \(2\)](#) performed on all clusters but synchronic periods revealed no differences among clusters 1, 3 and 4 in the 1990s (Quade's RANCOVA,  $F_{df} = 1.58_{2,}$ ,  $p = 0.208$ ), whereas it confirmed significant differences among clusters 1, 2 and 3 in the 2010s ( $F_{df} = 4.40_{2,}$ ,  $p = 0.013$ ). Specifically, fecundity was significantly higher in cluster 1 than cluster 3 (Tukey post hoc,  $p = 0.009$ ).



324

325 **Figure 5.** Fecundity (raw count of eggs), clutch length, hatching rate, sterility rate, abortion rate and  
 326 parasitism rate per cluster and sampling year. Black markers at the bottom show years when samples  
 327 have been collected, to distinguish true zeros from missing values. Error bars: SEM.

328

329

330 Hatching rate and egg Egg-mortality factors. Hatching rate did not differ Base-abortion and  
 331 parasitism rates differed significantly between clusters 1 and 3 (Analysis (1), Quade's RANCOVA,  $F_{dt} =$   
 332  $0.35_{1abortion-rate: F_{df} = 17.131, p = 0.566}$ ), yet it significantly differed between periods ( $F_{dt} = 35.65_{1 < 0.001}$ ; parasitism  
 333 rate:  $F_{df} = 4.531, p < 0.001$ ). As found for fecundity, the interaction term was significant ( $F_{dt} = 0.034$ ) and  
 334 periods (abortion rate:  $F_{df} = 50.92_{17, p < 0.001}$ ; parasitism rate:  $F_{df} = 20.48_{1361, p < 0.001}$ ). Cluster 1 was  
 335 the only cluster of the two where hatching rate decreased significantly between the past and recent  
 336 periods (pairwise t-test,  $t_{df} = 7.5_{1}$ ). However, the temporal increase in abortion and decrease in parasitism were similar in the two clusters since no  
 337 significant interaction was observed (abortion rate:  $F_{df} = 0.081, p < 0.001$  adjusted by Bonferroni correction). Particularly=  
 338  $0.776$ ; parasitism rate:  $F_{df} = 1.34_{17, p = 0.248}$ ). Yet, in 2017, hatching rate dropped to zero in cluster 1  
 339 with all eggs visually scored as sterile. Consequently, abortion and parasitism rates were also the  
 340 lowest (0 %) in this cluster that year since those mortality factors can only occur at a later

341 development stage (Figure 5, Table [SM8](#), [SM7](#)). ~~Cluster 1 was the only cluster where such a dramatic~~  
 342 ~~change was observed, and where hatching rate changed significantly between the past and recent~~  
 343 ~~periods (Analysis (1), pairwise t test,  $t_{df} = 7.5_{17}$ ,  $p < 0.001$  adjusted by Bonferroni correction).~~ This  
 344 average decrease over time can be attributed not only to the striking drop in 2017, but also to  
 345 decreases of lower magnitude in other recent years (Figure 5). Those changes correlate negatively  
 346 with the aforementioned trends in abortion rate and sterility rate. Of the two ~~clusters~~cluster, the  
 347 increase in abortion rate was most prominent in cluster 1 (Figure 5), although it may be  
 348 underestimated in recent years due to its null value in 2017 when eggs did not develop enough to  
 349 score abortion rate. The Quade's RANCOVA showed that base abortion and parasitism rates differed  
 350 significantly between clusters 1 and 3 (Quade's RANCOVA, abortion rate:  $F_{df} = 17.13_{17}$ ,  $p < 0.001$ ;  
 351 parasitism rate:  $F_{df} = 4.53_{17}$ ,  $p = 0.034$ ) and periods (abortion rate:  $F_{df} = 50.92_{17}$ ,  $p < 0.001$ ; parasitism  
 352 rate:  $F_{df} = 20.36_{17}$ ,  $p < 0.001$ ). However, the temporal increase in abortion and decrease in parasitism  
 353 were similar in the two clusters since no significant interaction was observed (abortion rate:  $F_{df} =$   
 354  $0.08_{17}$ ,  $p = 0.776$ ; parasitism rate:  $F_{df} = 1.34_{17}$ ,  $p = 0.248$ ). . ~~Analysis (2) showed synchronic differences~~  
 355 ~~among clusters in the rates of hatched, sterile, aborted and parasitized eggs (see Tables 2 and 3 for~~  
 356 ~~details).~~

357 Finally, to evaluate the influence of the 2017 peculiarity on overall trends in cluster 1 and investigate  
 358 other changes that may have been concealed by this heatwave, another Quade's RANCOVA has  
 359 similar analyses (1) and (2) have been performed on a subset of the data without cluster 1 in 2017  
 360 (see descriptive statistics in [Table SM9](#)). ~~It SM8). Analysis (1) revealed that hatching and abortion~~  
 361 rates still changed over time in the two clusters, to a lower extent than when considering data from  
 362 2017 in cluster 1 (hatching rate:  $F_{df} = 8.2_{17}$ ,  $p = 0.004$ ; abortion rate:  $F_{df} = 7.6_{17}$ ,  $p = 0.006$ ), whereas  
 363 sterility and parasitism did not change in any of the two clusters (sterility:  $F_{df} = 1_{17}$ ,  $p = 0.315$ ;  
 364 parasitism:  $F_{df} = 0.3_{17}$ ,  $p = 0.563$ ). ~~Analysis (2) revealed different patterns than those found when~~  
 365 ~~considering data from cluster 1 in 2017, since no difference among clusters appeared in the 2010s in~~  
 366 ~~any of the variables (hatching rate:  $F_{df} = 0.9_{27}$ ,  $p = 0.412$ ; sterility rate:  $F_{df} = 2.8_{27}$ ,  $p = 0.063$ ; abortion~~  
 367 ~~rate:  $F_{df} = 0.01_{27}$ ,  $p = 0.993$ ; parasitism rate:  $F_{df} = 1.5_{27}$ ,  $p = 0.218$ ).~~

368 **Table 2:** Results of Tukey's post hoc tests in the 1990s.

Variable	Clusters	Mean difference (I-J)	Standard error	p-value
Hatching rate	1 vs 3	43.5*	11.6	0.001
	1 vs 4	-43.9*	15	0.01
	3 vs 4	-87.4*	16.2	<0.001
Sterility rate	1 vs 3	9.8	11.8	0.68
	1 vs 4	32.1	15.3	0.09

Abortion rate	3 vs 4	22.3	16.5	0.37
	1 vs 3	-42.8*	11.8	0.001
Parasitism rate	1 vs 4	-13.9	15.3	0.64
	3 vs 4	29	16.5	0.19
	1 vs 3	-29.8*	11.4	0.03
	1 vs 4	72*	14.8	<0.001
	3 vs 4	101.4*	16	<0.001

369 \*. The mean difference is significant at the .05 level.

370

371 **Table 3:** Results of Tukey's post hoc tests in the 2010s.

Variable	Clusters	Mean difference (I-J)	Standard error	p-value
<b>Abortion</b> Hatching rate	1 vs 2	-18.7	13.1	0.33
	1 vs 3	-46.7*	16.8	0.02
	2 vs 3	-28	18.3	0.28
Sterility rate	1 vs 2	16.5	13.2	0.42
	1 vs 3	59.2*	16.9	<0.001
	2 vs 3	42.6	18.4	0.054
Abortion rate	1 vs 2	-34.3*	13.2	0.03
	1 vs 3	-35.2	17	0.10
	2 vs 3	-0.9	18.5	0.99
Parasitism rate	1 vs 2	-21.3	13.1	0.24
	1 vs 3	-48.1*	16.9	0.01
	2 vs 3	-26.8	18.4	0.31

372 \*. The mean difference is significant at the .05 level.

373

374

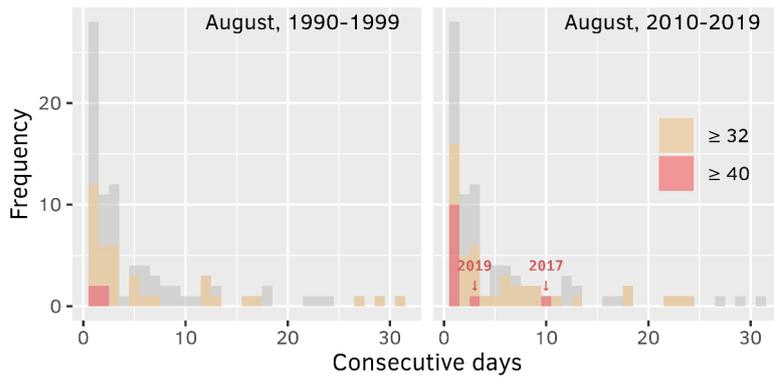
## 375 Discussion

376 Climate change has been recognized to be one of the major phenomena that may affect forest  
377 insect populations ([Jactel et al., 2019](#); Ramsfield et al., 2016). While many studies reported the  
378 occurrence of more frequent and larger insect outbreaks (Raffa et al., 2008; Robinet & Roques,  
379 2010), the opposite have also been observed (Pureswaran et al., 2018; Rozenberg et al., 2020).  
380 Ongoing global warming may exert mixed effects on population dynamics (Dreyer & Baumgärtner,  
381 1996; Huang et al., 2008), and ultimately have an impact on species distributions, as has been  
382 observed with the PPM northern range expansion in Europe (Battisti et al. 2005) and the southern

383 range retraction in Tunisia (Bourougaaoui et al. 2021). A report by the German Technical Cooperation  
384 Agency (GTZ et al. (2007)) has predicted more intense and longer heatwaves in Tunisia, with  
385 temperatures tending to rise even further in the coming century. To better understand potential  
386 adverse effects of climate change at the southern edge of the PPM range, the present study sought  
387 to explore variations in egg survival and hatching and their potential relationship with climate  
388 variations, based on a set of historical and recent field samplings across Tunisia.

### 389 **iii. Hatching failure and heatwaves**

390 The decrease in hatching rate observed in the 2010s period was caused by a [steep increase](#)  
391 ~~mixture of increases~~ in the rate of sterile eggs, ~~parasitized eggs~~, and a clear increase in abortion rate  
392 (i.e., fertile eggs with failed embryo development), the latter being possibly related to warmer  
393 conditions during embryonic development. The strikingly high sterility rate observed in 2017 could be  
394 related to an unusually long series of 10 consecutive days above 40 °C recorded that year (see Fig. 6  
395 and Fig. [SM10 for SM9 for](#) meteorological data from the closest station of the site sampled that year).  
396 While extreme compared to the last 30 years, this anomaly reflects the global increase in the total  
397 number of acute heat days recorded in August in this station (Fig. 6). This overall trend in turn  
398 corroborates the assumption that the likelihood of such stochastic events should increase with future  
399 climate change and cannot be neglected since they might represent a prime cause of mortality in the  
400 PPM, before the average warming. We found that July is the most rapidly warming month in Tunisia,  
401 but egg masses in sites within cluster 1 are mostly laid after July and occur in August. Since all 43 egg  
402 masses from cluster 1 in 2017 were collected in late August, after this long heatwave, egg  
403 development may have been directly impacted before any sign of embryogenesis could be detected  
404 (noted as “sterile” from visual inspections). Such acute heat may also have accelerated pheromone  
405 decay due to higher evaporation rate, hence affecting mating success and egg fecundation in the first  
406 place (Groot & Zizzari, 2019; Linn et al., 1988), or adult gametes (Sales et al., 2018). These results  
407 bear a close resemblance to those shown by Rocha et al. (2017), which revealed that negative effects  
408 appeared on Tunisian egg masses at 42°C after only 3 days of heatwaves, and no survivorship was  
409 noted at 44°C.



410

411 **Figure 6.** Frequency of consecutive days in August overreaching 32 (orange) and 40°C (red) over  
 412 1990-1999 and 2010-2019 in the Kasserine station. This station is situated less than two kilometers  
 413 away from Thélepte, the egg sampling site where 43 egg clutches (100 %) were sterile in 2017. Grey  
 414 histograms correspond to the total distribution across both periods.

415 Hatching rate was slightly higher in cluster 2 than cluster 1 in the 2010s (Table [SM8SM7](#)), which  
 416 can be attributed to the drop in 2017 in cluster 1 (Figure 5). Despite being true for all egg masses in  
 417 cluster 1 that year, caution must be taken before generalizing the low egg survival recorded in 2017  
 418 to a temporal trend in the whole cluster 1. First, all eggs of cluster 1 in 2017 originated from the  
 419 single site of Thélepte, however egg masses were collected on multiple trees scattered across the  
 420 area, thereby reducing the risk of biased sampling, and the sample size was one of the largest of the  
 421 whole data series (43 eggs masses; see Table 1). Second, inter-annual variability in climate and other  
 422 factors not measured in this study cannot be neglected, meaning that the recent years cannot be  
 423 summarized to the year of 2017 which has been shown to be extreme. However, global climatic  
 424 models as well as the data presented in this manuscript suggest that these types of events are likely  
 425 to increase in frequency with climate change, together with average warming, and therefore should  
 426 be explicitly taken into account when analyzing PPM dynamics because they represent prime causes  
 427 of lethality. By putting together long data series, the present study provides a broader view on the  
 428 spatial and temporal variations in the fecundity penalty that may result from these extremely high  
 429 temperatures, as well as how the timing of these heatwaves may differentially impact populations  
 430 from different areas. Beyond the dramatic drop in egg survival observed in 2017 in cluster 1, smaller  
 431 penalties on hatching rate have also been observed during other years of the 2010s. Those smaller  
 432 decreases of egg survival may have a snowball effect on whole colony success, as shown in Spain by  
 433 Pérez-Contreras et al. (2003) who found that 32 individuals is a threshold above which larval growth  
 434 reaches its maximum and mortality drops substantially. A similar result was observed in an  
 435 exploratory experiment in a French population during winter, where survival was null for colonies of  
 436 less than 50 individuals (Roques et al., 2015).

437 The probability to overreach biological thresholds was found to be the highest in cluster 2 and to  
438 significantly increase over time, but no historical egg data from this cluster are available to confirm  
439 whether egg survival used to be higher in the 1990s. Our finding ~~showingshowed~~ that August  
440 conditions in 2017 have likely been lethal in Thélepte (and possibly to a largest extent in cluster 1)  
441 may indicate that similar ~~or worse~~ dramatic effects associated with stochastic meteorological events  
442 are to be expected in cluster 2 where summers are already both the hottest and the most rapidly  
443 warming (Figures SM4). However, ~~despite a longer and more intense 2017 heatwave eggs occur later~~  
444 in cluster 2 (Figure SM11), ~~eggs occur later~~ (hatching starting in mid-October for recent samples) than  
445 in cluster 1 (hatching starting in early September), ~~which mayand may therefore~~ mitigate summer  
446 heatwave threats thanks to phenological avoidance. This contrasting situation warrants the high  
447 relevance of future research in populations from cluster 1 and 2 to observe in real-time and test how  
448 climate change may cause the retraction of the PPM at its southern edge (see Bourougaaoui et al.  
449 2021).

450 Temperature thresholds of 32°C and 40°C have been frequently used in the literature as pivotal  
451 for range limits of the PPM. They have been inferred from observations of survival in nature, but also  
452 appear consistent with more recent experiments in multiple populations of the PPM. Eggs from a  
453 French population were able to withstand a short transient exposure to a daily maximal temperature  
454 of 40°C during several consecutive days with no mortality impact (Robinet et al., 2013), while eggs  
455 from a nearby population could survive a single 6-hour-long exposure to up to 44°C (Poitou, 2021).  
456 However, mortality appeared on egg masses from a Tunisian population at 42°C after only three days  
457 of 4-hour daily exposures (Rocha et al., 2017). While slightly different methods have been used and  
458 make it difficult to compare populations, those results help narrowing down the tipping point at  
459 which PPM egg survival is impacted, depending on the duration of exposure. Regarding larvae, the  
460 survivorship of L1 and L2 from Portuguese populations started to drop after 4-hour exposures to  
461 36°C and 40°C, respectively (Santos et al., 2011), showing higher susceptibility in early larvae  
462 compared to eggs. Recently, Poitou et al. (2022) determined experimentally the thermal  
463 performance curves in development rate in the first four larval instars in a French population,  
464 highlighting that the 32°C threshold is above the optimal development temperature, within the  
465 decreasing performance phase. The 32°C and 40°C thresholds proposed by Démolin (1969) and  
466 (Huchon & Démolin, 1970) thus appear as conservative but consistent integrators of whether a  
467 population is facing stressful conditions regardless of the exact duration of exposure, which our  
468 results corroborate with full mortality after the 2017 heatwave but not after the intense yet less  
469 stringent 2019 heatwave (Figure SM10SM9).

470 In many parts of the world, climate warming more readily impacts nighttime than daytime,  
471 thereby contributing to a decrease in the diurnal thermal range (DTR; Higashi et al., 2020; see also  
472 e.g., Béja, Kef and Carthage in SM4). Several studies (e.g., Higashi et al., 2020; Zhao et al., 2014) have  
473 found that the impact of heat stress endured throughout the day on fitness can be exacerbated by  
474 increasing nighttime temperatures, since thermal fluctuations can help ectotherms repairing or  
475 buffering thermal injuries. In the pine processionary moth, however, warmer winters have facilitated  
476 larval feeding activity in France, which occurs when daytime and nighttime temperatures exceed 9°C  
477 and 0°C, respectively (Battisti et al., 2005). Likewise, the unusually warm night temperatures during  
478 the 2003 heatwave in southern Europe benefited to the PPM in the Alps by eliciting dispersal of  
479 imagoes, which are nocturnal (Battisti et al., 2006). This may be attributed to lower heat intensity due  
480 to altitude, and delayed phenology in the Alps which made adults the exposed stage. Due to their  
481 nocturnal activity and short lifespan, the beneficial influence of TN on flight activity may have  
482 prevailed in adults, whereas eggs or young larvae in other areas with a more advanced phenology are  
483 more likely to suffer from prolonged exposure to both high TX (causing thermal stress) and high TN  
484 (limiting recovery from heat injury; Zhao et al., 2014). Little is known about the impact of warming TN  
485 in Tunisia where the average temperature is higher, and warming nights may impede the capacity to  
486 recover from heat stress, but these findings suggest that local phenologies are key to determining  
487 the impact of reduced diurnal thermal fluctuations in the PPM.

#### 488 **iv. Thermal tolerance and phenology among populations**

489 Temperature is a crucial abiotic factor that can lead to local adaptations in insects and shape their  
490 geographic range (Bush et al., 2016; Hoffmann et al., 2002; Kellermann et al., 2012; Sinclair et al.,  
491 2012). Various thermal environments may therefore be associated with differences in behaviours  
492 and even physiological tolerance (Calosi et al., 2010). Numerous studies have focused on  
493 geographical variation in thermal tolerance in the fruit fly *Drosophila melanogaster*, considered as  
494 one of the most widely distributed insect species, highlighting local variation in the thermal tolerance  
495 and performance of distinct populations (see e.g., Sinclair et al., 2012). In this species, Hoffmann et  
496 al. (2002) found opposing clines in resistance to temperature extremes when comparing numerous  
497 Australian populations along a gradient from tropical to temperate latitudes, which suggests that  
498 thermal tolerance traits are under direct climatic selection. Likewise, summer temperature has been  
499 hypothesized as being a strong selection pressure in the PPM due to the mortality observed in eggs  
500 (Rocha et al. 2017) and neonate larvae (Santos et al. 2011) after experimental heat exposure,  
501 combined with the wide range of climates under which the PPM occurs. Variations in environmental  
502 parameters may induce phenological shifts in all stages of this species either as a consequence of  
503 altered developmental time or responses to stressors (Berardi et al., 2015; Robinet et al., 2015). In

504 areas where summers are the warmest, early mortality caused by heat stress may favour late-  
505 emerging individuals through natural selection, especially as adult females are shortlived and lay eggs  
506 only once (Rocha et al. 2017). A striking example of phenological differentiation among PPM  
507 populations was recently discovered in Portugal, where two sympatric forms exhibit contrasted life  
508 cycles: one with the typical overwinter larval development, and one with a summer larval  
509 development (Santos et al., 2011). While little is known on the causal factors that promoted the  
510 emergence of both forms in the same area, Godefroid et al. (2016) demonstrated that the range of  
511 the summer population is restricted to central-coastal Portugal due to climatic constraints, while  
512 winter populations expands northward. This may be due to the comparatively low thermal resistance  
513 found in eggs of the summer form, which develop into mature larvae before the peak of summer, as  
514 opposed to eggs and early instar larvae of the winter-developing form which usually occur in the  
515 middle of summer and are more likely to be exposed to heats (Rocha et al. 2017). The increase in  
516 climatic variability is expected to exert effects on insect species that differ from those caused by  
517 gradual global warming (Schreven et al., 2017). Large-scale heatwaves are known to have contrasted  
518 effects on different populations of the PPM depending on local climates and phenologies (Robinet et  
519 al., 2015). In Europe, the summer heatwave in 2003 led to a collapse of PPM populations in northern  
520 areas in France (Robinet et al., 2013), while it benefited to adult dispersal and altitudinal expansion in  
521 the Italian Alps, likely due to flight thermal thresholds more easily met than usual near the elevation  
522 edge (Battisti et al., 2006). These findings are congruent with the available data in Tunisia where  
523 phenology in cluster 2 is delayed compared to that in cluster 1, most likely due to the climatic and  
524 altitudinal differences found between those clusters. This fact could contribute to explain the lower  
525 hatching rate found in cluster 1 than in cluster 2 in 2017 (Figure 5), since eggs and neonate larvae  
526 occur after most summer heats in cluster 2 as a result of later adult emergences. This suggests that  
527 populations from the warmest areas of Tunisia may not be the most vulnerable to climate change  
528 thanks to phenological adaptation favouring heat avoidance, as opposed to populations from areas  
529 where individuals are close to their physiological limits but did not evolve differentiated phenology in  
530 response to heat lethality.

531       Despite the impacts of the PPM on Aleppo pine forests in Tunisia, little is known about how  
532 climate change can alter the phenology of Tunisian population and whether it is is spatially  
533 structured by climate heterogeneity. According to Robinet et al. (2015), predictable unfavourable  
534 conditions to which PPM populations are exposed can be alleviated by differentiated phenologies  
535 (phenological plasticity and/or adaptations), which is supported by later adult emergences in cluster  
536 2 on average. By contrast, unpredictable adverse conditions may be mitigated by bet-hedging  
537 strategies such as prolonged diapause, as it creates heterogeneity within populations and siblings by

538 expanding the life cycle of only a fraction of individuals that stay sheltered in the ground at the pupal  
539 stage and evade climate stochasticity ([Le Lann et al., 2021](#); Salman et al., 2019). Diapausing  
540 individuals may therefore contribute to reconstituting local population collapses after extreme  
541 events, but the cost is that survival decreases with the total duration of diapause due to other factors  
542 of mortality (fungi, pupal predation, etc.) (Salman et al. 2019). A significant relationship was found  
543 between the rate of prolonged diapause and both cold and warm winter temperatures, presumably  
544 because they serve as cues of the likelihood of either cold or hot lethal temperatures caused by inter-  
545 annual climate uncertainty (Salman et al. 2019). The acute heat stress exerted on PPM egg masses in  
546 Tunisia may help explain the retraction of this pest from southernmost regions, and further  
547 investigations on phenological differentiations and prolonged diapause strategies among populations  
548 incurring different levels climate harshness are now necessary to predict further distribution  
549 changes.

#### 550 **v. Host-parasitoid interactions and outbreaks in a warming climate**

551 A growing body of literature reveals that parasitoids are often more sensitive to climate warming  
552 than other trophic levels because of their higher position in the food web (Jefferies & Lewis, 2013;  
553 Rosenblatt & Schmitz, 2016). Climate change can lead to phenological asynchrony between  
554 parasitoids and their hosts in cases where the phenology of the interacting species respond variously  
555 to the same climatic cue (Visser & Holleman, 2001), or when the interacting species use different  
556 cues to initiate emergence or development (Jefferies & Lewis, 2013; Walther, 2010). Phenological  
557 asynchrony may also appear if one of the interacting partners rapidly develops or has a seasonal  
558 diapause in response to warming (Forrest, 2016). Parasitism rates therefore tend to decrease with  
559 increasing climatic variability that impedes parasitoids from tracking host populations (Chidawanyika  
560 et al., 2019). Alternatively, but not exclusively, eggs may escape parasitic attacks thanks to various  
561 counter-adaptations whose relative weight may differ among regions and populations. The  
562 identification of eggs by natural enemies may be hindered making egg masses inconspicuous, as PPM  
563 female covering eggs by greyish-brown scales similar to the colour of pine twigs (Battisti et al., 2015).  
564 These scales function not only as visual protection but also as factors impacting the microclimate of  
565 eggs and therefore their development rate (Milani, 1990), and as physical barriers limiting parasitoids  
566 from locating individual eggs in the clutch and greatly reducing their success (Pérez-Contreras &  
567 Soler, 2004). The chief defence against parasitoids, widely discussed in insects and in particular  
568 *Drosophila spp.*, is encapsulation [followed by melanisation](#), an immune responses which sequesters  
569 and kills foreign body (Cavigliasso et al., 2021; Wertheim et al., 2005). Such immune system with  
570 specific cells (hemocytes) is well-developed in larval stages as already observed in PPM larvae  
571 (Boudjahem et al., 2019), however, a study conducted by Reed et al. (2007) showed that hosts in the

572 egg stage can mount a cellular immune response against parasitoid eggs and larvae (Reed et al.,  
573 2007). Research on a wide range of species reveals that small changes in temperature can  
574 significantly shape insect immunity as well as parasitoid fitness (Murdock et al., 2012). Increases in  
575 temperature can consequently promote or repress the encapsulation process, which in turn may  
576 influence the outcome of parasitic success, (Cavigliasso et al., 2021). The outbreaks of phytophagous  
577 insects are expected to increase in the future as parasitism decreases (Stireman et al., 2005). At the  
578 southern edge of PPM distribution (North Africa), some studies showed that extremely high  
579 temperatures could both disrupt population regulation mechanisms and decrease the severity of  
580 outbreaks (Bouzar.Essaidi et al., 2021; Pureswaran et al., 2018). The lower PPM fecundity in this area  
581 (when compared to that at the northern edge, in southern Europe) due to lower plant quality  
582 (Bouzar.Essaidi et al., 2021; Pimentel et al., 2010) may explain why egg parasitism is also lower with  
583 climate warming. Our results showed that the mean number of eggs per egg mass ( $155 \pm 2.1$  in  
584 cluster 1,  $142 \pm 4.5$  in cluster 2,  $150 \pm 3.5$  in cluster 3,  $145 \pm 6.3$  in cluster 4;  $151 \pm 1.7$  when merging  
585 all clusters) was considerably lower than that found in Bulgaria ( $226 \pm 43.2$ ) and France ( $194.3 \pm 50.1$ )  
586 (Georgiev et al., 2020). The average rate of parasitism also showed a different pattern than what was  
587 found in northern parts of PPM distribution, with increases in coastal regions namely in cluster 1 in  
588 Tunisia, while it decreases in France and Spain from core to front populations and along an altitudinal  
589 gradient, respectively (Georgiev et al., 2020; Hódar et al., 2021). Although parasitoids account in egg  
590 mortality, their influence here was low compared to other factors (sterility and abortion) (Figure 5),  
591 and is therefore unlikely to be the main driver of PPM collapses at the southern edge of the  
592 distribution. Significant local warming in Tunisia appears as a prime candidate factor contributing to  
593 the sharp decrease of PPM populations (Bourougaaoui et al., 2021).

#### 594 **vi. Other factors influencing distribution**

595 Factors other than climate warming may putatively affect the survival and persistence of the PPM  
596 at its southern range edge. Embryonic mortality can be impacted by excessive exposure to intense  
597 solar radiation, particularly in southern parts of the distribution, as PPM females tend to lay their  
598 eggs exposed to the sun (Démolin, 1969). Another factor often modulating the spatial occurrence of  
599 insects is food availability. Nevertheless, it is rarely a limiting factor in the PPM because larvae feed  
600 on evergreen trees that are well distributed in the environment, from natural or semi-natural stands  
601 to urban areas where they often occur in relatively high numbers as ornamental trees (Martin, 2005).  
602 Natural enemies such as pathogens or predators (mostly insect parasitoids) at early larval stages  
603 have been suspected to cascade into increasing mortality during larval development because of the  
604 impact on the colony size and silk weaving effort to build a tent (Auger Rozenberg et al., 2015;  
605 Roques et al., 2015), however (1) there is no evidence that the enemy pressure would differ among

606 areas investigated here, and (2) temperature, particularly summer heat waves or early autumnal cold  
607 snaps, are often put forward as a major cause of early mortality (Robinet et al., 2015).

#### 608 vii. Conclusion

609 Heat tolerance has received close attention in insects, however its fluctuation throughout  
610 ontogeny and effects persisting from one developmental stage to another are still poorly  
611 documented. Besides the PPM, few case studies showed that the effects of acute heat stress  
612 received early in life cycle may be carried over to later instars. This was demonstrated in  
613 holometabolous insects such as the tropical butterfly, *Bicyclus anynana* (Klockmann et al., 2017).  
614 Beside consequences of heat on immediate mortality investigated in experimental work (e.g., Rocha  
615 et al 2017) or inferred in the present study by putting together long time series, the ultimate fitness  
616 of individuals that survive challenging heats at the egg stage or first larval instar would therefore be  
617 of great interest to understand the impacts of climate warming at the southern edge of the PPM.  
618 This insect remains one of the ideal models to study these questions owing to (i) the availability of  
619 historical data, (ii) its already demonstrated spatial and phenotypic causal response to climate  
620 change (Battisti et al., 2005; Robinet et al., 2007; Poitou et al., 2022), and (iii) ongoing processes at  
621 play in its southernmost distribution affecting population persistence (Bourougaaoui et al. 2021; this  
622 study).

623

624 **Acknowledgements**

625 We are grateful to Adel Ben Abada (INRGREF, Tunis, Tunisia) for his valuable help in the field. We  
626 acknowledge the National Institute of Meteorology (INM) in Tunis for providing temperature  
627 datasets.

628

629 **Funding**

630 This work was supported by the Tunisian Ministry of Higher Education of Scientific Research and  
631 Technology and the University of Carthage: the university provided two grants to AB for internships  
632 at INRAE URZF in France during her PhD.

633

634 **Conflict of interest disclosure**

635 The authors declare no conflict of interest.

636

637 **Author contributions**

638 Conceptualization: AB, CR, MLBJ, ML; Data curation: AB; Formal analysis: AB, ML; Funding  
639 acquisition: CR, MLBJ; Investigation: AB; Methodology: AB, CR, ML; Project administration: CR, MLBJ;  
640 Supervision: CR, MLBJ, ML; Writing – original draft: AB, CR, ML; Writing – review and editing: AB, CR,  
641 MLBJ, ML.

642

643 **Data, script and code availability**

644 Data and R scripts are publicly available at <https://doi.org/10.15454/RUEIOA>.

645

## 646 References

- 647 Allen, S., Cardona, O., Cutter, S., Dube, O. P., Ebi, K., Handmer, J., Lavell, A., Mastrandrea, M.,  
648 McBean, G., Mechler, R., & Nicholls, N. (2012). Managing the Risks of Extreme Events and  
649 Disasters to Advance Climate Change Adaptation. Special Report of Working Groups I and II  
650 of the Intergovernmental Panel on Climate Change. In.  
651 <https://doi.org/10.13140/2.1.3117.9529>
- 652 Auger Rozenberg, M. A., Barbaro, L., Battisti, A., Blache, S., Charbonnier, Y., Denux, O., Garcia, J.,  
653 Goussard, F., Imbert, C.-E., Kerdelhué, C., Roques, A., Torres Leguizamon, M., & Vetillard, F.  
654 (2015). Ecological Responses of Parasitoids, Predators and Associated Insect Communities to  
655 the Climate-Driven Expansion of the Pine Processionary Moth. In A. Roques (Ed.),  
656 *Processionary Moths and Climate Change : An Update* (pp. 311-357). Springer Netherlands.  
657 [https://doi.org/10.1007/978-94-017-9340-7\\_7](https://doi.org/10.1007/978-94-017-9340-7_7)
- 658 Battisti, A., Avci, M., Avtzis, D. N., Jamaa, M. L. B., Berardi, L., Berretima, W., Branco, M., Chakali, G.,  
659 El Alaoui El Fels, M. A., Frérot, B., Hódar, J. A., Ionescu-Mălăncuș, I., İpekdağ, K., Larsson, S.,  
660 Manole, T., Mendel, Z., Meurisse, N., Mirchev, P., Nemer, N., . . . Zamoum, M. (2015). Natural  
661 History of the Processionary Moths (*Thaumetopoea* spp.): New Insights in Relation to Climate  
662 Change. In A. Roques (Ed.), *Processionary Moths and Climate Change : An Update* (pp. 15-79).  
663 Springer Netherlands. [https://doi.org/https://doi.org/10.1007/978-94-017-9340-7\\_2](https://doi.org/https://doi.org/10.1007/978-94-017-9340-7_2)
- 664 Battisti, A., Stastny, M., Buffo, E., & Larsson, S. (2006). A rapid altitudinal range expansion in the pine  
665 processionary moth produced by the 2003 climatic anomaly. *Global Change Biology*, 12(4),  
666 662-671. <https://doi.org/https://doi.org/10.1111/j.1365-2486.2006.01124.x>
- 667 Battisti, A., Stastny, M., Netherer, S., Robinet, C., Schopf, A., Roques, A., & Larsson, S. (2005).  
668 Expansion of geographic range in the pine processionary moth caused by increased winter  
669 temperatures. *Ecol Appl*, 15(6), 2084-2096. <https://doi.org/https://doi.org/10.1890/04-1903>
- 670 Ben Jamâa, M., & Jerraya, A. (1999). Essai de lutte contre la processionnaire du pin, *Thaumetopoea*  
671 *pityocampa* Schiff.(Lep., Thaumetopoeidae), à l'aide de *Bacillus thuringiensis* Kurstaki  
672 (ECOTECH-PRO). *Annales de l'INRGREF*.
- 673 Berardi, L., Branco, M., Paiva, M., Santos, H., & Battisti, A. (2015). Development time plasticity of the  
674 pine processionary moth (*Thaumetopoea pityocampa*) populations under laboratory  
675 conditions. *Entomologia*, 3, 19-24. <https://doi.org/10.4081/entomologia.2015.273>
- 676 Boudjahem, I., Brivio Fransisco, M., Berchii, S., Mastore, M., & Aouati, A. (2019). Identification and  
677 Quantification of the Most Abondant Hemocytes in the Pine Processionary Caterpillar;  
678 *ThaumetopoeaPityocampa* (Notodontidae). *Energy Procedia*, 157, 992-998.  
679 <https://doi.org/https://doi.org/10.1016/j.egypro.2018.11.266>
- 680 Bourougaaoui, A., Jamâa, M. L. B., & Robinet, C. (2021). Has North Africa turned too warm for a  
681 Mediterranean forest pest because of climate change? *Climatic Change*, 165(3-4), 46.  
682 <https://doi.org/10.1007/s10584-021-03077-1>
- 683 Bouzar.Essaidi, K., Branco, M., Battisti, A., Garcia, A., Fernandes, M. R., Chabane, Y., Bouzemaarene,  
684 M., & Benfekih, L. (2021). Response of the egg parasitoids of the pine processionary moth  
685 to host density and forest cover at the southern edge of the range. *Agricultural and Forest*  
686 *Entomology*, 23(2), 212-221. <https://doi.org/https://doi.org/10.1111/afe.12423>
- 687 Bush, A., Mokany, K., Catullo, R., Hoffmann, A., Kellermann, V., Sgrò, C., McEvey, S., & Ferrier, S.  
688 (2016). Incorporating evolutionary adaptation in species distribution modelling reduces  
689 projected vulnerability to climate change. *Ecology Letters*, 19(12), 1468-1478.  
690 <https://doi.org/https://doi.org/10.1111/ele.12696>
- 691 Calosi, P., Bilton, D. T., Spicer, J. I., Votier, S. C., & Atfield, A. (2010). What determines a species'  
692 geographical range? Thermal biology and latitudinal range size relationships in European  
693 diving beetles (Coleoptera: Dytiscidae). *Journal of Animal Ecology*, 79(1), 194-204.  
694 <https://doi.org/10.1111/j.1365-2656.2009.01611.x>

- 695 Carus, S. (2009). Effects of defoliation caused by the processionary moth on growth of Crimean pines  
696 in western Turkey. *Phytoparasitica*, 37(2), 105-114. [https://doi.org/10.1007/s12600-008-](https://doi.org/10.1007/s12600-008-0018-z)  
697 [0018-z](https://doi.org/10.1007/s12600-008-0018-z)
- 698 Cavigliasso, F., Gatti, J. L., Colinet, D., & Poirié, M. (2021). Impact of Temperature on the Immune  
699 Interaction between a Parasitoid Wasp and Drosophila Host Species. *Insects*, 12(7), 647.  
700 <https://www.mdpi.com/2075-4450/12/7/647>
- 701 Charmantier, A., & Gienapp, P. (2014). Climate change and timing of avian breeding and migration:  
702 evolutionary versus plastic changes. *Evolutionary applications*, 7(1), 15-28.  
703 <https://doi.org/10.1111/eva.12126>
- 704 Chidawanyika, F., Mudavanhu, P., & Nyamukondiwa, C. (2019). Global Climate Change as a Driver of  
705 Bottom-Up and Top-Down Factors in Agricultural Landscapes and the Fate of Host-Parasitoid  
706 Interactions [Review]. *Frontiers in Ecology and Evolution*, 7(80).  
707 <https://doi.org/10.3389/fevo.2019.00080>
- 708 Chuine, I. (2010). Why does phenology drive species distribution? *Philosophical Transactions of the*  
709 *Royal Society B: Biological Sciences*, 365(1555), 3149-3160.  
710 <https://doi.org/doi:10.1098/rstb.2010.0142>
- 711 Chuine, I., de Cortazar-Atauri, I. G., Kramer, K., & Hänninen, H. (2013). Plant Development Models. In  
712 M. D. Schwartz (Ed.), *Phenology: An Integrative Environmental Science* (pp. 275-293).  
713 Springer Netherlands. [https://doi.org/10.1007/978-94-007-6925-0\\_15](https://doi.org/10.1007/978-94-007-6925-0_15)
- 714 Clark, B. R., & Faeth, S. H. (1997). The consequences of larval aggregation in the butterfly *Chlosyne*  
715 *lacinia*. *Ecological Entomology*, 22(4), 408-415.  
716 <https://doi.org/https://doi.org/10.1046/j.1365-2311.1997.00091.x>
- 717 Coumou, D., & Rahmstorf, S. (2012). A decade of weather extremes. *Nature Climate Change*, 2(7),  
718 491-496. <https://doi.org/10.1038/nclimate1452>
- 719 Crozier, L. (2004). WARMER WINTERS DRIVE BUTTERFLY RANGE EXPANSION BY INCREASING  
720 SURVIVORSHIP. *Ecology*, 85(1), 231-241. <https://doi.org/https://doi.org/10.1890/02-0607>
- 721 Démolin, G. (1965). Gréganisme et subsocialité chez *Thaumetopoea pityocampa* Schiff. Nids d'hiver-  
722 activité de tissage. Actes du V<sup>e</sup> Congress de L'Union Internationale pour L'étude des insectes  
723 Sociaux,
- 724 Démolin, G. (1969a). Bioécologie de la processionnaire du pin *Thaumetopoea pityocampa* Schiff.  
725 Incidences des facteurs climatiques. *Boletín del Servicio de Plagas Forestales*(23), 9-24.  
726 <https://hal.inrae.fr/hal-02732616>
- 727 Démolin, G. (1969b). Comportement des adultes de *Thaumetopoea pityocampa* Schiff. Dispersion  
728 spatiale, importance écologique. *Annales des sciences forestières*,
- 729 Démolin, G., & Rive, J. (1968). La processionnaire du pin en Tunisie. *Ann. I.N.R.F. Tunisie*, 1(1), 1-19.
- 730 Denno, R., & Benrey, B. (1997). Aggregation facilitates larval growth in the neotropical nymphalid  
731 butterfly *Chlosyne janais*. *Ecological Entomology*, 22(2), 133-141.  
732 <https://doi.org/https://doi.org/10.1046/j.1365-2311.1997.t01-1-00063.x>
- 733 Dreyer, H., & Baumgärtner, J. (1996). Temperature influence on cohort parameters and demographic  
734 characteristics of the two cowpea coreids *Clavigralla tomentosicollis* and *C. shadabi*.  
735 *Entomologia Experimentalis et Applicata*, 78(2), 201-213.  
736 <https://doi.org/https://doi.org/10.1111/j.1570-7458.1996.tb00783.x>
- 737 EPPO. (2004). EPPO Standards: *Thaumetopoea pityocampa*- PM7/37. *Bulletin OEPP/EPPO Bulletin*,  
738 34, 295-298.
- 739 Fischer, E. M., & Schär, C. (2010). Consistent geographical patterns of changes in high-impact  
740 European heatwaves. *Nature Geoscience*, 3(6), 398-403. <https://doi.org/10.1038/ngeo866>
- 741 Fontaine, B., Janicot, S., & Monerie, P.-A. (2013). Recent changes in air temperature, heat waves  
742 occurrences, and atmospheric circulation in Northern Africa. *Journal of Geophysical*  
743 *Research: Atmospheres*, 118(15), 8536-8552.  
744 <https://doi.org/https://doi.org/10.1002/jgrd.50667>
- 745 Forrest, J. R. K. (2016). Complex responses of insect phenology to climate change. *Current Opinion in*  
746 *Insect Science*, 17, 49-54. <https://doi.org/https://doi.org/10.1016/j.cois.2016.07.002>

- 747 Gardner, J. L., Peters, A., Kearney, M. R., Joseph, L., & Heinsohn, R. (2011). Declining body size: a third  
748 universal response to warming? *Trends in Ecology & Evolution*, 26(6), 285-291.  
749 <https://doi.org/https://doi.org/10.1016/j.tree.2011.03.005>
- 750 Georgiev, G., Rousselet, J., Laparie, M., Robinet, C., Georgieva, M., Zaemdzhikova, G., Roques, A.,  
751 Bernard, A., Poitou, L., Buradino, M., Kerdelhue, C., Rossi, J. P., Matova, M., Boyadzhiev, P., &  
752 Mirchev, P. (2020). Comparative studies of egg parasitoids of the pine processionary moth  
753 (*Thaumetopoea pityocampa*, Den. & Schiff.) in historic and expansion areas in France  
754 and Bulgaria. *Forestry: An International Journal of Forest Research*, 94(2), 324-331.  
755 <https://doi.org/10.1093/forestry/cpaa022>
- 756 Ghosh, S. M., Testa, N. D., & Shingleton, A. W. (2013). Temperature-size rule is mediated by thermal  
757 plasticity of critical size in *Drosophila melanogaster*. *Proceedings. Biological sciences*,  
758 280(1760), 20130174-20130174. <https://doi.org/10.1098/rspb.2013.0174>
- 759 Godefroid, M., Rocha, S., Santos, H., Paiva, M. R., Burban, C., Kerdelhué, C., Branco, M., Rasplus, J. Y.,  
760 & Rossi, J. P. (2016). Climate constrains range expansion of an allochronic population of the  
761 pine processionary moth. *Diversity and Distributions*, 22(12), 1288-1300.  
762 <https://doi.org/https://doi.org/10.1111/ddi.12494>
- 763 Groot, A. T., & Zizzari, Z. V. (2019). Does climate warming influence sexual chemical signaling? *Animal*  
764 *Biology*, 69(1), 83-93. <https://doi.org/https://doi.org/10.1163/15707563-20191103>
- 765 GTZ, MARH, & Exaconsult Gopa. (2007). *Stratégie nationale d'adaptation de l'agriculture tunisienne*  
766 *et des écosystèmes aux changements climatiques*. Rapport d'étude dans le cadre de la  
767 coopération Tuniso-allemande publié par Deutsche Gesellschaft für Internationale. [Hickling,  
768 R., Roy, D. B., Hill, J. K., & Thomas, C. D. \(2005\). A northward shift of range margins in British  
769 Odonata. \*Global Change Biology\*, 11\(3\), 502-506.  
770 <https://doi.org/https://doi.org/10.1111/j.1365-2486.2005.00904.x>](https://doi.org/https://doi.org/10.1111/j.1365-2486.2005.00904.x)
- 771 Higashi, C. H. V., Barton, B. T. [Hickling, R., Roy, D. B., Hill, J. K., & Oliver, K. M. \(2020\). Warmer nights  
772 offer no respite for a defensive mutualism. \*Journal of Animal Ecology\*, 89\(8\), 1895-  
773 1905. \[Thomas, C. D. \\(2005\\). A northward shift of range margins in British Odonata. \\*Global  
774 Change Biology\\*, 11\\(3\\), 502-506. \\[https://doi.org/https://doi.org/10.1111/1365-  
775 2656.13238j.1365-2486.2005.00904.x\\]\\(https://doi.org/https://doi.org/10.1111/1365-2656.13238j.1365-2486.2005.00904.x\\)\]\(https://doi.org/https://doi.org/10.1111/1365-2656.13238j.1365-2486.2005.00904.x\)](https://doi.org/https://doi.org/10.1111/1365-2656.13238j.1365-2486.2005.00904.x)
- 776 Hódar, J. A., Cayuela, L., Heras, D., Pérez-Luque, A. J., & Torres-Muros, L. (2021). Expansion of  
777 elevational range in a forest pest: Can parasitoids track their hosts? *Ecosphere*, 12(4),  
778 e03476. <https://doi.org/https://doi.org/10.1002/ecs2.3476>
- 779 Hoffmann, A. A., Anderson, A., & Hallas, R. (2002). Opposing clines for high and low temperature  
780 resistance in *Drosophila melanogaster*. *Ecology Letters*, 5(5), 614-618.  
781 <https://doi.org/https://doi.org/10.1046/j.1461-0248.2002.00367.x>
- 782 Huang, Z., Ren, S., & Musa, P. D. (2008). Effects of temperature on development, survival, longevity,  
783 and fecundity of the *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) predator,  
784 *Axinoscymnus cardilobus* (Coleoptera: Coccinellidae). *Biological Control*, 46(2), 209-215.  
785 <https://doi.org/https://doi.org/10.1016/j.biocontrol.2008.04.004>
- 786 Huchon, H., & Demolin, G. (1970). La bioécologie de la Processionnaire du pin : dispersion  
787 potentielle, dispersion actuelle.
- 788 Imbert, C. E. (2012). *Expansion d'un ravageur forestier sous l'effet du réchauffement climatique : la*  
789 *processionnaire du pin affecte-t-elle la biodiversité entomologique dans les zones*  
790 *nouvellement colonisées ?* PhD dissertation, Université d'Orléans (France), pp. 198.
- 791 Jacquet, J.-S., Bosc, A., O'Grady, A. P., & Jactel, H. (2013). Pine growth response to processionary  
792 moth defoliation across a 40-year chronosequence. *Forest Ecology and Management*, 293,  
793 29-38. <https://doi.org/https://doi.org/10.1016/j.foreco.2012.12.003>
- 794 [Jactel, H., Koricheva, J., & Castagnyrol, B. \(2019\). Responses of forest insect pests to climate change:  
795 not so simple. \*Current Opinion in Insect Science\*, 35, 103-108.  
796 <https://doi.org/https://doi.org/10.1016/j.cois.2019.07.010> Jeffs, C. T., & Lewis, O. T. \(2013\).  
797 Effects of climate warming on host-parasitoid interactions. \*Ecological Entomology\*, 38\(3\),  
798 209-218. <https://doi.org/https://doi.org/10.1111/een.12026>](https://doi.org/https://doi.org/10.1016/j.cois.2019.07.010)

- 799 Jones, P. D., Lister, D. H., Osborn, T. J., Harpham, C., Salmon, M., & Morice, C. P. (2012). Hemispheric  
800 and large-scale land-surface air temperature variations: An extensive revision and an update  
801 to 2010. *Journal of Geophysical Research: Atmospheres*, 117(D5).  
802 <https://doi.org/https://doi.org/10.1029/2011JD017139>
- 803 Karban, R., & Strauss, S. Y. (2004). Physiological tolerance, climate change, and a northward range  
804 shift in the spittlebug, *Philaenus spumarius*. *Ecological Entomology*, 29(2), 251-254.  
805 <https://doi.org/https://doi.org/10.1111/j.1365-2311.2004.00576.x>
- 806 Kellermann, V., Overgaard, J., Hoffmann, A. A., Fløjgaard, C., Svenning, J.-C., & Loeschcke, V. (2012).  
807 Upper thermal limits of *Drosophila* are linked to species distributions and strongly  
808 constrained phylogenetically. *Proceedings of the National Academy of Sciences*, 109(40),  
809 16228. <https://doi.org/10.1073/pnas.1207553109>
- 810 Kingsolver, J. G., Diamond, S. E., & Buckley, L. B. (2013). Heat stress and the fitness consequences of  
811 climate change for terrestrial ectotherms. *Functional Ecology*, 27(6), 1415-1423.  
812 <https://doi.org/https://doi.org/10.1111/1365-2435.12145>
- 813 Klockmann, M., Kleinschmidt, F., & Fischer, K. (2017). Carried over: Heat stress in the egg stage  
814 reduces subsequent performance in a butterfly. *PloS one*, 12(7), e0180968-e0180968.  
815 <https://doi.org/10.1371/journal.pone.0180968>
- 816 [Le Lann, C., van Baaren, J., & Visser, B. \(2021\). Dealing with predictable and unpredictable  
817 temperatures in a climate change context: the case of parasitoids and their hosts. \*Journal of  
818 Experimental Biology\*, 224\(Suppl\\_1\). <https://doi.org/10.1242/jeb.238626>](https://doi.org/10.1242/jeb.238626)
- 819 Linn, C. E., Campbell, M. G., & Roelofs, W. L. (1988). Temperature modulation of behavioural  
820 thresholds controlling male moth sex pheromone response specificity. *Physiological  
821 Entomology*, 13(1), 59-67. [https://doi.org/https://doi.org/10.1111/j.1365-  
822 3032.1988.tb00909.x](https://doi.org/https://doi.org/10.1111/j.1365-3032.1988.tb00909.x)
- 823 Liu, S. S., Zhang, G. M., & Zhu, J. (1995). Influence of Temperature Variations on Rate of Development  
824 in Insects: Analysis of Case Studies from Entomological Literature. *Annals of the  
825 Entomological Society of America*, 88(2), 107-119. <https://doi.org/10.1093/aesa/88.2.107>
- 826 Martin, J. (2005). La processionnaire du pin *Thaumetopoea pityocampa* (Denis et Schiffermüller).  
827 *Biologie et protection des forêts*. Avignon: Avignon Editions, INRA, 1-62.  
828 [http://www.prodinra.inra.fr/prodinra /pinra/index.xsp](http://www.prodinra.inra.fr/prodinra/pinra/index.xsp)
- 829 Milani, N. (1990). The temperature of the egg masses of *Thaumetopoea pityocampa* (Den. & Schiff.)  
830 (Lepidoptera, Thaumetopoeidae). *Redia*, 73(1), 149-161.
- 831 Murdock, C. C., Paaijmans, K. P., Cox-Foster, D., Read, A. F., & Thomas, M. B. (2012). Rethinking  
832 vector immunology: the role of environmental temperature in shaping resistance. *Nature  
833 Reviews Microbiology*, 10(12), 869-876. <https://doi.org/10.1038/nrmicro2900>
- 834 Nangombe, S. S., Zhou, T., Zhang, W., Zou, L., & Li, D. (2019). High-Temperature Extreme Events Over  
835 Africa Under 1.5 and 2 °C of Global Warming. *Journal of Geophysical Research: Atmospheres*,  
836 124(8), 4413-4428. <https://doi.org/https://doi.org/10.1029/2018JD029747>
- 837 Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J. K., Thomas, C. D., Descimon, H., Huntley, B., Kaila, L.,  
838 Kullberg, J., Tammaru, T., Tennent, W. J., Thomas, J. A., & Warren, M. (1999). Poleward shifts  
839 in geographical ranges of butterfly species associated with regional warming. *Nature*,  
840 399(6736), 579-583. <https://doi.org/https://doi.org/10.1038/21181>
- 841 Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across  
842 natural systems. *Nature*, 421(6918), 37-42.  
843 <https://doi.org/https://doi.org/10.1038/nature01286>
- 844 Pérez-Contreras, T., & Soler, J. J. (2004). Egg parasitoids select for large clutch sizes and covering  
845 layers in pine processionary moths (*Thaumetopoea pityocampa*). *Annales Zoologici Fennici*,  
846 41(4), 587-597. <http://www.jstor.org/stable/23735942>
- 847 Pérez-Contreras, T., Soler, J., & Soler, M. (2003). Why do pine processionary caterpillars  
848 *Thaumetopoea pityocampa* (Lepidoptera, Thaumetopoeidae) live in large groups? An  
849 experimental study. *Annales Zoologici Fennici*, 40, 505-515.

850 Pigliucci, M. (2001). *Phenotypic plasticity: beyond nature and nurture*. The John Hopkins University  
851 Press.

852 Pigliucci, M. (2005). Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology &*  
853 *Evolution*, 20(9), 481-486. <https://doi.org/10.1016/j.tree.2005.06.001>

854 Pimentel, C., Ferreira, C., & Nilsson, J. A. N. Å. (2010). Latitudinal gradients and the shaping of life-  
855 history traits in a gregarious caterpillar. *Biological Journal of the Linnean Society*, 100(1), 224-  
856 236. <https://doi.org/10.1111/j.1095-8312.2010.01413.x>

857 Pincebourde, S., Dillon, M. E., & Woods, H. A. (2021). Body size determines the thermal coupling  
858 between insects and plant surfaces. *Functional Ecology*, 35(7), 1424-1436.  
859 <https://doi.org/10.1111/1365-2435.13801>

860 Pincebourde, S., & Woods, H. A. (2020). There is plenty of room at the bottom: microclimates drive  
861 insect vulnerability to climate change. *Current Opinion in Insect Science*, 41, 63-70.  
862 <https://doi.org/10.1016/j.cois.2020.07.001>

863 Poitou, L. (2021). *Etude de l'impact du changement climatique sur la phénologie de la*  
864 *processionnaire du pin*. PhD dissertation, Université d'Orléans (France), pp.-300.

865 Poitou, L., Laparie, M., Pincebourde, S., Rousselet, J., Suppo, C., & Robinet, C. (2022). Warming  
866 ~~Causes Atypical Phenology~~ in a ~~Univoltine~~  
867 ~~Moth With Differentially Sensitive Larval Stages [Original Research]~~  
868 ~~univoltine moth with~~  
869 ~~differentially sensitive larval stages~~. *Frontiers in Ecology and Evolution*, 10—(in press).  
<https://doi.org/10.3389/fevo.2022.825875>

870 Pureswaran, D. S., Roques, A., & Battisti, A. (2018). Forest Insects and Climate Change. *Current*  
871 *Forestry Reports*, 4(2), 35-50. <https://doi.org/10.1007/s40725-018-0075-6>

872 Quade, D. (1967). Rank Analysis of Covariance. *Journal of the American Statistical Association*,  
873 62(320), 1187-1200. <https://doi.org/10.2307/2283769>

874 Raffa, K. F., Aukema, B. H., Bentz, B. J., Carroll, A. L., Hicke, J. A., Turner, M. G., & Romme, W. H.  
875 (2008). Cross-scale Drivers of Natural Disturbances Prone to Anthropogenic Amplification:  
876 The Dynamics of Bark Beetle Eruptions. *BioScience*, 58(6), 501-517.  
877 <https://doi.org/10.1641/B580607>

878 Ramsfield, T. D., Bentz, B. J., Faccoli, M., Jactel, H., & Brockerhoff, E. G. (2016). Forest health in a  
879 changing world: effects of globalization and climate change on forest insect and pathogen  
880 impacts. *Forestry: An International Journal of Forest Research*, 89(3), 245-252.  
881 <https://doi.org/10.1093/forestry/cpw018>

882 Reed, D. A., Lühring, K. A., Stafford, C. A., Hansen, A. K., Millar, J. G., Hanks, L. M., & Paine, T. D.  
883 (2007). Host defensive response against an egg parasitoid involves cellular encapsulation and  
884 melanization. *Biological Control*, 41(2), 214-222.  
885 <https://doi.org/10.1016/j.biocontrol.2007.01.010>

886 Reynolds, A. P., Richards, G., de la Iglesia, B., & Rayward-Smith, V. J. (2006). Clustering Rules: A  
887 Comparison of Partitioning and Hierarchical Clustering Algorithms. *Journal of Mathematical*  
888 *Modelling and Algorithms*, 5(4), 475-504. <https://doi.org/10.1007/s10852-005-9022-1>

889 Robinet, C., Baier, P., Pennerstorfer, J., Schopf, A., & Roques, A. (2007). Modelling the effects of  
890 climate change on the potential feeding activity of *Thaumetopoea pityocampa* (Den. &  
891 Schiff.) (Lep., Notodontidae) in France. *GLOBAL ECOL BIOGEOGR*, 16(4), 460-471.  
892 <https://doi.org/10.1111/j.1466-8238.2006.00302.x>

893 Robinet, C., Laparie, M., & Rousselet, J. (2015). Looking Beyond the Large Scale Effects of Global  
894 Change: Local Phenologies Can Result in Critical Heterogeneity in the Pine Processionary  
895 Moth. *Frontiers in Physiology*, 6(334). <https://doi.org/10.3389/fphys.2015.00334>

896 Robinet, C., & Roques, A. (2010). Direct impacts of recent climate warming on insect populations.  
897 *Integrative Zoology*, 5(2), 132-142. <https://doi.org/10.1111/j.1749-4877.2010.00196.x>

899 Robinet, C., Rousselet, J., Pineau, P., Miard, F., & Roques, A. (2013). Are heat waves susceptible to  
900 mitigate the expansion of a species progressing with global warming? *Ecol Evol*, 3(9), 2947-  
901 2957. <https://doi.org/10.1002/ece3.690>

902 Rocha, S., Kerdelhué, C., Ben Jamaa, M. L., Dhahri, S., Burban, C., & Branco, M. (2017). Effect of heat  
903 waves on embryo mortality in the pine processionary moth. *Bull Entomol Res*, 107(5), 583-  
904 591. <https://doi.org/https://doi.org/10.1017/S0007485317000104>

905 Ronnås, C., Larsson, S., Pitacco, A., & Battisti, A. (2010). Effects of colony size on larval performance in  
906 a processionary moth. *Ecological Entomology*, 35, 436-445. [https://doi.org/10.1111/j.1365-  
907 2311.2010.01199.x](https://doi.org/10.1111/j.1365-2311.2010.01199.x)

908 Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C., & Pounds, J. A. (2003). Fingerprints  
909 of global warming on wild animals and plants. *Nature*, 421(6918), 57-60.  
910 <https://doi.org/10.1038/nature01333>

911 Roques A, R. J., Avci M et al. (2015). Climate warming and past and present distribution of the  
912 processionary moths (*Thaumetopoea* spp.) in Europe, Asia Minor and North Africa. In R. A (Ed.),  
913 *Processionary moths and climate change : an update* (pp. 81-161). Springer.  
914 [https://doi.org/https://doi.org/10.](https://doi.org/https://doi.org/10.1007/978-94-017-9340-7_3)

915 [1007/978-94-017-9340-7\\_3](https://doi.org/https://doi.org/10.1007/978-94-017-9340-7_3)

916 Roques, L., Rossi, J.-P., Berestycki, H., Rousselet, J., Garnier, J., Roquejoffre, J.-M., Rossi, L.,  
917 Soubeyrand, S., & Robinet, C. (2015). Modeling the Spatio-temporal Dynamics of the Pine  
918 Processionary Moth. In (pp. 227-263). [https://doi.org/10.1007/978-94-017-9340-7\\_5](https://doi.org/10.1007/978-94-017-9340-7_5)

919 Rosenblatt, A. E., & Schmitz, O. J. (2016). Climate Change, Nutrition, and Bottom-Up and Top-Down  
920 Food Web Processes. *Trends in Ecology & Evolution*, 31(12), 965-975.  
921 <https://doi.org/https://doi.org/10.1016/j.tree.2016.09.009>

922 Rosenzweig, C., Casassa, G., Karoly, D., Imeson, A., Liu, C., Menzel, A., Rawlins, S., Root, T., Seguin, B.,  
923 & Tryjanowski, P. (2007). Assessment of observed changes and responses in natural and  
924 managed systems. . In O. F. C. M.L. Parry, J.P. Palutikof, and P.J. van der Linden (Ed.), *Climate  
925 Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the  
926 Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 79-131).  
927 Cambridge University Press.

928 Rozenberg, P., Pâques, L., Huard, F., & Roques, A. (2020). Direct and Indirect Analysis of the  
929 Elevational Shift of Larch Budmoth Outbreaks Along an Elevation Gradient [Original  
930 Research]. *Frontiers in Forests and Global Change*, 3(86).  
931 <https://doi.org/https://doi.org/10.3389/ffgc.2020.00086>

932 Sales, K., Vasudeva, R., Dickinson, M. E., Godwin, J. L., Lumley, A. J., Michalczyk, Ł., Hebberecht, L.,  
933 Thomas, P., Franco, A., & Gage, M. J. G. (2018). Experimental heatwaves compromise sperm  
934 function and cause transgenerational damage in a model insect. *Nature Communications*,  
935 9(1), 4771. <https://doi.org/10.1038/s41467-018-07273-z>

936 Salman, M. H. R., Bonsignore, C. P., El Alaoui El Fels, A., Giomi, F., Hodar, J. A., Laparie, M., Marini, L.,  
937 Merel, C., Zalucki, M. P., Zamoum, M., & Battisti, A. (2019). Winter temperature predicts  
938 prolonged diapause in pine processionary moth species across their geographic range. *PeerJ*,  
939 7, e6530-e6530. <https://doi.org/10.7717/peerj.6530>Santos, H., Paiva, M. R., Tavares, C.,  
940 Kerdelhué, C., & Branco, M. (2011). Temperature niche shift observed in a Lepidoptera  
941 population under allochronic divergence. *J. Evol. Biol*, 24(9), 1897-1905.  
942 <https://doi.org/https://doi.org/10.1111/j.1420-9101.2011.02318.x>  
943 Santos, H., Paiva, M. R., Tavares, C., Kerdelhué, C., & Branco, M. (2011). Temperature niche shift  
944 observed in a Lepidoptera population under allochronic divergence. *J. Evol. Biol*, 24(9), 1897-  
945 1905. <https://doi.org/https://doi.org/10.1111/j.1420-9101.2011.02318.x>

946 Sbay, H., & Zas, R. (2018). Geographic variation in growth, survival, and susceptibility to the  
947 processionary moth (*Thaumetopoea pityocampa* Dennis & Schiff.) of *Pinus halepensis* Mill.  
948 and *P. brutia* Ten.: results from common gardens in Morocco. *Annals of Forest Science*, 75(3),  
949 69. <https://doi.org/10.1007/s13595-018-0746-2>

- 950 Schreven, S. J. J., Frago, E., Stens, A., de Jong, P. W., & van Loon, J. J. A. (2017). Contrasting effects of  
 951 heat pulses on different trophic levels, an experiment with a herbivore-parasitoid model  
 952 system. *PloS one*, 12(4), e0176704-e0176704. <https://doi.org/10.1371/journal.pone.0176704>
- 953 Schubert, E., & Rousseeuw, P. J. (2019). Faster k-Medoids Clustering: Improving the PAM, CLARA, and  
 954 CLARANS Algorithms. In G. Amato, C. Gennaro, V. Oria, & M. Radovanović, *Similarity Search*  
 955 *and Applications* Cham.
- 956 Sheridan, J. A., & Bickford, D. (2011). Shrinking body size as an ecological response to climate change.  
 957 *Nature Climate Change*, 1(8), 401-406. <https://doi.org/10.1038/nclimate1259>
- 958 Sinclair, B. J., Williams, C. M., & Terblanche, J. S. (2012). Variation in Thermal Performance among  
 959 Insect Populations. *Physiological and Biochemical Zoology: Ecological and Evolutionary*  
 960 *Approaches*, 85(6), 594-606. <https://doi.org/10.1086/665388>
- 961 Stireman, J. O., Dyer, L. A., Janzen, D. H., Singer, M. S., Lill, J. T., Marquis, R. J., Ricklefs, R. E., Gentry,  
 962 G. L., Hallwachs, W., Coley, P. D., Barone, J. A., Greeney, H. F., Connahs, H., Barbosa, P.,  
 963 Morais, H. C., & Diniz, I. R. (2005). Climatic unpredictability and parasitism of caterpillars:  
 964 Implications of global warming. *Proceedings of the National Academy of Sciences of the*  
 965 *United States of America*, 102(48), 17384. <https://doi.org/10.1073/pnas.0508839102>
- 966 Thompson, R. M., Beardall, J., Beringer, J., Grace, M., & Sardina, P. (2013). Means and extremes:  
 967 building variability into community-level climate change experiments. *Ecology Letters*, 16(6),  
 968 799-806. <https://doi.org/https://doi.org/10.1111/ele.12095>
- 969 [Vasseur, D. A., DeLong, J. P., Gilbert, B., Greig, H. S., Harley, C. D. G., McCann, K. S., Savage, V.,](https://doi.org/https://doi.org/10.1111/ele.12095)  
 970 [Tunney, T. D., & O'Connor, M. I. \(2014\). Increased temperature variation poses a greater risk](https://doi.org/https://doi.org/10.1111/ele.12095)  
 971 [to species than climate warming. \*Proceedings of the Royal Society B: Biological Sciences\*,](https://doi.org/https://doi.org/10.1111/ele.12095)  
 972 [281\(1779\), 20132612. https://doi.org/doi:10.1098/rspb.2013.2612](https://doi.org/https://doi.org/10.1111/ele.12095)
- 973 Verner, D., Wilby, R., Breisinger, C., Al-Riffai, P., Robertson, R., Wiebelt, M., Kronik, J., Clement, V.,  
 974 Levine, T., Esen, F., & Roos, P. (2013). *Tunisia in a changing climate : assessment and actions*  
 975 *for increased resilience and development*. World Bank Publications.  
 976 <https://doi.org/https://doi.org/10.1596/978-0-8213-9857-9>
- 977 Visser, M. E., & Holleman, L. J. M. (2001). Warmer springs disrupt the synchrony of oak and winter  
 978 moth phenology. *Proceedings of the Royal Society of London. Series B: Biological Sciences*,  
 979 268(1464), 289-294. <https://doi.org/doi:10.1098/rspb.2000.1363>
- 980 Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., Fromentin, J.-M.,  
 981 Hoegh-Guldberg, O., & Bairlein, F. (2002). Ecological responses to recent climate change.  
 982 *Nature*, 416(6879), 389-395. <https://doi.org/https://doi.org/10.1038/416389a>
- 983 Walther, G. R. (2010). Community and ecosystem responses to recent climate change. *Philosophical*  
 984 *Transactions of the Royal Society B: Biological Sciences*, 365(1549), 2019-2024.  
 985 <https://doi.org/doi:10.1098/rstb.2010.0021>
- 986 Wertheim, B., Kraaijeveld, A. R., Schuster, E., Blanc, E., Hopkins, M., Pletcher, S. D., Strand, M. R.,  
 987 Partridge, L., & Godfray, H. C. J. (2005). Genome-wide gene expression in response to  
 988 parasitoid attack in *Drosophila*. *Genome Biology*, 6(11), R94. [https://doi.org/10.1186/gb-](https://doi.org/10.1186/gb-2005-6-11-r94)  
 989 [2005-6-11-r94](https://doi.org/10.1186/gb-2005-6-11-r94)
- 990 Woods, H. A., Dillon, M. E., & Pincebourde, S. (2015). The roles of microclimatic diversity and of  
 991 behavior in mediating the responses of ectotherms to climate change. *Journal of Thermal*  
 992 *Biology*, 54, 86-97. <https://doi.org/https://doi.org/10.1016/j.jtherbio.2014.10.002>
- 993 Wu, C.-H., Holloway, J. D., Hill, J. K., Thomas, C. D., Chen, I. C., & Ho, C.-K. (2019). Reduced body sizes  
 994 in climate-impacted Borneo moth assemblages are primarily explained by range shifts.  
 995 *Nature Communications*, 10(1), 4612. <https://doi.org/10.1038/s41467-019-12655-y>
- 996 [Zhao, F., Zhang, W., Hoffmann, A. A., & Ma, C. S. \(2014\). Night warming on hot days produces novel](https://doi.org/10.1038/s41467-019-12655-y)  
 997 [impacts on development, survival and reproduction in a small arthropod. \*Journal of Animal\*](https://doi.org/10.1038/s41467-019-12655-y)  
 998 [Ecology](https://doi.org/10.1038/s41467-019-12655-y), 83(4), 769-778. <https://doi.org/https://doi.org/10.1111/1365-2656.12196>
- 999 Zittis, G., Hadjinicolaou, P., Almazroui, M., Bucchignani, E., Driouech, F., El Rhaz, K., Kurnaz, L.,  
 1000 Nikulin, G., Ntoumos, A., Ozturk, T., Proestos, Y., Stenchikov, G., Zaaboul, R., & Lelieveld, J.  
 1001 (2021). Business-as-usual will lead to super and ultra-extreme heatwaves in the Middle East

1002 and North Africa. *npj Climate and Atmospheric Science*, 4(1), 20.  
1003 <https://doi.org/10.1038/s41612-021-00178-7>

1004 |

1005 |

## 1 Supplementary information

### 2 Table SM1. Coordinates of sampling sites.

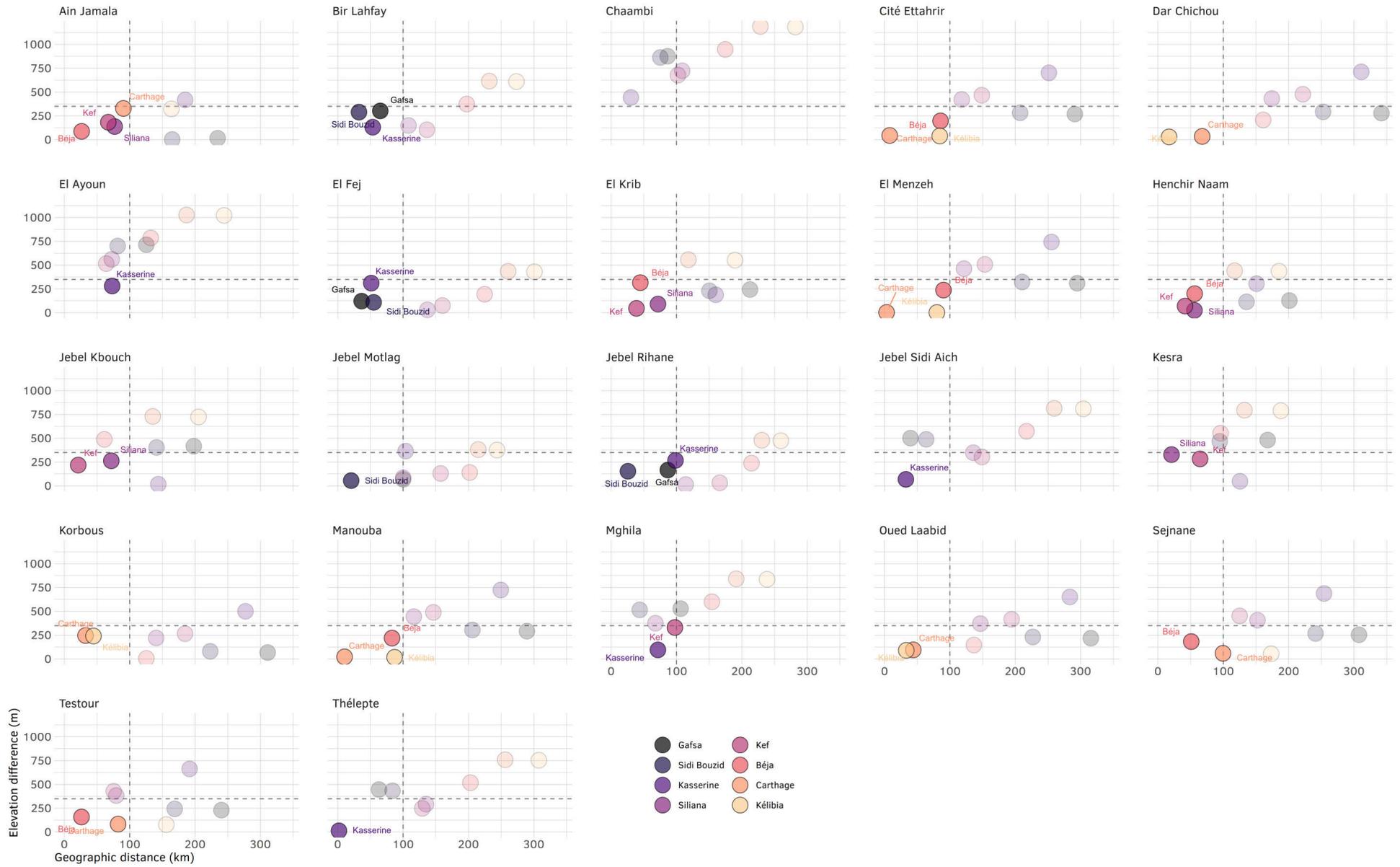
Field egg masses sampling sites	Latitude (°N)	Longitude (°E)
Chaambi	35.200000	8.700000
El Ayoun	35.557653	8.879097
Mghila	35.333333	9.200000
Thélepte	34.960611	8.583472
Bir Lahfay	34.944697	9.156361
El Fej	34.701582	9.043508
Jebel Motlag	34.954417	9.707389
Jebel Rihane	34.823708	9.636170
cit� Ettahrir	36.821750	10.135778
Dar Chichou	36.965594	10.964017
Korbous	36.833333	10.583333
Sejnane	37.183333	9.183333
Oued Laabid	36.816542	10.711303
El Menzeh	36.837850	10.184692
Manouba	36.814722	10.108361
Ain Jamala	36.503839	9.301167
Testour	36.544944	9.377444
Henchir Naam	36.216667	9.166667
Jebel Kbouch (Kef)	36.210000	8.930000
El Krib (Siliana)	36.332544	9.063128
Kesra (Siliana)	35.871833	9.366583
Jebel Sidi Aich (Gafsa)	34.783292	8.865861

3

4 **Table SM2.** Temperature datasets (combination of data from the Institut National de Météorologie,  
5 INM, and the NASA data in corresponding [grid cells of 0.5 degree × 0.625 degree \(roughly 50 × 60](#)  
6 [km\)8 km × 8 km grid](#)) and coordinates of meteorological stations.

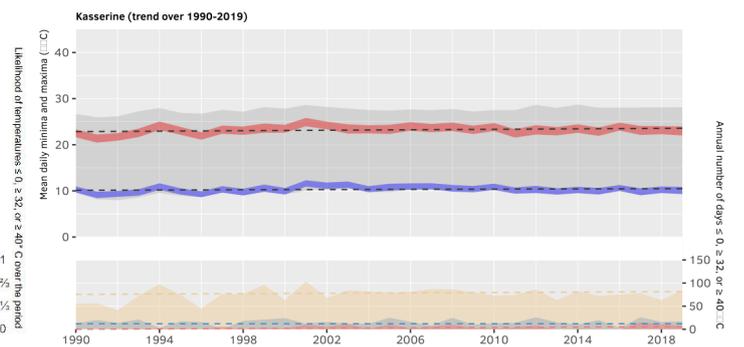
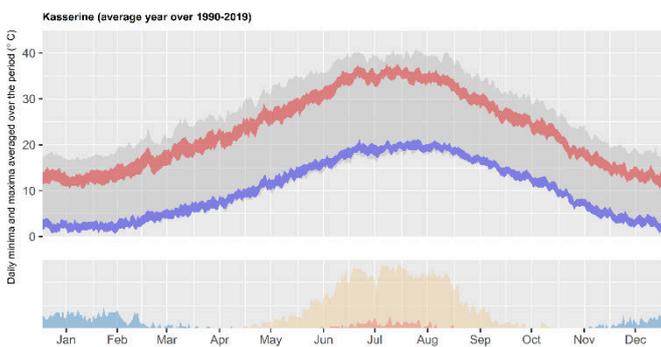
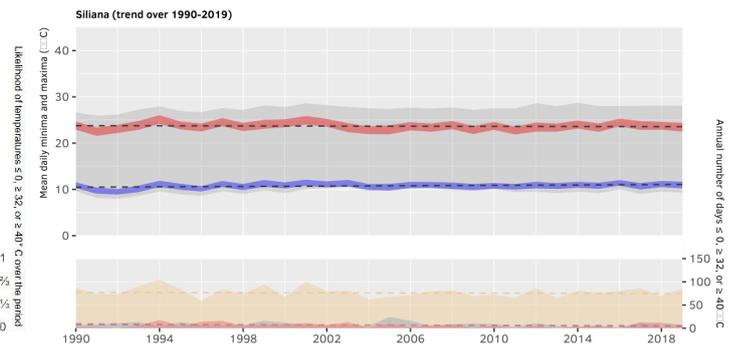
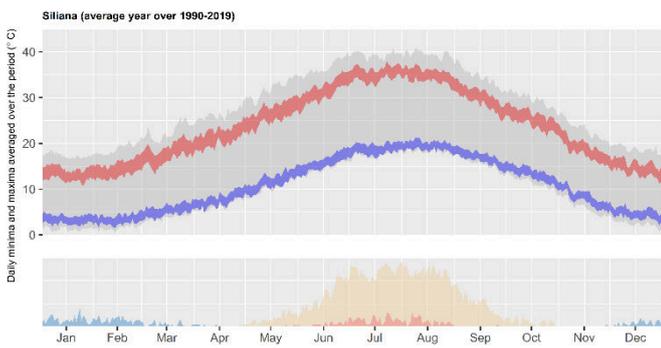
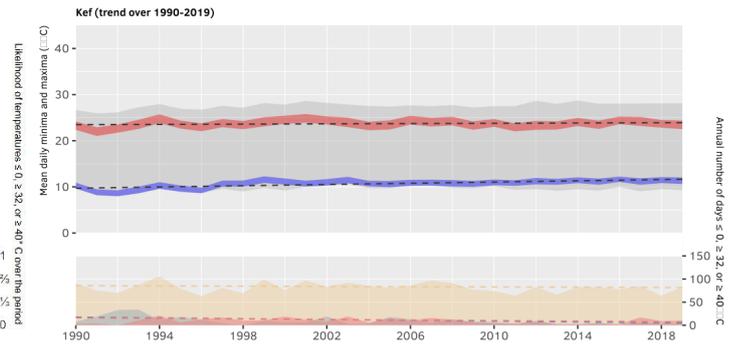
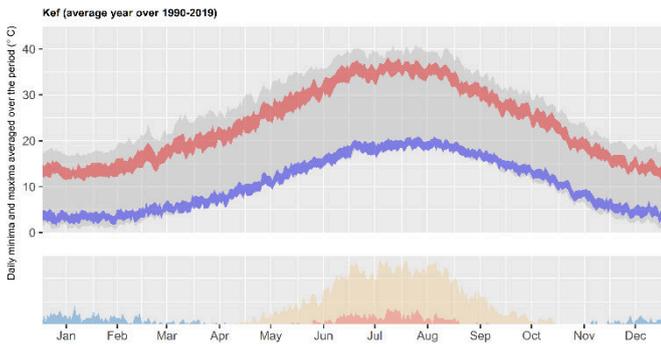
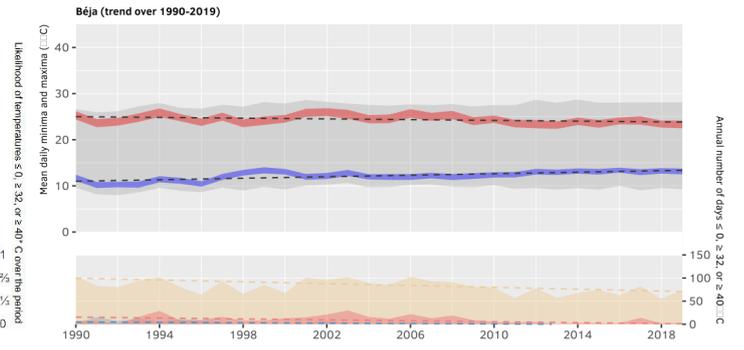
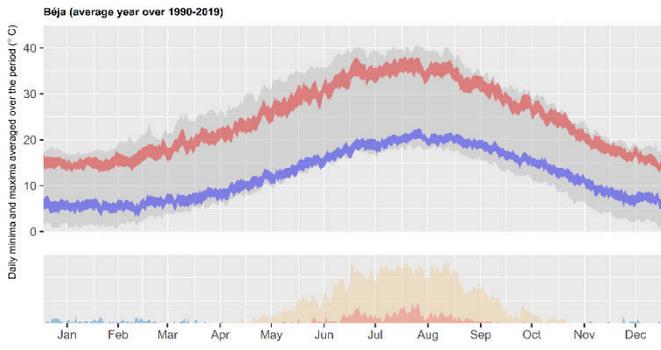
INM meteorological station	Latitude (°N)	Longitude (°E)	Available data (INM)	Data from NASA used to complete the INM datasets
Kélibia	36.844855	11.082701	2001-2011	1990-2000 2012-2019
Carthage	36.846081	10.219053	1990-2014	2015-2019
Béja	36.723338	9.184013	1990-1997 2001-2011	1998-2000 2012-2019
Siliana	35.851853	9.595147	1990-1997	1998-2019
Kef	36.120862	8.720267	1990-1997 2001-2011	1998-2000 2012-2019
Kasserine	34.948369	8.569550	2001-2011	1990-2000 2012-1019
Sidi Bouzid	35.025685	9.498840	1990-2014	2015-2019
Gafsa	34.427352	8.820959	1990-2014	2015-2019

**Geographic and elevational distances between meteorological stations and sampling sites (lower opacity if > 100 km and > 350 m, respectively)**



7 **Figure SM3.** Geographic and elevational distances between meteorological stations and egg sampling sites (lower opacity if >100 km and >350 m, respectively).

8

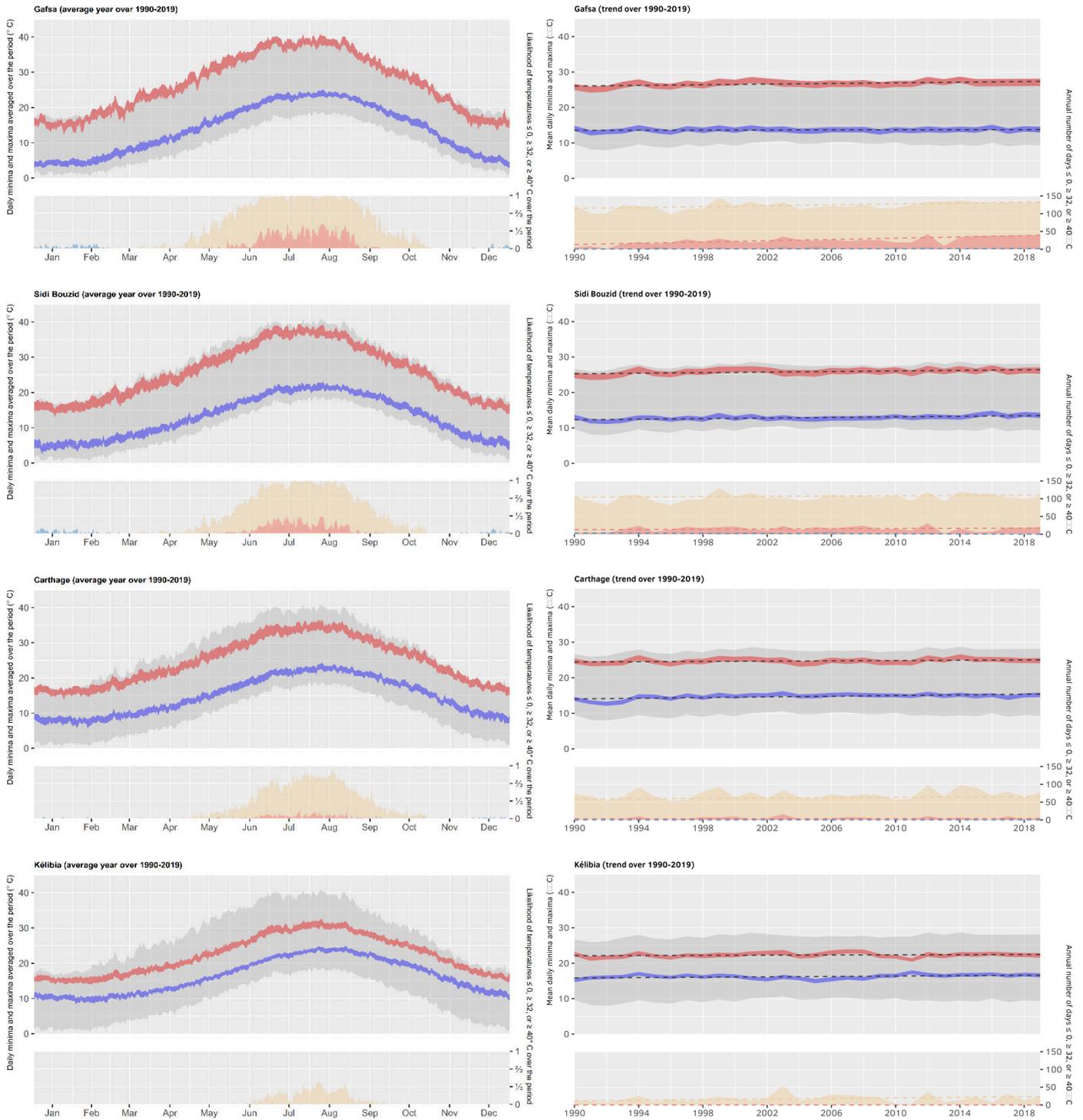


9

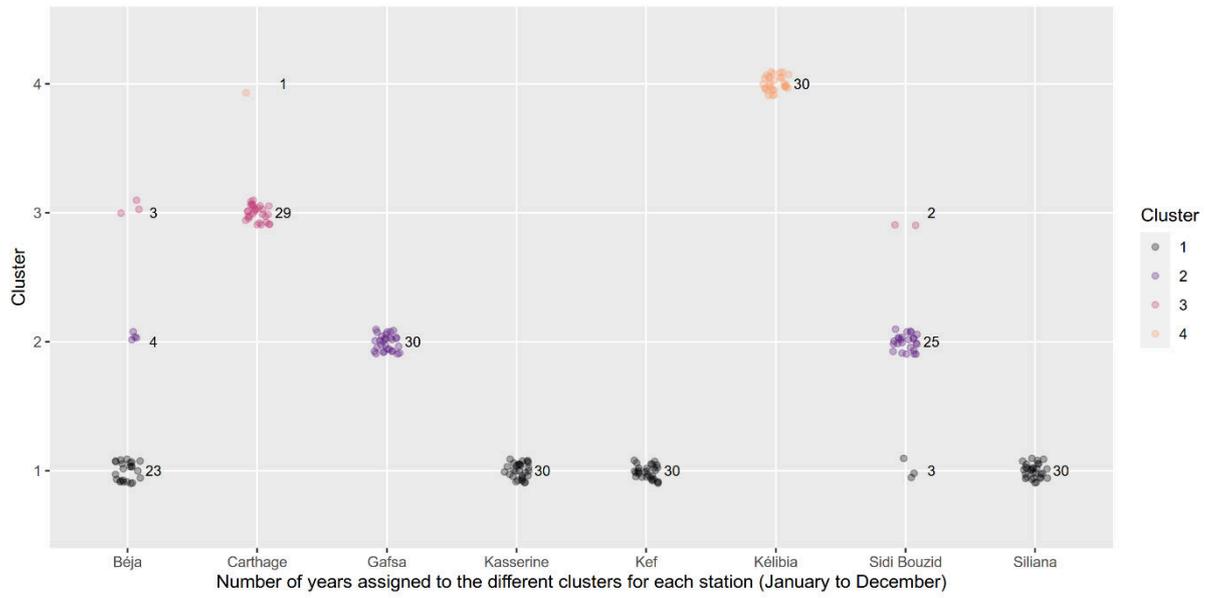
10

11

12



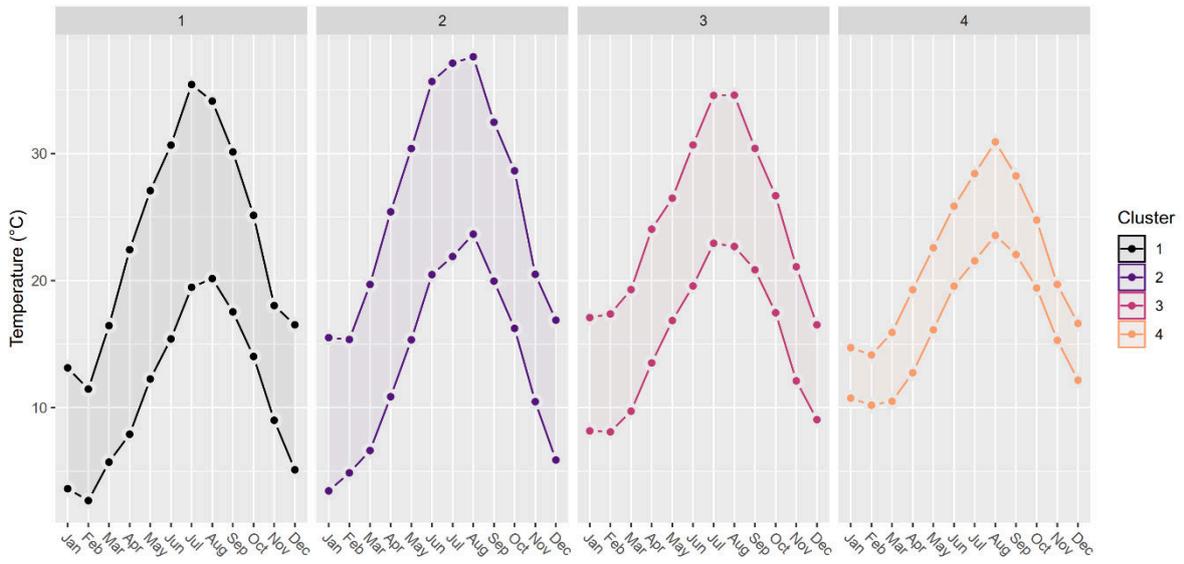
13 **Figure SM4.** Climate data in eight Tunisian regions between 1990 and 2019 (data source: INM and  
 14 NASA, see Table SM2). Left charts show the mean year in each region by averaging daily maxima  
 15 (red) and minima (blue) by day of the year over the period, represented as 95% CI ribbons. Right  
 16 charts show the yearly average of daily maxima (red) and minima (blue), represented as 95% CI  
 17 ribbons, and corresponding Theil-Sen estimators. Thick grey ribbons in the background show the  
 18 maximal thermal range across all nine regions depending on day of the year (left) or year (right). The  
 19 bottom part of left charts shows the likelihood of temperatures  $\leq 0$  (blue),  $\geq 32$  (beige) or  $\geq 40$  °C  
 20 (red), while the bottom part of right charts shows the annual number of days below or above those  
 21 thresholds. The 366<sup>th</sup> day during leap years was discarded due to its lower sample size.



22

23 **Figure SM5.** Number of years assigned to the different clusters for each meteorological series (PAM  
 24 clustering on data from January to December).

25



26

27 **Figure SM6.** TX and TN from January to December in each cluster medoid (PAM clustering on data  
 28 from January to December).

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48 SM7: Synchronic comparison of egg phenotypes among all four clusters.

49 A. Analysis with all data including cluster 1 in 2017.

50

51 A Quade's RANCOVA (see details on this method in subsection 3 of Materials and Methods) was used  
52 to compare egg phenotypes among all four clusters synchronically, i.e., only within the period(s)  
53 clusters have in common (no interaction term). It was followed by a Tukey post hoc procedure to  
54 investigate differences when a significant effect was found.

55 **Clutch size.** The analysis revealed no differences among clusters 1, 3 and 4 in the 1990s (Quade's  
56 RANCOVA,  $F_{df} = 1.58_2$ ,  $p = 0.208$ ), whereas it confirmed significant differences among clusters 1, 2 and  
57 3 in the 2010s ( $F_{df} = 4.40_2$ ,  $p = 0.013$ ). Specifically, fecundity was significantly higher in cluster 1 than  
58 cluster 3 (Tukey post hoc,  $p = 0.009$ ).

59 **Hatching rate and egg mortality factors.** The results showed synchronic differences among clusters  
60 in the rates of hatched, sterile, aborted and parasitized eggs (see the following two tables for  
61 details).

62

63 Results of Tukey's post hoc tests in the 1990s.

<u>Variable</u>	<u>Clusters</u>	<u>Mean difference (I-J)</u>	<u>Standard error</u>	<u>p value</u>
<u>Hatching rate</u>	<u>1 vs 3</u>	<u>43.5*</u>	<u>11.6</u>	<u>0.001</u>
	<u>1 vs 4</u>	<u>-43.9*</u>	<u>15</u>	<u>0.01</u>
	<u>3 vs 4</u>	<u>-87.4*</u>	<u>16.2</u>	<u>&lt;0.001</u>
<u>Sterility rate</u>	<u>1 vs 3</u>	<u>9.8</u>	<u>11.8</u>	<u>0.68</u>
	<u>1 vs 4</u>	<u>32.1</u>	<u>15.3</u>	<u>0.09</u>
	<u>3 vs 4</u>	<u>22.3</u>	<u>16.5</u>	<u>0.37</u>
<u>Abortion rate</u>	<u>1 vs 3</u>	<u>-42.8*</u>	<u>11.8</u>	<u>0.001</u>
	<u>1 vs 4</u>	<u>-13.9</u>	<u>15.3</u>	<u>0.64</u>
	<u>3 vs 4</u>	<u>29</u>	<u>16.5</u>	<u>0.19</u>
<u>Parasitism rate</u>	<u>1 vs 3</u>	<u>-29.8*</u>	<u>11.4</u>	<u>0.03</u>
	<u>1 vs 4</u>	<u>72*</u>	<u>14.8</u>	<u>&lt;0.001</u>
	<u>3 vs 4</u>	<u>101.4*</u>	<u>16</u>	<u>&lt;0.001</u>

64 \*. The mean difference is significant at the .05 level.

65

66 Results of Tukey's post hoc tests in the 2010s.

<u>Variable</u>	<u>Clusters</u>	<u>Mean difference (I-J)</u>	<u>Standard error</u>	<u>p value</u>
-----------------	-----------------	------------------------------	-----------------------	----------------

<u>Hatching rate</u>	<u>1 vs 2</u>	<u>-18.7</u>	<u>13.1</u>	<u>0.33</u>
	<u>1 vs 3</u>	<u>-46.7*</u>	<u>16.8</u>	<u>0.02</u>
	<u>2 vs 3</u>	<u>-28</u>	<u>18.3</u>	<u>0.28</u>
<u>Sterility rate</u>	<u>1 vs 2</u>	<u>16.5</u>	<u>13.2</u>	<u>0.42</u>
	<u>1 vs 3</u>	<u>59.2*</u>	<u>16.9</u>	<u>&lt;0.001</u>
	<u>2 vs 3</u>	<u>42.6</u>	<u>18.4</u>	<u>0.054</u>
<u>Abortion rate</u>	<u>1 vs 2</u>	<u>-34.3*</u>	<u>13.2</u>	<u>0.03</u>
	<u>1 vs 3</u>	<u>-35.2</u>	<u>17</u>	<u>0.10</u>
	<u>2 vs 3</u>	<u>-0.9</u>	<u>18.5</u>	<u>0.99</u>
<u>Parasitism rate</u>	<u>1 vs 2</u>	<u>-21.3</u>	<u>13.1</u>	<u>0.24</u>
	<u>1 vs 3</u>	<u>-48.1*</u>	<u>16.9</u>	<u>0.01</u>
	<u>2 vs 3</u>	<u>-26.8</u>	<u>18.4</u>	<u>0.31</u>

67 \*. The mean difference is significant at the .05 level.

68

69 B. Analysis without data from cluster 1 in 2017.

70 A Quade's RANCOVA (see details on this method in subsection 3 of Materials and Methods) was used  
71 to compare egg phenotypes among all four clusters synchronically, i.e., only within the period(s)  
72 clusters have in common (no interaction term), excluding data from cluster 1 in 2017. It was followed  
73 by a Tukey post hoc procedure to investigate differences when a significant effect was found.

74 The analysis revealed patterns different from those found when considering data from cluster 1 in  
75 2017, since no difference among clusters appeared in the 2010s in any of the variables (Quade's  
76 RANCOVA, hatching rate:  $F_{df} = 0.9_2$ ,  $p = 0.412$ ; sterility rate:  $F_{df} = 2.8_2$ ,  $p = 0.063$ ; abortion rate:  $F_{df} =$   
77  $0.01_2$ ,  $p = 0.993$ ; parasitism rate:  $F_{df} = 1.5_2$ ,  $p = 0.218$ ).

78

79 | **Table SM8SM7.** Descriptive statistics with all data including cluster 1 in 2017: observed mean (M),  
80 | Quade’s adjusted mean (Madj) and associated standard error (SE) for the different response  
81 | variables.

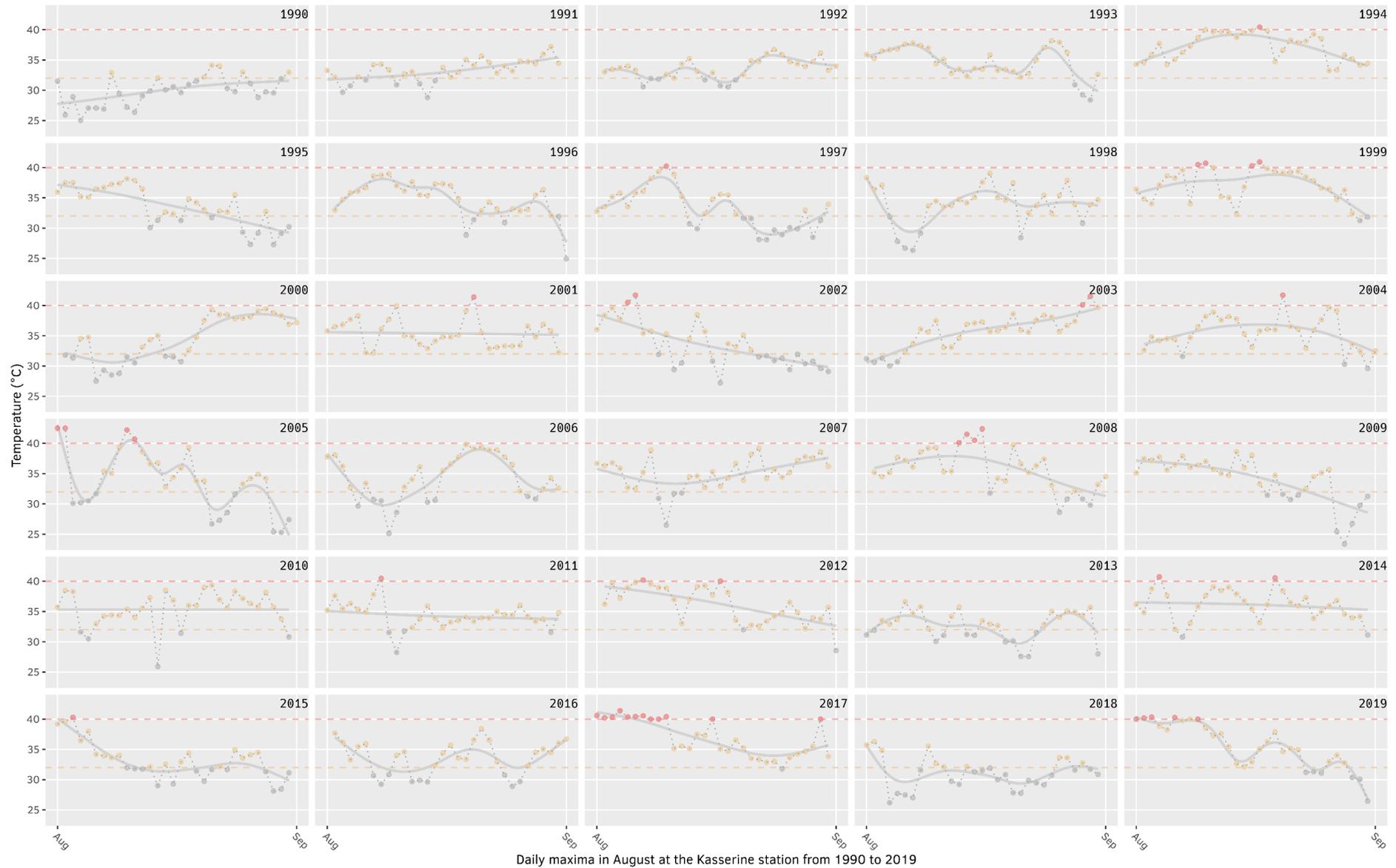
Variable	Period	Cluster	M (SE)	Madj
Fecundity	1990s	1	156.6 (3)	-0.2(6.9)
		2	NA	NA
		3	159.7 (4.3)	10 (10.2)
		4	145.4(6.3)	-20 (14.5)
	2010s	1	153 (3)	10.7 (7.8)
		2	142.1 (4.5)	-0.3 (10.8)
		3	134.5 (5.6)	-39.7 (14.7)
		4	NA	NA
Hatching rate	1990s	1	73.5 (1.7)	6.9 (7.1)
		2	NA	NA
		3	63.3 (2.5)	-36.6 (8.7)
		4	79.3 (3.1)	50.8 (14.1)
	2010s	1	46.8 (2.5)	-12.4 (8)
		2	48.9 (3.4)	6.2 (10.6)
		3	63.2 (3.6)	34.2 (12.6)
		4	NA	NA
Sterility rate	1990s	1	6.8 (0.6)	7.8 (7.3)
		2	NA	NA
		3	6.6 (0.7)	-2 (9.3)
		4	5.8 (0.7)	-24.3 (12.8)
	2010s	1	27.1 (2.5)	13.6 (8.4)
		2	17.3 (2.6)	-2.9 (9.9)
		3	6.6 (1.7)	-45.5 (11.7)
		4	NA	NA
Abortion rate	1990s	1	2.4 (0.2)	-15.3 (6.6)
		2	NA	NA
		3	9.3 (1.9)	27.5 (9.9)
		4	8.1 (2.8)	-1.4 (15.4)
	2010s	1	17.2 (1.8)	-15.4 (8)
		2	24.4 (2.9)	18.9 (10.7)
		3	17.4 (2.9)	19.8 (12.8)
		4	NA	NA
Parasitism rate	1990s	1	17.2 (1.3)	1.4 (6.8)
		2	NA	NA
		3	20.8 (1.7)	31.2 (9.4)
		4	6.8 (1.5)	-70.1 (13)
	2010s	1	8.9 (0.7)	-13.4 (8.3)
		2	9.4 (1.1)	7.9 (9.8)
		3	12.8 (1.8)	34.7 (13.3)
		4	NA	NA

82

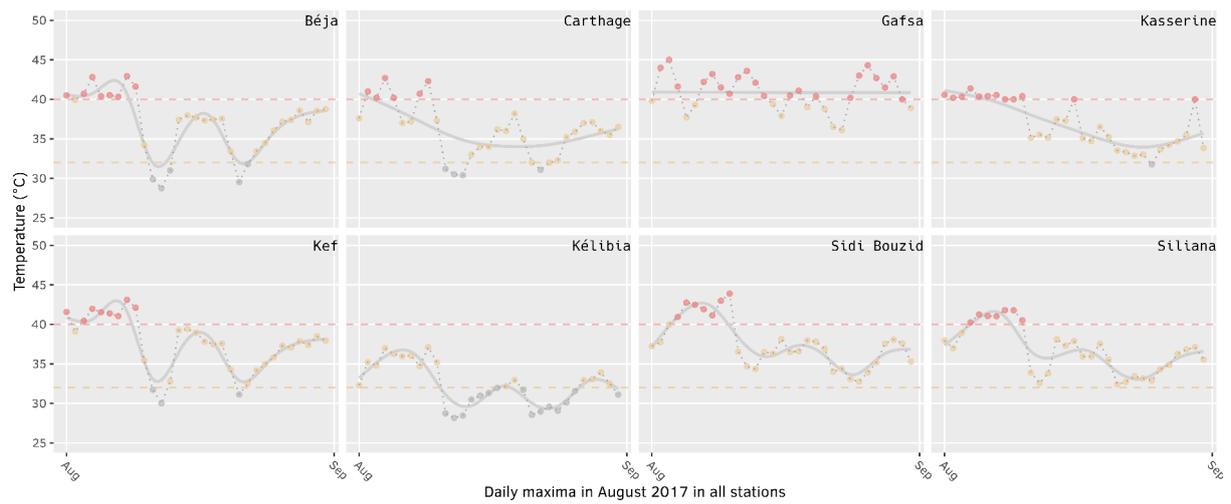
83

84 | **Table SM9SM8.** Descriptive statistics without data from cluster 1 in 2017: observed mean (M),  
85 | Quade's adjusted mean (Madj) and associated standard error (SE) for the different response  
86 | variables.

Variable	Period	Cluster	M (SE)	Madj (SE)
Fecundity	1990s	1	156.6 (3)	-0.2(6.9)
		2	NA	NA
		3	159.7 (4.3)	10 (10.2)
		4	145.4 (6.3)	-20 (14.5)
	2010s	1	155.9 (3.3)	13.6 (7.7)
		2	142.1 (4.5)	-2.5 (9.6)
		3	134.5 (5.6)	-36.6 (13.2)
		4	NA	NA
Hatching rate	1990s	1	73.5 (1.7)	6.9 (7.1)
		2	NA	NA
		3	63.3 (2.5)	-36.6 (8.7)
		4	79.3 (3.1)	50.8 (14.1)
	2010s	1	57.6 (2.5)	1.3 (7.8)
		2	48.9 (3.4)	-8.6 (9.9)
		3	63.2 (3.6)	13.2 (12.2)
		4	NA	NA
Sterility rate	1990s	1	6.8 (0.6)	7.8 (7.3)
		2	NA	NA
		3	6.6 (0.7)	-2 (9.3)
		4	5.8 (0.7)	-24.3 (12.8)
	2010s	1	10.2 (1.1)	-2.5 (8)
		2	17.3 (2.6)	15.4 (9.7)
		3	6.6 (1.7)	-23.1 (11.7)
		4	NA	NA
Abortion rate	1990s	1	2.4 (0.2)	-15.3 (6.6)
		2	NA	NA
		3	9.3 (1.9)	27.5 (9.9)
		4	8.1 (2.8)	-1.4 (15.4)
	2010s	1	21.2 (2.1)	-0.6 (7.8)
		2	24.4 (2.9)	0.8 (10.3)
		3	17.4 (2.9)	0.5 (12.3)
		4	NA	NA
Parasitism rate	1990s	1	17.2 (1.3)	1.4 (6.8)
		2	NA	NA
		3	20.8 (1.7)	31.2 (9.4)
		4	6.8 (1.5)	-70.1 (13)
	2010s	1	11 (0.8)	3 (8.1)
		2	9.4 (1.1)	-12.3 (9.3)
		3	12.8 (1.8)	15.4 (12.9)
		4	NA	NA



87 **Figure SM10.SM9:** August daily maximal temperature recorded in 1990-2019 at the Kasserine station, near the sampling site of Thélepte (cluster 1). Yellow points  
 88 correspond to daily maxima  $\geq 32$  °C, red points correspond to daily maxima  $\geq 40$  °C. Smooth lines are fitted with the “gam” ([generalized additive model](#))  
 89 method.



90

91 **Figure SM11.** Daily maximal temperature recorded in August 2017 in all stations. Béja, Kasserine, Kef  
 92 and Siliana are in Cluster 1; Gafsa and Sidi Bouzid are in Cluster 2; Carthage is in Cluster 3; Kélibia is in  
 93 Cluster 4. Yellow points correspond to daily maxima  $\geq 32$  °C, red points correspond to daily maxima  $\geq$   
 94  $40$  °C. Smooth lines are fitted with the “gam” (generalized additive model) method.