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

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Abstract

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

Keywords

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

Introduction

During the period 1901-2010, land temperature has risen by 1.12 and 0.84°C in the Northern and Southern hemispheres, respectively (Jones et al., 2012). This average climate warming already impacted phenology and distribution in many plant and ectotherm species (Parmesan & Yohe, 2003). Climate change is also expected to increase temperature variability and, in turn, increase the magnitude and frequency of stochastic extreme thermal events, which is already increasingly observed over most parts of the world (Allen et al., 2012; Coumou & Rahmstorf, 2012; Fischer & Schär, 2010), particularly northern Africa (Fontaine et al., 2013; Nangombe et al., 2019; Zittis et al., 2021). In Tunisia, the mean average temperature has risen by about 1.4°C since 1901, with a remarkable increase in the last 30 years with an average warming of 0.4°C per decade, and most substantial warming has been observed during summer in southern regions where average maximum temperatures can often exceed 40°C (Verner et al., 2013). Together with average warming, increasing thermal fluctuations and extreme events may impact organismal fitness (Kingsolver et al., 2013; Thompson et al., 2013), and their combination is expected to dramatically impact survival, distribution and life cycles of many organisms.

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

Contrary to beneficial effects of climate change demonstrated near the northern distribution edge of the PPM, adverse effects of climate change have been observed on the southern range edge (North Africa). Range retraction has been described in southern Tunisia and was found to result from increasing mortality rates of early life stages in a translocation experiment along a natural thermal gradient, which could be ascribed to local effects of climate warming (Bourougaaoui et al., 2021). Fecundity, hatching rate and predation at the egg stage (mostly from parasitoids) presumably play an

important role in the PPM because this species is gregarious. Several studies have emphasized how larval performance depends on the realised group size, i.e. the number of neonates, and ultimately the survival of the whole colony until the end of larval growth (Clark & Faeth, 1997; Denno & Benrey, 1997; Ronnås et al., 2010). Colony density has been suggested to influence feeding activity and feeding efficiency of individual larvae, which is particularly critical in early stages when individuals have little desiccation and starvation resistances. The number of larvae was also shown to impact silk weaving activity to build and maintain the nests that shelter larvae during the day until their pupation in spring (Démolin, 1965; Martin, 2005). Authors found that the number of surviving tents and the average proportion of living larvae per tent were positively correlated to colony size (Pérez-Contreras et al., 2003; Roques et al., 2015). Focusing on the main drivers of colony size in early development is therefore of key importance to understand distribution changes and responses to climate change.

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

In this study, we explored how climate warming over the last three decades may have impacted egg survival and hatching rate in Tunisia. To address this question, we combined historical and contemporary collections of egg masses originating from different Tunisian localities in the 1990s (1992, 1993, and 1995) and in the 2010s (2010, 2014, 2017, 2018, and 2019). Egg phenotypes and survival rate were investigated with regard to regional climatic features and contrasts analyzed from 30-year climatic data series across Tunisia.

Materials and Methods

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

A total of 755 egg masses from historical datasets and recent collections were analyzed in this study. Egg masses originated from 22 sites distributed across the PPM distribution in Tunisia (Table 1;

Figure 1; Table SM1). Historical datasets on egg masses collected in 1992, 1993, 1995, 2010 and 2014 on Aleppo pine stands, *Pinus halepensis* Miller, were retrieved from institutional reports (unpublished data, INRGREF). These datasets report the length of egg masses, the number of eggs per egg mass, and the phenotype of individual eggs (parasitized, aborted, sterile, hatched). In addition, we collected egg masses in various locations in 2017, 2018, and 2019. All these egg masses were also collected on Aleppo pine stands, and kept at ambient temperature 25 ± 2°C) at the INRGREF laboratory near Tunis where we followed a protocol similar to that used for historical collections of egg masses. Egg masses were kept individually in test tubes capped with cotton to allow ventilation. Egg hatching was checked daily. After a period of at least 40 days with no additional hatching, the protective scales that cover PPM egg masses were removed to observe individual eggs under a binocular magnifier and collect data similar to that available in historical datasets: length of egg masses, number of eggs per egg mass (fecundity), and egg phenotype. First, hatched eggs were distinguished from unhatched eggs based on the presence of the characteristic large jagged exit hole from which the neonate left the egg, and an empty transparent shell. Then, unhatched eggs were dissected to assess the cause of mortality (parasitized, *i.e.*, eggs with a small parasitoid exit hole and/or containing a dead parasitoid and/or containing parasitoid meconium; aborted, *i.e.*, dead embryo or dead PPM larva; and sterile, *i.e.*, undeveloped egg with dried-up yolk) (Imbert, 2012). Parasitism rate was calculated taking into account both emerged parasitoids found in the test tubes and dead ones found inside unhatched eggs.

Table 1 . Collection of PPM egg masses in Tunisia (see Table SM1 for coordinates of the sites). Calculated 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 2018	38 29	2	Sidi Bouzid; 20.6 km
Jebel Rihane	2017	25	2	Sidi Bouzid; 25.7 km
El Fej	2017	18	2	Gafsa; 36.6 km
El Menzeh	1992 1993 2014	19 57 10	3	Carthage; 3.2 km
Cité Ettahrir	2014	21	3	Carthage; 7.9 km
Manouba	2010	15	3	Carthage; 10.5 km
Korbous	1992 2010	30 15	3	Carthage; 32.5 km
Dar Chichou	1995	20	4	Kelibia; 17.1 km
Oued Laabid	1995	31	4	Kelibia; 33.3 km

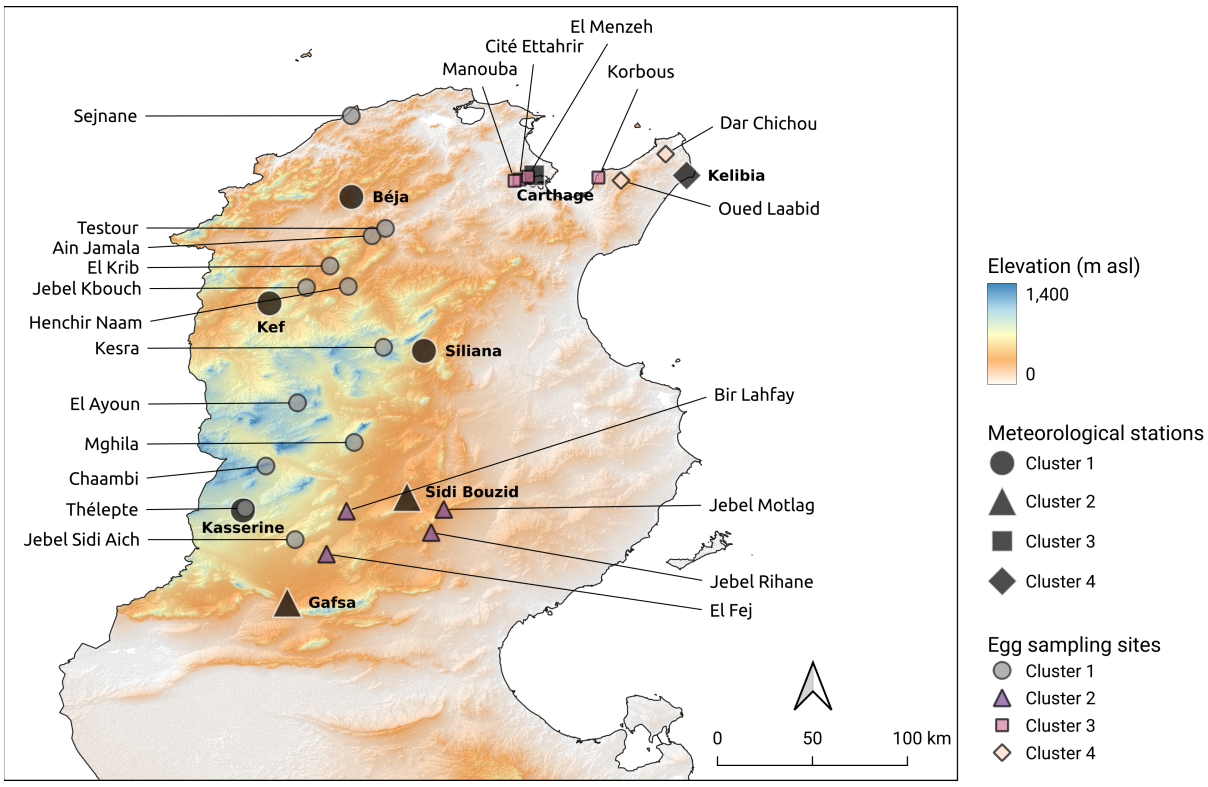

Figure 1. Location of egg sampling sites and meteorological stations, with associated calculated climate cluster.

2 Climate data

We used series of daily temperatures recorded (by the Institut National de Météorologie, INM, Tunis, Tunisia) in eight meteorological stations distributed within the PPM range in Tunisia (Fig. 1; Table SM2). To fill missing data in INM time series, satellite measurement of daily temperatures were

also retrieved from the NASA Prediction of Worldwide Energy Resources website (https://power.larc.nasa.gov/data-access-viewer/) on the grid cells of 8 km × 8 km matching the location of INM weather stations (Table SM2). The similarity of both sources of data was evaluated using Pearson correlations tests for daily maximal and daily minimal temperatures in Tunis, where the data series from INM since 1990 was the most comprehensive. Daily maximal and minimal temperatures from both data sources were found to be strongly correlated (Pearson tests, r= 0.95, $p < 0.001$ and r = 0.94, $p < 0.001$, respectively). The two types of datasets where therefore combined in case of missing data in other INM series to reconstruct uninterrupted series for the period 1990-2019 (Table SM2).

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

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

3 Statistical analyses

Climate clusters. The unbalanced egg sampling design throughout historical data and recent collections prevents allochronic comparisons of egg phenotypes within individual sampling sites. Therefore, we investigated climatic similarities and dissimilarities among meteorological series in order to identify regional climate clusters within which multiple meteorological series and associated egg sampling sites could be statistically grouped together. Climate-based grouping appeared more relevant and less arbitrary than using administrative regions because of the heterogeneous landscape and overall size of some regions. To do so, the monthly averages of TN and TX were calculated in each meteorological series over the period 1990-2019, resulting in a set of 24 variables (2 × 12 months) and 30 values per series (30 years). A Principal Component Analysis (PCA) was used on the covariance matrix of those variables to project the 30 years of data from each of the eight meteorological locations and better visualize their intra- and inter-group variance on reduced

185 dimensionality. The resulting multivariate object then fed a K-medoid clustering analysis using the
186 PAM method (Partitioning Around Medoids, see Reynolds et al. 2006, Schubert and Rousseeuw 2019)
187 to identify relevant climate clusters (listed in Table1). The PCA could be performed on unscaled
188 temperature variables since they were all measured in the same unit (covariance PCA), thereby
189 giving most weight to summer months due to generally higher temperature values, without
190 neglecting other months in the overall variance structure. As a consequence, the climate clusters
191 identified using all four seasons are mostly influenced by the season eggs are exposed to (roughly
192 June to September). Details of cluster assignation to individual points in each meteorological series
193 are detailed in SM5. Monthly means of TN and TX of the medoid of each cluster, *i.e.*, the individual
194 point that best represents its cluster due to low average dissimilarity to all other points, are
195 represented in SM6.

196 ***Interannual fluctuation of maximal summer temperature within clusters.*** To explore regional
197 warming trends to which eggs are subjected within clusters over 1990-2019, the monthly means of
198 daily maximal temperatures from meteorological series within each cluster were calculated from
199 June to September. A linear model was then built for each cluster and each month to plot regressions
200 over time and determine the slope for each cluster. The adequacy of residuals to Normality was
201 checked using QQ plots. Finally, the four months were averaged together per cluster and period to
202 obtain the mean maximal summer temperature in each cluster and period. Then, a two-way ANOVA
203 followed by a Tukey *post-hoc* procedure was performed to test the effects of cluster and period on
204 the maximal summer temperature.

205 ***Egg phenotype comparisons.*** Egg phenotype variables did not meet assumptions of
206 homoscedasticity and normality for parametric tests, therefore we used the non-parametric test
207 (RANCOVA) proposed by Quade (1967) to compare eggs sampled within each of the four clusters in
208 the 1990s or in the 2010s. First, the response variables (Fecundity, Hatching, Sterility, Abortion and
209 Parasitism rates) and the covariate (monthly means of TX averaged from June to September per year
210 per cluster) were ranked separately. Second, residuals from the respective linear regression of each
211 ranked response variable on the ranked covariate were calculated. Third, the effects of grouping
212 factors period (1990s vs 2010s) and Cluster (1, 2, 3, 4) on residuals were investigated for each
213 response variable using the Quade's RANCOVA. When a significant interaction was found, we
214 reported the pairwise comparisons in dependent variable between clusters at each period level, as
215 well as between period levels for each cluster using pairwise t-tests with Bonferroni corrections for
216 multiple testing. When no significant interaction was found between period and cluster, a Tukey post
217 hoc procedure was used to investigate pairwise differences between clusters.

Results

1 Climate clusters

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

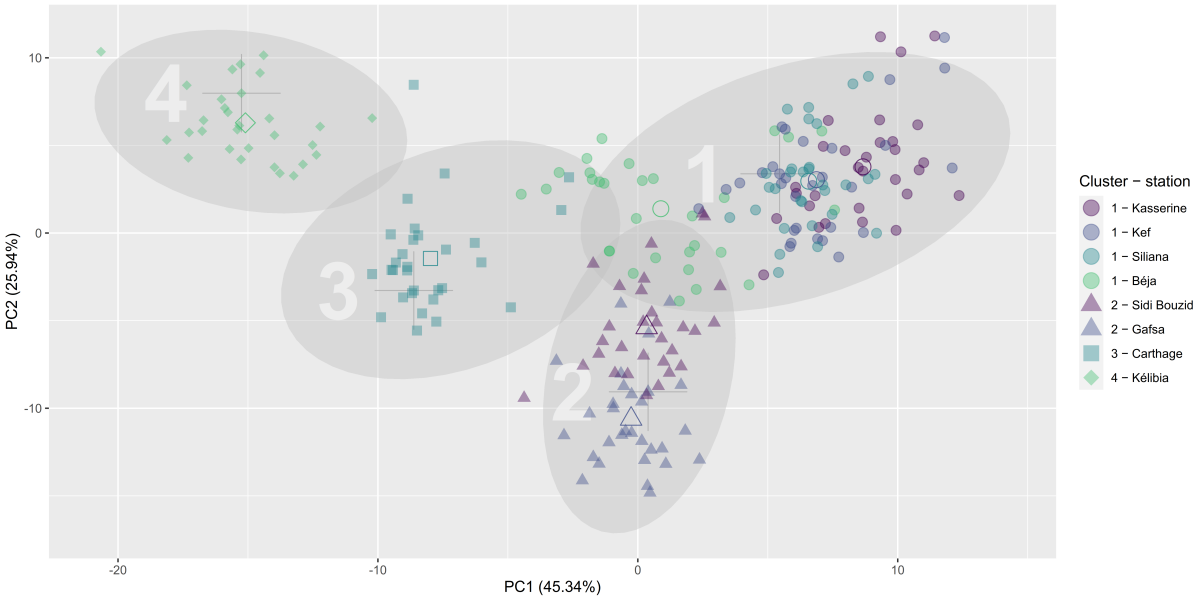

Figure 2. PCA scores for each year of data from the eight meteorological series (INM and NASA series, see table SM2) and 24 TN monthly average and TX monthly average variables. Each of the eight series are identified with different symbol and colour combinations. PAM clustering results are overlaid on the PCA scores with 95% confidence ellipses and different symbols for different clusters. Open points correspond to the centroids of each meteorological series, while large thin crosses mark the medoid point of each cluster.

Per-cluster climate reconstructions averaged from monthly means of TX over years in each cluster (Fig. 3) indicated comparatively cold winters and hot summers with a high interseasonal variability in cluster 1, a shift to warmer winters and summers in cluster 2 with similar interseasonal variability, no extreme winters or summers and lower interseasonal variability in cluster 3, and the lowest interseasonal variability with comparatively mild summers in cluster 4. Within-month variability also appeared to be the highest over the last 30 years in clusters 1 and 2. The probability to overreach 40°C in summer was found to be the highest in cluster 2, while cluster 4 showed the lowest probability of overreaching 32°C, with clusters 1 and 3 sitting in between those extremes. TN

monthly average within each medoid appeared to roughly reflect TX monthly average across each months of the year (SM6, SM9), indicating that similar trends can be inferred for per-cluster TN monthly average.

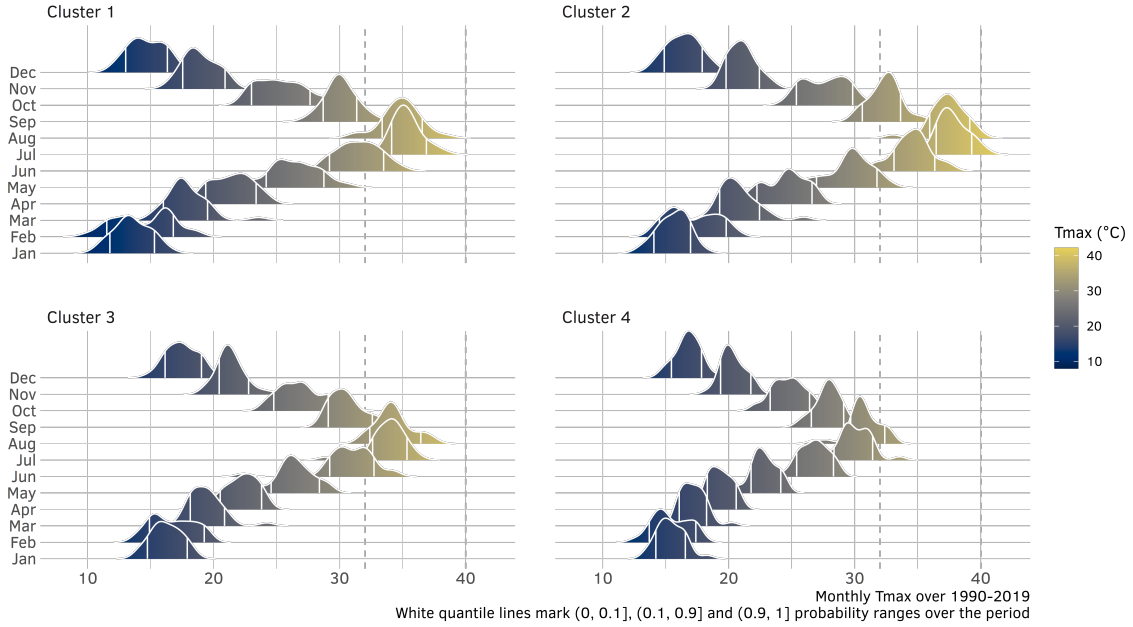

Figure 3. Ridge lines showing the distribution (kernel density) of monthly means of TX over 1990-2019 for each cluster. The area of each distribution equals 1 and white vertical lines mark 0.1 and 0.9 quantiles.

Averaging TX monthly means (from June to September each year) within clusters and periods (Fig. 4) confirmed significant differences among clusters (ANOVA, Fdf = 275.2453, $p < 0.001$; significant differences in all pairs of clusters but 1 and 3 (Tukey post hoc, $p < 0.0001$)). Period did not influence this summary summer variable significantly despite an increasing trend in clusters 2, 3 and 4 (ANOVA, Fdf = 2.5111, Fig. 4), and the interaction term between cluster and period was not significant (ANOVA, Fdf = 2.5111, $p = 0.116$).

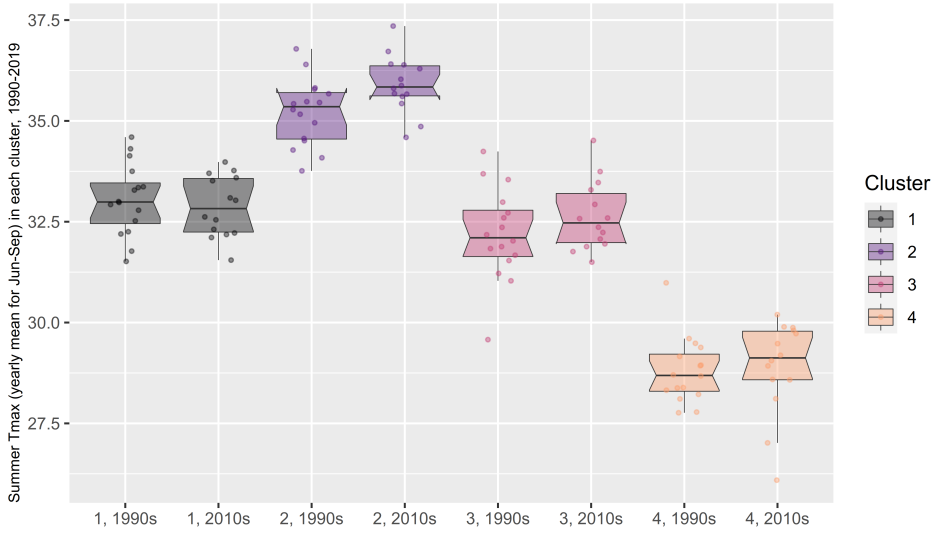

Figure 4. Maximal temperatures averaged from June to September each year, within each cluster and period. Year points in each group are slightly jittered along the x-axis to ease visualization. Cluster was the only significant factor, but period sub-groups are represented to reflect the two-way ANOVA we performed.

Fluctuations of monthly averaged TX during summer over the last three decades (Fig. 5) in each cluster indicated that September has not warmed significantly over time (no apparent slopes, non-significant linear regressions). Contrariwise, July got significantly warmer over time, particularly in cluster 2 and 3 (positive slopes, significant linear regressions). Cluster 2 appeared as the cluster with the most striking summer warming, with positive increases of TX monthly average spanning not only July, but also June (non-significant trend ($p < 0.1$), similar to cluster 3) and August (significant increase). Contrariwise, summers in clusters 1 and 4 appeared as the least subject to warming since 1990.

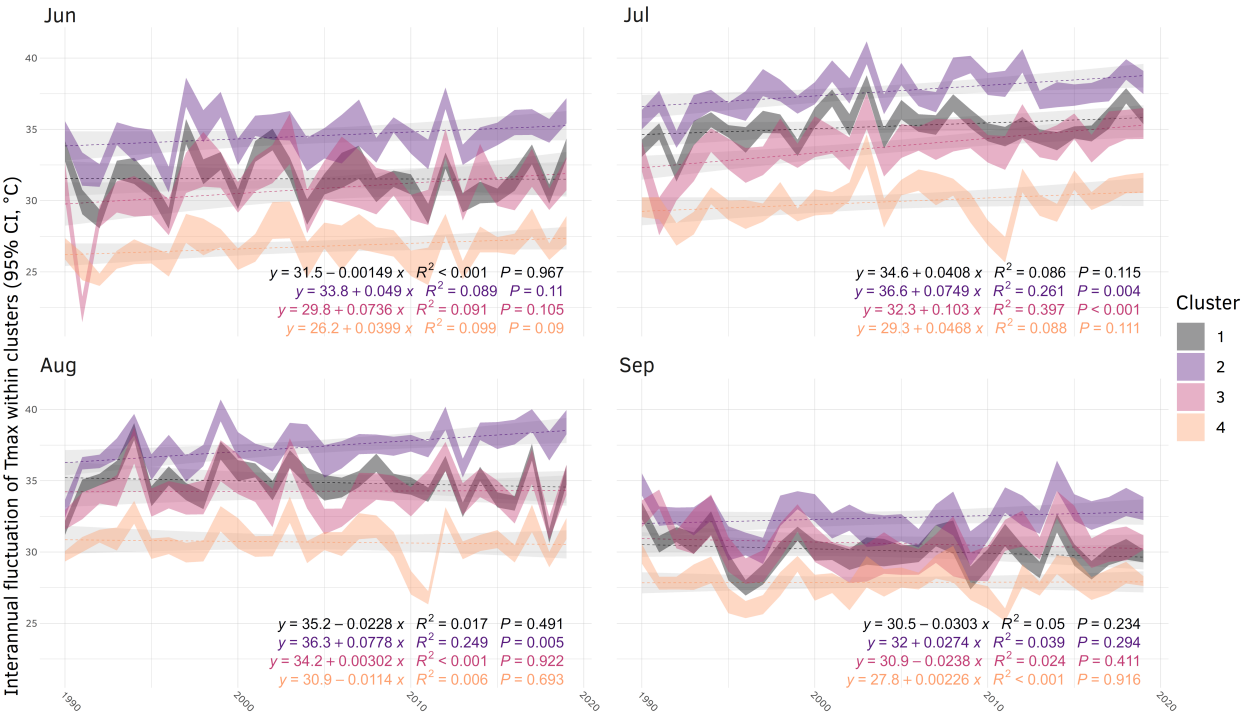

Figure 5. Fluctuations over time of monthly maximal temperature within each cluster, from June to September. The upper and lower lines of each ribbon correspond to 95% confidence intervals.

2 Egg comparisons

Fecundity and hatching rate. The length of egg masses was significantly and highly positively correlated to fecundity (Spearman correlation test, rs = 0.72, $p < 0.001$), therefore we focused further analyses on fecundity only. Fecundity across clusters and years ranged from 121 ± 8.2 SE to 174.6 ± 5.9 SE (Figure 6). Fecundity did not differ significantly between clusters (Quade's RANCOVA, $F_{df} = 2.36_3$, p = 0.071) and between periods ($F_{df} = 3.52_1$, p = 0.61). However, there was a crossover interaction (*i.e.*, the effect of periods on the fecundity is opposite, depending on the value in each cluster) (Quade's RANCOVA, $F_{df} = 6.41_1$, p = 0.012) suggested that significant differences among clusters can be found in at least one of the two periods, and evolved differentially over time. Specifically, no differences were found among clusters in the 1990s, whereas fecundity was significantly higher in cluster 1 than cluster 3 in the 2010s (pairwise t-test, $t_{df} = 3.2_2$, p = 0.005 adjusted by Bonferroni correction). Cluster 3 was the only cluster where fecundity significantly changed over time ($t_{df} = -3.1_1$, p = 0.002), with a decrease of 16 %.

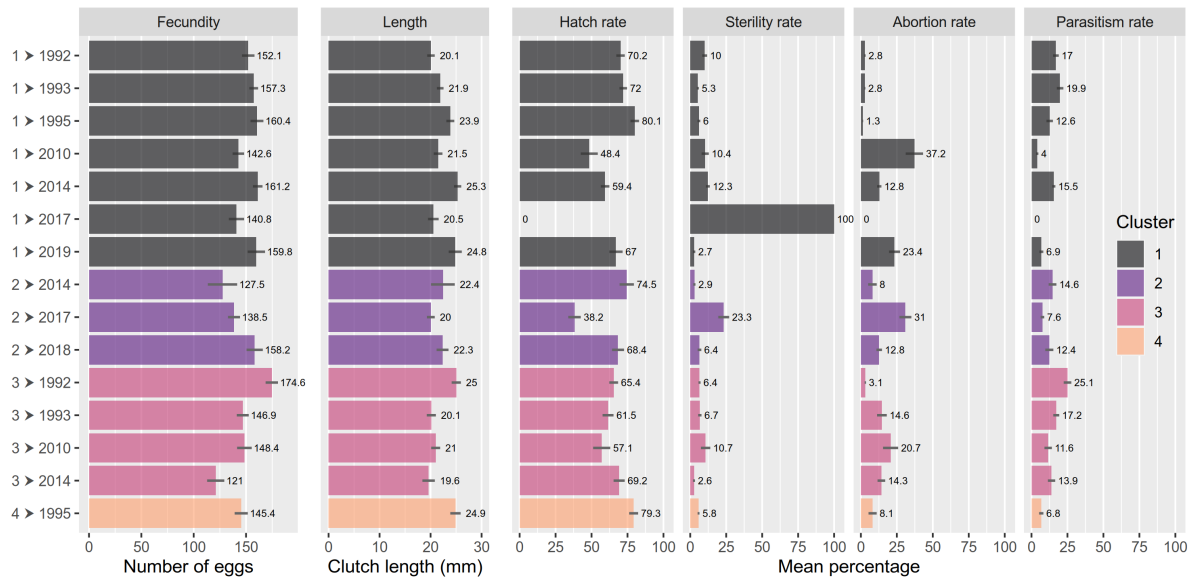

Figure 6. Fecundity (raw count of eggs), clutch length, hatching rate, sterility rate, abortion rate and parasitism rate in each egg sampling site and sampling year. Error bars: SEM.

Hatching rate significantly differed between clusters (Quade's RANCOVA, $F_{df} = 3.8_3$, p=0.01) and between periods ($F_{df} = 24.9_1$, p <0.001). As found for fecundity, differences in hatching rate among clusters varied between the 1990s and the 2010s (Quade's RANCOVA, $F_{df} = 21.7_1$, $p < 0.001$). Hatching rate was significantly lower in cluster 3 than cluster 1 (pairwise t-test, $t_{df} = -4.7_2$, $p < 0.001$ adjusted by Bonferroni correction) and cluster 4 ($t_{df} = -3.6_2$, p = 0.001) in the 1990s, and higher in cluster 2 than cluster 1 in the 2010s ($t_{df} = 2.4_2$, p = 0.05) (Table 2). The mean difference between periods showed that hatching rate in cluster 1 was significantly higher in the past ($t_{df} = 6.7_1$, $p < 0.001$) (Table 2). Particularly, in 2017, hatching rate dropped to zero in cluster 1, and largely decreased in cluster 2 compared to other years (Figure 6).

Table 2. Descriptive statistics: observed mean (M), Quade's adjusted mean (Madj) and associated standard error (SE) for the different response variables.

Variable	Period	Cluster	M (SE)	Madj (SE)
Fecundity	1990s	1	156.6 (3)	19.9 (1.1)
		2	NA	NA
		3	159.7 (4.3)	24.6 (2.1)
		4	145.4 (6.3)	-55.3 (4.3)
	2010s	1	153 (3)	14 (1)
		2	142.1 (4.5)	-12.8 (1.8)
		3	134.5 (5.6)	-84.2 (3.5)
		4	NA	NA
Hatching rate	1990s	1	73.5 (1.7)	76.8 (1)

		2	NA	NA
		3	63.3 (2.5)	-42.5 (1.7)
		4	79.3 (3.1)	82.2 (3.7)
	2010s	1	46.8 (2.5)	-59.8 (1)
		2	48.9 (3.4)	-4.9 (1.8)
		3	63.2 (3.6)	2.8 (3.3)
		4	NA	NA
Sterility rate	1990s	1	6.8 (0.6)	-30.8 (1)
		2	NA	NA
		3	6.6 (0.7)	-22.5 (1.7)
		4	5.8 (0.7)	-21.8 (3.2)
	2010s	1	27.1 (2.5)	46.7 (1.1)
		2	17.3 (2.6)	21.8 (1.9)
		3	6.6 (1.7)	-67.6 (3.2)
		4	NA	NA
Abortion rate	1990s	1	2.4 (0.2)	-96.2 (0.8)
		2	NA	NA
		3	9.3 (1.9)	22.2 (1.8)
		4	8.1 (2.8)	-2.6 (4.1)
	2010s	1	17.2 (1.8)	18.1 (1)
		2	24.4 (2.9)	36.2 (1.8)
		3	17.4 (2.9)	116.6 (3.3)
		4	NA	NA
Parasitism rate	1990s	1	17.2 (1.3)	60.2 (1)
		2	NA	NA
		3	20.8 (1.7)	89.5 (1.8)
		4	6.8 (1.5)	-173.4 (4.2)
	2010s	1	8.9 (0.7)	-55.3 (1)
		2	9.4 (1.1)	-1 (1.6)
		3	12.8 (1.8)	15.4 (3.3)
		4	NA	NA

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Egg mortality factors. Abortion and parasitism rates were the lowest (0%) in cluster 1 in 2017, while sterility was the highest, reaching 100% that year (Figure 6). The latter varied significantly between clusters (Quade's RANCOVA, F_{df} = 2.8_3, p = 0.037) and periods (F_{df} = 5.9_1, p = 0.016). Sterility differences among clusters varied between the 1990s and 2010s (Quade's RANCOVA, F_{df} = 9.1_1, *p* = 0.003). No differences were found between clusters in the 1990s, however, sterility was significantly lower in cluster 3 than cluster 1 (Pairwise t-test, t_{df} = -3.7_2, p = 0.001 adjusted by Bonferroni correction) and cluster 2 (t_{df} = -2.6_2, p = 0.025) in the 2010s. Cluster 1 was the only cluster where sterility significantly increased over time (t_{df} = 3.6_1, p <0.001) (Table 2).

Abortion and parasitism rates significantly differed between clusters (abortion rate: F_{df} = 9.2_3, p < 0.001; parasitism rate: F_{df} = 17.6_3, p < 0.001) and periods (abortion rate: F_{df} = 40.2_1, p <0.001; parasitism rate: F_{df} = 36.8_1, p < 0.001). However, unlike the other variables, no significant interactions were found between the main effects (abortion rate: F_{df} = 0.3_1, p = 0.607; parasitism rate: F_{df}= 1.2_1, p = 0.283). Pairwise differences for these variables between clusters are summarized in Table 3.

Table 3. Results of Turkey's post hoc tests at a threshold of 0.05 for abortion and parasitism rates.

Variable	Cluster	Observed mean (M)	Quade's adjusted mean (Madj)	Statistical significance for alpha = 0.05
Abortion	1	10.6	-33.1	a
	2	24.4	36.2	bc
	3	12.3	56.7	bd
	4	8.1	-2.6	ab
Parasitism	1	13	-3.5	b
	2	9	-1	bc
	3	18	62.5	d
	4	7	-173.4	a

Discussion

Climate change has been recognized to be one of the major phenomena that may affect forest insect populations (Ramsfield et al., 2016). While many studies report the occurrence of more frequent and larger insect outbreaks (Raffa et al., 2008; Robinet & Roques, 2010), diminishments in outbreak severity have also been observed (Pureswaran et al., 2018; Rozenberg et al., 2020). The ongoing global warming may exert mixed effects on population dynamics (Dreyer & Baumgärtner, 1996; Huang et al., 2008), and ultimately have an impact on species distributions, as has been observed with the PPM northern range expansion in Europe (Battisti et al. 2005) and the southern range retraction in Tunisia (Bourougaaoui et al. 2021). A report by the German Technical

333 Cooperation Agency (GTZ et al. (2007)) has predicted more intense and longer heatwaves in Tunisia
334 with temperatures tending to rise even further in the coming century. To better understand potential
335 adverse effects of climate change at the southern edge of the PPM range, the present study sought
336 to explore variations in egg survival and hatching and their potential relationship with climate
337 variations, based on a set of historical and recent field samplings across Tunisia.

338 Fluctuations of monthly averaged TX during summer months over the period 1990-2019 showed
339 that July and August are the warmest months overall (Fig 3, 5). The fastest warming was observed in
340 June and July with an increasing trend (although not always significant) in clusters 2, 3 and 4 for June,
341 and in all the clusters for July. In August, positive increase trends were noted in clusters 2 and 3, but
342 the trend was significant in cluster 2 only (Fig. 5).

343 Hatching rate tended to decrease between the 1990s and the 2010s in cluster 1 (for which the
344 number of egg samples were the highest). Particularly, in 2017, a sharp decrease to 0% in hatching
345 rate was observed (and reflected in an equally steep increase in sterility rate), with no PPM larva or
346 parasitoids (Fig. 6). The decrease in hatching rate observed during other years of the 2010s period
347 was less critical and caused by a mixture of sterile eggs, parasitized eggs, and most importantly, a
348 clear increase in the abortion rate (i.e., fertile eggs with failed embryo development), the latter being
349 possibly related to warmer conditions during embryonic development. The unusually high sterility
350 rate observed in 2017 could be explained by extreme temperatures during that particular summer,
351 suggesting that the trend is more than a simple correlation and might be causal. Maximal
352 temperature overreached 40°C during approximately 10 consecutive days in August, which matches
353 the usual timing of egg occurrence in sites within cluster 1. Such acute heat may have directly
354 damaged newly laid eggs during that period before any embryo could be visible (*i.e.,* not sterile per
355 se, but aborted very early), or alternatively, the heat stress may have impacted adult gametes and
356 fertility (Sales et al., 2018). These results bear a close resemblance to those shown by Rocha et al.
357 (2017), which revealed that negative effects appeared on Tunisian egg masses at 42°C after only 3
358 days of heatwaves, and no survivorship was noted at 44°C. Caution must be taken when interpreting
359 the hatching rate drop in 2017, however, because only one site (Thélepte) was sampled in cluster 1
360 during that peculiar year, and it remains unknown whether populations in the vicinity and similarly
361 exposed to the heatwave have also incurred a severe drop in hatching rate.

362 Hatching rate was slightly higher in cluster 2 than cluster 1 in the 2010s (Table 2), which can be
363 explained by the lowest hatching rate that dropped to 0% in 2017 in cluster 1 (Figure 6). This
364 dramatic decline contributed the most to the greater decrease in hatching observed in cluster 1 than
365 cluster 2 over the entire 2010s. Although the probability to overreach 32°C (proposed by Démolin

366 (1969) as a biological threshold above which physiological processes in eggs and/or larvae may be
367 altered and epizootic may be favoured, although it remains unclear whether this threshold was
368 experimentally verified and compared among populations) and 40°C in summer since the 1990s was
369 found to be the highest in cluster 2, no historical egg data from cluster 2 are available to confirm
370 whether hatching rate was lower in cluster 2 than cluster 1 in the 1990s. During uneven years with
371 unusually hot summers, the biological thresholds of the PPM summer instars may be overreached in
372 an unpredictable way and in turn negatively affect egg survival, as already demonstrated in southern
373 Tunisia (Bourougaaoui et al. 2021). According to this study, temperatures exceeded 35°C on more
374 than half of July and August months (nearly each year during the period 1980-2019), even 40°C for
375 few days in the southern regions. In September, temperatures above 35°C and 40°C were found to
376 be frequent in Tataouine over 1980-2019, which suggested the contribution of these high
377 temperatures to population declines and to the disappearance of the PPM from southernmost areas
378 (Bourougaaoui et al., 2021). The present study provides a broader view on the spatial and temporal
379 variations in the fecundity penalty that may result from these extremely high temperatures. Beyond
380 striking drops in fecundity observed in 2017 in cluster 1, lower fecundity penalties may have a
381 snowball effect on whole colony survival. In Spain, Pérez-Contreras et al. (2003) found that 32
382 individuals is a threshold above which larval growth reaches its maximum and mortality drops
383 substantially. A similar result was observed in an exploratory experiment in a French population
384 during winter, where survival was null for colonies of 25 or 50 individuals (Roques et al., 2015).

385 Temperature is a crucial abiotic factor that can lead to local adaptations in insects and shape their
386 geographic range (Bush et al., 2016; Hoffmann et al., 2002; Kellermann et al., 2012; Sinclair et al.,
387 2012). Various thermal environments may therefore be associated with differences in behaviours
388 and even physiological tolerance (Calosi et al., 2010). Numerous studies have focused on
389 geographical variation in thermal tolerance in the fruit fly *Drosophila melanogaster*, considered as
390 one of the most widely distributed insect species, highlighting local variation in the thermal tolerance
391 and performance of distinct populations (see e.g., Sinclair et al., 2012). In this species, Hoffmann et
392 al. (2002) found opposing clines in resistance to temperature extremes when comparing numerous
393 Australian populations along a gradient from tropical to temperate latitudes, which suggests that
394 thermal tolerance traits are under direct climatic selection.

395 Likewise, summer temperature has been hypothesized as being a strong selection pressure in the
396 PPM due to the mortality observed in eggs (Rocha et al. 2017) and neonate larvae (Santos et al.
397 2011) after experimental heat exposure, combined with the wide range of climates under which the
398 PPM occurs. Variations in environmental parameters may induce phenological shifts in all stages of
399 this species either as a consequence of altered developmental time or responses to stressors (Berardi

et al., 2015; Robinet et al., 2015). In areas where summer are the warmest, early mortality caused by heat stress may favour late-emerging individuals through natural selection, especially as adult females are shortlived and lay eggs only once (Rocha et al. 2017). A striking example of phenological differentiation among PPM populations was recently discovered in Portugal, where two sympatric forms exhibit contrasted life cycles: one with the typical overwinter larval development, and one with a summer larval development (Santos et al., 2011). While little is known on the causal factors that promoted the emergence of both forms in the same area, Godefroid et al. (2016) demonstrated that the range of the summer population is restricted to central-coastal Portugal due to climatic constraints, while winter populations expands northward. This may be due to the comparatively low thermal resistance found in eggs of the summer form, which develop into mature larvae before the peak of summer, as opposed to eggs and early instar larvae of the winter-developing form which usually occur in the middle of summer and are more likely to be exposed to heats (Rocha et al. 2017). The increase in climatic variability is expected to exert effects on insect species that differ from those caused by gradual global warming (Schreven et al., 2017). Large-scale heatwaves are known to have contrasted effects on different populations of the PPM depending on local climates and phenologies (Robinet et al., 2015). In Europe, the summer heatwave in 2003 led to a collapse of PPM populations in northern areas in France (Robinet et al., 2013), while it benefited to adult dispersal and altitudinal expansion in the Italian Alps, likely due to flight thermal thresholds more easily met than usual near the elevation edge) (Battisti et al., 2006) . These findings are congruent with the available data in Tunisia where phenology in cluster 2 is delayed compared to that in cluster 1, most likely due to the climatic and altitudinal differences found between those clusters. This fact could contribute to explain the lower hatching rate found in cluster 1 than in cluster 2 in 2017 (Figure 6), since eggs and neonate larvae occur after most summer heats in cluster 2 due to later adult emergences. This suggests that populations from the warmest areas of Tunisia may not be the most vulnerable to climate change thanks to phenological adaptation favouring heat avoidance, as opposed to populations from areas where individuals are close to their physiological limits but did not evolve differentiated phenology in response to heat lethality.

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

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

445 A growing body of literature reveals that parasitoids are often more sensitive to climate warming
446 than other trophic levels because of their higher position in the food web (Jeffs & Lewis, 2013;
447 Rosenblatt & Schmitz, 2016). Climate change can lead to phenological asynchrony between
448 parasitoids and their hosts in cases where the phenology of the interacting species respond variously
449 to the same climatic cue (Visser & Holleman, 2001), or when the interacting species use different
450 cues to initiate emergence or development (Jeffs & Lewis, 2013; Walther, 2010). Phenological
451 asynchrony may also appear if one of the interacting partners rapidly develops or has a seasonal
452 diapause in response to warming (Forrest, 2016). Parasitism rates therefore tend to decrease with
453 increasing climatic variability that impedes parasitoids from tracking host populations (Chidawanyika
454 et al., 2019). As a result, outbreaks of phytophagous insects are expected to increase in the future
455 (Stireman et al., 2005). At the southern edge of PPM distribution (North Africa), some studies
456 showed that extremely high temperatures could both disrupt population regulation mechanisms and
457 decrease the severity of outbreaks (Bouzar.Essaidi et al., 2021; Pureswaran et al., 2018). The lower
458 PPM fecundity in this area (when compared to that at the northern edge, in southern Europe) due to
459 lower plant quality (Bouzar.Essaidi et al., 2021; Pimentel et al., 2010) may explain why egg parasitism
460 is also lower with climate warming. Our results showed that the mean number of eggs per egg mass
461 (155 ± 2.1 in cluster 1, 142 ± 4.5 in cluster 2, 150 ± 3.5 in cluster 3, 145 ± 6.3 in cluster 4; 151 ± 1.7
462 when merging all clusters) was considerably lower than that found in Bulgaria (226 ± 43.2) and
463 France (194.3 ± 50.1)(Georgiev et al., 2020). Similarly, the average rate of parasitism in the four
464 clusters was low (13 ± 0.7% in cluster 1, 9 ± 1.1% in cluster 2, 18 ± 1.3% in cluster 3, 7 ± 1.5% in
465 cluster 4; 13 ±0.5% when merging all clusters) compared to northern parts of PPM distribution (24.4
466 % in France and 29% in Bulgaria) (Georgiev et al., 2020), and is therefore unlikely to be the main
467 driver of PPM collapses at the southern edge of the distribution. Our findings confirm Bouzar et al.

468 (2021)'s results showing that fecundity and parasitism were lower in Algeria than in southern Europe.
469 Significant local warming in Tunisia appears as a prime candidate factor contributing to the sharp
470 decrease of PPM populations (Bourougaaoui et al., 2021).

471 Heat tolerance has received close attention in insects, however its fluctuation throughout
472 ontogeny and effects persisting from one developmental stage to another are still poorly
473 documented. Besides the PPM, few case studies showed that the effects of acute heat stress
474 received early in life cycle may be carried over to later instars. This was demonstrated in
475 holometabalous insects such as the tropical butterfly, *Bicyclus anynana* (Klockmann et al., 2017).
476 Beside consequences of heat on immediate mortality investigated in experimental work (e.g., Rocha
477 et al 2017) or inferred in the present study by putting together long time series, the ultimate fitness
478 of individuals that survive challenging heats at the egg stage or first larval instar would therefore be
479 of great interest to understand the impacts of climate warming at the southern edge of the PPM.
480 This insect remains one of the ideal models to study these questions owing to the availability of
481 historical data and its already demonstrated spatial and phenotypic causal response to climate
482 change (Battisti et al., 2005; Robinet et al., 2007).

483

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Conflict of interest disclosure

The authors declare no conflict of interest.

Author contributions

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

Data, script and code availability

Data and R scripts are available as a public git repository at *https://sourcesup.renater.fr/projects/ppm-eggs* (see *https://doi.org/10.15454/RUEIOA*).

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Supplementary information

A. Available temperature data from INM and NASA sources, GPS coordinates of meteorological stations and sites of egg sampling.

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
cité 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

Table SM2. Temperature datasets (combination of data from the Institut National de Météorologie, INM, and the NASA data in corresponding 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

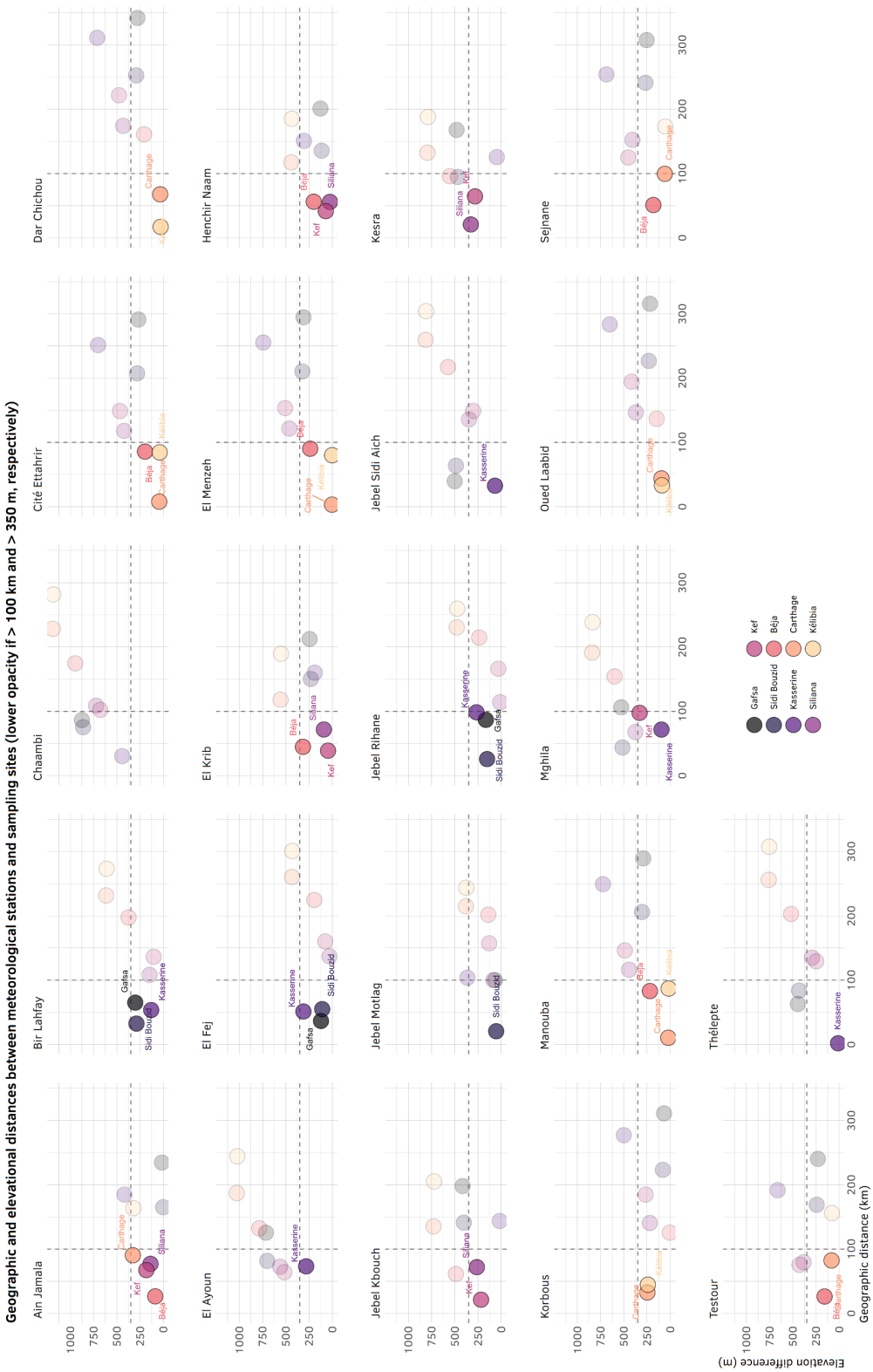
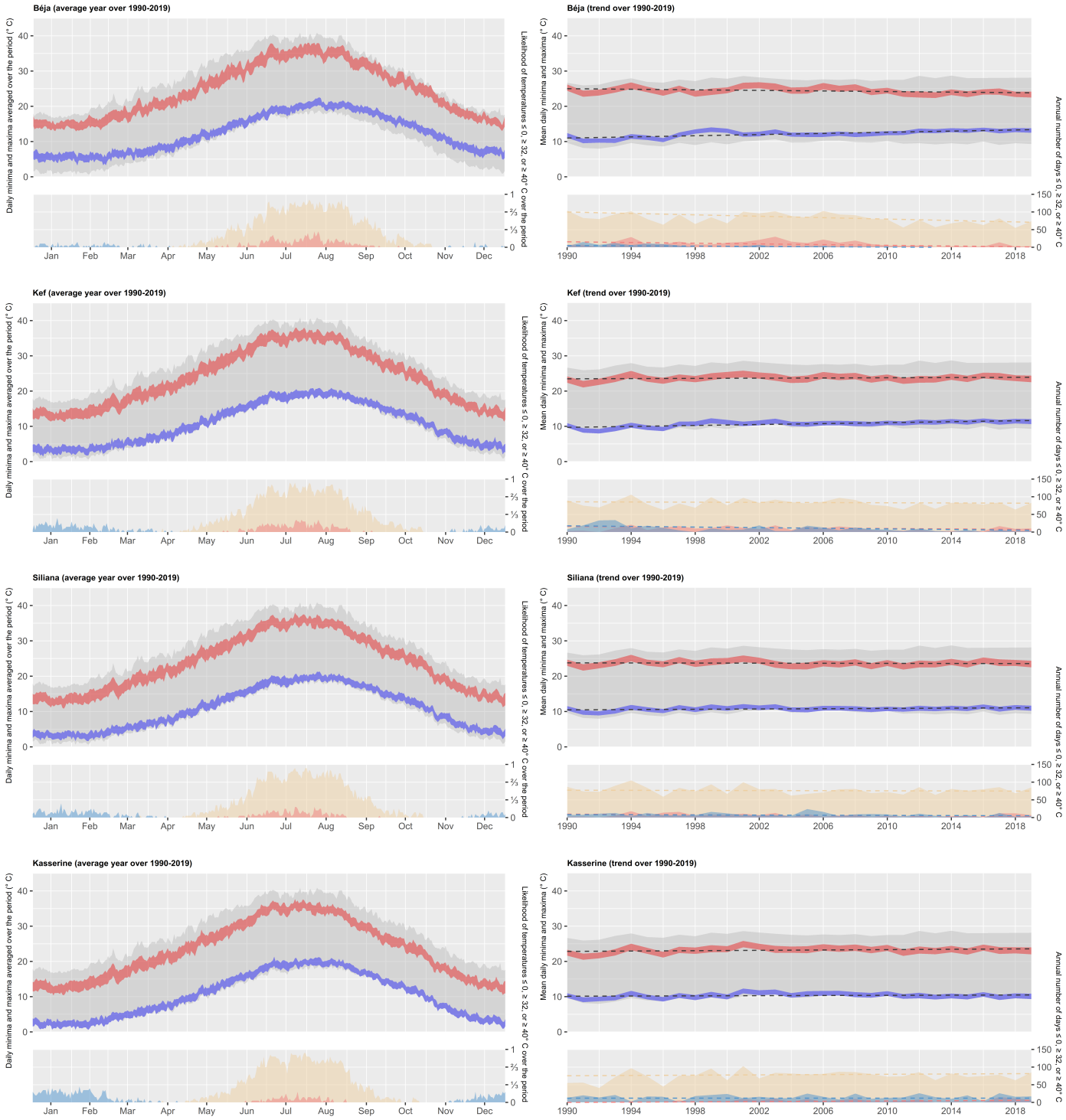


Figure SM3. Geographic and elevational distances between meteorological stations and egg sampling sites (lower opacity if >100 km and >350 m, respectively).


Béja (average year over 1990-2019)
Béja (trend over 1990-2019)
Kef (average year over 1990-2019)
Kef (trend over 1990-2019)
Siliana (average year over 1990-2019)
Siliana (trend over 1990-2019)
Kasserine (average year over 1990-2019)
Kasserine (trend over 1990-2019)
Daily minima and maxima averaged over the period (° C)
Mean daily minima and maxima (° C)
Likelihood of temperatures ≤ 0, ≥ 32, or ≥ 40° C over the period
Annual number of days ≤ 0, ≥ 32, or ≥ 40° C
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
1990
1994
1998
2002
2006
2010
2014
2018

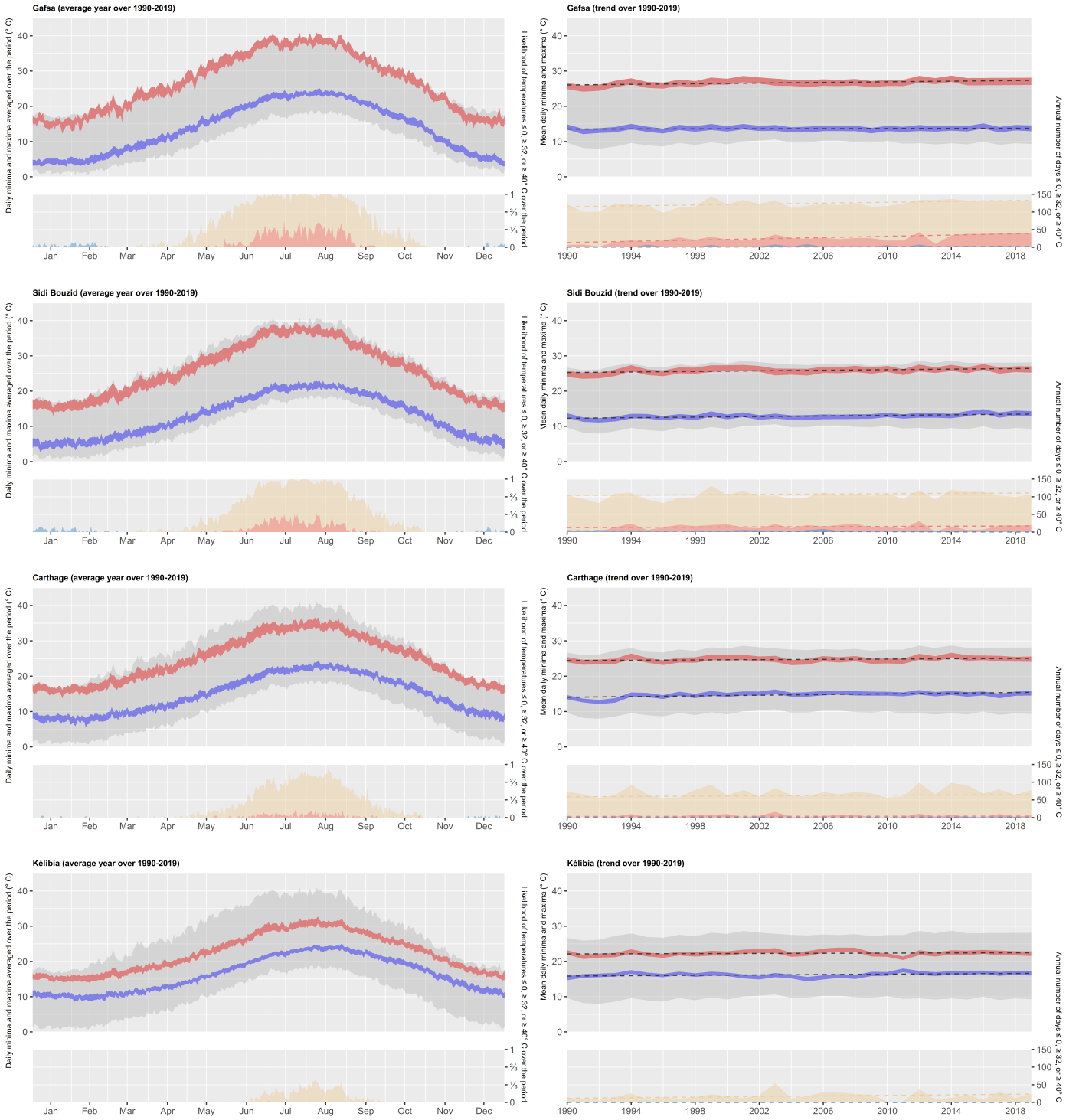


Figure SM4. Climate data in eight Tunisian regions between 1990 and 2019 (data source: INM and NASA, see Table SM2). Left charts show the mean year in each region by averaging daily maxima (red) and minima (blue) by day of the year over the period, represented as 95% CI ribbons. Right charts show the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

B. Multivariate analyses on climate data and K-medoids clustering.

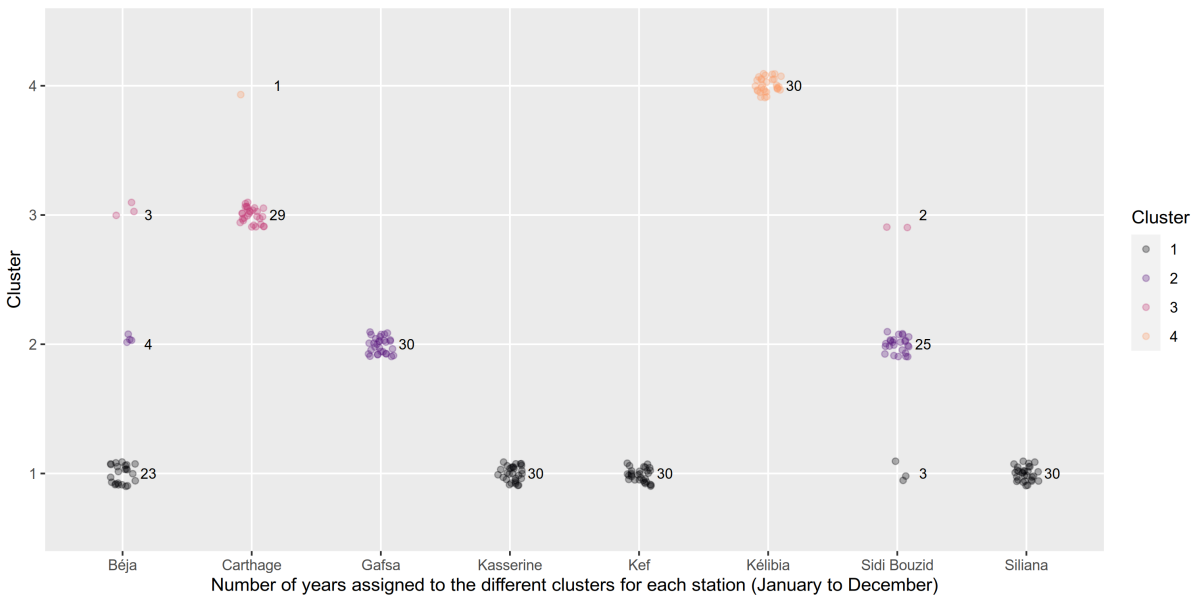


Figure SM5. Number of years assigned to the different clusters for each meteorological series (PAM clustering on data from January to December).

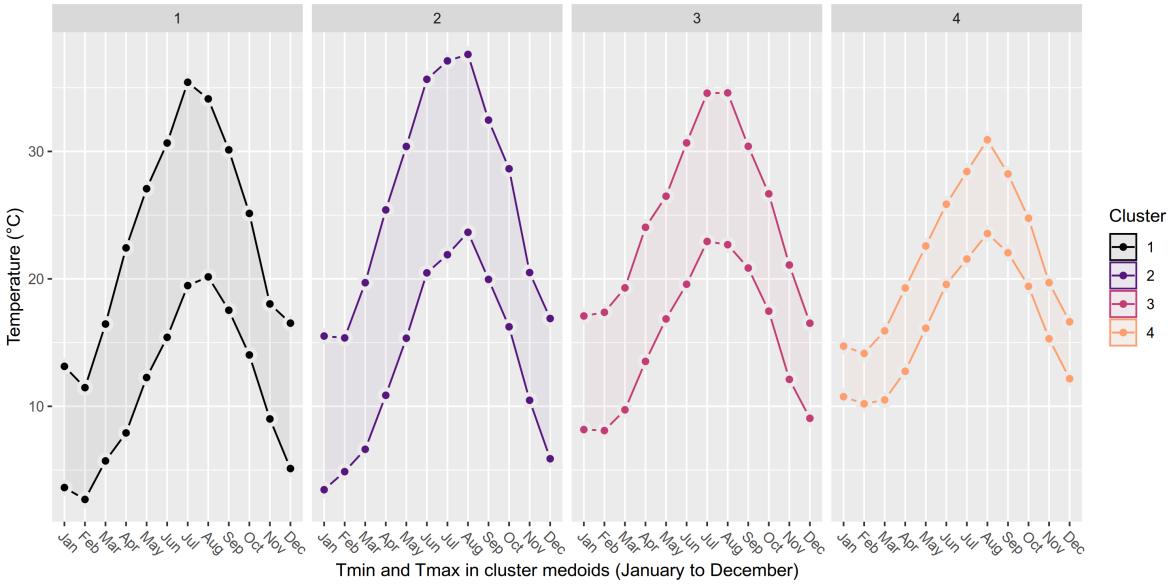
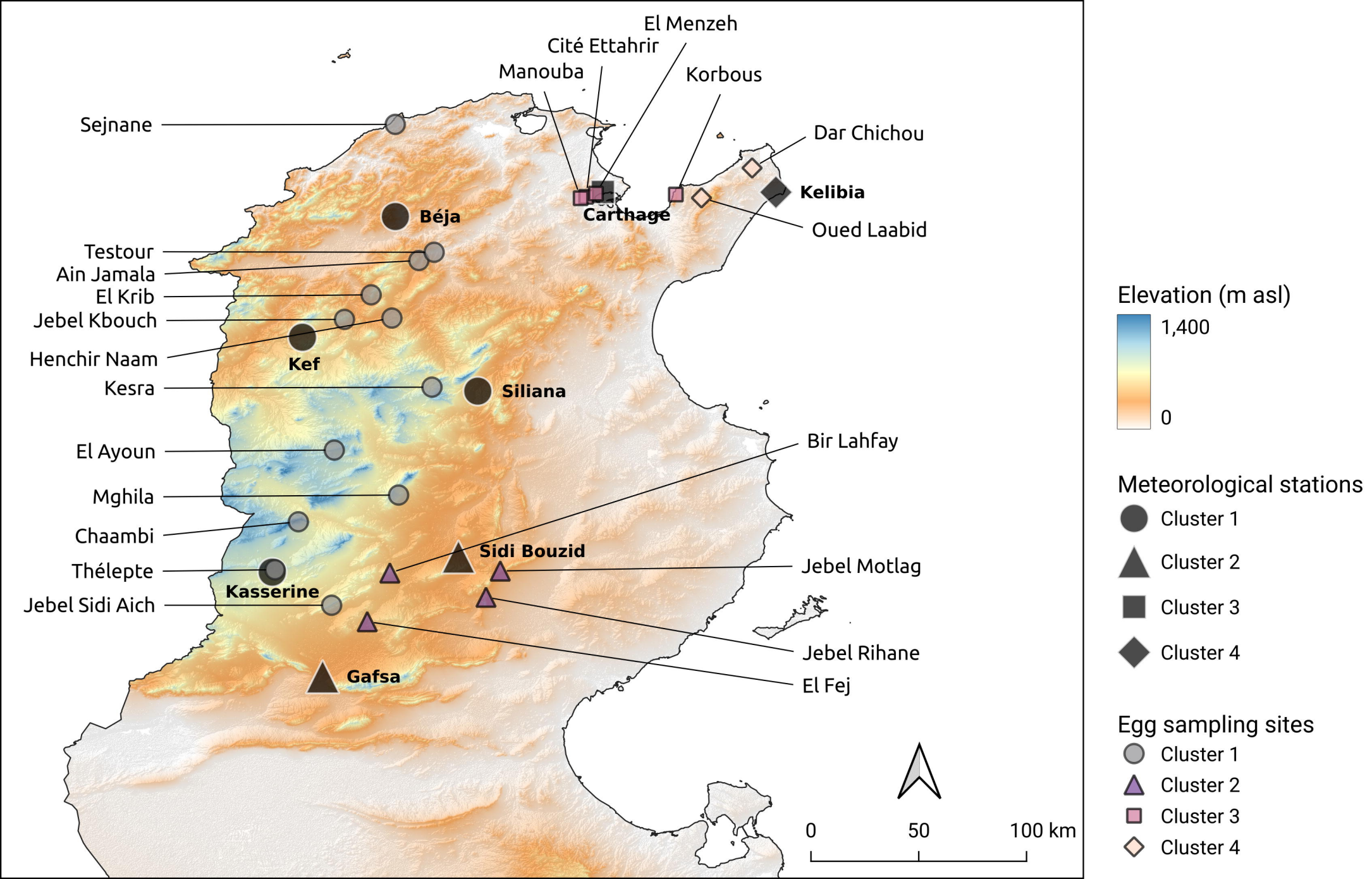
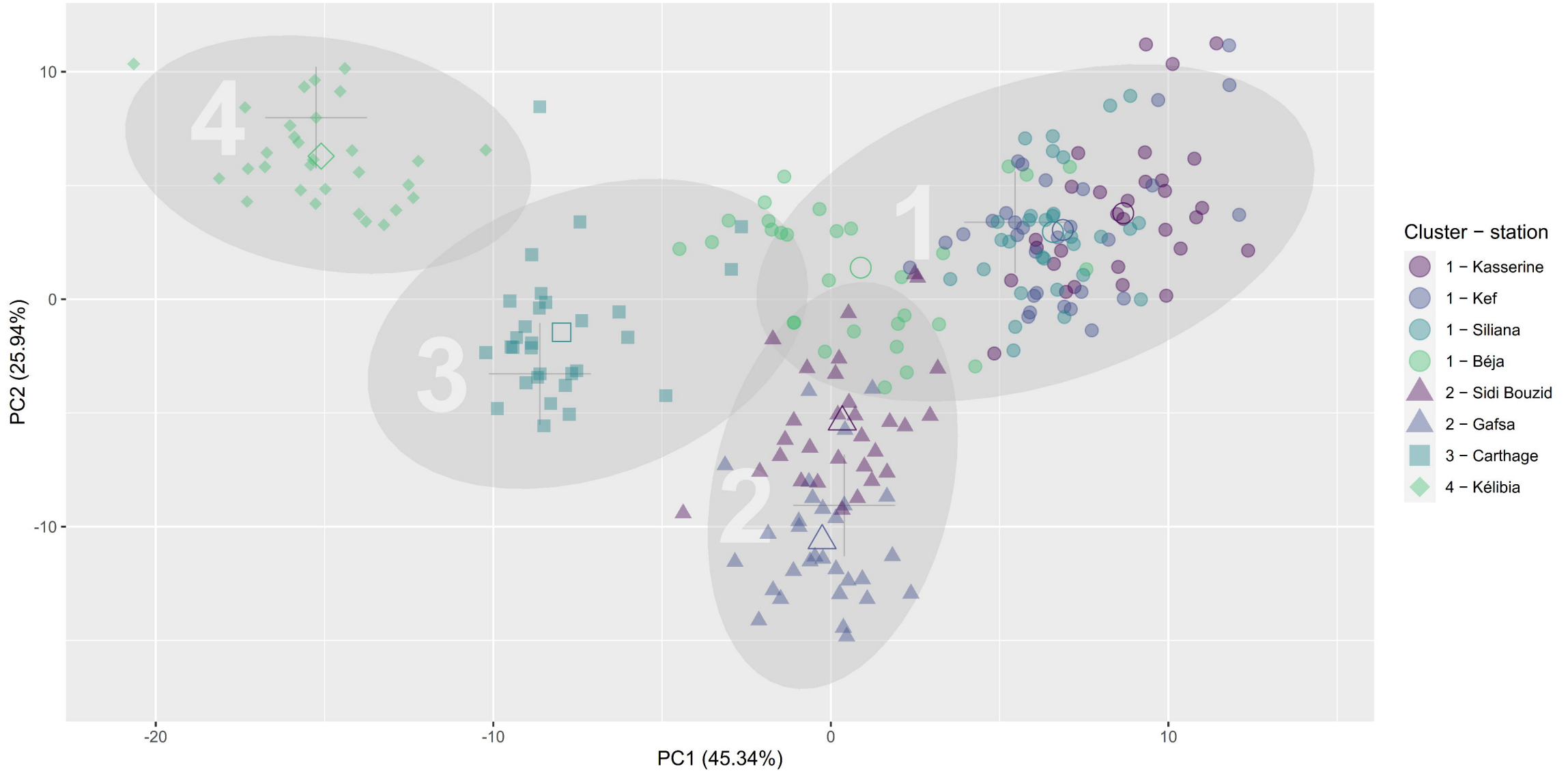
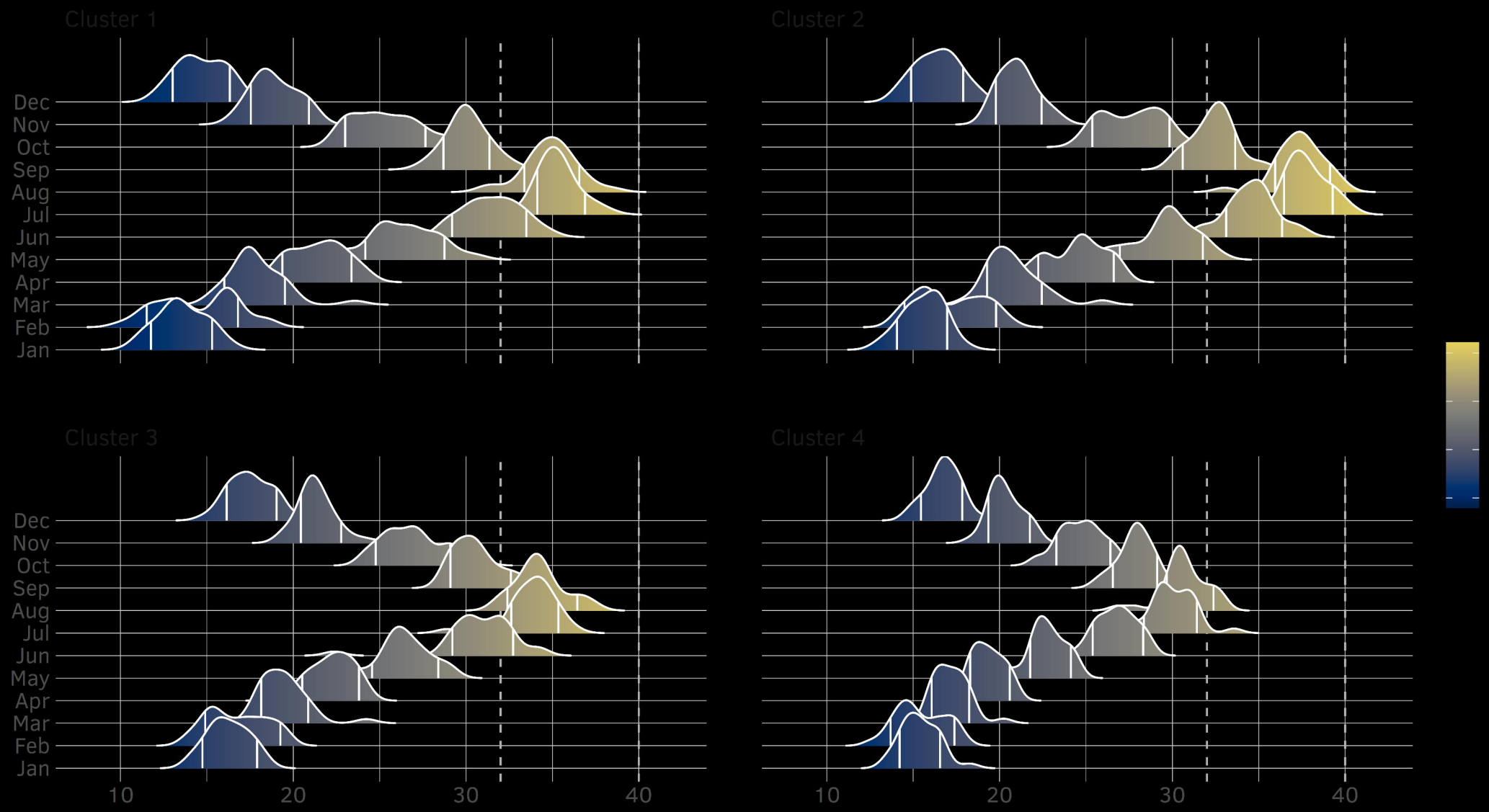
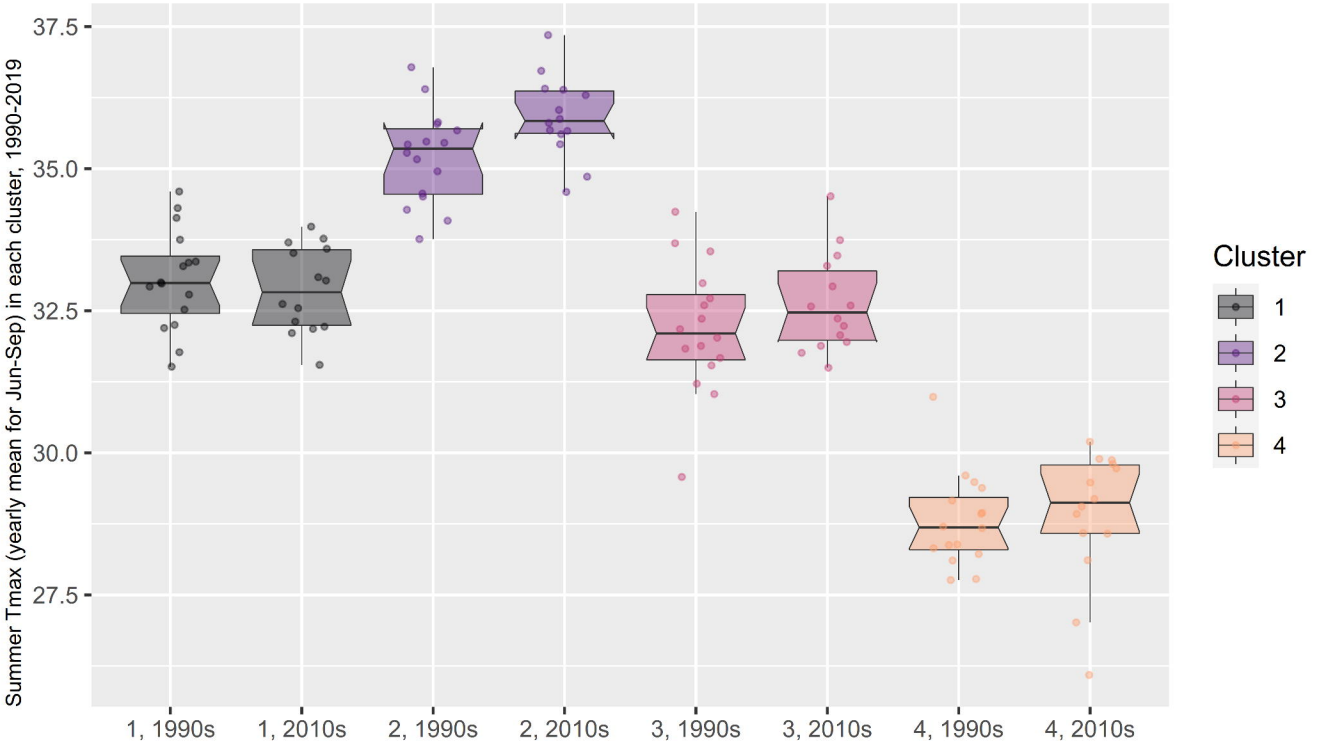


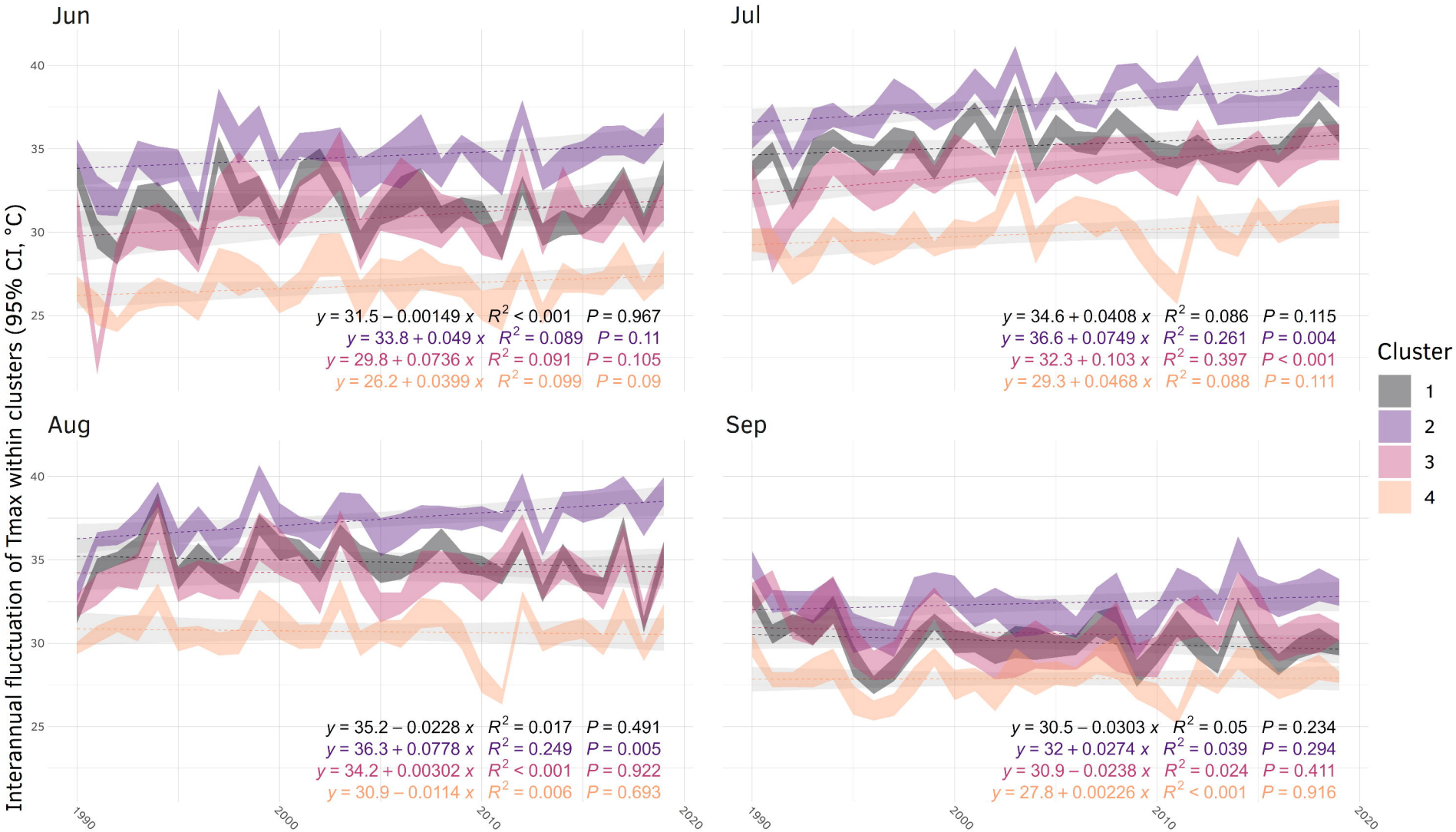
Figure SM6. Tmin and Tmax from January to December in each cluster medoid (PAM clustering on data from January to December).

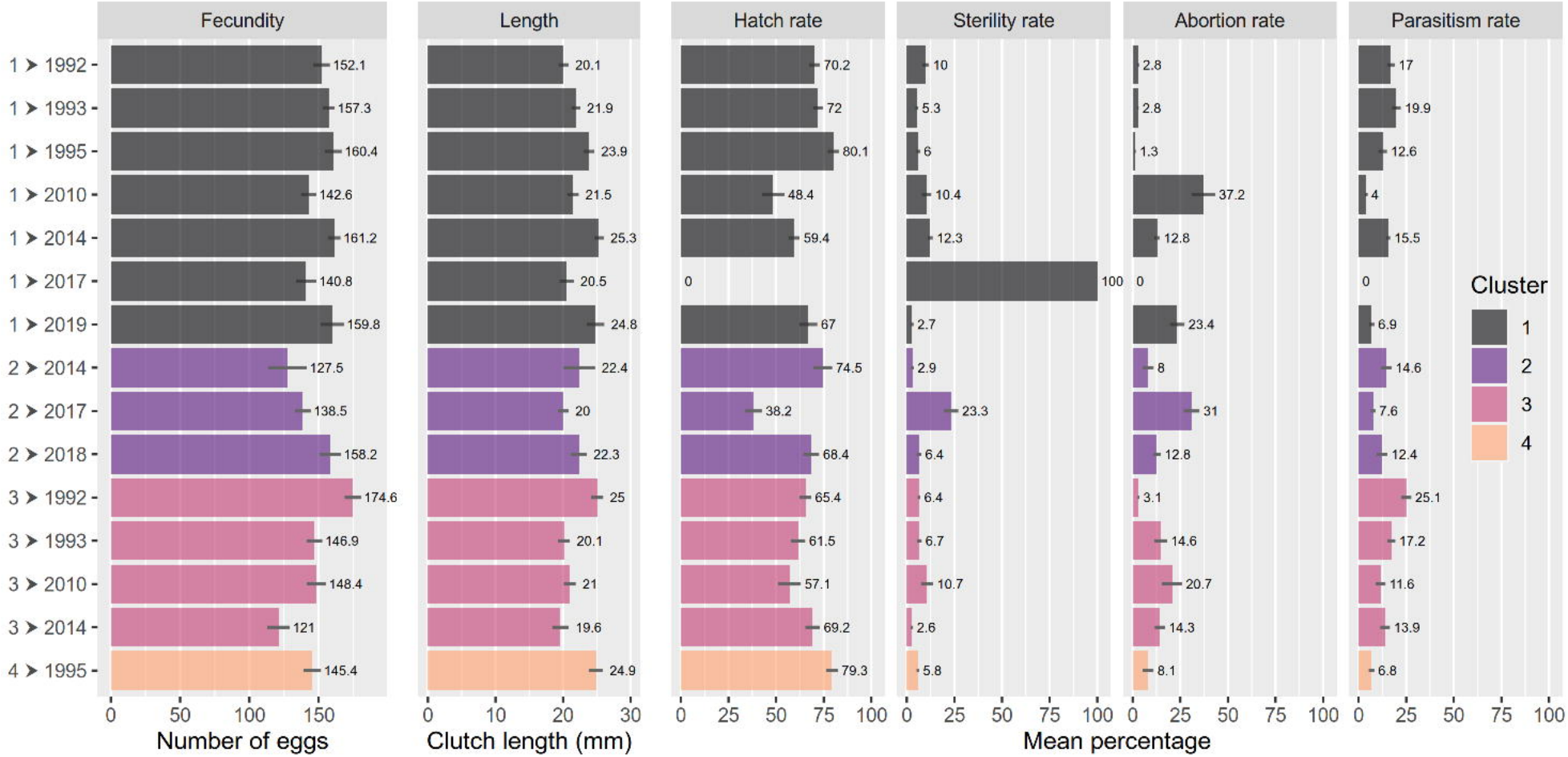

El Menzeh
Cité Ettahrir
Manouba
Korbous
Sejnane
Dar Chichou
Kelibia
Béja
Carthage
Oued Laabid
Testour
Ain Jamala
El Krib
Jebel Kbouch
Henchir Naam
Kef
Kesra
Siliana
El Ayoun
Bir Lahfay
Mghila
Chaambi
Sidi Bouzid
Thélepte
Jebel Motlag
Kasserine
Jebel Sidi Aich
Jebel Rihane
Gafsa
El Fej
0
50
100 km
Elevation (m asl)
1,400
0
Meteorological stations
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Egg sampling sites
Cluster 1
Cluster 2
Cluster 3
Cluster 4


4
3
1
2
10
0
-10
-20
-10
0
10
PC2 (25.94%)
PC1 (45.34%)
Cluster − station
1 − Kasserine
1 − Kef
1 − Siliana
1 − Béja
2 − Sidi Bouzid
2 − Gafsa
3 − Carthage
4 − Kélibia


Cluster 1
Cluster 2
Cluster 3
Cluster 4
Dec
Nov
Oct
Sep
Aug
Jul
Jun
May
Apr
Mar
Feb
Jan
10
20
30
40


Summer Tmax (yearly mean for Jun-Sep) in each cluster, 1990-2019
37.5
35.0
32.5
30.0
27.5
1, 1990s
1, 2010s
2, 1990s
2, 2010s
3, 1990s
3, 2010s
4, 1990s
4, 2010s
Cluster
1
2
3
4


Jun
Jul
Aug
Sep
Interannual fluctuation of Tmax within clusters (95% CI, °C)
40
35
30
25
1990
2000
2010
2020
y = 31.5 – 0.00149 x R2 < 0.001 P = 0.967
y = 33.8 + 0.049 x R2 = 0.089 P = 0.11
y = 29.8 + 0.0736 x R2 = 0.091 P = 0.105
y = 26.2 + 0.0399 x R2 = 0.099 P = 0.09
y = 34.6 + 0.0408 x R2 = 0.086 P = 0.115
y = 36.6 + 0.0749 x R2 = 0.261 P = 0.004
y = 32.3 + 0.103 x R2 = 0.397 P < 0.001
y = 29.3 + 0.0468 x R2 = 0.088 P = 0.111
y = 35.2 – 0.0228 x R2 = 0.017 P = 0.491
y = 36.3 + 0.0778 x R2 = 0.249 P = 0.005
y = 34.2 + 0.00302 x R2 < 0.001 P = 0.922
y = 30.9 – 0.0114 x R2 = 0.006 P = 0.693
y = 30.5 – 0.0303 x R2 = 0.05 P = 0.234
y = 32 + 0.0274 x R2 = 0.039 P = 0.294
y = 30.9 – 0.0238 x R2 = 0.024 P = 0.411
y = 27.8 + 0.00226 x R2 < 0.001 P = 0.916
Cluster
1
2
3
4


Fecundity
Length
Hatch rate
Sterility rate
Abortion rate
Parasitism rate
1 ➤ 1992
1 ➤ 1993
1 ➤ 1995
1 ➤ 2010
1 ➤ 2014
1 ➤ 2017
1 ➤ 2019
2 ➤ 2014
2 ➤ 2017
2 ➤ 2018
3 ➤ 1992
3 ➤ 1993
3 ➤ 2010
3 ➤ 2014
4 ➤ 1995
152.1
157.3
160.4
142.6
161.2
140.8
159.8
127.5
138.5
158.2
174.6
146.9
148.4
121
145.4
20.1
21.9
23.9
21.5
25.3
20.5
24.8
22.4
20
22.3
25
20.1
21
19.6
24.9
70.2
72
80.1
48.4
59.4
0
67
74.5
38.2
68.4
65.4
61.5
57.1
69.2
79.3
10
5.3
6
10.4
12.3
100
2.7
2.9
23.3
6.4
6.4
6.7
10.7
2.6
5.8
2.8
2.8
1.3
37.2
12.8
0
23.4
8
31
12.8
3.1
14.6
20.7
14.3
8.1
17
19.9
12.6
4
15.5
0
6.9
14.6
7.6
12.4
25.1
17.2
11.6
13.9
6.8
Cluster
1
2
3
4
0 50 100 150
Number of eggs
0 10 20 30
Clutch length (mm)
0 25 50 75 100
Mean percentage