



# Effect of summer temperature on prolonged diapause of Tettigoniidae (Orthoptera) under realistic field conditions

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## ABSTRACT

To face recurrent temperature changes, tettigoniids inhabiting temperate climates overwinter as eggs in a diapause stage, being able to postpone embryogenesis for one or more years. To date, it is unclear if species living in warm regions, especially under the Mediterranean climate, could exhibit a diapause for a single year or enter a prolonged diapause due to higher summer temperatures experienced by eggs immediately after oviposition. In this two-year study, we tested the effect of summer temperatures on diapause of six Mediterranean tettigoniid species under natural field conditions. We found that five species can exhibit a facultative diapause depending on mean summer temperatures. For two species, a substantial shift in egg development from 50 to 90% occurred over an interval of c. 1 °C after the first summer period. All the species increased considerably their development (nearly 90%) after the second summer period irrespective of temperatures. Overall, this study suggests that diapause strategy and the different thermal sensibility of embryonic development varies considerably across species potentially affecting their population dynamics.

## 1. Introduction

Diapause favors adaptation of organisms to their environment, synchronizing growth and reproduction to seasonal changes, allowing to overcome adverse meteorological conditions (Tauber, & Tauber, 1976, Denlinger, 2022). In insects, diapause is characterized by a cessation of morphological development and is genetically controlled and regulated by environmental cues such as temperature, photoperiod and moisture (Denlinger, 2002; Ingrisch, 1984; Ingrisch, 1985; Ingrisch, 1986b). Although many insects terminate diapause within one seasonal cycle, some species can extend diapause period for two or more years (Hartley and Warne, 1972).

To face recurrent temperature changes, tettigoniids (Orthoptera, Ensifera, Tettigoniidae) inhabiting temperate climates overwinter as eggs in a diapause stage, being able to postpone embryogenesis for one or more years (Ingrisch, 1986a, Srygley, 2014). According to the time required for development, life cycle of Western Palearctic tettigoniid species can be divided in: (i) annual, (ii) annual or biennial with facultative diapause, (iii) biennial or longer (up to 8 years due to a prolonged diapause) (Ingrisch, 1986a). However, it is unclear if species living in warmer regions could diapause for a single year or enter a prolonged diapause due to higher summer temperatures. Warmer temperatures

experienced by eggs immediately after oviposition could trigger embryonic development, increasing the number of eggs able to develop in just one summer, potentially affecting population dynamics (Ortis et al., 2022). After oviposition (usually between July and October), embryonic development can either be interrupted, even for several years, in a state called initial diapause in the young embryo or triggered until reaching the final diapause stage, where the embryo is mature and occupies the whole egg space (Warne, 1972; Bailey & Rentz, 1990). When final diapause is reached, the egg overwinters for only one winter before completing development and hatching in spring.

Because diapause may influence population dynamics, affect voltinism (Steinbauer et al., 2004) and modify interactions between pathogens and their hosts (Corley et al., 2004), understanding diapause dynamics can help to predict the adaptation of insects to climate change (Tobin et al., 2008; Tougeron, 2019). Most of the previous studies on diapause were carried out under laboratory constant temperatures, that do not reflect the effect of diapause incidence under natural conditions (Colinet et al., 2015; Ortis et al., 2022). The objective of this work was to test the effect of summer temperatures on diapause of several Mediterranean tettigoniid species under natural field conditions. In a two-year experiment, we want to clarify the adaptive strategies of species to seasonal changes that may lead to population fluctuation in certain

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years.

## 2. Methods

### 2.1. Bush-cricket rearing

During summer 2021, six species of Ensifera (Orthoptera) were collected from multiple sites located in the Euganean Hills and Berici Hills (north-east of Italy): *Eupholidoptera schmidti* (Fieber, 1861), *Pholidoptera fallax* (Fischer, 1853), *Pholidoptera aptera* (Fabricius, 1793), *Pholidoptera littoralis* (Fieber, 1853), *Tettigonia viridissima* (Linnaeus, 1758), *Pachytrachis striolatus* (Fieber, 1853). Species were selected for the facultative diapause trait previously reported within the genus (Ingrisch, 1986a) although for certain species no data were available. Adults of both sexes were reared in a greenhouse with natural photoperiod and temperature fluctuating between 19 °C and 35 °C and relative humidity cycling between 70% and 80%. During June and July 2021, bush-cricket rearing was split in six cages (150 × 50 × 50 cm). Each cage contained at the bottom a tray filled with clean sand for egg laying. Adults were fed with branches of *Rubus* sp., fruits, vegetables and dry food for cats. Because egg laying period can change among species, eggs were sifted from the sand at different dates. Eggs of *P. aptera* and *T. viridissima* were collected on the 24th of June 2021, while eggs of the remaining species were collected on the 6th of August 2021.

### 2.2. First summer experiment

To test the effect of summer temperature experienced by eggs, we monitored embryonic development of eggs during two subsequent years. Ten sites were selected to obtain a mean temperature range from 18 °C to 23 °C, at which embryonic development of most species could occur. In 2021 and 2022, we chose the sites in two areas in northeastern Italy: one in the Euganean Hills and one in Eastern Hills (Table S1). In each site, newly laid eggs of each species were randomly selected and placed in one plastic cup (10 × 10 × 5 cm) filled with soil and covered with a nylon net of 0.9 mm mesh to prevent damage by predators. The bottom of each cup was removed and replaced with a nylon net to allow the rainwater flow. Eggs were buried approximately 2 cm beneath the surface. In each site, temperature was recorded every 15 min using a datalogger (Extech TH10) buried approximately 2 cm under the surface in the proximity of the cups. Cups and dataloggers were placed in soil for the same period.

At the end of summer 2021, we counted for each cup i) the number of eggs with embryonic development in progress (i.e. final diapause), ii) the number of eggs that did not develop (i.e. initial diapause), and iii) the number of eggs that were strongly flattened or damaged (mortality). Eggs in final diapause can be easily recognized with a stereoscope by their turgescence following Warne (1972) and for the typical green coloration of the embryo visible by increasing the transparency of the chorion with water (Ortis et al., 2020). All of the vital eggs (in initial diapause) retrieved were placed in soil in one site to allow overwintering under the same environmental conditions.

### 2.3. Second summer experiment

To quantify how many eggs that did not develop from 2021 developed in 2022, we buried in the ground eggs in initial diapause recovered from the experiment carried out in 2021. Because for some species it was not possible to assign to each site a reasonable number of eggs, we redistributed an equal number of eggs in initial diapause randomly selected from all eggs retrieved at the end of summer 2021 (Table S2). Eggs were placed in soil on the 13th of April 2022 and recovered at the end of summer 2022 (20th September). We checked eggs as in the first year experiment described above.

## 3. Statistical analyses

For the first summer experiment, we related the proportion of eggs that reached final diapause on total viable eggs (final diapause + initial diapause) with the mean temperature registered by data-loggers in the period between the 24th of June to 31st of August (*P. aptera* and *T. viridissima*: 24th of June to 31st of August; *E. schmidti*, *P. fallax*, *P. littoralis* and *P. striolatus*: 6th of August to 31st of August). For the second summer experiment, we related the proportion of eggs that reached final diapause with the mean temperature from the 1st of June to the 31st of August.

First, we visually checked the relationship between temperature and the proportion of eggs that reached final diapause. For the species that exhibited a logistic trend, we fitted the following three-parameters logistic curve:

$$y = \frac{d}{1 + e^{b \cdot (\log(x) - \log(a))}}$$

where  $b$  is the slope at the inflection point,  $d$  is the maximum asymptote, and  $a$  is the inflection point. For the species exhibiting an asymptotic trend, we fitted an asymptotic curve using the following function:

$$y = c + (d - c) \left( 1 - \exp\left(-\frac{x}{e}\right) \right)$$

where  $c$  is the lower limit,  $d$  is the upper limit, and the parameter  $e > 0$  is determining the steepness of the increase as  $x$ . Finally, for species exhibiting a linear relationship we fitted a linear regression model. For non-significant models we computed the average proportion of eggs that reached final diapause on total viable eggs over the different temperatures.

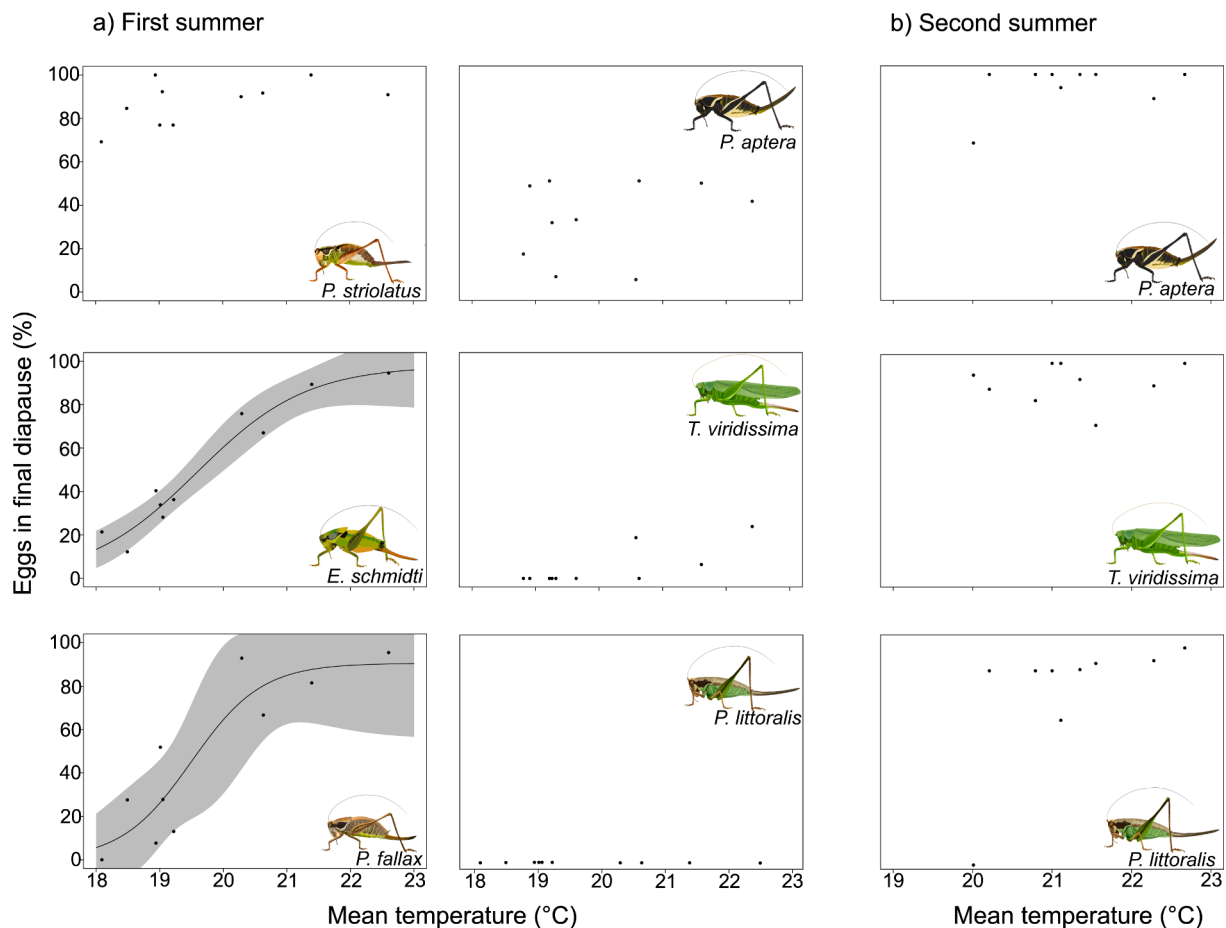
All analyses were performed using R 4.2.1 (R Core Team, 2020).

## 4. Results

During the two years, we recorded an average summer temperature between approximately 18 and 23 °C. Our results showed that summer temperatures can influence differently embryonic development across the tested species (Fig. 1, Table S2). Two species, *Eupholidoptera schmidti* and *Pholidoptera fallax*, followed a logistic model, with percentages of development from nearly 0 to 90% across the thermal gradient (Table S3). For all other species exhibiting qualitatively an asymptotic or linear trend, we chose to show only the raw data as models were not significant. During the first year, eggs of *Pholidoptera aptera* developed with percentages up to 50% (32.69% ± 18.06 SD) irrespective of temperature, while in the second year all remaining eggs showed percentages of development around 90% (94.58% ± 10.56 SD). All eggs of *Pachytrachis striolatus* developed in large number irrespective of temperature during the first year (87.25% ± 10.19 SD). *Tettigonia viridissima* showed a facultative incidence of diapause, as eggs developed in few numbers (4.73% ± 8.57 SD) if temperatures were high enough during the first year, while in the second year all remaining eggs developed in large number irrespective of temperature (91.02% ± 9.47). Finally, *Pholidoptera littoralis* showed to be an obligate biennial species, as eggs developed only during the second summer period (79.11% ± 31.03 SD). However, no development occurred at the coldest mean temperature tested (20 °C).

## 5. Discussion

We described for the first time diapause responses of six tettigoniid species under realistic field conditions, showing how mean summer temperatures affected embryonic development during two years. We found that at least five species tested could exhibit a facultative diapause, developing just after one single year. However, species had different responses depending on the temperature experienced by eggs



**Fig. 1.** Proportion of eggs (%) that reached final diapause at the end of a) first and b) second summer. Observed (points) and predicted (lines) values are also reported. Shaded lines indicate the intervals of confidence (95%).

during first summer. In particular, in *E. schmidti* and *P. fallax* only c. 20% of eggs developed when field average temperatures were under 19 °C, suggesting that those temperatures represent the lowest limit where inception of embryonic development could occur. Egg development change from 50 to 90% occurred over an interval of 1 °C, from 20 to 21 °C. This narrow thermal range was already reported for an outbreaking bush-cricket (Martinez-Sañudo et al., 2021; Ortis et al., 2022), highlighting how recurrent years with temperatures over this thermal threshold could increase the number of eggs able to develop in one year, leading to a rapid increase in population abundances.

While eggs of the bush-crickets described above developed according to a logistic function following a thermal gradient even with a short summer period (i.e. August), other species that laid eggs earlier (i.e. June) had different diapause response, demonstrating different adaptive strategies. In particular, eggs of *P. aptera* developed with rates around 50% regardless of summer temperature. In a previous study Ingrish (1986a) reported *T. viridissima* as a slow developing species with an initial diapause occurring independently from temperature. Here, we found that a low number of eggs could reach final diapause even during first summer period but only if temperatures were over 20 °C. While possible effects mediated by the mother (e.g. age, food supply, photoperiod) could affect prolonged diapause, in this study these factors could not be investigated as female were reared under the same environmental conditions.

Generally, phenological responses to climate change can vary among taxa (Thackeray et al., 2016) and shifts in diapause expression could potentially modify multi-trophic interactions (Davis et al., 1998). In this study, we demonstrated how small variations in temperature over the thermal range of each species were able to regulate incidence of

diapause, potentially shaping population dynamics. All the species increased considerably their egg development (nearly 90%) after the second warm period independently of the temperatures tested. Hence, it might be possible that high abundances in certain years could be due to a synchronization of mass hatchings. It was reported that climate warming can lead to different responses in alpine species that show cyclical fluctuations in the abundance due to the occurrence of prolonged diapause, favoring widespread generalist species while penalizing specialist species (Illich & Zuna-Kratky, 2022). In this view, species with a facultative diapause could take advantage of on-going environmental changes, anticipating developing and hatching period and increasing population density over time (Cavaletto et al., 2019). Furthermore, antagonistic interactions with natural enemies can change due to a shorter life cycle, as eggs remain in soil for a shorter period reducing the impact of predation and/or parasitism and other mortality factors related to environmental conditions such as lack of water (Corley et al., 2004).

While fluctuations of tettigoniid populations are expected to be related to the variable duration of the initial diapause (Ingrisch, 1986c), we clarified that the occurrence of a facultative diapause is triggered by summer temperatures experienced by eggs after oviposition. Further studies on biology and ecology of tettigoniids should take into account their diapause strategy and the different thermal sensibility of embryonic development to temperature.

#### CRediT authorship contribution statement

**Giacomo Ortis:** Conceptualization, Investigation, Formal analysis, Writing – original draft, Visualization. **Luca Mazzon:** Visualization,

Writing – review & editing, Resources. **Lorenzo Marini**: Conceptualization, Writing – review & editing, Supervision.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jinsphys.2023.104499>.

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