

## ECOLOGY

# Development time plasticity of the pine processionary moth (*Thaumetopoea pityocampa*) populations under laboratory conditions

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## Abstract

The pine processionary moth *Thaumetopoea pityocampa* (Lepidoptera, Notodontidae) is a univoltine defoliator that is active over a wide range of latitudes and elevations, being largely influenced by temperature variations, especially during larval development across the winter. This work compares field development time with that observed in the laboratory rearing under controlled conditions, in four *Th. pityocampa* populations characterized by different life history phenology: two populations from the Italian Alps characterized by early and late adult emergence, and two populations from Portugal, the first characterized by winter feeding and late adult emergence, the second by a switch of the larval feeding from winter to summer. The rearing started from the egg stage and was maintained in the laboratory at 20-25°C under natural light in transparent boxes. In spite of the different geographic origins and asynchrony of the period of larval development, all populations maintained an annual life cycle under laboratory conditions, as well as a phenology similar to that

of the field populations. Such an outcome was possible due to a trade-off in the duration of the larval and pupal stages, the latter being identified as the phase of development when an efficient regulatory mechanism is acting to maintain the univoltine life cycle.

## Introduction

Regulating phenology to be active and reproduce at the most favourable season is a common evolutionary mechanism in animals and plants (Tauber *et al.*, 1986; Danks, 1987; Whitman & Agrawal, 2009). Many insect species, in particular the herbivores, are univoltine and semelparous. The single reproductive event occurs normally in the season which allows for the optimization of offspring survival. Seasonality thus appears as a strong factor maintaining the univoltine cycle and insects use environmental variables to track the right time of activity (Saunders, 1982; Danks, 1987). For herbivore insects, the univoltine life cycle is often explained by a precise synchronization with a particular developmental phase of the host plant. For example, several early-feeding herbivores of temperate forest ecosystems depend on the ingestion of young foliage, which is available during a restricted time window in the spring, while less specialized feeders are less constrained in time and can develop over longer periods or alternatively become multivoltine (Dajoz, 2000). Evergreen coniferous host plants offer a good opportunity to test this hypothesis providing a relatively stable food supply *i.e.* mature leaves, which remain available year round. For example, in the diprionid sawflies, some species have a fixed univoltine cycle whereas others may have univoltine cycles interchanged with multivoltine ones (Larsson *et al.*, 1993). The latter species when reared in the laboratory under optimal conditions of temperature and photoperiod may develop continuous generations, as in the case of *Diprion pini* (Eichhorn, 1976).

However, shifts in the phenology may occur in any life stage depending on alterations of the environmental parameters. Saunders (1982) showed that the effects of day length on the induction and termination of diapause may be modified by rising temperatures. This happens because the photoperiod is not affected by climate change whereas temperature is, especially in temperate and boreal zones and particularly in the winter more frequently than in the summer (Hodkinson, 2005). Evidence may be obtained by demonstrating that conditions exceeding the currently observed thresholds of species survival will either impose prohibitive mortality, or preclude the completion of the life cycle.

The winter pine processionary moth, *Thaumetopoea pityocampa* (Lepidoptera, Notodontidae), provides a classic example of an insect feeding on mature foliage that does not need to be synchronized with

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the spring leaf flush. In addition, the larvae feed during the winter, usually while in the third to fifth instar, and respond positively even to a slight increase of temperature (Battisti *et al.*, 2005). As *Th. pityocampa* is univoltine, favourable conditions could promote faster development and thus the possibility to develop continuously. Larvae are night feeders and gregarious, with a development time in the field varying between four and nine months (Démolin, 1969; Battisti *et al.*, 2015), depending on the winter conditions. The timing of adult emergence is generally inversely proportional to the length of the larval development, *i.e.* populations with early adults show the longest larval development, and vice versa (Démolin, 1969; Battisti *et al.*, 2015). A phenological plasticity is typical among populations of this species, being considered as an adaptive strategy to the wide gradients of latitude and elevation over which *Th. pityocampa* range extends. Such plasticity consists of a complementary duration of the larval and pupal stages, for example a long feeding period coupled with a short pupal development in cooler areas and vice versa (Huchon & Démolin, 1970).

Interestingly, in 1997 a population with shifted phenology was discovered in Portugal, which is characterized by larval feeding in the summer, instead of in the winter. In this population, the adults emerge in the late spring (April-June), instead of in the summer (August-September), as locally observed for the typical populations. Previous research established that the shifted population is genetically differentiated from the sympatric winter pine processionary moth (Santos *et al.*, 2007; 2011a). The summer and winter populations co-occur in the same area, being however to a large extent isolated, due to asynchronous reproductive periods (Santos *et al.*, 2011a).

Considering that *Th. pityocampa* presents a large phenotypic plasticity regarding the duration of the different life stages, it would be important to determine its development under constant laboratory conditions. Although the pine processionary moth was reared by many entomologists under controlled conditions, in the literature two data sets only are available. The first one relates to a population of the winter feeding *Th. pityocampa* from Kassandra peninsula, in northern Greece (Devkota & Schmidt, 1990). This population was collected in the egg stage and transferred to a laboratory in Germany (Hannover) where it was raised at 17–20°C. Adult emergence was observed exactly one year later, although larval development lasted between 86 and 109 days only. The second data set concerning another Greek population from Kalogria (southern Greece) provided similar results (Schmidt, 1990; Douma-Petridou, 1990). The difficulty to rear urticating larvae which pose serious health risks to researchers, thus implying the availability of dedicated rearing chambers (Battisti *et al.*, 2011), probably contributes to explain this gap.

Therefore, the objective of this study was to obtain fundamental data pertaining to the duration of *Th. pityocampa* development under controlled conditions. We had access to two independent data sets which are collated in this paper in order to provide the reader with the first synthesis on the development time of the pine processionary moth under laboratory conditions. Four distinct populations were studied, two from Italy characterized by winter feeding and early versus late moth emergence in the field, and two from Portugal characterized by summer *versus* winter feeding. Although methods differed between the two data sets available, findings allow for comparisons in the duration of the developmental periods and mortality of the different life stages among populations having different phenology. Such knowledge may contribute to understanding the *Th. pityocampa* adaptation to different climatic conditions.

## Materials and Methods

### Data set from Italy

Populations previously identified in north-eastern Italy as having early and late moth emergence (Stastny *et al.*, 2006) were selected for

egg batch collection (early population: Cimolais, 12°27'E 46°19'N, 650 m, 12 egg batches collected in July 2012; late population: Cinto Euganeo, 11°39'E 45°16'N, 200 m, and Tregnago, 11°09'E 45°30'N, 450 m, 16 egg batches collected in August 2012). The population density of the pine processionary moth was very low at all sites.

The rearing was carried out in the laboratory of Padova University at Agripolis Legnaro at room temperature (average 22.0°C, interval 20–25°C) and natural light. Egg batches of all populations were stored individually in plastic vials until hatching. All hatched larvae were transferred to aerated boxes (size 15×10×5 cm) with freshly collected pine twigs carrying needles. After that, each egg batch was then cleaned from scales and the number of eggs and hatching holes counted. Colonies originating from different egg batches were reared in separate boxes. *Pinus sylvestris* was used instead of the original host *P. nigra* because the needles are shorter and easier to handle in the rearing boxes, while the larvae perform equally well on the two hosts (Stastny *et al.*, 2006). The rate of larval mortality was calculated for each instar of each colony as the total number of dead larvae in a given instar over the number of larvae that entered that instar. At the end of the larval development, sand was added to the box to allow pupation. The boxes were inspected twice a week for the whole development period.

### Data set from Portugal

Populations previously known to be characterised by larval feeding in winter and summer (Santos *et al.*, 2007) on maritime pine *Pinus pinaster* in the National Forest of Leiria, Portugal (39°47'N, 8°58'W, 50 m) were selected for the laboratory rearing. Egg batches of both populations were obtained in the laboratory from late instar larvae collected in the field and reared to adult stage. Therefore, eggs were not exposed to parasitoids or harsh climate and egg survival was 100%. Egg batches were obtained for five consecutive years, totalizing 12, 6, 46, 46, 52 for the summer population, and 4, 34, 45, 60, 26 for the winter population, respectively. Number of eggs per egg batch ranged from 38 to 110. Egg batches were used to start the rearing in the laboratory of the University of Lisbon at room temperature (average 21.5°C, interval 20–24°C) and natural light. Larvae from the same egg batch were fed in small cages with fresh maritime pine needles, which is the main host species in Leiria pine forest. Fresh twigs were provided every one or two days during the feeding period by gently removing the colony from the old twig, as well as the faeces and the feeding debris. Food was added *ad libitum*. On that occasion, the developmental stage as well as the larval mortality was checked. These observations were carried out at colony level, *i.e.* indication of the average instar of the larvae and whether or not the whole colony has died. The mean duration of each larval instar was calculated as the time when half of the larvae moulted to the next instar, excluding those that died during each specific period. The late fifth instar larvae were transferred to a box (size 25×15×5 cm) containing a bottom layer of 4 cm of sterilized sand, providing an adequate environment for pupation. About five weeks after pupation, the cocoons were sorted out from the sand and the pupae extracted. Each pupa was then kept individually in a plastic vial, under identical room temperature conditions. The pupae were inspected once a week until the beginning of the emergence of the moths, after which they were inspected daily.

### Statistical analyses

To test for differences in the number of individuals and duration of development between Italian populations, a t-test was used. Differences in the developmental time of the colonies of summer and winter populations from Portugal were tested by one-way ANOVA. Mortality of Italian populations was compared with a  $\chi^2$  test while that of Portuguese populations was analysed by logistic regression, considering the proportion of dead families as dependent variable and the factors population and larval stage.

## Results

### Data set from Italy

The eggs of the Italian populations hatched in August in the early population and in September in the later one. The egg batches from the early populations had fewer eggs per batch (225.2, SD=50.0, n=12) than those of the late population (251.7, SD=35.4, n=16), although the difference was not significant (t-test=1.24, P=0.23) and the number of hatched eggs was similar for both populations (57.2, SD=19.9, n=12, and 65.1, SD=36.3, n=16 for the early and late population, respectively) (t-test=0.57, P=0.58). The remaining eggs did not hatch mainly because of egg parasitism which occurred in the field prior to collection.

The overall duration of the larval development did not differ significantly between the two populations (t-test=0.51, P=0.69) (Table 1). Early instar larvae developed faster than later instars, with a slight variation between populations for each instar (Figure 1A). All mature larvae buried in the sand for pupation between December and January. The duration of the pupal development did not differ between the two populations (t-test=0.45, P=0.80) (Table 1). The adults of the early population started emerging at the beginning of June and those from the late population one month later.

Larval mortality was associated with the arrest of feeding and the desiccation of the larvae. Symptoms clearly associated with microbial infections were not observed. For the early and late Italian populations, four out of 12 and four out of 16 colonies, respectively, reached the adult stage, although with few individuals. Larval mortality was rather high for the young larvae and declined progressively in the following instars (Figure 2), with no significant differences between the populations (test  $\chi^2=4.3$ , P=0.63). Colony disappearance due to 100% larval mortality was not associated with a specific stage of development. Individual mortality in the colonies that survived to complete pupal development reached only 5.6% in the early and 11.3% in late population, that is much lower values than the mean colony mortality 71%, calculated by pooling data for all colonies of both populations.

### Data set from Portugal

Results of the five year monitoring of the Portuguese populations showed that eggs hatched from May 8<sup>th</sup> to June 2<sup>nd</sup> in the summer population and from August 3<sup>rd</sup> to September 10<sup>th</sup> in the winter population. Development thus began about three months earlier in the population with a shifted phenology, than in the typical one. The larval development in the summer population was significantly shorter than in the winter population ( $F_{1,53}=39.07$ ,  $P<0.001$ ) (Table 1). Accordingly, the duration of instars was also shorter in the summer population (Figure 1B). For the summer population, all surviving larvae pupated during the months of August and September while in the winter population they pupated between the second week of November and the end of

December. Emergences occurred from the first week of May to the end of June in the summer population, and from the end of July to mid-August in the winter population. Therefore the pupal stage was significantly longer in the summer population than in the winter population ( $F_{1,36}=14.35$ ,  $P=0.001$ ) (Table 1).

Larval mortality, estimated at colony level only, was higher for the early

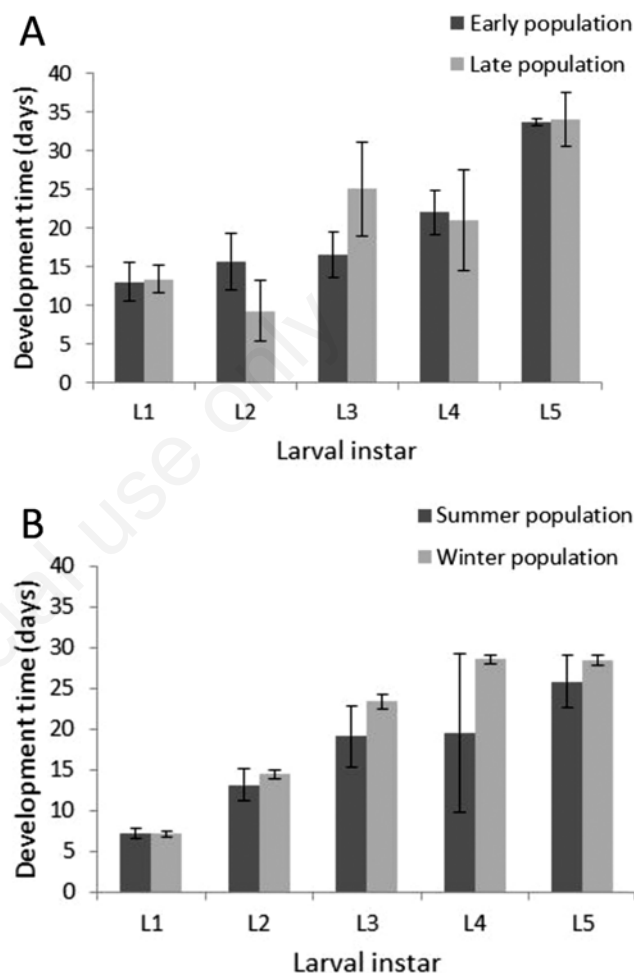


Figure 1. Duration of larval development of each instar of early and late populations of *Thaumetopoea pityocampa* from the Italian Alps (A) and of summer and winter populations of *Thaumetopoea pityocampa* from Portugal (B). Bars indicate standard deviation. Numbers of colonies are given in the text.

Table 1. Duration of larval and pupal development of populations of *Thaumetopoea pityocampa* under laboratory conditions. Data from literature are added (\*).

Population	Larval development (days±SD)	Larval T, °C	Pupal development (days±SD)	Pupal T, °C
Italy-early emergence	100.7±3.25	21.5	223±8.5	21.5
Italy-late emergence	102.5±4.42	21.5	229±11.3	21.5
Portugal-summer population	88.6±9.4	24.9	258±30.0	22.0
Portugal-winter population	100.4±3.9	22.6	234±27.1	22.0
Greece-Kassandra Devkota & Schmidt (1990)*	93.3±6.73	18.5	241	NA
Greece-Kalogria Schmidt (1990)*	>100	21.0	248	NA

SD, standard deviation; NA, not available.



than for the late instars in the summer population (Wald  $\chi^2=12.94$ ,  $P=0.012$ ), while it was similar across instars in the winter population (Table 2). Marginally significant differences between populations were found on the larval stage (Wald  $\chi^2=3.60$ ,  $P=0.058$ ), with higher mortality in the winter population, which was particularly strong in the pupal stage ( $F_{1,41}=5.89$ ,  $P<0.01$ ) (Table 2). The overall mean colony mortality during larval development, pooled from five years, was 64% and 72%, for summer and winter populations, respectively.

## Discussion and Conclusions

A striking result was achieved with the present work, namely that the pine processionary moth will maintain an annual life cycle even under conditions that could lead to continuous development. Raising larvae at a room temperature of 20-25°C, either under short or long day-length, resulted in a developmental time variable between 88 and 102 days only, while larval development under field conditions usually takes between 180 and 300 days for the winter feeding populations and around 90 days for the summer feeding populations (Battisti *et al.*, 2015). The maintenance of an annual life cycle is therefore made possible by a flexible duration of the pupal stage, which in the typical and widespread winter feeding populations was much longer under laboratory conditions than in the field. In addition, the duration of the pupal stage can be much longer in the field in case of prolonged diapause, while such an extension has never been reported under laboratory conditions for this species.

For the typical winter populations, the duration of the larval period has been shown to be strictly dependent on temperature, since continuous larval feeding takes place as long as two conditions are met: night temperatures above 0°C and previous day temperatures remaining above 9°C inside the tent (Battisti *et al.*, 2005). In addition, colonies may cope with extended periods of starvation and repeated freezing across the winter (Hoch *et al.*, 2009). Interestingly, the switching to summer feeding recently observed in Portugal (Santos *et al.*, 2007; Santos *et al.*, 2011a), resulted in larval and pupal developmental times

which resemble those obtained in the laboratory, at 20-25°C, for the winter populations. This agrees with the fact that average monthly temperatures are normally within 20-25°C during larval development of the summer population. Thus, it seems that the timing of adult emergence is regulated by factors which are relatively independent from temperature and determined by the pupae diapause, while the duration of both the adult and egg stages does not show major variations among populations (Battisti *et al.*, 2015).

Data obtained from laboratory rearing confirm the general pattern described by Démolin (1969), based on the collation of a large number of observations across elevation and latitude gradients. According to this author, a negative association can be observed between winter temperatures and the duration of larval development and a positive relationship with the length of the pupal stage. Such a mechanism is basically dependent on variations of the timing of adult emergence, which can extend over several months depending on the location, as confirmed by the survey of Pimentel *et al.* (2010).

Data available for six populations of *Th. pityocampa* reared under laboratory conditions show little variation regarding the duration of the period of larval development at room temperature, in spite of their different geographic origins. Still, slight variations observed in the laboratory may be in part justified by differences in temperature. Although the correlation was not significant, developmental time tended to decrease with increasing temperature ( $r=-0.27$ ,  $P=0.61$ ), consistently with the expected effect of temperature on larval development which is generally observed in the field. Such results are in accordance with the general predictions of temperature-based models of insect development (Chapman, 1998).

Within the larval stage, longer durations of the L3, L4, and L5 instars were observed with respect to the first two instars. The higher biomass of the later instars may justify their longer development time, as the biomass of L5 larvae may be 22 times greater than that of the L2 (Branco *et al.*, 2008). For all larval instars, the developmental time was shorter in the summer population than in the Portuguese winter population. This result might be justified by slight temperature differences between the two periods (Table 2). Otherwise, we can hypothesize that the higher egg size found in the summer population, compared to that of the winter population (Santos *et al.*, 2013), may have an adaptive significance resulting in lower mortality and shorter development time (Zovi *et al.*, 2008). Quantitative and/or qualitative variations of the diet could also have affected larval development, as low growth rates are associated with poor diets (Chapman, 1988). However, this does not seem to be the case of the studied populations, as they were raised on a preferred host species and regularly provided with fresh pine twigs.

The pupal development times show much higher variation which does not seem to be explained by temperature. This physiological

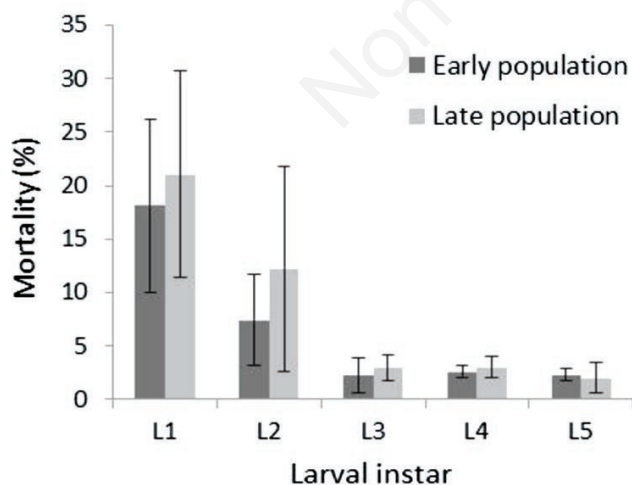


Figure 2. Average individual mortality of larvae of early and late Italian populations of *Thaumetopoea pityocampa*. Mortality has been evaluated by counting all the individuals which died in a given instar in each colony. Bars indicate standard deviation (number of starting colonies: 12 for early and 16 for late populations).

Table 2. Average colony mortality percentage (%) of larvae and pupae of the Portuguese populations of *Thaumetopoea pityocampa*. The averages indicate the percentage of colonies which died in each instar during the five years of the study.

Instars	Summer population (mean±SD)	Winter population (mean±SD)
L1	32±3.6	38±3.4
L2	32±4.4	38±4.3
L3	42±5.6	30±5.2
L4	13±5.1	24±5.7
L5	18±6.1	45±7.7
Pupae	41±6.0	69±10.2

SD, standard deviation.

process is probably influenced by interaction between environmental, hormonal and genetic signals (Tauber *et al.*, 1986; Danks, 1987; Denlinger, 2002) that lead to the maintenance of the univoltine cycle and emergence of the adults in the correct season similarly to what happens in the field, for each specific population. A recent study demonstrated that adults under laboratory conditions emerge one month earlier than in the field (Zhang *et al.*, 1998). Still, in the present study the rearing was started from eggs and a good synchronization of the emergences times with those expected for the respective field populations was observed.

The percentage of mortality of *Th. pityocampa* larvae clearly decreased with the progression of the larval stages, although the causes of death could not be identified due to the lack of specificity of the symptoms. In the field, mortality is mostly due to pathogenic agents such as viruses, bacteria, and fungi. Pupae in the soil are mostly attacked by fungi which are a key factor causing the decline of the populations, especially in closed stands where soil moisture is high (Markalas, 1989; Battisti *et al.*, 1998). However, the larval mortality assessed at individual level for the Italian populations of *Th. pityocampa* reflected the typical decreasing survivorship trend with larval growth (Speight *et al.*, 1999). In the field, the young larvae of all populations are affected by a strong mortality, showing a direct influence from the daunting task of establishing themselves on a food plant (Zalucki *et al.*, 2002). In the laboratory, the young larvae were found to have higher mortality in comparison with older larvae when exposed to disturbances such as thermal stress (Santos *et al.*, 2011b).

Development times under laboratory conditions are also available for other species of *Thaumetopoea*. The larvae of *Th. processionea* of a population from Romania, were maintained at 20-22°C and developed in 76-79 days, instead of the 96-100 days observed in the field (Dissescu *et al.*, 1968 in Battisti *et al.*, 2015), although the annual life cycle was maintained. The larvae of *Th. jordana* developed faster in the laboratory than in the field and much faster at high temperatures, taking 70 days at 20°C, 48 days at 25°C, and 40 days at 30°C (Furth & Halperin, 1979); still, in this case no anticipation of the adult emergence occurred. Studies of a Spanish population of *Th. pinivora* (Galapagar, Madrid, Spain) (Montoya & Robredo, 1972) indicated similar developmental times in the laboratory as in the field although the extended diapause of the pupal stage was missing.

Although the available data were obtained under different conditions, it can be concluded that processionary moths are well synchronized with the seasonality of their habitats, despite the thermal variations that the larvae might experience, probably resulting from a long co-evolutionary process which has defined the development patterns. Yet, it is surprising that such pattern can be completely reversed, as in the case of the summer processionary moth of Portugal, while univoltinism is maintained. Since this event is considered to be recent, as far as the genetic distances indicate (Santos *et al.*, 2007; 2011a), a large flexibility in the responses to environmental changes may be expected.

While the effect of temperature on larval development is quite clear, the mechanisms regulating pupal development need to be thoroughly considered. Due to the reduced metabolic activity of the pupal stage, it is likely that responses to temperature are different from those of the active phases. The flexible duration of the pupal stage ensures that most of the environmental stochasticity may be buffered. Therefore, it is expectable that a reliable signal, allowing the insects to break this stage and develop further, must have evolved. However, such signal cannot rely exclusively on temperature, due to the reduced thermal amplitudes experienced by the pupae in the soil. In short, the mechanisms regulating the physiology of diapause in *Th. pityocampa* must be further investigated, since this stage may provide the key to an understanding of the regulation of the phenology and thus the synchronization of emergences.

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