



Behavioural plasticity and tree architecture shapes tent and foraging locations of pine processionary larval colonies

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With 6 figures and 2 tables

Abstract: Establishing in the right location is particularly important for larval insects. Lepidopteran females are generally selective when choosing oviposition sites to ensure the best survival for their offspring. Unlike most social and shelter-building Lepidoptera, egg batches of the pine processionary moth, *Thaumetopoea pityocampa* Denis & Schiffermüller (1775) (Lepidoptera: Notodontidae), are oviposited randomly on the host tree. *Thaumetopoea pityocampa* is gregarious throughout all larval instars and live in silken tents, spun repeatedly and maintained by the colony during larval development. In this study, a single *T. pityocampa* egg batch was transplanted on *Pinus nigra* trees free from natural egg batches in one of four compass orientations: north, east, south or west. The orientation of transplanted egg batches had no significant effect on the final survival of the larvae. *Thaumetopoea pityocampa* larvae were behavioural thermoregulators from first to final instar and tended to position their tent in a southerly orientation for maximum sun exposure. Thermoregulation was the utmost priority for *T. pityocampa* larvae as they feed through the winter. Feeding behaviour and number of tents built by early instars changed as larvae became older, and this could be explained by natural enemy avoidance and/or evasion of plant defence. The results can help predict larval movement and assist in pest management strategies for *T. pityocampa* at a microhabitat level. This study is the first to investigate detailed movement behaviour of all *T. pityocampa* larval instars on a host tree, describing the transition of larvae starting as patch-restricted foragers to central place foragers.

Keywords: Insect-plant relationships, Lepidoptera, Notodontidae, pest, *Pinus*, shelter-building, *Thaumetopoea pityocampa*

1 Introduction

Herbivorous insects' decision where to feed and live on the plant depends on various abiotic and biotic factors. Insect movement is influenced by abiotic factors such as light and temperature, and biotic factors such as plant defences, plant architecture, pathogens and natural enemies (Perkins et al. 2008; Cribb et al. 2010). It is common to have a combination of factors that impact insect movement. For example, after herbivore attack, the plant releases herbivore-induced plant volatiles which are reliable olfactory cues used by invertebrate predators and parasitoids to locate their herbivore prey (Frost et al. 2008). Therefore, herbivores should keep moving to different parts of the plant or change host plant to avoid detection by natural enemies. Herbivores such as Lepidoptera larvae have evolved various strategies to avoid detection, and repel or escape from natural enemies through chemical, physiological, morphological, and behav-

oural defences (Greeney et al. 2012). Lepidoptera larvae may enhance the effectiveness of their defence strategies by aggregation (Greeney et al. 2012). Gregarious behaviour is defined as individuals feeding, moving or living in tight groups made up of siblings or conspecifics (Fitzgerald & Costa 1999).

There are at least 300 species from 27 families of Lepidoptera with gregarious larvae (Costa & Pierce 1997). Gregarious larvae can benefit from a lower risk of attack by natural enemies because of the dilution effect and enhanced chemical and behavioural defences (Pérez-Contreras et al. 2003). Larval aggregation can also maximise growth of individuals through feeding facilitation, because as a group, they establish new feeding sites more efficiently and can overcome plant defences (Ruf & Fiedler 1999). There are three feeding patterns of gregarious larvae: patch-restricted foraging, nomadic foraging and central-place foraging (Fitzgerald & Costa 1999). Patch-restricted foraging is when colonies

confine their feeding within a single contiguous patch or sequentially exploited patches of leaves. Nomadic foraging is when all individuals of the colony synchronize their activity and wander widely to search for feeding and resting sites. Lastly, central-place foraging is when a colony establishes a permanent/semi-permanent resting site from which they perform intermittent forays in search of food. Many of these gregarious Lepidoptera construct and live in shelters during part or whole larval life. Shelters are multi-functional and assist in larval thermoregulation, development, and defence against predators (Fitzgerald & Costa 1999). However, living in an aggregation comes with costs, such as enhanced levels of disease transmission, intraspecific competition for food and conspicuous shelters attracting natural enemies (Costa 1997). Not only are the shelters visually apparent to natural enemies, the olfactory cues arising from the shelter is enhanced by the numerous individuals and their by-products such as frass (Mondor & Roland 1997). Despite the costs, many Lepidoptera species continue to live in communal shelters and must therefore benefit from the presence of other individuals (Reader & Hochuli 2003).

The pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775), is a well-studied social Lepidoptera species that creates silken shelters (tents or nests) throughout its larval life (L1 – 5). *Thaumetopoea pityocampa* is a widespread pest of pine and cedar trees in the Mediterranean basin and Southern Europe, and in outbreak years the larvae can defoliate numerous tree stands (Battisti et al. 2015). Another important aspect of the pest is their detachable urticating microscopic hairs (setae) present from L3 onwards, and thought to be a form of defence strategy against mammalian and avian predators (Battisti et al. 2011). Contact with urticating setae can cause skin lesions of varying severity, respiratory problems and allergic reactions in humans (Battisti et al. 2011). *Thaumetopoea pityocampa* has a univoltine lifecycle and remain gregarious from the egg batch in summer to pupation in spring. Younger instar larvae start off as patch-restricted foragers feeding on pine needles near the tent and later become central-place foragers, making feeding forays further afield (Fabre 1898; Balfour-Browne 1925; Fitzgerald & Costa 1999). This description, first reported by Fabre (1898), was then interpreted by Balfour-Browne (1925) and later by Fitzgerald & Costa (1999); however, this reporting was not supported by quantitative analyses. When *T. pityocampa* are eggs and early larval instars, temperatures are warm and many invertebrate predators are active during this time, which corresponds to high mortality (Pimentel et al. 2006). The temperature starts to drop in autumn when *T. pityocampa* larvae are in third to fourth instars. At the same time, activity of invertebrate predators decline while avian predation starts to increase due to the low numbers of insect prey (Jactel et al. 2015). Therefore, *T. pityocampa* may adjust its defence strategies and behaviour depending on the predators they encounter

at different larval instars, as described in the social caterpillar *Malacosoma disstria* Hübner (1820) (Lepidoptera: Lasiocampidae) (McClure & Despland 2011). Throughout early larval instars, *T. pityocampa* colony continues to abandon and build multiple tents until the older instars build a ‘winter tent’, which is their final and largest tent (Balfour-Browne 1925; Halperin 1990). Larvae survive the winter by thermoregulating inside the winter tent that is generally positioned at the upper branches or tops of the tree crown (Démolin & Rivé 1968). As older instar larvae gather to the top of the canopy, it is not uncommon to have several hundreds of individuals from multiple egg batches/colonies in a single winter tent (Rouques et al. 2015a).

Despite the importance of *T. pityocampa* as a pest, there are still some gaps in understanding the larval behaviour and movement on the tree. A study on the social caterpillar *Hemileuca lucina* H. Edwards (1887) (Lepidoptera: Saturniidae), showed movement of more than 3 m for thermoregulation after being attacked by predators or after moulting (Cornell et al. 1988). Therefore, natural predators and larval instar may also influence movements and tent-building behaviour of *T. pityocampa*. It is known that *T. pityocampa* colonies, along with many other tent-building Lepidoptera species (*Malacosoma americanum* (Fabricius, 1793) (Lasiocampidae) Fitzgerald & Willer 1983; *Malacosoma californicum phivialis* (Dyar, 1893) (Lasiocampidae) Moore et al. 1988 and Sarfraz et al. 2013; *Yponomeuta mahalebella* Guenée (1845) (Yponomeutidae) Alonso 1997; *Eriogaster catax* (Linnaeus, 1758) (Lasiocampidae) Ruf et al. 2003), build their tents in a southerly orientation where the tree gets the most sun exposure (Breuer et al. 1989; Sebti & Chakali 2014). However, the latter two studies only focused on the final winter tent orientation built by the older *T. pityocampa* instars; the location of tents built by younger instars and position of the tent on the tree branch is still unknown. Jactel et al. (2015) assumed that females are selective in oviposition location on the tree, so that it will be more favourable for larval development. If so, one would expect *T. pityocampa* females to oviposit in a southerly orientation to facilitate egg development and tent establishment, however this was not the case. *Thaumetopoea pityocampa* females oviposit on the tree in all orientations almost equally (Tiberi 1983; Zamoum et al. 2015). This may suggest that caterpillars are plastic in their behaviour and build their tents according to their abiotic and biotic requirements.

This study investigated the survival, detailed movements and choices of where *T. pityocampa* colonies established on the tree from where their egg batch location was set, i.e. four main compass orientations, to their final winter tent location. First instar *T. pityocampa* larvae build a tent by spinning and attaching silk to pine needles near the egg batch. Given the reduced mobility of younger instars, egg batches placed in the south should have better performance because of the increased absorption of thermal radiation by the tent. Colonies from egg

batches placed on other orientations should move to south-erly facing part of the host tree for maximum insolation, and this may result in a cost for the colony. Additionally, the tent position in respect to the tree architecture was investigated to determine if *T. pityocampa* larvae prefer a certain age and density of the pine needles. By exploring how abiotic conditions and architectural heterogeneity within the plant affects the behaviour of *T. pityocampa* larvae, it can assist with the development of new methods for managing this species.

2 Materials and methods

2.1 Study site and design

From the end of August through to September 2018, 88 *T. pityocampa* egg batches with pine needles attached were collected from Austrian pine *Pinus nigra* at Precastio, Verona Italy (45° 31'N, 11° 10'E, 530 m). The field site is a 13,546 m² scattered *P. nigra* stand with trees approximately 1–6 m in height, growing as isolated trees or small groups in a dry meadow (Supplementary Fig. 1). *Thaumetopoea pityocampa* egg batches were collected in a bag and the oviposition orientation of a sub-sample of egg batches were recorded (N = 35). In total, 57 *P. nigra* host trees of 1.5–3 m in height (average: 2.3 ± SE 0.05 m) free of natural egg batches were used as a transplant recipient of *T. pityocampa* egg batches. Host trees higher than 3 m were not used because of the difficulty to perform observational analyses of the larvae and their tent. One egg batch was randomly selected from the bag and deployed in one of four orientations: north, east, south or west at mid height of the recipient host tree. As branches were not always available at specific compass orientations (N, E, S or W), the nearest branch was used. Maximum deviation from a specific compass orientation was 45 degrees in one case, the average deviation was 15.2 ± SE 1.8 degrees. Only one egg batch was attached to the periphery of each host tree around the current-year (2018) needles using a garden tie wire; these are the normal recipient needles for natural egg batches (Tiberi 1983; Zamoum et al. 2015). To identify the egg batch, a tag was attached close to it, reporting the identification code of the colony. Each host tree was free of other *T. pityocampa* egg batches and if present, the egg batches were removed at initial sighting and relocated according to the experimental design. Each colony was monitored once every one to three days up to the third instar (Sept-Oct) and then weekly to fortnightly thereafter until March 2019 when the larvae left the trees to pupate in soil. At each monitoring, the condition and location of the colony were recorded (Table 1) (see *Survival of Thaumetopoea pityocampa colonies* for more information). Air temperature at Precastio was recorded every 15 min with HOBO Temperature/RH data loggers (Onset Computer Corporation, Macquarie, USA) for the duration of the experiment.

2.2 Survival of *Thaumetopoea pityocampa* colonies

The activity of each *T. pityocampa* colony was monitored by observing if fresh frass was present/absent at the tent site and if there was feeding damage on the surrounding pine needles. The larval instar was determined based on the size of frass (Battisti et al. 1986; Battisti 1988a; Grison et al. 1951) and presence of exuviae inside the tent. Any sign of larval activity was recorded, such as silk spinning, moulting, etc. The date, time and weather condition of each monitoring day was recorded. If *T. pityocampa* colonies did not survive past the egg or L1, a new unhatched egg batch was transplanted to replace the previous colony on the same tree. The egg batch was placed in either the same or different orientation as the previous colony. The orientation of the egg batch depended on which one of the four compass orientations had the lowest number of successful transplants, to balance the total number of colonies on the four orientations. After egg hatching and presence of a tent, the transplanted egg batches were removed for analyses. Larval emergence holes from eggs were counted and the length of the egg batch was measured to determine how many larvae hatched and the total number of eggs oviposited by the female, respectively. Number of larvae in each colony was not counted throughout the monitoring period because this was not possible without destruction of the tent. At the end of April 2019, when final instar larvae abandoned their 'winter tent' in search of a pupation site, the tents were collected to estimate how many survived to the final instar. Tents were dissected and L4 exuviae and dead final instar larvae were counted.

2.3 Movement of *Thaumetopoea pityocampa* colonies at tree scale

The first tent was always spun close to the eggs, so there were no issues with locating it because of the tag. When larvae abandoned the tents, the new tents were located by a thorough search around the same branch, then of the branches of the same/closer whorl(s) (see Fig. 1A for pine tree characteristics). In some cases, the search was facilitated by finding the pine needles on which the larvae had fed after the movement. To analyse the movement of *T. pityocampa* colonies, the distance from the previous to the current tent was measured by following the silk trail on the branch (if not visible, the shortest route was measured) using a ruler. This was repeated for each tent that was built by the colony and to avoid confusion, a new identification tag was taped on the branch of the tent. The compass orientation of each new tent was measured using the Apple iPhone iOS application Compass, by pointing the phone away from the closest branch bifurcation where the larvae could have made a choice. If the final winter tent was spun on the top of the tree, orientation was not taken.

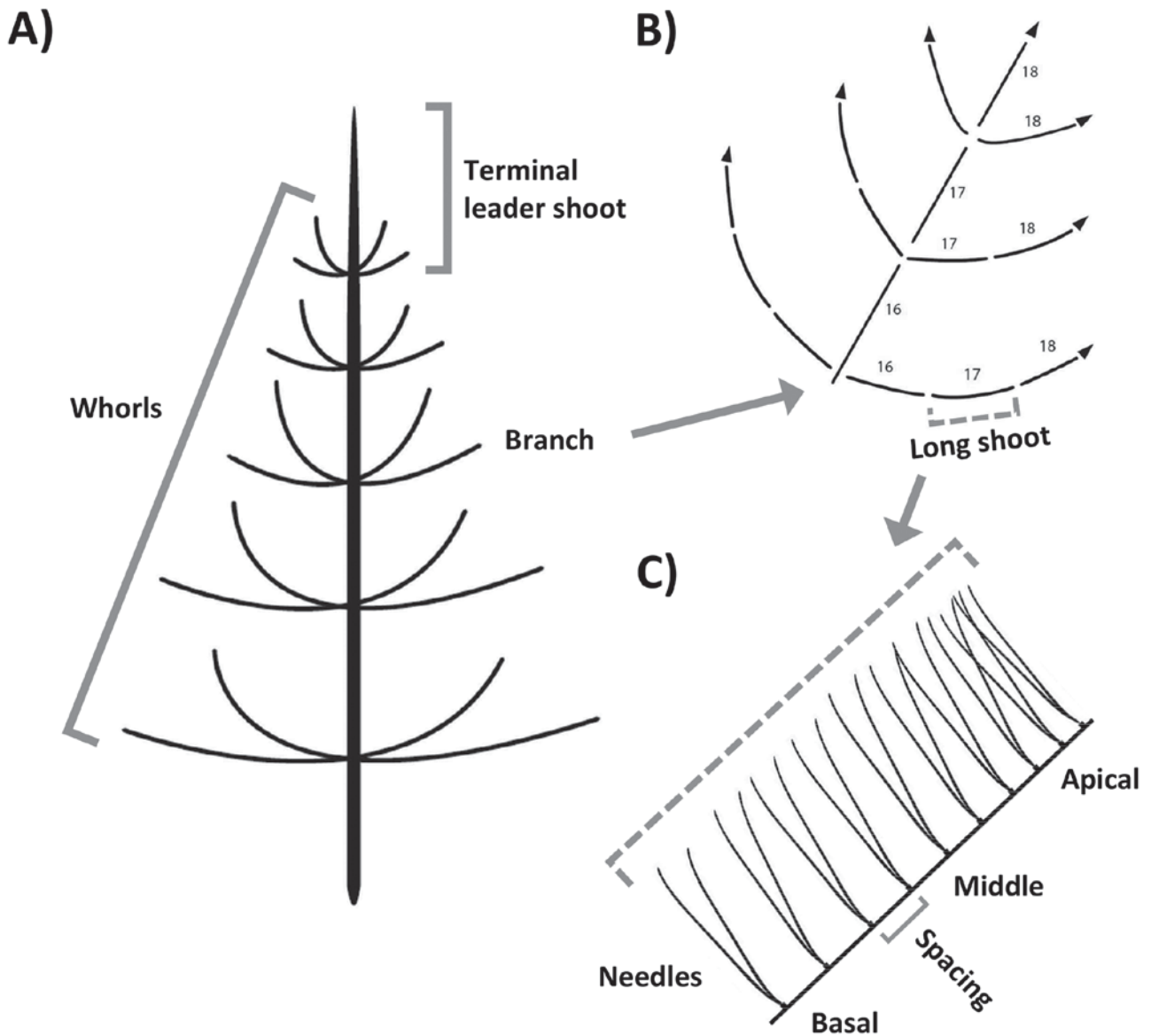


Fig. 1. Simplified schematic diagrams of Austrian pine *Pinus nigra* tree and its characteristics. **A)** Pine tree with lateral branches organized in whorls, each whorl is formed in a specific year. The most apical shoot at the top is growth from the current-year and referred as the terminal leader shoot. **B)** A branch with lateral long shoots, each long shoot represents one year (numbers 16, 17 and 18 refers to the year of the shoot growth, i.e. 2016, 2017 and 2018, respectively). Shoots older than 3 years drop their pine needles. **C)** A long shoot with dwarf shoots containing needle pairs. The long shoot has three sections: basal, middle and apical. Spacing refers to the minimum distance between one needle pair to the next. Diagrams not drawn to scale, drawn by Paolo Paolucci.

2.4 Movement of *Thaumetopoea pityocampa* colonies at branch scale

In mid-September 2018, the characteristics of pine branches where younger *T. pityocampa* (L1 – 2) colonies settled to build a tent after the first movement were analysed. The long shoot year was identified from the outer to the inner part of the branch as: 2018 (current-year shoot during the study), 2017, and 2016; any older shoots were without needles (Fig. 1B). Characteristics of the pine shoots where new tents were established were: long shoot year, shoot posi-

tion (basal, middle or apical), length of the pine needles and distance between each dwarf shoot (pine needle pair) at the three shoot positions (Fig. 1C). The length and spacing of the pine needles were measured with a 10 cm ruler. Additionally, the position and number of pine needles with feeding scars at each tent settlement were counted. In older instars, characteristics of the established tent sites on the pine shoot were not possible because the colony were settled in the tent permanently and measurements were difficult due to their urticating setae.

Table 1. Terminology used to characterise the condition and location of *Thaumetopoea pityocampa* colonies (egg batch or tent) at each monitoring.

Characteristic	Description
Hatched	Caterpillars hatched and resting on/nearby the egg batch
Predated	Egg batch or tent destroyed by predators (e.g. bush cricket)
No hatch	No caterpillars hatched from the egg batch
Moulted	Caterpillars moulted to the next instar, moult determined by frass size and presence of exuviae
Abandoned	No fresh frass or feeding damage near the tent and no caterpillars remained inside the tent
Extinct	No caterpillars survived on the host tree
New tent	New tent establishment after moving (orientation and distance) from the previous tent location
Shoot year	Age of the shoot at the established tent location

2.5 Statistical analyses

2.5.1 Survival of *Thaumetopoea pityocampa* colonies

To determine the survival of the colonies, a generalized linear model (GLM) with a binomial distribution was used. The survival of *T. pityocampa* colonies were given a score of 0 and 1, 0 being no L5 survival and 1 being at least one larva survived to the 5th instar. The categorical and continuous explanatory variables, egg batch orientation (N, E, S or W) and total eggs hatched, respectively, were used to describe the survival of the colony. A linear model (LM) was used to determine if the total eggs hatched was affected by the orientation of the transplanted egg batches on the tree. Kuiper's test of uniformity was used with the R software package "CircStats" (Lund & Agostinelli 2018) to analyse if female moths preferred to oviposit in a particular orientation on the tree based on locations of naturally laid egg batches.

2.5.2 Movement of *Thaumetopoea pityocampa* colonies at tree scale

The continuous response variables 'total distance travelled by each colony to build tents' and 'number of days a colony took for the first movement (new tent) away from the egg batch', were modelled against egg batch orientation using a LM. Oviposition of *T. pityocampa* female moths and movements of the colonies on the host tree were plotted as circular plots using the R software package "ggplot2" (Wickham 2016). Circular plots were divided into 16 sections (slices) of 22.5 degrees, which gave the most appropriate representation of the distribution of oviposition and larval movements. Movements of the larval colonies were grouped into younger (L1 – 2) and older (L3 – 4) instars because they are characterized by different abiotic conditions: younger instars are active in summer and early autumn while older larvae are active in late autumn and winter. The pooling allowed to increase sample size and statistical power, especially in the older instar larvae that generally moved much less. No L5 moved to build new tents during the study. To determine if

the colonies had a preference(s) for tent orientation, Kuiper's test of uniformity was used. Chi-square goodness-of-fit (GOF) test was used to analyse if L1 remained in the same egg batch orientation, i.e. if the new tent establishment was the same orientation as the transplanted egg batch. An exact binomial test was used to analyse the expected and observed frequencies of the first tent orientation for each of the four egg batch compass orientations. Chi-square GOF test was used to determine if average proportion of movements by each instar (L1 – 4) was the same throughout all larval instars. For each colony, average proportion of movements was calculated by counting the number of movements (tents) made by each instar larva divided by the total number of movements for that colony. The proportions for each instar larva from all the colonies were calculated to get the average. The average proportions of movements for each larval instar for the whole population were plotted with the average daily temperature when the larvae moulted. Average daily temperature for each colony when the larvae moulted was calculated from the HOBO temperature data logger recordings in Precastio and then all colonies were pooled to get the population average. Average distance travelled by each instar larva (L1 – 4) from the four egg batch orientations were compared using the chi-square GOF test. The average distance travelled by all egg batch orientations combined were compared by each larval instar using an analysis of variance (ANOVA).

2.5.3 Movement of *Thaumetopoea pityocampa* colonies at branch scale

To assess whether younger instars (L1 – 2) had a preferred shoot year and position to build a tent, a chi-square GOF was also used. The spacing and length of the pine needles of the tent location were analysed with an ANOVA test using the shoot year and position as categorical explanatory variables.

All statistical analyses were performed using the program RStudio version 1.2.5033 (RStudio Team 2019) and an alpha value of $P < 0.05$ was determined as statistically significant.

3 Results

3.1 Survival of *Thaumetopoea pityocampa* colonies

A total of 85 egg batches were transplanted on 57 host trees (28 egg batches hatched then became extinct from predation/unknown causes), and out of the colonies that hatched, 48 egg batches/colonies had at least one larva that survived until L5. All host trees were not completely defoliated, maximum defoliation on available pine needles on a tree was approximately 20% (Supplementary Fig. 2). The life history duration for *T. pityocampa* larvae in Precastio are displayed in Table 2. The average number of eggs hatched per egg batch from Precastio was $108 \pm \text{SE } 6.9$ eggs ($N = 59$). Survival of *T. pityocampa* colonies until L5 was not affected by egg batch orientation (binomial GLM: $P > 0.6$). However, the total number of eggs hatched had a marginal effect on the survival; i.e. colonies with more hatched eggs were more likely to survive until L5 than colonies with less hatched eggs (binomial GLM: $z = 1.89$, $N = 52$, $P = 0.059$). Egg batch orientation had no effect on the total eggs hatched (LM: $P > 0.05$). Female oviposition location of a sub-sample of egg batches did not show any pattern in orientation (Kuiper's test: $P > 0.15$; Supplementary Fig. 3).

3.2 Movement of *Thaumetopoea pityocampa* colonies at tree scale

Egg batch orientation had no effect on the total distance each colony travelled to build tents (LM: $P > 0.1$) or the number of days for L1 to leave the egg batch and build a new tent (LM: $P > 0.1$). Colonies that survived until the final instar (L5), made an average of 3.6 tents ($\text{SE} \pm 0.25$, $N = 44$). After hatching, all colonies build their first tent beside or in very close proximity to the egg batch which is almost always on the 2018 shoot. After the first tent, the colonies abandoned and built a new tent usually on a different branch. Younger instars (L1–2) built their tents across all orientations but significantly more towards the east and south (Kuiper's test statistic: 1.83, $N = 90$, $P < 0.05$; Fig. 2a). Older instars (L3–4) built their tents significantly more towards the east and south (Kuiper's test statistic: 3.14, $N = 44$, $P < 0.01$; Fig. 2b). Older instars also built 12 winter tents at the tree top compared to one tent by younger instars. At the first movement, L1 *T. pityocampa* larvae left their initial egg batch orientation and built a tent elsewhere (GOF: $X^2_3 = 10.96$, $P < 0.05$). The north orientation was the least utilised by the colonies (Exact binomial test: observed: 0.13 and expected: 0.29; $P < 0.05$) and other orientations were equally established with expected frequencies (GOF: $P > 0.05$). Orientations of the tents built

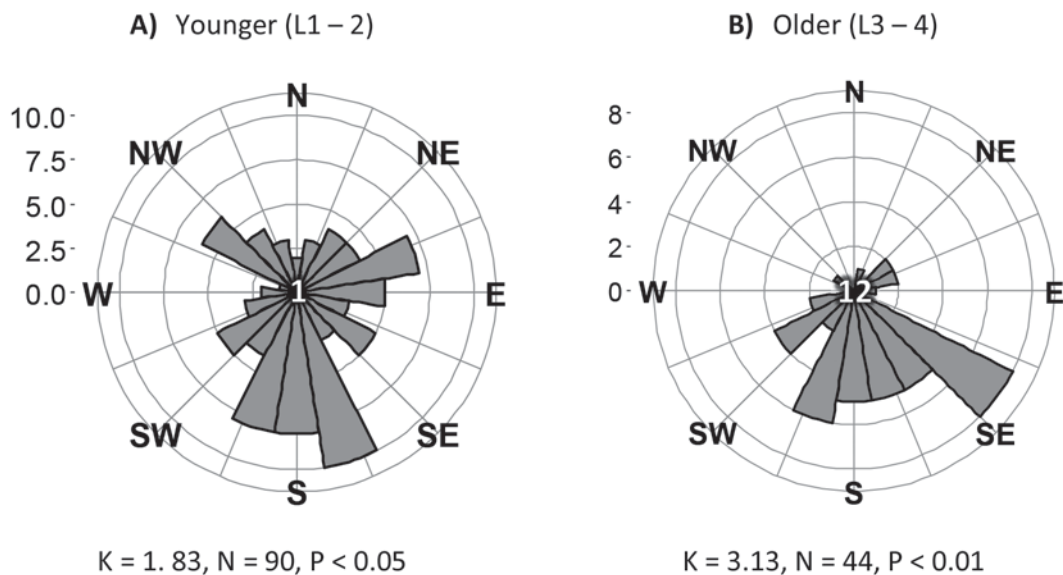


Fig. 2. Distribution and frequency of where and how many **A)** younger (L1–2) and **B)** older (L3–4) *T. pityocampa* larvae built a tent on the host tree irrespective of transplanted egg batch orientation. The numbers on the left of each diagram correspond to the rings inside the circle and it represents the frequency of movements by larval colonies; starting from the smallest number in the centre to the largest number in the second last outer ring. Number in the centre represents the number of tents that were built at the most apical part of the tree canopy (leader shoot) which had no orientation. K and N represents the Kuiper's test statistic and number of colonies, respectively.

Table 2. Life history duration for *T. pityocampa* from Precastio, Italy 2018–2019.

Larval instar	L1	L2	L3	L4	L5
Duration (d)	19	21	34	74	53
Standard error	1.3	1.5	1.5	2.6	2.3
<i>N</i>	44	42	49	44	48

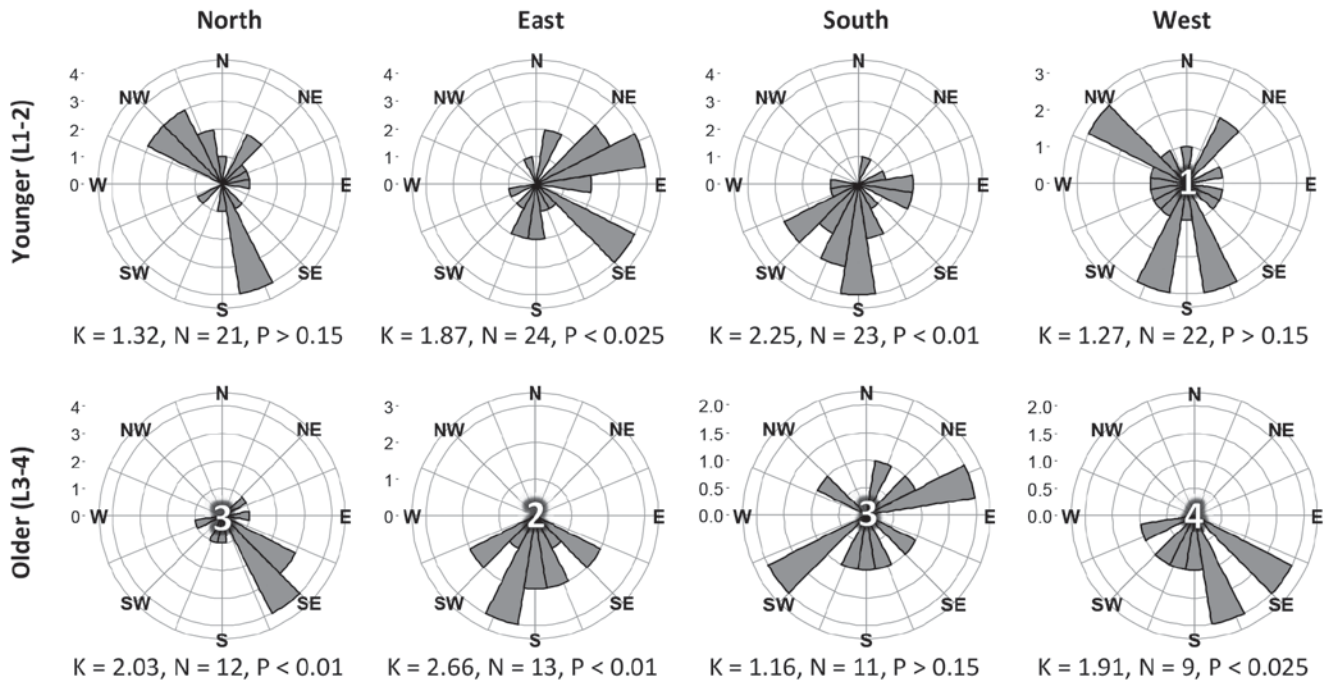


Fig. 3. Distribution and frequency of tent orientations built by younger (L1–2) and older (L3–4) *T. pityocampa* larvae from different initial transplanted egg batch orientations (North, East, South and West). The numbers on the left of each diagram correspond to the rings inside the circle and it represents the frequency of movements by larval colonies; starting from the smallest number in the centre to the largest number in the second last outer ring. Number in the centre represents the number of tents that were built at the most apical part of the tree canopy (leader shoot) which had no orientation. *K* and *N* represents the Kuiper's test statistic and number of colonies, respectively.

by younger and older instars of each egg batch orientation are shown in Fig. 3 (for detailed movements of each larval instar see Supplementary Fig. 4). Older instars built the tents in a more southerly orientation compared to younger instars. The average proportions of movements were significantly different across the four larval instars, with L2 moving the most and L4 moving the least (GOF: $X^2_3 = 17.54$, $P < 0.001$; Fig. 4). Total average distance travelled by colonies that survived until L5 was $341 \pm \text{SE } 33.5$ cm ($N = 36$). Average distance travelled by each instar larva were not different across the four egg batch orientations (GOF: $P > 0.4$). When all egg batch orientations were combined, distance travelled by each larval instar was significantly different; with L1 traveling the least and L4 traveling the most distance (ANOVA: $F_{3, 145} = 28.14$, $P < 0.001$; Fig. 5).

3.3 Movement of *Thaumetopoea pityocampa* colonies at branch scale

Thaumetopoea pityocampa larvae at the first movement preferred to build a tent on the previous-year 2017 shoot compared to other years 2016 and 2018 (GOF: $X^2_2 = 41.82$, $P < 0.001$). Needle length of the 2017 shoot was 38% shorter than the average of other years ($54.2 \pm \text{SE } 2.37$ mm, ANOVA: $F_{2, 45} = 21.32$, $P < 0.001$). Majority of the colonies (92%) chose to establish in the apical position of the shoot compared to the middle and basal positions (GOF: $X^2_2 = 77.56$, $P < 0.001$; Fig. 6). Spacing between the dwarf shoots (pine needle pairs) in the apical position of the shoot was approximately a half of the basal position (i.e. pine needles were more dense in the apical than basal position) which was the next choice by the larvae (3.28 ± 0.17 mm, ANOVA:

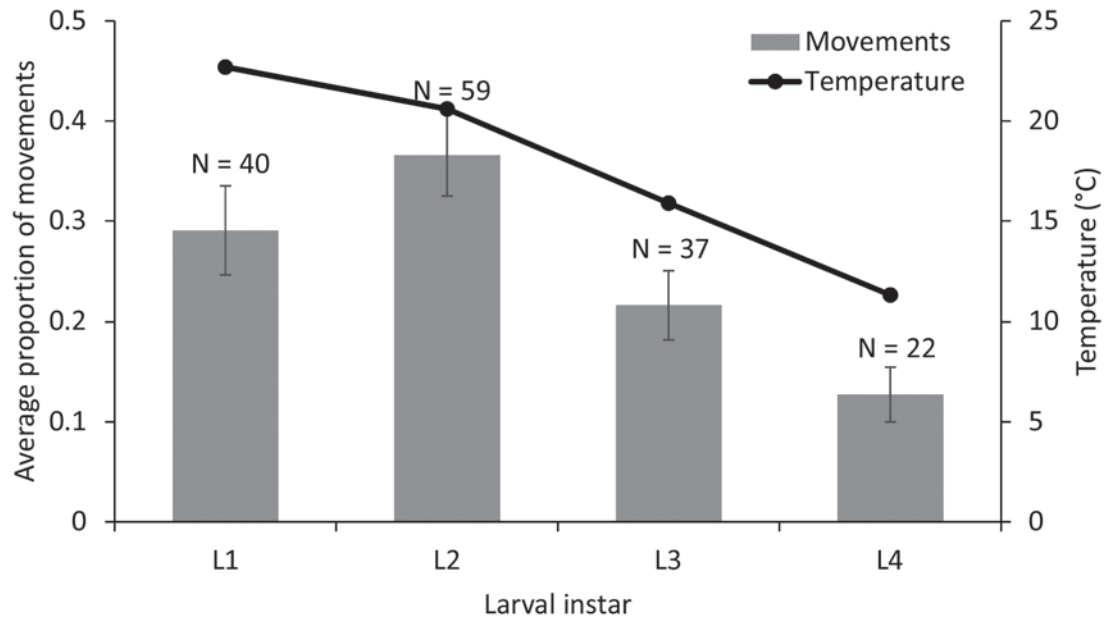


Fig. 4. Average proportion of movements (158 tents built in total) by each *T. pityocampa* colony (N = 57) throughout different instars by all egg batch orientations. Number above each bar represents the total number of movements/tents built from all colonies within the instar. Average daily temperature for each instar is displayed as a black line. There were no movements/tents built by the final instar larva (L5).

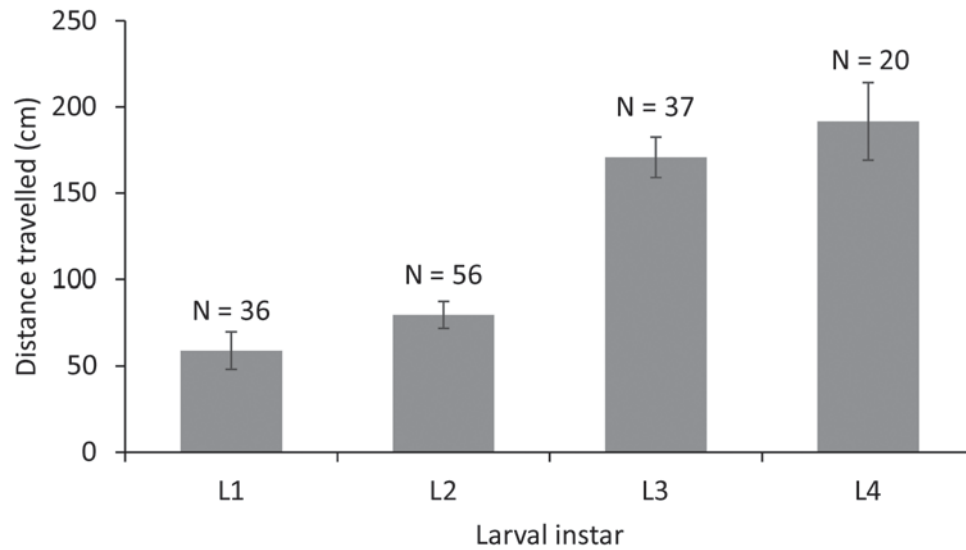


Fig. 5. Average distance travelled (cm) to build a new tent by various larval instars of *T. pityocampa* colonies (N = 57) from all egg batch orientations. Number above each bar represents the total number of movements/tents built from all colonies within the larval instar. There were no movements/tents built by the final instar larva (L5).

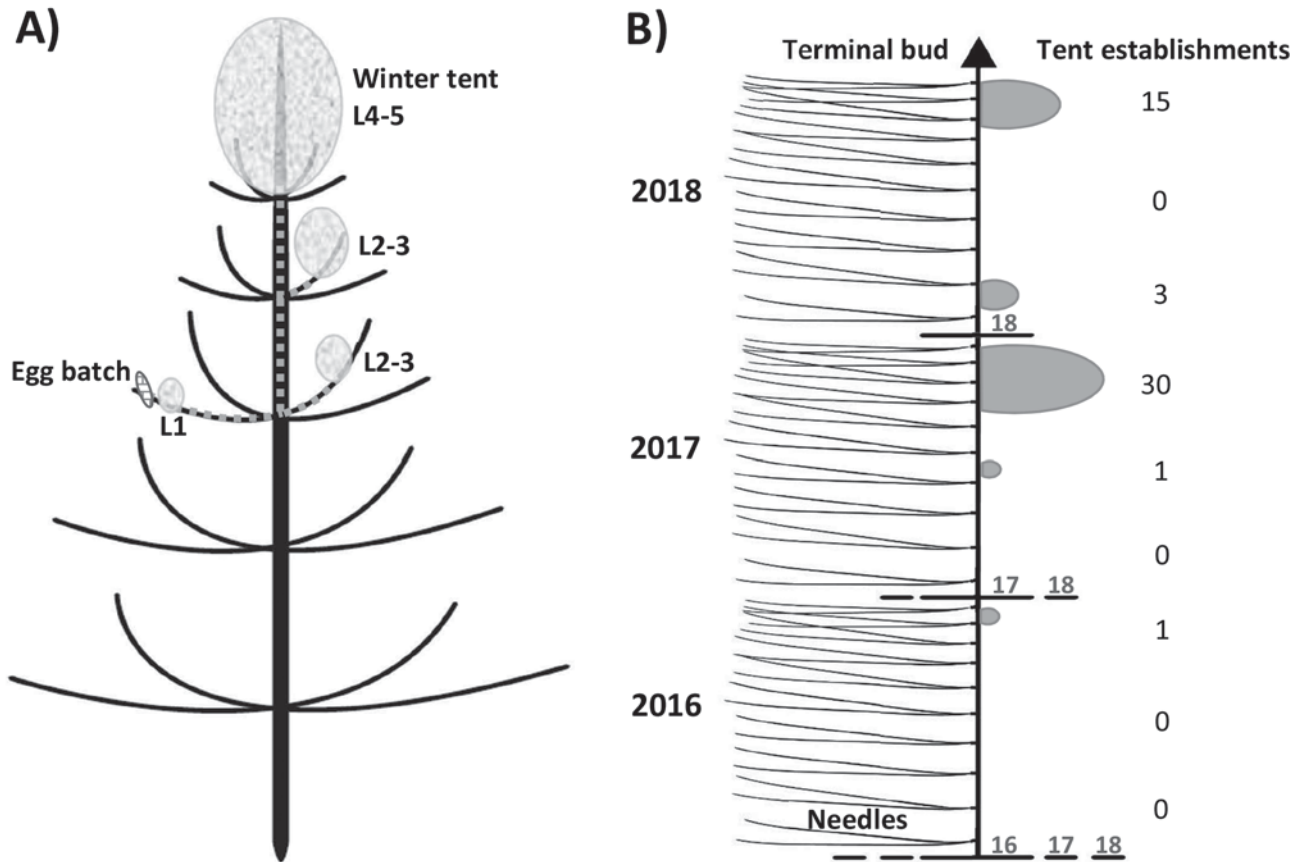


Fig. 6. **A)** Schematic diagram of *T. pityocampa* colony movements and tent establishments (light grey ovals) on *P. nigra*. Dashed grey line represents the movements between abandoned and new tents, starting from the tent near the egg batch to the final winter tent at the most apical part of the tree. Size of tents get progressively larger through larval age. **B)** Schematic diagram of a *P. nigra* branch divided by dashed horizontal lines which represents the three years of growth (long shoots) going vertically and horizontally. The numbers 16, 17 and 18 in grey represents 2016, 2017 and 2018 (current-year of the study) shoots, respectively. The triangle at the end of the 2018 shoot represents the terminal bud. The semicircles on the right of the pine needles are the total numbers of tents that were built on that shoot position (basal, middle or apical) and year by younger instar (L1–2) *T. pityocampa* larvae. Diagrams not drawn to scale, drawn by Paolo Paolucci.

$F_{2, 45} = 12.48$, $P < 0.001$). Younger instar *T. pityocampa* larvae ate an average of 21 needle pairs ($SE \pm 1.35$) at each tent location.

4 Discussion

During larval development, the active movements of *T. pityocampa* larvae on the tree may compensate for the disadvantages associated with abiotic and biotic factors. *Thaumetopoea pityocampa* females oviposit in all orientations on the tree (Tiberi 1983; Zamoum et al. 2015), which has been confirmed in this study. The orientation of transplanted *T. pityocampa* egg batches had no significant effect on the survival of final instar larvae. From an early age, the larvae were able adjust and choose where to establish according to their abiotic and/or biotic requirements. It is

widely accepted that Lepidoptera females have an oviposition preference between and within host plants to ensure the best survival for the offspring (Awmack & Leather 2002). In many social shelter-building Lepidoptera, where females lay eggs is where the larvae construct the shelter; for example: *M. californicum* (Moore et al. 1988), *Ochrogaster lunifer* Herrich-Schäffer (1855) (Notodontidae) (Floater 1996), *Y. mahalebella* (Alonso 1997) and *Eriogaster lanestrus* Linnaeus (1758) (Lasiocampidae) (Ruf & Fiedler 1999). In most of these species, oviposition location is not random. If *T. pityocampa* also built the shelter at the natal location, females would be expected to have a preferred orientation for oviposition, however this was not observed. As for group size, *T. pityocampa* behaved like other social Lepidoptera (*M. disstria*, McClure & Despland 2011; *Ascia monuste orseis* (Godart, 1819) (Pieridae), Santana et al. 2017). The total number of hatched eggs had a marginal effect on the

survival of *T. pityocampa* colonies, and colonies with more individuals were more likely to have larvae that survived until L5. Increase in group size has been shown to increase larval survival by cooperative defence strategies and by dilution effects (McClure & Despland 2011; Santana et al. 2017). In winter, it may be particularly important for *T. pityocampa* as more individuals enables the colony to spin more silk for better insulation (Breuer et al. 1989) and to maintain a well-protected tent from avian predators.

Within-tree heterogeneity seems to play an essential role in the plant-herbivore relationships studied. Plant structure creates different microclimates including differing wind exposure, temperature and insolation at different orientations and height (Bernays & Chapman 1994). Younger and older *T. pityocampa* larval colonies established their tents in a southerly orientation on *P. nigra* trees. Previous studies have also reported *T. pityocampa* tents in a southerly orientation (Breuer et al. 1989), including a study that surveyed over 5500 tents (Sebti & Chakali 2014). However, in these studies, the orientation was only measured for the final winter tent and other temporary tents built by younger larvae were not explored. In the northern hemisphere, southerly orientation is the most sun exposed part of the host plant and therefore the preferred shelter location for many spring feeding Lepidoptera species when temperatures are not high; *M. americanum* (Fitzgerald & Willer 1983), *M. californicum* (Moore et al. 1988), *Y. mahalebella* (Alonso 1997) and *E. catax* (Ruf et al. 2003). The sun is a vital external heat source for larvae to raise their body temperature and achieve faster development rate and larger body size which corresponds to higher survival (Knapp & Casey 1986). Irrespective of different egg batch orientations, there was no significant difference in survival because *T. pityocampa* larvae are behavioural thermoregulators; and their movement on the tree correlates closely with the position of the sun. Populations in Algeria, which is the south of *T. pityocampa* occurrence record, also had nests positioned in the southerly orientation (Zamoum 1998). Which is contrary to what Roques et al. (2015b) stated about the sun not having a role in winter tent location and how the tents were less architected in southern populations. Younger *T. pityocampa* larvae also experience warmer temperatures similar to those of the southern population and most colonies moved and established their tents in a southerly orientation. These younger larvae may seek higher body temperature to accelerate their growth and develop into older instars earlier, when larvae are larger but protected by defensive structures (setae) from avian predators (Barbaro & Battisti 2011). Reducing the time spent as younger instar larvae can minimize the overall risk of mortality by invertebrate predators (Werner & Gilliam 1984). A further study should be done to confirm if this concept applies for *T. pityocampa* larvae. Additionally, more observational analyses should be done to determine tent locations of southern *T. pityocampa* populations to confirm the statements either by Zamoum (1998) or Roques et al. (2015b).

A novel aspect of this study is the quantitative assessment of the changes in shelter-building and foraging behaviour of a social Lepidoptera at different larval instars. The study can affirm that younger *T. pityocampa* make several tents as a patch-restricted forager and become central-place foragers living in a permanent tent as they reach older instars (Fabre 1898; Balfour-Browne 1925; Fitzgerald & Costa 1999). Younger *T. pityocampa* larvae had less days to develop between moults and moved more (i.e. built more tents) than older instars but travelled less in distance. This behaviour of abandoning and re-building multiple new tents has not been described in any other social Lepidoptera species. Fabre (1898) mentioned *T. pityocampa* larvae have this behaviour suggesting several factors: the temporary tents are weak structures and prone to wind damage, tent loses rigidity due to the needles used in infrastructure become dried and weak, and/or the larvae ate all available fresh needles on the shoot. These statements were not supported by our quantitative analyses. Alternative explanations for this transition from patch-restricted forager to central-place forager may be the different natural enemies/mortality factors, as seen in *M. distria* (McClure & Despland 2011), plant defences and/or weather that *T. pityocampa* colonies encounter throughout different larval instars. Their small size with no defensive structures (setae) or behaviour make younger instars vulnerable to predation compared to older instars. When younger *T. pityocampa* larvae are developing, invertebrate predators are also active during these warm temperatures therefore, having a tent nearby is possibly a quick way of getting protection. Younger instars may have evolved to move more (abandon and rebuild tents) to reduce olfactory cues arising from the tent, their by-products and herbivore-induced plant volatiles that initially attract predators and parasitoids. At each established tent site, younger *T. pityocampa* larvae ate an average of 21 needle pairs which is approximately 15% of the total 150 pine needle pairs available on the shoot; which leaves 85% of uneaten pine needles (percentages calculated from Battisti 1988b). Therefore, lack of food is not an influencing factor for the movement of younger colonies. Secondary metabolites produced by the plant could possibly affect the number of needles eaten and correspond to the frequent movements of changing tent/foraging sites. Secondary metabolites such as resin acid inhibited sawfly larvae (*Neodiprion sertifer* (Geoffroy, 1785)) from feeding on *Pinus* spp. and negatively affected larval metabolism (Saikkonen et al. 1995). However, there was no clear relationship between secondary metabolites in pine needles and *T. pityocampa* larval survival (Jactel et al. 2015). As *T. pityocampa* become older, the ambient temperature is too low for most invertebrates to be active. Vertebrate predators such as birds feed on *T. pityocampa* larvae when invertebrate prey numbers are low (Barbaro & Battisti 2011). However, the larvae are well protected by their urticating setae and can survive through the winter in their well-insulated permanent tent. This behaviour implies the cost of traveling longer distances to forage.

The results suggest that thermal and architectural heterogeneity of the tree encouraged plastic behavioural movements of *T. pityocampa* larvae. Majority of younger *T. pityocampa* larvae established on the apical part of 2017 shoots (previous-year shoot) of *P. nigra* trees. The apical part of the shoot had the shortest spacing between pine needles compared to the middle and especially the basal position. Therefore, the apical position is denser with pine needles, which could provide more cover and protection for the younger larvae. Additionally, because of their small size, the shorter distance between needles could be easier for younger instars to attach silk to the needles when building a tent. Further experiments are required to test these hypotheses. A study on a Lepidoptera pine defoliator *Dendrolimus punctatus* Walker (1855) (Lasiocampidae), found higher survivorship because of faster development and heavier body mass when larvae were fed with previous-year pine needles of *Pinus massoniana* (Luo et al. 2018). The study suggested that previous-year pine needles had higher nutritional value for the larvae (Luo et al. 2018). Younger *T. pityocampa* instars are patch restricted foragers, which means they only feed on needles immediately near the tent. Nitrogen levels and energetic values were higher in the previous-year needles compared to the current-year needles in undefoliated *P. nigra* trees (Battisti 1988a). Therefore, establishing a tent on the previous-year shoot could possibly increase survivorship as described for *D. punctatus*. However, *T. pityocampa* larval survival and pine needle traits showed no clear relationship (Jactel et al. 2015). Establishing a tent in the 2017 shoot could also be associated with the visibility of the tent and shade cover. Constructing a tent at the most periphery of pine branches (current-year shoot) could make the tent more conspicuous to predators and parasitoids. Additionally, younger *T. pityocampa* larvae could easily experience the upper temperature threshold (36–40 °C) on the branch surface that lowers their survival (Santos et al. 2011). Therefore, establishing a tent towards the inner part of the branch could help with escaping heat waves. In late autumn and winter, older *T. pityocampa* larvae may have evolved to build their winter tents at the most apical part of the host plant for maximum insolation; because in a dense forest, all sides of the tree except the top is shaded by other trees.

This study is the first to investigate detailed movements of all *T. pityocampa* larval instars at tree and branch scale. Previous studies have only focused on female oviposition location and final location of the winter tent. These results can help predict larval movement and assist pest management strategies at microhabitat level. Current pest management practices for this species is aerial application of an entomopathogenic bacterium (*Bacillus thuringiensis kurstaki*) on infested host trees (Roques et al. 2015b). Younger caterpillars are more susceptible than older caterpillars (Battisti et al. 1998) therefore, application of bacteria will be more effective when they are younger. However, when *T. pityocampa* are younger instars, there are many non-

target species that are active in summer through to autumn. New technologies such as drones (unmanned aerial vehicles) could assist with precise bacteria application on L1 colonies and knowledge about where tents are constructed is helpful to limit consequences on non-target species. Furthermore, many future ecological and behavioural experimental opportunities could arise from this study to understand more about the movement behaviour of *T. pityocampa*. Experimental manipulation of *T. pityocampa* tent establishments can be done by shading southern parts of the host tree and by removing pine needles from previous-year's growth and observe the establishments of *T. pityocampa* colonies. How younger and older *T. pityocampa* larvae navigate on the tree and decide where to establish a tent will be beneficial to understand the abiotic and/or biotic cues they may use. The larvae of the shelter-building *Epargyreus clarus* Cramer (1775) (Lepidoptera: Hesperidae) use their body length as a ruler (Weiss et al. 2003). It will be interesting to determine if younger *T. pityocampa* larvae also use body length to determine that they have reached the apical part of the shoot for tent establishment. *Thaumetopoea pityocampa* larvae are behavioural thermoregulators and their movements on the tree were highly correlated with the orientation of the sun. This study provides a detailed description of *T. pityocampa* larval movements and their preferences to micro-habitat host characteristics. If herbivory constantly occurs in specific locations of the host, it could affect the reproduction and growth of those individual branches and further affect the general health of the host tree (Alonso 1997). This study helps to understand why larvae feed and establish on specific locations of the tree and the possible consequences for the host and herbivore.

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References

- Alonso, C. (1997). Choosing a place to grow. Importance of within-plant abiotic microenvironment for *Yponomeuta mahalebella*. *Entomologia Experimentalis et Applicata*, 83(2), 171–180. <https://doi.org/10.1046/j.1570-7458.1997.00169.x>
- Awmack, C. S., & Leather, S. R. (2002). Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, 47(1), 817–844. <https://doi.org/10.1146/annurev.ento.47.091201.145300>
- Balfour-Browne, F. (1925). The evolution of social life among caterpillars. In Jordan, K., & Horn, W. (Eds.) *The Proceedings of*

- the Third International Congress on Entomology* (pp. 334–339). Zurich.
- Barbaro, L., & Battisti, A. (2011). Birds as predators of the pine processionary moth (Lepidoptera: Notodontidae). *Biological Control*, 56(2), 107–114. <https://doi.org/10.1016/j.biocontrol.2010.10.009>
- Battisti, A. (1988a). Phytophagous insects in the energy flow of an artificial stand of *Pinus nigra* Arnold in Northern Italy. *Redia (Firenze)*, 71, 139–159.
- Battisti, A. (1988b). Host-plant relationships and population dynamics of the pine processionary caterpillar *Thaumetopoea pityocampa* (Denis & Schiffermüller). *Journal of Applied Entomology*, 105(1-5), 393–402. <https://doi.org/10.1111/j.1439-0418.1988.tb00202.x>
- Battisti, A., Dell’Agnola, G., & Masutti, L. (1986). L’attività di *Thaumetopoea pityocampa* (Denis et Schiffermüller) nel ciclo della sostanza organica in popolamenti artificiali di *Pinus nigra* Arnold. *Frustula Entomologica*, 7–8, 507–520.
- Battisti, A., Longo, S., Tiberi, R., & Triggiani, O. (1998). Results and perspectives in the use of *Bacillus thuringiensis* Berl. var. *kurstaki* and other pathogens against *Thaumetopoea pityocampa* (Den. et Schiff.) in Italy (Lep., Thaumetopoeidae). *Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz*, 71, 72–76.
- Battisti, A., Avci, M., Avtzis, D. N., Jamaa, M. L. B., Beradi, L. ... Zamoum, M. (2015). Natural history of the processionary moths (*Thaumetopoea* spp.): new insights in relation to climate change. In A. Roques (Ed.), *Processionary Moths and Climate Change: An Update* (pp. 15–79). Dordrecht: Springer. https://doi.org/10.1007/978-94-017-9340-7_2
- Battisti, A., Holm, G., Fagrell, B., & Larsson, S. (2011). Urticating hairs in arthropods: Their nature and medical significance. *Annual Review of Entomology*, 56(1), 203–220. <https://doi.org/10.1146/annurev-ento-120709-144844>
- Breuer, M., Devkota, B., Douma-Petridou, E., Koutsaftikis, A., & Schmidt, G. H. (1989). Studies on the exposition and temperature of nests of *Thaumetopoea pityocampa* (Den. & Schiff.) (Lep., Thaumetopoeidae) in Greece. *Journal of Applied Entomology*, 107(1-5), 370–375. <https://doi.org/10.1111/j.1439-0418.1989.tb00271.x>
- Cornell, J. C., Stamp, N. E., & Bowers, M. D. (1988). Variation and developmental change in activity of gregarious caterpillars, *Hemileuca lucina* (Saturniidae). *Psyche*, 95(1-2), 45–58. <https://doi.org/10.1155/1988/12962>
- Costa, J. T. (1997). Caterpillars as social insects: Largely unrecognized, the gregarious behavior of caterpillars is changing the way entomologists think about social insects. *American Scientist*, 85, 150–159.
- Costa, J. T., & Pierce, N. E. (1997). Social evolution in the Lepidoptera: ecological context and communication in larval societies. In J. C. Choe & B. J. Crespi (Eds.), *The evolution of social behavior in insects and arachnids* (pp. 407–442). Cambridge: Cambridge University Press. <https://doi.org/10.1017/CBO9780511721953.021>
- Cribb, B. W., Hanan, J., Zalucki, M. P., & Perkins, L. E. (2010). Effects of plant micro-environment on movement of *Helicoverpa armigera* (Hübner) larvae and the relationship to a hierarchy of stimuli. *Arthropod-Plant Interactions*, 4(3), 165–173. <https://doi.org/10.1007/s11829-010-9097-0>
- Démolin, G., & Rivé, J. L. (1968). La processionnaire du pin en Tunisie. *Annales d’INRF Tunisie*, 1, 1–19.
- Fabre, J. (1898). Souvenirs Entomologiques. 6^{ème} Série (pp. 298–392). Paris: Delagrave.
- Fitzgerald, T., & Willer, D. E. (1983). Tent-building behavior of the eastern tent caterpillar *Malacosoma americanum* (Lepidoptera: Lasiocampidae). *Journal of the Kansas Entomological Society*, 56, 20–31.
- Fitzgerald, T., & Costa, J. (1999). Collective behavior in social caterpillars. In C. Detrain, J. L. Deneubourg, & J. M. Pasteels (Eds.), *Information processing in social insects* (pp. 379–400). Basel: Birkhäuser; https://doi.org/10.1007/978-3-0348-8739-7_20
- Fitzgerald, T. D., & Underwood, D. L. A. (2000). Winter foraging patterns and voluntary hypothermia in the social caterpillar *Eucheira socialis*. *Ecological Entomology*, 25(1), 35–44. <https://doi.org/10.1046/j.1365-2311.2000.00236.x>
- Floater, G. J. (1996). Life history comparisons of ground- and canopy-nesting populations of *Ochrogaster lunifer* Herrich-Schaffer (Lepidoptera: Thaumetopoeidae): Evidence for two species? *Australian Journal of Entomology*, 35(3), 223–230. <https://doi.org/10.1111/j.1440-6055.1996.tb01395.x>
- Frost, C. J., Mescher, M. C., Carlson, J. E., & Moraes, C. M. (2008). Plant defense priming against herbivores: Getting ready for a different battle. *Plant Physiology*, 146(3), 818–824. <https://doi.org/10.1104/pp.107.113027>
- Greeney, H. F., Dyer, L. A., & Smilanich, A. M. (2012). Feeding by lepidopteran larvae is dangerous: A review of caterpillars’ chemical, physiological, morphological, and behavioral defenses against natural enemies. *Invertebrate Survival Journal: ISJ*, 9, 7–34.
- Grison, P., Sylvestre, R de S., & Galichet, P. F. (1951). La processionnaire du pin (*Thaumetopoea pityocampa* Schiff.). Mœurs, dégâts, moyens de lutte. *Revue du Zoologie Agricole et Appliquée*, 50, 26–33.
- Halperin, J. (1990). Life history of *Thaumetopoea* spp. (Lep., Thaumetopoeidae) in Israel. *Journal of Applied Entomology*, 110(1-5), 1–6. <https://doi.org/10.1111/j.1439-0418.1990.tb00088.x>
- Jactel, H., Barbaro, L., Battisti, A., Bosc, A., Branco, M. ... Schlyter, F. (2015). Insect – Tree interactions in *Thaumetopoea pityocampa*. In A. Roques (Ed.), *Processionary Moths and Climate Change: An Update* (pp. 265–310). Dordrecht, Versailles: Springer.
- Knapp, R., & Casey, T. M. (1986). Thermal ecology, behavior, and growth of gypsy moth and eastern tent caterpillars. *Ecology*, 67(3), 598–608. <https://doi.org/10.2307/1937683>
- Lund, U., & Agostinelli, C. (2018). R package ‘CircStats’: Circular Statistics (version 0.2-6). <https://cran.r-project.org/web/packages/CircStats/CircStats.pdf>. Accessed on 23 July 2019.
- Luo, D., Lai, M., Xu, C., Shi, H., & Liu, X. (2018). Life history traits in a capital breeding pine caterpillar: Effect of host species and needle age. *BMC Ecology*, 18(1), 1–8. <https://doi.org/10.1186/s12898-018-0181-0>
- McClure, M., & Despland, E. (2011). Defensive responses by a social caterpillar are tailored to different predators and change with larval instar and group size. *Naturwissenschaften*, 98(5), 425–434. <https://doi.org/10.1007/s00114-011-0788-x>
- Mondor, E. B., & Roland, J. (1997). Host locating behaviour of *Leschenaultia exul* and *Patelloa pachypya*: Two tachinid parasitoids of the forest tent caterpillar, *Malacosoma disstria*. *Entomologia Experimentalis et Applicata*, 85(2), 161–168. <https://doi.org/10.1046/j.1570-7458.1997.00246.x>

- Moore, L. V., Myers, J. H., & Eng, R. (1988). Western tent caterpillars prefer the sunny side of the tree, but why? *Oikos*, *51*(3), 321–326. <https://doi.org/10.2307/3565313>
- Pérez-Contreras, T., Soler, J. J., & Soler, M. (2003). Why do pine processionary caterpillars *Thaumetopoea pityocampa* (Lepidoptera, Thaumetopoeidae) live in large groups? An experimental study. *Annales Zoologici Fennici*, *40*, 505–515.
- Perkins, L. E., Cribb, B. W., Hanan, J., Glaze, E., Beveridge, C., & Zalucki, M. P. (2008). Where to from here? The mechanisms enabling the movement of first instar caterpillars on whole plants using *Helicoverpa armigera* (Hübner). *Arthropod-Plant Interactions*, *2*(4), 197–207. <https://doi.org/10.1007/s11829-008-9047-2>
- Pimentel, C., Calvão, T., Santos, M., Ferreira, C., Neves, M., & Nilsson, J.-Å. (2006). Establishment and expansion of a *Thaumetopoea pityocampa* (Den. & Schiff.) (Lep. Notodontidae) population with a shifted life cycle in a production pine forest, Central-Coastal Portugal. *Forest Ecology and Management*, *233*(1), 108–115. <https://doi.org/10.1016/j.foreco.2006.06.005>
- Reader, T., & Hochuli, D. F. (2003). Understanding gregariousness in a larval Lepidopteran: The roles of host plant, predation, and microclimate. *Ecological Entomology*, *28*(6), 729–737. <https://doi.org/10.1111/j.1365-2311.2003.00560.x>
- Roques, L., Rossi, J., Berestycki, H., Rousselet, J., Garnier, J. ... Robinet, C. (2015a). Modeling the spatio-temporal dynamics of the pine processionary moth. In A. Roques (Ed.), *Processionary Moths and Climate Change: An Update* (pp. 227–263). Dordrecht, Versailles: Springer. https://doi.org/10.1007/978-94-017-9340-7_5
- Roques, A., Rousselet, J., Avci, M., Avtzis, D. N., Basso, A. ... Delb, H. (2015b). Climate warming and past and present distribution of the processionary moths (*Thaumetopoea* spp.) in Europe, Asia Minor and North Africa. In A. Roques (Ed.), *Processionary Moths and Climate Change: An Update* (pp. 81–161). Dordrecht, Versailles: Springer. https://doi.org/10.1007/978-94-017-9340-7_3
- RStudio Team (2019). RStudio: Integrated for R. RStudio, Inc.
- Ruf, C., & Fiedler, K. (1999). Colony survivorship of social caterpillars in the field: A case study of the small egg moth (Lepidoptera: Lasiocampidae). *Journal of Research on the Lepidoptera*, *38*, 15–25.
- Ruf, C., Freese, A., & Fiedler, K. (2003). Larval sociality in three species of central-place foraging lappet moths (Lepidoptera: Lasiocampidae): A comparative survey. *Zoologischer Anzeiger*, *242*(3), 209–222. <https://doi.org/10.1078/0044-5231-00099>
- Saikkonen, K., Neuvonen, S., & Kainulainen, P. (1995). Oviposition and larval performance of European pine sawfly in relation to irrigation, simulated acid rain and resin acid concentration in scots pine. *Oikos*, *74*(2), 273–282. <https://doi.org/10.2307/3545657>
- Santana, A. F. K., Rodrigues, D., & Zucoloto, F. S. (2017). Larval aggregation in a Neotropical butterfly: Risky behaviors, per capita risk, and larval responses in *Ascia monuste orseis*. *Behavioral Ecology and Sociobiology*, *71*(12), 174. <https://doi.org/10.1007/s00265-017-2403-4>
- Santos, H., Paiva, M. R., Tavares, C., Kerdelhué, C., & Branco, M. (2011). Temperature niche shift observed in a Lepidoptera population under allochronic divergence. *Journal of Evolutionary Biology*, *24*(9), 1897–1905. <https://doi.org/10.1111/j.1420-9101.2011.02318.x>
- Sarfraz, R. M., Kharouba, H. M., & Myers, J. H. (2013). Tent caterpillars are robust to variation in leaf phenology and quality in two thermal environments. *Bulletin of Entomological Research*, *103*(5), 522–529. <https://doi.org/10.1017/S0007485312000892>
- Sebti, S., & Chakali, G. (2014). Distribution and importance of the pine processionary moth winter nests *Thaumetopoea pityocampa* (Denis & Schiffmüller) (Lepidoptera: Notodontidae) in the forests cedar of the national park of Chréa (Algeria). *International Journal of Agricultural Science and Research (IJASR)*, *4*, 77–84.
- Tiberi, R. (1983). Sulla distribuzione delle ovature di *Thaumetopoea pityocampa* (Den. & Schiff.) in un giovane impianto di *Pinus pinaster* e *P. insignis*. *Redia (Firenze)*, *66*, 603–614.
- Weiss, M. R., Lind, E. M., Jones, M. T., Long, J. D., & Maupin, J. L. (2003). Uniformity of leaf shelter construction by larvae of *Epargyreus clarus* (Hesperiidae), the silver-spotted skipper. *Journal of Insect Behavior*, *16*(4), 465–480. <https://doi.org/10.1023/A:1027399122333>
- Werner, E. E., & Gilliam, J. F. (1984). The ontogenetic niche and species interactions in size-structured populations. *Annual Review of Ecology and Systematics*, *15*(1), 393–425. <https://doi.org/10.1146/annurev.es.15.110184.002141>
- Wickham, H. (2016). ggplot2: Elegant graphics for data analysis. Springer International Publishing, New York.
- Zamoum, M. (1998). Données sur la bioécologie, les facteurs de mortalité et la dynamique des populations de *Thaumetopoea pityocampa* Denis et Schiffmüller. (Lep., Thaumetopoeidae) dans les pineraies subsahariennes de la région de Djelfa (Algérie). Thèse de Doctorat, Univ. des Sciences de Rennes I, France, pp 247.
- Zamoum, M., Martin, J. C., & Bensidi, A. (2015). Fecundity of the pine processionary moth *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae) at the southern edge of its distribution range. *Biologia*, *70*(3), 386–392. <https://doi.org/10.1515/biolog-2015-0036>

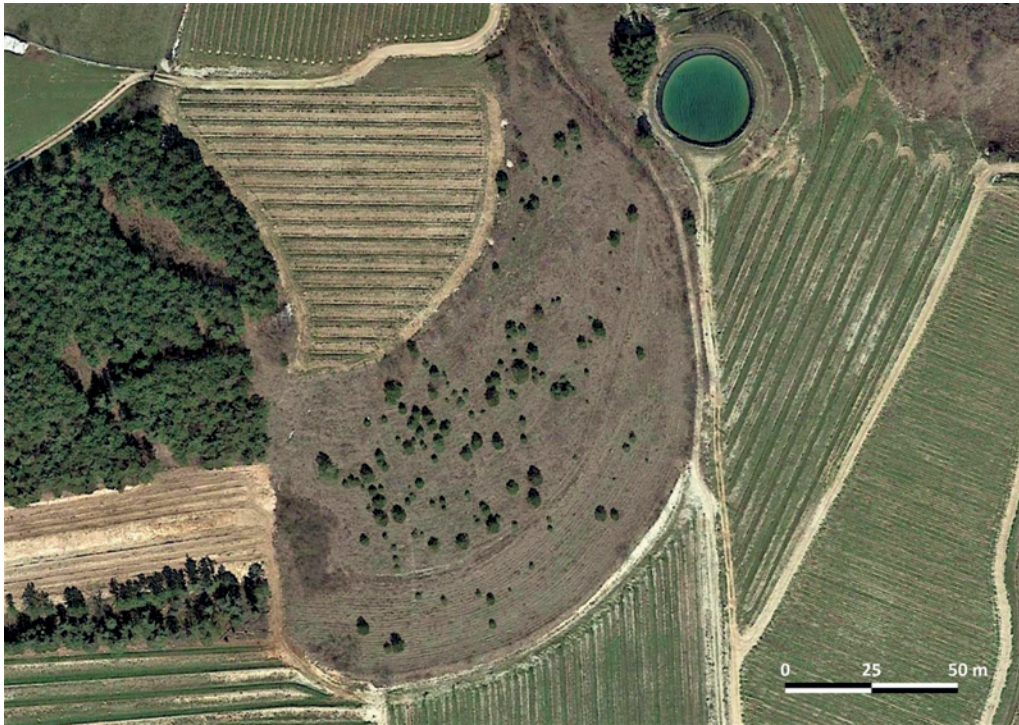
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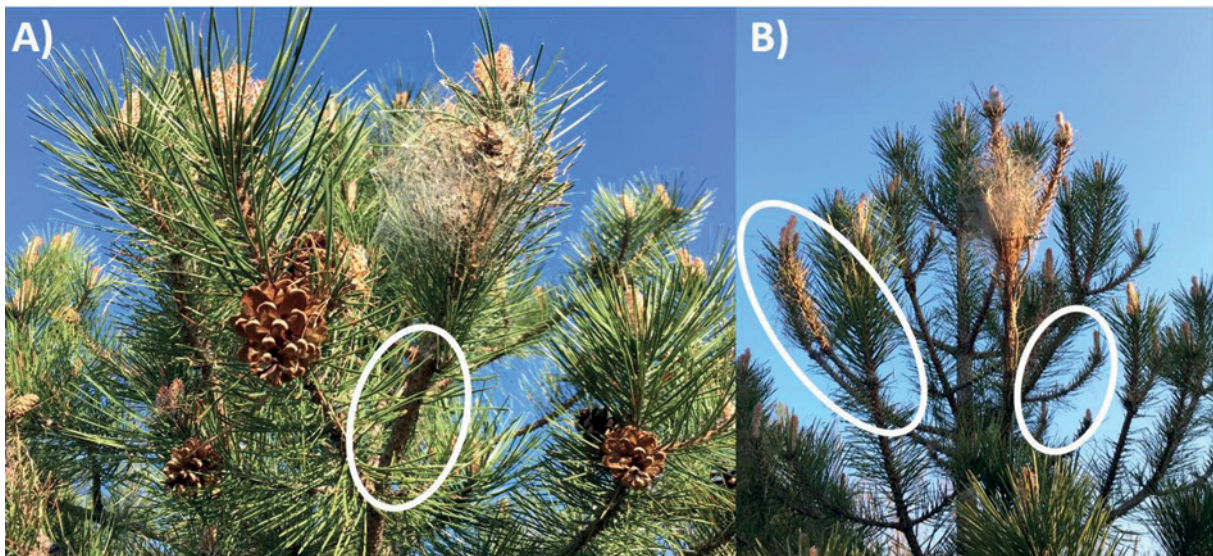
Modified version received: 29 June 2020

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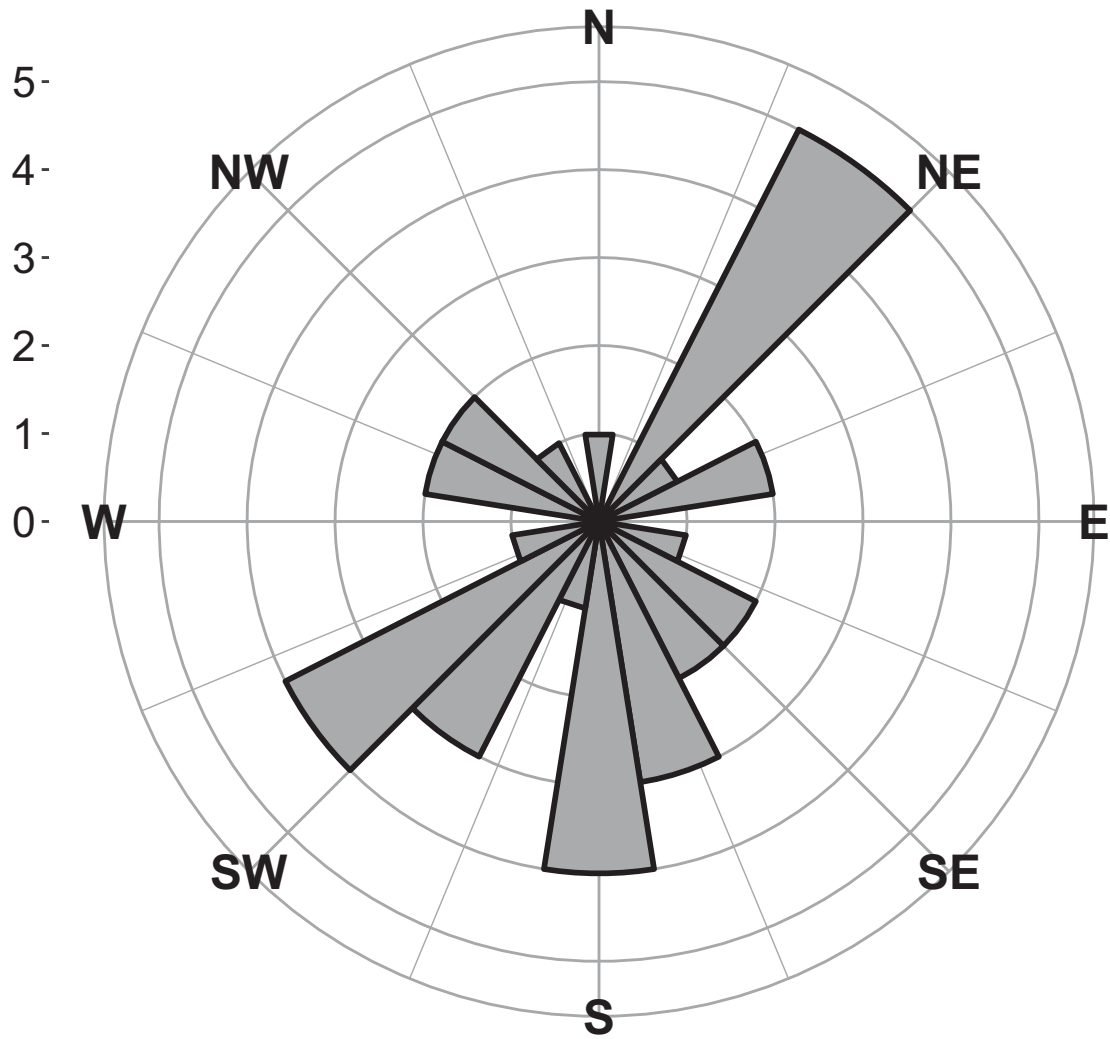
Appendix



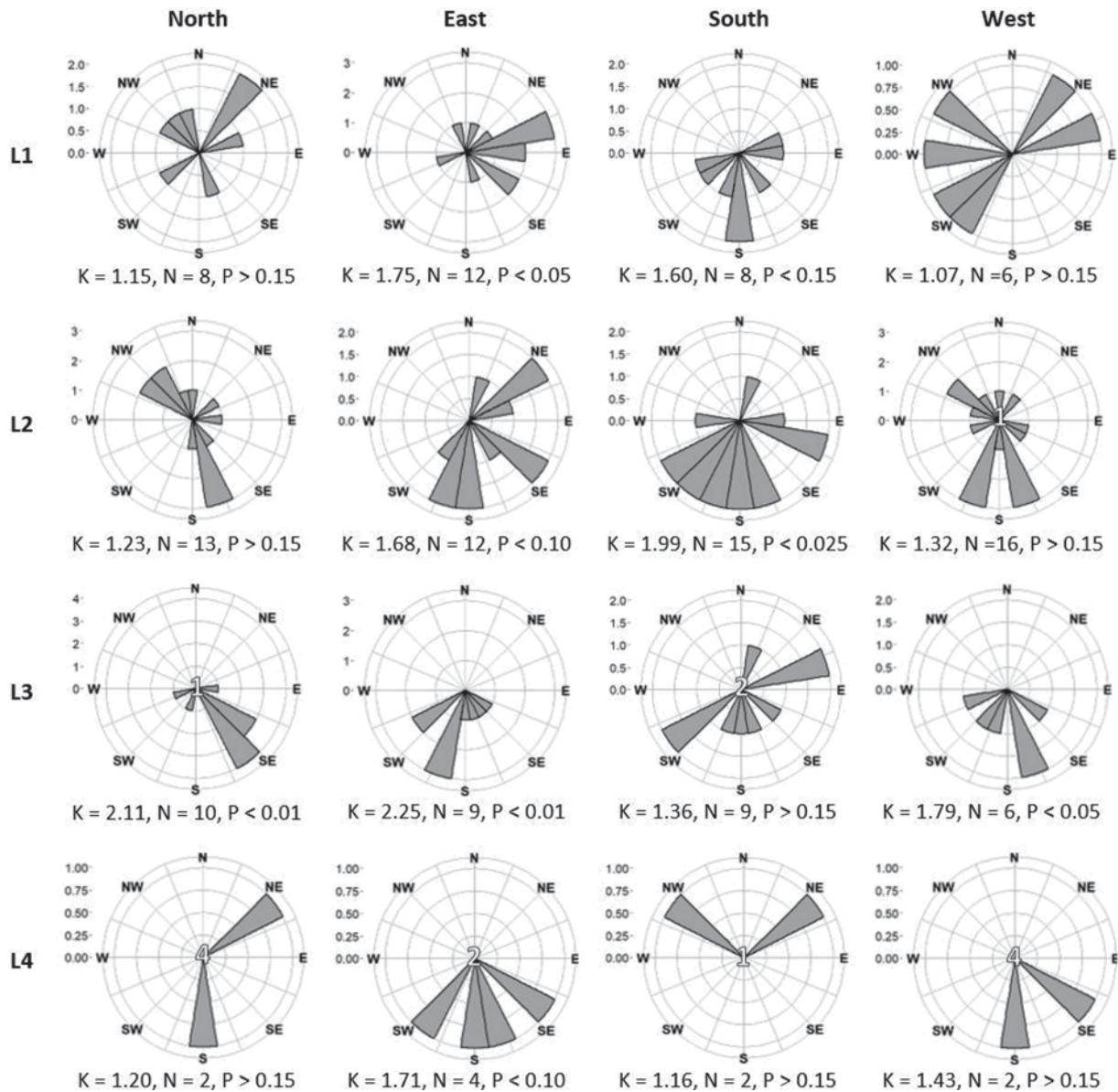
Supplementary Fig. 1. *Pinus nigra* stand in Precastio, Verona Italy, a study site where *T. pityocampa* egg batches were transplanted on isolated trees to investigate larval development and movement. Satellite image was taken on 22/03/2018 by Google Earth Pro, image accessed 06/03/2020.



Supplementary Fig. 2. *Thaumetopoea pityocampa* tents and feeding damage (white circles) on *P. nigra* at Precastio, Verona Italy. **A)** Young instar temporary tent with feeding damage underneath the tent. **B)** Older instar winter tent with feeding damage directly next to the tent, and other branches of different whorls. Images taken by Andrea Battisti.



Supplementary Fig. 3. Egg batch orientation on *P. nigra* oviposited by *T. pityocampa* females at the end of summer 2018 in Precastio, Verona Italy (N = 35). The numbers on the left side of the diagram correspond to the rings inside the circle and it represents the frequency of egg batches; starting from the smallest number in the centre to the largest number in the second last outer ring.



Supplementary Fig. 4. Distribution and frequency of tent orientations built by each *T. pityocampa* instar larva from different transplanted egg batch orientations (north, east, south and west). The numbers on the left of each diagram correspond to the rings inside the circle and it represents the frequency of movements by larval colonies; starting from the smallest number in the centre to the largest number in the second last outer ring. Number in the centre represents the number of tents that were built at the most apical part of the tree canopy (leader shoot) which had no orientation. K and N represents the Kuiper's test statistic and number of colonies, respectively.