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Movement ecology and social behaviour of processionary caterpillars from Australia and Europe

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Padua, 30 September 2021 Mizuki Uemura

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Dichiarazione

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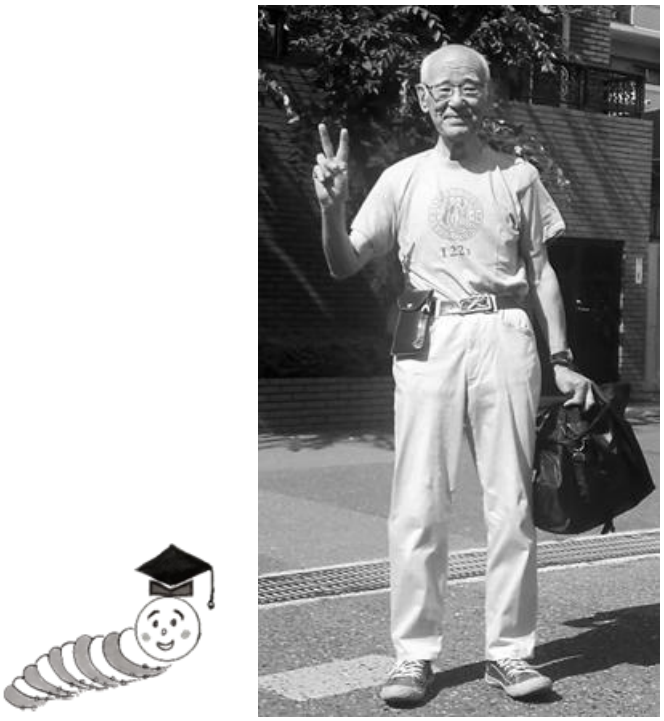

I dedicate this thesis to my grandfather, Takeshi Hirano, my number one fan. Thank you for inspiring me to become who I am today and for being so proud of me every step of the way. To my parents, Kayoko and Shinji Uemura for your loving support. 有り難う, 感謝の気持ちで一杯です。To all my mates from Australia and amici from Padova for keeping me sane and celebrating my milestones.

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Contribution of Authors

Three chapters within this thesis resulted in publications in peer-reviewed international journals. I was the first author and principal researcher for collecting and analysing the results under the supervision of Prof. Battisti and Prof. Zalucki. Other contributors of Chapters 2 and 3, Dr. Lynda Perkins, and A/Prof. Belušič and Dr. Meglič, respectively, were supervisors who helped collect data and guided me with the research.

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Summary

Pest insects are an ongoing issue to human society. Containment of pests can become problematic, especially when the species are gregarious and are a health risk to humans and animals. A species of recent interest is the processionary caterpillar, *Ochrogaster lunifer*, an urticating species found in Australia and associated with defoliation of acacias and eucalypts. A similar European species is the pine processionary moth, *Thaumetopoea pityocampa*. The caterpillars are destructive defoliators of pine and cedar trees in the Mediterranean Basin and Southern Europe. Both *O. lunifer* and *T. pityocampa* caterpillars live gregariously in a communal nest from egg to final instar larva. Setae (detachable urticating hairs) from the abdominal segments of *O. lunifer* and *T. pityocampa* caterpillars are responsible for various health issues to humans and animals and therefore require management actions. Comparing the two species of processionary caterpillars from opposite hemispheres will enable unique management strategies suitable for two different climates and environmental conditions.

Ochrogaster lunifer and *T. pityocampa* have a univoltine lifecycle with similar periods of developmental stages throughout the year but in completely opposite seasons (summer and winter, respectively). During my Ph.D., I investigated the behaviours and mechanisms of social group living, by comparing morphological, physiological, and ecological drivers of sociality of the two species of processionary caterpillars. Knowledge of these drivers in these important pest species will facilitate in building models to understand their population dynamics, predict future outbreaks, and aid in pest management.

Pre-pupation procession behaviour of *O. lunifer* and *T. pityocampa* caterpillars were compared by observing behaviour in the field. *Ochrogaster lunifer* caterpillars oriented towards the northern and southern shaded areas of the environment when leaving their natal host tree to a potential pupation site. *Ochrogaster lunifer* caterpillars may have avoided environments where it was bright because of overheating, which is unsuitable for pupation/development. In contrast, *T. pityocampa* caterpillars had no preferred orientation and specifically travelled towards brightly lit areas of the environment. The brightly lit areas of the environment may reduce the chances of pathogens attacking pupae that may diapause up to 8 years. The differences in pupation site preference between the two species may be explained by their own environmental and physiological requirements for optimal development.

Behavioural experiments and anatomical analysis on final instar *O. lunifer* and *T. pityocampa* caterpillars were used to determine how these species orientate in the

environment. The heading orientation of larval processions of both species could be manipulated with linear polarising filters held above the leading caterpillar. Exposing caterpillars to changes in the angle of polarisation resulted in corresponding changes in heading angles of the procession. Anatomical analysis indicated specialisations for polarisation vision of stemma I in both species. The study concluded that polarised light cues are important for larval orientation and detected using simple visual structures.

The tent and foraging locations of *T. pityocampa* caterpillars from first to final instar were explored through behavioural experiments. The first two larval instars start off as patch-restricted foragers, feeding directly near the tent, and transitioned to central place foragers from third instar onwards. Throughout larval development, the caterpillars built multiple tents positioned in a southerly orientation for maximum sun exposure.

Tent building and maintenance behaviour of *T. pityocampa* caterpillars were investigated through observational studies. The final tent built by older instar larvae had considerably more layers of silk laid on the southern side of the tent compared to other orientations. The few caterpillars that spin silk on the tent at sunset (early active caterpillars) are possibly using the strong polarised band of the sky along the azimuth to determine where is south. Amongst the early active caterpillars, there were more males spinning silk than females. Consequently, the early active males that were maintaining the tent had the highest proportion of Tachinid parasitism compared to early active females and late active males and females.

I have identified and filled several gaps in our current knowledge about social caterpillars. To fully understand the movement ecology and social behaviour of *T. pityocampa* and *O. lunifer*, it required a multiple factor approach. The thesis outlined several hypotheses which were explained by quantitative and manipulative behavioural, physiological, and ecological studies. Such information is important in building a model to predict possible future outcomes and aid in pest management. We have suggested targeted pest management strategies of the two processionary caterpillars using: pesticide and/or nematode application in predicted pupation sites, manipulation of pre-pupation processions through the obstruction/interference of polarisation vision in caterpillars, precise application of entomopathogenic bacterium (*Bacillus thuringiensis kurstaki*) on vulnerable younger larval instar colonies using drones (unmanned aerial vehicles) or manual sprays, and releasing natural enemies or spraying pesticide at exactly when caterpillars are outside maintaining the tent. Filling the gaps of the well-studied *T. pityocampa* and understudied *O. lunifer* and doing a comparison of the two species bring out new concepts and patterns about sociality in Lepidopteran caterpillars.

Riassunto

Alcuni insetti rappresentano una minaccia per la società umana fin da tempi immemorabili e la loro gestione appare spesso problematica, in particolare nel caso di organismi gregari in grado di causare danni non solo alle piante ma anche ad uomini ed animali. In tal senso, una specie di interesse emergente è la processionaria australiana *Ochrogaster lunifer*, specie associata ad acacia ed eucalipto, stretta parente dell' europea *Thaumetopoea pityocampa*, la ben nota processionaria del pino, diffusa nel bacino del Mediterraneo ed in Europa meridionale e vivente su *Pinus* e *Cedrus*. Entrambe le specie sono gregarie, vivendo in un nido dallo stadio di uovo a quello di larva matura. A partire dal terzo stadio, le larve producono setole staccabili urticanti di importanza medico-sanitaria per uomo ed animali, e che quindi richiedono importanti misure per il loro contenimento. Il confronto tra le due specie ha lo scopo di individuare analogie nella biologia ed ecologia che possono essere utili nel migliorarne la gestione ed i metodi di controllo.

Ochrogaster lunifer e *T. pityocampa* hanno un ciclo di sviluppo annuale, molto simile nella durata dei diversi stadi ma con stagionalità opposta, rispettivamente estate e inverno. In questa mia tesi di dottorato ho investigato il comportamento e la vita sociale di entrambe le specie, confrontandone i caratteri morfologici, fisiologici ed ecologici, sia in un'ottica di ricerca di base che applicata. Le conoscenze acquisite sono di importanza fondamentale per comprendere la dinamica di popolazione di entrambe le specie, per la previsione di possibili attacchi e pullulazioni e per favorirne una gestione più efficiente.

Il comportamento delle processioni di impupamento al suolo di entrambe le specie è stato confrontato mediante lavoro sul campo in entrambi gli emisferi. Le larve di *O. lunifer* si orientano di preferenza verso le zone ombreggiate situate a nord e a sud del nido per evitare ambienti surriscaldati che potrebbero ostacolare lo sviluppo pupale. Al contrario, quelle di *T. pityocampa* non hanno una preferenza per la direzione ma sono attratte dalle aree ricche di luce. Queste aree riducono la probabilità che gli agenti patogeni attacchino le pupe, che possono rimanere in diapausa fino a 8 anni. Tali differenze sono spiegate da particolari esigenze ecologiche legate al clima e sono indirizzate a ottimizzare la sopravvivenza delle pupe.

Esperimenti sul comportamento ed osservazioni anatomiche delle larve all'ultimo stadio di *O. lunifer* e *T. pityocampa* hanno mostrato come le larve si orientano nell'ambiente. L'orientamento della colonia in processione può essere modificato utilizzando filtri per luce polarizzata lineare, i quali, una volta posti in determinato modo sopra il capofila della processione, ne causano la deviazione per l'angolo corrispondente. L'analisi anatomica ha

confermato come lo stemma 1 delle larve sia responsabile della percezione della luce polarizzata in entrambe le specie. Questi risultati rappresentano una novità per gli ocelli larvali e dimostrano come strutture relativamente semplici siano in grado di assicurare la lettura di un fattore importante di orientamento come la luce polarizzata.

La costruzione del nido e i movimenti delle larve di *T. pityocampa* sono stati indagati con esperimenti comportamentali dal primo all'ultimo stadio di sviluppo. I primi due stadi sono perlopiù limitati alla zona dove sono state deposte le uova mentre i successivi si spostano nel cosiddetto nido invernale, situato in posizione centrale rispetto all'albero. Tale posizionamento permette loro di accedere agevolmente al substrato alimentare. In questa fase il nido viene sempre costruito con esposizione a sud per massimizzare l'esposizione al sole.

Le larve di *T. pityocampa* presentano uno speciale comportamento per la costruzione del nido invernale in quanto questo è fortemente asimmetrico con larga prevalenza di strati seta nel settore rivolto a sud. La costruzione del nido è opera di un numero limitato di larve che iniziano l'attività all'imbrunire, utilizzando quindi la luce polarizzata che in quel momento è massima in direzione nord-sud. Le larve tessitrici sono prevalentemente di sesso maschile e questa attività le espone maggiori attacci da parte del tachinide parassitoide *Phryxe caudata*, attivo nelle ore di luce.

Con questo studio ho portato un piccolo contributo allo studio dei lepidotteri sociali che dovrà essere ulteriormente sviluppato con esperimenti volti a confermare quanto osservato e completare le conoscenze sull'ecologia del movimento e il comportamento sociale di *O. lunifer* e *T. pityocampa*. I risultati possono tuttavia essere di interesse immediato per 1) l'adozione di misure gestionali quali l'individuazione delle aree dove utilizzare metodi di lotta biologica sul terreno (ad esempio funghi e nematodi entomopatogeni), 2) possibili interventi di manipolazione dell'orientamento durante le processioni attraverso l'alterazione della percezione della luce polarizzata, 3) individuare con precisione la posizione dei nidi invernali in fase di costruzione su cui applicare insetticidi biologici come i preparati a base di *Bacillus thuringiensis kurstaki* mediante droni teleguidati, 4) rilasciare antagonisti naturali quando l'esposizione dell'insetto bersaglio è massima, cioè durante la costruzione del nido invernale. Queste opzioni dovranno ovviamente essere testate e sostenute da ulteriori esperimenti volti al miglioramento della comprensione della socialità in queste interessanti specie di lepidotteri.

Chapter 1

General introduction

1.1 Sociality in insects

Alongside various animals, some insects have adopted sociality into their lifestyle. Social insects are individuals that display reciprocal and communication between each other that results in cooperative behaviour (Wilson, 1971). Being social comes with advantages such as overcoming plant defences, enhanced defence against natural enemies and through larger aposematic signals, foraging efficiency, and thermoregulation (Fitzgerald and Peterson, 1988). On the other hand, there are disadvantages including an increased risk of infectious diseases, intraspecific competition for food, and conspicuousness to natural enemies (Santana et al., 2017). Social insects have been categorised into three main groups: eusocial, quasi/sub-social, and communal (Costa and Fitzgerald, 1996; Fig. 1.1). Eusocial insects display cooperative brood care, overlapping generations, sterile castes and aggregation (Costa and Fitzgerald, 1996). Eusocial insects occur in the Hymenoptera (ants and bees) and Blattodea/Isoptera (termites) (Leonhardt et al., 2016). Quasi/sub-social insects display overlapping generations and aggregation (Costa and Fitzgerald, 1996), including insects from the Hemiptera (true bugs), Coleoptera (beetles), Thysanoptera (thrips), Blattodea (cockroaches), Dermaptera (earwigs), etc. (Leonhardt et al., 2016). Finally, communal insects only display aggregation within the same generation (Costa and Fitzgerald, 1996), and occur in the Lepidoptera (moth caterpillars), Orthoptera (crickets and katydids) and Hymenoptera (sawfly larvae) (Leonhardt et al., 2016).

Division of labour is fundamental to the organisation of social insects and one of the principal factors for their ecological success (Robinson, 1992). Division of labour is characterised by two features: (1) different activities are performed simultaneously by (2) groups of specialised individuals (Robinson, 1992). The most complex insect societies that display this division of labour are eusocial insects from the Blattodea/Isoptera (termites) and Hymenoptera (ants and bees), associated with a caste system. However, division of labour is a recurrent characteristic of other non-eusocial insects, including aphids (Rhoden and Foster, 2002), thrips (Crespi, 1992) and caterpillars (Underwood and Shapiro, 1999).

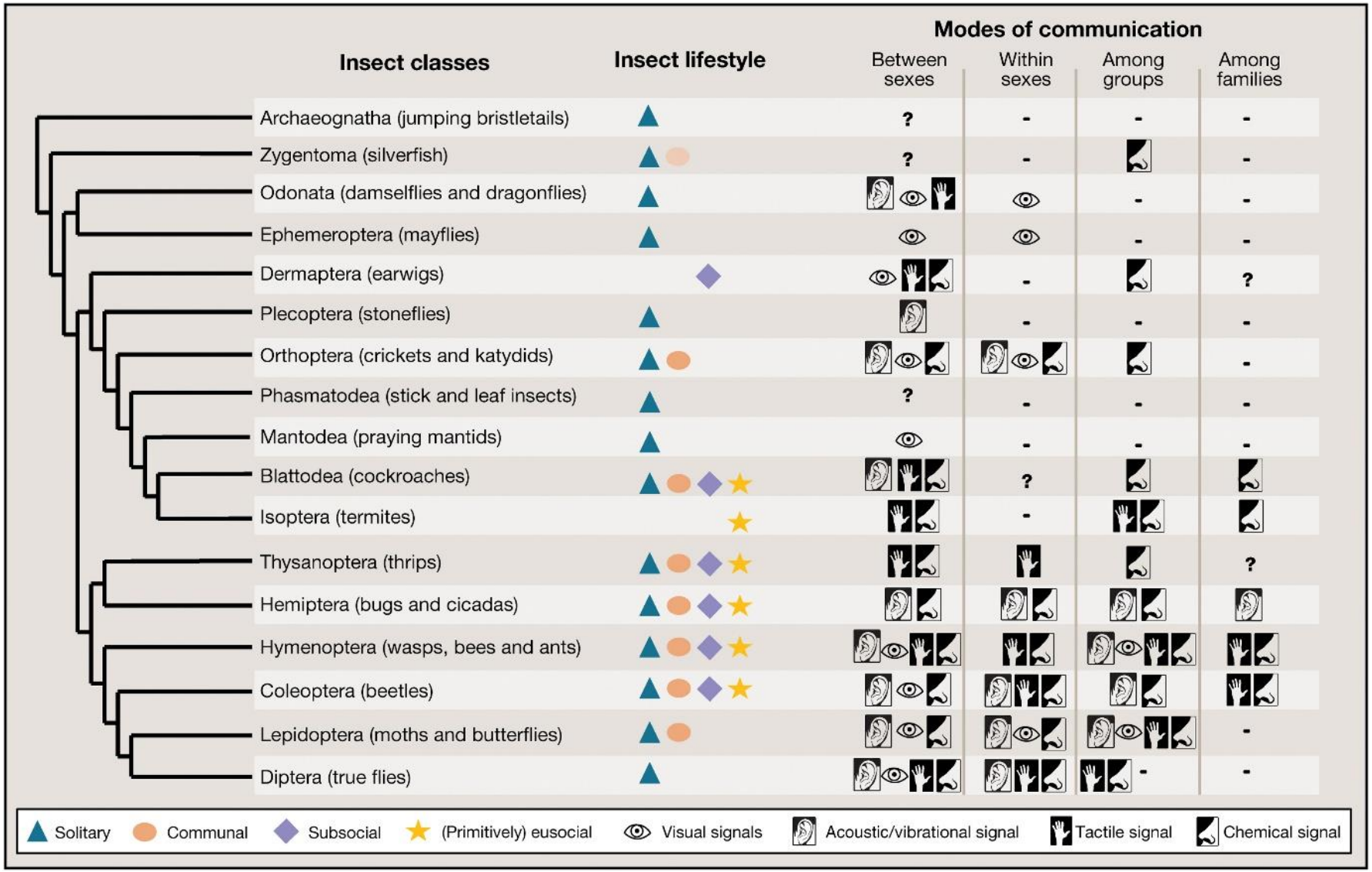

Figure 1.1. Types of social organisation and communication modes employed by major insect clades; figure from Leonhardt et al. (2016).

1.2 Social Lepidoptera

Although social caterpillars lack the complexity and richness of social interactions that characterise eusocial insects (Fitzgerald, 1995), studies have found that they display division of labour (Wellington, 1957; Underwood and Shapiro, 1999). Male *Eucheira socialis* caterpillars actively spun more silk on the nest for maintenance and foraged less compared to females (Underwood and Shapiro, 1999). This behaviour displayed by male *E. socialis* caterpillars can benefit females by conserving their energy and resources for later egg production (Underwood and Shapiro, 1999). Variability of activities/specialisation for tasks within a colony is beneficial because it promotes collective flexibility and resiliency, and therefore enhances colony efficiency and productivity (Jeanson and Weidenmüller, 2014).

Sociality is widespread within the Lepidoptera, including 27 or more families and has evolved numerous times in response to various selective pressures (Fitzgerald and Costa, 1999). Of those 27 or more families, there are over 300 species of caterpillars which live gregariously as a group for some or the whole larval stage (Costa and Pierce, 1997). For caterpillars to be regarded as social, they must cooperate in defence, nest building and foraging (Costa, 1997). The complexity of social behaviour in Lepidoptera varies depending on the number of communication characters, which include: (1) group defence, (2) aggregation, and (3) foraging (Costa and Pierce, 1997; Fig. 1.2). The social complexity is essentially defined by three levels of foraging behaviours which is associated with changes in group defence and signals employed in aggregation (Costa and Pierce, 1997). Fitzgerald and Peterson (1988) identified the three levels of foraging behaviour from least to most complex: patch-restricted, nomadic, and central-place foraging (Fig. 1.3). Patch-restricted foraging is the most common and it is when the colony obtains food from a single leaf or confine their feeding within a single contiguous patch. Nomadic foraging is when the colony is unrestrained from nests and frequently wander widely to search for new feeding and resting sites. Central-place foraging is the least common and is associated with the colony establishing a permanent/semi-permanent nest, from which they perform intermittent forays in search for food. Central-place foraging set the stage for the evolution of cooperative interactions that go beyond the social complexity seen in patch-restricted and nomadic foragers (Fitzgerald and Costa, 1999).

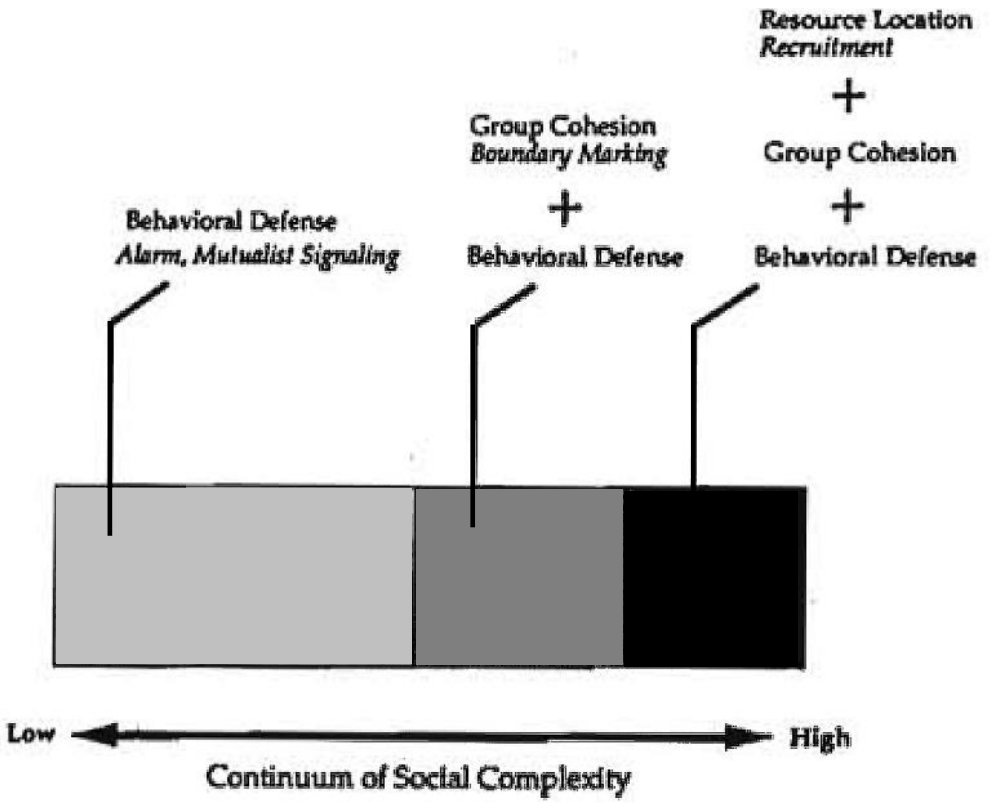

Figure 1.2. Social complexity of Lepidoptera, modified figure and figure caption from Costa and Pierce (1997). Complexity, indicated by intensity of shading, is defined in terms of communication characters: defensive, cohesion, and recruitment. Moving from low to high along the continuum, weakly or facultatively social species exhibit group defence only, more complex social species exhibit group cohesion in addition to group defence, and the most complex lepidopteran societies exhibit recruitment, cohesion, and defence. The continuum is intended to illustrate the range of extant lepidopteran social complexity and does not represent explicit evolutionary transitions.

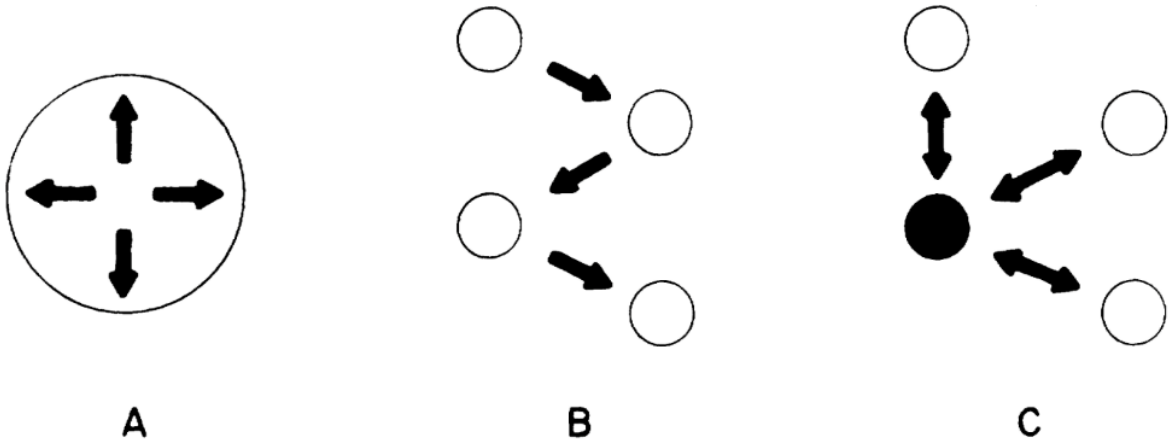

Figure 1.3. A) Patch-restricted, **B)** nomadic, and **C)** central-place foraging patterns of social caterpillars, figure and figure caption from Fitzgerald and Peterson (1988). Arrows indicate movement of caterpillars **A)** within patches, **B)** between patches, and **C)** between feeding sites and resting site. The complexity of communication systems associated with these foraging strategies is likely to increase from **A** to **B** to **C**.

1.3 Model species

Nest building is a major advance in the evolution of sociality among caterpillars because it requires a high degree of communication among colony members (Fitzgerald, 1995). Caterpillars of Notodontidae, particularly species from the subfamily Thaumetopoeinae, are amongst one of the most socially complex within Lepidoptera, as they are central-place foragers and remain gregarious from egg to final instar larva. The caterpillars build and remain in silken nests for long periods on the host tree, and each colony can include several tens to hundreds of individuals. Having a home base and dispersed food source requires some of the most sophisticated forms of communications (Costa, 1997). Caterpillars may communicate through the use chemical, tactile and acoustical cues (Costa and Pierce, 1997), and are commonly used in conjunction with one another.

The well-known example of Notodontidae is the pine processionary moth, *Thaumetopoea pityocampa* Denis & Schiffermüller (1775), a widespread pest present throughout the Mediterranean basin and southern Europe (Battisti et al., 2015; Fig. 1.4). Early larval instars start off as patch-restricted foragers, feeding in the immediate area of their semi-permanent communal tent on *Pinus* and *Cedrus* spp. host trees (Fabre, 1898; Balfour-Browne, 1925; Fitzgerald and Costa, 1999; Uemura et al., 2020). Later instar larvae become central-place foragers, they feed on dispersed foliage and come back to the tent (Fabre, 1898; Balfour-Browne, 1925; Fitzgerald and Costa, 1999; Uemura et al., 2020). As *T. pityocampa* caterpillars move on the host plant or on the ground in search of pupations sites or new host tree, they form head-to-tail processions (Fabre, 1898; Démolin, 1967). As the caterpillars move, they mark their pathways with silk and a trail pheromone (Fitzgerald and Blas, 2003). The trail pheromone is volatile which allows caterpillars to distinguish new from aged trails, to and from the tent to foraging sites (Fitzgerald and Blas, 2003). This is especially important for caterpillars which may get isolated from the procession and need to find a way back to the tent, foraging site or conspecifics (Fitzgerald and Blas, 2003). However, the connection of caterpillars in the procession is dependent on tactile cues associated with the setae from the tip of the abdomen of the precedent caterpillar and the setae from the head of the following caterpillar (Démolin, 1971). Being gregarious greatly increases survival due to enhanced predator defence, thermoregulation, and foraging efficiency (Fitzgerald, 1993).

A similar species to *T. pityocampa* in the same family, is the processionary caterpillar or bag-shelter moth, *Ochrogaster lunifer*, Herrich-Schäffer, 1855 from Australia. This species

also forms processions when they leave the nest to forage, pupate or change nest location (Floater, 1996a). Unlike its northern hemisphere relative, *O. lunifer* (ground nesting form) is a central place forager from the second instar larva; the first instar larva does not feed (Floater, 1996b). Both *T. pityocampa* and *O. lunifer* have a univoltine lifecycle with similar periods of developmental stages throughout the year but in completely opposite seasons (winter and summer, respectively). From third larval instar onwards, the caterpillars possess millions of urticating hairs on the abdominal segments called true setae; and when in contact, it causes inflammation and allergic reactions in mammals and birds (Battisti et al. 2011). Humans and animals are at highest risk of exposure to the urticating setae during pre-pupation processions when caterpillars disperse over the ground (Perkins et al., 2015). Pest management is becoming more important in caterpillars that cause serious health problems in humans and animals, especially in outbreaks. There are efforts to reduce populations of urticating Lepidoptera worldwide (Battisti et al., 2011).

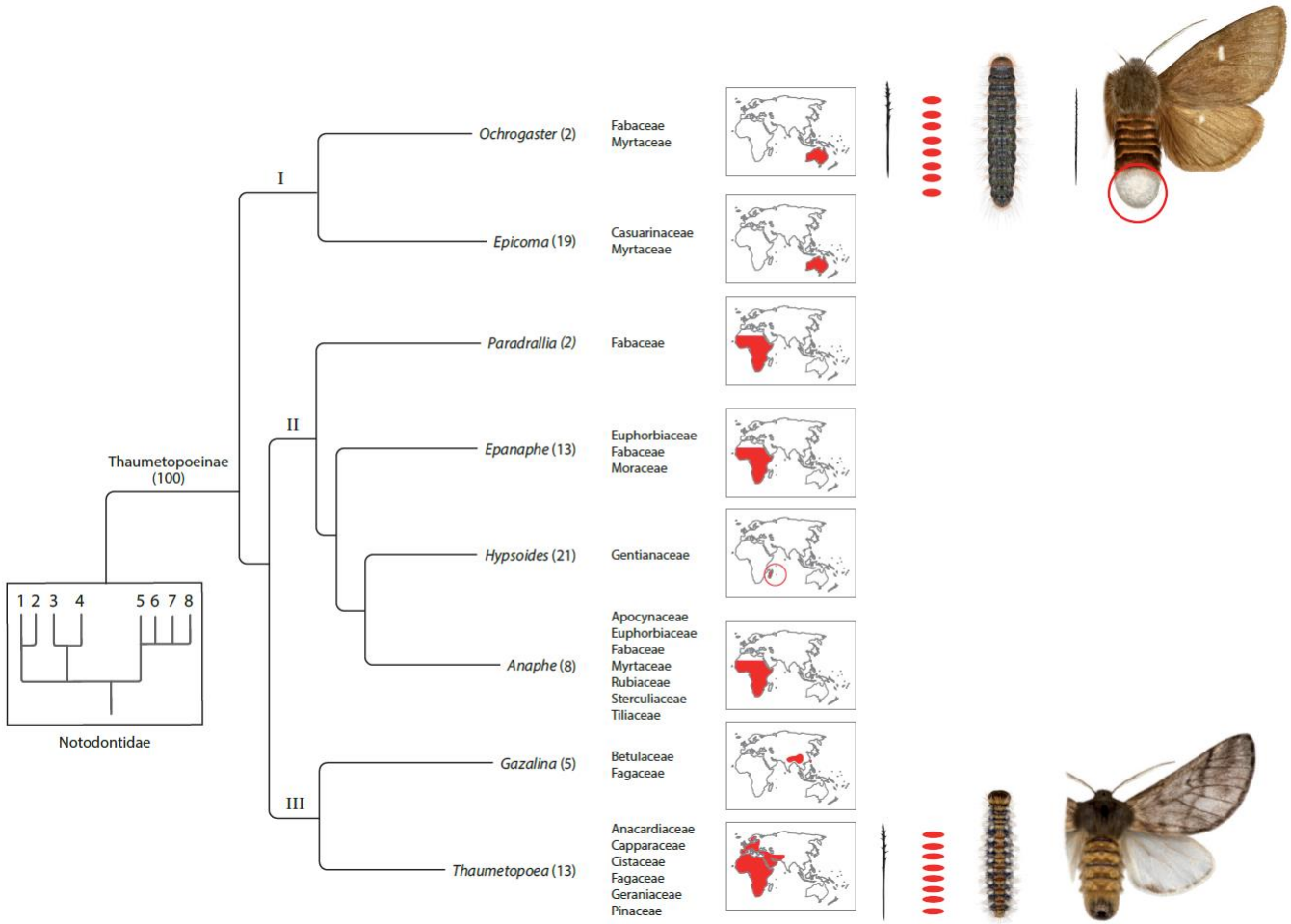

Figure 1.4. Cladogram of the Thaumetopoeinae within Notodontidae showing the three major clades (roman numerals), the main genera (N = number of species), main host-plant families, and their geographic distribution. In the genus *Ochrogaster* and *Thaumetopoea*, the true seta, number of setal fields (mirrors) on the abdominal tergites from the final instar larva, larva and adult moth are pictured and not to scale. The adult females of *Ochrogaster* have urticating setae in the tuft scales (circled). Figure and figure caption modified from Battisti et al. (2017). Phylogenetic tree of Notodontidae: 1 = Pygaerinae, 2 = Dudusinae, 3 = Phalerinae, 4 = Thaumetopoeinae, 5 = Heterocampinae, 6 = Notodontinae, 7 = Nystaleinae, 8 = Dioptinae.

1.4 Research gaps and aims

In a suitable habitat, gregarious caterpillars living in communal shelters can often achieve numerical dominance in an ecosystem (Fitzgerald, 1993). During outbreaks, they may defoliate a vast number of host plants causing economic loss and increased cases of health problems to humans and animals if they are urticating. Determining the behaviour of these social caterpillars, can contribute to a better understanding of their population dynamics. Dispersal movements of caterpillars is one element that has a great impact on population fluctuations (Floater, 1995).

Measuring dispersal behaviour of animals in the field is usually difficult (Floater, 1996a). However, there is increased interest in understanding the movement ecology and the manipulation of dispersal behaviour in caterpillars as a pest management strategy (Rieske and Townsend, 2005). The phenomenon of pre-pupation dispersal on the ground has been described in *T. pityocampa* and *O. lunifer* however, there were no quantitative data presented to describe where or how they find pupation sites. On the host tree, it is known that *T. pityocampa* colonies make multiple tents throughout their larval stage however, dispersal within the host tree at microhabitat level has never been investigated. Furthermore, the behaviour of tent construction in *T. pityocampa* larvae has not been studied using quantitative data. Here I investigate and determine various unknown aspects of the movement ecology and social behaviour of *T. pityocampa* and *O. lunifer* and compare some of their key characteristics.

This thesis explores three main questions:

1. Where do *T. pityocampa* and *O. lunifer* caterpillars pupate and how do they navigate in the environment?
2. Where do *T. pityocampa* caterpillars develop on the host tree at within tree/microhabitat level?
3. How do *T. pityocampa* caterpillars construct the tent and what social behaviours are involved?

By answering these key questions and comparing the closely related Australian and European species, it will help us understand more about population dynamics of these destructive species as well as insect behaviour in general (Chapter 6). Knowledge of key behavioural movements in these important pest species, will facilitate in building models to predict possible future outcomes and aid in pest management. Pre-pupation behaviour have

been described in both species (see Démolin, 1971; Fitzgerald, 2003; Floater, 1996a), but no quantitative data have been collected on how far the caterpillars move, the orientation they take, and what environmental cues they use (Chapters 2 and 3). Furthermore, *O. lunifer* ecology and behaviour in general is poorly understood and there is a deep gap in our knowledge of their larval attributes. Earlier studies on *T. pityocampa* have determined egg (Tiberi, 1983; Zamoum et al., 2015) and tent (Breuer, et al., 1989) orientation relative to the host tree, but have not explored the details at the within tree, microhabitat level (Chapters 4 and 5). The ability to anticipate the direction and distance of dispersing caterpillars on the tree and on the ground is a key step in minimizing exposure to urticating setae. Overall, by researching further on *T. pityocampa* and *O. lunifer* will gain a deeper insight into the validity of some conclusions by previous studies and about social behaviour in larval insects.

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Chapter 2

Pupation site preference of processionary caterpillars from Europe and Australia

The following chapter is based on the published manuscript: Uemura, M., Perkins, L.E., Zalucki, M.P., Battisti, A. 2020. Movement behaviour of two social urticating caterpillars in opposite hemispheres. Movement Ecology. 8: 1–11. doi:10.1186/s40462-020-0189-x.

The manuscript was edited according to the thesis evaluator's feedback and merged with the original text and supplementary material in this thesis chapter.

2.1 Abstract

Introduction: Investigating movement ecology of organisms has economic, societal, and conservation benefits. Larval movement of insects for example, plays many significant ecological roles, and with the expansion of the human population and development, encounters and conflicts with insects have increased. Urticating caterpillars are a health concern to people and animals, especially when they disperse in a gregarious and synchronised manner in areas frequented by humans. *Ochrogaster lunifer* and *Thaumetopoea pityocampa* from the southern and northern hemispheres respectively, are two geographically isolated species of moth with similar gregarious urticating caterpillars that can outbreak causing defoliation and medical issues.
Materials and Methods: Each year from March to May, *O. lunifer* and *T. pityocampa* caterpillars leave their nesting sites and form head-to-tail processions on the ground in search of pupation sites. This pre-pupation procession behaviour and its associated risk of human contact with *O. lunifer* and *T. pityocampa* caterpillars were studied and compared in Australia and Italy, respectively. The distance, duration, orientation and response to visible light of the pre-pupation processions were studied in both species to determine general patterns.
Results: In the morning, *O. lunifer* and *T. pityocampa* processions travelled on average 40 and 16m per day from the nest in 153 and 223 min respectively, in search for potential pupation sites. *Ochrogaster lunifer* pre-pupation processions travelled generally to the north or south when leaving the nest, as was their final orientation to the bivouac/pupation site. Whereas *T. pityocampa* processions had no preference in orientation. *Ochrogaster lunifer* and *T. pityocampa* pre-pupation processions travelled towards the darker and the lighter areas of the environment, respectively. During our observations, 27% of *O. lunifer* and 44% of *T. pityocampa* processions had contact with humans driving, cycling or walking.
Conclusion: The amount of human contact is surprising and alarming, because of the serious health implications they cause to humans and animals. The processionary dispersal on the ground risks further spread of urticating hairs that can be easily detached, and particular during inadvertent contact. Our limited sample size of *T. pityocampa* processions may benefit from more observations to make conclusive remarks on their pre-pupation behaviour. Understanding the movement behaviour of *O. lunifer* and *T. pityocampa* pre-pupation processions around populated areas is crucial for predicting exposure risk and application of management strategies.

Keywords: Australia, Europe, Lepidoptera, medical importance, *Ochrogaster lunifer*, processionary caterpillars, pupation site, *Thaumetopoea pityocampa*

2.2 Introduction

The need to understand animal movement has increased over the years, whether it is for pest management, novel ecological discoveries, conservation and protection of a species, or impacts of anthropogenic factors and climate change on organisms, just to name a few. As humans modify the environment and expand its use for agriculture, housing and recreation, encountering wildlife is not out of the ordinary. Investigating movements of black bears by Zeller et al. (2019) highlighted the importance of conservation and management of these species in human development. On the other hand, human-induced environmental change has exacerbated the spread of the highly invasive fire ants in United States (Forys et al., 2002). The occurrence and spread of the red imported fire ant and its impacts on humans and endangered species native to the area were examined to protect and help recover endemic populations of organisms that were affected (Forys et al., 2002). Further understanding of movement ecology has economic, societal, and conservation benefits.

Larvae of many Lepidoptera (butterflies and moths) leave their larval feeding sites to find suitable pupation sites and this stage is when the maximal larval movement occurs (Hagstrum and Subramanyam, 2010). Larval movement plays many important ecological roles, and with the expansion of the human population and development, encounters and conflicts with pest insects have increased. Pest insects are defined as organisms that cause harm to humans, animals, crops or property (Dent, 2000). One of which includes gregarious caterpillars associated with defensive structures such as urticating hairs which can be in the form of true setae, modified setae, or spines (Battisti et al., 2011). Urticating hairs are used as a defence against natural enemies and living in large groups has been hypothesised to enhance this mechanism (Pérez-Contreras et al., 2003). True setae are a characteristic of processionary caterpillars of Notodontidae and tussock moths (Erebidae) and can also occur on the adult of a few species (Kawamoto and Kumada, 1984). The setae are released by disturbance and/or mechanical stimulation and are harmful to humans and animals in several ways, including urticaria and allergic reactions in humans, tongue necrosis in dogs (Mullen and Durden, 2009), and miscarriages in horses (Cawdell-Smith et al., 2012). Occurrences of urticaria in humans from processionary moths accounts for 6 to 18% of the population in Europe (Battisti et al., 2011). In Australia, numerous cases of urticaria in humans by *O. lunifer* larvae have been reported by Southcott (1978) and in literature as early as 1911 (Froggatt, 1911) (in both cases as *Teara contraria*).

Humans and animals can be exposed to urticating setae by direct contact with larvae, indirectly via the environment (e.g. on the ground and carried by wind), and by ingestion through contaminated feed and water (Mullen and Durden, 2009). The dispersal of urticating caterpillars during pre-pupation processions is one of the highest risk times for direct contact with humans and animals (Perkins et al., 2019, 2016). In a pre-pupation procession, gregarious caterpillars ready to pupate travel head-to-tail in a line from their nest to a pupation site. Caterpillars maintain a procession by following silk threads, trail pheromone and through thigmotaxis (Fitzgerald, 2003; Steinbauer, 2009). The leader (first larva of the procession) and the following caterpillars are kept in contact by the long posterior hairs from the last abdominal segment and the head (Démolin, 1971; Steinbauer, 2009). Caterpillars aggregating in groups for at least part of their larval stage is common, however being gregarious at all instars from neonate to pre-pupae is uncommon (Fitzgerald, 1993). Social species from the Notodontidae, *Ochrogaster lunifer* Herrich-Schäffer and *Thaumetopoea pityocampa* (Denis & Schiffermüller) are two examples of the latter. *Ochrogaster lunifer* and *T. pityocampa* have a univoltine lifecycle, with larvae feeding throughout the summer and winter, respectively. Every year, from March to May, pre-pupation processions of *O. lunifer* and *T. pityocampa* occur in southern and northern hemispheres, respectively. Although geographically isolated, the two species share a similar period of development through the year but in opposite seasons (Floater, 1996; Battisti et al., 2017).

Pre-pupation dispersal has interested entomologists but where they go has raised recurring questions (Wellington et al., 1951). Behaviour of pre-pupation processions of *O. lunifer* (Mills, 1951; Floater, 1996; Floater and Zalucki, 1999) and *T. pityocampa* (Robredo, 1963; Démolin, 1971; Franck and Hailman, 1972) have been described however, all studies contained no quantitative data and analyses of the processions. *Ochrogaster lunifer* pre-pupation processions travel several days until they find a suitable pupation site (Floater, 1996) which can be up to 200 m from the host tree (Floater and Zalucki, 1999). After leaving the host tree, the procession can split into smaller groups containing 1 to 10 larvae that disperse and pupate over a large area (Floater, 1996). There is more literature on the behaviour of *T. pityocampa* pre-pupation processions compared to *O. lunifer*. It is known that *T. pityocampa* larvae descend from the tent in the tree canopy and reach the ground by 07:00 (solar time), with most activity on the ground between 07:00 and 09:00 (Robredo, 1963). There is little to no pre-pupation procession activity during cold and rainy days (Robredo, 1963). The number of larvae in a procession varies and splitting into smaller groups occurs for undetermined

reasons but occurs more frequently when larvae are traveling on rough ground (Robredo, 1963). The leader of the procession is positively phototactic and will therefore lead the following caterpillars to a location where it is lighter (Démolin, 1971), but not directly towards the sun (Franck and Hailman, 1972). *Thaumetopoea pityocampa* processions travel on average 16.5 m at a speed of 4.1 m/h to their pupation site across 2 days (Robredo, 1963). When the leader finds a suitable pupation site, all the larvae from the procession burrow together and spend 1 or 2 days in the top layer of the soil before they go further underground to pupate (Robredo, 1963).

Here we investigate the behavioural movement of *O. lunifer* and *T. pityocampa* pre-pupation processions and the occurrences of human contact. The study focused on quantitative observational and environmental data associated with pre-pupation procession movements. It is important to understand if the two model species disperse in a predictable way and how general this behavioural pattern may be in the same taxonomical group. Knowledge of dispersal movements in these important pest species will facilitate building models to predict exposure risk and application of pest management strategies.

2.3 Materials and Methods

Field sites and data collection

Ochrogaster lunifer

Ochrogaster lunifer is a widespread univoltine species found across coastal and inland Australia where its *Acacia*, *Eucalyptus* and *Corymbia* spp. host trees occur (Perkins et al., 2016; Steinbauer, 2018). Within the species, there are five nesting types (Perkins et al., 2016) with different ecology, morphology and genetics (Mather et al., 2019). In our study, we focused only on the ground nesting form in which larvae create a silken nest at the trunk base of various *Acacia* and *Eucalyptus* spp. host trees (Floater, 1996). The larvae feed throughout the summer until they reach their final instar in autumn, when they leave their nest permanently in search of a suitable pupation site underground. In our study, *O. lunifer* pre-pupation processions were followed in two seasons, one over seven and the other over ten non-consecutive days from end of March until first week of April 2017 and 2019, respectively. Field visits occurred on other days during the season but there were no active processions. Processions came from various *Acacia* spp. tree ground nests at The University of Queensland (UQ), Gatton campus, Queensland, Australia (−27°56’ S, 152°34’ E; Fig. 2.1). The campus is a semi-urban environment with buildings and patches of vegetation throughout. Larvae generally left the

nest after sunrise, between 06:00 to 07:00 local time. Time at which the leader of the procession left the nest and the time at which the last larva of the procession (or singleton) went underground (bivouac) were recorded. A bivouac is not necessarily the final site where the larvae pupate, especially for the first day of travel (M. Uemura, personal observation 2017). Most often, the larvae leave the bivouac at the following sunrise to a new bivouac/pupation site. Any disruptions during the procession were recorded, e.g. larvae run over by cars or pedestrians and were classified as human contact. Every time a procession broke into sub-processions or changed direction, coloured flags with codes were used as markers. Once the procession and/or singleton went underground, the GPS coordinates of flags and where caterpillars went underground were recorded with Garmin GPS Etrex10 (in 2017) or iPhone application ViewRanger (in 2019). In-situ environmental temperatures were recorded for *O. lunifer* populations using Tinytag Plus 2 data-logger TGP-4505 (Hastings Data Loggers, Port Macquarie, Australia) in 2017 but not in 2019. Environmental temperatures for 2019 during the pre-pupation processions were collected from the UQ Gatton station 040082 (Commonwealth of Australia, 2019).

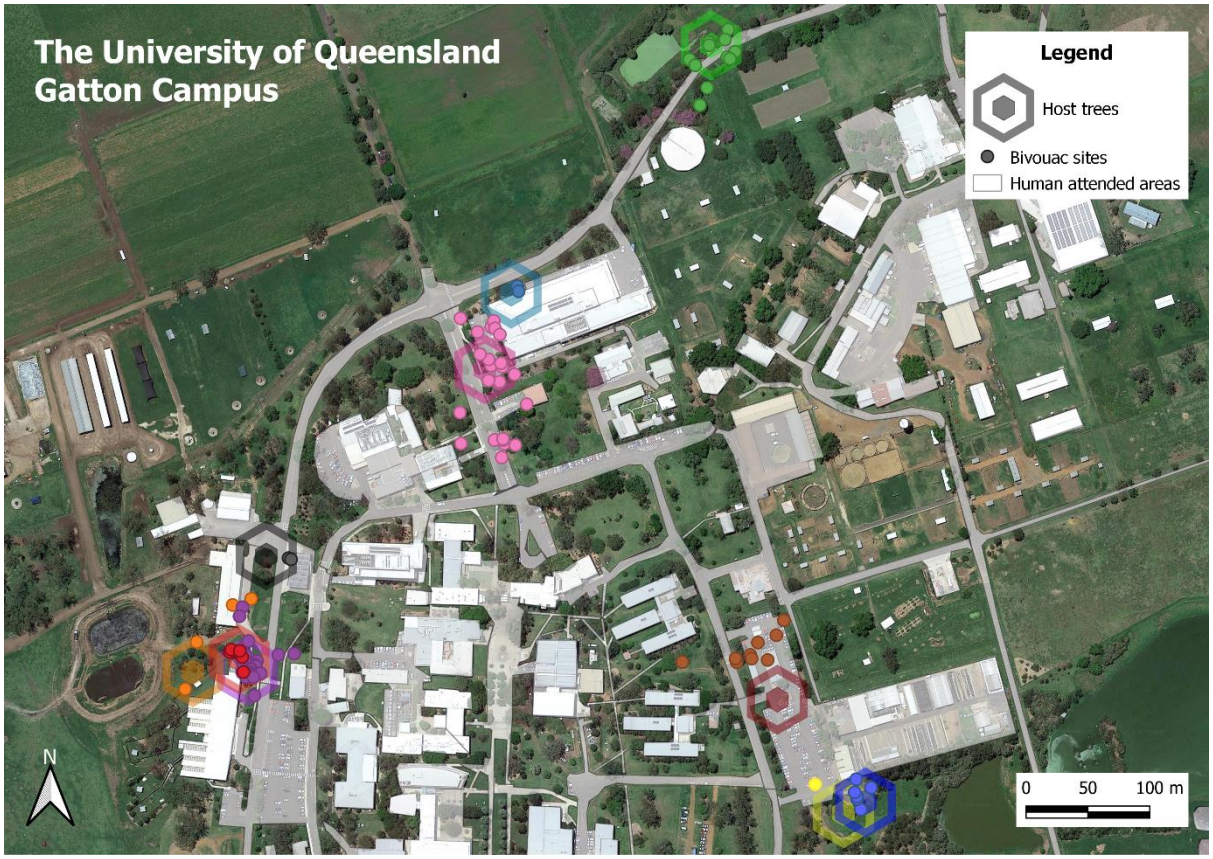


Figure 2.1. The University of Queensland, Gatton campus, Australia, the fieldsite where *Ochrogaster lunifer* pre-pupation processions were studied. Pre-pupation processions were followed from ten *Acacia* spp. host trees that are represented as different coloured hexagons. Processions were followed until the larvae went into a bivouac which are represented as circles (colour coordinated with the host tree). Human attended areas are shaded in white and are not suitable for bivouac/pupation sites because it is made of concrete; with the exception of some areas that had leaf litter.

Thaumetopoea pityocampa

Thaumetopoea pityocampa larvae are destructive defoliators of pine and cedar trees in the Mediterranean Basin and Southern Europe (Zamoum et al., 2015). *Thaumetopoea pityocampa* larvae feed throughout winter until spring in northern Italy, when the final instar larvae leave their tent (nest) in the canopy and descend to the ground to find a pupation site (Robredo, 1963). *Thaumetopoea pityocampa* pre-pupation processions were followed over seven and two non-consecutive days from the first until the third week of April 2018 and last week of March until first week of April 2019, respectively. Field visits occurred on other days during the season but there were no active processions. The field sites were at three pine forests in Veneto, Italy: Monte Garzon (45°30’ N, 11°11’ E; Fig. 2.2), Precastio (45°31’ N, 11°10’ E) and Carbonari (45°32’ N, 11°10’ E). The selected field sites are planted forest for soil conservation and are also used for recreational purposes such as hiking, cycling and picnics. Processions could not

be followed from the tent because they are situated in the canopy of *Pinus nigra* host trees that can be more than 6 m high. Therefore, procession data could only be collected from the first sighting along the footpath of the field sites, i.e. all *T. pityocampa* pre-pupation procession data are approximate measures. Once a procession was found and followed until the bivouac, GPS of the bivouac location was recorded using iPhone application ViewRanger and any disruption/movement of the procession were recorded in a similar manner to the *O. lunifer* processions. Continuous environmental temperatures for *T. pityocampa* field sites were measured from HOBO Temperature/RH data loggers (Onset Computer Corporation, Macquarie, USA) placed in a village approximately 1 km away from each field site (Tregnago, Veneto, Italy).

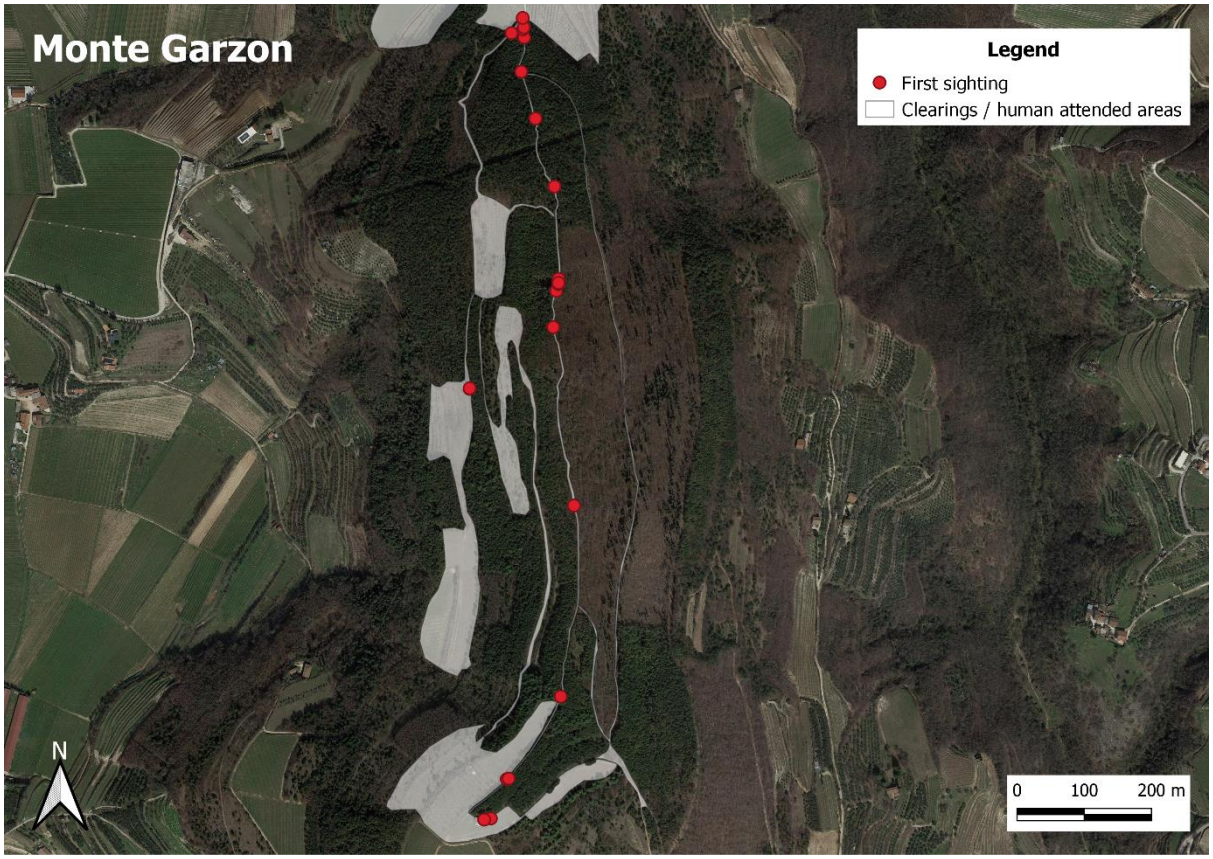


Figure 2.2. Monte Garzon, Veneto, Italy, one of the three field sites where *Thaumetopoea pityocampa* pre-pupation processions were studied. Quantitative data were collected from processions at first sighting along the footpath, represented as red circles. Clearings and human attended areas shaded in white, are suitable and preferred bivouac/pupation sites for *T. pityocampa* larvae.

Distance, duration and speed of pre-pupation processions

Topographic distance and travel duration by *O. lunifer* were calculated from processions leaving the nest until completing a bivouac. Once all the larvae from a nest were underground, the distance travelled was measured with a measuring tape or trundle wheel, retracing every movement of the procession. Speed was calculated from the duration and distance travelled from the nest or first sighting to the bivouac. Distance and duration travelled by *T. pityocampa* pre-pupation processions are an underestimate because it was not feasible to follow processions from *T. pityocampa* tents that are in the tree canopy. The distance travelled by *T. pityocampa* was calculated by addition of the height of the nearest host tree with a visible viable nest (estimated from the orientation at first sighting of the procession before it formed a bivouac) and distance from that host tree until the bivouac site. Duration of travel was calculated by dividing the estimated distance travelled by the average speed of *T. pityocampa* pre-pupation processions. Speed was calculated from measurements of 15 processions on the ground (e.g. travelled x m in x mins).

Orientation of pre-pupation processions

Orientation of every procession is determined by the leader. Each directional change of the procession leaving from the nest to the bivouac was recorded using a handheld compass (in Australia 2017) or iPhone application Compass (in Italy 2018/19 and Australia 2019). For *O. lunifer*, orientation of all pre-pupation processions leaving the nest and final orientation to the bivouac following the last turn of the leader were used for the data analyses. For *T. pityocampa*, orientation of processions at first sighting were used for the data analyses.

Light preference of leading larvae

Solar radiation/light (W/m^2) was measured for a sub-sample of 2018 *T. pityocampa* and 2019 *O. lunifer* processions using the Solar Power Meter (SPM) ISM410 (RS Pro, 2016, London, England). Solar radiation was measured for *O. lunifer* processions when they left the nest and at final orientation to the bivouac. For *T. pityocampa* processions, the solar radiation was measured at first sighting. Three solar radiation measurements were taken: directly beside at the same orientation as the leader, 90° left and right of the leader. Standardised light was calculated by dividing light (W/m^2) from the leader's position by the average of left and right of the leader. Value of 1 means there is no difference between the light intensity at the orientation of the leader/procession and the surrounding. Values more or less than 1 means the

leader/procession travelled to the lighter or darker relative to the surrounding environment, respectively.

Risks of pre-pupation processions contacting humans

For *O. lunifer*, 2019 data for caterpillar contacts with humans were used, because in 2017, some processions were protected from being run over by cars and walked over by pedestrians. Processions for *T. pityocampa* were not protected from encounters by humans. A human attended area is defined as areas where people travel to and from places by walking, cycling and driving. It was calculated so the two field sites, Australia and Italy can be compared. For *O. lunifer*, the average human attended area in percent at UQ Gatton campus was calculated by the amount of urban area (e.g. concrete footpath, buildings, roads, etc.) surrounding each host tree at a 10 m radius using QGIS version 3.6.2 Noosa (QGIS Development team, 2019) (10 host trees in total). Human attended areas at UQ are not suitable for bivouac/pupation sites because it is made of concrete; with the exception of some areas that had leaf litter. For *T. pityocampa*, the average human attended area in percent was calculated at Monte Garzon by the amount of gravel foot path surrounding each procession at first sighting within a 10 m radius using QGIS (24 processions in total). Human attended areas in Italian field sites are the preferred and suitable bivouac/pupation sites for *T. pityocampa* because there is exposed dirt/soil and loose gravel (A. Battisti, personal communication 2018).

Statistical analyses

All statistical analyses were performed using R Studio version 1.1.419 (RStudio Team, 2019) and an alpha value of $P < 0.05$ was taken as statistically significant. Mapping was performed using QGIS with satellite images from Google Earth Pro version 7.3.2 (Google, 2019). Data for *O. lunifer* populations collected in 2017 and 2019 were combined in the analyses. Linear models were used to determine if the number of larvae in a procession and environmental temperatures influenced the distance travelled and/or speed of *O. lunifer* and *T. pityocampa* pre-pupation processions. To determine if human attended areas affected the distance travelled by *O. lunifer* processions, a linear model was used, with the variables: distance travelled and human attended area of the host tree where the procession originated (Results in Distance, duration and speed of pre-pupation processions). Distance travelled by *T. pityocampa* processions were not modelled against human attended areas because the habitat is a pine forest. Procession orientations were represented as rose diagrams made in R Studio using the software package "Circular" (Agostinelli and Lund, 2017). To determine if processions had a

preference(s) in orientation, Kuiper's test of uniformity was used for each rose diagram with the R software package "CircStats" (Lund and Agostinelli, 2018). Each *O. lunifer* procession was nested within its host tree (10 host trees in total from 2017 and 2019 combined) therefore, Kuiper's test of uniformity was also used to determine if host trees affected the orientation of processions. Host trees that had more than 10 processions were selected for the Kuiper's test of uniformity. Light preference of *O. lunifer* and *T. pityocampa* pre-pupation processions were analysed using Chi-square Goodness-of-Fit test for a 50:50 distribution. A linear model was used to determine if host tree and number of larvae in the procession affected light preference by *O. lunifer* and *T. pityocampa* processions. Host tree could not be tested for light preference in *T. pityocampa* pre-pupation processions because the exact host trees and timing at which they were at the tree base were unknown.

2.4 Results

Distance, duration and speed of pre-pupation processions

Ochrogaster lunifer processions travel approximately three times further and four times faster than *T. pityocampa* (Table 2.1). *Ochrogaster lunifer* processions left the ground nest between 05:43–09:25 h (median = 06:15 h, N = 115; Fig. 2.3) and finished the bivouac between 06:23–14:00 h (median = 08:53 h, N = 86). *Thaumetopoea pityocampa* processions were found along the footpath between 07:45–14:29 h (median = 10:12 h, N = 38; Fig. 2.3) and finished the bivouac between 08:22–15:37 h (median = 13:23 h, N = 11). *Ochrogaster lunifer* processions travelled significantly further from the nest to the bivouac when there was more human attended area (concrete/building) surrounding the nest (LM: $t_{85} = 2.810$, adjusted $R^2 = 0.07505$, $P = 0.00616$). In both species, number of larvae in a procession did not affect the total distance travelled or the speed (all $P > 0.1$). Environmental temperature at the field sites had no influence on procession speed for *O. lunifer* and *T. pityocampa* ($P > 0.05$ and $P > 0.2$, respectively) and distance travelled by *T. pityocampa* ($P > 0.8$). Environmental temperature in Gatton influenced the distance travelled by *O. lunifer*, with processions travelling less at higher temperatures (LM: $t_{85} = -3.551$, adjusted $R^2 = 0.119$, $P = 0.000628$).

Table 2.1. Comparison of the average distance travelled, duration, speed and environmental temperature between *Ochrogaster lunifer* and *Thaumetopoea pityocampa* pre-pupation processions

Species	Average distance travelled from nest to bivouac (m)	Average duration travelled from nest to bivouac (min)	Average speed of procession (m/h)	Average environmental temperature (°C)
Ochrogaster lunifer	40.3 (±SE 3.4, N = 87)	153 (±SE 11, N = 86)	17.4 (±SE 0.8, N = 129)	19.1 (±SE 0.4, N = 87)
Thaumetopoea pityocampa	15.8 [a] (±SE 2, N = 11)	223 [a] (±SE 30, N = 11)	4.2 (±SE 0.7, N = 15)	13.7 (±SE 0.9, N = 11)

[a] Approximate measurements

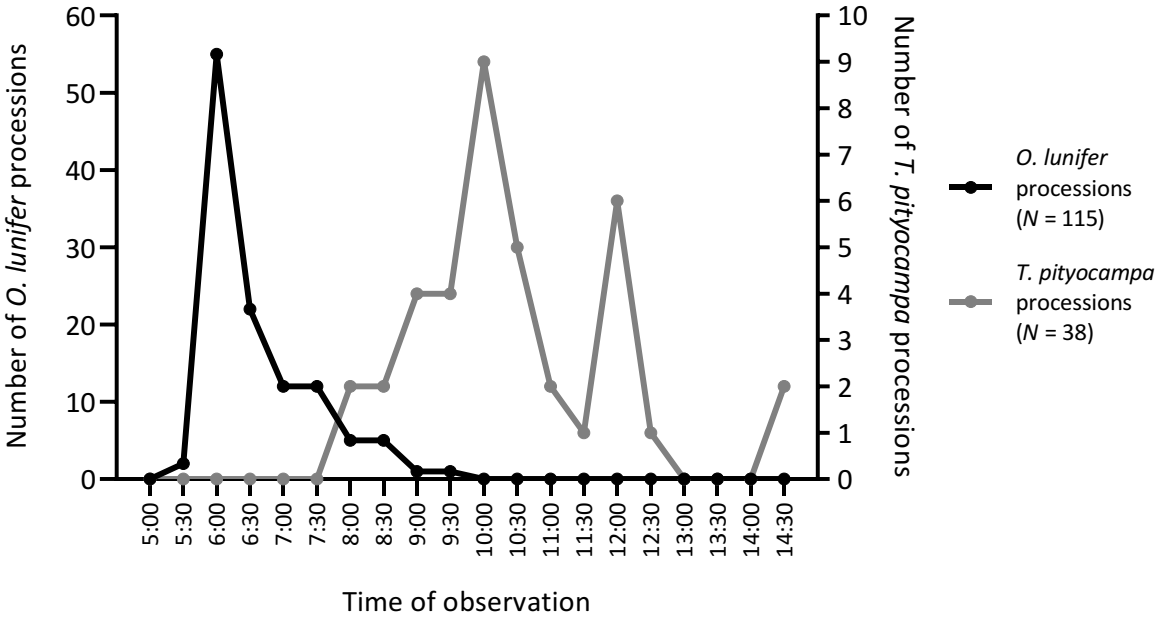


Figure 2.3. Comparison of the observed pre-pupation procession times of *Ochrogaster lunifer* and *Thaumetopoea pityocampa* in Australia and Italy, respectively. Time of observation for *O. lunifer* (black line) was from the time the procession left the nest and *T. pityocampa* (grey line) was first sighting on the footpath.

Orientation of pre-pupation processions

Ochrogaster lunifer pre-pupation processions travelled more often towards the north and south, and less to the east and west when leaving the nest and to the final orientation to the bivouac following the last turn of the leader (Fig. 2.4 a and b, respectively; Table 2.2). There were six

and four host trees with more than 10 *O. lunifer* processions, for orientations from the nest and to the final orientation to the bivouac, respectively. These host trees were tested for uniformity, and the tests showed that eight of the ten host trees had processions with a preferred orientation (all north and/or south, except one host tree with processions that headed to the north and west) i.e. procession orientations were not uniform and therefore clumped (all $P < 0.025$). In contrast, *T. pityocampa* pre-pupation processions had no preferred orientation at first sighting (Fig. 2.4 c, Table 2.2).

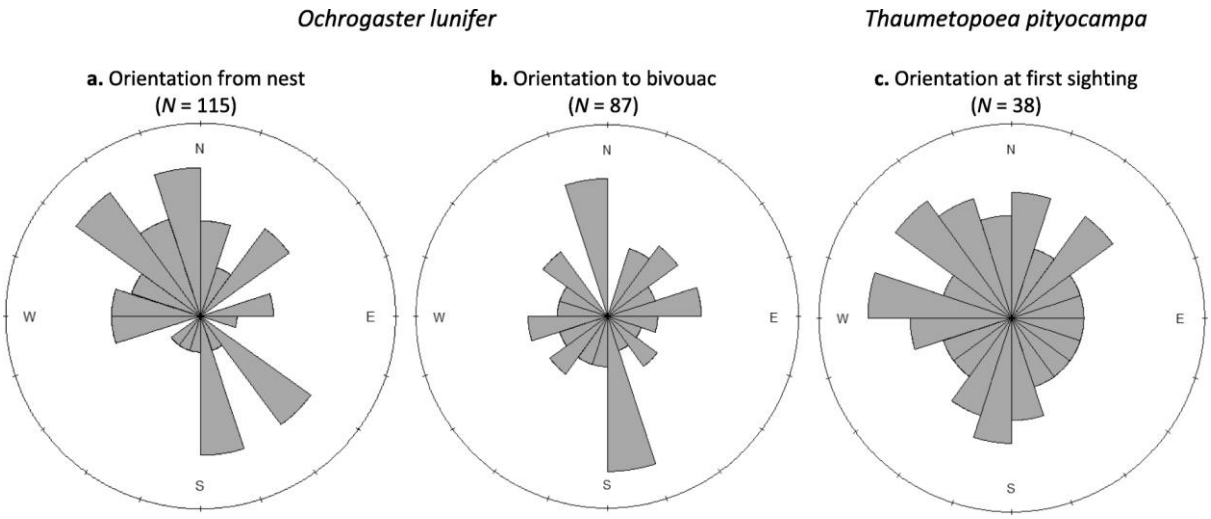


Figure 2.4. Rose diagrams of the orientation of pre-pupation processions of *Ochrogaster lunifer* leaving the nest (**a**) and final orientation to the bivouac following the last turn of the leader (**b**), and of *Thaumetopoea pityocampa* at first sighting (**c**).

Table 2.2. Comparison of Kuiper's test of uniformity for the orientation of *Ochrogaster lunifer* and *Thaumetopoea pityocampa* pre-pupation processions

Species	Kuiper's test statistic	P	N
Ochrogaster lunifer			
Orientation from nest	2.9109	< 0.01	115
Orientation to bivouac	1.7765	< 0.05	87
Thaumetopoea pityocampa			
Orientation at first sighting	1.2345	> 0.15	38

Light preference of leading larvae

More *O. lunifer* pre-pupation processions travelled to the dark (shaded areas of the environment) than to the light when leaving from the nest ($X^2 = 8.1667$, df = 1, P = 0.004267, N = 24; Fig. 2.5 a) and when arriving to the bivouac ($X^2 = 16.03$, df = 1, P = $6.234e^{-05}$, N = 33; Fig. 2.5 b), irrespective of the number of larvae in a procession (from nest: P > 0.2; to bivouac: P > 0.1) and host tree (P > 0.5). Whereas *T. pityocampa* pre-pupation processions travelled more to the light (brighter areas of the environment) than the dark ($X^2 = 5.7692$, df = 1, P = 0.01631, N = 38; Fig. 2.5 c), irrespective of the number of larvae in a procession (P > 0.4).

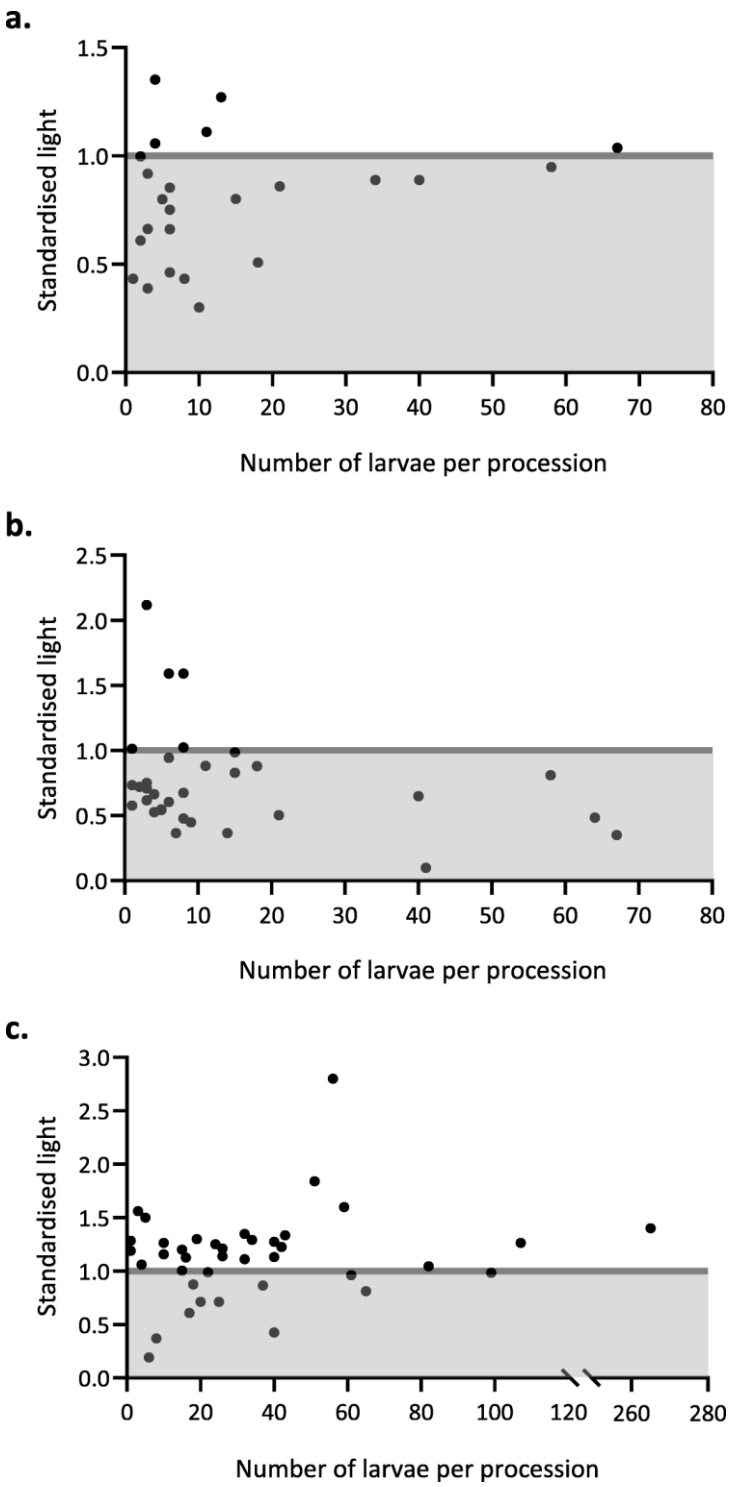


Figure 2.5. a) Standardised light choice by *Ochrogaster lunifer* pre-pupation processions (N = 24) at initial orientation when leaving the ground nest. **b)** Standardised light choice by *O. lunifer* pre-pupation processions (N = 33) at final orientation to the bivouac following the last turn of the leader. **c)** Standardised light choice by *Thaumetopoea pityocampa* pre-pupation processions (N = 38) at first sighting. Each point represents one procession. Area shaded in grey (standardised light value less than 1) represents processions that travelled to the darker in comparison to its surroundings. Unshaded area (standardised light value above 1) represents processions that travelled to the lighter compared to its surroundings. There is no correlation between number of larvae in each procession and the choice for standardised light.

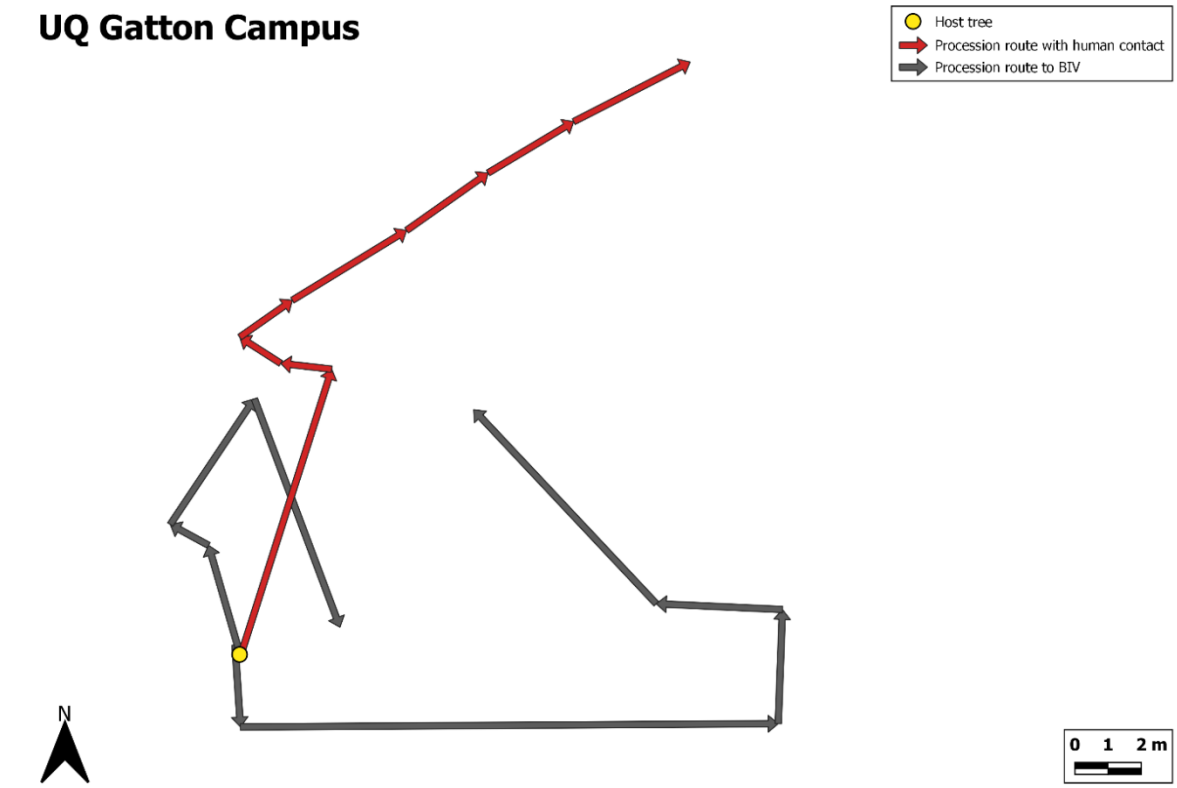
Risks of pre-pupation processions contacting humans

People were 1.5 times more likely to be in contact with *T. pityocampa* than *O. lunifer* pre-pupation processions in our field sites (Table 2.3). On average, human attended area at UQ Gatton campus was two times larger than pine forests in Veneto, Italy. Visual representation of the procession route taken by selected *O. lunifer* and *T. pityocampa* processions from a host tree/first sighting are presented in Fig. 2.6 and 2.7, respectively.

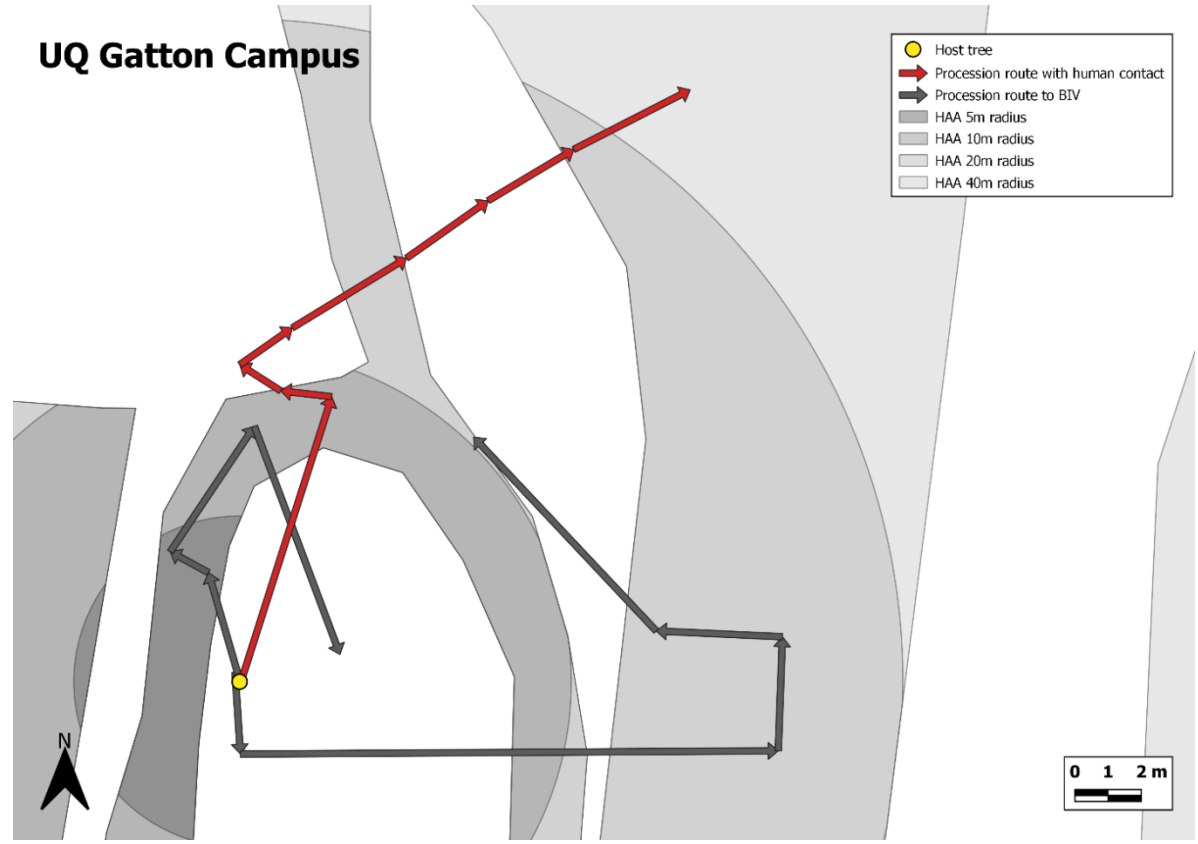
Table 2.3. Comparison of the number of human contacts with *Ochrogaster lunifer and Thaumetopoea pityocampa* pre-pupation processions

Species	Number of processions with human contact	Total number of processions	Human contact (%)	Human attended area (%)
Ochrogaster lunifer	22	82	26.8	33 (±SE 2.0)
Thaumetopoea pityocampa	23	53	44	15 (±SE 0.7)

A


UQ Gatton Campus
Host tree
Procession route with human contact
Procession route to BIV
N
0 1 2 m

B


UQ Gatton Campus
Host tree
Procession route with human contact
Procession route to BIV
HAA 5m radius
HAA 10m radius
HAA 20m radius
HAA 40m radius
N
0 1 2 m

C

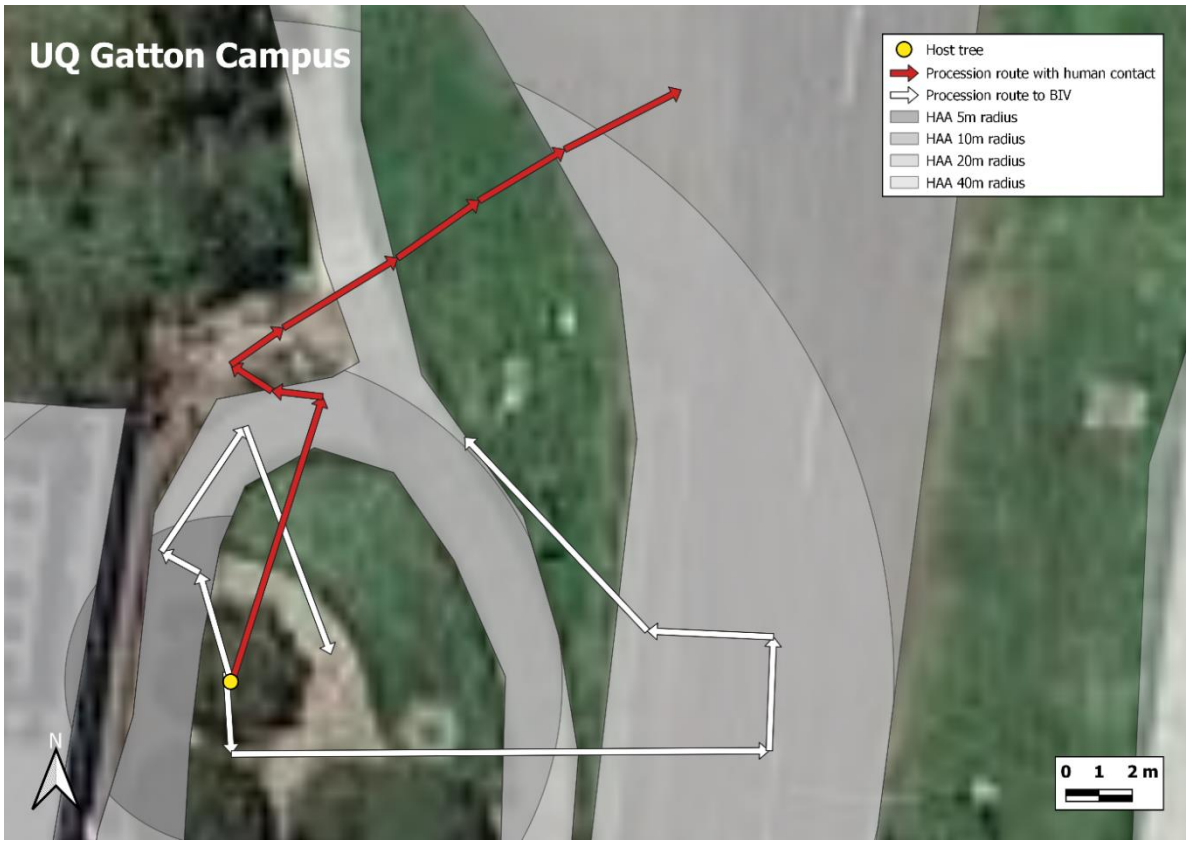
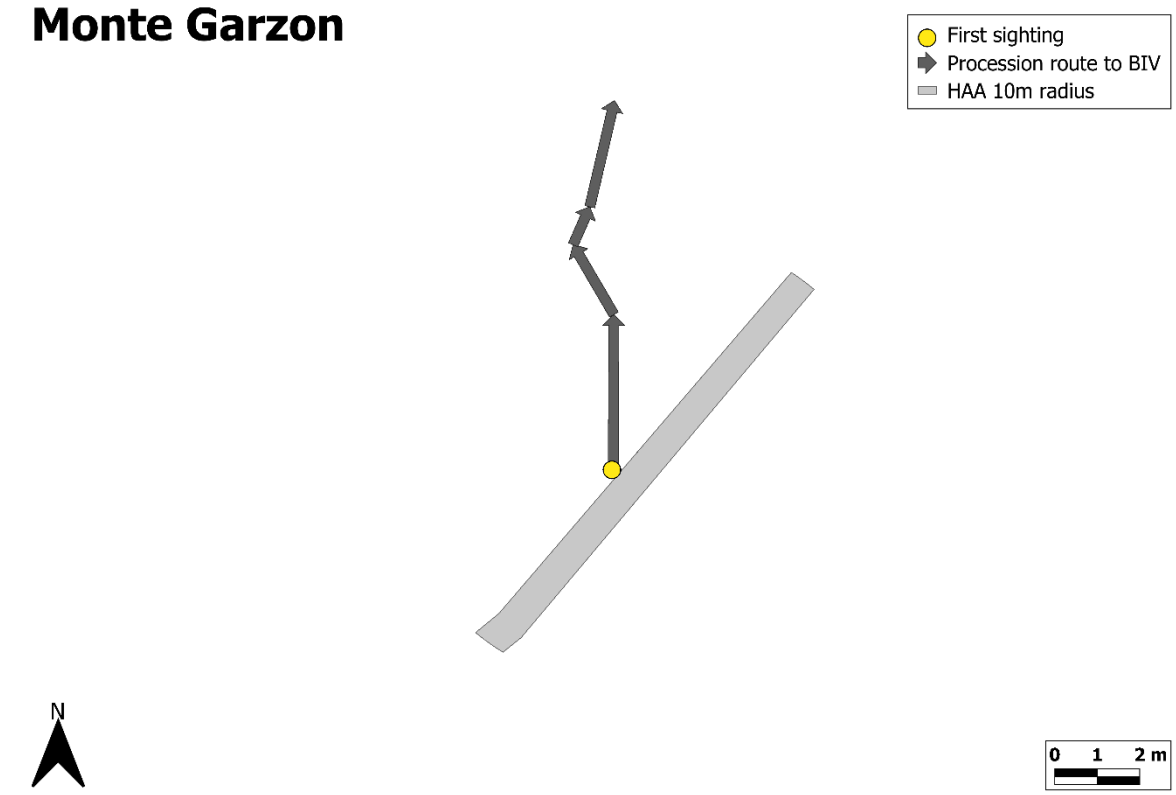


Figure 2.6. A sub-sample of three *Ochrogaster lunifer* pre-pupation procession routes from one host tree at the University of Queensland, Gatton campus, Australia. Every procession starts from the host tree (yellow circle) and each arrow represents a change in procession orientation in search for a pupation site (grey/white arrows)/had human contact (red arrows). The tip of the last arrow is the last point. Red arrows represent the procession that was run over by a car on the road (human contact). Grey (**A** and **B**)/white (**C**) arrows represent processions that successfully went into a bivouac. In **B** and **C**, various shades of grey circles starting from the host tree is the amount of human attended areas (urban structures) there are for a given radius of various increments (5, 10, 20, 40 m). Figure 2.6 **A**, **B** and **C** represents the same three *O. lunifer* pre-pupation processions with different geographic layers, starting from **A** being the simplest to **C** being the most complex with the satellite image.

A


Monte Garzon
First sighting
Procession route to BIV
HAA 10m radius
N
0 1 2 m

B


Monte Garzon
First sighting
Procession route to BIV
HAA 10m radius
N
0 1 2 m

Figure 2.7. *Thaumetopoea pityocampa* pre-pupation procession route from first sighting along the footpath at Monte Garzon, Veneto Italy. The procession was studied from the first sighting (yellow circle) and every grey (**A**)/white (**B**) arrow represents a change in orientation in search for a pupation site. The thick grey line represents the human attended area of a 10m radius from the first sighting of the procession. Figure 2.7 **A** and **B** represents the same *T. pityocampa* pre-pupation procession with different geographic layers, **A** being the simplest and **B** being the most complex with the satellite image.

2.5 Discussion

The expansion of human growth and development has increased the likelihood of insect-pest human interactions. This includes processionary caterpillar species *O. lunifer* and *T. pityocampa*, which can cause serious health problems to humans and animals in Australia and Europe, respectively. In our study, we focused and compared the movements of *O. lunifer* and *T. pityocampa* during the pre-pupation period to determine the risks associated to humans.

Ochrogaster lunifer and *T. pityocampa* pre-pupation processions travelled tens of metres per day in search for pupation sites over two or more days (Robredo, 1963; Floater, 1996). *Ochrogaster lunifer* processions travelled 2.5 times further and 4 times faster than *T. pityocampa*. These differences could be explained by the final instar body size; *O. lunifer*: 35–50 mm (Floater, 1996) vs. *T. pityocampa*: 33 mm (Pérez-Contreras et al., 2003); environmental temperatures (Table 2.1, see Wellington (1960), number of instar stages (*O. lunifer*: 8 instars vs. *T. pityocampa*: 5 instars), environmental terrain (*O. lunifer*: mostly flat and smooth grounds vs. *T. pityocampa*: loose gravel ground) and possibly other physiological and morphological differences. At the UQ Gatton campus, *O. lunifer* larvae travelling several tens of metres per day could enter paddocks containing farm animals and ingress further into human settlement; which means an increased risk for spreading setae and exposure to people and animals.

Ochrogaster lunifer pre-pupation processions left the nest at sunrise and travelled for approximately 2.5 h. On working days and possibly weekends, students and staff were in contact with these larvae in the morning, especially because some nests are in close vicinity to classrooms, footpaths, car parks, etc. This corresponded to the 27% of human contact with *O. lunifer* processions. In contrast, 44% of *T. pityocampa* pre-pupation processions in Italy had human contact. The chance of human contact is higher in *T. pityocampa* compared to *O. lunifer*; possibly due to the number of people that attended the area, but most likely can be explained by the behaviour of *T. pityocampa*. Human attended area in Italian field sites are approximately

a half of the human attended area in Australia, and people are limited to walk only on footpaths. Additionally, *T. pityocampa* larvae do not travel far, are slow walkers and prefer lighter areas (see below) which is almost always the footpath. The longer the larvae stay on the footpath, the higher chance of contact with humans and pets. The amount of human contact in both field sites are concerning because setae are released upon disturbance (Mullen and Durden, 2009) and the surviving larvae in the procession after contact may be very reactive. Not only do setae become airborne after release (Perkins et al., 2019), it can contaminate bicycle wheels, shoes and clothing and be brought back to the car, home, classroom, etc. The toxins inside the seta can remain active for at least a year (Battisti et al., 2011) and the microscopic scale of the seta makes it difficult to determine what/where is contaminated.

In general, there was a tendency for *O. lunifer* pre-pupation processions to travel north or south when leaving the nest and also to their final orientation to the bivouac. In contrast, *T. pityocampa* pre-pupation processions had no preference in orientation at first sighting. The orientation preference/absence could be explained by the light preference by the leading larvae of both species. *Ochrogaster lunifer* larvae orientated towards darker areas while *T. pityocampa* orientated towards lighter areas of the environment for potential pupation sites. At the Australian field site, it is generally warmer at this time of year and host trees were patchy with a lot of clearings. Therefore, orienting more to the north or the south could avoid direct sunlight which came from the east to the west. Italian field sites were cooler because of the homogenous dense pine forest, and lighter areas are restricted to footpaths and nearby meadows. Orientation may be insignificant for *T. pityocampa* because the orientation chosen may only depend on where is lighter in relation to where the host tree is. *Thaumetopoea pityocampa* processions search for sunny exposed soil in gaps and edges of forests for optimal pupae survival (Jactel et al., 2015). Therefore, *O. lunifer* and *T. pityocampa* pre-pupation processions may have been in search for 'cooler' and 'warmer' places, respectively for optimal development in suitable thermal niches. It may be particularly important for *T. pityocampa* to find warmer places because the pupae can diapause up to 8 years (Salman et al., 2016) with possibly less chance of fungal attack if the pupation site is dryer. Polarised light vision for directed larval movement in the environment has been recorded in Lepidoptera (Wellington et al., 1951; Doane and Leonard, 1975; Gilbert, 1994). More research is necessary to determine if polarised light is used by the leading larva in the procession as a cue for pupation sites with suitable temperature characteristics. The adaptive value of pre-pupation processions to move away from the host tree was not explored however, there are several hypotheses. By

aggregating in similar pupation site conditions, conspecifics may increase the chances of finding a mate and facilitating gene flow. Additionally, dispersing further away from the host may decrease the chances of natural enemies finding pre-pupae/pupae underground (Camazine 1986). Female *O. lunifer* moths are strong flyers but consistently oviposit on the same trees year after year (Floater and Zalucki, 2000); which suggests that pre-pupal dispersal away from the original host tree is not for female moths to choose a new host tree. Further research is required to understand the behavioural evolutionary trait of pre-pupal dispersal.

Ochrogaster lunifer and *T. pityocampa* processions preferred different environmental cues to navigate to the bivouac, and this may be a result of urbanisation in the environment. Organisms living in urban environments often differ in behaviour compared to those from rural environments, and having adjusted to anthropogenic disturbances (Jactel et al., 2015). This includes changes in food resources, nesting site, pedestrian and vehicle traffic, artificial light and industrial noise (Jactel et al., 2015). As environments/microhabitats for *O. lunifer* and *T. pityocampa* vary within the species, the data we present may only be representative for the field sites chosen (semi-urban and pine forest/rural, respectively). It would be beneficial to repeat the same behavioural analyses on *O. lunifer* populations in a rural/natural environment and also for *T. pityocampa* populations in a semi-urban/urban environment. This can determine whether the pre-pupation movement by the two species has indeed changed with urbanisation. *Thaumetopoea pityocampa* also has a population of summer feeding larvae in Portugal, that have pre-pupation processions occurring at the end of summer every year (Pimentel et al., 2006). A comparison of winter and summer feeding *T. pityocampa* population processions could provide further information on whether or not the same behaviour and environmental preferences exists within the species.

Our results on pre-pupation processions are comparable with the findings from previous published studies. Mills (1950, 1951) observed *O. lunifer* processions leaving the nest in the morning in a northerly or southerly direction, consistent with our observations. Average speed of our *O. lunifer* processions (17.4 m/h at 19 °C) in the field differed from the procession work by Steinbauer (2009) (approx. 23.3 m/h at 19 °C, calculated from regression of speed over temperature 'all processions combined'). This is possibly explained by the physical handling of study organisms (posterior or anterior hairs of the larva cut or brushed) by the experimenters and/or the short observation duration of 1 min per procession (see Steinbauer (2009) for more information). In contrast to *O. lunifer*, *T. pityocampa* pre-pupation processions travelled to

lighter areas consistent with Démolin (1971); he stated that *T. pityocampa* larvae are phototactic and pupated in the open. Additionally, the distance travelled and speed of *T. pityocampa* pre-pupation processions documented by Robredo (1963) were similar to our measurements (cf. Introduction and Table 2.1). However, the timing of *T. pityocampa* pre-pupation processions on the ground in our study was different to that reported by Robredo (1963). This could be explained by several cloudy and rainy days during our study which delayed the timing of caterpillars leaving the tent. Because *T. pityocampa* tents were high in their host trees, we were not able to observe caterpillars leaving their tents. Therefore, we had to use measurements from processions when they were first sighted on the ground sometime later. More observations and future investigations of *T. pityocampa* pre-pupation processions at sites with shorter host trees where the tents could be observed directly would give more accurate data on their behaviour.

The risk of exposure to *O. lunifer* and *T. pityocampa* processions are particularly high because of the tent/nest distribution in the environment. Both species are edge species, thus female moths restrict their oviposition mostly to the outer edges of forests or road verges where larval colonies later develop (van Schagen et al., 1992; Floater and Zalucki, 2000; Jactel et al., 2015). Clumped distribution of urticating caterpillars in close vicinity to human settlement, in combination with their procession movements on the ground make these species an important medical concern. Seasonal abundance or outbreaks of urticating caterpillars are recorded in other social Lepidoptera genera such as *Euproctis* (Erebidae), and *Hylesia* and *Hemileuca* (Saturniidae) (Hossler, 2010). These species may not form processions or travel en masse but they can disperse over the ground after defoliating a host plant or to find a pupation site. Precautions are needed to reduce cutaneous or systemic symptoms that can be caused by medically important Lepidoptera species (see below).

Much of the information for *O. lunifer* and *T. pityocampa* pre-pupation processions presented here is novel. Understanding the movement of urticating caterpillars in human settlement plays an important role in interpreting its ecological and medical significance. With this information, we can forewarn people to avoid infested areas especially in the morning or stay at least 80 m and 30 m away from host trees with *O. lunifer* and *T. pityocampa* nests respectively, during the pre-pupation procession period. If it is necessary to go to these high-risk areas, people should be visually alert where they are walking or cycling and wear appropriate protective clothing and eyewear. Awareness is important for prevention, because

these urticating caterpillars will disperse from the host tree to pupation sites every year. With more sampling and monitoring of infested host trees, it can further improve the predictability of the movements of urticating processionary caterpillars.

2.6 Conclusion

Anthropogenic changes in the environment are increasing and expanding, therefore more people are at risk of coinciding with insects of medical importance. We investigated and compared pre-pupation procession behaviours of two urticating caterpillar species from opposite hemispheres. Our aim was to understand their movement to determine the contact risk with humans. Differences in movement behaviour of *O. lunifer* and *T. pityocampa* pre-pupation processions may be explained by their own environmental and physiological requirements for optimal development. Our data on human contact with both species, in addition to the alarming numbers of urticaria cases in Europe, raises the need for preventative measures. The research presented here highlights the importance of investigating movement patterns of organisms to mitigate harmful impacts.

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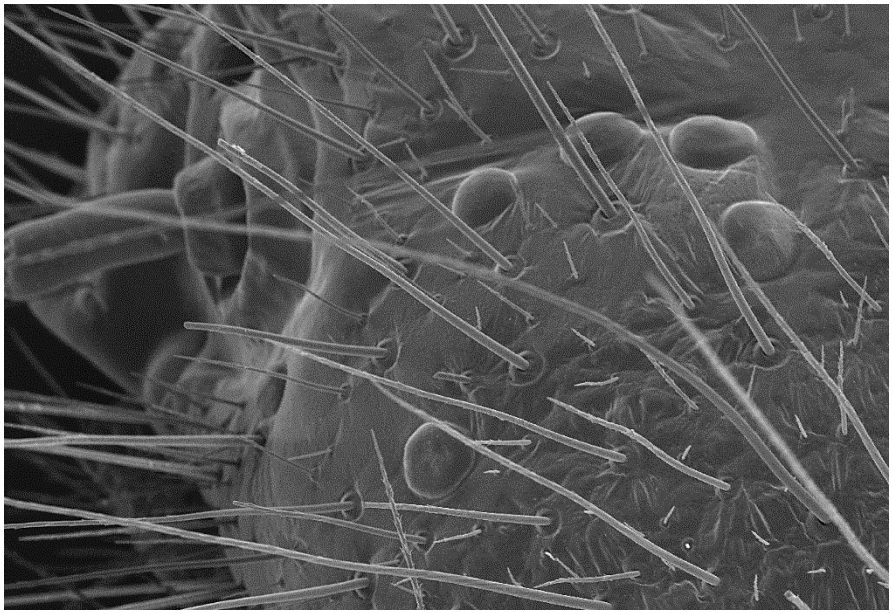
Chapter 3

Spatial orientation of social caterpillars is influenced by polarised light



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doi: 10.1098/rsbl.2020.0736

The manuscript was merged with the original text and supplementary material in this thesis chapter.

3.1 Abstract

Processionary caterpillars of *Thaumetopoea pityocampa* (in Europe) and *Ochrogaster lunifer* (in Australia) (Lepidoptera: Notodontidae) form single files of larvae crawling head-to-tail when moving to feeding and pupation sites. We investigated if the processions are guided by polarisation vision. The heading orientation of processions could be manipulated with linear polarising filters held above the leading caterpillar. Exposure to changes in the angle of polarisation around the caterpillars resulted in corresponding changes in heading angles. Anatomical analysis indicated specialisations for polarisation vision of stemma I in both species. Stemma I has a rhabdom with orthogonal and aligned microvilli, and an opaque and rugged surface, which are optimisations for skylight polarisation vision, similar to the dorsal rim of adult insects. Stemmata II-VI have a smooth and shiny surface and lobed rhabdoms with non-orthogonal and non-aligned microvilli; thus, optimised for general vision with minimal polarisation sensitivity. Behavioural and anatomical evidence reveal that polarised light cues are important for larval orientation and can be robustly detected with a simple visual system.

Keywords: Larval vision, Lepidoptera, orientation, polarisation vision, stemma

3.2 Introduction

Movement of animals through the environment is a fundamental part of life history, success, adaptation and evolution. Animals must be able to detect and interpret external cues to navigate to a food and water source, mating ground, shelter and for predator avoidance (Åkesson et al., 2014). External cues include odours, landmarks, celestial bodies, polarised light and magnetic field (Berdahl et al., 2018). Polarised pattern of the sky is used by many insects as a stable spatial reference, which helps them to maintain an orientation and navigate or simply keep a straight line (Wehner, 1984; Heinloth et al., 2018). Insects have evolved many adaptations of their visual system for detecting polarised light (Horváth and Varjú, 2004; Mathejczyk and Wernet, 2017; Heinloth et al., 2018).

Extensive research has been done on visual structures that detect polarised light in adult insects from several orders (Åkesson et al., 2014). Compound eyes of most adult insects have a specialised region for detecting skylight polarisation pattern, called the dorsal rim area (DRA) (Meyer and Labhart, 1981). Each ommatidium in the DRA has photoreceptor pairs that sample a common visual angle using orthogonal rhabdomeres with straight and aligned microvilli that are sensitive to two planes of polarisation (Wehner, 1976). This arrangement is crucial for achieving high polarisation sensitivity (PS) (Roberts et al., 2011). Polarised light vision has been indicated in larvae of four orders of holometabolous insects (Hymenoptera, Lepidoptera, Trichoptera and Diptera) (Gilbert, 1994), with behavioural (Wellington et al., 1951; Wellington, 1955; Doane and Leonard, 1975; Dethier, 1989) and anatomical (Meyer-Rochow, 1974; Singleton-Smith, 1980; Li and Chang, 1991) evidence dating 30-70 years ago.

Processionary caterpillars (Lepidoptera: Notodontidae) from Europe and Australia, *Thaumetopoea pityocampa* and *Ochrogaster lunifer* respectively, have a directed behaviour when mature larvae are ready to pupate (Uemura et al., 2020). The larvae form a single file crawling head-to-tail and lay down a silk trail when moving to feeding and pupation sites (Mills, 1950; Fitzgerald, 2003). Once the leader of the procession establishes an orientation, the larvae travel along that orientation many metres (10-100 m) per day with minimal deviation (Uemura et al., 2020). Pheromone trails and physical contact between larvae keep the procession together however, neither could serve as a guide to a suitable pupation site (Fitzgerald, 2003). So how do these larvae maintain orientation through the environment? We used manipulative behavioural field experiments to determine if processionary caterpillars will react to changes in the angle of polarisation. Morphological analyses of larval stemmata through scanning

electron microscopy (SEM), light microscopy (LM) and transmission electron microscopy (TEM) helped identify the likely organ responsible for polarised light vision. Here we show that the caterpillars strongly react to the presentation of polarised light, and we identify the likely visual organ for its detection, stemma I.

3.3 Materials and Methods

Behavioural analyses

In August 2018 and 2020, outdoor experiments with first instar (L1) *T. pityocampa* larvae were conducted on sunny days between 0930 – 1130 h (GMT +2) at Tregnago, Veneto, Italy (45°51' N, 11°17' E). A sheet of 50 cm^2 white paper was used as the experimental arena where 10 first instars at a time were released in the middle of the sheet and behavioural observations were made; N = 58 observations (31 single larvae (singletons) and 29 two or more larvae (processions)). After release, the larvae clustered for a few minutes, then formed processions or travelled as a singleton in various orientations. Four treatments were applied to the processions/singletons after the larva(e) established a course. A flexible 25 cm^2 linear polarising filter (PF) for visible light (XP42HE-40, ITOS, Mainz, Germany) was bent into a tunnel and held above the procession leader or singleton (Fig. 3.1). The PF that filtered the light incident to both sides of the head was held either (1) 'horizontally' or (2) 'vertically' by rotating the filter 90°; so the horizontally or vertically polarised light was transmitted at low elevations, respectively, creating two orthogonal polarisation patterns around the larvae. After application, the larvae proceeded under (3) unobstructed sky without a filter. The PF created shade (transmission to unpolarised light 40%), and additionally decreased the incident light by maximum 30%, depending on the angle with respect to the polarised skylight (Pomozi et al., 2001). As the stemmata are non-image forming organs with large fields of view, the possible intensity artefact slightly affected the total, but not the differential signal in the orthogonal photoreceptor pairs. Thus, (4) control experiments were performed using a 45% neutral density filter (NDF) (Lee 298 and 209 combined; Lee Filters, Hampshire, UK), in place of PF. Each treatment lasted for 2 min, and the larval orientation was recorded 20 s after commencement (Fig. 3.2). The larvae were changed after every trial.

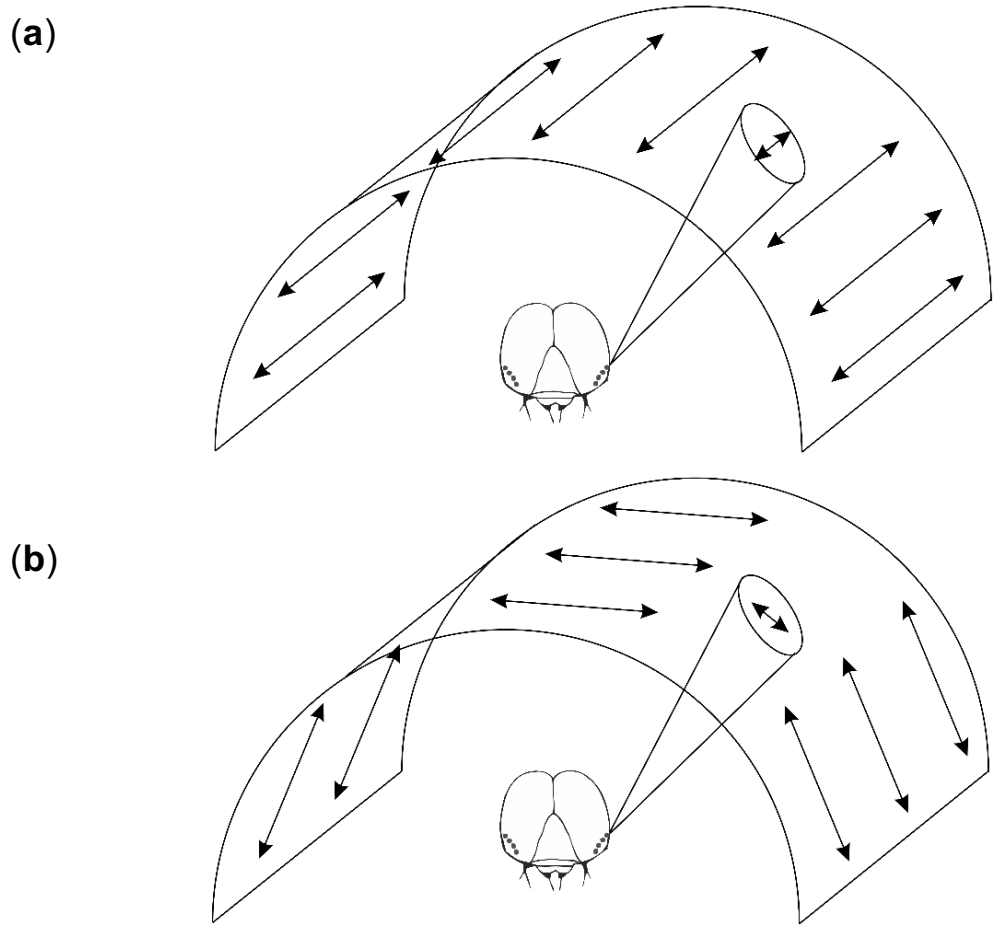
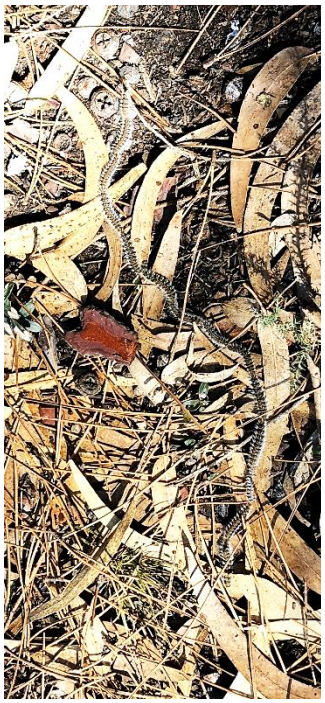


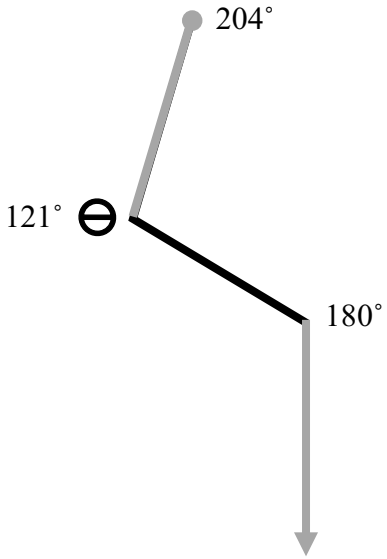
Figure 3.1. Schematic diagram of the application of the linear polarising filter (PF) above the procession leader or single caterpillar. The PF was held (**a**) horizontally and (**b**) vertically to manipulate the larval heading orientation.

(*a*)

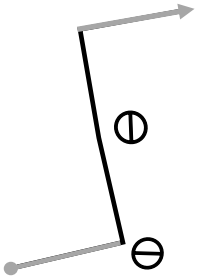


(*b*)

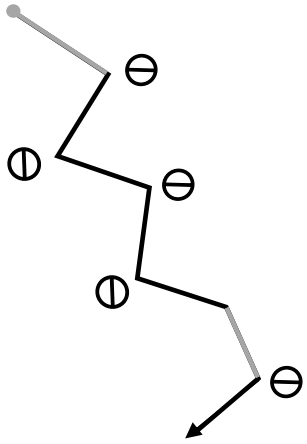
(*c*)



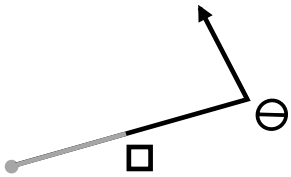
(*d*) *Thaumetopoea pityocampa* neonate (L1)

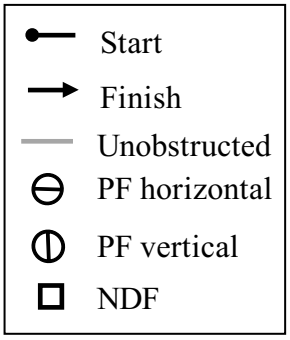


(*e*) *Thaumetopoea pityocampa* final instar larvae (L5)

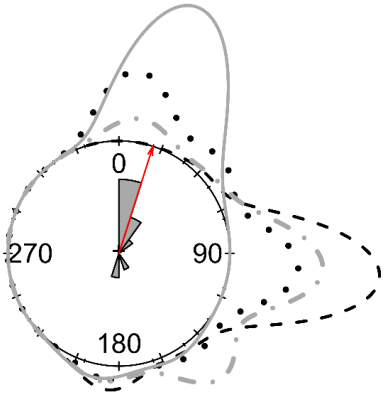


(*f*) *Ochrogaster lunifer* final instar larvae (L8)

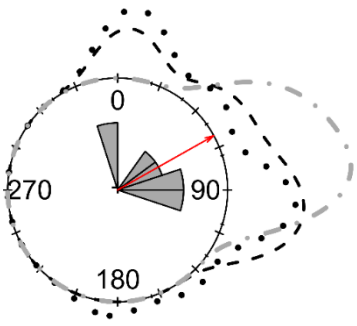




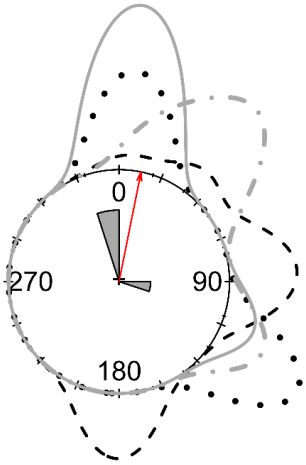
(*g*) *T. pityocampa* L1 N = 113



(*h*) *T. pityocampa* L5 N = 15



(*i*) *O. lunifer* L8 N = 26



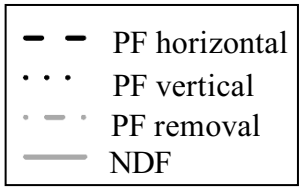


Figure 3.2. Influence of polarised light on caterpillar heading direction. **(*a*)** Final instar *Thaumetopoea pityocampa* pre-pupation procession after the experiment with/without a polarising filter (PF). **(*b*)** Procession from (*a*) without background. **(*c*)** Schematic path from (*b*). Numbers indicate the orientation of the procession. **(*d-f*)** Schematic path of *T. pityocampa* L1, L5 and *O. lunifer* L8, respectively. (*c-f*) Each line segment represents 2 min duration of locomotion. ***(g-i*)** Circular plots with kernel density estimations (KDE) of summarised angular differences of *T. pityocampa* L1, L5 and *O. lunifer* L8 caused by PF and NDF. The KDE are represented as lines extruding in the outer circle. The rose histogram of *(g,i*) in the middle represents the angular difference after NDF application. In (*h*), NDF was unavailable therefore, data from PF horizontal was plotted. The red arrow represents the mean of the histogram data.

The same experiments were conducted on final 8th instar (L8) *O. lunifer* (N = 12 processions) and 5th instar (L5) *T. pityocampa* (N = 7 processions) larvae in their natural habitat without the arena. *Ochrogaster lunifer* were observed in late March 2019 at 0600 – 1200 h (GMT+10) at The University of Queensland, Gatton campus, Queensland, Australia (-27°56' S, 152°34' E). Summer feeding *T. pityocampa* (Santos et al., 2007) were observed in September 2019 at Leiria, Portugal (39°31' N, 9°07' W) at 0800 – 1300 h (GMT+1).

Morphological analyses

Preparation of stemmata for scanning electron (SEM), light (LM) and transmission electron (TEM) microscopy was performed as described previously (Belušič et al., 2017). Details can be found online (Uemura and Belušič, 2020).

Statistical analyses

All analyses were performed using R Studio version 1.1.419 and an alpha value of $P < 0.05$ was taken as statistically significant. In some trials, the PF was applied and removed repeatedly on the same procession/singleton. Before pooling the data for analyses, we tested if the orientation of processions was affected by previous exposure to the PF. The Wallraff Test of Angular Distances was performed using the RStudio software package 'circular' (Agostinelli and Lund, 2017) on all processions/singletons. There were no significant differences (all $P > 0.1$), therefore, the data were used as independent for each treatment. To determine if the procession/singleton reacted to the treatments, the angular difference was calculated by subtracting the initial orientation of travel from the manipulated orientation after PF/NDF exposure/removal. Summarised angular differences of each group after the treatments were graphed as circular plots with a kernel density estimation (Figs. 3.2 g-i). The angular difference

was given a score of 0 or 1 according to the difference being less (no change) or greater than 22.5° (change; 22.5° is the minimal cardinal value on the compass). Circular Logistics Regression Model (CLRM) for Binomial Data (Al-Daffaie and Khan, 2017) was applied on the angular difference using the variables: Treatment, Azimuth angle (degrees), Procession/singleton ID, Number of larvae in the procession and Starting orientation. The models were reduced to the model of best fit by removing non-significant covariables and by lowering the Akaike's information criterion value. Environmental temperatures of the study sites were collected, but not used as a variable because of collinearity ($r > 0.70$) with the Azimuth angle. Details of R codes used can be found online (Uemura and Belušič, 2020).

3.4 Results and Discussion

Behavioural analyses

Application of PF either 'horizontally' or 'vertically' resulted in directional change of the leader and the rest of the procession, creating a zig-zag column (Figs. 3.2 a-f), by 58-103° (Table 3.1 a). However, the PF attenuated the incident light. We tested for this effect by using a NDF, which caused a minor heading change of 18-32° (Table 3.1 a). None of the other explanatory variables contributed to the angular difference (all $P > 0.1$) for all three groups: L1 and L5 *T. pityocampa*, and L8 *O. lunifer*. The angular differences for NDF were significantly different to all other treatments in *T. pityocampa* L1 and for PF horizontal in *O. lunifer* L8 (Table 3.1 b; Fig. 3.3); NDF produced minimal deviation in orientation. For *T. pityocampa* L5, the NDF was unavailable; therefore, the next variable in alphabetical order, PF horizontal, was compared against the angular difference of PF vertical and removal. Angular differences between the treatments were not statistically significant, suggesting any PF angle produced a change in larval orientation. Our results support findings (Wellington, 1953, 1955; Doane and Leonard, 1975) that holometabolous larvae change their orientation of travel proportionally to the PF angle. The polarised pattern alone is an ambiguous cue and its rotation by 90° should lead to directional changes by -90° or +90°. The zig-zag path of the procession indicates that the directional changes were roughly consistent between trials with and without PF (including NDF). Thus, the caterpillars were probably orienting using additional cues such as the solar position or landmarks. Skylight polarisation cues may be particularly important for processionary caterpillars because individuals separated from processions could re-join their colony at pupation or nesting sites. Being gregarious is beneficial for both species because increase in group size can increase larval survival through cooperative defence strategies and

by dilution effects (McClure and Despland, 2011; Santana et al., 2017). Doane and Leonard (1975) found that after PF removal, larvae returned to their original direction. In our study, this was observed in 21% of the trials, suggesting that most larvae set a new heading orientation after each PF treatment, similar to e.g. tethered fruit flies, flying below PF (Warren et al., 2018; Mathejczyk and Wernet, 2019).

Table 3.1. a) Mean ± standard error of the angular difference (degrees) after exposure to each treatment.

Groups	NDF	PF horizontal	PF vertical	PF removal	N
Thaumetopoea pityocampa L1	32 ± 10	102 ± 6	62 ± 11	96 ± 10	113
Thaumetopoea pityocampa L5	-	59 ± 15	101 [a]	72 ± 6	15
Ochrogaster lunifer L8	18 ± 18	103 ± 17	58 ± 58	49 ± 22	26
N	34	55	31	34	

- data unavailable.

[a] SE was not calculated, not enough data.

b) Results from circular logistics regression model for binomial data. Binomial data from the angular difference of NDF was compared against other treatments.

Groups	PF horizontal		PF vertical		PF removal		N
	z	*P*	*z*	*P*	*z*	*P*	
Thaumetopoea pityocampa L1	3.99	< 0.001	2.62	0.009	3.89	< 0.001	113
Thaumetopoea pityocampa L5 [b]	-	-	0	1	0.001	1	15
Ochrogaster lunifer L8	2.74	0.006	0.003	1	0.006	1	26

[b] NDF data unavailable, PF horizontal was compared against other treatments.

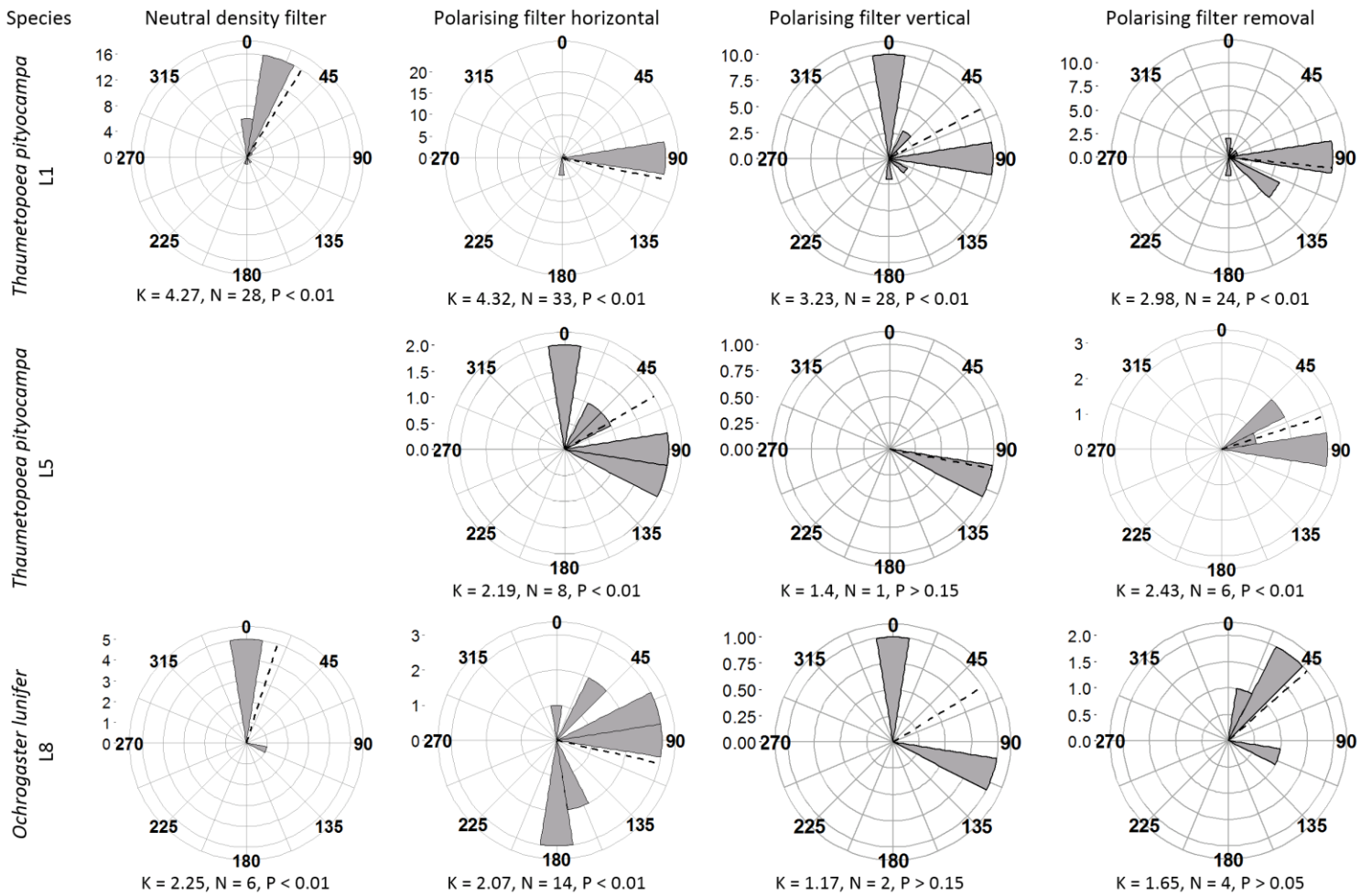


Figure 3.3. Circular histogram of the angular differences after subjecting first and final instar *Thaumetopoea pityocampa* and final instar *Ochrogaster lunifer* processions/single larva (singleton) to four treatments. Four treatments are: neutral density filter 40% transmission, linear polarising filter (PF) held horizontally, PF held vertically and PF removal after PF exposure. The angular difference plotted is the difference between the initial orientation a procession/singleton was traveling and after treatment exposure. The angular difference ranged from 0˚ (no change) to 180˚ maximum. The numbers on the left of each circular histogram correspond to the rings inside the circle and it represents the frequency of a procession/singleton changing/unchanging angles. The dashed black line represents the mean of the frequency. K and N represents the Kuiper's test statistic and number of processions/singletons, respectively.

Morphological analyses

Larvae of both species have six stemmata on each side of the head (Fig. 3.4 a). Stemmata II – VI appear shiny, while stemma I appears opaque (Fig. 3.4 b). SEM revealed that ⅔ of the surface of stemma I pointing upwards is rugged and ⅓ pointing downwards is smooth (Fig. 3.4 c); whereas stemmata II – VI are smooth all around (Fig. 3.4 d and Fig. 3.5). The dark pigment in the lower third indicates that stemma I samples the dorsal part of the visual field (Fig. 3.4 b). The rugged surface of stemma I is a diffuser and a spatial low-pass filter for incident light. A similar opaque optical structure has evolved in the DRA ommatidia of honeybees and locusts (Meyer and Labhart, 1981; Homberg and Paech, 2002). These structures enlarge visual fields, decrease acuity and are thought to reduce the visual clutter caused by clouds, thereby stabilising skylight polarisation vision (Meyer and Labhart, 1981). We hypothesised that stemma I could harbour photoreceptors optimised for polarisation vision. Indeed, LM and TEM images showed a single-tiered, cushion-shaped light-sensitive structure (rhabdom), formed of two pairs of photoreceptors with orthogonal and aligned microvilli (Figs. 3.4 e and f, 3.6 and 3.7). Other stemmata have flower-shaped rhabdoms, formed of > 3 photoreceptors in the distal and proximal tier; their microvilli are neither aligned nor orthogonal (Figs. 3.4 g and h, 3.6 and 3.7). The rhabdom in stemma I is similar to rhabdoms in DRA ommatidia of Noctuid and Crambid moths, while the rhabdoms in stemmata II-VI resemble the lobed rhabdoms in the main retina of adult moths (Meinecke, 1981; Belušič et al., 2017). The conspicuous dioptrical apparatus and rhabdom clearly indicate that stemma I is optimised for polarisation vision, while other stemmata do not show such optimisations and are thus suitable for general vision, such as intensity or colour contrast detection.

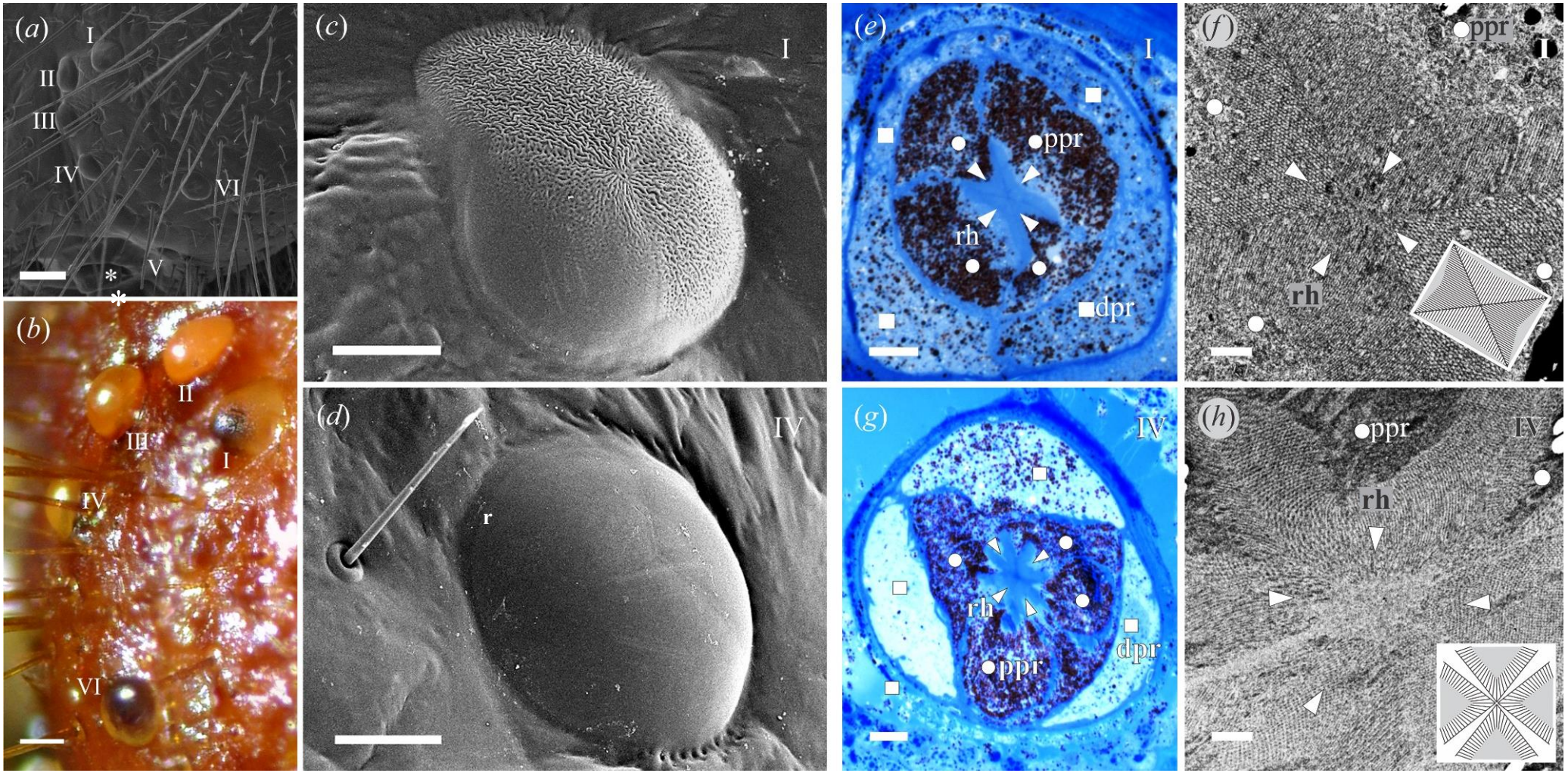


Figure 3.4. Anatomy of *Ochrogaster lunifer* (***a – c, f***) and *Thaumetopoea pityocampa* (***d, e, g, h***) stemmata. **(*a, b*)** Side view of left head capsule of final instar larva; scanning electron micrograph (SEM) (***a***), stereomicrograph (***b***). Numbers refer to stemma I-VI and * refers to the antenna base. **(*c - e*)** Stemma I; SEM (***c***), light micrograph (LM; ***d***), transmission electron micrograph (TEM; ***e***). **(*f - h*)** Stemma IV; SEM (***f***), LM (***g***), TEM (***h***). The top right corner in (***e***) and (***h***) are schematic diagrams of the rhabdom illustrating the microvillar orientation. Scale bars: (*a*) 200 µm; (*b*) 100 µm; (*c*,*f*) 50 µm; (*d, g*) 10 µm; (*e, h*) 1 µm.

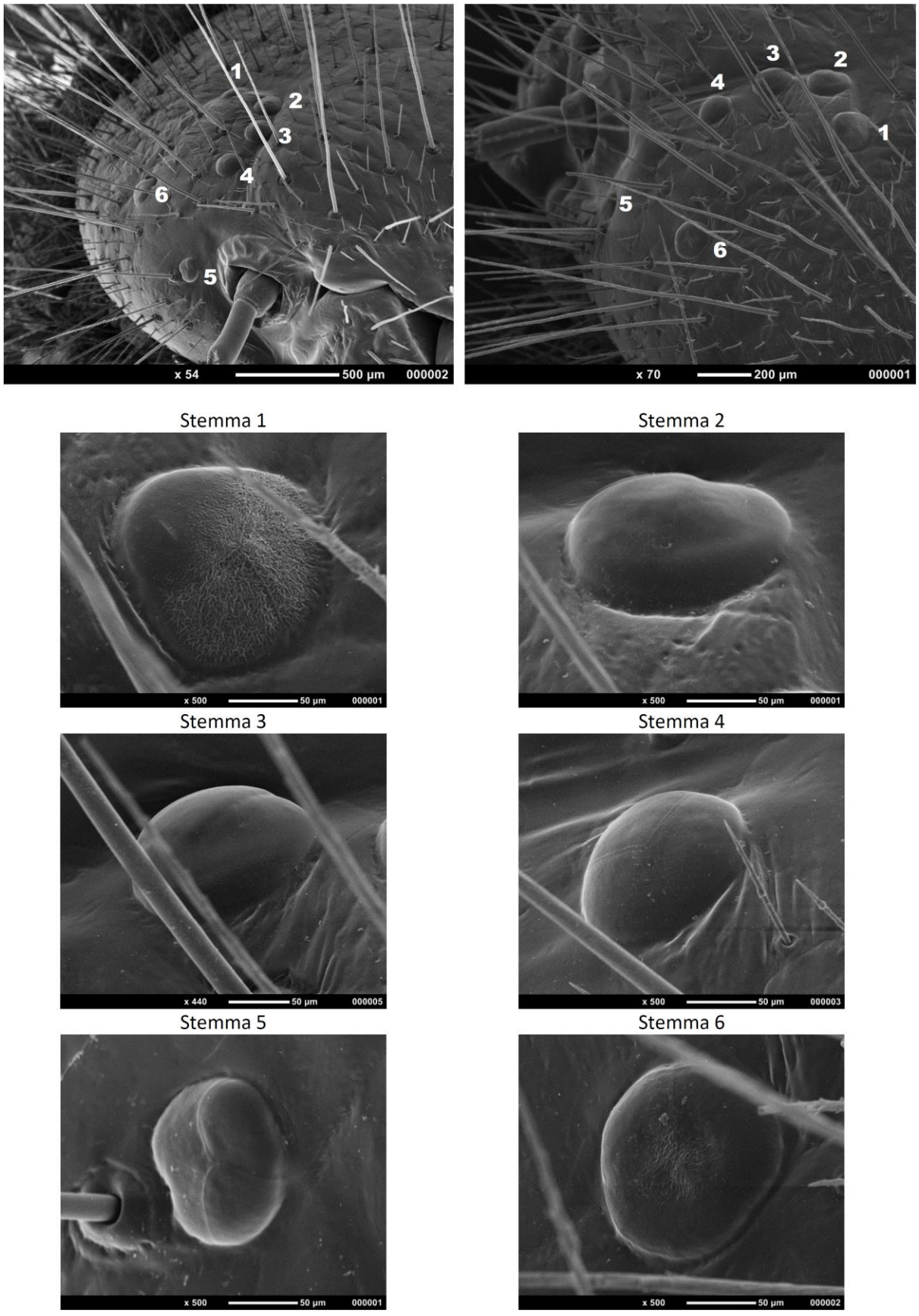


Figure 3.5. Scanning electron micrographs of final instar *Ochrogaster lunifer* caterpillar. Images taken by Erica Lovas from The Centre for Microscopy and Microanalysis, The University of Queensland.

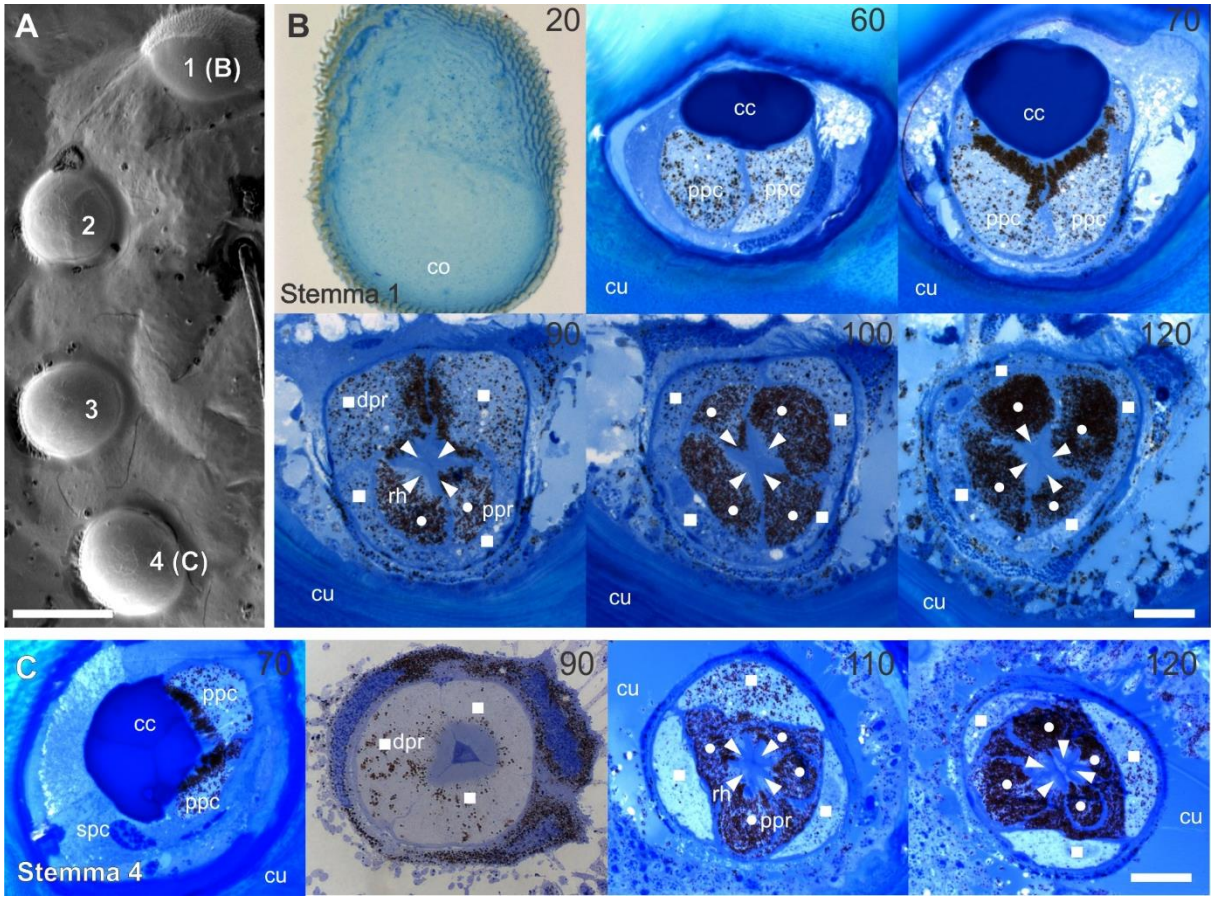


Figure 3.6. Anatomy of stemmata 1 and 4 in *Thaumetopoea pityocampa* caterpillar; **A)** Scanning electron micrographs, **B)** and **C)**, light micrographs at depths, indicated in top right corner of each panel. **A)** Last instar stemmata 1-4 **B)** Serial sections of stemma 1 (20 µm, corneal lens; 60 µm and 70 µm, crystalline cone and pigment cells; 90-120 µm, rhabdom; note the alignment of the rhabdom along the z-axis). **C)** Serial sections of stemma 4 (70 µm, crystalline cone and pigment cells; 90 µm, tri-lobed rhabdom, distal tier; 110-120 µm, four-lobed rhabdom, proximal tier).

Annotations: **co**, cornea; **cc**, crystalline cone; **ppc**, primary pigment cells; **spc**, secondary pigment cells; **cu**, cuticle; **dpr**, distal photoreceptors; **ppr**, proximal photoreceptors; **rh**, rhabdomere.

Scale bars: A) 100 µm, B) and C) 20 µm.

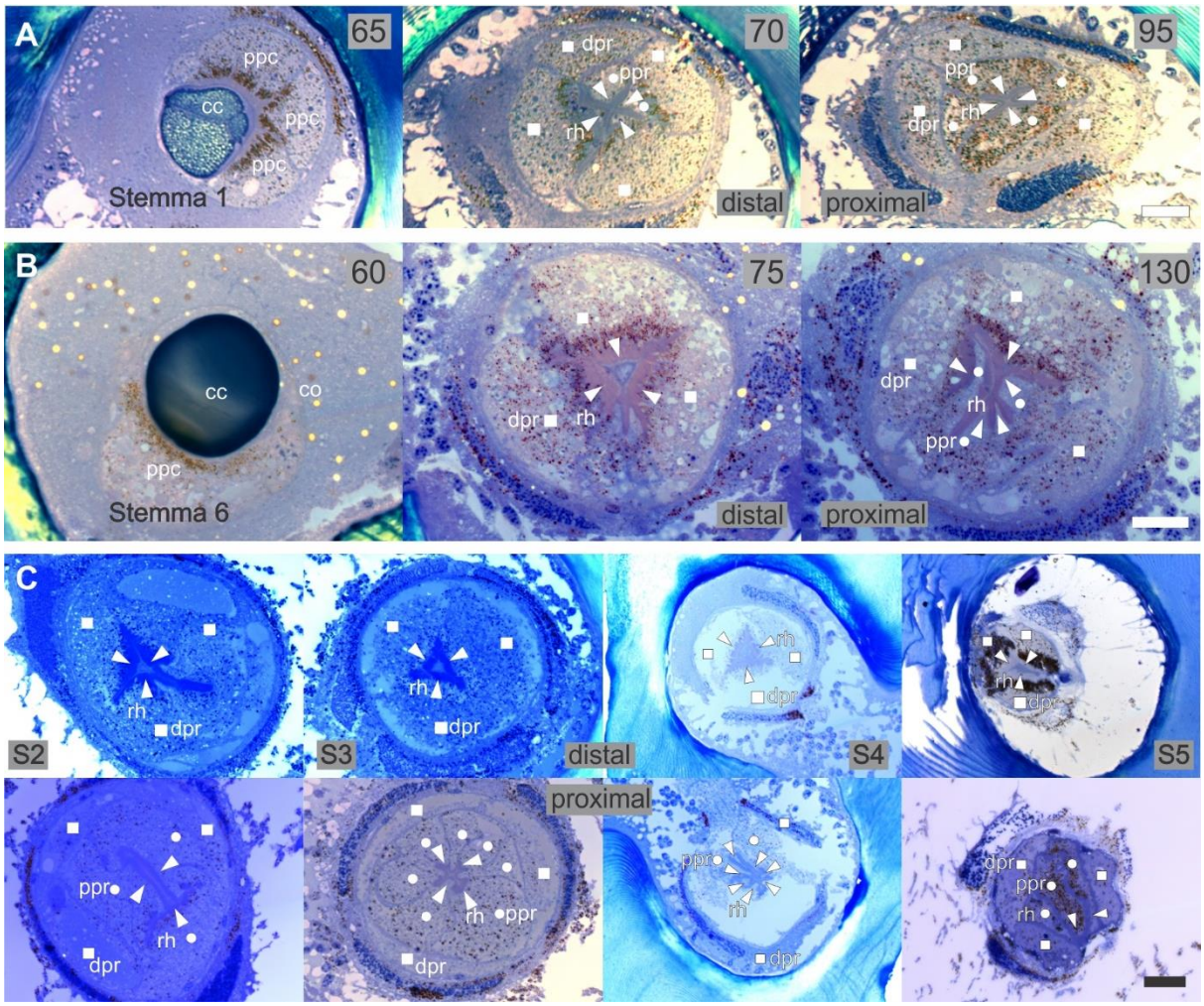


Figure 3.7. Light micrographs of stemmata 1 - 6 in *Ochrogaster lunifer* at depths, indicated in top right corner of each panel. **A)** Serial sections of stemma 1 (65 µm, crystalline cone and pigment cells; 70 and 95 µm, rhabdom; note the alignment of the rhabdom along the z-axis). **B)** Serial sections of stemma 6 (60 µm, crystalline cone and pigment cells; 75 µm, tri-lobed rhabdom, distal tier; 130 µm, four-lobed rhabdom, proximal tier). **C)** Sections of the distal and proximal tiers of stemmata 2-5 with tri-lobed (distal) and multi-lobed (proximal) rhabdoms.
Annotations: **co**, cornea; **cc**, crystalline cone; **ppc**, primary pigment cells; **spc**, secondary pigment cells; **cu**, cuticle; **dpr**, distal photoreceptors; **ppr**, proximal photoreceptors; **rh**, rhabdomere.
Scale bars: A), B) and C) 20 µm.

3.5 Conclusion

We have demonstrated that stemma I in social caterpillars is anatomically similar to a single ommatidium in the DRA of adult moths. Although it is a non-imaging polarisation detector that samples only a fraction of the skylight pattern, a pair of stemmata is still capable of assisting spatial orientation, similar to the specialised simple eyes in certain spiders (Dacke et al., 1999). Larval locomotion was manipulated with a PF, which showed that polarisation

vision is one of the mechanisms that guided their social behaviour. Skylight polarisation pattern enables pre-pupation processions to have a constant heading away from the nest to disperse further and to avoid drift, which could result in a loop. Stemmata with a rugged surface are absent in solitary caterpillar species studied to date (Ichikawa and Tateda, 1982; Lin et al., 2002). It will be interesting to investigate if this trait is conserved in Notodontidae or evolved independently in different families. Social behaviour and organised locomotion exert strong selective pressure on the visual organs, which in turn robustly convey visually guided behaviour, even in the simplest structural form.

3.6 Acknowledgements

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Chapter 4

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

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The manuscript was edited according to the thesis evaluator's feedback and merged with the original text and supplementary material in this thesis chapter.

4.1 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 in all orientations on the host tree branches. *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*

4.2 Introduction

Herbivorous insects' decision where to feed and live on the plant depends on various abiotic and biotic factors. Insect movement on plants 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 hide or move to different parts of the plant or change host plant to avoid detection by natural enemies. Herbivores such as caterpillars have evolved various strategies to avoid detection, and repel or escape from natural enemies through chemical, physiological, morphological, and behavioural defences (Greeney et al. 2012). Caterpillars 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 and Costa, 1999).

There are at least 300 species from 27 families of Lepidoptera with gregarious larvae (Costa and 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 and Fiedler, 1999). There are three feeding patterns of gregarious larvae: patch-restricted foraging, nomadic foraging and central-place foraging (Fitzgerald and 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 synchronise 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 the whole larval life. Shelters are multi-functional and assists in larval thermoregulation, development, and defence against predators (Fitzgerald and 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 and Roland, 1997). Despite the costs, the evolutionary benefit from the presence of other individuals in a communal shelter is still greater (Reader and 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; moths oviposit in mid to late summer and the larvae hatch ca. 4 weeks after and continue larval development through to spring when the final instar larvae procession to find a pupation site. The pupae remain underground until the following summer or may diapause up to 8 years (Salman et al. 2016). The species remain gregarious from the hatching to pupation. 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 and Costa, 1999). This description, first reported by Fabre (1898), was then interpreted by Balfour-Browne (1925) and later by Fitzgerald and Costa (1999); however, this reporting was not supported by quantitative analyses. In summer, when *T. pityocampa* are eggs and early larval instars, temperatures are warm and many invertebrate predators and parasitoids 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 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 and 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 and 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 (Roques et al. 2015a). A similar species to *T. pityocampa, T. pinivora*, have spring-summer feeding larvae that do not construct tents (Ronnås et al. 2010). This further explains the importance for *T. pityocampa* larval colonies to build winter tents when temperature decreases.

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 and Willer, 1983; *Malacosoma californicum pluvialis* (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 and 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 *T. pityocampa* females are selective in oviposition location on the tree, so that it will be more favourable for larval development, as predicted by the preference performance hypothesis (Gripenberg et al., 2010; Rivas-Ubach et al., 2015). 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 southerly facing part of the host tree for maximum insolation, and this may result in energetic costs and increased risk of predation 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.

4.3 Materials and Methods

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 m^2 scattered *P. nigra* stand with trees approximately 1 – 6 m in height, growing as isolated trees or small groups in a dry meadow (Fig. 4.1). *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 the same stand at Precastio, a total of 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. In this study site, defoliation was uncommon however, any host trees that showed previous defoliation were not used for the experiment. 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 4.1) (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.

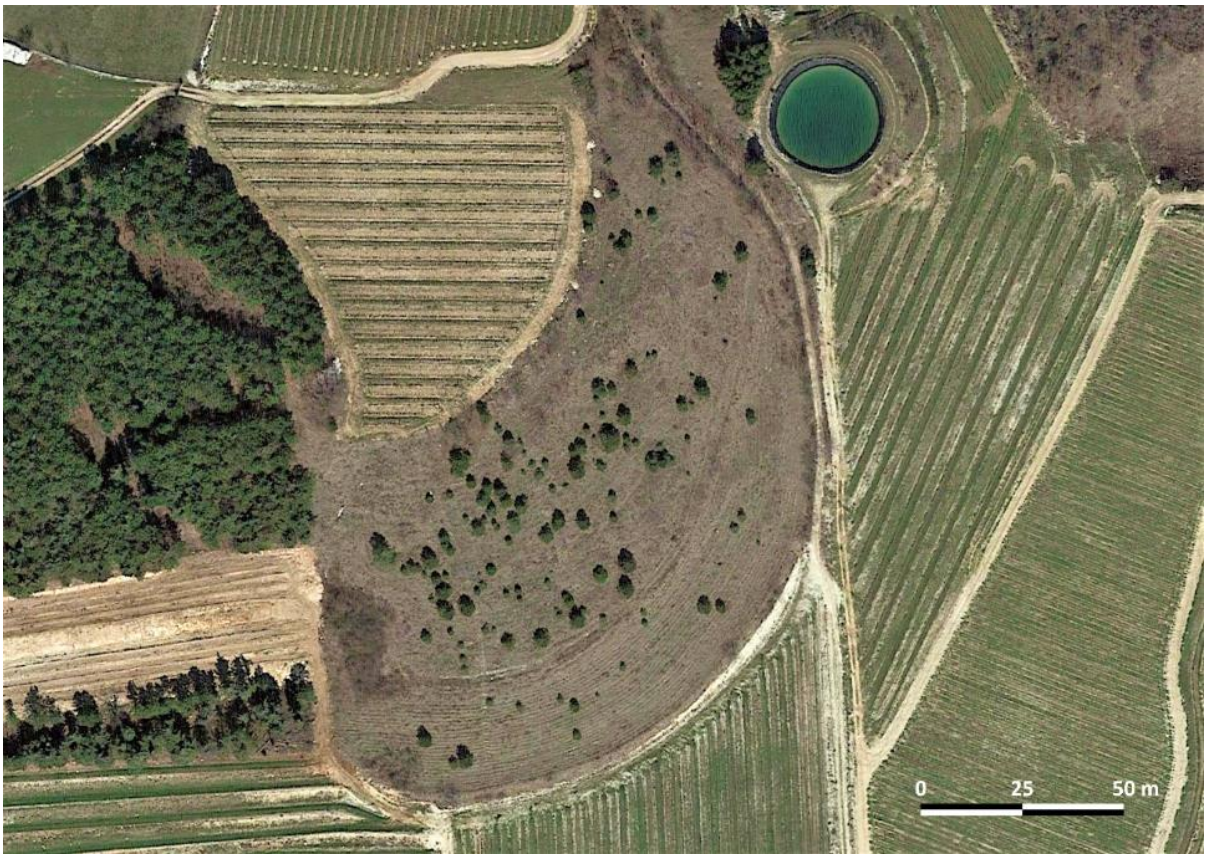

Figure 4.1. *Pinus nigra* stand in Precastio, Verona Italy, a study site where *Thaumetopoea pityocampa* egg batches were transplanted on isolated trees to investigate larval development and movement. Satellite image was taken on 22/03/2018 by Google Earth Pro, image accessed 06/03/2020.

Table 4.1. 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

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 (Grison et al., 1951; Battisti et al., 1986; Battisti, 1988a) 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 collected earlier on site 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 (colonies per orientation ranged from 17 to 21, numbers are unequal due to extinct colonies). 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.

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. 4.2 A 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.

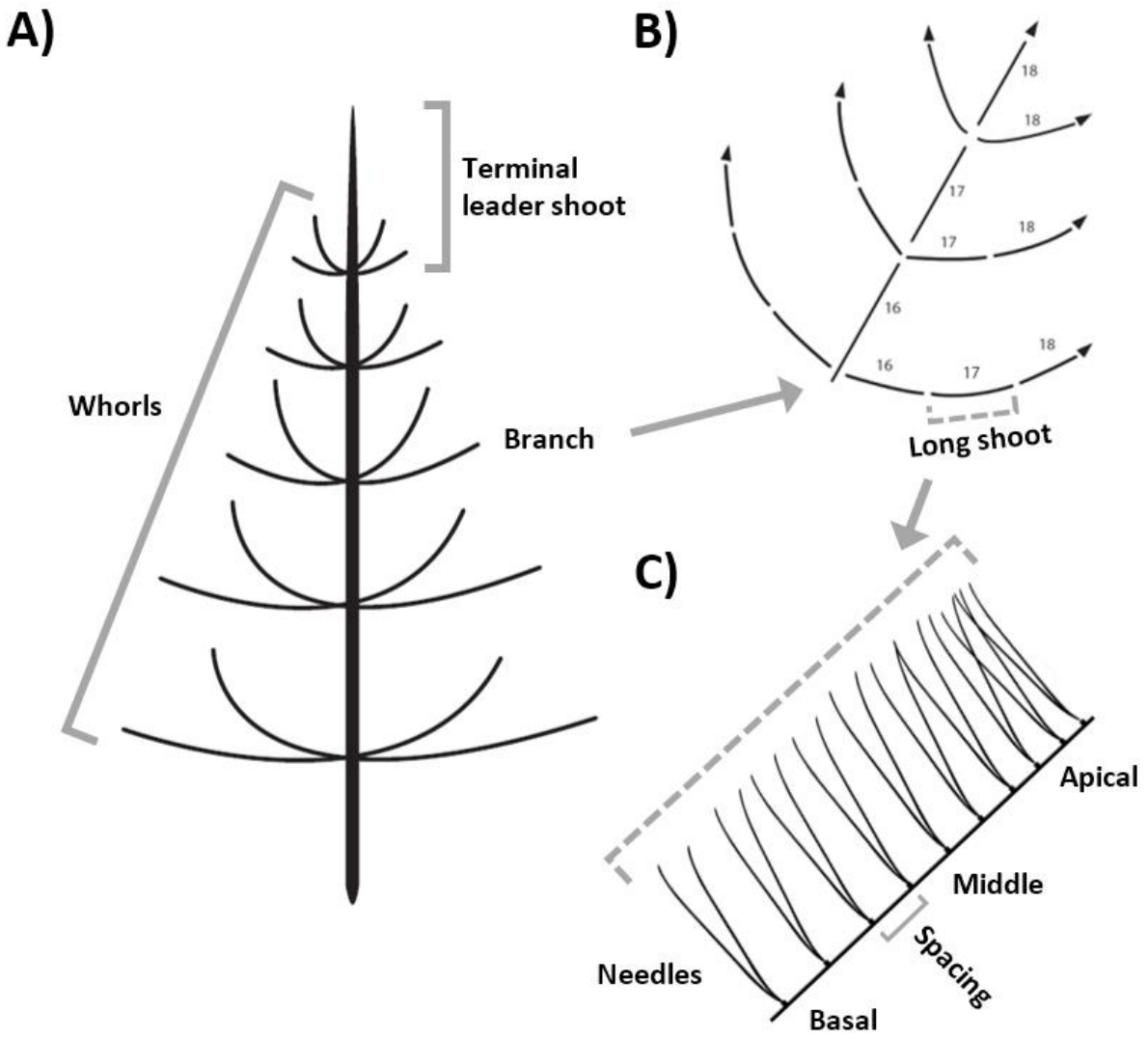

Fig. 4.2. Simplified schematic diagrams of Austrian pine *Pinus nigra* tree and its characteristics. **A)** Pine tree with lateral branches organised in whorls, each whorl is formed in a specific year. The most apical shoot at the top is growth from the current-year and referred as the terminal leader 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.

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. 4.2 B).

Characteristics of the pine shoots where new tents were established were: long shoot year, shoot position (basal, middle or apical), length of the pine needles and distance between each dwarf shoot (pine needle pair) at the three shoot positions (Fig. 4.2 C). 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.

Statistical analyses

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.

Survival of Thaumetopoea pityocampa colonies

To determine the survival of the colonies, a generalised 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 and 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.

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 characterised by different abiotic conditions: younger instars are active in late 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 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 larval instar divided by the total number of movements for that colony. The proportions for each larval instar from all the colonies were calculated to get the average. The average proportion 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 larval instar (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).

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.

4.4 Results

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 57 colonies that hatched, 48 egg batches/colonies had at least one larva that survived until L5. None of the host trees were completely defoliated, and the maximum defoliation on available pine needles on a tree was

approximately 20% (Fig. 4.3). The life history duration for *T. pityocampa* larvae in Precastio are displayed in Table 4.2. The average number of eggs hatched per egg batch from Precastio was 108 ± 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 statistic: 1.48, N = 35, P > 0.15; Fig. 4.4).

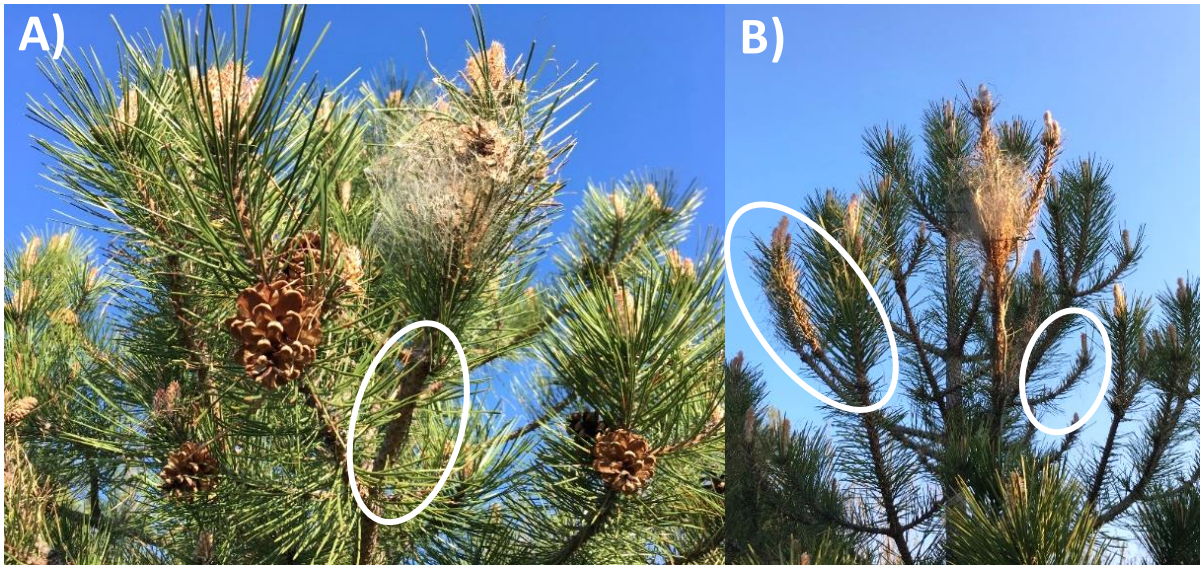

Figure 4.3. *Thaumetopoea pityocampa* tents and feeding damage (white circles) on *Pinus nigra* at Precastio, Verona Italy. **A)** Young larval instar temporary tent with feeding damage underneath the tent. **B)** Older larval instar winter tent with feeding damage directly next to the tent, and other branches of different whorls. Images taken by Andrea Battisti.

Table 4.2 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
N	44	42	49	44	48

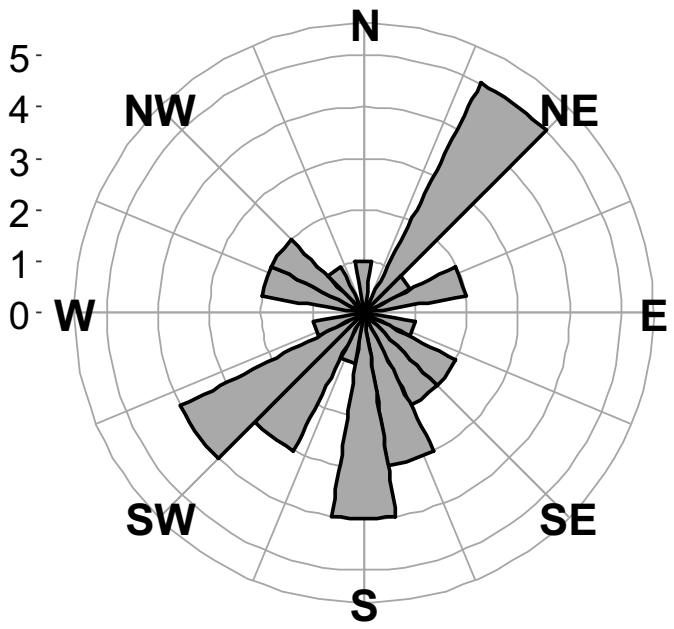

Figure 4.4. Natural egg batch orientation on *Pinus nigra* oviposited by *Thaumetopoea pityocampa* females at the end of summer 2018 in Precastio, Verona Italy (N = 35). The numbers on the left side of the diagram correspond to the rings.

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 (SE ± 0.25, $N = 48$). After hatching, all colonies build their first tent beside or in very close proximity (ca. 2 cm) to the egg batch, which is almost always on the 2018 shoot. After the first tent, the colonies abandoned and built a new tent almost always on a different shoot (90% of colonies). 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. 4.5 a). 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. 4.5 b). 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: $X^2 = 10.96$, df = 3, $P < 0.05$). 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 by younger and older instars of each egg batch orientation are shown in Figure 4.6 (for detailed movements of each larval instar see Fig. 4.7). Older instars built the tents in a more southerly orientation compared

to younger instars. The average proportion of movements were significantly different across the four larval instars, with L2 moving the most and L4 moving the least (GOF: $X^2 = 17.54$, df = 3, $P < 0.001$; Fig. 4.8). Total average distance travelled by colonies that survived until L5 was 341 ± SE 33.5 cm (N = 36). Average distance travelled by each larval instar 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. 4.9).

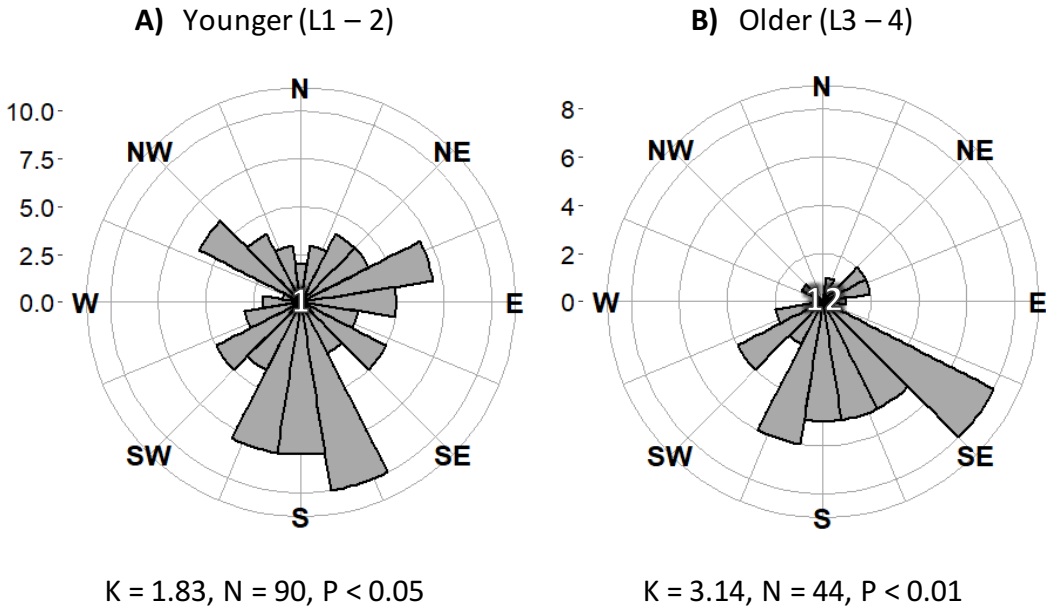

Figure 4.5. Distribution and frequency of where and how many **A)** younger (L1–2) and **B)** older (L3–4) *Thaumetopoea pityocampa* larvae built a tent on the host tree irrespective of transplanted egg batch orientation. The numbers on the left of each diagram correspond to the rings inside the circle 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.

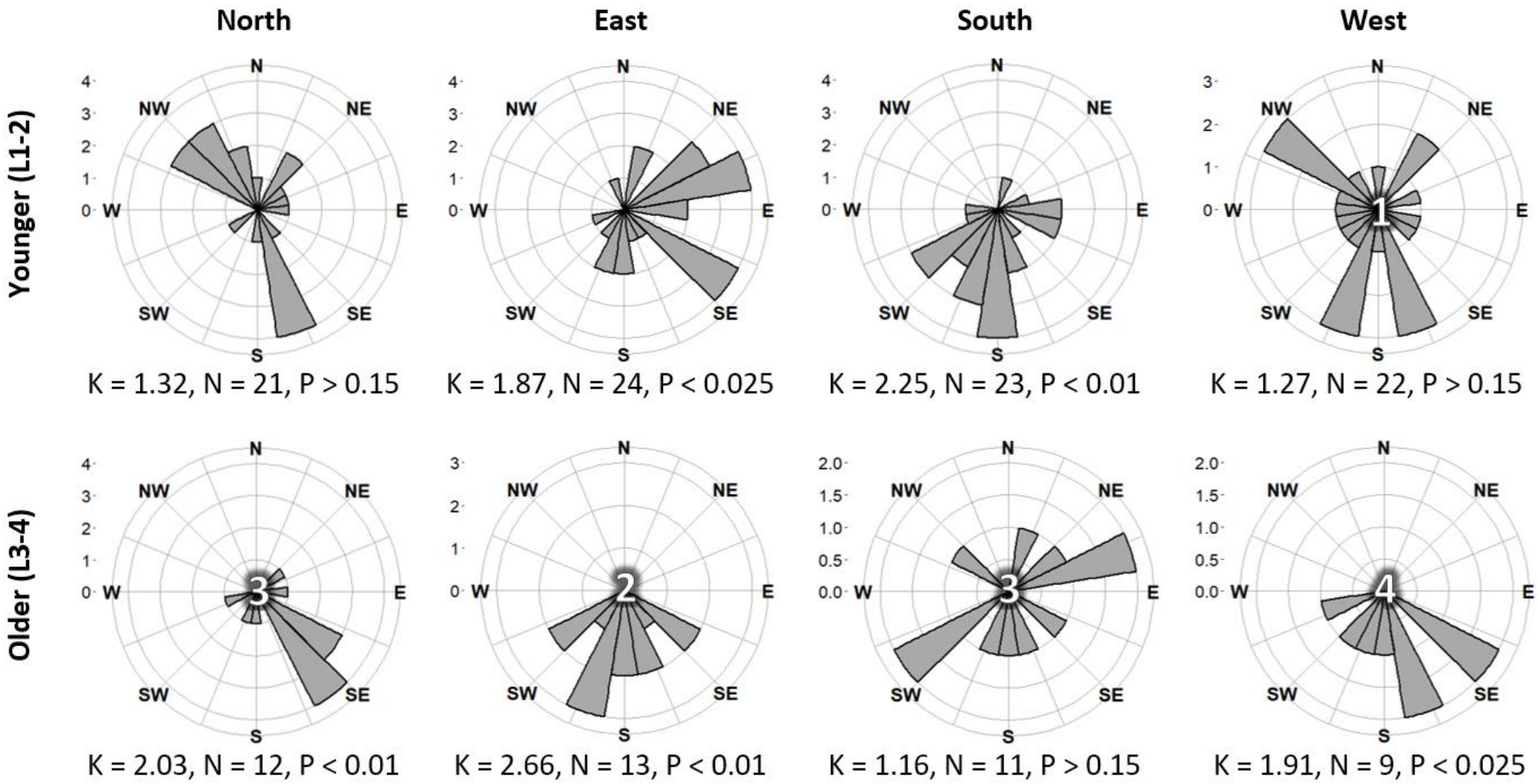

Figure 4.6. Distribution and frequency of tent orientations built by younger (L1–2) and older (L3–4) *Thaumetopoea pityocampa* larvae from different initial transplanted egg batch orientations (North, East, South and West). The numbers on the left of each diagram correspond to the rings inside the circle 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.

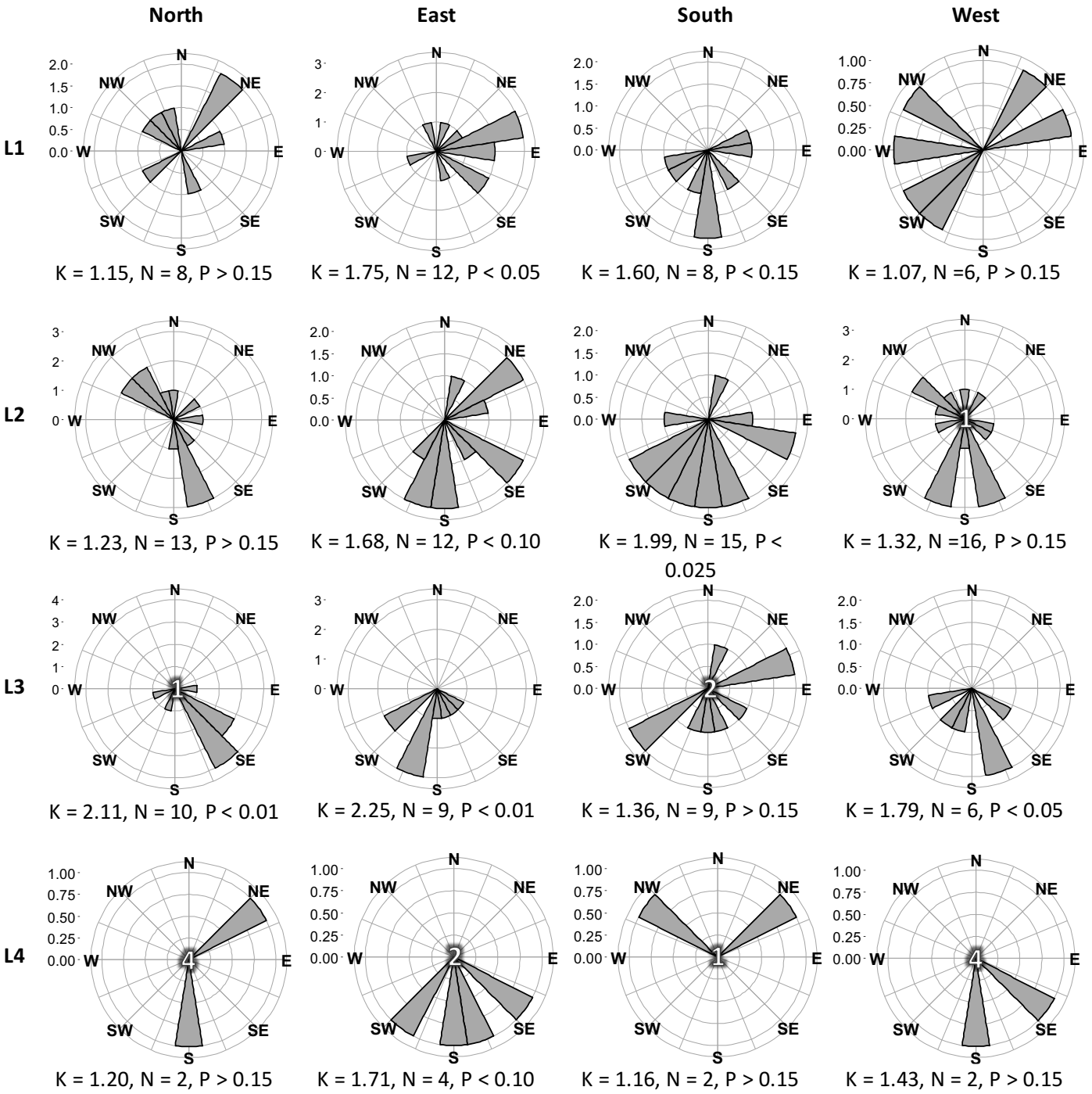

Figure 4.7. Distribution and frequency of tent orientations built by each *Thaumetopoea pityocampa* larval instar from different transplanted egg batch orientations (north, east, south and west). The numbers on the left of each diagram correspond to the rings inside the circle and it represents the frequency 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.

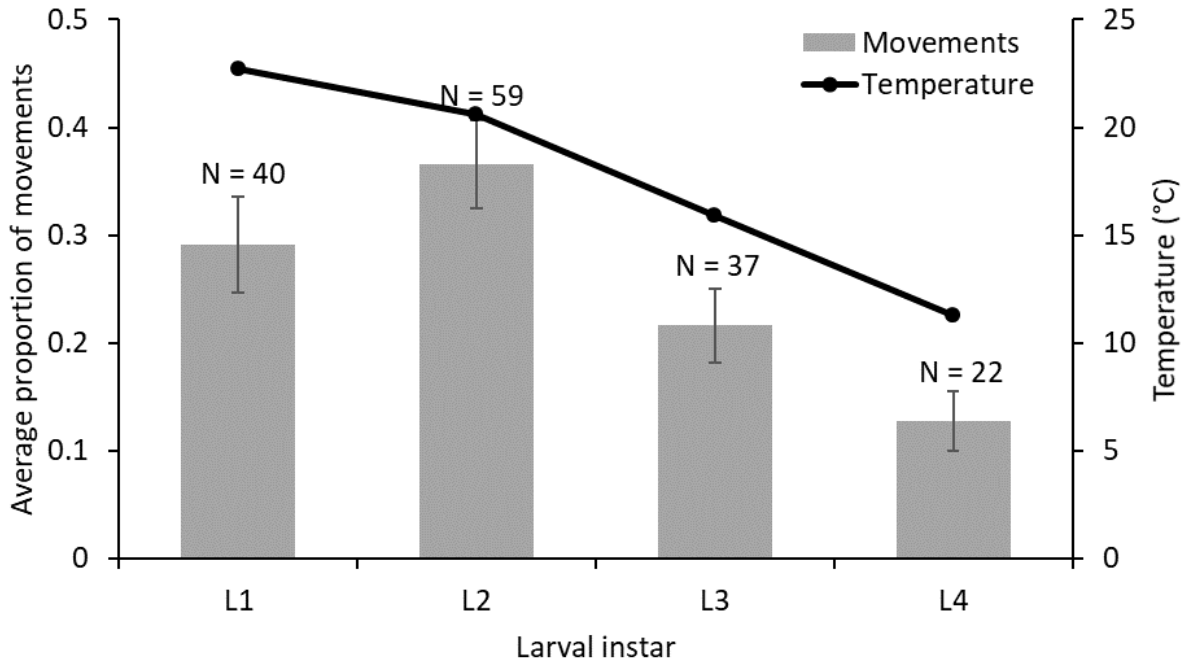

Figure 4.8. Average proportion of movements (158 tents built in total) by each *Thaumetopoea pityocampa* colony (N = 57) throughout different instars by all egg batch orientations. Number above each bar represents the total number of movements/tents built from all colonies within the instar. Average daily temperature for each instar is displayed as a black line. There were no movements/tents built by the final instar larva (L5).

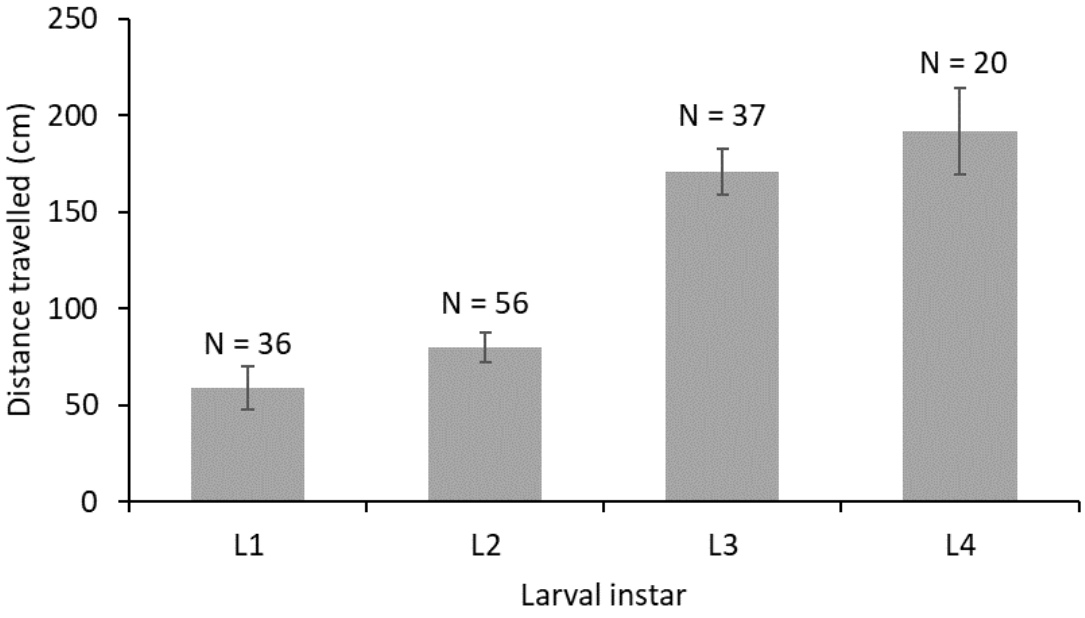

Figure 4.9. Average distance travelled (cm) to build a new tent by various larval instars of *Thaumetopoea pityocampa* colonies (N = 57) from all egg batch orientations. Number above each bar represents the total number of movements/tents built from all colonies within the larval instar. There were no movements/tents built by the final instar larva (L5).

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 ± 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. 4.10). 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: $F_{2,45} = 12.48$, $P < 0.001$). Younger instar *T. pityocampa* larvae ate an average of 21 needle pairs (SE ± 1.35) at each tent location.

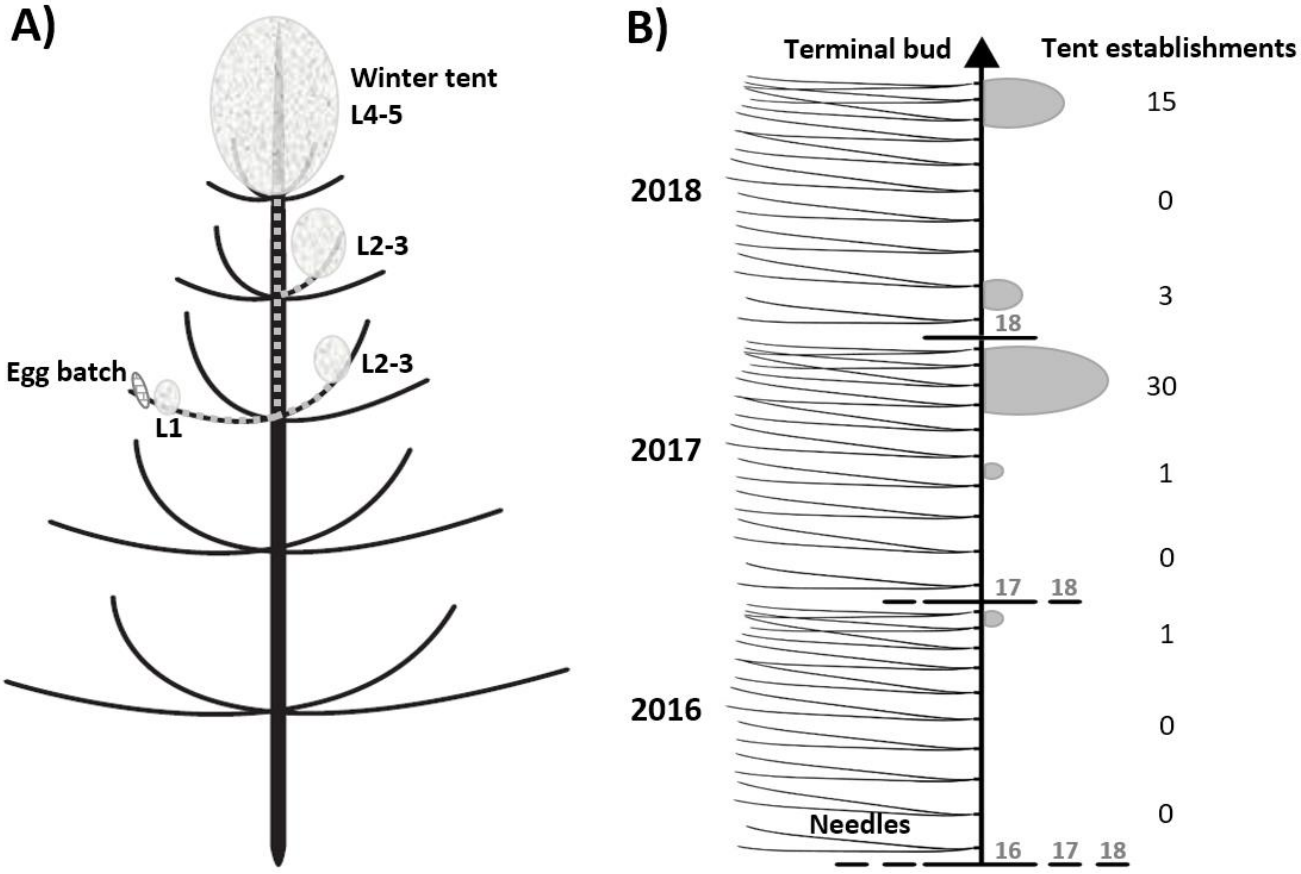

Figure 4.10. A) Schematic diagram of *Thaumetopoea pityocampa* colony movements and tent establishments (light grey ovals) on *P. nigra*. Dashed grey line represents the movements between abandoned and new tents, starting from the tent near the egg batch to the final winter tent at the most apical part 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.

4.5 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 to 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 and 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 lanestris* Linnaeus (1758) (Lasiocampidae) (Ruf and 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 and 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 and 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 and 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 and 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; such as *M. americanum* (Fitzgerald and 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 and 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 southernmost range of *T. pityocampa* occurrence record, also had nests positioned in the southerly orientation (Zamoum, 1998). That 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 and Battisti 2011). Reducing the time spent as younger instar larvae can minimise the overall risk of mortality by invertebrate predators (Werner and 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 (Balfour-Browne, 1925; Fabre, 1898; Fitzgerald and 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. The first tents built by younger instar larvae were still present on the tree throughout the season and only the eaten needles became dried (M. Uemura, personal observation 2018); therefore, losing rigidity making it unsuitable for tent construction was not supported. 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. 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. disstria* (McClure and Despland 2011), plant defences and/or weather that *T. pityocampa* colonies encounter throughout different larval instars. Younger instars are more vulnerable to predation compared to older instars because they are more suitable prey for invertebrate predators, for example: spiders, lacewing larvae, and syrphid larvae (Auger-Rozenberg et al. 2015). 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. Previous studies found that feeding damage by *T. pityocampa* larvae caused the pine to produce increased concentrations of monoterpenes (Achotegui-Castells et al. 2013), polyphenolics (Rivas-Ubach et al. 2015) or both (Moreira et al. 2013). Terpenes and phenolics have herbivore-deterrent properties however, more research is needed to confirm if these chemicals impact *T. pityocampa* survival. Emissions of volatile terpenes produced by the pine after folivory can attract parasitoids and predators (Mumm and Hilker, 2006); and in turn may have driven younger instar *T. pityocampa* larvae to move frequently. 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). Tent relocation may decrease the risk of disease transmission derived from

frass and dead larvae which may thrive from warmer temperatures and younger instar larvae are more immunologically vulnerable compared to older instar larvae. 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 and 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 compensates for 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. The 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 the 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: Hesperiidae) 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.

4.6 Acknowledgements

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Chapter 5

Polyethism in tent construction and maintenance in a social caterpillar (*Thaumetopoea pityocampa*)

The following chapter is currently in preparation for publication as: Uemura, M., Zalucki, M.P., Battisti, A. Polyethism in tent construction and maintenance in a social caterpillar (*Thaumetopoea pityocampa*).

5.1 Abstract

Social organisms require organisation to be efficient and functional as a colony. Throughout the whole larval life, *Thaumetopoea pityocampa* live gregariously in their communal tent with siblings and even conspecifics from other colonies. In this study, we explored how *T. pityocampa* caterpillars determine orientation on the tree and the social behaviour associated with tent construction. The location of the tent built on the host tree and silk application on the tent were both predominant in the south. The caterpillars can determine spatial orientation/direction from early larval instar through a pair of specialised stemmata that detect skylight polarisation patterns, which they used to determine where is south for tent construction and maintenance. When environmental conditions are optimal, the tent is maintained daily at around sunset by a few individuals which are predominantly male. The male caterpillars emerge from the tent first and spin silk on the tent for expansion and strength. During this time, these caterpillars are exposed to parasitoids and consequently, nearly half of parasitised caterpillars from the colony were male caterpillars maintaining the tent. Using non-destructive field collection methods with wildlife cameras and a novel frass counting apparatus, we were able to determine the foraging behaviour of early instar *T. pityocampa* colonies. The use of wildlife cameras on small-sized insects in the field was effective, and this opens opportunities to other researchers to simultaneously monitor insects over long time periods without disturbance. Exploration into tent construction behaviour of *T. pityocampa* caterpillars indicated inter-individual differences which benefitted the colony.

Keywords: Animal behaviour, colony, cooperation, larva, nest, processionary caterpillar, sex differences, social organisation

5.2 Introduction

Living in social groups is fundamental for the success of some species. A social group is defined as a set of conspecifics that interact and remain together for a period of time (Wilson, 1975). Sociality has interested many scientists and has been researched extensively in invertebrates and vertebrates, each with various levels of sophistication. In social organisms, cooperation provides a benefit to other individuals and has evolved because of this benefit (West et al., 2021). When relatedness among individuals is higher and the females mate monogamously or with few males, they are more likely to cooperate (West et al., 2021). There are two theoretical explanations for cooperation: direct fitness benefits and indirect fitness benefits. Direct fitness benefits occur when cooperation can increase the reproductive success of the actor (West et al., 2007; 2021). Indirect fitness benefits is when cooperation is directed towards related individuals and increases reproductive success of the other individual, known as kin selection (West et al., 2007; 2021).

Cooperation may come in the form of some individuals performing specific behavioural tasks/activities for the colony (polyethism). Wellington (1957) reported polyethism in social caterpillars of *Malacosoma pluviale* and described that different levels of activity were performed by caterpillars with various personality traits. Three types of larval traits were recognised, Type I and two Type II. Type I were described as caterpillars that are capable of independent directed movements. One Type II consisted of caterpillars incapable of independent directed movement (Type IIa). The other Type II were caterpillars that were 'sluggish' and moved less often than the other types (Type IIb). Type I caterpillars fed more regularly, had faster development and constructed several tents which benefited the colony compared to colonies with more Type IIa and IIb caterpillars (Wellington, 1957). Edgerly and Fitzgerald (1982) reported that in a closely related species to *M. pluviale*, *Malacosoma americanum,* caterpillars did not have traits as described by Wellington (1957).

Polyethism (individual differences) within colonies have also been described in social spiders (Keiser et al., 2014), sawfly larvae (Tostowaryk, 1971; Weinstein and Maelzer, 1997; Hodgkin et al. 2014) and other caterpillar species (Underwood and Shapiro 1999a; Fitzgerald 2003; McClure et al. 2011). A handful of individuals within a sawfly colony displayed leadership roles, where leaders position themselves on the periphery of the aggregation and were more exposed to predators and parasitoids compared to others positioned on the inside (Weinstein and Maelzer, 1997). Similarly, in pine processionary caterpillars, *Thaumetopoea*

pityocampa, the female caterpillars take the role of leading processions (single file head-to-tail formation) more often than males, exposing themselves to more risk (Fitzgerald, 2003). In another tent-building social caterpillar species, *Eucheira socialis*, more male caterpillars spun silk and tended nest maintenance than females (Underwood and Shapiro 1999a). Polyethism in these species show little resemblance to the hard-wired division of labour found among eusocial insects. However, having behavioural/individual differences within a colony could possibly be an important driver for group performance (Keiser et al., 2014).

In this study, we wanted to explore polyethism in *T. pityocampa* caterpillars which spend majority of their life in a nesting structure (tent) with their siblings and conspecifics of other colonies. In a species with gregarious behaviour from egg to final instar larva, it is expected that individuals have highly cooperative organised activities to perform well as a colony. It is known that *T. pityocampa* larval colonies spin a voluminous amount of silk to build their tent to protect the colony from predators and severe environmental conditions. However, quantitative analyses on the mechanism and behaviour of building a tent has not been recorded in *T. pityocampa*. Wellington (1957) described different behavioural traits of *M. pluviale* caterpillars, which may also be the case for *T. pityocampa* colonies to drive group survival. We asked the following question: do certain *T. pityocampa* individuals perform specific tasks/activities to benefit the colony? We investigated the tent building behaviour of first (L1) to fourth (L4) larval instar *T. pityocampa* colonies in the field through time-lapse images taken with wildlife cameras. Orientation of the tent and silk application, caterpillar vision, foraging activity, frass production and interactions with parasitoids were also investigated.

5.3 Materials and Methods

Model organism

In summer, female *T. pityocampa* moths oviposits all her eggs in one batch (150 - 350 eggs) on the outer foliage of a pine tree and the eggs hatch in a month (Battisti et al., 2015). The species has a univoltine lifecycle and is gregarious from egg to final instar larva, with five larval instars, and later instars feed throughout the winter. Neonates build their first temporary tent at the oviposition site, and the second generally on the same branch (Uemura et al., 2020a). The tent acts as protection against predators, parasitoids and environmental elements such as wind, rain and the cold (Hódar et al., 2003; Branco et al., 2008). Later larval instars (L3-4) construct a thick-walled permanent tent (winter tent) at the most outer parts of the tree, where they spend the winter until spring when they move to the ground for pupation (Uemura et al., 2020b). From approximately third instar onwards, caterpillars become nocturnal foragers and return to the tent before sunrise. The winter tent facilitates thermoregulation during the day and assists aggregated caterpillars to digest the food they consumed throughout the night (Pérez-Contreras et al., 2003). When final instar larva are ready to pupate, they leave the tent and crawl in a procession on the ground to search for a pupation site, which is usually in brightly lit areas of the environment (Uemura et al., 2020b).

Preliminary experiments on larval tent construction and feeding

From late August to end of September 2018, experiments to monitor feeding activity of early instar larvae (L1-2) were conducted at an indoor facility close to the study site (Precastio, Italy (45°31′ N, 11°10′ E)) in Tregnago, Italy (45°51’ N, 11°17’ E). Three *Pinus nigra* branches with one L1 *T. pityocampa* colony each were cut from pine trees at Precastio using secateurs. The cutting was placed into a conical flask containing water to prolong the freshness of the pine needles for *T. pityocampa* caterpillars to feed on. A 21 cm diameter rotating disc was placed directly underneath the tent of each colony to collect the fallen frass produced by the caterpillars. The rotating disc was built by attaching a circular plastic plate on a 24 h mechanical plug-in timer using Scotch clear extreme fastener strips. The plate had a circular paper template with 24 equal slices to represent each hour of the day and the position of the tent was marked with a pencil on one of the slices. The timer was on continuously and a picture of the disc was taken from above at 17:00 (GMT +1) every day using an iPhone (Apple, California, USA). After capturing the photo, the disc was taken off from the timer and cleaned to remove the frass

and placed back on the timer for the next 24 h. This was repeated every day for 38 consecutive days. The images were cropped to each slice and the frass on each slice counted digitally using ImageJ version 1.53 e with the Analyze Particles function (function and results in Supplementary Materials S1 and S2, respectively).

From November 2018 to February 2019, outdoor trials using a Hyperfire wildlife camera (RECONYX, Wisconsin, USA) were conducted to capture L4 *T. pityocampa* colony activity every 15 min. At the onset of darkness, an infrared beam is automatically activated when a photograph is taken, and this had no impact on larval behaviour. The images from preliminary trials were clear and suitable for detecting relatively small insects and analysing larval activity. Therefore, this methodology was used for *T. pityocampa* field experiments described later. Successive photographs of *T. pityocampa* tents from a few colonies were taken with an iPhone during field visits in Precastio from October 2018 to April 2019 (Fig. 5.1). From these images, it was clear that most of the silk application/tent maintenance occurred when caterpillars were L3, when they build the winter tent (October to November). The detailed tent maintenance and foraging activity of L3 caterpillars were recorded and analysed in *Tent construction*.

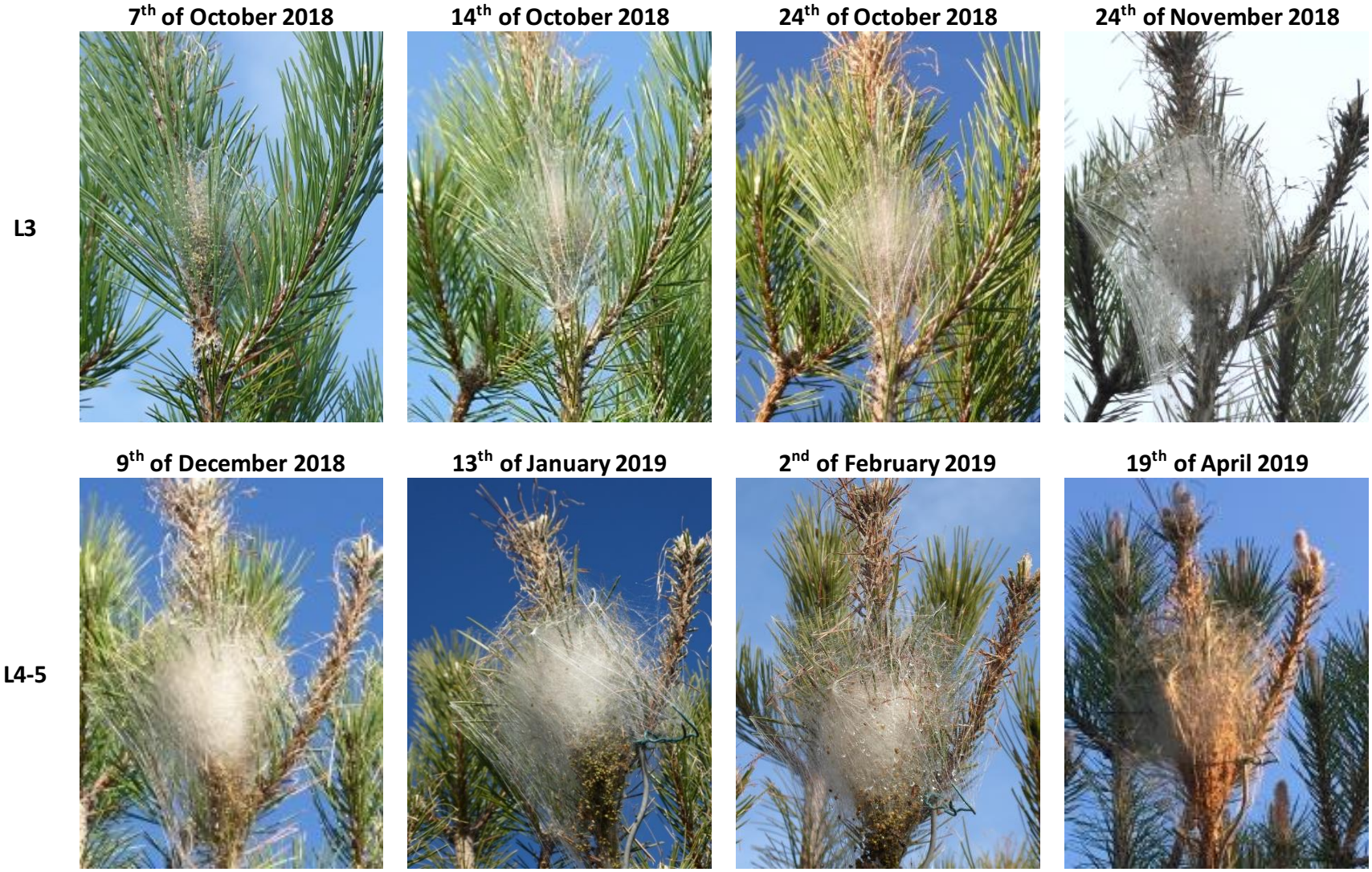


Figure 5.1. Successive photographs of one *Thaumetopoea pityocampa* tent from third to final (L5) instar larva. Photographs taken by Andrea Battisti.

Tent construction

Tent construction/maintenance behaviour of 45 *T. pityocampa* colonies of first (L1) to fourth (L4) larval instar were monitored in the field at Precastio from July 2020 to February 2021. Final instar (L5) caterpillars were not used in the experiment because they are less involved in tent construction (Breuer et al., 1989), and was confirmed by observations carried out by one of the authors (A. Battisti) in the same site in previous years. Colonies were randomly selected from ten Austrian pine *Pinus nigra* host trees growing isolated at Precastio (Fig. 5.2). Protective gear against urticating setae was worn when handling colonies L3 and older to minimise the experimenter's exposure.

Figure 5.2. *Pinus nigra* stand in Precastio, Verona Italy, a study site where *Thaumetopoea pityocampa* colonies were monitored for foraging and tent construction behaviour. Satellite image was taken on 17/03/2020 by Google Earth Pro, image accessed 18/05/2021.

In mid-August to start of November 2020 (L2-L4), two wildlife cameras were set up on a tripod and focused to take images of a single colony each, every 15 min for 24 h. Every two to three days, the cameras were moved to capture different colonies and the same colony was not used again. The images were stored on a SD card and viewed on a computer. The time

stamp and larval activity captured on each image, such as spinning silk/tent maintenance, feeding and returning to the tent, were noted. Spinning silk/tent maintenance was determined when caterpillars were visible through the silk strands of the tent and moving on the tent surface. Feeding activity was determined when caterpillars moved in a procession from the tent to pine needles nearby and engaged in feeding on the pine needle. Caterpillars returning to the tent was determined when caterpillars moved in a procession from the pine needles back to the tent and remained inside. Larval activity times determined from the images were further confirmed by checking the colonies in the field daily at different times of the day and night. When observing *T. pityocampa* colonies at night, a red-light headlamp was used to not disturb their behaviour. HOBO Temperature data logger (Onset Computer Corporation, Macquarie, USA) probes were placed next to the tent being monitored to measure the environmental temperature every 15 min.

In the beginning of October-November 2020, when caterpillars are L3 and spin the most amount of silk for tent construction/maintenance, we analysed the wildlife camera images of eight colonies. From the images, we distinguished three types of activity: (1) first constructors of the tent (first constructors) were caterpillars that were spinning silk in the internal layers of the tent, first spinning behaviour on the tent surface (first spinners) were caterpillars that were spinning silk on the most outer surface of the tent and first foraging behaviour (first foragers) were caterpillars that moved from the tent to forage on the pine needles. The time, environmental temperature, average daily temperature, colony identification (ID), weather condition and wildlife camera position at which the three types of activity occurred were recorded.

Final tent structure

The spiral characteristics (phyllotaxis) of *P. nigra* needles on the shoots provide a consistent spherical platform for silk attachment/tent construction for *T. pityocampa* caterpillars. To determine if *T. pityocampa* build an isodiametric tent according to the spiral phyllotaxis of *P. nigra*, we measured the radii of 100 winter tents built by later instar larvae from Monte Garzon, Italy (45°31′ N, 11°10′ E) in January 2021. We determined the four cardinal orientations of the tent radii by using the iPhone application Compass. The pine shoot was used as the central reference point and the radii were measured with a ruler going outwards towards N, E, S and W. Orientation of the tents on the tree was also documented. The tent measurements were done from the ground or by using a ladder or tree climbing equipment.

A previous study by Uemura et al. (2021) determined that final instar *T. pityocampa* caterpillars have a pair of specialised stemma for polarised vision to orient in the environment. Third instar larvae and onwards come out of the tent to spin silk at sunset when the polarised band is the strongest along the zenith (Cronin et al., 2006). This may suggest that the polarised skylight pattern is important for tent construction in *T. pityocampa* caterpillars. Therefore, scanning electron micrographs (SEM) of stemmata from all instars L1-L5 were imaged (N = 34) to determine if they share the same morphology of the polarised sensitive stemmata described in Uemura et al. (2021).

Task division

In mid-August to end of September 2020, when *T. pityocampa* caterpillars are L1 to L2, frass production of 12 colonies in the field at Precastio were measured and analysed using the same experimental design from *Preliminary experiments*. The larval instar was determined by the body and frass size. All colonies that were maximum 1 m off the ground on 10 *P. nigra* host trees were selected for the experiment. The plastic plate and the paper template were unsuitable for field conditions due to rain and strong winds. Therefore, rotating discs were created using a 21 cm diameter polypropylene corrugated sheet covered with a 21 cm diameter circular transparent sheet of plastic printed with the same 24-slice template. Coloured paperclips were used as a marker to determine where the tent was positioned on the disc. The disc was placed directly underneath the tent where the caterpillars feed in the immediate area (Uemura et al., 2020). The disc was secured on a 24 h mechanical plug-in timer using Scotch clear extreme fastener strips. In the field, the wind caused the frass to move therefore, the plastic sheet was coated with liquid Vaseline to keep the frass in place. The discs with the timer were attached to a metal ring of an 80 cm steel stand with heavy duty tape (Figs. 5.3 A and B). The timers were powered using the Wolf PRO Power Bank (Litionite, Ancona, Italy) and the power bank was sheltered from the rain and wind by an umbrella tied to the tree branches. One rotating disc was placed under each colony and two colonies were surveyed for 24 h. No more than two discs could operate at one time because of the power bank battery capacity. The same colony was not used to measure the frass of more than one instar. The experiment started in the afternoon, generally when no larval activity is observed, and stopped after approximately 24 h. Then a photo of the disc was taken from above using an iPhone (Fig. 5.3 C), and the disc was cleaned and recoated with liquid Vaseline for the next collection. The images were cropped and analysed in the same way as the preliminary laboratory experiment.

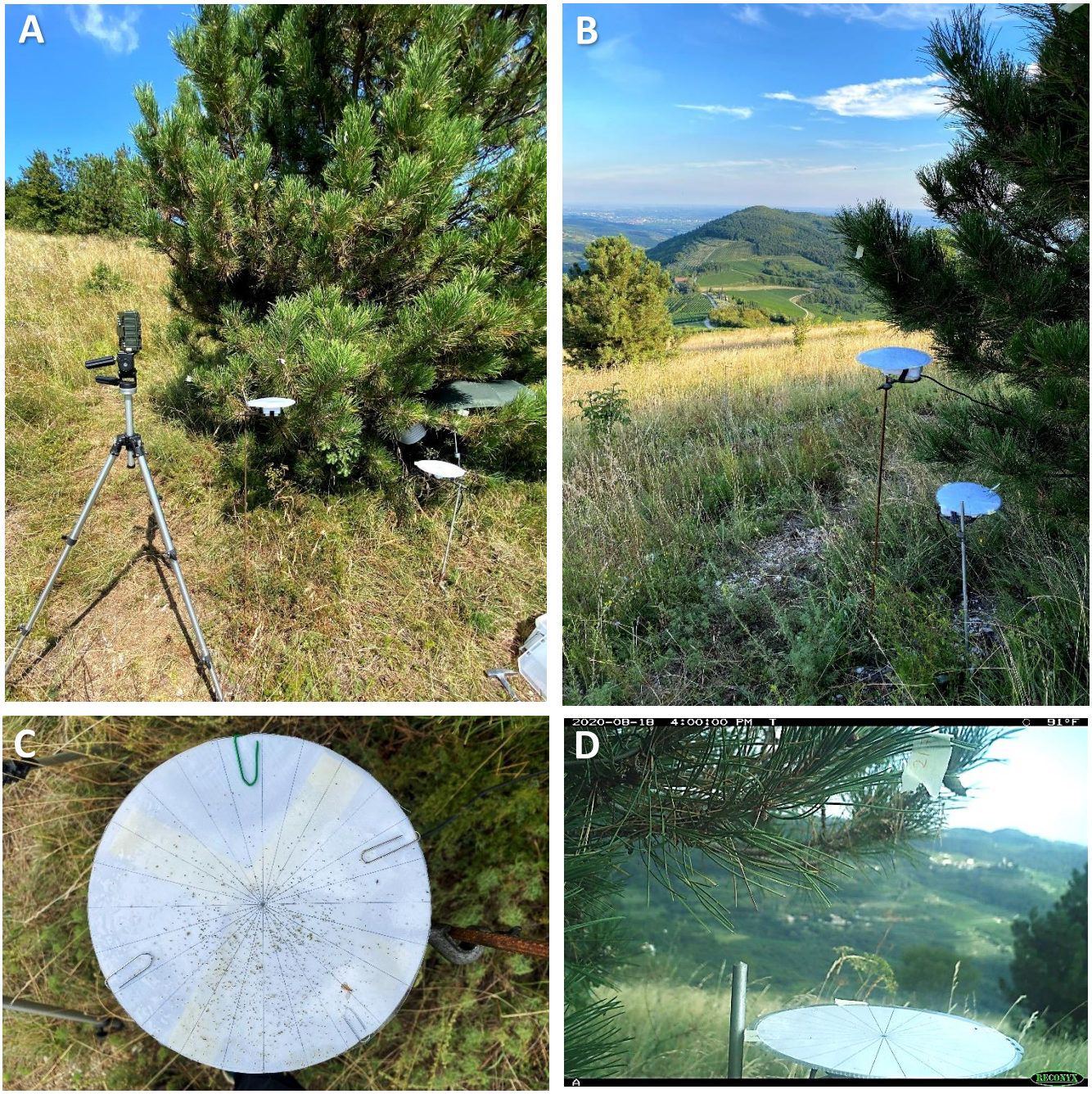


Figure 5.3. A and B Experimental set up for counting frass from *Thaumetopoea pityocampa* colonies in Precastio, Verona Italy. Rotating discs were positioned directly underneath a L2 colony. **C** Image of the rotating disc after 24h of collecting frass from a L2 colony. **D** Image taken from the wildlife camera showing the rotating disc underneath a L1 colony. Photographs taken by Mizuki Uemura.

We observed that not all caterpillars come out of the tent at sunset to spin silk to enlarge and maintain the tent. Thus, we hypothesised that there could be division of labour within colonies and specific individuals come out to spin. During our observations from wildlife camera images and field visits at sunset and in the evening, we were able to identify early and late active caterpillars in all *T. pityocampa* colonies. At sunset, the early active caterpillars spun silk on the tent and foraged immediately after the cessation of spinning activity. Whereas late active caterpillars emerged out of the tent later and foraged around midnight to early morning. Across two nights in November to December 2020, we collected L4 caterpillars from two experimental groups: early and late active caterpillars. Once the early and late active caterpillars initiated their behaviour, 20-25 caterpillars were collected with forceps from 10 tents for each group and stored in 70% ethanol. Protective clothing and eyewear were worn during the collection to eliminate contact with urticating setae from the caterpillars. From the onset of darkness, we used a red headlamp to determine and differentiate the behaviour of the caterpillars. Differentiating sex using external morphology (Lavenseau, 1982; Underwood, 1994) was difficult on *T. pityocampa* caterpillars. Therefore, each caterpillar was dissected, and the presence or absence of testis located dorsally on the 5th abdominal segment, determined if the caterpillar was male or female, respectively (Battisti, 1988). The dorsal side of the maximum head capsule width from all dissected caterpillars were measured using a Leica S9i Digital Stereo Microscope at 20× magnification with a micrometre eyepiece.

Larval parasitism

Throughout our field experiments, the tachinid parasitoid *Phryxe caudata* was frequently observed ovipositing on *T. pityocampa* caterpillars spinning silk on the tent (Fig. 5.4 and see Supplementary Material S3 for the synthesis on *P. caudata*). To determine if these early active caterpillars were more prone to parasitism, during the dissection to differentiate the sex of early and late active *T. pityocampa* caterpillars, we also examined for the presence/absence of the tachinid larva(e) inside the body cavity. The parasitoid larva(e) was present as diapausing second instar larva (length < 1 mm) in the trachea of the host's posterior abdomen. All Tachinid larvae were collected and preserved in 96% ethanol at – 20 °C for molecular analysis to confirm the species identification. Extraction of DNA samples was performed on one third instar maggot and seven second instar maggots found inside *T. pityocampa* caterpillars using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. A region of the mitochondrial DNA corresponding to a fragment

of the Cytochrome C Oxidase subunit I (COI) was amplified using the universal primer pairs LCO-1490/HCO-2198, following procedures as in Folmer et al. (1994). PCR products were purified using Exonuclease and Antarctic Phosphatase (GE Healthcare) and sequenced at the BMR Genomics Service (Padova, Italy). Sequences obtained were then compared with the software MEGA X (Kumar et al., 2018) and identified through a nucleotide BLAST analysis (http://blast.ncbi.nlm.nih.gov) on the GenBank database as well as by using the integrated bioinformatics platform Barcode of Life Data (BOLD) System database (http://www.barcodinglife.org).

Figure 5.4. A and B *Phryxe caudata* on a newly established L3 and well-established L4 *Thaumetopoea pityocampa* tent (circled), respectively, at Precastio, Verona Italy. Photographs taken by Andrea Battisti.

Statistical analyses

The following statistical analyses were performed using R Studio version 4.1.0 and GraphPad Prism 9. An alpha value of $P < 0.05$ was taken as statistically significant.

Tent construction

The time at which *T. pityocampa* caterpillars went out to spin silk, forage and return to the tent was determined and noted for each colony by visually analysing the wildlife camera images. The time of activity was converted to frequency by averaging the counts of activity per hour from all colonies within a larval instar. Hourly temperatures were calculated by averaging environmental temperatures collected from data loggers during the developmental period that each larval instar experienced. Hours of darkness was calculated by averaging the sunrise and sunset times retrieved from SunCalc (2021) for the developmental period that each larval instar

experienced. The data (L2: N = 11; L3: N = 15; L4: N = 5) was plotted using GraphPad Prism version 9.0.0 for Windows. Tent maintenance behaviour in L3 caterpillars was analysed using a generalised linear model (GLM) to determine if the time of the three activities (first constructors, spinners and foragers) were associated with various independent variables. To take visible light into account, the time at sunset must be considered because it differs day by day. Therefore, the time of activity was standardised to minutes before and after sunset. The sunset times of each day when caterpillars were L3, were retrieved from SunCalc (2021). The difference in time of activity and sunset were calculated and rounded to the nearest 15 min because the wildlife camera images were taken every 15 min. The standardised time of activity was used as the dependent variable and tested against the independent variables: activity, average daily temperature, colony ID, weather condition and wildlife camera position. Akaike information criterion (AIC) values for all possible models were compared to determine the best model to explain the data. The environmental temperature was not used as an independent variable as it is correlated with time of activity. However, Analysis of variance (ANOVA) was used to assess if the environmental temperature of when the three activities occurred differed with date of the sampling period (Oct to Nov).

Final tent structure

The radii of 100 *T. pityocampa* winter tents were averaged for each cardinal direction: N, E, S and W. The average and SD of the tent radii were plotted using RStudio version 1.2.5033 software package 'ggplot2'. ANOVA was used to assess if there were differences in radii lengths of the final winter tent to determine if the tents are isodiametric or not. To determine if *T. pityocampa* winter tents were distributed in a particular orientation on the tree, Kuiper's test of uniformity was performed using the RStudio software package 'CircStats'.

Task division

The amount of frass produced by a *T. pityocampa* colony was digitally counted for each slice (24 slices in a 24 h day) from a disc, which represented an hour out of that day. The frass production for each colony varies because of the different sizes/number of caterpillars in each colony therefore, the data was standardised. The frass count for each hour/slice of the day was divided by the total frass count from the same 24 h disc. The standardised hourly frass count was then averaged from all colonies within a larval instar. The frass data was plotted with the average feeding activity from the wildlife camera using GraphPad Prism. For graphical reasons,

the standardised frass count was multiplied by 10 to easily compare with the average feeding activity.

To determine if *T. pityocampa* male or female caterpillars were active earlier or later, sex and activity (early or late active) were analysed using a Chi-square Goodness-of-Fit test for a 50:50 distribution. Additionally, a GLM was used to determine if head capsule widths differed by sex and activity. AIC values for all possible models were compared to determine the best model to explain the data.

Larval parasitism

To determine if parasitism proportions differed between sex or activity, a Chi-square Goodness-of-Fit test for a 50:50 distribution was performed.

5.4 Results

Final tent structure

The winter tents constructed by later instar *T. pityocampa* were oriented on the southern facing branches of the host trees ($K = 5.82$, $P < 0.01$, $N = 100$; Fig. 5.5 A). Additionally, the tents were not isodiametric. Radii lengths between the four compass orientations were significantly different (ANOVA: $F_{3, 396} = 268.93$, $P < 2.2e^{-16}$; $N = 100$), with a thicker silk layer observed on the southern side of the tent compared to other orientations (Fig. 5.5 B). The SEM images of stemma 1 (stemma responsible for polarised vision) of L1-L5 *T. pityocampa* caterpillars affirmed that all instars had ⅔ rugged and ⅓ smooth surface (Fig. 5.6). This is a morphological trait characteristic of stemma with polarisation sensitivity in processionary caterpillars (Uemura et al., 2021). Tent construction/maintenance behaviour commenced at sunset when the polarised band is the strongest along the zenith (Cronin et al., 2006). Therefore, *T. pityocampa* caterpillars were able to determine where the southern side of the tent is using the position of the sun and skylight polarisation pattern at sunset for silk application on the tent (see Uemura et al., 2021).

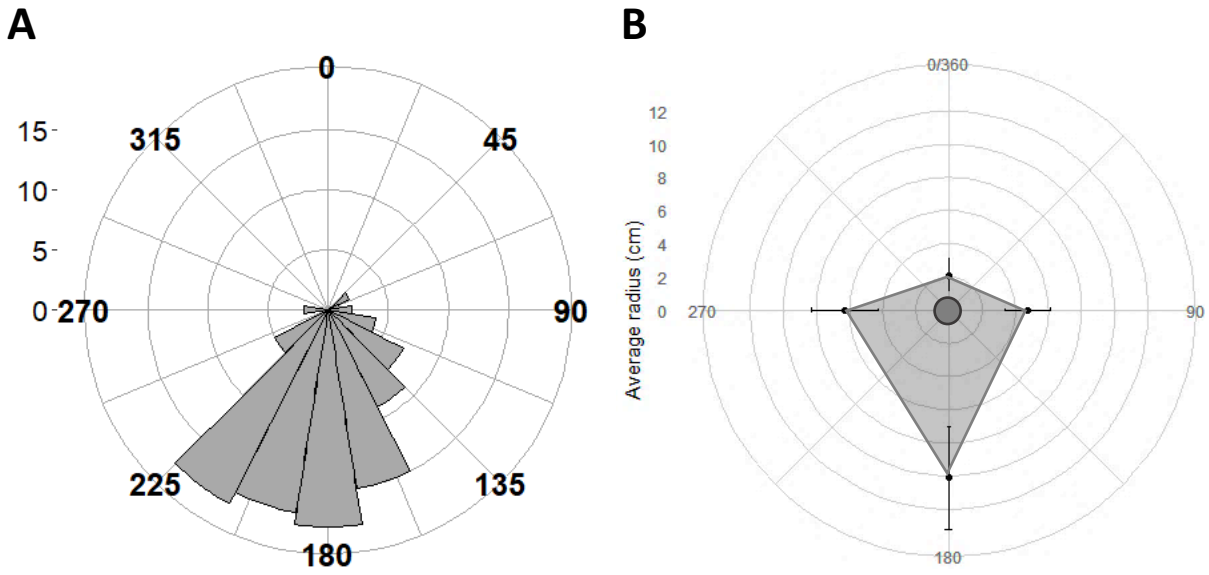
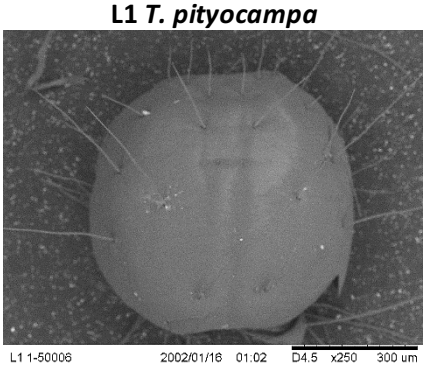
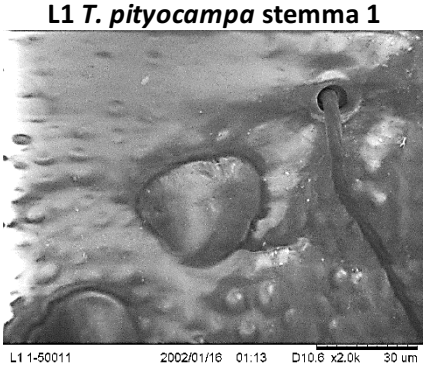


Figure 5.5. A) Rose diagram of the frequency and orientation of *Thaumetopoea pityocampa* winter tents on the host tree *Pinus nigra* at Monte Garzon, Verona Italy (N = 100). Each grey ring represents the number of tents shown on the left, starting from the least in the centre to the most in the second last outer ring. **B)** Average tent radii (± SD) from the pine branch in the centre (dark grey circle) outwards towards North (0/360), East (90), South (180) and West (270) of final instar *T. pityocampa* tents (N = 100). Each grey ring represents the distance away from the branch on the left, starting from the shortest distance in the centre to the furthest in the second last outer ring.

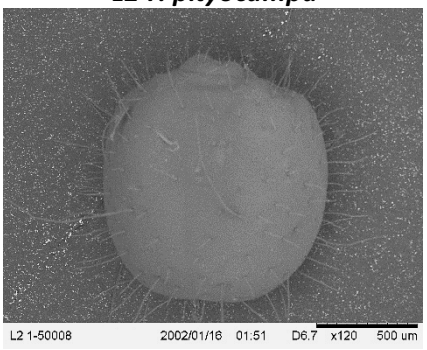
L1 *T. pityocampa*



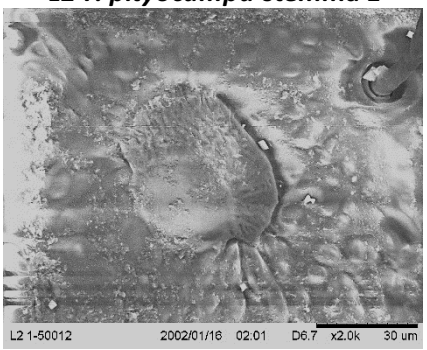
L1 *T. pityocampa* stemma 1



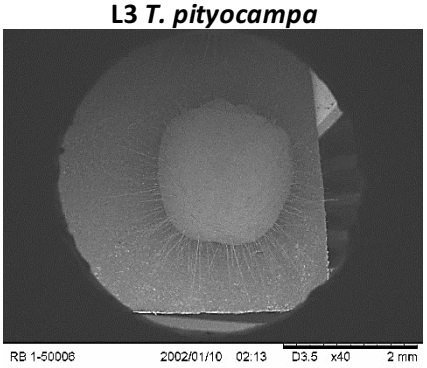
L2 *T. pityocampa*



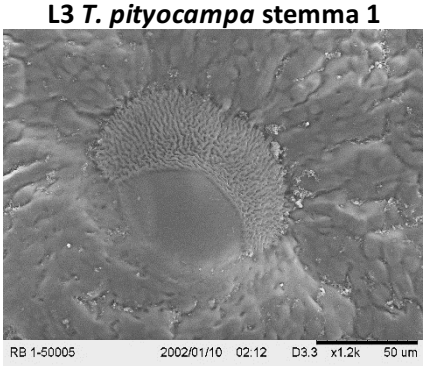
L2 *T. pityocampa* stemma 1



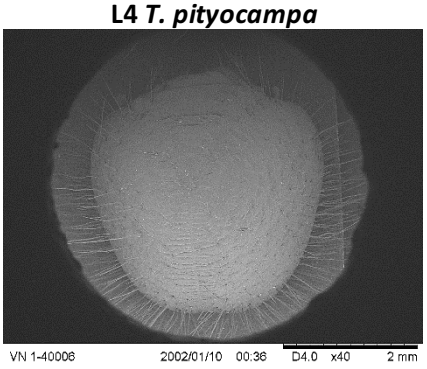
L3 *T. pityocampa*



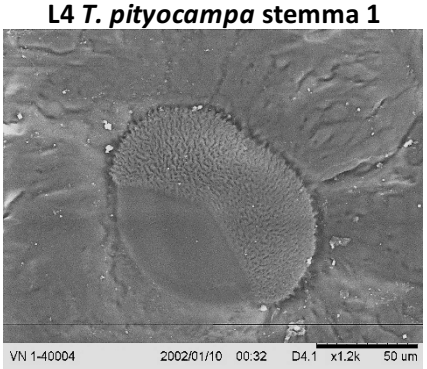
L3 *T. pityocampa* stemma 1



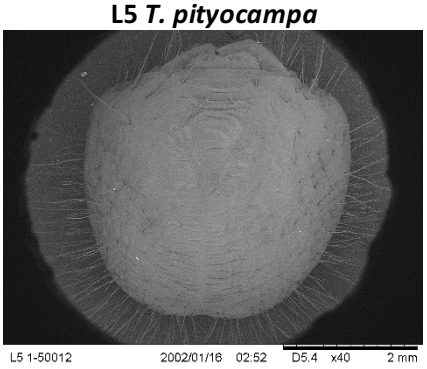
L4 *T. pityocampa*



L4 *T. pityocampa* stemma 1



L5 *T. pityocampa*



L5 *T. pityocampa* stemma 1

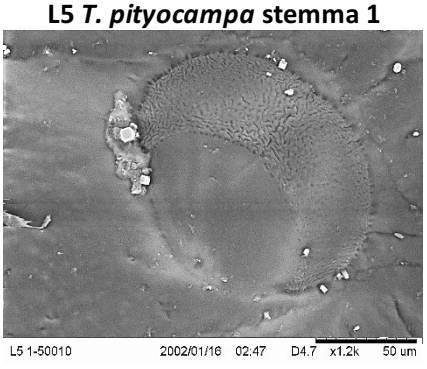


Figure 5.6. Scanning electron micrographs (SEM) of *Thaumetopoea pityocampa* caterpillars from L1-L5: head capsules on the left and stemma 1 on the right. In all larval instars, 1/3 of the stemma is smooth and 2/3 with a rugged surface. SEM taken by Paolo Paolucci.

Tent construction

The differentiation between spinning and feeding behaviour of L1 caterpillars were undetectable from the wildlife camera images because of their small size; therefore, the two activities were recorded and analysed from L2 onwards. The colonies from L2-L4 fed predominantly at night in darkness (Fig. 5.7). Some L2 colonies occasionally foraged in the middle of the day and there was minimal spinning behaviour. Once the environmental temperature started to cool and when caterpillars moulted to L3, the silk spinning behaviour was conspicuous.

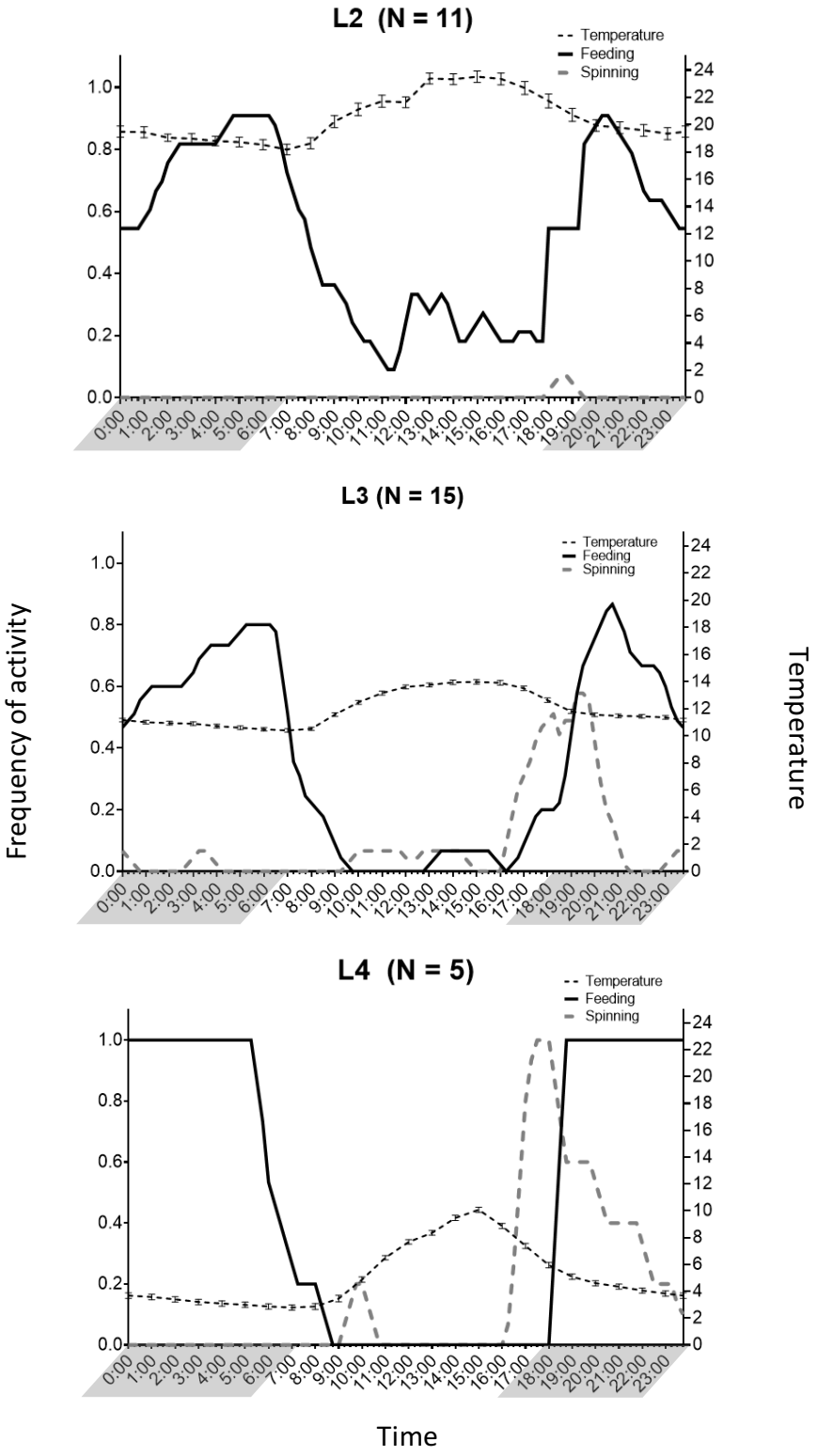


Figure 5.7. Average daily feeding and spinning activity of 2nd, 3rd, and 4th instar *Thaumetopoea pityocampa* caterpillars (L2, L3 and L4). The feeding and spinning activity of caterpillars is represented as a solid black line and grey dashed line, respectively and has been averaged, standardised, and plotted against the left Y axis. The average ambient temperature of each hour for each instar has been plotted against the right Y axis. The shaded times on the X axis represents the hours of darkness.

The few L3 individuals that were spinning silk (spinners) commenced maintaining the outside of the tent around sunset and continued for approximately an hour (Fig. 5.8). After spinning silk, the spinners left the tent and followed the foraging trails to feed and were joined by the rest of the colony that remained inside the tent. The colony came back from foraging to the tent around sunrise. *Thaumetopoea pityocampa* caterpillars had specific timing for spinning silk to maintain the tent and for foraging. Tent maintenance (constructing and silk spinning) occurred when environmental temperatures were > 10 °C whereas, foraging generally occurred when it was < 10 °C in complete darkness (Fig. 5.8). Peak tent maintenance started 45 min after sunset when there was minimal light and peak foraging activity started approximately 135 min after peak tent maintenance behaviour (i.e. 180 min after sunset). Other independent variables: average daily temperature, colony ID, weather condition and wildlife camera position had no significant effect on the timing of activity (all $P > 0.1$). Timing of L3 activity was significantly different with activity. Time at which the first constructors started maintaining the tent was significantly different with the time of the first spinners (GLM: $t = 2.40$, $P = 0.0181$, $N = 133$; Fig. 5.8) and foragers (GLM: $t = 9.29$, $P = 4.70e^{-16}$, $N = 133$; Fig. 5.8). The environmental temperature of when the three activities occurred was significantly different (ANOVA: $F_{1, 131} = 77.83$: $P = 6.11e^{-15}$) as the season progressed, with decrease in temperature from October to November.

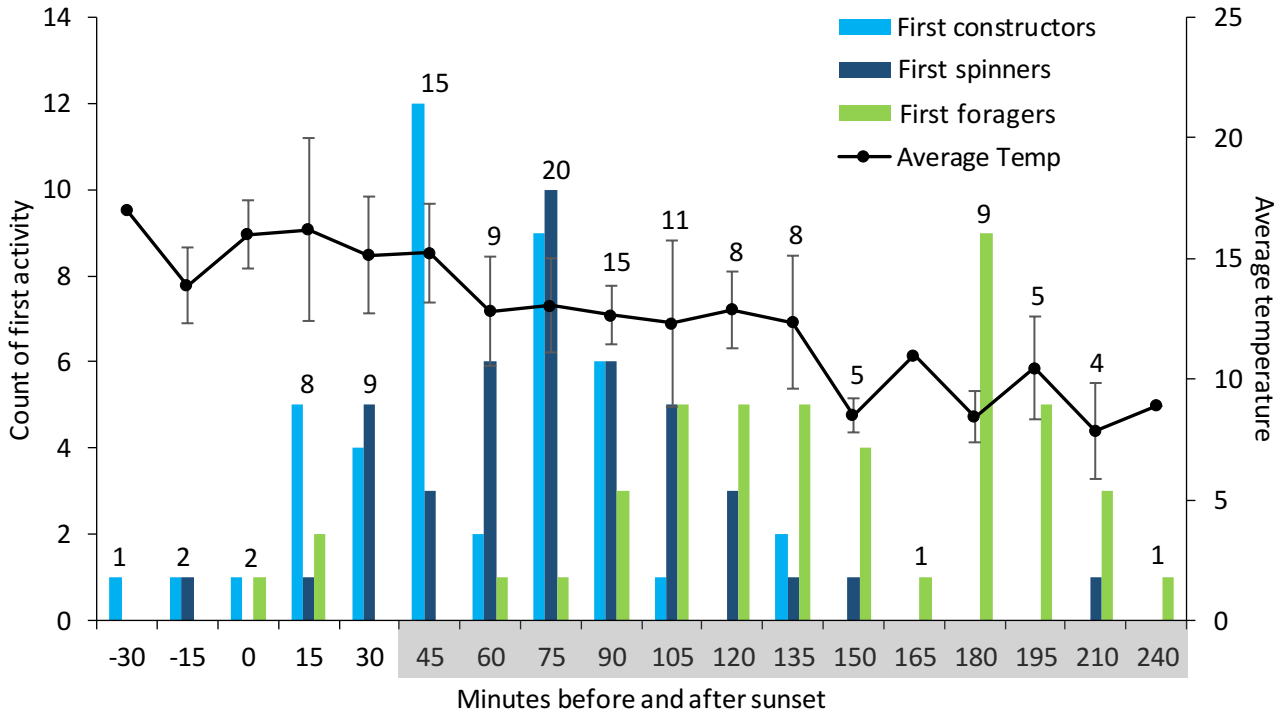


Figure 5.8. Counts of first activity of tent construction (light blue), tent silk spinning (dark blue) and foraging behaviour (green) of third instar (Oct-Nov) *Thaumetopoea pityocampa* colonies (N = 8) plotted against minutes before and after sunset. The shaded grey bar in the minutes before and after sunset, indicates darkness in the environment that gradually starts 30 min after sunset. The average temperature ± SD (black line) of the time of the single activity events across the period of observations is displayed on the right Y-axis. The numbers above the bars are the number of observations per minutes before and after sunset.

Task division

Frass was produced relatively in sync with the foraging activity recorded from the wildlife camera images. The frass production by L1 and L2 had fluctuations throughout the day (Fig. 5.9). This could be explained by the environmental temperatures the caterpillars experienced. The warm environmental temperatures when *T. pityocampa* are L1 and L2, would have assisted with metabolism and digestion.

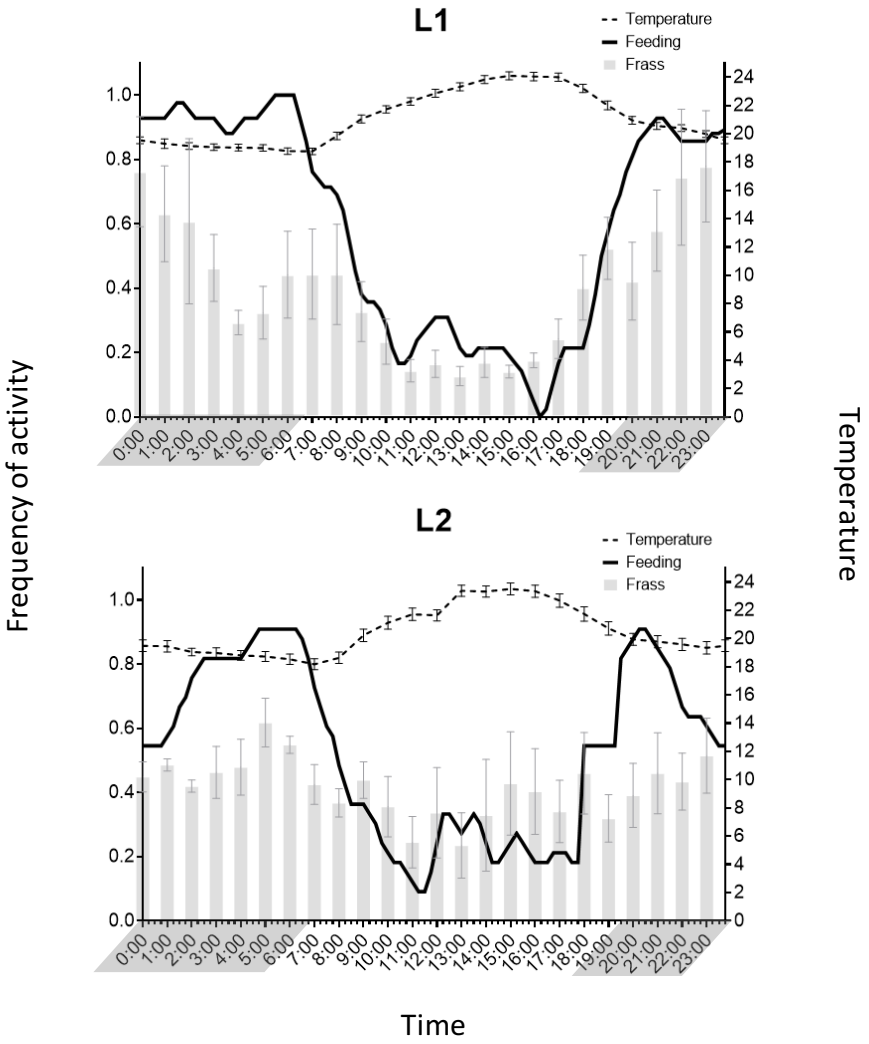


Figure 5.9. Average daily feeding activity and frass production of 1st (L1, N = 14 and 7) and 2nd (L2, N = 11 and 5) instar *Thaumetopoea pityocampa* caterpillars. The feeding activity and frass production of caterpillars is represented as a solid black line and grey box plots, respectively and has been averaged, standardised, and plotted against the left Y axis. For graphical reasons, the proportion of frass production has been altered to the relative scale by 10 times. The average ambient temperature of each hour for each instar has been plotted against the right Y axis. The shaded times on the X axis represents the hours of darkness.

A total of 474 early (first constructors, first spinners and first foragers, N = 238) and late (foragers, N = 236) active L4 *T. pityocampa* caterpillars were dissected to determine the sex of individuals for each activity over time. Of the 474 caterpillars dissected, 52% were female and 48% male (Fig. 5.10). The early active caterpillars were 59% male and 41% female (N = 238) whereas, the late active caterpillars were 36% male and 64% female (N = 236). Both frequencies are significantly different from the expected 50:50 ratio (Early: $X^2 = 8.13$, df = 1, P = 0.004343, N = 238; Late: $X^2 = 17.36$, df = 1, P = $3.1e^{-05}$, N = 236). Head capsule measurements of all dissected caterpillars showed that female L4 caterpillars were significantly

larger than males irrespective of activity (GLM: t = 9.14, P = $2e^{-16}$, N = 474; Fig. 5.11). Additionally, the head capsule widths of early active caterpillars were significantly larger than late active caterpillars (GLM: t = -5.59, P = $3.79e^{-08}$, N = 474; Fig. 5.11). These findings suggest that larger males were active early in the night and smaller females emerged later in the night/early morning.

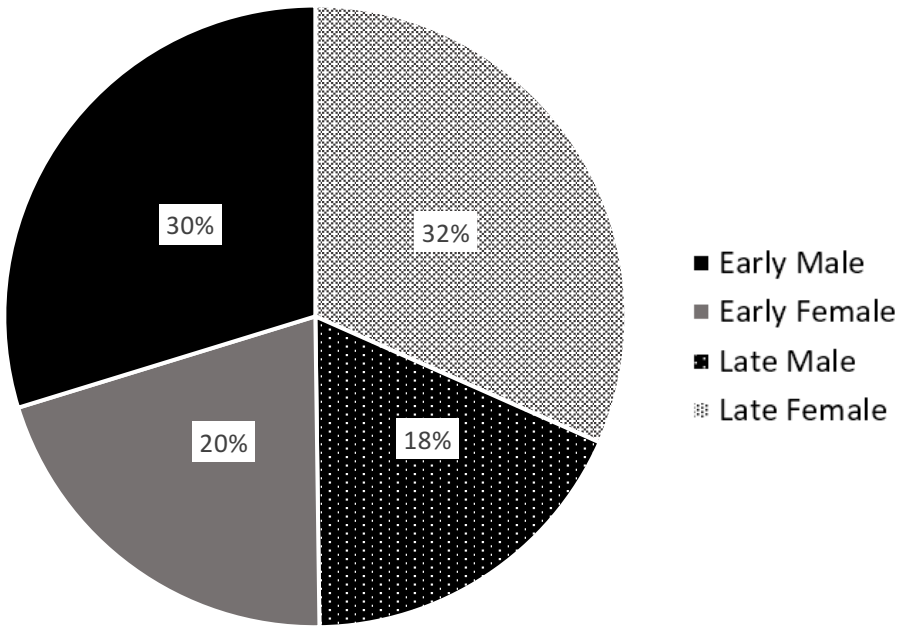


Figure 5.10. Proportions of male and female *Thaumetopoea pityocampa* caterpillars that were active early (first constructors, first spinners and first foragers) and late (foragers) in the night (N = 474). Solid coloured black and grey represents early active male and female caterpillars, respectively. Black with white dots and grey with many dots represent early active male and female caterpillars, respectively.

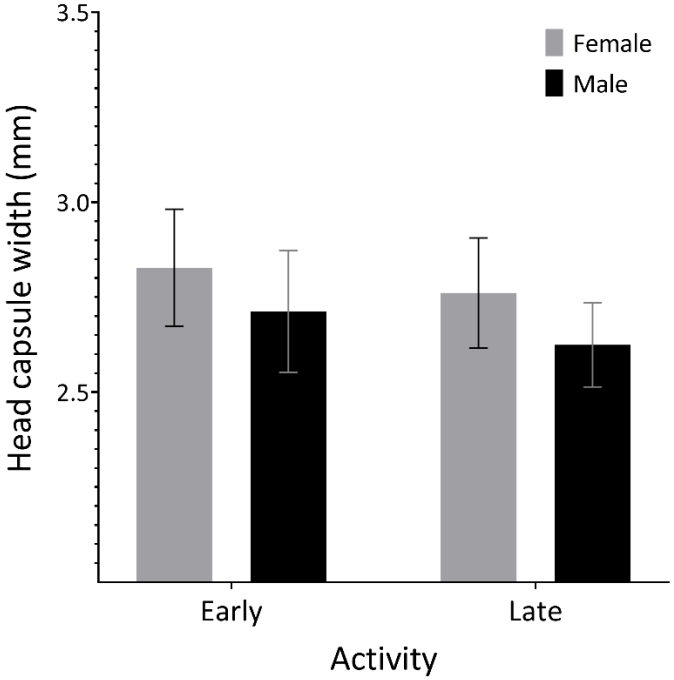


Figure 5.11. Average head capsule widths (± SD) of *Thaumetopoea pityocampa* caterpillars that were active early (first constructors, first spinners and first foragers; N = 236) and late (foragers; N = 237). Females and males represented as grey and black, respectively.

Larval parasitism

During the dissection, 95 *T. pityocampa* caterpillars contained a tachinid parasitoid larva(e) inside the body cavity. The sequencing of the COI region yielded a 618-bp-long fragment and the samples of maggots analysed showed identical sequences. A comparison with BoldSystem and NCBI databases showed a similarity of 96.6% and 95.8% respectively with *Phryxe pecosensis*; the only species from the genus *Phryxe* that had deposited sequences before this study. The sequence obtained from our samples was deposited in the NCBI database with the GenBank accession number MW834331 as *Phryxe caudata*. There was one, occasionally two, *P. caudata* larva per caterpillar. There were more parasitised *T. pityocampa* caterpillars in early active individuals compared to late active caterpillars ($X^2 = 10.12$, df = 1, P = 0.00147, N = 95). Of those early active parasitised individuals, 19% were females and 48% males. In late active parasitised individuals, 29% were females and 5% males (Fig. 5.12). Overall, parasitism proportion between sexes was not significantly different ($X^2 = 0.26$, df = 1, P > 0.5, N = 95). *Phryxe caudata* was not detected in the wildlife camera images in the evenings, which indicates that they are day-active and require light for host finding and oviposition.

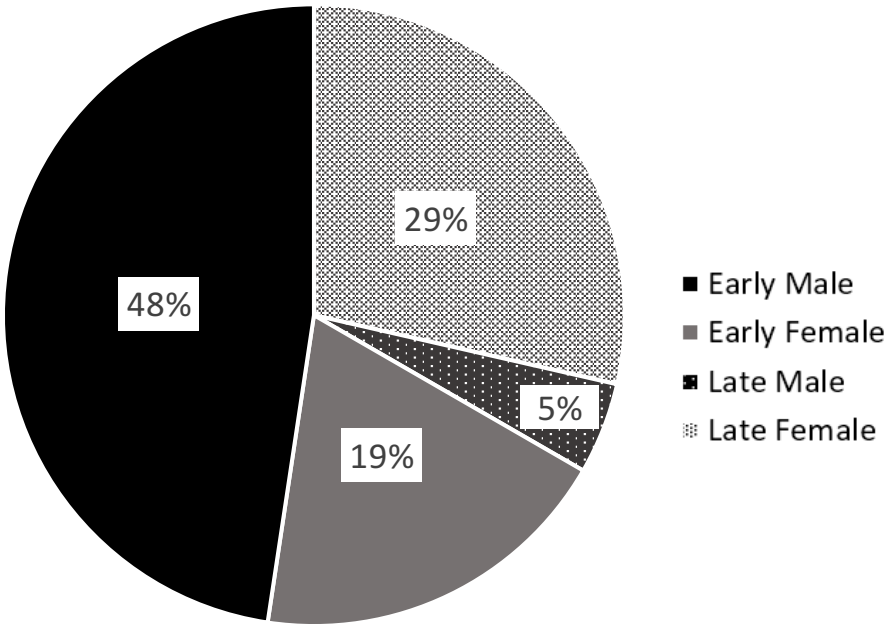


Figure 5.12. Proportions of parasitised *Thaumetopoea pityocampa* caterpillars that were active early and late differentiated by sex (N = 95). Solid coloured black and grey represents early active male and female caterpillars, respectively. Black with white dots and grey with many dots represent early active male and female caterpillars, respectively.

5.5 Discussion

The south was the preferred orientation for *T. pityocampa* caterpillars to build a tent on the host tree and for silk application on the tent. Southerly facing final winter *T. pityocampa* tent orientation on the host tree from this study confirm findings from previous studies (Breuer et al., 1989; Sebti and Chakali, 2014; Uemura et al., 2020a). However, the comparison of silk application on the tent of the four cardinal orientations has not been measured for *T. pityocampa* before. Larval preference for the south for tent orientation and silk application clearly demonstrates that it is to receive maximum solar radiation. Maximum insolation is necessary especially for later instar *T. pityocampa* caterpillars when they must endure cold winter temperatures. So how do *T. pityocampa* caterpillars determine where is south? The most outer stemma positioned in the semi-circle away from the mandibles (stemma I) was identified to be the stemma responsible for polarisation vision in *T. pityocampa* (Uemura et al., 2021). Stemma I SEM images of L1 to L4 showed the same polarised sensitive morphological characteristics described in L5 in Uemura et al. (2021). Stemma I from all instars had ⅔ of the surface rugged and ⅓ smooth (for more details see Uemura et al., 2021). This explains that not only is stemma I responsible for guiding final instar larvae on the ground during pre-pupation processions (see Uemura et al., 2021), it can also help them orientate on the tree and the tent

from first larval instar. The skylight polarisation pattern is particularly strong along north and south at sunset (and sunrise) (Heinloth et al., 2018) when the caterpillars initiate tent maintenance/construction. The combination of the skylight polarisation pattern and the sun position enable caterpillars to distinguish where is south. Studies on tent construction in *M. americanum* caterpillars also identified concentrated spinning activity on the most intensely illuminated side of the tent (Fitzgerald and Willer, 1983). The authors mention that the caterpillars were influenced by the position of the sun during morning and afternoon spinning bouts but did not explore polarisation vision in these social caterpillars. Future studies may explore the disruption of polarisation vision in *T. pityocampa* by using a polarised filter above caterpillars maintaining the tent or by 'blinding' the caterpillar by coating stemma I with paint. This will enable us to verify that the caterpillars use the skylight polarisation patterns at sunset to determine the southern side of the tent for silk application.

At sunset, not all *T. pityocampa* caterpillars came out of the tent to spin silk for tent maintenance. From the dissections, we determined that the caterpillars that were maintaining the tent were predominantly larger males and some larger females. While most of the smaller sized individuals which were mainly females were active later in the evening/early morning when it was time to forage. Démolin (1967) mentioned that *T. pityocampa* caterpillars spin and attach silk by arching its anterior part of its body backwards to a substrate (Supplementary Fig. S4). Fitzgerald and Willer (1983) described similar tent construction behaviour in *M. americanum* (Supplementary Fig. S5). Larger individuals spinning silk for tent construction can be beneficial as they can cover a larger surface area or perhaps have a larger reservoir in the silk gland. Polyethism and energy expenditure endured by male caterpillars during tent maintenance was also demonstrated in *E. socialis* caterpillars. Male *E. socialis* caterpillars were active first in the colony, spent more time spinning silk on the nest and were first to embark on foraging forays (Underwood and Shapiro, 1999a, 1999b; Yayalar, 2009). Silk is proteinaceous and involves elevated metabolic expenditures and nitrogen investment (Berenbaum et al., 1993). It was suggested that male *E. socialis* take on the role as 'workers' (Yayalar, 2009), and therefore allow female caterpillars to conserve resources for later use in egg production (Underwood and Shapiro, 1999a). Silk production is a costly investment and constituted 18% of ingested nitrogen in parsnip webworms (Berenbaum et al., 1993). Therefore, individuals with higher nitrogen levels may maintain/construct the tent and individuals with lower levels may only forage. Future studies could explore this in *T. pityocampa* caterpillars by providing pine needles with differing levels of nitrogen content. The stimulus that triggers caterpillars to

spin silk for tent maintenance is unknown. However, at the onset of darkness, male caterpillars may be more sensitive to environmental cues to initiate nest maintenance tasks and emerge from the nest earlier than females (Yayalar, 2009). Female caterpillars that emerge later may lack silk spinning stimuli and immediately go out foraging (Underwood and Shapiro, 1999a). The combination of task preference and response thresholds to stimuli may generate polyethism within a colony (Yayalar, 2009). In highly social caterpillars, tent-building can only be achieved through effective cooperation and communication between individuals in a colony (Ruf and Fiedler, 1999). *Malacosoma americanum* caterpillars engage in tent maintenance at specific times to delay the departure of the whole colony from emerging and allows time for individuals to assemble on the tent surface (Fitzgerald and Willer, 1983). Coordinated group assembly and spinning may be essential for tent expansion and foraging efficiency (Fitzgerald and Willer, 1983). Unlike *M. americanum* tents where there is one exit hole (Fitzgerald and Willer, 1983), in *T. pityocampa* tents, the caterpillars can exit from any direction within the tent. Therefore, by limiting the number of caterpillars being stimulated to spin silk and emerging from the tent all at once, it could help retain the integrity of the tent structure, a form of social order within the colony. Additionally, by having a few individuals responding to the stimuli for tent maintenance/construction, it will allow the spinners to lay down the silk efficiently without overcrowding. With the majority of the colony members resting deeply inside the tent, those caterpillars are protected from predators and parasitoids that may still be active. Future studies on the silk gland size between sexes may provide further information as to why males are more likely to construct the tent than females. Investigating larval activities that occur deeply inside the tent using a borescope may explain the tasks of the other caterpillars that are 'resting' while the spinners maintain the tent. Identifying if the same individuals perform tent maintenance day after day using coloured tags (e.g. paint on head capsule) will be crucial to better understand the social structure of *T. pityocampa* colonies. Marked individuals may then be tracked automatically using video tracking colour-tagged insects.

All larval instars of *T. pityocampa* fed predominantly at night in darkness. During bad weather, e.g. heavy rain and wind, the caterpillars would not emerge from the tent for tent maintenance nor to forage. If the bad weather is not continuous, the caterpillars start foraging after the rain has surpassed and the regular foraging pattern is disrupted. When weather conditions are optimal, early instars (L1-2) which are patch-restricted foragers (Uemura et al., 2020a), fed at night and occasionally in day light. The short distance the caterpillars travelled for food sources and their small size may permit them to forage during the day when predators

and parasitoids are present. It was frequently observed that early instars had multiple foraging bouts throughout 24 h. The relatively warm environmental temperatures experienced by the early instars would have facilitated their digestion and metabolism, which was represented by the frass production that was in sync with foraging activity. Therefore, the early instar larvae can empty their gut and go out foraging again in a short amount of time. Whereas in later instars (L3-4), the decrease in temperature increased the duration of foraging and locomotion. It was common for caterpillars to feed throughout the night and return to the tent before sunrise. This behaviour has also been described in the winter-feeding social caterpillars *E. lanestris* (Ruf and Fiedler, 2002). Tent maintenance by later instar *T. pityocampa* caterpillars occurred when temperatures were above 10 °C. After 1-2 h, the caterpillars that were spinning silk starts foraging and joined by the rest of the caterpillars that were resting inside the tent. Caterpillars start foraging when the temperature had dropped below 10 °C and it is complete dark. This drop in temperature and complete darkness may be a stimulus for caterpillars to cease tent maintenance and initiate foraging. Future studies should manipulate environmental temperature and visible light in laboratory conditions to determine if it affects the timing of tent maintenance and foraging in *T. pityocampa* colonies. Underwood and Shapiro (1999a) hypothesised that *E. socialis* caterpillars that first emerge from the nest are stimulated to spin silk until a threshold amount of silk or until a chemical marker is deposited. The stimuli that trigger the change of behaviour from spinning silk on the tent to foraging in social caterpillars is unknown and needs to be further explored. Later instar *T. pityocampa* caterpillars are central place foragers (Uemura et al., 2020a) and follow previously laid foraging trails in a procession to pine needles further away from the tent. Due to their larger size and longer distance to travel to food sources, this may have influenced their nocturnal foraging behaviour for parasitoid and predator evasion.

Of those parasitised *T. pityocampa* caterpillars, nearly half were the early active males that were spinning silk for tent maintenance. Maintaining the tent makes these individuals an easy target for *P. caudata* parasitoids that are still active around sunset. Therefore, not only are male caterpillars expending energy for tent construction, but they are also exposing themselves to higher chances of parasitism and predation. This indirect fitness benefit by male social caterpillars to construct the tent and protect the colony from predation/parasitism is new and has not been described in this species. Overall, the parasitism proportion between the sexes were not significantly different, with late and early active females coming in second and third after early active males. Late active males contributed the least proportion of parasitism, most

likely because there were less males active later in the evening/early morning. Nearly 70% of the caterpillars parasitised were active early in the night and *P. caudata* were undetected in the wildlife camera images, which suggest that visible light is a significant factor to the parasitism proportion. A relatively high proportion of late active females parasitised could perhaps be explained by those individuals that were foraging through the early morning at sunrise. Further behavioural and ecological assays need to be done to understand the relationship between *T. pityocampa* and *P. caudata*. A future study could use laboratory colonies of *T. pityocampa* that were exposed to *P. caudata* and compare the behaviour of parasitised and unparasitised groups. It will be beneficial to investigate if tent construction behaviour is altered in parasitised *T. pityocampa* caterpillars, which has not been described in this species or similar.

5.6 Conclusion

Investigating the tent building behaviour of *T. pityocampa* colonies has opened our knowledge about how they achieve order and structure in their day-to-day life. It has given us a further understanding on how non-eusocial insects live socially and how they organise themselves within the colony. Specialised stemma for detecting polarised light found in *T. pityocampa* caterpillars as early as neonates, provides new insight in spatial orientation of insect larvae and caterpillar vision. The sacrifices made by male *T. pityocampa* caterpillars for tent construction is yet another example of how cooperation and polyethism can be beneficial for a social organism. Future studies may consider automated detection of caterpillar activity (e.g. tent maintenance) of certain individuals by using modern video tracking analyses. Investigating how *T. pityocampa* caterpillars apply silk to build the tent using macro slow motion cameras will be interesting to understand if the caterpillars communicate with each other or if it is a continuous and synchronous innate behaviour done individually. Further investigations on tent structure and the procedures used for construction by *T. pityocampa* caterpillars may be beneficial for bio-inspired engineering. In this study, the use of wildlife cameras and the novel frass counting apparatus demonstrated non-destructive field collection methods which captures the natural behaviour of these insects. This opens opportunities to other researchers to simultaneously monitor insects over long time periods without disturbance.

5.7 Acknowledgements

We thank Isabel Martinez and Paolo Paolucci from University of Padova for the genomic sequencing of *Phryxe caudata* and for the scanning electron micrography of *Thaumetopoea*

pityocampa samples, respectively. We thank European Union's Horizon 2020 Program for Research and Innovation 'HOMED' [grant no. 771271] for M.U., M.Z. and A.B. and 'Fondazione Cassa di Risparmio di Padova e Rovigo PhD programme 2018' for M.U.

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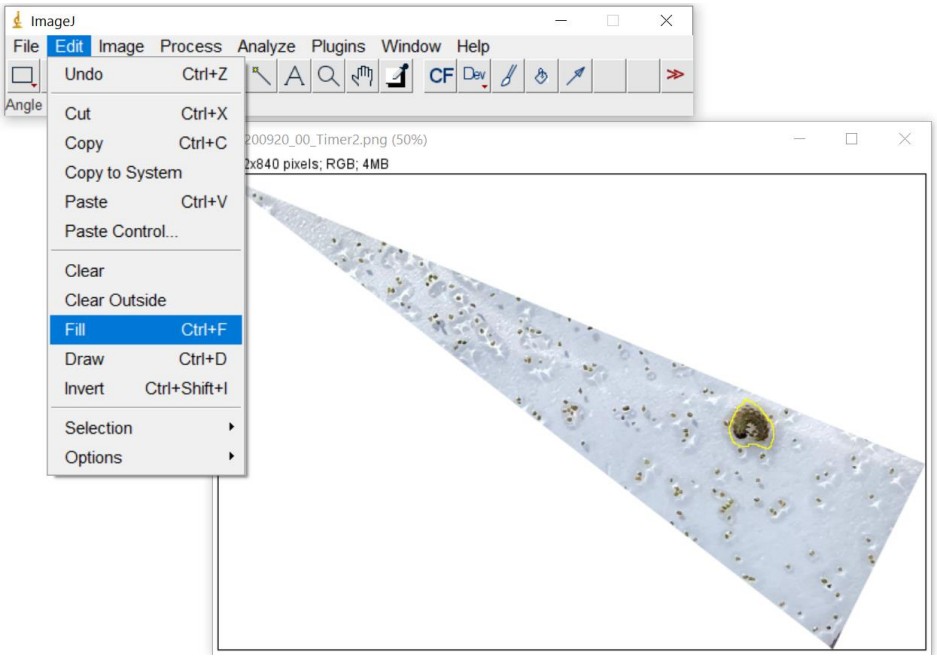
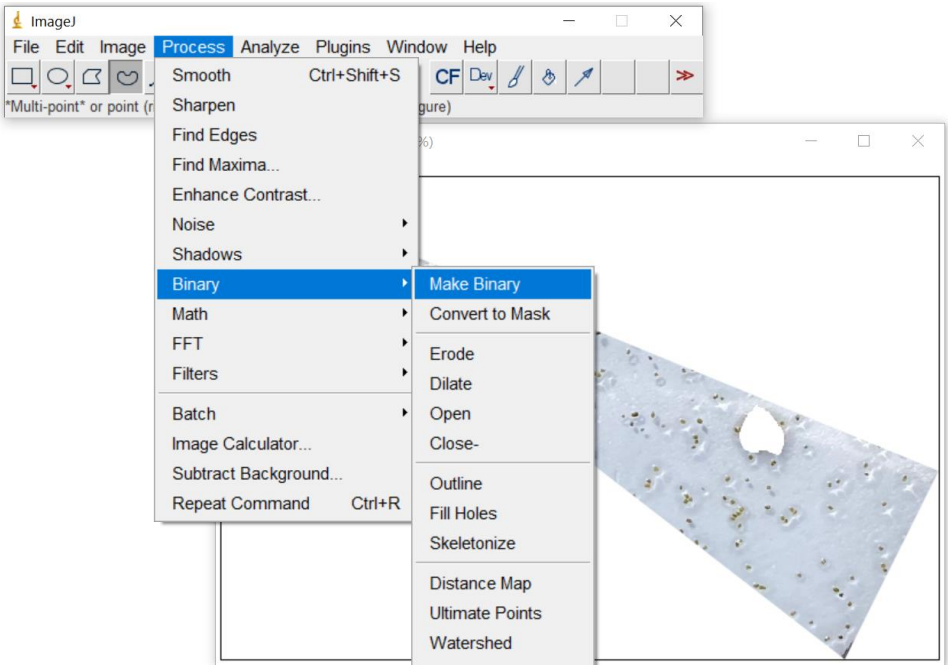
5.9 Supplementary Materials

Supplementary material functions S1

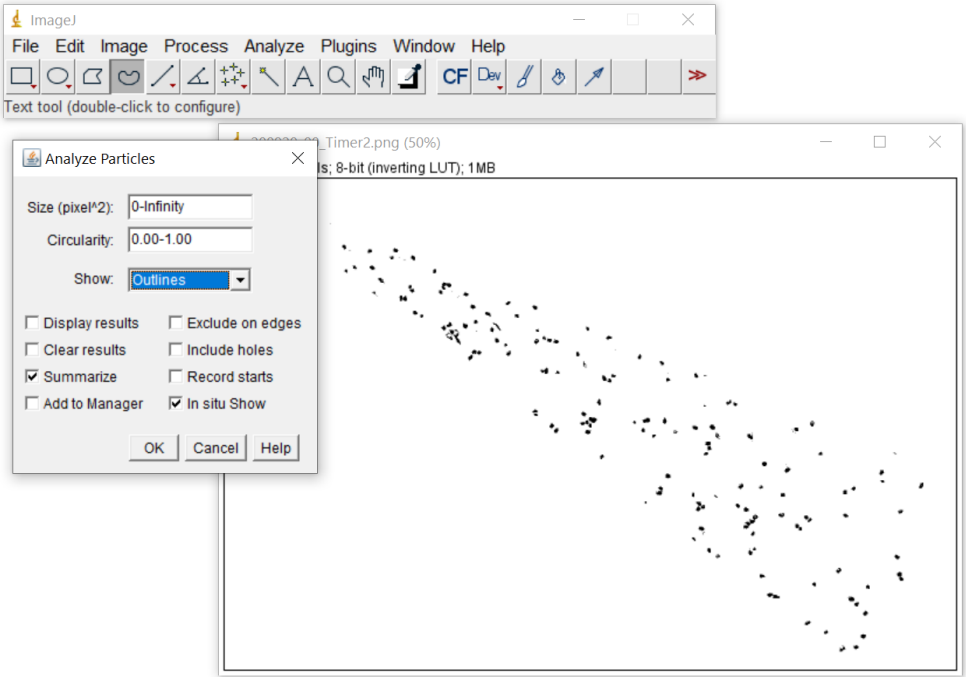
1. Open a cropped image (one slice of 24 slices) in imageJ and remove big objects (e.g. pine needle, fallen *Thaumetopoea pityocampa* caterpillars, etc.) that are not frass by circling the image using any of the shapes in the toolbar → click Edit → click Fill



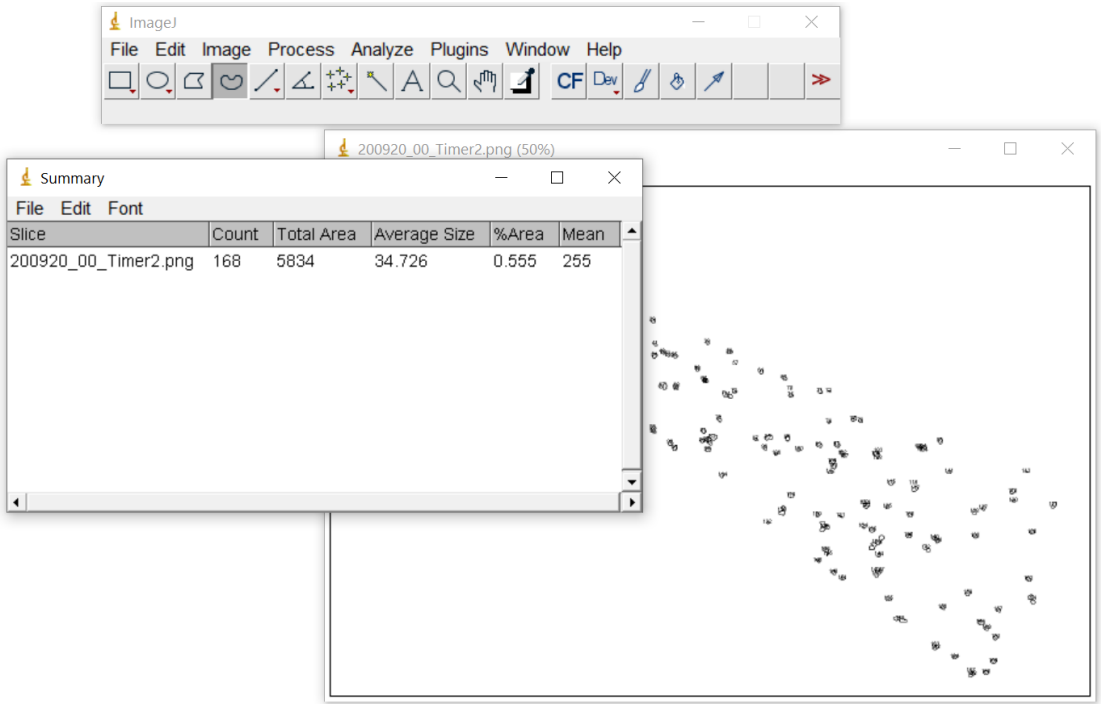
2. Format image to easily view the frass (particles) by clicking: Process → Binary → Make Binary

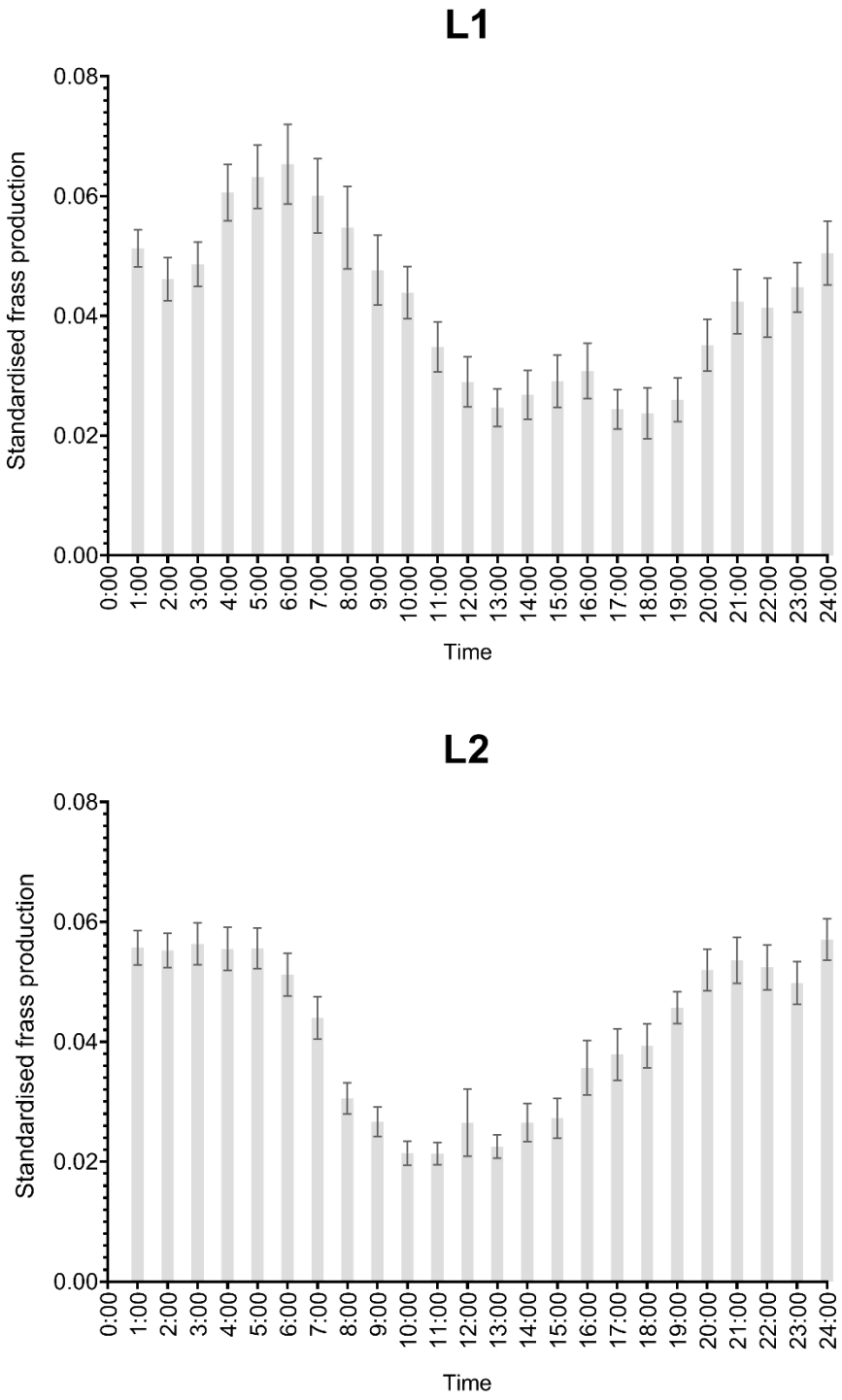


3. Count caterpillar frass in the image by clicking Analyze → Analyze particles → In the pop-up window set "Show:" to Outlines → tick Summarize and In situ Show → OK



4. Continue this for each slice/cropped image (24 slices in total for one image) from the same image and the Summary table will accumulate with all the results → Press Ctrl + A → Press Ctrl + C to copy all data in the Summary Table and input in Excel file. The 'Count' is the number of frass (particles) that were counted in the image.



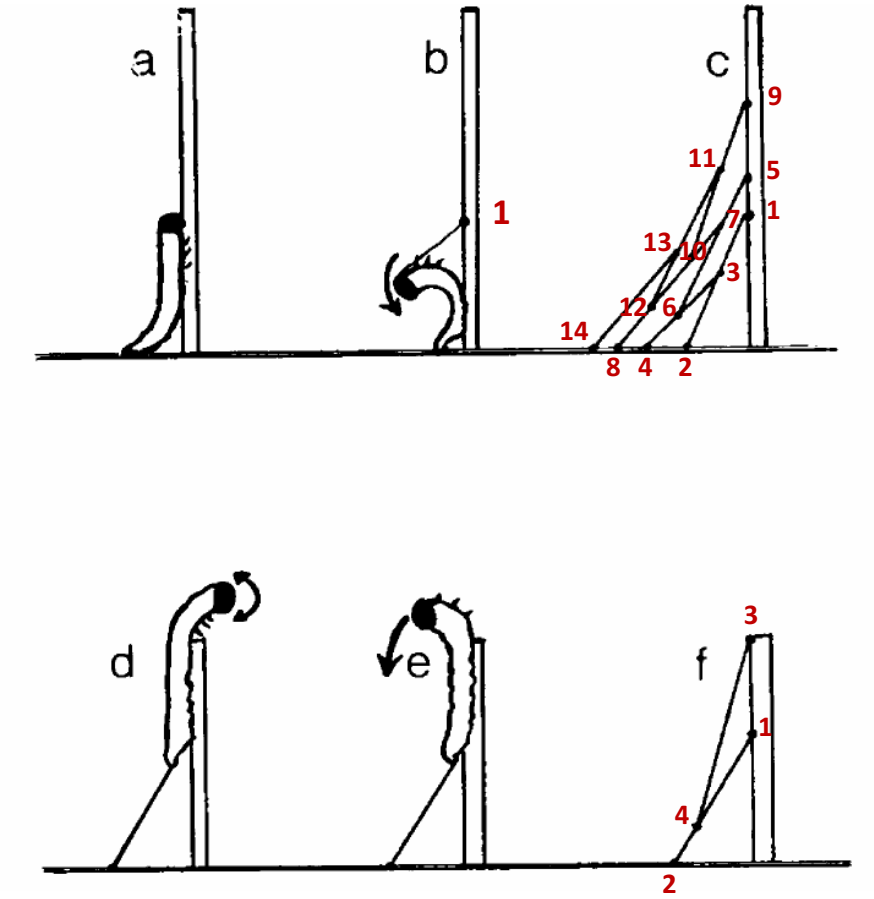


Supplementary Figure S2. Average standardised daily frass production of first (L1, N = 3 colonies) and second (L2, N = 3 colonies) instar *Thaumetopoea pityocampa* caterpillars in indoor laboratory conditions over 8 and 30 consecutive days, respectively.

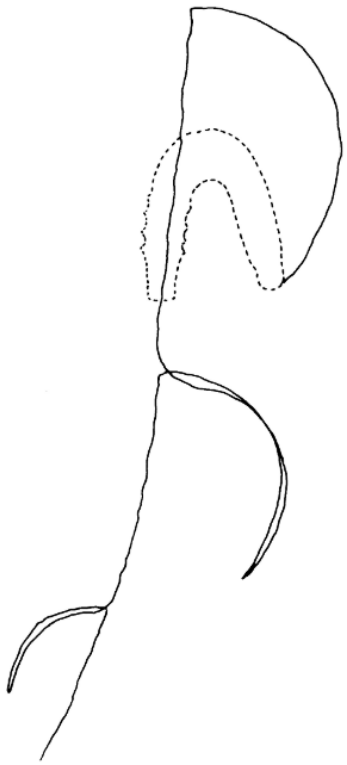
Supplementary Material S3. Life history of *Phryxe caudata* based on Biliotti (1956).

Phryxe caudata has two generations a year on *Thaumetopoea pityocampa* caterpillars. The first generation starts with oviposition on third to fourth instar *T. pityocampa* caterpillars, generally in October. The egg is laid on the larval body at daytime. The parasitoid larva hatches immediately and burrows into the epidermis of the host and navigate inside the haemolymph to reach the trachea in the posterior part of the abdomen. Here the parasitoid larva moults to the second instar in a tracheal vesicle and enter diapause. Diapause is broken with the moulting of the host larva from fourth to fifth (final) instar, which generally happens at the end of winter. The parasitoid larva then moults to third (final) instar, leaves the trachea vesicle and devours the host larva. The parasitoid larva leaves the dead host and moves to the surface of the tent to form the puparium. Adult flies emerge rapidly, mate and search for new *T. pityocampa* caterpillars to parasitise. These flies are observed during the pupation procession period of the host caterpillars (typically March to April), where the eggs of the second generation are laid. The parasitoid larvae develop quickly inside the pre-pupae and pupae of *T. pityocampa* and form a puparium that later becomes the flies starting the first generation.

Biliotti, E., 1956. Biologie de *Phryxe caudata* Rondani (Dipt. Larvaevoridae) parasite de la chenille processionnaire du pin (*Thaumetopoea pityocampa* Schiff.). Rev. Pathol. Veg. d'Entomologie Agric. Fr. 35, 50–65.



Supplementary Figure S4. Illustration of how a *Thaumetopoea pityocampa* caterpillar spin and attach silk during tent construction, figure from Démolin (1967). **a – c** *Thaumetopoea pityocampa* caterpillar applying multiple strands of silk by grasping the platform with its prolegs and bending its anterior body backwards as much as possible. The length of the silk strands is the limit of how far the caterpillar can bend. The numbers in red represent the order of silk attachment. **d-e** Silk attachment by *T. pityocampa* on a smaller platform.



Supplementary Figure S5. Illustration of how *Malacosoma americanum* caterpillar spin silk during tent construction, figure from Fitzgerald and Willer (1983). The anterior body of the caterpillar is swung as far to the side as possible. The solid line represents the silk strand and the dotted line represents the caterpillar

Chapter 6

Synopsis and future work

Photograph taken by Anthony Hearsey.

6.1 Synopsis

For this thesis, I had the unique opportunity to study a species of processionary caterpillar in two hemispheres, one species from Australia and one from Europe. *Ochrogaster lunifer* and *Thaumetopoea pityocampa* belong to the Notodontidae (Fig. 6.1) and shares similar behavioural, ecological, and morphological characteristics even after the separation of the continents millions of years ago. The retainment of these characteristics illustrates how important these functions are for the survival of the species. The thesis explored various behavioural, ecological, and biological hypotheses of the two processionary caterpillar species that remained unknown until now.

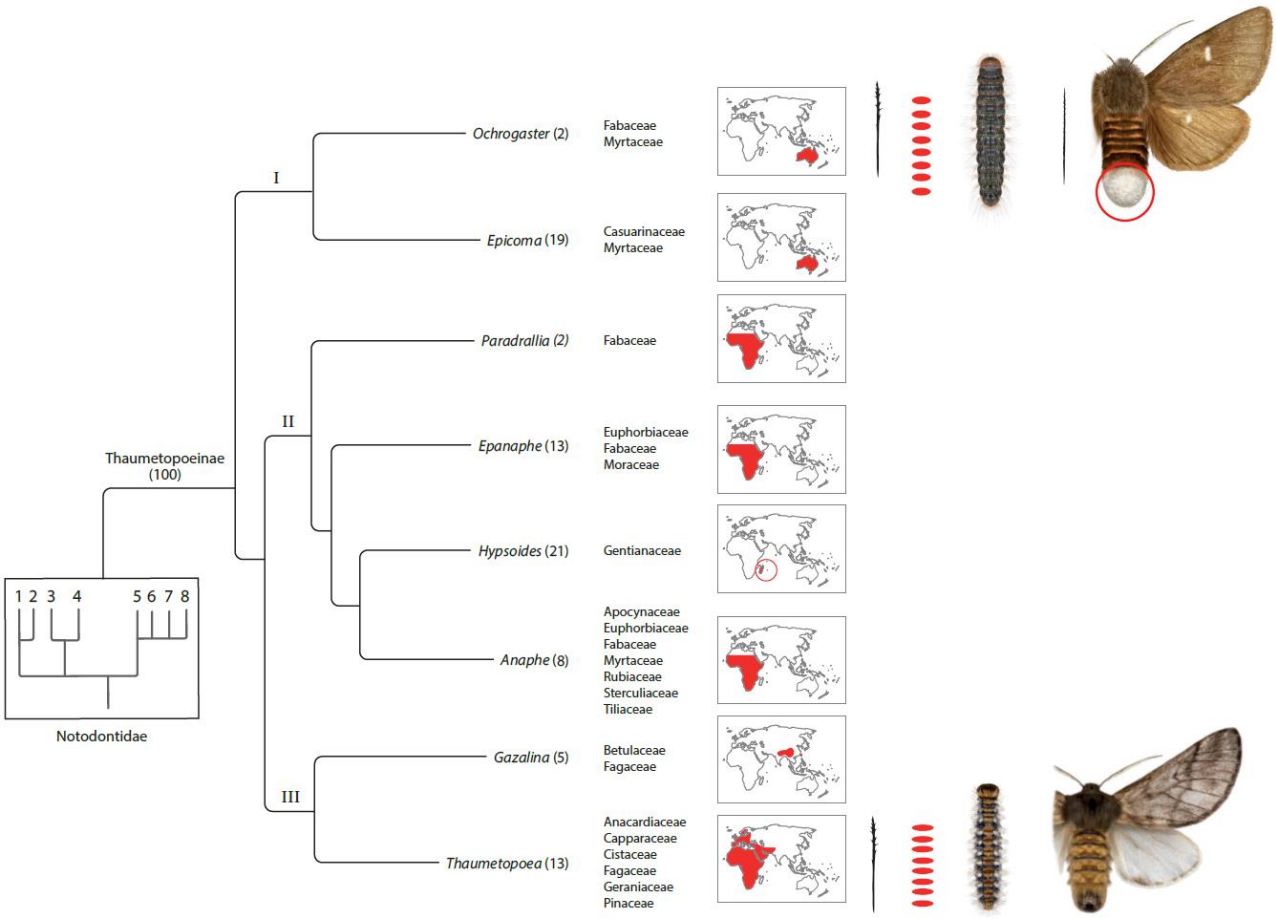


Figure 6.1. Cladogram of the Thaumetopoeinae within Notodontidae showing the three major clades (roman numerals), the main genera (N = number of species), main host-plant families, and their geographic distribution. In the genus Ochrogaster and Thaumetopoea, the true seta, number of setal fields (mirrors) on the abdominal tergites from the final instar larva, larva and adult moth are pictured and not to scale. The adult females of Ochrogaster have urticating setae in the tuft scales (circled). Figure and figure caption modified from Battisti et al. (2017). Phylogenetic tree of Notodontidae: 1 = Pygaerinae, 2 = Dudusinae, 3 = Phalerinae, 4 = Thaumetopoeinae, 5 = Heterocampinae, 6 = Notodontinae, 7 = Nystaleinae, 8 = Dioptinae.

I discovered the environmental preferences for potential pupation sites of *O. lunifer* and *T. pityocampa* caterpillars (Chapter 2). This period of development is the most critical as humans and animals may be exposed to the urticating setae of caterpillars as they crawl across the ground. The processionary caterpillars can move on the ground for a considerable distance before finding a suitable pupation site. From the nest, *O. lunifer* caterpillars travelled on average 40 m per day to the north and south (Fig. 6.2 a and b) towards environments where there was shade. From the initial sighting in the field, *T. pityocampa* caterpillars travelled on average 16 m per day in all orientations (Fig 6.2 c) towards brightly lit areas of the environment. The pupation sites for both species are located at some distance away from the host tree where the caterpillars developed and fed. These results have two implications: 1) the control of the caterpillar/pre-pupae/pupae with natural enemies (e.g. entomopathogenic fungi and nematodes) and/or insecticide can be improved by a better localisation of the application of these control agents; 2) the area where urticating setae are dispersed during the procession and at the pupation sites can be better defined and measures can be taken to avoid an overlap with the activity of humans and animals.

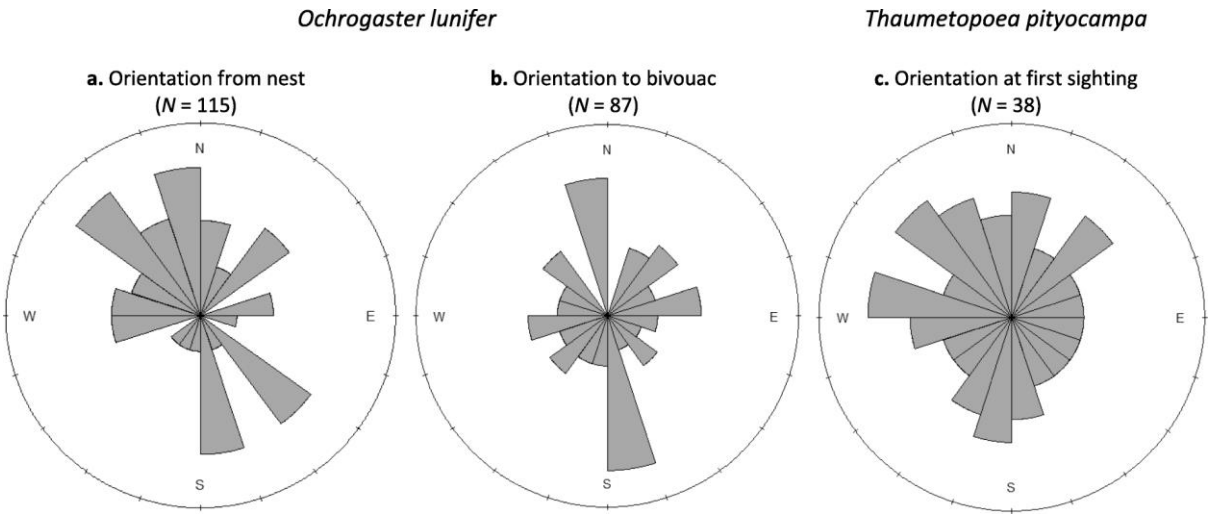


Figure 6.2. Rose diagrams of the orientation of pre-pupation processions of *Ochrogaster lunifer* leaving the nest **a**) and final orientation to the bivouac following the last turn of the leader **b**), and of *Thaumetopoea pityocampa* at first sighting **c**). Figure from Uemura et al. (2020a).

I determined that *O. lunifer* and *T. pityocampa* caterpillars orientate in the environment using a pair of specialised stemmata (stemma I) and the skylight polarisation pattern as a compass (Chapter 3). The compass is based on the detection of polarisation patterns in the sky, and the mechanism is based on the anatomical structure and functions of stemma I found on

the most upper position of both sides of the head capsule. With this knowledge, we can 1) predict the direction taken by caterpillars on the ground, which is essential to deploy measures to prevent contact of humans and animals with urticating caterpillars when they are highly mobile; and 2) produce devices to interfere with the movement of caterpillars via external obstruction of the larval visual field and to manipulate caterpillars to unsuitable pupation grounds for poor development. This study was the first to use both behavioural and anatomical evidence to determine caterpillar polarisation vision. Additionally, the unique external morphological characteristics (⅔ rugged and ⅓ smooth surface) of stemma I responsible for polarisation vision from the two species has not been reported previously in other Lepidoptera species (Fig. 6.3). This morphological trait may help quickly distinguish if other caterpillar species are capable of polarisation vision.

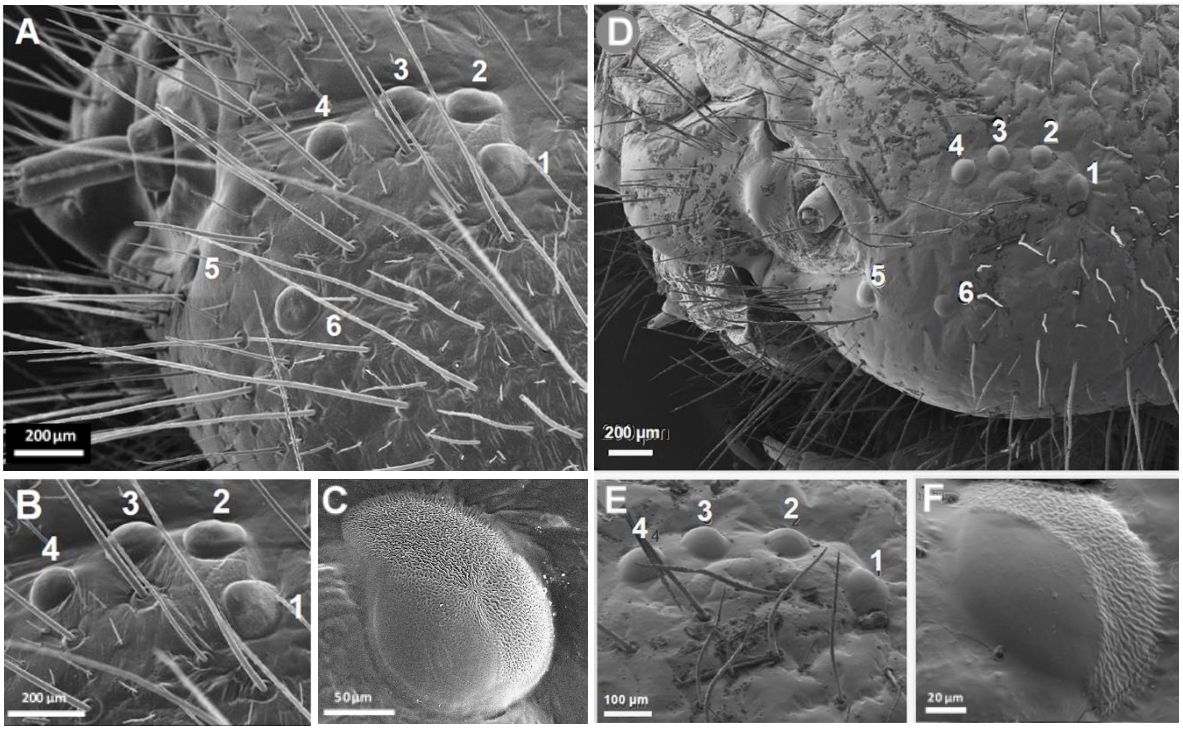


Figure 6.3. Scanning electron micrographs (SEM) of the left head capsule of final instar *Ochrogaster lunifer* (**A-C**) and *Thaumetopoea pityocampa* (**D-F**) caterpillars. **B and E** SEM of stemma I-IV arranged in a semicircle. **C and F** SEM of stemma I with rough and smooth surface covering ⅔ and ⅓ of the stemma, respectively. *Ochrogaster lunifer* SEM images taken by Erica Lovas and *T. pityocampa* SEM images taken by Gregor Belušič.

I identified the larval movement and tent locations of *T. pityocampa* from first to final larval instar on the host tree (Chapter 4). *Thaumetopoea pityocampa* colonies were consistent with the preferred tent locations and orientation on the host tree (Fig. 6.4). The initial tents of first instar larval colonies are distributed homogenously in the tree canopy but tend to converge

to the south facing side of the tree as they moult to the second and third instar. The tendency to move to the south becomes stronger in the fourth instar, when the final winter tent is built. This knowledge is important for 1) the detection of *T. pityocampa* colonies in the field, especially when an automatic image analysis is used for a rapid census of the population density; 2) for the application of a biopesticide (e.g. entomopathogenic bacterium *Bacillus thuringiensis kurstaki*) that can be restricted to the tent and the surrounding foraging area. The biopesticide may be sprayed by drones (unmanned aerial vehicles) to limit consequences on non-target species.

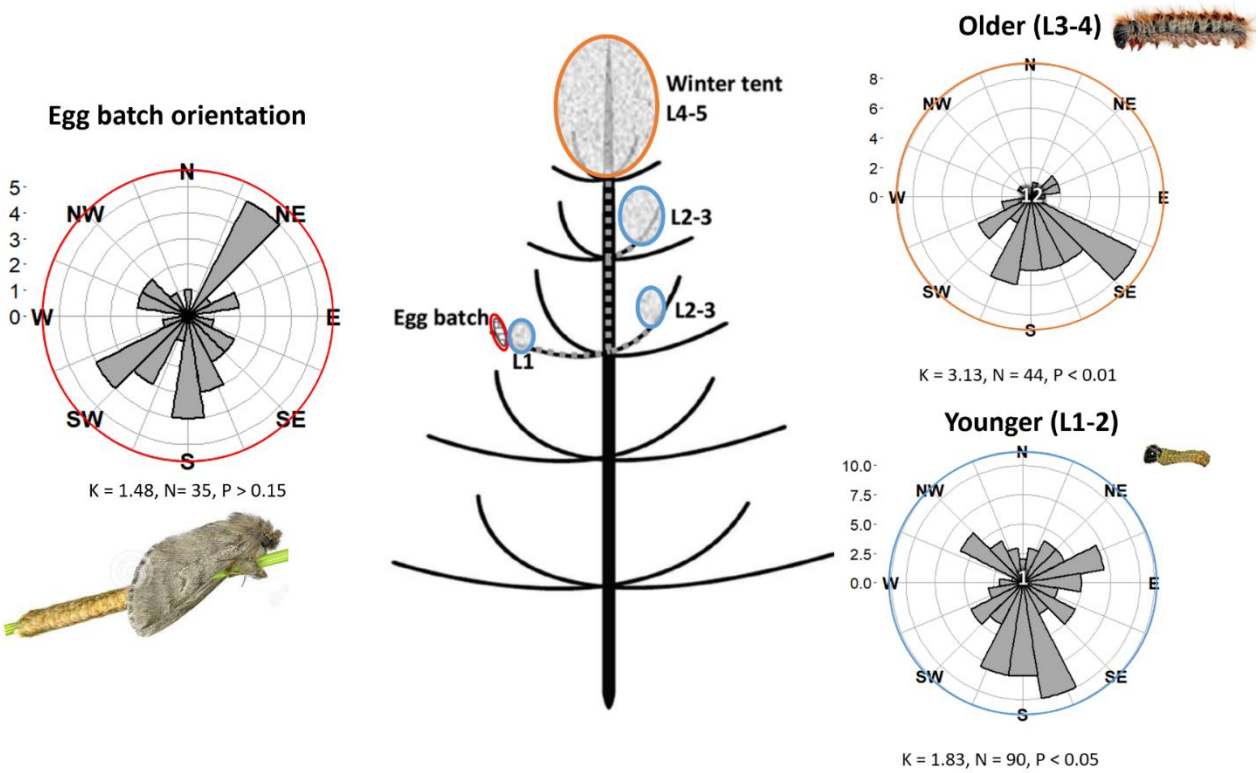


Figure 6.4. Rose diagrams of the orientations of *Thaumetopoea pityocampa* egg batches (red) and younger (blue) and older (orange) larval tents on *P. nigra* host trees; modified from Uemura et al. (2020b). The numbers on the left of each rose diagram correspond to the rings inside the circle and it represents the number of observations, starting from the smallest number in the centre to the largest number in the second last outer ring. Number in the centre of the rose diagrams of younger and older larval colonies 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. The schematic diagram in the middle represents *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.

Finally, I described the tent construction and foraging behaviour of *T. pityocampa* colonies from first to fourth larval instar (Chapter 5). Quantitative behavioural data on division

of labour in *T. pityocampa* caterpillars during tent construction has not been studied until now. At sunset, larger male caterpillars (early active males) were more likely to construct and maintain the tent, while most of the female caterpillars (late active female) remained inside the tent until foraging time (Fig. 6.5). Consequently, nearly half of the males that were actively spinning silk to maintain the tent were parasitised by *Phryxe caudata*. Tent maintenance occurred at sunset, possibly for predator/parasitoid evasion and/or for the detection of the strong polarised skylight pattern present at that time of the day (Fig. 6.6). In addition to building tents on the south facing side of the host tree (see Chapter 4), more silk was applied on the south facing side of the tent (Fig. 6.7). SEM results showed that from first instar larva, caterpillars have specialised morphological characteristics in stemma I that would enable polarisation vision. Therefore, by utilising the polarisation pattern of the sky at sunset with stemma I, the caterpillars were able to recognise where is south on the tree and tent. Determining the timing of larval tent construction and foraging patterns may enable more effective pest management strategies by 1) manipulating natural enemies and releasing them or timing the spray of pesticide during the hours of when caterpillars are vulnerable and outside of their tent; 2) obstructing the larval visual field so silk application and construction of the tent could be disrupted and therefore produce an ineffective tent for survival/thermoregulation. Additionally, the cooperative behaviours described in this species highlights the intricacy of social organisations experienced in simpler non-eusocial insects.

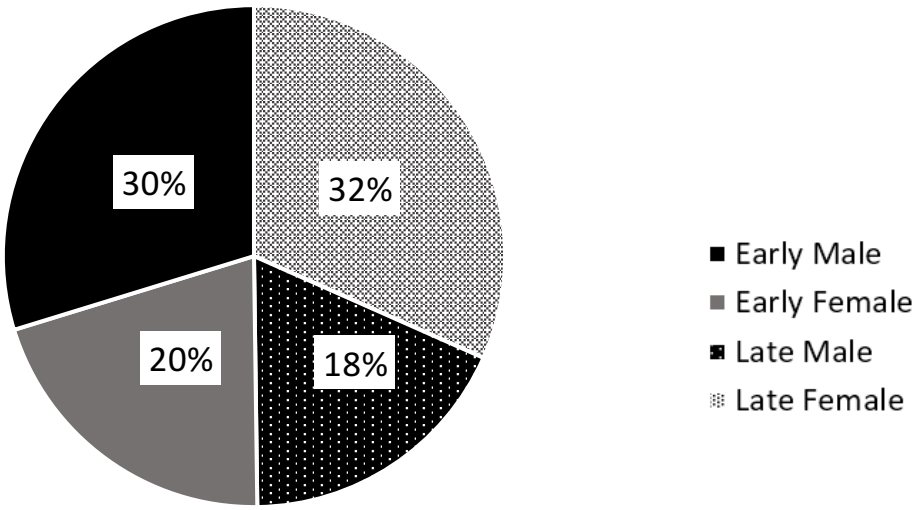


Figure 6.5. Proportions of male and female *Thaumetopoea pityocampa* caterpillars that were active early and late in the night (N = 474). Solid coloured black and grey represents early active male and female caterpillars, respectively. Black with white dots and grey with many dots represent early active male and female caterpillars, respectively.

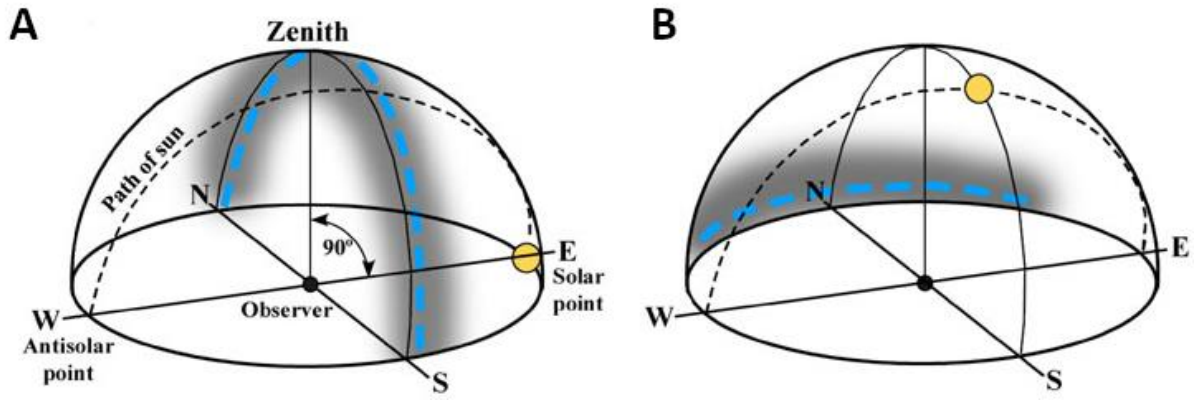


Figure 6.6. Pattern of polarised light across the sky at sunrise (**A**) and noon (**B**), figure and figure caption from Bradbury and Vehrencamp (2011). At sunrise and sunset, the polarised band arcs across the middle of the sky (described as Zenith in figure A). At noon, the polarised band is close to the horizon, north in northern hemisphere and south in southern hemisphere. Blue dashed lines show the orientation of the e-vectors within the strongly polarised band. Less strong bands of polarised light form concentric circles around the sun.

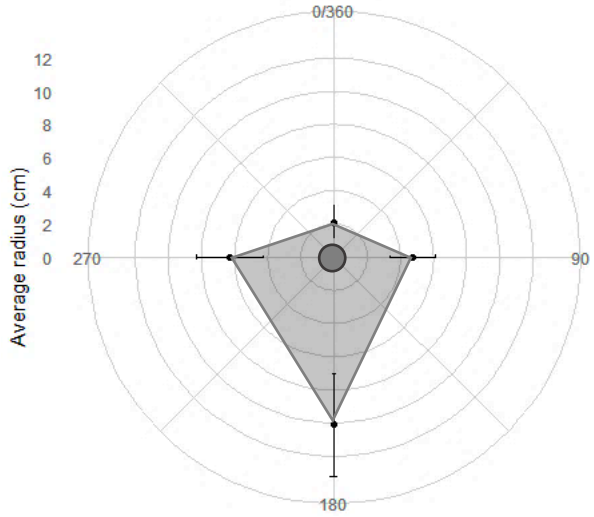


Figure 6.7. Average tent radii (± SD) from the pine branch in the centre (dark grey circle) outwards towards North (0/360), East (90), South (180) and West (270) of final instar *Thaumetopoea pityocampa* tents (N = 100). Each grey ring represents the distance away from the branch on the left, starting from the shortest distance in the centre to the furthest in the second last outer ring.

6.2 Future work

Advances in technology, tools and facilities in science provides opportunities for innovative methods to better understand biological systems. Throughout this thesis, I have developed novel ways of investigating various unknowns in two species of processionary caterpillars of pest concern. The research has provided prospects for improved management of *O. lunifer* and *T. pityocampa* which could also be applied to other species of concern. Further studies that follow from our research can conclude various unresolved hypotheses that I could not address in this thesis.

In our research, polarisation vision in processionary caterpillars was determined through behavioural and anatomical analyses. Despite the large effort, we were unable to assess the physiological response of stemma 1 to polarised light because of complications with finding the optical nerve of the stemma. Using electrophysiology in larval vision experiments is hi ghly important as it provides direct data on the spectral sensitivities of the stemma to polarised light and visible and non-visible light wavelengths. Direct readings from each stemma to test the response of polarised light and various wavelengths of light could be made possible in the future studies with optical indicators of membrane voltage or calcium activity. This knowledge would contribute to a better understanding of the larval behaviour under different environmental conditions on the tree and on the ground.

The data collected on *T. pityocampa* egg batch distribution and larval movement on the tree is based on the most common host plant *Pinus nigra*. Female *T. pityocampa* moths have several different hosts, therefore, it would be valuable to understand whether or not the pattern is similar in other host species. For example, *Cedrus* is another host genus for *T. pityocampa,* and they have a different architecture and needle age distribution to *Pinus*. *Cedrus* spp. are important ornamental conifers in Europe and are often oviposited by the moth. Therefore, it would be beneficial to use *Cedrus* as a target host for future behavioural analyses and to compare with *P. nigra*.

Our observations and experiments have opened new possibilities to identify social organisation in processionary caterpillars. Both *O. lunifer* and *T. pityocampa* are gregarious and highly social from egg to final instar larva, and there is still so much to learn from their sophisticated social structure. The challenge now is to explore the role of gender and size in caterpillars in the organisation of the colony; especially for nest construction as it involves fine-

tuned communication systems. Division of labour/polyethism is possibly the underlying advantages for successful social living in *T. pityocampa*. I identified the importance of male *T. pityocampa* caterpillars in the colony as they expend their energy on silk production and tent construction whilst exposing themselves to natural enemies. Detection of caterpillar activity (e.g. tent maintenance) of certain individuals may be automated through the use of video tracking colour-tagged insects. A comparative study of parasitised and unparasitised caterpillar colonies would be interesting to determine if caterpillars alter their behaviour and if there is an organisational imbalance of the colony. Further comparative studies on various model caterpillar species can identify the trends of social organisation in non-eusocial insects which are currently unstudied.

The comparative approach of the Australian and European model species of processionary caterpillars was shown to be very successful. I believe that carrying it further would shed light on the social systems of processionary moths, whilst providing useful insights for the management of these species that can be harmful to trees, humans, and animals. I hope that our findings can promote and inspire future research on the two species and beyond.

6.3 References

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Chapter 7

Appendices

Photograph taken by Anthony Hearsey.

7.1 Dissemination

The following is a dissemination about *O. lunifer* as a potential pest in Europe and published as: Uemura, M., Battisti, A., 2019. Overview and preliminary risk assessment of the processionary caterpillar *Ochrogaster lunifer* (Lepidoptera: Notodontidae), a Eucalyptus pest from Australia. Atti Accademia Nazionale Italiana Di Entomologia 143–150.

Atti Accademia Nazionale
Italiana di Entomologia
Anno LXVII, 2019: 143-150

OVERVIEW AND PRELIMINARY RISK ASSESSMENT OF THE PROCESSIONARY CATERPILLAR *OCHROGASTER LUNIFER* (LEPIDOPTERA: NOTODONTIDAE), A *EUCALYPTUS* PEST FROM AUSTRALIA

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ORCID 0000-0002-2497-3064
Lettura tenuta durante la Tavola Rotonda "Avversità e interesse apistico dell'eucalipto in ambiente mediterraneo". Seduta pubblica dell'Accademia - Firenze, 14 giugno 2019.

Overview and preliminary risk assessment of the processionary caterpillar Ochrogaster lunifer *(Lepidoptera: Notodontidae), a* Eucalyptus *pest from Australia*

As human population increases, so does the demand for resources from domestic and international suppliers for their quality and/or quantity. Increase in international trade, has exacerbated the introduction of non-native invasive pests causing ecological, societal and economic damage. In order to reduce this outcome, it is crucial to consider all possibilities of potential pests from foreign countries that could establish in Europe. *Eucalyptus* and *Acacia* spp. are used as ornamental plants in Europe, commonly planted in parks, suburban and recreational areas frequented by humans and pets/animals in the Mediterranean region. Here, we explore the potentials of a *Eucalyptus* and *Acacia* pest, *Ochrogaster lunifer* Herrich-Schäffer (Lepidoptera, Notodontidae), a medically important species from Australia, entering and establishing in Europe. In outbreak years, *O. lunifer* larvae completely defoliate host plants but more importantly, it can increase the number of cases of urticaria and allergic reactions in humans and domestic animals because each larva possess millions of urticating setae. The species may enter Europe as eggs, larva, pre-pupa and pupa found on the host plant, in the soil or in packaging material. Establishment and spread of *O. lunifer* will depend on the host plant availability, efforts by inspectors during quarantine checks and phytosanitary measures. *Ochrogaster lunifer* is only found in Australia, but with the severity of various health implications that they cause to humans and animals, and with the frequent introduction of eucalypt pests from that continent, it is necessary to assess risks for the introduction of this species into Europe.

KEY WORDS: Defoliator, medical importance, setae, social Lepidoptera

INTRODUCTION

As human population increases, so does the demand for resources from domestic and international suppliers for their quality and/or quantity. Increase in international trade, has introduced invasive pests causing ecological, societal and economic damage. Plant-insect pest management is economically demanding; however, it becomes more problematic when serious health implications are involved with humans and animals. A species of recent interest is the processionary caterpillar, *Ochrogaster lunifer* Herrich-Schäffer (Lepidoptera, Notodontidae), an urticating species found throughout Australia. The name 'processionary caterpillar' arises from their dispersal behaviour when larvae travel in a single file head-to-tail from the nest to search for a new host plant or a pupation site (FITZGERALD, 2003). Populations of *O. lunifer* are concentrated in coastal and inland habitats where *Acacia* and *Eucalyptus* spp. host plants occur (FLOATER, 1996; Fig. 1). There are other plants which *O. lunifer* feed on but are less recognised as primary host plants, therefore, more confirmation is needed. In outbreak years, *O. lunifer* are known to cause severe damage to plants through complete defoliation (FROGGATT, 1911; VAN SCHAGEN *et al*., 1992a; Fig. 2). Outbreak years can also increase the number of cases of urticaria and allergic reactions in humans and various medical problems in animals (see *Damage and health impacts of* Ochrogaster lunifer *on humans, animals and plants*).

Medical risks associated with *O. lunifer* is derived from their urticating microscopic hairs called setae, which are found on the abdominal segments of mid to late instars (BATTISTI *et al*., 2011). From third instar, each larva produces approximately 4000 setae and progressively increases until they reach final instar (VIII) where the number of setae increases up to approximately 2.0-2.5 million (PERKINS *et al*., 2016). Setae can be easily detached during inadvertent contact with the larva but can also be released and spread throughout the environment by wind (PERKINS *et al*., 2019). *Ochrogaster lunifer*

– Pubblicato marzo 2020

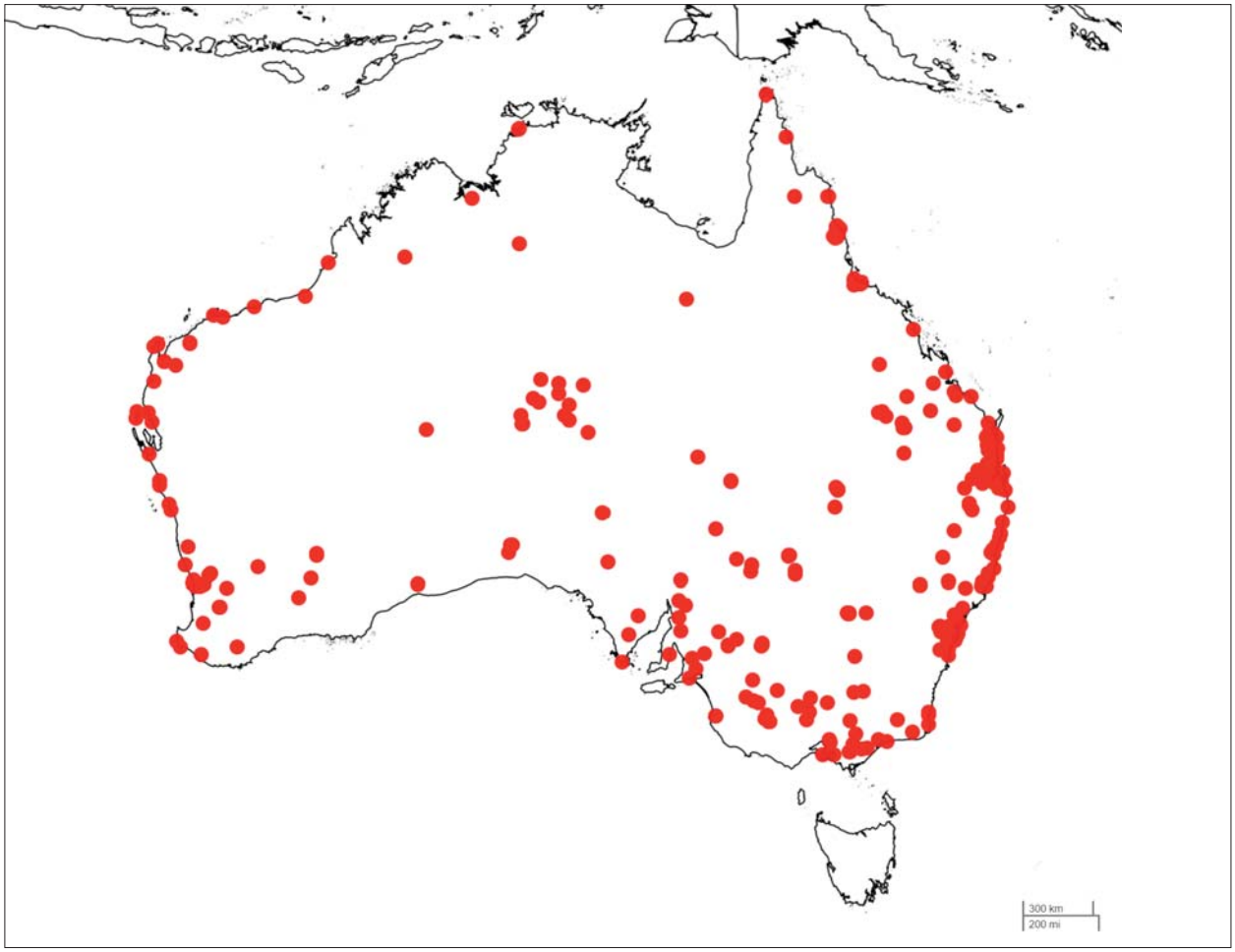

Fig 1 – Occurrence records of *Ochrogaster lunifer* in Australia represented as red dots (Atlas of Living Australia, 2020).

larvae live in a communal nest throughout all instar stages, with some nests easily exceeding a few hundred individuals. Within a nest, at each larval moult, there is an accumulation of exuviae containing millions of setae from each individual (Perkins *et al*., 2016). When the colony of final instar larvae abandon the nest in search for a pupation site, the structure of the nest deteriorates. As the nest breaks down over time, millions of setae that can remain active for at least a year (Battisti *et al*., 2011) are now exposed and dispersed in the environment (Perkins *et al*., 2016; Fig. 3).

Life history of *Ochrogaster lunifer*

Ochrogaster lunifer are a univoltine species and remains gregarious from egg to pupa (Floater and Zalucki, 1999; Fig. 4). Adult moths emerge in Austral spring (October - November), and mated females deposit an egg mass consisting of 150-550 eggs on the host tree (Floater and Zalucki, 1999). The eggs are covered with material from the female moth's anal tuft, composed of filamentous scales and long urticating setae which are thought to provide protection from natural enemies (Floater and Zalucki, 1999). Eggs hatch after a month and the neonates remain within the egg mass and do not feed until second instar (Floater, 1996). The neonates moult to second instar after approximately 14 days, and leave the egg mass and ascend to the canopy to feed on the foliage during the day (Floater, 1996). Later instars feed almost exclusively at night, leaving the nest at approximately sunset every day (Floater, 1996). The gregarious larvae continuously spin silk throughout all larval stages to build a communal nest within the tree (Floater, 1996). Within the species *O. lunifer*, there are five distinct nesting forms and they differ by phenotypic, genotypic and ecological characteristics (more information in *Nesting forms of* Ochrogaster lunifer). *Ochrogaster lunifer* has eight larval instars, with later instars showing sexual dimorphism with females being larger than males (Floater, 1996). As the larvae get older, the nest expands around the original egg batch (Floater, 1996). In *O. lunifer* that feeds exclusively on *Corymbia tessellaris* (syn. *Eucalyptus tessellaris*), at third instar, the larvae

Fig. 2 – Completely defoliated *Corymbia tessellaris* by *Ochrogaster lunifer* larvae in Gatton, Australia. Image taken by Mizuki Uemura.

Fig. 3 – Deteriorating abandoned *Ochrogaster lunifer* nest tangled on a branch of *Corymbia tessellaris* and blown by the wind, in Gatton, Australia. Image taken by Andrea Battisti.

Fig. 4 – Life history of *Ochrogaster lunifer* in south-east Australia. *Ochrogaster lunifer* has a univoltine lifecycle: 1 month as an egg in Oct-Dec, larva for 5 months in Dec-May, pre-pupal diapause for 4 months in May-Sep, pupa for 1 month in Sep-Oct, and adult for a few days in Oct-Nov. Going clockwise from the image in the top middle: **1)** a golden above ground nest *O. lunifer* egg mass with L2 larvae, **2)** above ground nest egg mass in the canopy of a eucalypt, **3)** procession of late instar larvae on a eucalypt, **4)** pre-pupation procession of last instar (VIII) larvae leaving the host tree to search for a pupation site, **5)** uncovered pre-pupa in diapause, **6)** cocoon, pupa and larval exuvia of a male *O. lunifer*, **7)** *O. lunifer* female. Images 1 and 6 taken by Lynda Perkins, and images 2-5 and 7 taken by Mizuki Uemura.

move to the trunk and build a nest on the surface of the tree at approximately mid height (M. Uemura, pers. obs. 2017). If there is more than one egg mass on a host tree, different colonies may gather in one nest consisting of a few hundred individuals (Floater, 1996). In May, when final instar *O. lunifer* larvae are fully fed, the colony leaves the nest in a procession to find a pupation site underground (Floater, 1996). Processions may spend several days to find a suitable site, creating temporary 'bivouacs' during their journey (Uemura *et al*., 2020). When the colony finds a suitable pupation site, the larvae burrow together to a depth approximately 10-20 cm from the surface (M. Uemura, pers. obs. 2017) and goes into diapause for approximately three months as a pre-pupa (Floater and Zalucki, 1999). In September, pre-pupa spins a cocoon that is embedded with larval setae and emerges as an adult in October-November (Floater, 1996). Moths have reduced mouthparts and do not feed during the few days they are alive (Floater, 1996). Mated females find a suitable host tree by 'sampling' the leaves and branches of a tree, which may take a few attempts to find the right one (Floater, 1996).

Larval numbers decreased as *O. lunifer* development progressed, due to natural enemies such as predators and parasitoids and from other natural causes (van Schagen *et al*., 1992b). There are various invertebrate predators and parasitoids that prey on *O. lunifer* at different life stages. Highest mortality occurred during the egg and first instar stages by dermestid larvae (Coleoptera: Dermestidae) predation (Floater, 1996). Two species of dermestid larvae present in *O. lunifer* egg masses were *Dermestes ater* De Geer and *Trogoderma apicipenne* Reitter however, the prevalence of dermestid predation was variable (Floater, 1996). Other predators recorded feeding on *O. lunifer* are predatory pyrrhocorid bug, *Dindymus circumcinctus* Stål (Hemiptera: Pyrrhocoridae) (Floater, 1996), predatory moth, *Titanoceros* sp. (Lepidoptera: Pyralidae) and spiders (van Schagen *et al*., 1992b). Egg parasitism by chalcid wasps *Anastatus fuligispina* Girault (Hymenoptera: Chalcidoidea) was a common occurrence with 95% of egg masses parasitised in Gatton, Australia (Uemura *et al*., 2019). However, this is possibly due to a concentration effect of the local *A. fuligispina* population in Gatton, since Floater (1996) found low prevalence of *A. fuligispina* in 1.2 - 3.6 % of egg masses surveyed (Uemura *et al*., 2019). An important mortality in *O. lunifer*, is larval parasitism by *Carcelimyia dispar* Macquart (Diptera: Tachinidae) and less commonly by sarcophagid flies (Diptera: Sarcophagidae) (Floater, 1996). In the case of *C. dispar*, mated female flies deposit one or several eggs on the head capsule of *O. lunifer* larvae (Floater, 1996). As for vertebrate predators, adult moths were recorded to be predated by pied butcher bird *Craticus nigrogularis* Gould (Passeriformes: Artamidae) (van Schagen *et al*., 1992b) and noisy miner bird *Manorina melanocephala* Latham (Passeriformes: Meliphagidae) (M. Uemura, pers. obs. 2018). Vertebrate predators of larval *O. lunifer* are likely to be less abundant because of the inflammatory reaction to the short urticating setae, which are considered a defence mechanism against mammalian and avian predators (Battisti *et al*., 2011). However, in addition to setae, the volatile chemicals present on the body of *O. lunifer* larva deterred predatory ants from attacking (Uemura *et al*., 2017). Therefore, the combination of setae and volatile chemicals produced by *O. lunifer* larvae may deem unpalatable to vertebrate predators (Perkins *et al*., 2019).

Nesting forms of *Ochrogaster lunifer*

Ochrogaster lunifer have five different nesting forms: canopy, trunk, tree-hugger, hanging and ground nests (Perkins *et al*., 2016; Fig. 5). The variation of nesting forms found within *O. lunifer* depend on the location of oviposition site, morphology and ecology of the species (Mather *et al*., 2019). Female moths from all nesting forms except for the ground nest, oviposits in the canopy of the host tree. Females from ground nests oviposit on the base of the host tree trunk (Floater, 1996), whereas the canopy nest females oviposit on the branches and twigs of the host tree. Additionally, the white colouration of the egg mass is found only in ground nests, which differs from other nesting forms which are golden (Floater, 1996; Fig. 6). The egg mass colouration is derived from the colour of the anal tuft scales present on the female moth. Correspondingly, the morphology of adult moths of both sexes differ between the nesting forms (Fig. 7). Size and colour of larvae appear to differ between above ground and ground nest forms (Mills, 1951). Colouration of the silk produced by the larvae to build the nest is white in ground nests and yellow to golden in above ground nests (M. Uemura, pers. obs. 2017). Nesting forms found both on the ground and above ground feed on various *Acacia* spp., however, *O. lunifer* nests above ground feed on eucalypts (*Eucalyptus* and *Corymbia*) (Floater, 1996). Numerous scientists have raised the question if *O. lunifer* is a cryptic species. Mather *et al*. (2019) have identified at

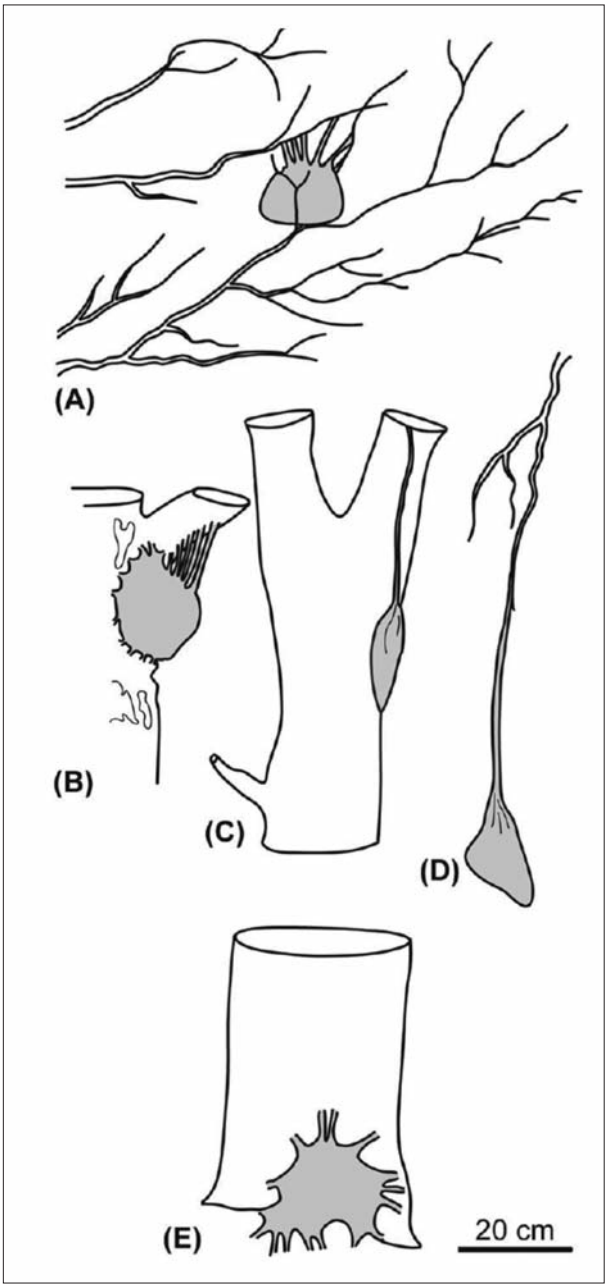

Fig. 5 – Schematic diagrams of the five nest types built by *Ochrogaster lunifer* larvae in Australia. (A) Canopy nest. (B) Trunk nest. (C) Tree-hugger nest. (D) Hanging nest. (E) Ground nest. (PERKINS *et al*., 2016).

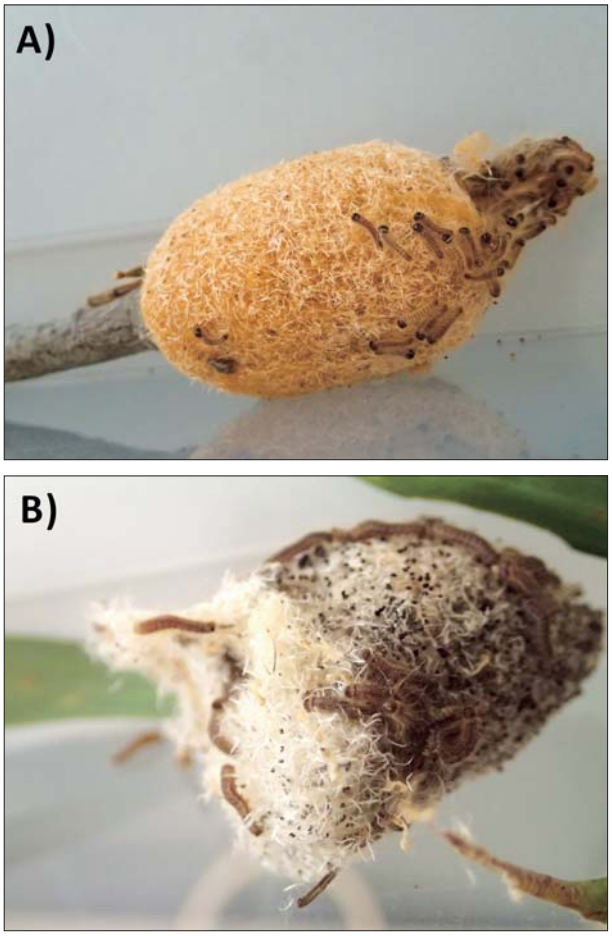

Fig. 6 – **A)** Golden above-ground nest and **B)** white ground nest *Ochrogaster lunifer* egg mass with second instar larvae crawling on the surface. Images taken by Lynda Perkins.

least two reproductively isolated species within the current concept of *O. lunifer* and is under further taxonomic review.

DAMAGE AND HEALTH IMPACTS OF *OCHROGASTER LUNIFER* ON HUMANS, ANIMALS AND PLANTS

Recently, more research efforts on *O. lunifer* arose after their role in equine amnionitis foetal loss (EAFL), a medical condition in pregnant mares which can result in miscarriage after accidental setae ingestion (CAWDELL-SMITH *et al*., 2012). What follows is based on the setae of the larvae, whereas those produced by the female moth to protect the eggs are still to be studied in detail. Urticating setae are used as a defence against natural enemies, and this defence is enhanced by living gregariously as seen in this species. Setae contain proteins which the mammalian immune system recognizes as foreign, which then results in inflammatory or immunological defence reactions of varying severities (BATTISTI *et al*., 2011). In Australia, urticaria cases in humans from *O. lunifer* have been reported in literature as early as 1911 (FROGGATT, 1911) (as *Teara contraria*). Humans and animals can be exposed to urticating setae by direct contact with larvae, by wind carrying the setae, and by ingestion of contaminated feed and water (MULLEN, 2009). Direct contact with larvae may occur when humans and animals are near infested host trees but most commonly when the larvae leave the nest permanently to search for a pupation site (PERKINS *et al*., 2016). During a study at the university campus in Australia, there were 22 cases out of 82 *O. lunifer* pre-pupation processions which came into contact

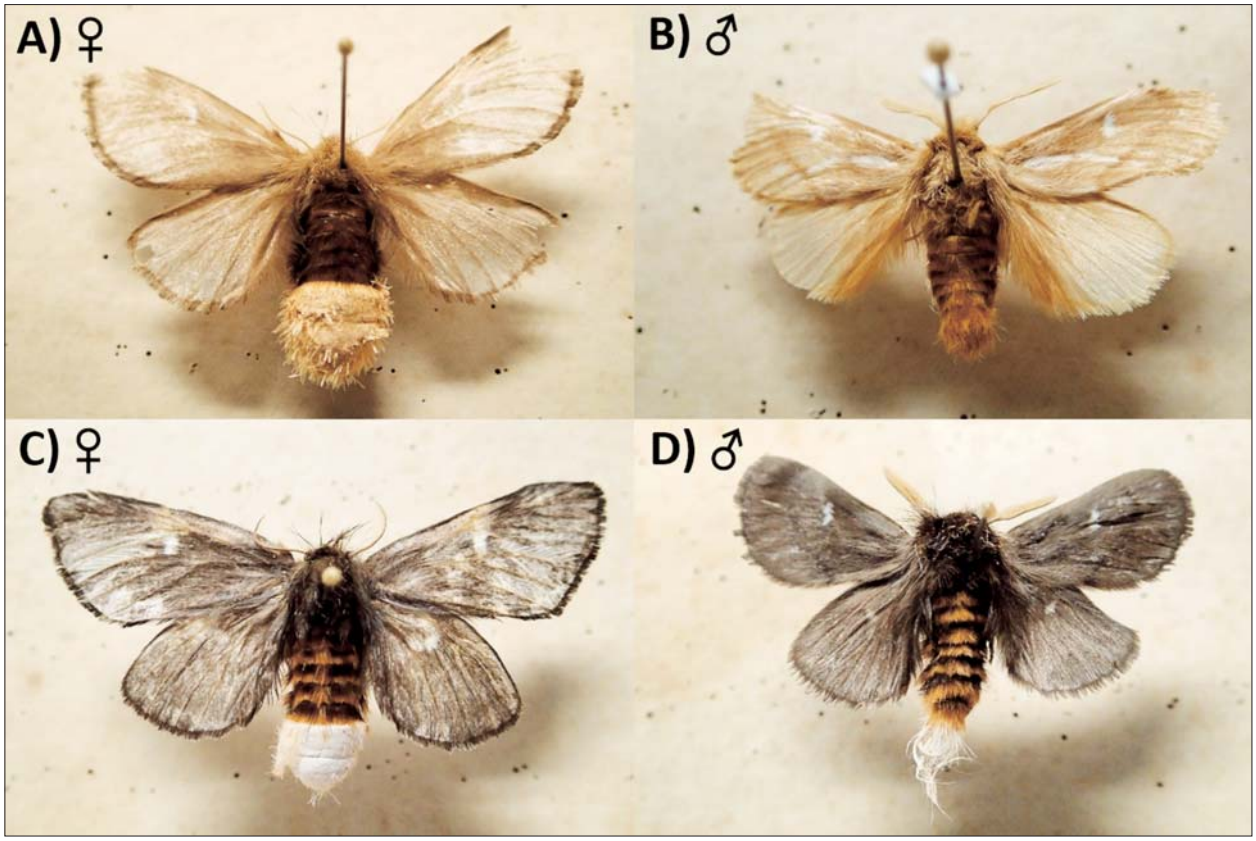

Fig. 7 – *Ochrogaster lunifer* female and male from above ground (A & B) and ground nests (C, D). There are variations of wing pattern and colouration within and between nest types and sex. Images taken by Mizuki Uemura.

with pedestrians or by vehicles (Uemura *et al*., 2020). After contact, the shoes, clothing and/or wheels may be contaminated with setae and brought back to the car, home, classroom, etc. (Uemura *et al*., 2020). Irritation and other reactions may occur even after some time, since the materials in the setae remain active and the microscopic scale of the setae can be undetected (Uemura *et al*., 2020). The health impacts and contamination are exacerbated because of the gregarious behaviour throughout all life stages, especially as larvae. Devastating impacts are not limited to humans and animals, it can also affect the host trees of *O. lunifer*, as larvae defoliate large stands of trees (Floater, 1996) which can then have an ecological and economic impact. Defoliation of trees may impact cattle and other animals which used the trees as shade to protect them from the harsh Australian sun. Complete defoliation of the host occurs most frequently at later instar stages when larvae need to feed more and when the cohort is large from merging of multiple egg masses (M. Uemura, pers. obs. 2017). The cohort of larvae move during the day to find another host and subsequently, people may encounter these processioning larvae.

Preliminary risk assessment of *Ochrogaster lunifer* introduction to Europe

Pest distribution and occurrence

Ochrogaster lunifer occur throughout Australia, with higher density around the coastlines of the country (see Fig. 1). Within the occurrence areas, populations of *O. lunifer* are found in disturbed and non-disturbed environments. These environments include university campus, home and backyard, public parks, national parks, bushlands and roadsides. *Ochrogaster lunifer* are edge species therefore, female moths restrict their oviposition on isolated trees on the outer edges of forests, road verges, etc. (van Schagen *et al*., 1992a; Floater and Zalucki, 2000). The species is only found in Australia where it is not regulated.

Entry, establishment and spread in Europe

Ochrogaster lunifer may enter Europe through cargo ships and flight vessels for international trade and tourism. The species may enter as eggs, larva, pupa or pre-pupal larva found on the host plant, in the soil or in packaging. Adults are unlikely to survive because of the short life span of a few days (Floater, 1996). Eggs of *O. lunifer* may be

undetected in the canopy or the trunk of the host plant being shipped for planting. Eggs and neonates can survive without feeding/care for one and a half months, until the larvae moult to second instar which they can feed on the host foliage. Tree-hugger nest for example, are well camouflaged on the trunk of the tree therefore, the larvae living inside the nest may go unnoticed. Pupa and pre-pupal larva may be found within bare root host trees and/or contained in the soil of host plant liners. *Ochrogaster lunifer* processions searching for pupation sites were commonly found entering in human settlement (Uemura *et al*., 2020), including buildings which contained storage for dairy products (M. Uemura, pers. obs. 2019). Larvae are also capable of crawling up vertical surfaces such as walls (M. Uemura, pers. obs., 2018). Therefore, pre-pupal larvae may end up in packaging and commodities during this pre-pupation procession period. From the life stage of pre-pupal larva onwards, *O. lunifer* do not feed and remain in diapause as a pre-pupa for three months, pupa for a month and adult for a few days (Floater, 1996).

After successful entry of *O. lunifer* into Europe, the species may establish where host plants *Acacia*, *Eucalyptus*, *Corymbia* spp. and others occur (see Floater [1996]). There may be a higher possibility of establishment if *O. lunifer* are exposed to host plants that are readily available at harbors and airports. The Mediterranean basin has a similar climate to Australia, which can be favourable for *O. lunifer* development. This species occurs throughout all Australian landscapes therefore, climate may not be a limiting factor except for cold stress. However, normal development of *O. lunifer* may be complicated by day length and opposite seasonality (in northern and southern hemispheres).

Spread of *O. lunifer* will depend on the density and location of where host trees are present. *Eucalyptus* and *Acacia* spp. are used as ornamental plants in Mediterranean Europe, commonly planted in parks, suburban and recreational areas frequented by humans and pets/animals. Therefore, once established, *O. lunifer* could spread in areas with high human population density. This will create a higher risk for humans and animals affected by various medical problems associated with the setae (see *Damage and health impacts of* Ochrogaster lunifer *on humans, animals and plants*).

Availability and limits of mitigation measures

Currently in Australia, there are no mitigation measures for *O. lunifer* populations. Australian farmers and landowners with cattle, horses and other animals have used various methods to reduce/eliminate *O. lunifer* colonies from their own property (land) by: removing host trees, cutting bag nests from trees, burning nests, removing nests and burying it underground, and pouring gasoline and/or burning egg masses (L. Perkins, pers. comm. 2017).

Many eucalypt pests have been introduced to Europe from international trading and *O. lunifer* could also be added to the list. Therefore, strict phytosanitary measures and quarantine checks should be done to mitigate the introduction of *O. lunifer* into Europe. However, with thousands of cargo ships and flights coming into Europe every day, it is unfeasible to check everything with the limited number of staff.

Ochrogaster lunifer is highly social throughout all life stages and the chance of survival decreases with fewer number of larvae. Additionally, *O. lunifer* is not an 'hitch-hiker' and will not be attracted to commodities of food or wood, nor can they disperse far in the environment. Therefore, the possibilities of *O. lunifer* introduction to Europe are limited to host plants shipped from infested places and possibly inside packaging of commodities from where *O. lunifer* nests occur nearby.

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RIASSUNTO

L'aumento degli scambi commerciali va di pari passo con la popolazione umana e comporta una continua esposizione al rischio di introduzione di specie esotiche di parassiti delle piante, che possono talvolta presentare un rischio per la salute dell'uomo e degli animali. La coltivazione di specie esotiche di piante per uso forestale e ornamentale, come gli eucalitti e le acacie nella regione mediterranea, rappresenta un caso tipico di esposizione

al rischio di introduzione di specie esotiche. In questo lavoro viene presentato il caso del rischio di introduzione di una specie di lepidottero notodontide, *Ochrogaster lunifer* Herrich-Schäffer, più noto come processionaria dell'eucalitto e dell'acacia, noto per causare danni ingenti agli alberi e alla salute pubblica in Australia. Negli anni di pullulazione le larve defogliano completamente le piante ospiti e inoltre producono, analogamente alle processionarie europee, ingenti numeri di setole urticanti che vengono disperse nell'ambiente e sono causa di rilevanti disturbi a uomini e animali allevati. La specie può essere introdotta in Europa come uovo, larva, prepupa e pupa associati a piante ospiti e relativo substrato di sviluppo, oppure come prepupa e pupa casualmente incluse in spedizioni di vario materiale. L'insediamento dipende dalla presenza di piante ospiti e dall'accuratezza dei controlli eseguiti dalle autorità specifiche. Al momento *Ochrogaster lunifer* è presente solo in Australia dove è causa di ingenti danni e disturbi. L'insediamento in Europa di molte specie di insetti legati all'eucalitto consiglia un'attenta valutazione dei rischi associati a una possibile introduzione.

Parole chiave: defogliatore, importanza medica, setole urticanti, socialità.

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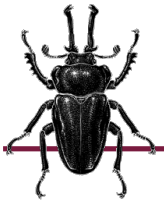
7.2 Dissemination

The following is a dissemination about the movement ecology of *O. lunifer* and *T. pityocampa* pre-pupation and how the caterpillars determine orientation in the environment. Published as: Uemura, M., Zalucki, M.P., Battisti, A., 2021. Why did the caterpillar cross the road? A case study of two processionary caterpillars from Australia and Europe. Entomological Society of Queensland News Bulletin, 49, 44-48.



Why did the caterpillar cross the road? A case study of two processionary caterpillars from Australia and Europe

Presented by Mizuki Uemura
PhD candidate
University of Queensland & University of Padova

In the past month or two, you may have seen or heard about hairy caterpillars crawling in a uniform line that can reach several meters along footpaths, roads, in backyards, etc. (Figures 1 & 2). Every year in March–April, final 8th instar processionary caterpillars of *Ochrogaster lunifer* Herrich-Schäffer (Lepidoptera, Notodontidae) leave their nest in the early morning to search for an over-wintering/pupation site. *Ochrogaster lunifer* is an urticating species found in Australia and is associated with defoliating acacias and eucalypts (Perkins et al., 2016). Setae (detachable urticating hairs) from the abdominal segments of *O. lunifer* larvae are responsible for foetal loss in pregnant mares (Cawdell-Smith et al., 2012). Various severity of allergic reactions and rashes may arise in humans when in contact with larval setae and from adult females which have urticating anal tuft scales used to cover the egg mass (Perkins et al., 2016). *Ochrogaster lunifer* is a gregarious species from egg to pre-pupa and live in a communal nest made of silk (Figure 3). For more information on the life history of *O. lunifer* see Floater & Zalucki (1999), Perkins et al. (2016), Steinbauer (2018) and Mather et al. (2019).

Figure 1. Final instar *Ochrogaster lunifer* pre-pupation procession crossing the footpath at Gatton, Australia. Photo: Mizuki Uemura.

A species similar to *O. lunifer* is the European pine processionary moth, *Thaumetopoea pityocampa* Denis & Schiffermüller (Lepidoptera, Notodontidae). *Thaumetopoea pityocampa* larvae also have setae which cause urticaria, dermatitis and allergic reactions when in contact with mammals (Battisti et al., 2017). A great deal of research has been done on this species because they are destructive defoliators of pine and cedar trees in the Mediterranean Basin and Southern Europe (Roques, 2015). Although geographically isolated, *O. lunifer* and *T. pityocampa* have a univoltine lifecycle with similar periods of development throughout the year but in completely opposite seasons (Battisti et al., 2017; Floater, 1996). The same phenomenon of ground-dispersing processions occurs in Europe in March-May, where final 5th instar *T. pityocampa* larvae leave their communal tent and crawl in search for pupation sites (Figure 4).

Figure 2. *Ochrogaster lunifer* procession comprised of 66 caterpillars stretching over 3 m. Photo: Mizuki Uemura.

Figure 3. Final instar *O. lunifer* larvae spinning silk to maintain the nest structure. Photo: Anthony Hearsey.

Figure 4. Final instar *Thaumetopoea pityocampa* pre-pupation procession at Verona, Italy. Photo: Mizuki Uemura.

Where do these processionary caterpillars go?

Although the two species share the same dispersal behaviour, the environmental preference for pupation sites was dissimilar. *Ochrogaster lunifer* larvae orientate towards the north and south (Figures 5 a & b) and seek out shade (Uemura et al., 2020). Whereas, *T. pityocampa* larvae had no preference for direction (Figure 5c) but pupated in brightly lit areas of the environment (Uemura et al., 2020). The differences in pupation site preference likely reflects the dissimilar environmental conditions that the pupae face during development. The sun is harsh in Australia therefore, *O. lunifer* larvae may seek shaded environments to avoid desiccation, whereas, *T. pityocampa* can diapause underground as a pupa for up to 8 years (Salman et al., 2016) and there is possibly less chance of fungal attack in drier environments (areas with more sun). The most concerning issue during this study was that *T. pityocampa* and *O. lunifer* caterpillars travelled several tens and hundreds of meters, respectively, per day in search of pupation sites. During our observations, 25% of *O. lunifer* (N = 82 processions) and 44% of *T. pityocampa* (N = 53 processions) processions had contact with humans driving, cycling or walking (Uemura et al., 2020). This pre-pupation dispersal on the ground is alarming and risks the spread of urticating hairs that can be easily detached particularly during inadvertent contact. My research

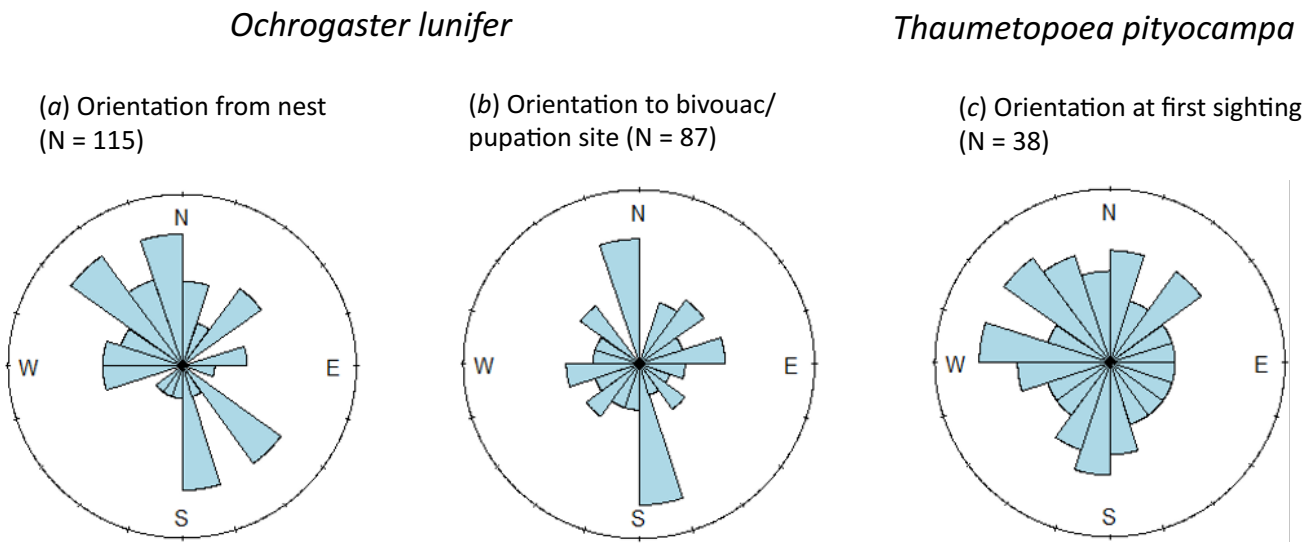


Figure 5. Rose diagrams of the orientation of pre-pupation processions of **(*a*)** *O. lunifer* leaving the nest and **(*b*)** final orientation to the bivouac/pupation site following the last turn of the leader, and **(*c*)** of *T. pityocampa* at first sighting. Figures modified from Uemura et al. (2020).

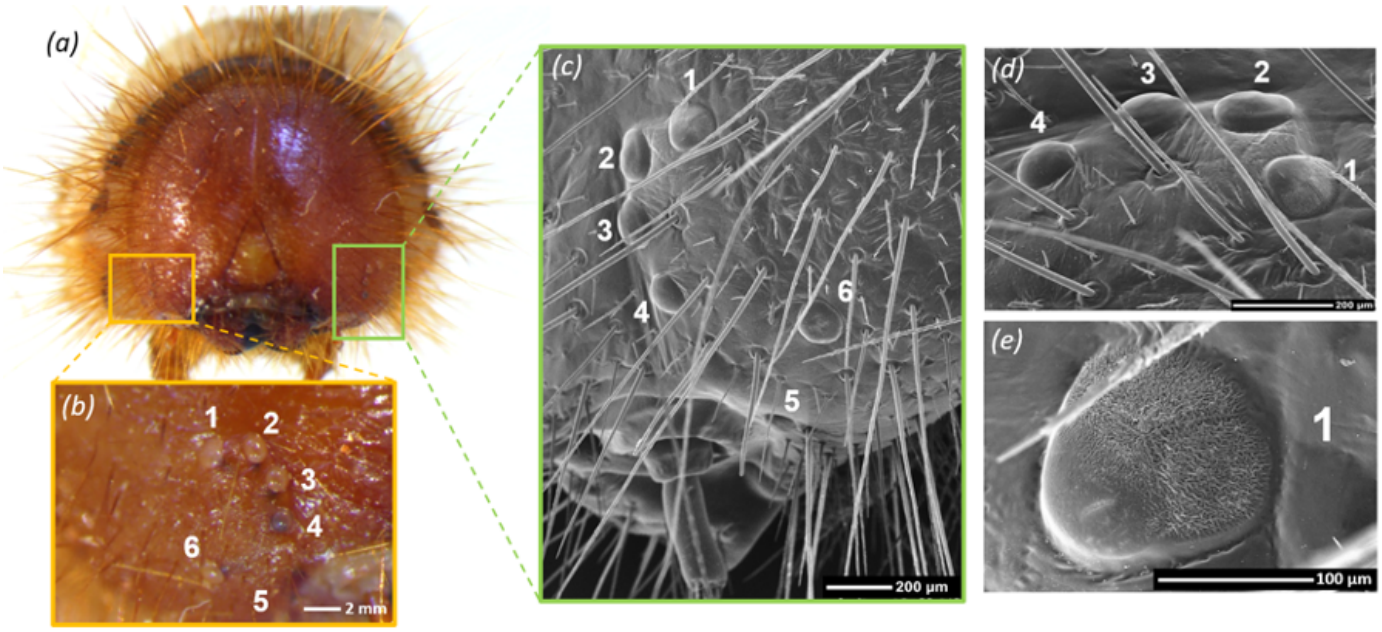


Figure 6. Final instar *O. lunifer* stemmata. ***(a, b)*** Stereomicrographs of head capsule. ***(c-e)*** Scanning electron micrographs (SEM) of left head capsule and stemmata. Images modified from Uemura et al. (2021)

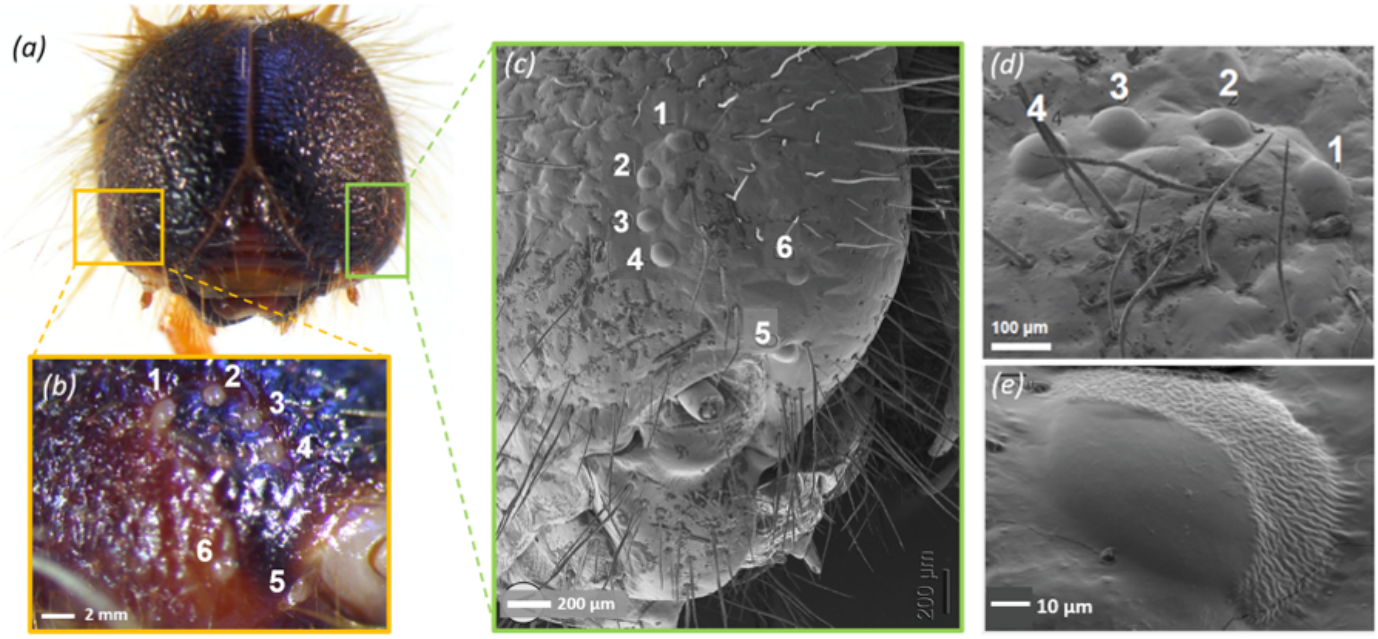


Figure 7. Final instar *T. pityocampa* stemmata. ***(a, b)*** Stereomicrographs of head capsule. ***(c-e)*** Scanning electron micrographs (SEM) of left head capsule and stemmata. Images modified from Uemura et al. (2021).

highlights the importance of investigating movement patterns of organisms to mitigate harmful impacts.

How do these processionary caterpillars orientate in the environment?

On cloudy and rainy days, there is little to no pre-pupation procession activity by *O. lunifer* and *T. pityocampa* larvae. On days when it is environmentally suitable, the larvae leave their nest/tent at sunrise and venture out. Both species move in daylight (particularly in the morning) and need clear skies to travel because they use the polarised skylight pattern to navigate in the environment (Uemura et al., 2021). Both species have six pairs of stemmata (simple eyes) on the sides of the head (Figures 6 & 7). Of these six, stemma I which is located at the most upper part of the head capsule, is responsible for polarized vision (Figures 6e & 7e; Uemura et al., 2021). Stemma I has a cushion-shaped rhabdom (light-sensitive structure) with orthogonal and aligned microvilli (Figures 8 b & c), and an opaque and rugged surface which are optimisations for skylight

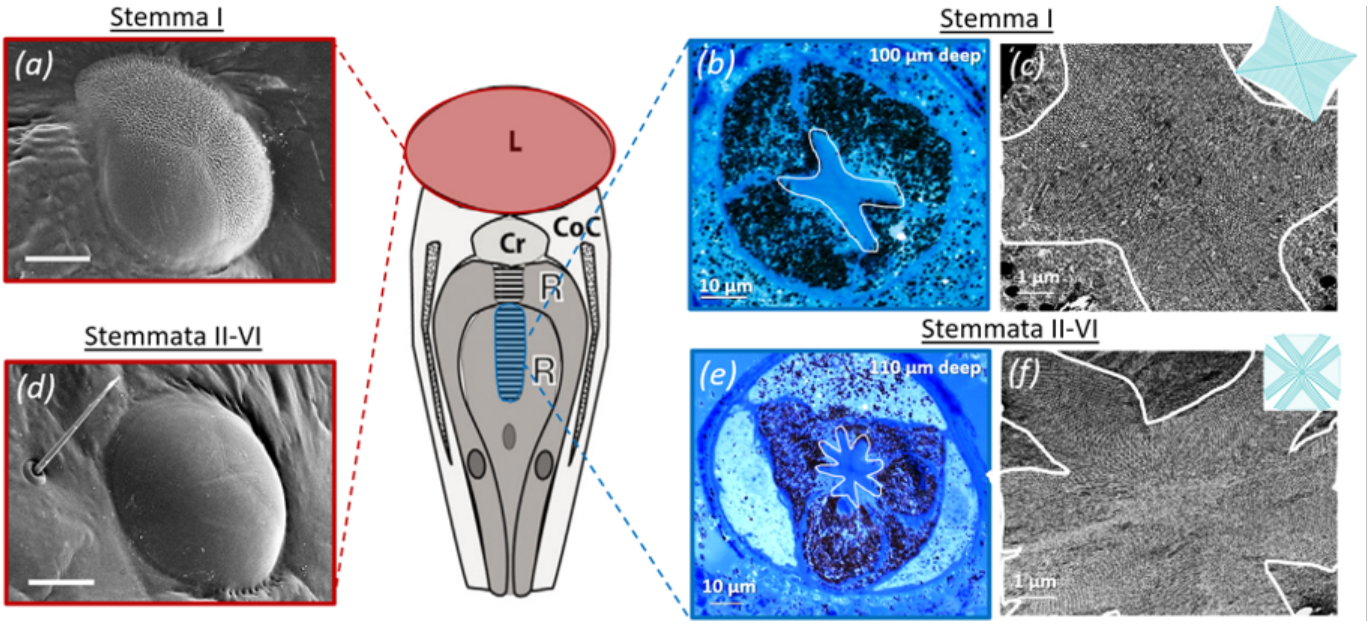


Figure 8. Comparison of stemma I and stemmata II-VI in relation to polarization sensitivity. **(*a-c*)** Stemma I; *(a)* SEM, (*b*) light micrograph (LM) of pillow-shaped rhabdom, (*c*) transmission electron micrograph (TEM) of the rhabdom with orthogonal and aligned microvilli and schematic diagram of stemma I microvillar orientation in top right corner. **(*d-f*)** Stemma IV; *(d)* SEM, *(e)* LM of flower-shaped rhabdom, *(f)* TEM of rhabdom with non-orthogonal and non-aligned microvilli and schematic diagram of stemma II-VI microvillar orientation in top right corner.
Schematic diagram of a Lepidoptera stemma in the middle. **L**, lens; **Cr**, crystalline cone; **R**, photoreceptors; **CoC**, corneagenous cell. Images altered from Uemura et al. (2021).

polarisation vision, similar to the dorsal rim area of adult insects (Uemura et al., 2021). This rugged surface covering two-thirds of stemma I (Figures 6e & 7e) has only been described in *O. lunifer* and *T. pityocampa* larvae and is absent in other caterpillar species studied to date. Stemmata II-VI have a smooth and shiny surface and flower-shaped rhabdoms with non-orthogonal and non-aligned microvilli (Figures 8 e & f) which are optimized for general vision with minimal polarization sensitivity (Uemura et al., 2021). The social behaviour and organised locomotion of processionary caterpillars exert strong selective pressure on the visual organs, even in the simplest structural form.

The overall purpose of this research on *O. lunifer and T. pityocampa* is to investigate and determine their behavioural movements and to compare their morphological, physiological, and ecological drivers. Knowledge of movement ecology in these important pest species, will facilitate building models to predict possible future outcomes, aid in pest management and understand their population dynamics.

Our past and current research helps us and the scientific community to:

- Identify areas in the environment where the urticating setae are most likely to be present
- Understand habitat preferences and location of *O. lunifer* and *T. pityocampa* colonies at various population densities
- Manage plantation and nursery designs to minimise the risk of exposure to urticating caterpillars
- Deepen the knowledge on the social behaviour of Lepidoptera in the larval stages
- Explore how natural enemies are associated with the social behaviour of caterpillars and how they can be best exploited for population regulation
- Understand the ecological role of the urticating setae as a defence against predators

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A bit about Mizuki:
I am in my last few months of my Ph.D., studying the social behaviour and ecology of processionary caterpillars from Europe and Australia, supervised by Prof. Andrea Battisti (University of Padova) and Prof. Myron Zalucki (The University of Queensland). I am interested in understanding insect-plant and predator-prey interactions to propose novel pest management strategies. I was living in Padova, a city in Northern Italy close to Venice, for about 2 years, researching pine processionary moth caterpillars, *Thaumetopoea pityocampa*. I returned to UQ early this year to continue my research with the Zalucki lab on processionary caterpillars, *Ochrogaster lunifer*. It has been a rewarding experience to do research on these fascinating insects and to travel around the world doing what I love!