Author Manuscript

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/eth.12598](https://doi.org/10.1111/eth.12598)

This article is protected by copyright. All rights reserved

DR. EUNICE JINGMEI TAN (Orcid ID : 0000-0003-1667-3958)

Received Date : 07-Dec-2016

Revised Date : 05-Jan-2017

Accepted Date : 10-Jan-2017

Article type : Research Paper

Corresponding author email id; eunice.t@gmail.com

Predators, parasites and heterospecific aggregations in chrysomeline larvae

Running title: Heterospecific chrysomeline larval aggregations

Abstract

Gregariousness is a common feature in aposematic insect prey and offers the additional benefit of enhancing the effectiveness of their toxic defences. Aggregations of the aposematic larvae of two species of leaf beetles, *Paropsis atomaria* and *Paropsisterna variicollis*, occur together on the same Eucalyptus trees over spring and summer. Conventionally, the colouration of these larvae is thought to provide aposematic protection against vertebrate predators, but supporting evidence is limited. We determine if environmental preferences contribute to the heterospecific aggregations, and the potential costs for larvae of living in heterospecific aggregations. We surveyed natural aggregations of the larvae of both species in the field and recorded environmental variables of these aggregations. This revealed that heterospecific aggregations occurred more commonly at higher leaf temperatures, and in less visually conspicuous locations. *P. atomaria* larvae were twice as likely to be found in heterospecific aggregations than *Pa. variicollis*. Next, we manipulated larval aggregations in the field to investigate the survival and gregarious behaviour of larvae in heterospecific aggregations. The gregarious behaviour of both species of larvae did not differ between heterospecific and monospecific aggregations. Further, the survival of larvae did not significantly differ between heterospecific and monospecific aggregations. We suggest that the preference for *P. atomaria* larvae to aggregate with

Pa. variicollis at higher leaf temperature results in the observed heterospecific aggregations, with a potential benefit of lowerer parasitoidism rates for P. atomaria.

Keywords: Heterospecific aggregations, aposematic colouration, parasitoids, chrysomelines

Introduction

Gregariousness is widespread in insect larvae (Ghent 1960; Tsubaki 1981; Tsubaki & Shiotsu 1982; Wade & Breden 1986), and particularly benefits aposematic insects enhancing the effectiveness of their toxic defences (Cott 1940; Rettenmeyer 1970; Harvey et al. 1982; Sillen-Tullberg et al. 1982). Avian predators typically learn to avoid aposematic insects more rapidly and more strongly if they occur in aggregations (Gagliardo & Guilford 1993; Alatalo & Mappes 1996; Gamberale & Tullberg 1996). Accordingly, aposematic signalling has often evolved in gregarious prey species (Gagliardo & Guilford 1993; Alatalo & Mappes 1996; Alatalo & Mappes 2000), perhaps through a collective increase in the effectiveness of the aposematic signal (Gagliardo & Guilford 1993; Riipi et al. 2001). However, the reproducibility of colour patterns of individuals is expected to be high, if predators are to learn to avoid certain colour patterns, and deviations from these colour patterns are therefore not expected (Brower 1960; Brower et al. 1960; Rettenmeyer 1970; Sillen-Tullberg et al. 1982; Joron & Mallet 1998) but see Arias et al. (2016).

Aggregations with mixed phenotypes are unusual because individuals are not necessarily subject to the same risks of predation (Morse 1977). Heterospecific associations, reported in a range of taxa from phytoplankton to insects to dolphins (Terborgh 1990; Ohtsuka et al. 1995; Glos et al. 2007; Kiszka et al. 2011) see also review by Stensland et al. (2003), are thought to increase foraging efficiency and predator avoidance (Powell 1974; Barnard 1978; Kiszka et al. 2011; Bryer et al. 2013). However, these benefits and costs may not be similar for all individuals within heterospecific groups, and large deviations in colours and patterns among a few individuals within aggregations may lead to the so-called ‘oddity effect’ (Tosh et al. 2007; Rodgers et al. 2013). This occurs where a minority of individuals possess a distinctive (odd) phenotype that may reduce a predator’s sensory confusion brought on by a large group of uniform-looking prey (Landeau & Terborgh 1986). As a result, the probability of the predator targeting odd individuals increases, and they consequently suffer proportionately higher rates of predation (Landeau & Terborgh 1986; Krakauer 1995). This may lead

to size-assortative grouping: fish, for example, are more likely to join shoals of individuals that match their size (Peuhkuri 1997; Peuhkuri et al. 1997; Peuhkuri 1999). However, different classes of individuals (e.g. small versus large) may reduce oddity by altering their foraging activities (Peuhkuri 1998), suggesting that other factors such as competition for food may also be involved in the decision to shoal with individuals of particular sizes.

However, predation is not the only factor that affects larval survival in the field. While a predator's decision to attack a particular prey is influenced by the immediate nutritional returns, the decision for parasitoids will be influenced by the potential longer-term nutritional value for her offspring. Parasitoids may select hosts depending on their size, nutritional content and ease of digestability (Elgar & Jebb 1999; Costa et al. 2004) and use a combination of olfactory and visual cues to locate hosts (Köpf et al. 1997; Stireman 2002; Depalo et al. 2010; Ichiki et al. 2011; Depalo et al. 2012; Bora & Deka 2014). As larger group size can increase visual detection risk by parasitoids (Low 2008), larger aggregations do not necessary increase individual survival in chemically defended prey (Lindstedt et al. 2011; Daly et al. 2012).

Most macrohabitats are thermally heterogeneous, and an animal can modify its temperature through activity and the choice or manipulation of their microhabitat (Huey 1991; Anne & Rasa 1995; Ruf & Fiedler 2000; Ohashi et al. 2005; De Los Santos et al. 2006). Unsurprisingly, gregarious behaviour increases the temperature of individuals across a range of species (Villa & Rinderer 1993; Klok & Chown 1999; Ruf & Fiedler 2000; Fletcher 2009). This has implications for larval growth, as heat gains may reduce developmental time (Ruf & Fiedler 2000; Ronnås et al. 2010; Aubernon et al. 2016) and improve immune responses (Catalán et al. 2012), ultimately improving survival (Wade & Breden 1986; Ronnås et al. 2010; Aubernon et al. 2016). Larval aggregation also improve establishment and feeding efficacy, particularly in younger instars (Ghent 1960; Tsubaki 1981; Weed 2010). Increased group size can have a positive effect on the size attained (Costa & Ross 2003), which is positively

correlated to adult size at emergence (Bartlett & Ashworth 1988; Smith 2002) and larger sizes are generally associated with higher fecundity (Bartlett & Ashworth 1988; Tammaru et al. 1996) and lower mortality (Smith 2002).

Aggregations of paropsine leaf beetle larvae (Coleoptera, Chrysomelidae) of different ages and species have been documented for decades (Cumpston 1939; Carne 1966; Grégoire 1988). The adults and larvae of two species of Australian leaf beetles, *Paropsis atomaria* and *Paropsisterna variicollis*, occur together on the same *Eucalyptus* trees over spring and summer (Cumpston 1939; Mo & Farrow 1993). The larvae of both species may aggregate through random processes, or they could be driven by similar preferences for microhabitats, thus resulting in a shared space. As the nutrition content of leaves declines with leaf maturation numerous species, including *Eucalyptus* (Schultz et al. 1982; Coley 1983; Ayres & MacLean 1987; Wendler et al. 1995; Diaz et al. 2008; Balemi 2011), most herbivorous insects, including of *P. atomaria* and *Pa. variicollis*, prefer young leaves (Cumpston 1939; Gherlenda et al. 2016). The aposematic colouration of these larvae is viewed as protection against vertebrate predators (Moore 1967; Matthews & Reid 2002), but evidence is limited (Elek 2012). The larvae of *P. atomaria* and *Pa. variicollis* have contrasting colour patterns of yellow and black that vary according to instar. First instars are minute and yellowish but with many small black spots and black ends of the body. Second and third instars are yellow, with a black head and abdominal apex (hereafter referred to as the ‘early instar colour pattern’), and the fourth instars of both species additionally have a black prothorax and three black dorsal and lateral abdominal stripes (‘late instar colour pattern’) (Cumpston 1939). In addition to similar colour patterns, the larvae of both species respond similarly to disturbance by rearing up their abdominal apices and everting a pair of glands. These glands release volatile secretions that include hydrogen cyanide, benzaldehyde and glucose (Moore 1967). Cyanide is toxic, largely due to the inhibition of cytochrome oxidase-producing cytotoxic anoxia (Chen & Rose 1952). Moore (1967) observed that *Iridomyrmex* ants, potential predators found naturally in the same habitat, were usually dead within 30 mins of coming into contact with the secretions of *P. atomaria* larvae. Disturbed larvae also regurgitate and defecate

114 digested Eucalyptus leaves, containing anti-herbivore metabolites such as terpenoids and phenolics
115 (Brophy et al. 1991; Batish et al. 2008).

116
117 Paropsine larvae have a number of invertebrate enemies, including tachinid and hymenopteran
118 parasitoids (Cumpston 1939; Tanton & Khan 1978; Tanton & Epila 1984a; Selman 1994), and
119 predatory coccinellid beetles and bugs (Tanton & Khan 1978). Tachinids are the predominant
120 parasitoids of chrysomelids in Australia and lay eggs on the cuticle of host larvae, from which the
121 parasitoid larvae subsequently emerge and burrow into the host body (Colless 2012). Tachinids are
122 not strongly host-specific, attacking all similar eucalypt leaf feeding beetle larvae, including the
123 weevil genus *Gonipterus*. The presence of hymenopteran parasites is not externally visible but these
124 are either hyperparasitoids of tachinids or form a small percentage of larval parasitoids (Tanton &
125 Epila 1984b).

126
127 The existence of mixed age groups and heterospecific aggregations of *P. atomaria* and *Pa. variicollis*
128 is puzzling, because of the different signals contributed by larvae of different instars. A critical issue
129 is whether the benefits of being in an aggregation outweigh the costs of presenting conflicting signals
130 to potential predators. Field experiments reveal that larval models without stripes were preyed on
131 more heavily than models with stripes (Tan et al. 2016), suggesting that i) the survival of larvae with
132 the early instar colour pattern will be compromised when they are in the presence of other larvae with
133 the late instar colour patterns, and ii) 'odd' individuals in an aggregation will suffer a greater
134 predation rate. Evaluating these predictions is challenging, and our approach was to consider how
135 larval survival prospects might vary according to the composition of the aggregation.

136
137 This study aims to determine if environmental preferences contribute to the heterospecific
138 aggregations, and the potential costs for larvae of living in heterospecific aggregations. We

hypothesized that the two species may have similar environmental preferences, and heterospecific aggregations may be driven by preferences for similar habitats. We surveyed natural aggregations of the larvae of both species in the field, and recorded environmental variables of these aggregations. Next, we conducted field experiments of larvae to investigate the survival and gregarious behaviour of larvae in heterospecific aggregations. We hypothesized that i) the survival of larvae with the early instar colour pattern will be compromised when they are in the presence of other larvae with the late instar colour patterns, and ii) gregarious behaviour of the larvae should be similar across aggregation types (monospecific and heterospecific), in order for heterospecific aggregations to persist.

Materials and methods

The observations and experiments took place at a Eucalyptus plantation at the Koala Conservation Centre, Phillip Island, Australia (38°29'6.6"S, 145°15'39.8"E).

Habitat preference

During November–December 2012, we surveyed aggregations of larvae on the eucalypt trees, and recorded corresponding environmental variables: ambient light at the aggregation, leaf surface temperature, ambient temperature and height from ground. Eucalyptus trees were systematically surveyed from ground up to a height of 1.90 m, for the presence of larvae. The observed aggregations were categorised based on the species composition – *P. atomaria*, *Pa. variicollis* or heterospecific aggregation; and the colour patterns of the larvae – early or late instar, or a mixture of both. *Paropsis atomaria* larvae have yellow legs while *Pa. variicollis* larvae have black legs, and this feature was used to distinguish between the species. Legs are mostly hidden under the larval body, so this colour difference is not likely to be a significant part of the aposematic system. We performed the Shapiro-Wilk test to test for normality in the distribution of aggregation sizes. As the aggregation sizes across the three aggregation types were not normally distributed, we performed Kruskal-Wallis tests to examine if the distribution of aggregation sizes was different across the three aggregation types, and a post-hoc Wilcoxon test with Bonferroni correction to examine differences between the aggregation types. Next, we examined if the number of larvae of *P. atomaria* and *Pa. variicollis* in heterospecific groups was correlated. Using Fisher's exact test, we examined if species composition (*P. atomaria*, *Pa. variicollis*, heterospecific) had an effect on the colour patterns of aggregations observed.

In contrast to many cryptic species, mimics and models may be conspicuous by staying on the upper surfaces of leaves rather than underneath (Rettenmeyer 1970). Thus, we recorded the location of the larval aggregations (under the leaf, on top of the leaf, on leaves that were sideways, or on the petiole)

to estimate the visibility of the larval aggregations by visually seeking predators. We analysed the effects of group size, position of larvae and environmental variables – ambient temperature, leaf surface temperature, ambient light and height from ground – on the species composition of naturally occurring larval aggregations using multinomial logistic regressions. The initial model with all the interactions was explored, and non-significant ($P > 0.05$) interaction terms were subsequently dropped from the model and not reported. We performed multinomial logistic regressions using the *nnet* package (Venables & Ripley 2002) and the analysis of deviance using the *car* package (Fox & Weisberg 2011). Models were visualised using the *effects* package (Fox 2003; Fox & Hong 2009). All analyses were performed in R (R Development Core Team 2016).

Gregarious behaviour and survival of larvae in heterospecific aggregations

We collected larvae of *P. atomaria* and *Pa. variicollis* from trees in the plantation and used them in four treatments that varied in both species and colour patterns. These included monospecific treatments of *P. atomaria* and *Pa. variicollis*, which were used to compare the gregarious behaviour and the survival of larvae in heterospecific aggregations. Two heterospecific treatments included aggregations in which both species had either similar or different colour patterns.

Each treatment comprised ten individuals drawn from two ‘sets’ of five larvae from a naturally existing aggregation. The four treatments had the following combinations of larval ‘sets’: I) two sets of third instar *P. atomaria* larvae from different aggregations; II) two sets of fourth instar *Pa. variicollis* larvae from different aggregations; III) one set of third instar *P. atomaria* larvae and one set of third instar *Pa. variicollis* larvae; IV) one set of third instar *P. atomaria* larvae and one set of fourth instar *Pa. variicollis* larvae. We selected *P. atomaria* larvae that were of similar length to the larvae of *Pa. variicollis* in each treatment. A total of 10 replicates involving 100 larvae were performed for all four treatments. However, after the first day of the experiment, larvae of two replicates for treatment I, and one replicate for treatment III had disappeared and the data for these

replicates were disregarded. We predicted no differences in the survival of larvae of *P. atomaria* and *Pa. variicollis* when they had similar colour patterns (i.e. third instar of *Pa. variicollis* species, and second instar of *P. atomaria* species). However, we predicted differences in survival when the larvae had different markings: following (Tan et al. 2016), fourth instar larvae of *Pa. variicollis* larvae with black stripes should suffer lower predation than the third instar larvae of *P. atomaria*.

The groups of larvae for each treatment were placed on Eucalyptus saplings that had been previously cleared of all other externally feeding larvae. Björkman et al. (2000) suggested that the complexity of plant architecture can play a role in the predation rates of heteropterans, but all the plants used in this study across the treatments were Eucalyptus saplings with similar, simple architecture. Paropsine larvae feed on immature leaves (Carne 1966; Ohmart & Edwards 1991), so the choice of saplings simulates the natural microhabitats of the two species. Eggs of both beetle species are typically oviposited on or near the terminal shoots (Cumpston 1939; Carne 1966), and the larvae were relocated onto sub-terminal leaves for the experiment. The presence and behaviour of the larvae on the saplings was observed an hour after their introduction, and at 0900 hours, 1300 hours and 1700 hours daily for the following four days. Instances of predation, presence of potential predators and parasitic eggs were recorded during these observations. Predators such as heteropterans can be a major source of larval mortality in chrysomelids (Phillips 1977; Björkman et al. 2000; Björkman et al. 2003)(**Fig. S1a**). Our observations of predation events may underestimate the true rate of predation, as they are derived from point sampling. However, we used this method as it causes minimal disruption to the fauna and thus, potential predators. Parasitoidism of the larvae was recorded as the number of larvae with tachinid eggs, which were clearly visible (**Fig. S1b**). Prior parasitoid attacks on the larvae before commencement of the experiment would not affect the results because ovipositing tachinid females apparently do not discriminate against previously attacked host individuals (Belshaw 1994).

Analysis of gregarious behaviour and larval survival

The gregarious behaviour of larvae was scored to ascertain how likely they were to be in a monospecific or heterospecific group. Larvae were considered to be part of a group when they were in physical contact with another individual as described in Cumpston (1939). The overall gregarious behaviour of larvae was scored as a function of the number of larvae (i) observed in aggregations and the number of neighbours each larva had ($i - 1$). For j number of aggregations for a total of k larvae, the overall gregarious score was:

$$\text{Overall gregarious score} = \frac{i_1(i_1-1)+i_2(i_2-1)+\dots+i_j(i_j-1)}{k}$$

Monospecific gregarious scores were calculated in the same way as the overall gregarious score.

A measure of the gregarious behaviour of each species in heterospecific treatments (III and IV) was estimated as:

$$\text{Heterospecific gregarious score} = \frac{l_1/i_1+l_2/i_2+\dots+l_j/i_j}{m}$$

where l is the number of like-species neighbours in each aggregation of i larvae, and m is the total number of like-species larvae present in j aggregations. The gregarious scores were then square root-transformed to improve the distribution of the data.

A generalised linear model was used to determine the effect of treatment and day on the gregarious behaviour of the larvae. The initial full model with interactions of treatment and day with the gregarious scores was explored, and non-significant ($P > 0.05$) interaction terms subsequently removed. Non-significant variables that did not have significant interaction terms were also removed and the reduced model only is reported. A generalised linear model was also used to determine the effect of *P. atomaria* and *Pa. variicollis* monospecific gregarious score, treatment type and day on

heterospecific gregarious score. The initial model with interactions of the heterospecific behaviour scores with all of the above factors was explored, and non-significant ($P < 0.05$) interaction terms subsequently removed. As above, non-significant variables that did not have significant interaction terms were also removed and the reduced model only is reported.

Survival analysis of the larvae was performed in R, using the Survival package (Therneau 2013). The survival of larvae was compared across treatments.

Results

Habitat preference

We observed a total of 3041 larvae in 312 aggregations: 150 aggregations of *P. atomaria* comprising 563 individuals; 133 aggregations of *Pa. variicollis* comprising 2085 individuals, and a total of 393 larvae in 29 heterospecific aggregations of *P. atomaria* ($n = 134$) and *Pa. variicollis* ($n = 261$). 23.8% of the total number of *P. atomaria* larvae surveyed was found in heterospecific aggregations, compared with 12.5% of *Pa. variicollis* larvae. **Fig. 1** shows the group size of naturally found aggregations with respect to species composition. A Kruskal-Wallis test revealed a significant effect of aggregation type on the number of larvae ($\chi^2_2 = 105.7$, $P < 0.001$). A post-hoc Wilcoxon test with Bonferroni correction showed that *P. atomaria* aggregations were significantly smaller than that of *Pa. variicollis* aggregations ($P < 0.001$) and heterospecific aggregations ($P < 0.001$). The number of larvae of *P. atomaria* and *Pa. variicollis* in heterospecific groups was not correlated ($R^2 = < 0.001$, $P = 0.98$, $df = 27$). The observed colour patterns of aggregations depended upon species composition (*P. atomaria*, *Pa. variicollis*, heterospecific) ($P < 0.001$; **Fig. 2**). Heterospecific aggregations had less variation in patterns compared with monospecific *P. atomaria* or *Pa. variicollis* aggregations.

The gregarious behaviour of larvae at rest (i.e. larvae that were not feeding) varied between species: *P. atomaria* larvae grouped in a compact series of overlapping rows, resting longitudinally on the leaf, with the heads of larvae in each row touching the ventral thoracic regions of anterior larvae, while *Pa. variicollis* larvae arranged themselves on the leaf in a circular fashion, overlapping when numerous, with their heads pointed towards a central focus, i.e. cycloalexy (Weinstein 1989). Larvae in heterospecific aggregations tended to arrange themselves in either fashion (**Fig. 3**).

The mean environmental parameters for aggregations, classified according to their species composition, are listed in Table 1. Multinomial logistic regression of the effects on species composition showed that the position of the larval aggregation, leaf surface temperature, ambient light, and group size were significantly different across the species composition types (Table 2, Fig. 4). Heterospecific aggregations occurred in relatively less visually conspicuous locations overall. Monospecific aggregations of *P. atomaria* and *Pa. variicollis* were more likely to occur on the petioles, compared to heterospecific aggregations (Fig. 4a). Heterospecific aggregations were less likely to occur in brighter ambient light conditions (Fig. 4b). While monospecific aggregations of *P. atomaria* and *Pa. variicollis* were more likely to occur at lower leaf temperatures, heterospecific aggregations were more likely to occur at higher leaf temperatures (Fig. 4c). *Pa. variicollis* and heterospecific aggregations were more likely to be larger than *P. atomaria* aggregations (Fig. 4d). The height of larval aggregation from the ground and ambient temperature did not have a significant effect on species composition (Table 2, Fig. S2). Further details of the model are reported in Table 2.

Gregarious behaviour and survival of larvae in heterospecific aggregations

A total of 10 replicates involving 100 larvae were included in each of four treatments. After the first day of the experiment, larvae of two replicates for treatment I, and one replicate for treatment III had disappeared and the data for these replicates were disregarded in the analyses, leaving eight replicates for treatment I, ten replicates for treatment II, nine replicates for treatment III and ten replicates for treatment IV. The larval aggregation behaviour ranged from solitary individuals to monospecific and heterospecific aggregations. There was no difference in the overall gregarious behaviour of larvae across the four treatments ($F_{3,176} = 2.034$, $P = 0.111$) but there was an effect of day on the overall gregarious behaviour ($F_{4,179} = 6.084$, $P < 0.001$;). Post-hoc comparisons using the Tukey HSD test indicated that day 2 had significantly higher gregarious behaviour than days 4 and 5 (Table 3).

The overall and heterospecific gregarious scores of *P. atomaria* and *Pa. variicollis* larvae did not indicate that the larvae behaved differently in heterospecific compared with monospecific aggregations. There was no difference in the overall and heterospecific gregarious behaviour (measured by gregarious scores) of *P. atomaria* or *Pa. variicollis* larvae in heterospecific aggregations in days 1, 2 and 4 (*P. atomaria*, day 1: $F_{1,18} = 1.10$, $P = 0.31$; day 2: $F_{1,18} = 0.44$, $P = 0.52$; day 4: $F_{1,18} = 3.68$, $P = 0.07$; *Pa. variicollis*, day 1: $F_{1,18} = 2.69$, $P = 0.12$; day 2: $F_{1,18} = 0.11$, $P = 0.75$; day 4: $F_{1,18} = 1.80$, $P = 0.20$). However, the gregarious behaviour of *P. atomaria* larvae on days 3 and 5 were significantly different from *Pa. variicollis*. On day 3, the overall gregarious behaviour of heterospecific groups was higher when *P. atomaria* larvae were more gregarious (Day 3: $F_{1,18} = 7.58$, $P = 0.019$; Day 4: $F_{1,18} = 11.62$, $P = 0.004$). There were also interaction effects on these days: on Day 3 between gregarious behaviour of *P. atomaria* and *Pa. variicollis* and the treatment type ($F_{1,18} = 7.60$, $P = 0.019$), and on day 5 between the gregarious behaviour of *P. atomaria* and the treatment type ($F_{1,18} = 6.34$, $P = 0.025$).

The survival of larvae did not significantly differ across the treatments ($\chi^2_3 = 4.80$, $P = 0.19$; **Fig. 5a**), nor was there a significant interaction effect between treatment and species. *Pa. variicollis* larvae had a higher survival rate (mean = 0.485) than *P. atomaria* larvae (mean = 0.347). The survival of *P. atomaria* did not differ significantly across the treatments ($\chi^2_2 = 2.95$, $P = 0.23$; **Fig. 5b**). The survival of *Pa. variicollis* larvae was not comparable across treatments III and IV as the larvae in the two treatments were of different instars. For treatments II and IV where *Pa. variicollis* larvae were in the fourth instar, the survival was not significantly different ($\chi^2_1 = 0.23$, $P = 0.89$).

Specific predation events were not recorded, but predators included the assassin bug, *Gminatus* sp. There was one observed instance of predation in each of treatments I, II and IV, while three instances of predation were observed for treatment III. Parasitoidism rates by tachinid flies depended on the treatment and species. The parasitoidism rate on *Pa. variicollis* larvae was similar throughout the

329 treatments, while that of *P. atomaria* varied depending on treatment (Table 4). However, the instances
330 of parasitoidism are too few for formal statistical analysis.

Discussion

Our survey of natural aggregations suggest that environmental preferences contribute to the heterospecific aggregations, with heterospecific aggregations occurring more commonly at higher leaf temperatures, and in less visually conspicuous locations. Manipulation of larval aggregations in the field indicate that predation rates and survival of larvae did not significantly differ across heterospecific and monospecific aggregations, providing no evidence for the ‘oddity effect’ in the heterospecific aggregations of mixed phenotypes, or that ‘odd’ individuals confuse potential predators.

There may be other benefits driving aggregation that we did not measure in our study. For instance, animals in aggregations experience lower water loss compared with solitary individuals (Klok & Chown 1999). However, evaporative water loss is unlikely to be a major factor favouring aggregating behaviour, since the larvae in our study are transpiring in an open space in the field, and individual transpiration is unlikely to affect the surrounding relative humidity of adjacent aggregating individuals.

Larval survival was not influenced by colour pattern composition (treatment IV), which is surprising because previous experiments with larval models indicated that between individual differences in signals increased mortality rates (Tan et al. 2016). Presumably, the persistence of heterospecific groups in these two species of chrysomeline larvae reflects the benefits of aggregating outweighing the costs of presenting diverse signals to potential predators. Heterospecific aggregations may be driven by environmental preferences. As poor leaf nutrition content can negatively affect larval growth (Ayres & MacLean 1987), the preference of *P. atomaria* and *Pa. variicollis* larvae for young Eucalyptus leaves may predispose the larvae to exploit similar microhabitats. The benefits of larval aggregations in establishment, feeding efficacy and development (Ghent 1960; Tsubaki 1981; Wade & Breden 1986; Ronnås et al. 2010; Weed 2010; Catalán et al. 2012; Aubernon et al. 2016) may

further favour heterospecific aggregations, leading to similar gregarious behaviour of both species of larvae across monospecific and heterospecific aggregations. Avian predators typically learn to avoid aposematic prey more rapidly and more strongly if they are aggregated rather than solitary (Gagliardo & Guilford 1993; Alatalo & Mappes 1996; Gamberale & Tullberg 1996; Riipi et al. 2001). The costs of presenting diverse signals may be mitigated by the preference of larvae in natural heterospecific aggregations for less visually conspicuous locations, such as leaf petioles, even at higher temperatures. Preference for particular habitats according to species composition in groups could be responsible for the lack of difference in survival.

Studies in aposematic colouration of gregarious animals predominantly focus on the effects of visually seeking vertebrate predators (Gagliardo & Guilford 1993; Alatalo & Mappes 1996; Gamberale & Tullberg 1996; Alatalo & Mappes 2000). Our study highlights the potential significance of parasitoids as a selection pressure acting on aposematic colouration: both *P. atomaria* and *Pa. variicollis* are hosts to several parasitoid species (Tanton & Khan 1978; Tanton & Epila 1984b; Colless 2012), and although parasitoidism rates in this study were lower than those observed elsewhere (Tanton & Epila 1984b), the brief timeframe of our experiments may underestimate the true rates. Parasitoidism by tachinid flies in particular may be a significant force shaping the coexistence of heterospecific aggregations comprising *P. atomaria* and *Pa. variicollis*, because parasitoids can influence the coexistence of different host species ((Renaud et al. 1996). Our unexpected finding that parasitoidism rates on monospecific larval aggregations of *P. atomaria* was two to four times that of larvae in heterospecific treatments is consistent with this view. Perhaps heterospecific aggregations arise from the preference of *P. atomaria* larvae to aggregate with *Pa. variicollis*: *Pa. variicollis* and heterospecific aggregations were typically larger than *P. atomaria* aggregations, and heterospecific aggregations were more likely to occur at higher leaf temperatures than that for monospecific aggregations of both species. Individuals of *P. atomaria*, by joining aggregations of *Pa. variicollis*, may reduce the risk of parasitoid attacks, especially when they are more active, such as during higher temperatures (López et al. 1997).

383

384 If large host size benefits parasitoid larval development (Stenberg 2012; Stenberg 2015), then tachinid
385 parasitoids may favour *P. atomaria* larvae over *Pa. variicollis* larvae because the former are larger
386 (Reid 1992). Tachinid parasitoids use a combination of olfactory and visual cues to locate hosts
387 (Stireman 2002; Depalo et al. 2010; Ichiki et al. 2011; Depalo et al. 2012; Bora & Deka 2014), and
388 while tachinids target a variety of lepidopterans, aposematic hosts tend to support less diverse tachinid
389 assemblages (Stireman & Singer 2003). Nevertheless, defensive larval secretions may act differently
390 on parasitoids compared with predators. Tachinid larvae burrow into the host body after hatching
391 (Colless 2012), and the growing tachinid larvae may evade the chemical defences of the host larvae
392 by avoiding ingestion of the glands containing the defensive secretions. On the other hand, predators
393 may not be able to avoid ingesting the defensive glands if the chrysomeline larvae are swallowed
394 whole.

395

396 Closely related species are usually more phenotypically different when in sympatry because of
397 ecological character displacement (Brown & Wilson 1956). As competition for resources leads to
398 divergence in resource use or acquisition (Schulter et al. 1985), sympatric species that exist in
399 heterospecific aggregations present opportunities to examine the mechanisms that drive the
400 association (Landeau & Terborgh 1986). Similarities in colour pattern can be the result of (i)
401 convergent evolution; (ii) parallel evolution through Müllerian advantage; or (iii) parallel evolution
402 without Müllerian advantage (Brower et al. 1963). It is possible that the similar colour patterns in the
403 larvae of *P. atomaria* and *Pa. variicollis* represent Müllerian mimicry, as both species are
404 unpalatable; both are found at the same place and time; and they are not distinguished by predators.
405 Heterospecific aggregations of the same instars of *P. atomaria* and *Pa. variicollis* could arise in
406 response to intense predation, which then selects for a Müllerian advantage.

Acknowledgements

We thank the Holsworth Wildlife Endowment for funding the research, and Philip Island Nature Parks for permitting the experiments and providing lodging at the volunteer house. EJT thanks the Australian Postgraduate Award and the International Postgraduate Research Scholarship for funding her postgraduate candidacy. We also thank Johanna Mappes, Birgitta Tullberg and anonymous reviewers for their valuable comments on earlier versions of this manuscript.

Literature cited

- Alatalo, R. V. & Mappes, J. 1996: Tracking the evolution of warning signals. *Nature* **382**, 708-710.
- Alatalo, R. V. & Mappes, J. 2000: Initial evolution of warning coloration: comments on the novel world method. *Anim. Behav.* **60**, F1-F2.
- Anne, O. & Rasa, E. 1995: Ecological factors influencing burrow location, group size and mortality in a nocturnal fossorial Kalahari detritivore, *Parastizopus armaticeps* Peringuey (Coleoptera: Tenebrionidae). *J. Arid Environ.* **29**, 353-365.
- Arias, M., Mappes, J., Théry, M. & Llaurens, V. 2016: Inter-species variation in unpalatability does not explain polymorphism in a mimetic species. *Evol. Ecol.* **30**, 419-433.
- Aubernon, C., Boulay, J., Hédouin, V. & Charabidzé, D. 2016: Thermoregulation in gregarious dipteran larvae: evidence of species-specific temperature selection. *Entomol. Exp. Appl.* **160**, 101-108.
- Ayres, M. P. & MacLean, S. F. 1987: Development of Birch Leaves and the Growth Energetics of *Epirrita Autumnata* (Geometridae). *Ecology* **68**, 558-568.
- Balemi, T. 2011: Effect of phosphorus (P) nutrition and leaf age on physiological growth parameters in a P-inefficient potato genotype. *The journal of horticultural science & biotechnology* **86**, 384-390.
- Barnard, C. J. 1978: Predation and the evolution of social mimicry in birds. *Am. Nat.* **113**, 613-618.
- Bartlett, J. & Ashworth, C. M. 1988: Brood size and fitness in *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Behav. Ecol. Sociobiol.* **22**, 429-434.
- Batish, D. R., Singh, H. P., Kohli, R. K. & Kaur, S. 2008: Eucalyptus essential oil as a natural pesticide. *For. Ecol. Manage.* **256**, 2166-2174.
- Belshaw, R. 1994: Life history characteristics of Tachinidae (Diptera) and their effect on polyphagy. In: *Parasitoid Community Ecology*. (Hawkins, B. A. & Sheehan, W., eds). Oxford University Press, New York. pp. 145-162.
- Björkman, C., Bengtsson, B. & Häggström, H. 2000: Localized outbreak of a willow leaf beetle: plant vigor or natural enemies? *Popul. Ecol.* **42**, 91-96.
- Björkman, C., Dalin, P. & Eklund, K. 2003: Generalist natural enemies of a willow leaf beetle (*Phratora vulgatissima*): abundance and feeding habits. *J. Insect Behav.* **16**, 747-764.
- Bora, D. & Deka, B. 2014: Role of visual cues in host searching behaviour of *Exorista sorbillans* Widemann, a parasitoid of muga silk worm, *Antheraea assama* Westwood. *J. Insect Behav.* **27**, 92-104.
- Brophy, J. J., House, A. P. N., Boland, D. J. & Lassak, E. V. 1991: Digests of the essential oils of 111 species from northern and eastern Australia. In: *Eucalyptus leaf oils: use, chemistry, distillation and marketing*. (Boland, D. J., Brophy, J. J. & House, A. P. N., eds). Inkata Press, Melbourne. pp. 29-155.
- Brower, J. V. Z. 1960: Experimental studies of mimicry. IV. The reactions of starlings to different proportions of models and mimics. *Am. Nat.* **94**, 271-282.
- Brower, L. P., Brower, J. V. Z. & Collins, C. T. 1963: Experimental studies of mimicry. 7. Relative palatability and Mullerian mimicry among neotropical butterflies of the sub-family Heliconiinae. *Zoologica* **48**, 65-84.
- Brower, L. P., Brower, J. V. Z. & Westcott, P. W. 1960: Experimental studies of mimicry. 5. The reactions of toads (*Bufo terrestris*) to bumblebees (*Bombus americanorum*) and their robberfly mimics (*Mallophora bomboides*), with a discussion of aggressive mimicry. *Am. Nat.* **94**, 343-355.
- Brown, W. L. & Wilson, E. O. 1956: Character displacement. *Systematic Zoology* **5**, 49-64.
- Bryer, M. A. H., Chapman, C. A. & Rothman, J. M. 2013: Diet and polyspecific associations affect spatial patterns among redtail monkeys (*Cercopithecus ascanius*). *Behaviour* **150**, 77-293.
- Carne, P. B. 1966: Ecological characteristics of the Eucalypt-defoliating chrysomelid *Paropsis atomaria* OL. *Aust. J. Zool.* **14**, 647.
- Catalán, T. P., Wozniak, A., Niemeyer, H. M., Kalergis, A. M. & Bozinovic, F. 2012: Interplay between thermal and immune ecology: Effect of environmental temperature on insect immune response and energetic costs after an immune challenge. *J. Insect Physiol.* **58**, 310-317.

- Chen, K. K. & Rose, C. L. 1952: Nitrite and thiosulfate therapy in cyanide poisoning. *Journal of the American Medical Association* **149**, 113-119.
- Coley, P. D. 1983: Herbivory and Defensive Characteristics of Tree Species in a Lowland Tropical Forest. *Ecol. Monogr.* **53**, 209-229.
- Colless, D. H. 2012: The *Froggattimyia*-*Anagonia* genus group (Diptera: Tachinidae). *Rec. Aust. Mus.* **64**, 167-211.
- Costa, F. G., Pérez-Miles, F. & Mignone, A. 2004: Pompilid wasp interactions with burrowing tarantulas: *Pepsis cupripennis* versus *Eupalaestrus weijenberghi* and *Acanthoscurria suina* (Araneae, Theraphosidae). *Studies on Neotropical Fauna and Environment* **39**, 37-43.
- Costa, J. T. & Ross, K. G. 2003: Fitness Effects of Group Merging in a Social Insect. *Proceedings: Biological Sciences* **270**, 1697-1702.
- Cott, H. B. 1940: Adaptive coloration in animals. Methuen, London.
- Cumpston, M. 1939: Observations on the bionomics and morphology of seven species of the tribe Paropsini (Chrysomelidae). *Proc. Linn. Soc. N.S.W.* **64**, 353.
- Daly, D., Higginson, A. D., Chen, D., Ruxton, G. D. & Speed, M. P. 2012: Density-dependent investment in costly anti-predator defences: an explanation for the weak survival benefit of group living. *Ecol. Lett.* **15**, 576-583.
- De Los Santos, A., Ferrer, F., De Nicolás, J. P. & Crist, T. O. 2006: Thermal habitat and life history of two congeneric species of darkling beetles (Coleoptera: Tenebrionidae) on Tenerife (Canary Islands). *J. Arid Environ.* **65**, 363-385.
- Depalo, L., Dindo, M. & Eizaguirre, M. 2012: Host location and suitability of the armyworm larvae of *Mythimna unipuncta* for the tachinid parasitoid *Exorista* larvarum. *BioControl* **57**, 471-479.
- Depalo, L., Marchetti, E., Baronio, P., Martini, A. & Dindo, M. L. 2010: Location, acceptance and suitability of *Spodoptera littoralis* and *Galleria mellonella* as hosts for the parasitoid *Exorista* larvarum. *Bull. Insectol.* **63**, 65-69.
- Diaz, C., Lemaître, T., Christ, A., Azzopardi, M., Kato, Y., Sato, F., Morot-Gaudry, J.-F., Le Dily, F. & Masclaux-Daubresse, C. 2008: Nitrogen Recycling and Remobilization Are Differentially Controlled by Leaf Senescence and Development Stage in *Arabidopsis* under Low Nitrogen Nutrition. *Plant Physiol.* **147**, 1437-1449.
- Elek, J. 2012: Are birds important predators of leaf beetles in Tasmania eucalypt plantations? *The Tasmanian Naturalist* **134**, 71-84.
- Elgar, M. & Jebb, M. 1999: Nest provisioning in the mud-dauber wasp *Sceliphron laetum* (F. Smith): Body mass and taxa specific prey selection. *Behaviour* **136**, 147-159.
- Fletcher, L. E. 2009: Examining potential benefits of group living in a sawfly larva, *Perga affinis*. *Behav. Ecol.* **20**, 657-664.
- Fox, J. 2003: Effect displays in R for generalised linear models. *Journal of Statistical Software* **8**, 1-27.
- Fox, J. & Hong, J. 2009: Effect displays in R for multinomial and proportional-odds logit models: extensions to the effects package. *Journal of Statistical Software* **32**, 1-24.
- Fox, J. & Weisberg, S. 2011: An R companion to applied regression, 2.0-22 edn. Sage, Thousand Oaks CA.
- Gagliardo, A. & Guilford, T. 1993: Why do warning-coloured prey live gregariously? *Proc. R. Soc. Lond. B. Biol. Sci.* **251**, 69-74.
- Gamberale, G. & Tullberg, B. S. 1996: Evidence for a peak-shift in predator generalization among aposematic prey. *Proc. R. Soc. Lond. B. Biol. Sci.* **263**, 1329-1334.
- Ghent, A. W. 1960: A study of the group-feeding behaviour of larvae of the Jack pine sawfly, *Neodiprion pratti banksianae* Roh. *Behaviour* **16**, 110-148.
- Gherlenda, A. N., Moore, B. D., Haigh, A. M., Johnson, S. N. & Riegler, M. 2016: Insect herbivory in a mature Eucalyptus woodland canopy depends on leaf phenology but not CO₂ enrichment. *BMC Ecol.* **16**, 47.
- Glos, J., Dausmann, K. H. & Linsenmair, E. K. 2007: Mixed-species social aggregations in Madagascan tadpoles—determinants and species composition. *J. Nat. Hist.* **41**, 1965-1977.
- Grégoire, J.-C. 1988: Larval gregariousness in the Chrysomelidae. Kluwer Academic Publishers, Dordrecht.

- Harvey, P. H., Bull, J. J., Pemberton, M. & Paxton, R. J. 1982: The evolution of aposematic coloration in distasteful prey: a family model *Am. Nat.* **119**, 710-719.
- Huey, R. B. 1991: Physiological Consequences of Habitat Selection. *Am. Nat.* **137**, S91-S115.
- Ichiki, R. T., Kainoh, Y., Yamawaki, Y. & Nakamura, S. 2011: The parasitoid fly *Exorista japonica* uses visual and olfactory cues to locate herbivore-infested plants. *Entomol. Exp. Appl.* **138**, 175-183.
- Joron, M. & Mallet, J. L. B. 1998: Diversity in mimicry: paradox or paradigm? *Trends Ecol. Evol.* **13**, 461-466.
- Kiszka, J., Perrin, W. F., Pusineri, C. & Ridoux, V. 2011: What drives island-associated tropical dolphins to form mixed-species associations in the southwest Indian Ocean? *J. Mammal.* **92**, 1105-1111.
- Klok, C. J. & Chown, S. L. 1999: Assessing the benefits of aggregation: thermal biology and water relations of anomalous Emperor Moth caterpillars. *Funct. Ecol.* **13**, 417-427.
- Köpf, A., Rank, N., Roininen, H. & Tahvanainen, J. 1997: Defensive larval secretions of leaf beetles attract a specialist predator *Parasyrphus nigratarsis*. *Ecol. Entomol.* **22**, 176-183.
- Krakauer, D. C. 1995: Groups confuse predators by exploiting perceptual bottlenecks: a connectionist model of the confusion effect. *Behav. Ecol. Sociobiol.* **36**, 421-429.
- Landeau, L. & Terborgh, J. 1986: Oddity and the 'confusion effect' in predation. *Anim. Behav.* **34**, 1372-1380.
- Lindstedt, C., Huttunen, H., Kakko, M. & Mappes, J. 2011: Disentangling the evolution of weak warning signals: high detection risk and low production costs of chemical defences in gregarious pine sawfly larvae. *Evol. Ecol.* **25**, 1029-1046.
- López, E. R., Roth, L. C., Ferro, D. N., Hosmer, D. & Mafra-Neto, A. 1997: Behavioral ecology of *Myiopharus doryphorae* (Riley) and *M. aberrans* (Townsend), tachinid parasitoids of the colorado potato beetle. *J. Insect Behav.* **10**, 49-78.
- Low, C. 2008: Grouping increases visual detection risk by specialist parasitoids. *Behav. Ecol.* **19**, 532-538.
- Matthews, E. G. & Reid, C. A. M. 2002: A guide to the Genera of Beetles of South Australia Part 8. South Australian Museum, Adelaide.
- Mo, J. H. & Farrow, R. A. 1993: Estimation and correction of egg mortality rates from sample data of two coexisting leaf beetles (Coleoptera: Estimation and correction of egg mortality rates from sample data of two coexisting leaf beetles (Coleoptera: Chrysomelidae: Paropsini). *Aust. J. Entomol.* **32**, 85-92.
- Moore, B. P. 1967: Hydrogen cyanide in the defensive secretions of larval Paropsini (Coleoptera: Chrysomelidae). *Aust. J. Entomol.* **6**, 36-38.
- Morse, D. H. 1977: Feeding behavior and predator avoidance in heterospecific groups. *Bioscience* **27**, 332-339.
- Ohashi, K., Sakuratani, Y., Osawa, N., Yano, S. & Takafuji, A. 2005: Thermal Microhabitat Use by the Ladybird Beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae), and Its Life Cycle Consequences. *Environ. Entomol.* **34**, 432-439.
- Ohmart, C. P. & Edwards, P. B. 1991: Insect herbivory on Eucalyptus. *Annu. Rev. Entomol.* **36**, 637-657.
- Ohtsuka, S., Inagaki, H., Onbe, T., Gushima, K. & Yoon, Y. H. 1995: Direct observations of groups of mysids in shallow coastal waters of western Japan and southern Korea. *Mar. Ecol. Prog. Ser.* **123**, 33-44.
- Peuhkuri, N. 1997: Size-assortative shoaling in fish: the effect of oddity on foraging behaviour. *Anim. Behav.* **54**, 271-278.
- Peuhkuri, N. 1998: Shoal composition, body size and foraging in sticklebacks. *Behav. Ecol. Sociobiol.* **43**, 333-337.
- Peuhkuri, N. 1999: Size-assorted fish shoals and the majority's choice. *Behav. Ecol. Sociobiol.* **46**, 307-312.
- Peuhkuri, N., Ranta, E. & Seppä, P. 1997: Size-assortative Schooling in Free-ranging Sticklebacks. *Ethology* **103**, 318-324.
- Phillips, W. M. 1977: Observations on the biology and ecology of the chrysomelid genus *Haltica* Geoff, in Britain. *Ecol. Entomol.* **2**, 205-216.

- Powell, G. V. N. 1974: Experimental analysis of the social value of flocking by starlings (*Sturnus vulgaris*) in relation to predation and foraging. *Anim. Behav.* **22**, 501-505.
- R Development Core Team. 2016: R: A language and environment for statistical computing.
- Reid, C. A. M. 1992: Descriptions of the pupae of nine genera of Australian Paropsine Chrysomelinae (Coleoptera: Chrysomelidae). *Proc. Linn. Soc. N.S.W.* **113**, 311-337.
- Renaud, F., Clayton, D. & De Meeüs, T. 1996: Biodiversity and evolution in host-parasite associations. *Biodiversity & Conservation* **5**, 963-974.
- Rettenmeyer, C. W. 1970: Insect mimicry. *Annu. Rev. Entomol.* **15**, 43-75.
- Riipi, M., Alatalo, R. V., Lindstrom, L. & Mappes, J. 2001: Multiple benefits of gregariousness cover detectability costs in aposematic aggregations. *Nature* **413**, 512-514.
- Rodgers, G. M., Kimbell, H. & Morrell, L. J. 2013: Mixed-phenotype grouping: the interaction between oddity and crypsis. *Oecologia* **172**, 59-68.
- Ronnås, C., Larsson, S., Pitacco, A. & Battisti, A. 2010: Effects of colony size on larval performance in a processionary moth. *Ecol. Entomol.* **35**, 436-445.
- Ruf, C. & Fiedler, K. 2000: Thermal Gains Through Collective Metabolic Heat Production in Social Caterpillars of *Eriogaster lanestris*. *Naturwissenschaften* **87**, 193-196.
- Schulter, D., Price, T. D. & Grant, P. R. 1985: Ecological character displacement in Darwin's finches. *Science* **227**, 1056-1059.
- Schultz, J. C., Nothnagle, P. J. & Baldwin, I. T. 1982: Seasonal and Individual Variation in Leaf Quality of Two Northern Hardwoods Tree Species. *Am. J. Bot.* **69**, 753-759.
- Selman, B. J. 1994: The biology of the paropsine eucalyptus beetles of Australia. In: Novel aspects of the biology of Chrysomelidae. (Jolivet, P., Cox, M. L. & Petitpierre, E., eds). Kluwer Academic Publishers, Dordrecht. pp. 555-565.
- Sillen-Tullberg, B., Wiklund, C. & Jarvi, T. 1982: Aposematic coloration in adults and larvae of *Lygaeus equestris* and its bearing on Müllerian mimicry: an experimental study on predation on living bugs by the great tit *Parus major*. *Oikos* **39**, 131-136.
- Smith, R. J. 2002: Effect of larval body size on overwinter survival and emerging adult size in the burying beetle, *Nicrophorus investigator*. *Canadian Journal of Zoology* **80**, 1588-1593.
- Stenberg, J. A. 2012: Plant-mediated effects of different *Salix* species on the performance of the braconid parasitoid *Perilitus brevicollis*. *Biol. Control* **60**, 54-58.
- Stenberg, J. A. 2015: Outbreking herbivore escapes parasitoid by attaining only a small body size. *Ecosphere* **6**, 1-10.
- Stensland, E. V. A., Angerbjörn, A. & Berggren, P. E. R. 2003: Mixed species groups in mammals. *Mamm. Rev.* **33**, 205-223.
- Stireman, J. O. 2002: Host location and selection cues in a generalist tachinid parasitoid. *Entomol. Exp. Appl.* **103**, 23-34.
- Stireman, J. O. & Singer, M. S. 2003: Determinants of parasitoid–host associations: Insights from a natural tachinid–lepidopteran community. *Ecology* **84**, 296-310.
- Tammaru, T., Kaitaniemi, P., Ruohomäki, K. 1996: Realized Fecundity in *Epirrita autumnata* (Lepidoptera: Geometridae): Relation to Body Size and Consequences to Population Dynamics. *Oikos* **77**, 407-416.
- Tan, E. J., Reid, C. A. M. & Elgar, M. A. 2016: Colour pattern variation affects predation in chrysomeline larvae. *Anim. Behav.* **118**, 3-10.
- Tanton, M. & Khan, S. 1978: Aspects of the biology of the Eucalypt-defoliating chrysomelid beetle *Paropsis atomaria* Ol. in the Australian Capital Territory. *Aust. J. Zool.* **26**, 113-120.
- Tanton, M. T. & Epila, J. S. O. 1984a: Description of the planidium of *Perilampus tasmanicus* Cameron (Hymenoptera: Chalcidoidea), a hyperparasitoid of larvae of *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae). *Aust. J. Entomol.* **23**, 149-152.
- Tanton, M. T. & Epila, J. S. O. 1984b: Parasitization of larvae of *Paropsis atomaria* Ol. (Coleoptera: Chrysomelidae) in the Australian Capital Territory. *Aust. J. Zool.* **32**, 251-259.
- Terborgh, J. 1990: Mixed flocks and polyspecific associations: Costs and benefits of mixed groups to birds and monkeys. *Am. J. Primatol.* **21**, 87-100.
- Therneau, T. 2013: A package for survival analysis in S. R package version 2.37-4.

- Tosh, C. R., Jackson, A. L. & Ruxton, G. D. 2007: Individuals from different-looking animal species may group together to confuse shared predators: simulations with artificial neural networks. *Proc. R. Soc. Lond. B. Biol. Sci.* **274**, 827-832.
- Tsubaki, Y. 1981: Some beneficial effects of aggregation in young larvae of *Pryeria sinica* Moore (Lepidoptera: Zygaenidae). *Res. Popul. Ecol.* **23**, 156-167.
- Tsubaki, Y. & Shiotsu, Y. 1982: Group feeding as a strategy for exploiting food resources in the burnet moth *Pryeria sinica*. *Oecologia* **55**, 12-20.
- Venables, W. N. & Ripley, B. D. 2002: Modern applied statistics with S, 3.0-2 edn. Springer, New York.
- Villa, J. D. & Rinderer, T. E. 1993: Cold room thermoregulation, store consumption, and survival of Africanized and European honey bees (*Apis mellifera* L). *Apidologie* **24**, 411-423.
- Wade, M. J. & Breden, F. 1986: Life history of natural populations of the imported willow leaf beetle, *Plagioderma versicolora* (Coleoptera: Chrysomelidae). *Ann. Entomol. Soc. Am.* **79**, 73-79.
- Weed, A. S. 2010: Benefits of larval group feeding by *Chrysolina aurichalcea asclepiadis* on *Vincetoxicum*: improved host location or feeding facilitation? *Entomol. Exp. Appl.* **137**, 220-228.
- Weinstein, P. 1989: Cycloalexy in an Australian Pergid sawfly (Hymenoptera, Pergidae). *Bull. Ann. Soc. R. Belge Entomol.* **125**, 53-60.
- Wendler, R., Carvalho, P. O., Pereira, J. S. & Millard, P. 1995: Role of nitrogen remobilization from old leaves for new leaf growth of *Eucalyptus globulus* seedlings. *Tree Physiology* **15**, 679-683.

Fig. 1 Bar plot of mean group size of larvae in monospecific and heterospecific aggregations with standard error values reflected in the error bars

Fig. 2 Frequency of colour patterns observed in aggregations of larvae. The grey areas represent aggregations with early instar colour pattern; open areas represent aggregations with late instar colour pattern; black areas represent aggregations with mixed instar colour pattern.

Fig. 3 *Paropsis atomaria* and *Paropsisterna variicollis* larvae in the field. a) Monospecific aggregation of *P. atomaria*; b) Monospecific aggregation of *Pa. variicollis*; c) Heterospecific aggregation with larval arrangement after that of *P. atomaria*; d) Heterospecific aggregation with larval arrangement after that of *Pa. variicollis*. White spots indicate *P. atomaria* larvae and the white bar in each image represents 5mm.

Fig. 4 Visualisation of multinomial logistic regression model of the effects of variables. a. Larval position on leaf (P: on petiole; S: on side of leaf; T: on top of leaf; U: under leaf); b. ambient light environment measured in lux; c. leaf surface temperature (°C); d. number of larvae, on the species composition of larval aggregations. The y-axis represents the probability of each aggregation type (*P. atomaria*, *Pa. variicollis*, or heterospecific aggregations) occurring for each variable. Grey areas in graphs b-c indicate the standard deviation, while the solid line indicate the mean.

Fig. 5 Kaplan-Meier survival curves of larvae across treatments. A. Overall survival of larvae was not different across the four treatments ($X^2_3 = 4.80$, $p = 0.19$). B. Survival of *P. atomaria* was not different across the three treatments ($X^2_2 = 2.95$, $p = 0.23$). Treatment I: $n = 8$; Treatment II: $n = 10$; Treatment III: $n = 9$; Treatment IV: $n = 10$. The number of larvae at the start of each replicate was 10.

Table 1 Environmental parameters based on aggregation composition with

standard error values

Species composition	Ambient light (lux)	Ambient (°C)	Height from ground (m)	Leaf surface temperature (°C)
P. atomaria	36.50 ± 7.37	23.40 ± 2.91	1.29 ± 0.28	14.44 ± 0.16
Pa. variicollis	34.70 ± 7.66	23.03 ± 3.08	1.36 ± 0.25	13.76 ± 0.26
Heterospecific aggregations	33.05 ± 10.54	22.37 ± 2.52	1.43 ± 0.30	16.10 ± 0.47

Table 2 Multinomial logistic regression of the effects on larval aggregation composition (P. atomaria, Pa. variicollis, or heterospecific aggregations)

Variable(s)	df	χ^2	P
Ambient light	8.43	2	0.015
Ambient temperature	1.97	2	0.37
Group size	63.38	2	<0.001
Height from ground	3.25	2	0.20
Leaf surface temperature	14.63	2	<0.001
Position on leaf	16.15	6	0.013
Ambient light*leaf temperature*ambient temperature	24.60	2	<0.001
Height*ambient temperature*group size	8.99	2	0.011
Height*leaf temperature*ambient temperature	7.96	2	0.019
Leaf temperature*ambient light*position*group size	13.88	6	0.031
Height*leaf temperature*ambient light*ambient temperature*group size	26.61	2	<0.001

Table 3 Mean overall gregarious scores of the four treatments with standard error mean values values

Treatment	n	Day				
		1	2	3	4	5
I	8	1.80 ±	1.80 ±	1.26 ±	1.03 ±	1.05 ±
		0.33	0.18	0.30	0.25	0.26
II	10	1.67 ±	2.23 ±	1.94 ±	1.48 ±	1.20 ±
		0.17	0.23	0.28	0.21	0.25
III	9	1.51 ±	1.78 ±	1.59 ±	1.15 ±	1.28 ±
		0.20	0.11	0.22	0.29	0.27
IV	10	1.18 ±	1.81 ±	1.51 ±	1.39 ±	1.29 ±
		0.20	0.21	0.24	0.22	0.14

Table 4 Parasitoidism rates of larvae across treatments

Treatment	Larval species	No. of larvae	No. of larvae parasitised	Parasitoidism rate (%)
I	<i>P. atomaria</i>	78	7	8.97
II	<i>Pa. variicollis</i>	100	2	2.00
III	<i>P. atomaria</i>	45	2	4.44
	<i>Pa. variicollis</i>	46	1	2.17
IV	<i>P. atomaria</i>	48	1	2.08
	<i>Pa. variicollis</i>	49	1	2.04

Frequency of colour patterns

This article is protected by copyright. All rights reserved

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Tan, EJ;Reid, CAM;Elgar, MA

Title:

Predators, Parasites and Heterospecific Aggregations in Chrysomeline Larvae

Date:

2017-04-01

Citation:

Tan, E. J., Reid, C. A. M. & Elgar, M. A. (2017). Predators, Parasites and Heterospecific Aggregations in Chrysomeline Larvae. ETHOLOGY, 123 (4), pp.293-306. <https://doi.org/10.1111/eth.12598>.

Persistent Link:

<http://hdl.handle.net/11343/292469>