Mimicry in the ant attended leafhopper species *Eurymela rubrolimbata*

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Declaration

I certify that the work in this thesis, entitled 'Mimicry in the ant attended leafhopper species *Eurymela rubrolimbata*' has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree at any other university or institution other than Macquarie University. I certify that work presented in this thesis contains only original work that has been written by me. In addition, I also certify that all literature and sources used during preparation of this thesis have been acknowledged.

All research described in this report is my own original work.

Alexander Edward Harvey Burton

Supervisors: Dr Matthew Bulbert, professor Mariella Herberstein

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Abstract

Mimicry is a diverse and well-studied topic. However, little literature exists regarding why animals adopt mimicry over other defence strategies. Juvenile *Eurymela rubrolimbata* leafhoppers both mimic *Dolichoderus clarki* ants, and provide them with honeydew in return for protection. Interestingly, other ant attended species have not been described to resemble their ant partners. Considering this, I first determined whether *E. rubrolimbata* mimics *D. clarki* or just displays similar aposematic colouration. To do so I took a multi-trait approach applying recently developed techniques and incorporating multiple life stages and backgrounds. This approach revealed that *E. rubrolimbata* display mimetic colour and colour distribution at size appropriate instar stages, possess a more ant-like shape due to background interactions and move rapidly like *D. clarki*. To understand why mimicry may be displayed, I assessed *E. rubrolimbata's* relationship with *D. clarki* and with key resources. Like other species, the mimic and model display a strong relationship. However, *E. rubrolimbata* regularly travels between spatially separated resources, setting it apart from other ant attended species which are primarily stationary. Hence, I propose movement requirements contributed to *E. rubrolimbata* evolving mimicry. Indeed, the need for movement may be a determining factor in the evolution of mimicry among other species.

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Chapter 1

Ant mimicry in *Eurymela rubrolimbata* leafhoppers depends on spatial and temporal context

1. Introduction

Ants are a well defended group of invertebrates. Many predators avoid ants as they are aggressive and carry defensive weaponry such as mandibles, spines, venomous stings and/or chemical sprays [1]. Ants also occur in high densities and recruit conspecifics *en masse* via alarm pheromones when threatened [1-3]. Consequently, ants have relatively few predators and such predators often possess specialised morphologies or capture strategies [4-6]. For example, species of *Zenodorus* spider use ambush techniques when hunting ants rather than actively pursuing them as they do for other prey [7]. The abundance of ants in all terrestrial environments except the extremely cold means that encounters between ants and other animals are inevitable [1]. As a result, predators which are not innately averse to ants [8, 9] have plenty of opportunities to sample them and learn their unpalatability [10, 11]. Due to the formidable nature of ants and the suite of predators they deter, other invertebrates have evolved ant-based defence strategies for protection. Two of the most common forms of ant-based defence are ant mimicry and ant attendance.

Ant mimicry is a defence strategy in which the mimic replicates visual cues of a defended ant model, resulting in predators misclassifying the mimic as an unpalatable ant [12]. Currently, over 2000 ant mimicking species have been described from 11 different invertebrate orders [13] and the strategy is effective at deterring a range of insectivorous predators, including both invertebrates and vertebrates [14-16]. Among ant mimics, Batesian mimicry [17], in which an undefended mimic resembles a defended model, is the most prevalent [13, 14, 18, 19] as opposed to the Mullerian ant mimicry [20] where the mimic and model are co-defended [16].

Strategies used to mimic ants vary from simply resembling colour, to extreme modification of body structure and behaviour [13, 21]. Ants have a characteristic body plan with a defined head, alitrunk and gaster which is separated by a constricted waistline [1]. Ants, unlike other unpalatable mimics, do not generally display the vibrant aposematic colouration of other unpalatable species

(although see [16] for exception). However, some species have distinct colour combinations. Thus, the simplest form of mimicry is to replicate the ant models colour and pattern [13, 21, 22]. Some ant mimics go further and use deceptive colouration to create the illusion of possessing a more ant like form. For example, colour patches at the waist have been reported to create the illusion of a constricted, ant-like petiole [13, 23-26]. While colour tactics do not require morphological alterations, colour deception relies on features of the environment and predator perception to be successful [10]. The most iconic examples of ant mimicry occur when invertebrates possess physical modifications that produce an ant-like form. For insects, this shift is not that extreme given they possess a three-segmented body plan like ants. However, spiders only have two body segments and often rely on constrictions to achieve an ant-like appearance [21, 27, 28]. Mimics not only resemble an ants physical form but also their behaviour. This may include similar movement patterns [21, 29, 30], spending more time moving around like a foraging ant [31], and in some spiders, waving their front pair of legs to resemble antenna [23, 24, 27, 30]. These modifications may increase the likelihood of survival but at a cost of mobility, such as loss of wings [13, 32], or a reduction in fecundity due to body constrictions [21, 33].

Ant attendance is another ant-related defence that is distinct from that of ant mimicry. While ant mimics generally have a commensal relationship with ants, ant attended species (known as trophobionts) rely on a mutualistic exchange. Trophobionts defend themselves from predation and parasitism by encouraging ant attendance through the provision of food resources [34, 35]. Lepidopteran trophobionts produce food from dorsal nectary organs [36] while hemipteran provide honeydew [37-39], a waste product of sap feeding that contains carbohydrates, amino acids, amides and water [40-43]. By encouraging ants to gather around them, trophobionts effectively create a shield of ant bodyguards. These attendant ants deter predators and parasitoids both passively with their presence, and actively by attacking threats [34, 37, 39, 44-46]. Ant attendance has additional benefits for honeydew producers as a build-up of the excrement can cause the growth of mould [37, 39] and can drown low-mobility individuals producers [47, 48]. The strength of relationships between trophobionts and ants varies considerably. Facultative relationships occur when ants opportunistically attend trophobionts found within their range [37, 49-51], providing varying levels of protection depending on ant species [52, 53] and colony size [54]. In stronger relationships, ants occasionally carry trophobionts to better feeding locations, out of harm's way or even inside the ant nest [34, 37, 39, 55]. Additionally, some ant species construct physical shelters to protect trophobionts from predators, parasitoids and adverse weather [56-59].

In obligate relationships, ants and/or trophobionts heavily rely on their partners. For example, populations of obligate trophobionts can quickly decline and even die out if their ant partners are excluded [60-62]. Irrespective of association level, ant attended species are generally more successful than those which are unattended [37, 39, 44, 53, 63-65].

The mechanisms by which ant attendance and ant mimicry repel threats vary along with the traits required to maintain each strategy. Both strategies rely on predators and parasitoids being ant-adverse. However, ant mimics require their model to be within their environment, but do not require a close association as threats are deterred by the mimic's ant-like appearance [13]. In contrast, trophobionts require a close association and for ants to be in the immediate vicinity as ant presence repels predators and parasitoids [34]. So theoretically, trophobionts should not need to invest in traits to look like ants, and mimics should not need to invest in traits to appease them.

Trophobionts generally do not resemble their ant associates. Ant attendance is common among caterpillars of Lycaenidae butterflies [36, 66]. These caterpillars have extensive adaptions for life among ants and use chemical cues to attract and reward ants [36, 67]. Despite the diverse array of relationships displayed, physical resemblance has not been described. The sap-feeding hemipteran suborders Auchenorrhyncha and Sternorrhyncha, make up the largest percentage of ant-attended species [1, 34] however, ant mimicry appears to be non-existent among Sternorrhyncha, and rare in Auchenorrhyncha [68-74]. Among ant mimicking Auchenorrhyncha, ant attendance has only been described in three species of treehopper [69].

Ant mimicry has primarily been described in adult Auchenorrhyncha. However, mimicry in juvenile stages may be underreported as descriptions of nymph morphology and behaviour are uncommon, particularly in the tropics, despite the diversity present in the suborder [although see 75-77]. A lack of focus on nymphs may be due to identification keys primarily focusing on adult organisms [78, 79], the difficulty in raising nymphs to adulthood for identification, and the soft bodies of nymphs making them difficult to maintain in museum collections [69]. Hence, the true number of species engaging in a combination of ant attendance and ant mimicry is unknown.

Recently, an Australia wide mimetic complex has been described in which over 140 species either are, or resemble an ant and possess some coverage of a golden, aposematic sheen [16]. One of the members reported to sit within this mimicry complex is the leafhopper species *Eurymela rubrolimbata*, Kirkaldy 1906 (*Auchenorrhyncha: Cicadellidae*). Leafhoppers are a group of highly diverse, sap sucking insects [80] with around 22 000 species described worldwide [81] and

approximately 450 species described in Australia [82]. Adult *E. rubrolimbata* have black and white elytra much like their relatives [48] and a powerful jump to avoid capture [83]. In comparison, juvenile *E. rubrolimbata* cannot jump (pers obs). Instead, some instar stages appear to mimic the gold and black ant species *Dolichoderus clarki*. This mimetic colour resemblance is the basis for the species' inclusion in the golden mimicry complex. An important requirement of mimicry is that both mimic and model occur within the same environment [10, 84]. Descriptions of *E. rubrolimbata's* geographical distribution are limited [82]. However, the few recorded locations overlap the range of *D. clarki* which is restricted to NSW and the ACT [82, 85], supporting the notion of mimicry. A variety of leafhopper species have been described as ant attended [50, 59, 61, 63, 86-89] and ant attendance appears to be obligatory in other species from *E. rubrolimbata's* tribe Eurymelini [48, 90]. Much like its relatives, *E. rubrolimbata* also appears to be ant attended, in this case by the same species the juveniles mimic, *D. clarki*. The leafhoppers have been observed providing *D. clarki* with honeydew, traveling in the ant's foraging trails on eucalypt trunks and forming ant attended aggregations (pers obs).

If *E. rubrolimbata* does indeed display ant mimicry, it would be one of the very few trophobiotic species to do so [69, 72] and may be a valuable model for understanding the factors contributing to the development of mimicry as a defence strategy. However, *E. rubrolimbata's* mimetic display appears to be primarily colour based, with little morphological similarity when viewed by a human observer. While inaccurate mimicry has been described in many species [91, 92] and *E. rubrolimbata* can occasionally be mistaken for *D. clarki* in the field, the lack of a resemblance beyond colour may indicate that *E. rubrolimbata* juveniles are mimicking an aposematic signal, rather than being true ant mimics. Indeed, gold colouration appears to be an established aposematic colour in the Australian environment [16] and other leafhopper species are known to exhibit aposematic colouration [90].

The goal of this study was to examine the likelihood that *E. rubrolimbata* is a mimic of the ant species *D. clarki* by assessing mimetic fidelity. I used a multi-trait approach comparing both physical and behavioural traits, placing them in the context of a predator's visual sensitivities where possible. Unlike other mimicry studies, I took into account *E. rubrolimbata's* developmental stages and natural backgrounds to better contextualise mimicry fidelity. I hypothesised that if *E. rubrolimbata* are truly ant mimics then; 1) The colour intensity and pattern of gold-bearing juveniles would be comparable to that of *D. clarki* and more similar than to any other ant species in the environment; 2) Not all instars would display gold, only those within the size range of the

ant; 3) Body shape would be closer to that of *D. clarki* than to other ants or a typical bug shape; and 4) The leafhoppers would behave more like *D. clarki* than other species by traveling at a similar speed.

2. Methods

(a) Field collection

All specimens and field data were collected from Duckmaloi crown reserve (-33.7131S, 149.971E) between January and March 2017 unless otherwise specified. This site is open Eucalypt woodland dominated by *Eucalyptus viminalis.* These trees are characterised by a smooth pale trunk with rough bark at the base. The bark region varies in height and includes thick, brittle bark held tight against the trunk superseded by thin loose bark which peels away in ribbons.

I haphazardly collected 30 adult *E. rubrolimbata* and 191 juveniles from 10 *E. viminalis* trees. A larger number of juveniles were collected to allow for instar determination. As the true bug species *Notius depressus* was commonly observed at the field site, 30 individuals were collected to represent a typical bug species. These specimens were collected from both Duckmaloi and from Macquarie University campus (-33.7737S, 151.1127E). Thirty ants from 11 different species were collected and identified to species or genus using the Atlas of Living Australia [82] and Bulbert (2012)[93]. The species collected included; *D. clarki, Dolichoderus scabridus, Camponotus claripes, Camponotus consobrinus, Camponotus innexus, Camponotus intrepidus, Crematogaster sp., Iridomyrmex calvus, Leptomyrmex erythrocephalus, Myrmecia pilosula* and *Myrmecia tarsata* (appendix 1). These species were collected as they are common within the leafhoppers local environment. The ant species *Camponotus consobrinus* is known to occur at Duckmaloi crown reserve (pers obs) but was not collected from the site. *Camponotus consobrinus* ant nests can be difficult to locate due to their subtle presentation and the workers foraging at dusk. Despite searching during dusk, an obvious nest was not located during the collection phase so specimens from Macquarie University campus were used. In the laboratory, *E. rubrolimbata* were provided with *E. viminalis* branch clippings while ants were given dilute honey water (25%). Fresh water was also provided to all insects. Specimens were kept alive in the laboratory for a maximum of four days before being processed.

Bark samples were collected to represent the leafhoppers natural backgrounds. From 10 *E. viminalis* trees hosting *E. rubrolimbata*, I haphazardly collected 50 samples of bark which were roughly 50x150 millimetres (mm) in size. Bark was collected from both the very base of the tree

(dark bark) as well as from the tips of bark ribbons which share the colouration of the tree trunk (pale bark).

(b) Imaging specimens

Imaging was required for colour, size and shape analyses. All specimens were imaged using a tripod mounted Nikon DSL 7100 digital camera fitted with a Novoflex Noflexar 35mm macro lens. The camera was quartz converted prior to use to allow both ultraviolet (UV) and visible light spectra to be captured. Prior to imaging, *E. rubrolimbata* specimens were euthanised in a -30°C freezer for 3 minutes and then thawed. Unfortunately, upon freezing, ants curled up meaning they could not be positioned naturally for images. My lab has found that treating ants with ethyl acetate results in them standing in their natural resting posture. Hence, all ants were euthanised in a kill jar with ethyl acetate fumes (Sally Hanson acetone free nail polish remover). Preliminary tests suggest the colour of *E. rubrolimbata* should be comparable when killed by freezing or with ethyl acetate. Bark samples were trimmed into 30x30 mm flat squares.

Two RAW format images were taken of each specimen; the first within the visible spectrum (400 to 700 nm) and the second immediately afterwards in the UV spectrum (300 to 400nm). Control over the captured spectrum was achieved using 2-inch Baader circular filters housed in a sliding bracket mounted to the camera lens. The first, a U-Venus filter, for capturing reflectance in the UV spectrum (320-380nm) and the second, a UV/IR cut filter, for the visible spectrum (400-680nm). The visible and UV spectrum images were taken with shutter speeds of 1/25 of a second and 2.5 seconds respectively. These settings were previously determined to avoid both under and over exposure which can lead to the loss of colour information [94]. All images were taken under light produced by an Iwasaki Eye Colour Arc MT70D light bulb positioned 300 mm away from the specimen. The outer coating of UV filtering film was removed from the bulb with a steel brush prior to use. This created a wide spectrum light source emitting both visible and UV light which simulates illuminant D65 i.e. standard daylight. Specimens were placed on a 1.5 mm thick piece of white Polytetrafluoroethylene (PTFE) and positioned so their dorsal plane was horizontal. A cylinder of semi-transparent, 0.5mm thick, white PFTE was placed around the specimen to defuse the light. I used PTFE as it is spectrally flat, equally reflecting visible and UV light. The PTFE diffuser cools the temperature of the light, reducing the illuminant to an approximation of shady conditions on a sunny day. All images were taken of the specimen's dorsal surface with a 5x5 mm scale and a 5% grey standard for colour calibration included in each image. The standard was a 4x4 mm piece of 5% SphereOptics Zenith Polymer diffuse target (SG 3155). This material is diffuse and

spectrally flat, reflecting the same amount of light over the 300-700nm wavelength range required for this study.

(c) Determining instar stages

To understand how *E. rubrolimbata's* developmental stage relates to mimetic display, I first needed to differentiate instar stages. I attempted to raise the leafhoppers under laboratory conditions to make direct morphological measurements of each instar stage. Based on previous descriptions of leafhopper breeding experiments, I constructed specialised housing containers and provided *E. rubrolimbata* with bark shelter and sugar water (10%) [80]. Housing containers were stored in a specimen room at 24°C, 22% humidity and with lights on a 12 - 12 cycle. Unfortunately, *E. rubrolimbata* did not survive, suggesting nutritional requirements and/or environmental requirements were not met. Instead, I applied a frequency distribution method using transocular width (distance across the eyes) to differentiate instar stages [95-98]. To assess whether all stages were sampled, I applied the Brooks-Dyar rule [99, 100] which assumes that the size ratio between successive stages is a constant. As arthropods generally increase in size via regular geometric progression, any deviations from a constant would indicate that not all instars were collected (figure 1).

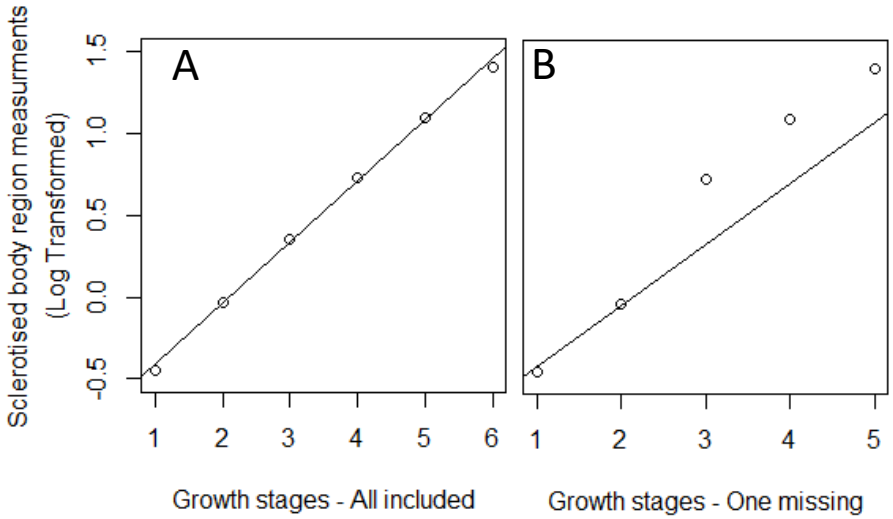

Figure 1. Hypothetical data depicting the deviation caused by omitting a growth stage. All growth stages are accounted for in plot (A) while the hypothetical 3rd growth stage has been omitted from plot (B).

The transocular widths of adult and juvenile *E. rubrolimbata* were measured in ImageJ [101] from images taken for colour analyses. A frequency distribution histogram with 100 bins, each with a

0.05 mm range, was used to plot the measured transocular widths. From the resulting plot, peaks were interpreted as separate growth stages.

(d) Comparing *E. rubrolimbata's* body colour

For colour comparisons I used a technique which relies on images taken with a digital camera. A common problem that is faced when working with colour in invertebrates is actually measuring it. Even the smallest probes of traditional spectrophotometry are often too large for the whole specimen, let alone any particular body region. The colour analysis approach I took is the first attempt at using this technique for an ant mimic-model comparison and so I had to adapt it for this particular purpose. The advantage of this photographic approach over traditional spectrometry is that whole body regions can be measured as well as any particular body region or pattern. Colour was quantified using the Image Calibration and Analysis Toolbox [94], a collection of recently developed ImageJ plugins. This toolbox allows images taken with consumer digital cameras to be linearized and calibrated from an included standard, and for colour measurements to be made using the visual model of a predator. For each specimen, the toolbox was used to create multispectral image stacks of the cameras red, green, blue, UV-red and UV-blue wavelength channels.

Many ant mimics appear to replicate an ant-like head, alitrunk and gaster through either morphology or colour. Considering this, I took colour measurements from these regions as well as from the legs. Equivalent body regions were selected for *E. rubrolimbata* and *N. depressus*. For the remainder of this study the term 'thorax' will be used to indicate the alitrunk of ants and the pronotum of *E. rubrolimbata* and *N. depressus* while 'abdomen' will be used to indicate the ant's gaster, the leafhoppers abdomen and the elytra of *N. depressus* as they conceal the bugs abdomen from predators. For each multispectral image and body region, two regions of interest (ROI) were selected, one on either side of the body. The body centre was avoided due to reflection artefacts. When a body region consisted of more than one colour, the colour covering the largest amount of area was chosen. For example, in gold-bearing *E. rubrolimbata*, the gold region of the abdomen was selected and the black outline was avoided.

Analysis of raw colour output is not biologically relevant so colours were converted relative to the visual sensitivities of a potential predator group. I chose an avian visual model for analysis as a variety of birds are common at the field site, including many insectivorous treecreepers (Order: Passeriformes, family: Climacteridae). In comparison, reptiles have rarely been sighted and thus, were not considered (pers obs, pers com). Invertebrate predators, such as spiders or predatory

ants, were also considered but visual models for these animals are not yet available in the ImageJ toolbox. I converted all images to avian colour space using a polynomial mapping technique within the ImageJ toolbox. The model I used for cone mapping was that of the blue tit (*Cyanistes caeruleus*), a small, insectivorous passerine bird with known relative cone abundances (UV = 0.46, sw = 0.85, mw = 1, lw = 0.96)[102]. Cone abundances are known for other insectivorous passerine birds including blackbirds (Turdus merula) [102] however, blackbirds are ground feeders while blue tits feed in trees which aligns with where *E. rubrolimbata* are located [103]. Following conversion, the average UV, short wave (sw), medium wave (mw) and long wave (lw) photon catches of each body region were measured in the toolbox with the two ROI making up each body region measured as is they were a single area.

Units of just noticeable differences (JND) were used to approximate the ability of the modelled predator to discriminate between colours. To calculate JND I used a log form of the Vorobyev-Osorio model [104] with a weber fraction of 0.05 [105-108]. However, I did not perform colour analysis on 1st and 2nd *E. rubrolimbata* instars or *I. calvus* as these specimens were too small for accurate colour capture. In general, a JND less than 1 is considered to indicate that two colours are practically indistinguishable. There is no fixed JND above which two colours can unmistakably be differentiated. However, for this study I use a value of 3 as JND below this threshold have been considered indicate that colours can only be discriminated under good viewing conditions [109-112]. Above this threshold, increasingly larger JND has been suggested to lead to an increase in discrimination ability [105, 110].

The body colour of *E. rubrolimbata* juveniles appears to vary with instar stage so between instar colour comparisons were performed. To assess colour variation I compared head, thorax, legs and abdomen colour between the leafhoppers 3rd, 4th and 5th instars. For each body region, if multiple instars shared the same colour (mean JND < 3) then the colours were considered non-unique and only the colour of the oldest instar was used in further analysis. In comparing leafhopper body colour to that of other species, I started from the premise that the *E. rubrolimbata* should resemble its ant model and do so more than it resembles other species within the environment. To assess this, I compared the unique colours on each of the leafhoppers body regions to the mean colour of the respective region for each other species I collected.

(e) Shape morphometrics

Colour was also considered for the shape analyses. Animals are generally not observed in isolation on a white background. Instead, they occur on backgrounds that have colour properties of their

own. Hence, the perceived appearance of an animal can vary depending on interactions between background colours and the animal's own colouration [10]. Considering this, I took into account possible variation in *E. rubrolimbata's* shape when viewed by predators in the context of its natural environment.

Gold-bearing *E. rubrolimbata* juveniles possess abdomen colour patches that may interact with the leafhoppers main backgrounds, pale and dark bark, changing the shape predators perceive. These regions include a black outline surrounding the gold abdomen colouration and pale patches encroaching from the extremities of the abdomen, anterior to the black outline (figure 2).

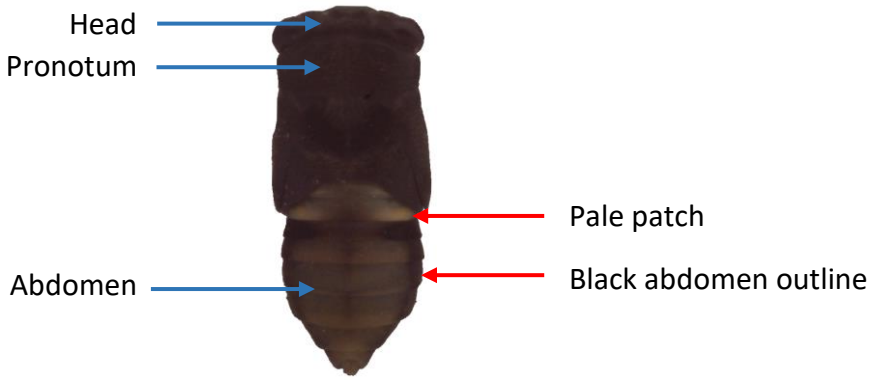

Figure 2. Diagram of a 5th instar *E. rubrolimbata* juvenile (legs erased). Blue arrows indicate the three body regions colour measurements were taken from. Red arrows indicate the colour regions which were compared to the leafhoppers backgrounds.

Prior to colour comparisons, the pale patches and black outline of 5th instars were selected as ROI and a single ROI was selected for each bark sample. To allocate bark into pale and dark categories, luminance values based on avian double cones were calculated in the ImageJ toolbox. For pale and dark bark, the mean colour of the five lightest and five darkest pieces of bark were used respectively.

For shape comparisons, I examined whether *E. rubrolimbata* instars possessed a shape closer to that of *D. clarki* as opposed to the other ants or the typical bug shape of *N. depressus*. Both overall body shape and the shape of the instars after considering background interactions were examined. Images of specimens in the most natural posture were selected for this exercise. Up to fifteen images per species/instar were chosen, depending on availability. Using the software program Paint.net [113], the images were aligned vertically along the specimen's mid-line. Background colour was removed using thresholding tools while appendages (legs, stings, antenna, stylets and mandibles not held against the head) were erased by hand as I chose to focus on the most basic shape that constitutes a specimen's appearance. The appendages are also problematic

in that they can be held in multiple orientations. For gold-bearing instars, additional silhouettes were created that excluded the pale waist patches as they may be difficult to discriminate from the pale bark.

Analyses on abdomen shape were also performed as some predators appear to only use highly salient cues, such as *D. clarki's* gold abdomen, for mimetic discrimination [114, 115]. *Dolichoderus clarki* have gold on their entire abdomen while *E. rubrolimbata* only have partly gold abdomens. Hence, I predict that *E. rubrolimbata's* gold region, rather than the entire abdomen, will resemble the shape of *D. clarki's* abdomen. Only *E. rubrolimbata* life stages bearing gold were included for abdomen shape analysis. For some instars, the leafhoppers scutellum conceals part of the abdomen from view. For these individuals, only the region of the abdomen visible to predators was included. Two silhouettes were created for *E. rubrolimbata*, one of the whole visible abdomen and a second of only the gold abdomen region. A single silhouette was created for each other species.

(f) Size morphometrics

If *E. rubrolimbata* are mimetic, then *D. clarki*-like colours would be expected in instars of comparable size to the model ant. To determine whether only gold-bearing *E. rubrolimbata* are within the size range of *D. clarki* and whether they are closer to *D. clarki* than to other ant species, I measured body length and abdomen area in ImageJ from images taken for colour analysis. Measurements were made in millimetres after first calculating pixels per millimetre in ImageJ from the scale included in each image. Body length was measured centrally from the tip of the abdomen to the tip of the head. Stings, stylets and mandibles not held against the head were excluded as I chose to primarily focus on the insects 3 body segments. Abdomen area was measured by carefully outlining each specimen's abdomen using the free-hand tool in ImageJ. As with shape, only the area of the leafhoppers abdomen not concealed from view by the scutellum was selected. Furthermore, I measured the leafhoppers gold region as it is the area reminiscent of *D. clarki's* golden signal. Thresholding could not separate *E. rubrolimbata's* golden region from the black outline so the area of the golden signal was selected by hand. The gold region of adult leafhoppers was also selected to assess whether it is constrained to the size of *D. clarki's* gold region, despite the adults not engaging in ant mimicry

(g) Movement comparisons

Movement mimicry has been noted as a key attribute for ant mimics and so I expected gold-bearing instars to move at comparable speeds to *D. clarki*, despite variation in body size between instars, and to possess a similar movement pattern. Additionally, leafhopper movement speed and pattern would be expected to more closely resemble that of *D. clarki* than other ants or the bug species *N. depressus*. To film movement, a tripod mounted Casio Exilim Pro EX-F1 digital camera was directed at foraging trails on *E. viminalis*. Filming was conducted at 30 frames per second on high definition settings. Shortly after filming began, a checkerboard scale (each checker = 1.53 x 1.53 mm) was briefly placed in the shot parallel to the tree surface. Trails of *E. rubrolimbata*, *D. clarki*, *M. pilosula*, *I. calvus* and *Crematogaster sp.* were located in the field. For the other species included in this study, specimens were collected and their movement was filmed *ex-situ* in the laboratory. The camera system was set up in the same configuration as that used in the field and was directed at either a vertical log or a vertical, tactile, plastic surface onto which specimens were released. However, when introduced into this unfamiliar environment, ants moved in slow exploratory patterns, as opposed to the more purposeful and direct movement shown by individuals on foraging trails. In contrast, *N. depressus* readily and consistently walked directly up the log. Due to the discrepancy between *in-situ* and *ex-situ* movement among ants, only *E. rubrolimbata*, *D. clarki*, *M. pilosula*, *I. calvus*, *Crematogaster sp.* (*in situ*) and *N. depressus* (*ex-situ*) were used for movement analysis. Leafhopper instars 1, 2 and 3 were not included as they were no longer present during the timeframe when filming was conducted.

Filmed specimens were required to meet certain criteria to be included in the movement analysis. Individuals had to; 1) be travelling upward; 2) not interact with any other insect and; 3) engage in a continuous bout of movement over 40 consecutive frames. Thirty individuals were used to represent each group except for 4^{th} instars in which only 21 individuals met the required criteria. Using the program Tracker [116], the tip of each specimen's head was marked five times in 10 frame successions and a list of x, y coordinates for each location were recorded. Pixels per mm were calculated for each video and the distance between each successive pair of coordinates was calculated. Each specimen's speed (mm/second) was calculated by taking the average distance between successive marked locations and multiplying by 3 (time between marked locations was 1/3 of a second). Relative movement rate (body lengths per second) was also calculated for gold-bearing instars. If rates are not the same then the instars may be controlling their speed to better match that of *D. clarki*. To quantify movement pattern, I assessed directional change over the

recorded bout of movement. The direction of vectors between successive x, y coordinates were calculated and the angle between consecutive vectors were measured. Mean angle change for each specimen was then calculated.

(h) Data Analyses

All statistical analyses were conducted in 'R' version 3.3.2 [117] within 'R Studio' version 1.0.136 [118]. To determine whether all of *E. rubrolimbata's* instar stages were collected, I performed a linear regression to test for a linear relationship between instar stage and the log transformation of mean transocular width of each instar stage.

Colour similarities were determined by assessing the magnitude of JND values. To determine whether *E. rubrolimbata* are more similar in colour to *D. clarki* than other species I used linear mixed models ('lme4' package [119]) comparing JND log transformed for normality. For these analyses, JND was set as the response variable, comparison species as a fixed factor and, *E. rubrolimbata* individual ID as a random factor. To assess whether *E. rubrolimbata* are more similar in colour to *D. clarki* than to other species, I set the *D. clarki* comparison as the reference level to which all other levels of the fixed factor were compared. Significant differences are reported when 95% confidence intervals (CI) do not contain 0. Fixed effect estimates were used to assess the direction and magnitude of any significant differences.

All shape analyses were performed in R using the package 'Momocs' [120]. This is the first application of an Elliptical Fourier approach to ant mimicry research. I chose this approach over other traditional methods that require landmarks as it accounts for an organism's entire outline. This removes the subjective judgements required in landmarking regarding what structures of the mimic are comparable to that of the model. From body shape silhouettes, outlines were extracted as a list of x, y coordinates. These outlines were then centred, scaled and aligned horizontally along their longest axis. A 20 harmonic, Elliptical Fourier analysis [120, 121] was then performed on the outlines and a principle component analyses (PCA) applied to the output. The PCA axes which explained 99% of variation were then analysed using a MANOVA to compare each species shape. Multidimensional Euclidean distances were calculated between *E. rubrolimbata* instars and *E. rubrolimbata* adults, *N. depressus* and ants using the same PCA axis as MANOVA analysis. A nearly identical procedure was performed for abdomen shapes. However, due to the longest axis of some rounder abdomen not being directly through the midline, abdomens could not be aligned using the default function provided by 'Momocs'. Instead abdomens were all manually aligned vertically in Paint.net. Paired t-tests were used to assess how morphospace distance to *D. clarki*

changed upon removal of the leafhoppers pale patches and when only the gold region was included in abdomen shape analysis. Following Shapiro-Wilk and Levene's test's, Euclidean distance values for 5th instar abdomen analysis were log transformed to achieve homogeneity of variance.

Fisher-Pitman permutation tests ('coin' package [122]) were performed to compare body size measurements of each leafhopper life stage to that of the other species collected. For each test, 2000 permutations were performed. A "holm" correction was applied due to multiple comparisons [123].

T-tests were used to compare movement speed and relative movement rate between gold-bearing instars following analysis with Shapiro-Wilk and Levene's test which identified a normal distribution and homogeneity of variance respectively. A t-test was also used to compare turning angle of gold bearing instars. Turning angle data were square-root transformed to achieve normality. Regression models were used to assess whether the movement speed of these instars was significantly different to that of adult *E. rubrolimbata*, *D. clarki*, *M. pilosula*, *I. calvus*, *Crematogaster sp.* or the other hemipteran, *N. depressus*. For each regression model, the instar stage being assessed was set as the reference level to which the different groups were compared. A "holm" correction was applied for multiple comparisons [123].

3. Results

(a) Instar stage determination

Six distinct development stages consisting of 5 instars plus the adult were evident from transocular width measurements (figure 3). The clear distinction between each developmental stage allowed designation with a high degree of certainty based on mean transocular widths (appendix 1). Instar stages 1, 2 and 3 have completely black bodies while instars 4 and 5 are black with golden abdomens (figure 4). Adult *E. rubrolimbata* are black with white markings on the wing casings and gold colouration on the pronotum. The gold of the adult appears in two forms: a broad "T" shape or a thin line (figure 4).

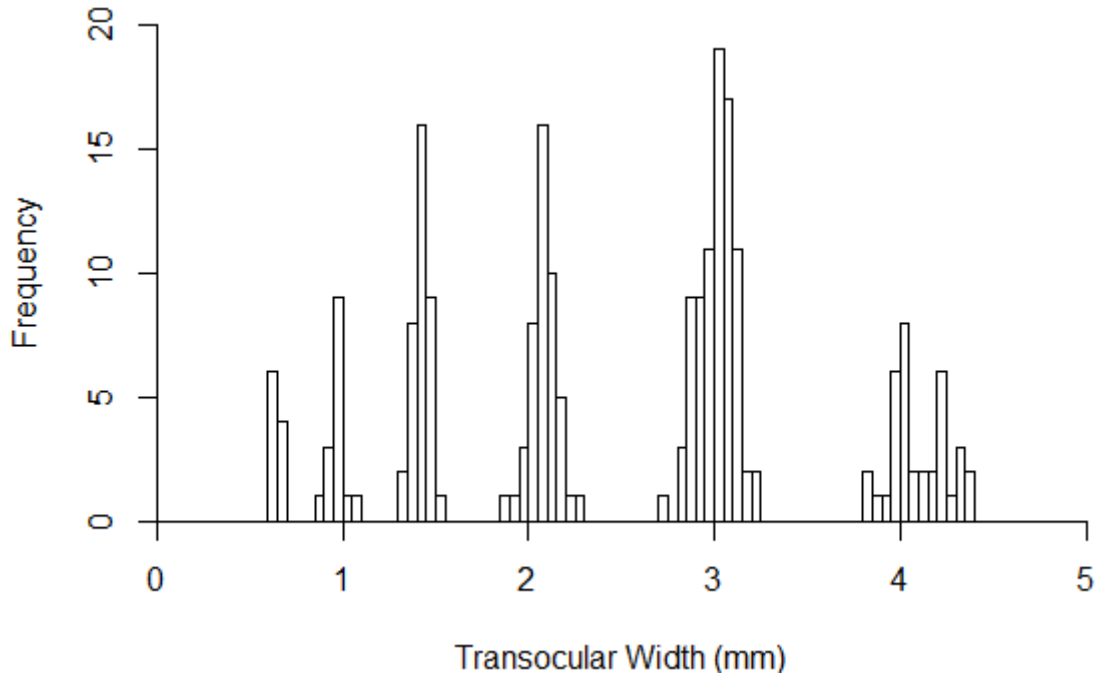

Figure 3. Frequency distribution plot of *E. rubrolimbata* transocular widths (mm) placed into 1 of 100 bins. Each bin has a range of 0.05 mm and the y-axis indicates the number of measurements falling within each bin. Six distinct peaks are clearly visible indicating five separate instar stages plus adults. A linear regression on log transformed mean transocular width was significant ($F_{(1,4)}$ = 1993, $p < 0.001$) and was a strong fit (adjusted R2 = 0.9975), confirming that all instar stages were collected.

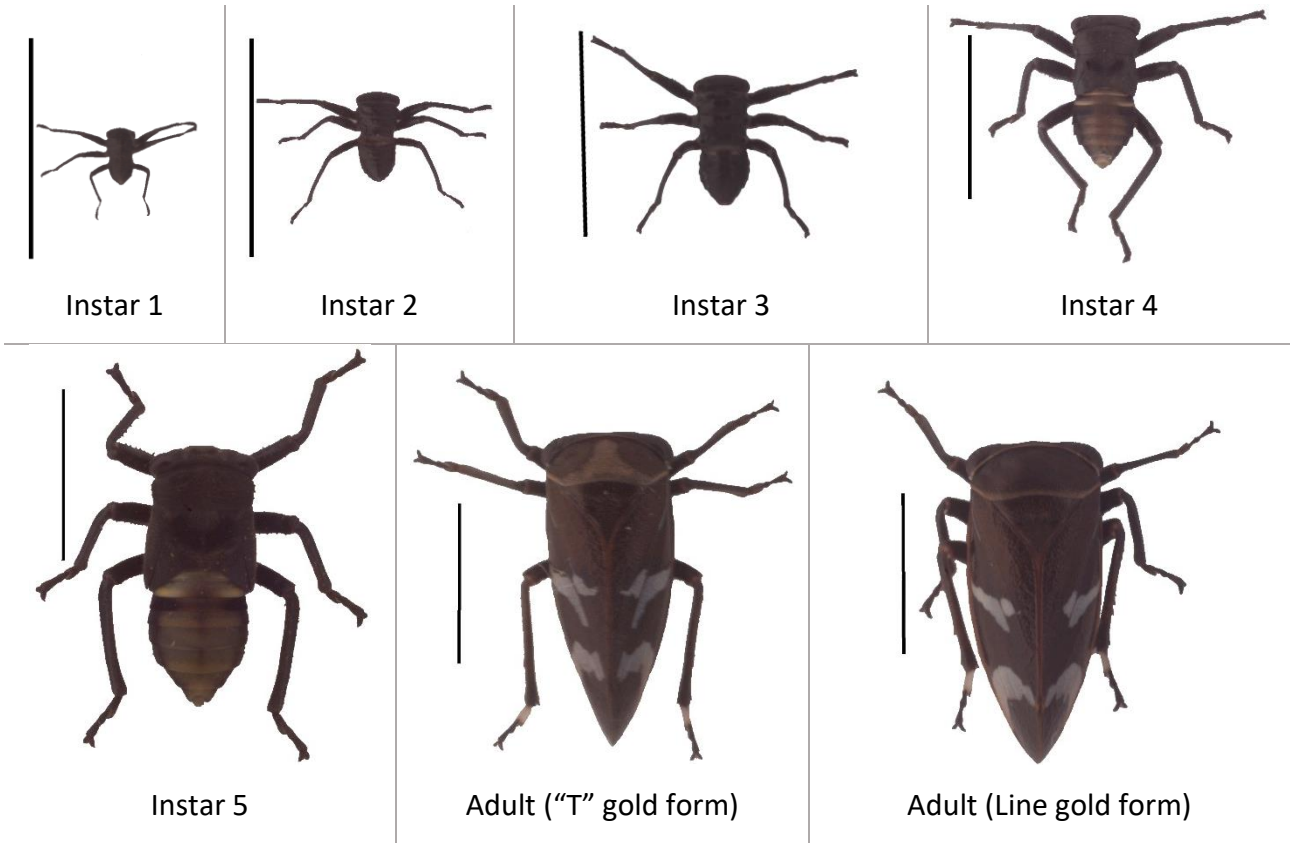

Figure 4. Images of all of *E. rubrolimbata's* life stages. Black line scales indicate 5 mm. Images not to the same scale.

(b) Body colour comparisons

Colour comparisons revealed that only abdomen colour varied between instars. Values of just noticeable differences (JND) calculated using an avian visual model indicate that the leafhoppers head, thorax and leg colour are comparable between instar stages 3, 4 and 5 and lack distinction (mean JND < 3) (figure 5). In comparison, an avian predator is predicted to be able to discriminate between the black abdomen of 3rd instars and the gold abdomen of 4th and 5th instars (figure 5). The mean JND comparisons of the 4th and 5th instars to the 3rd instar were between 2-3 times that of the discriminability threshold of 3. In contrast, the mean JND for 4th and 5th was right on the threshold (mean JND = 3.15) with an overlapping range suggesting the ability to discriminate the gold between the instar stages is variable (figure 5).

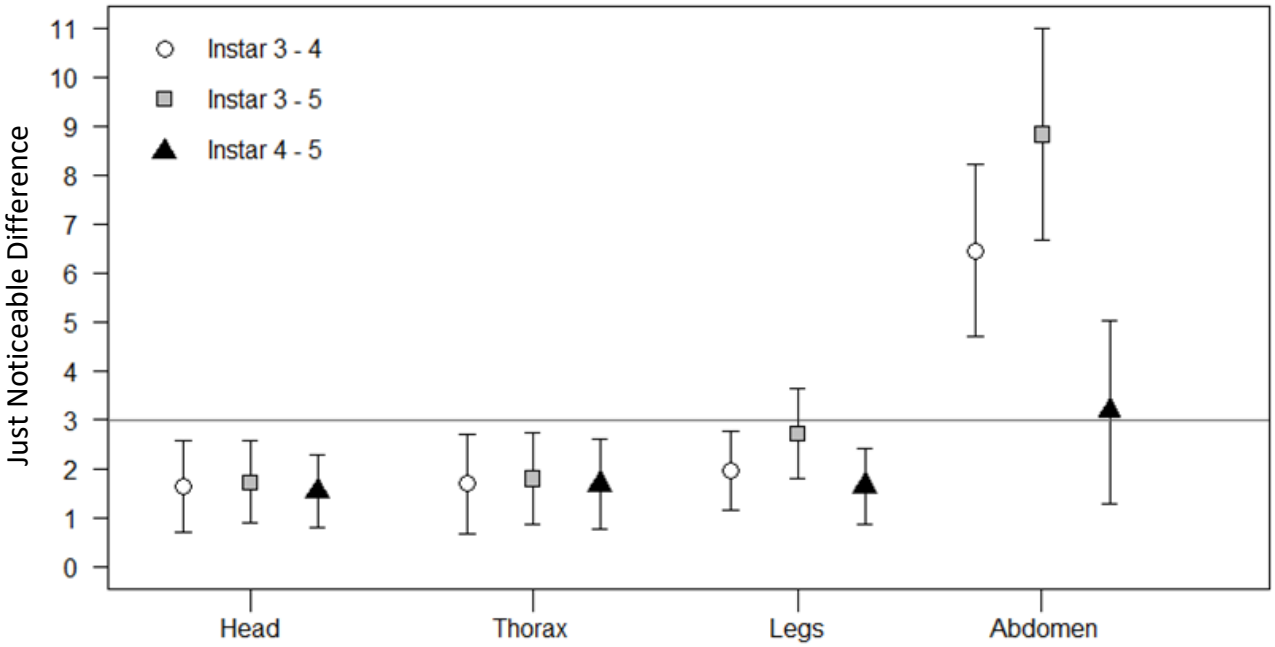

Figure 5. Colour comparisons between *E. rubrolimbata* instars presented as mean JND ± 1 SD. JND calculated for a blue tit visual model. Colour comparisons are between instars 3, 4 and 5 within four body regions: head, thorax, legs and abdomen. The horizontal line at 3 JND indicates the theoretical threshold below which two colours are difficult to distinguish.

Comparisons of *E. rubrolimbata* and *D. clarki* head, thorax and leg colour indicate that avian predators are unlikely to be able to distinguish between them (mean JND < 3) (Head: figure 6 - Thorax: figure 7 - Legs: figure 8). Linear mixed models revealed that several species have significantly smaller head, thorax and leg JND than *D. clarki* (Head: figure 6 - Thorax: figure 7 - Legs: figure 8) (appendix 2, 3, 4). However, considering mean JND values for *D. clarki* for these body regions are below the threshold of 3, avian predators are very unlikely to perceive these species as being noticeably more similar in colour to *E. rubrolimbata* than *D. clarki*. Indeed, many of the sampled species have black heads, thoraxes and legs like that of *E. rubrolimbata* instars and this is reflected in the number of species with JND values less than 3.

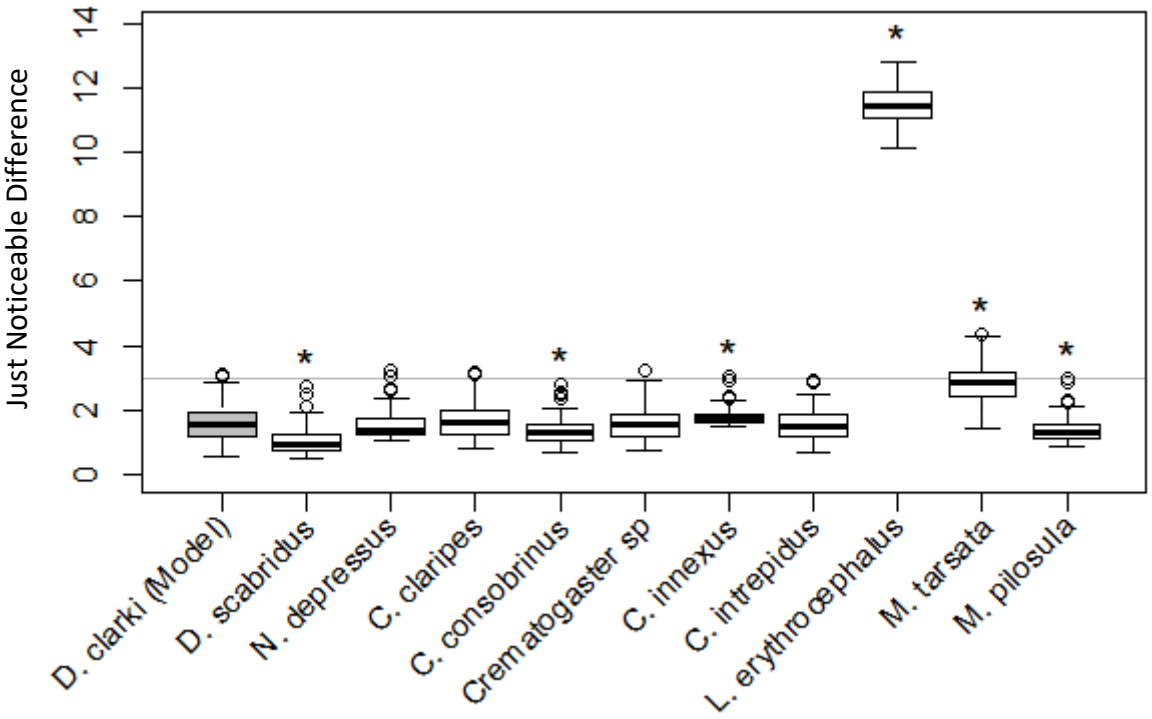

Figure 6. Boxplots comparing the head colour of 5th instar *E. rubrolimbata* to that of *D. clarki* (grey) and other species (white). JND calculated for a blue tit visual model. The horizontal line at 3 JND indicates the theoretical threshold below which two colours are difficult to distinguish. Species with JND significantly different to *D. clarki* (Linear mixed model: 95% confidence intervals do not include 0) are indicated with '*'.

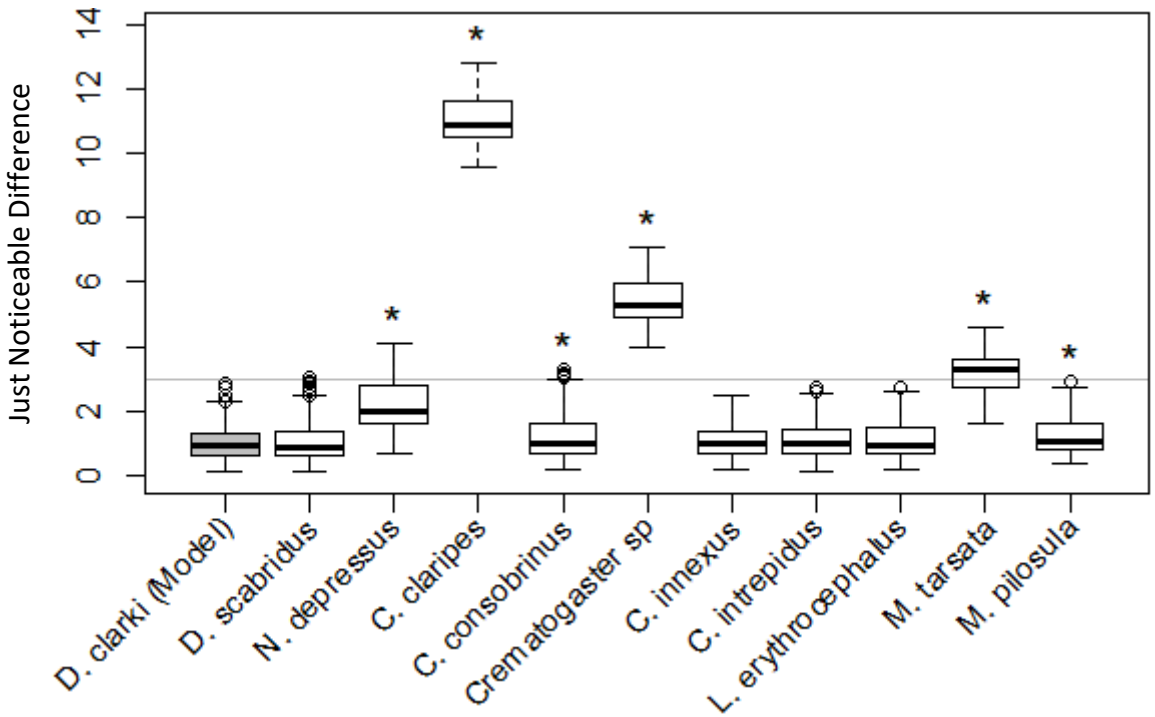

Figure 7. Boxplots comparing the thorax colour of 5th instar *E. rubrolimbata* to that of *D. clarki* (grey) and other species (white). JND calculated for a blue tit visual model. The horizontal line at 3 JND indicates the theoretical threshold below which two colours are difficult to distinguish. Species with JND significantly different to *D. clarki* (Linear mixed model: 95% confidence intervals do not include 0) are indicated with '*'.

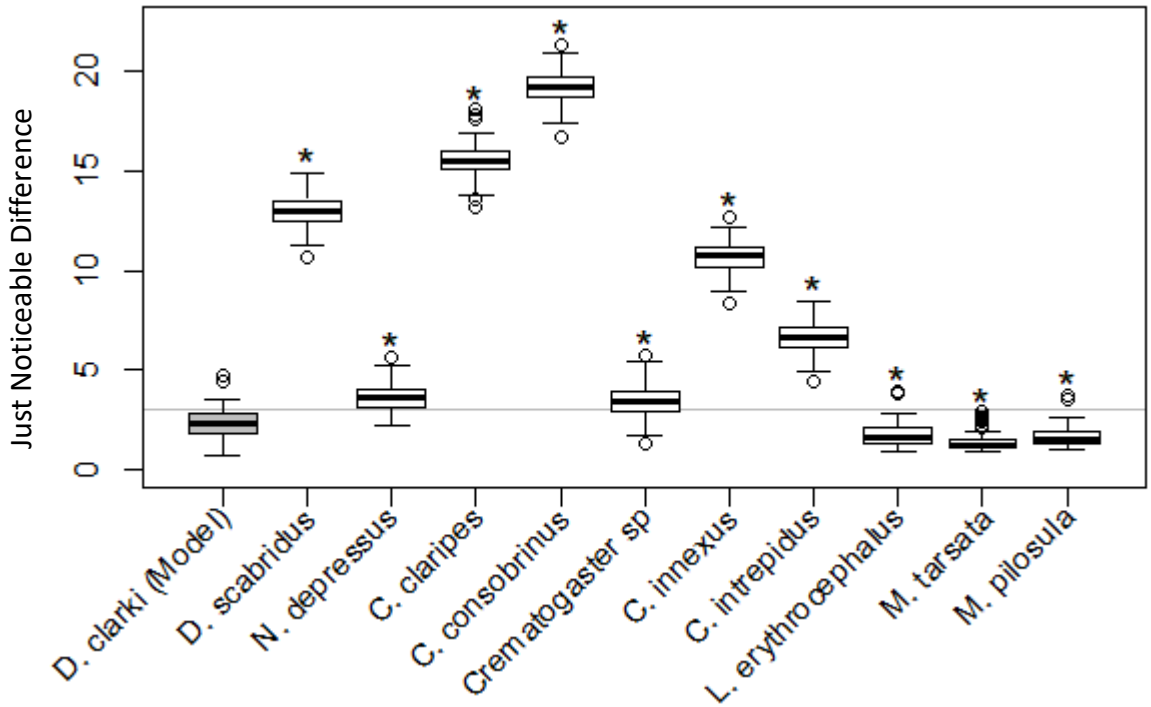

Figure 8. Boxplots comparing the leg colour of 5th instar *E. rubrolimbata* to that of *D. clarki* (grey) and other species (white). JND calculated for a blue tit visual model. The horizontal line at 3 JND indicates the theoretical threshold below which two colours are difficult to distinguish. Species with JND significantly different to *D. clarki* (Linear mixed model: 95% confidence intervals do not include 0) are indicated with '*'.

The colour of the abdomen of 3rd instars compared with *D. clarki* exceeded the discriminability threshold (mean JND = 4.92). In comparisons to other ants, the JND of 3rd instars for *D. clarki* were significantly greater than those of all other species (figure 9) (appendix 5). This suggests the 3rd instars are a closer match to all other species. There was greater comparability between the colour of the 4th and 5th instar abdomen with *D. clarki* with mean JNDs of 2.23, 4.09 respectively. The value for the 5th instars was larger than anticipated but the range of JNDs overlaps the threshold which is not the case for the 3rd instars. In addition, the abdomens of both the 4th instars (figure 10) and 5th instars (figure 11) were predicted to be more difficult to discriminate from *D. clarki* compared to other species (appendix 6). All ant species except *D. scabridus* had JNDs 2-4 times larger than those predicted for *D. clarki* relative to the 4th and 5th instar hoppers. *Dolichoderus scabridus* also share the similar colour arrangement and correspondingly the golden rump was also comparable to the 4th and 5th instars (figure 10, figure 11) along with low JND's for the head and thorax (figure 6, figure 7). However, *D. scabridus* leg JND are much higher than the threshold of 3 (mean JND = 12.95) due to the red legs of this species. Indeed, apart from body size the colour of the legs is a key distinguishing feature.

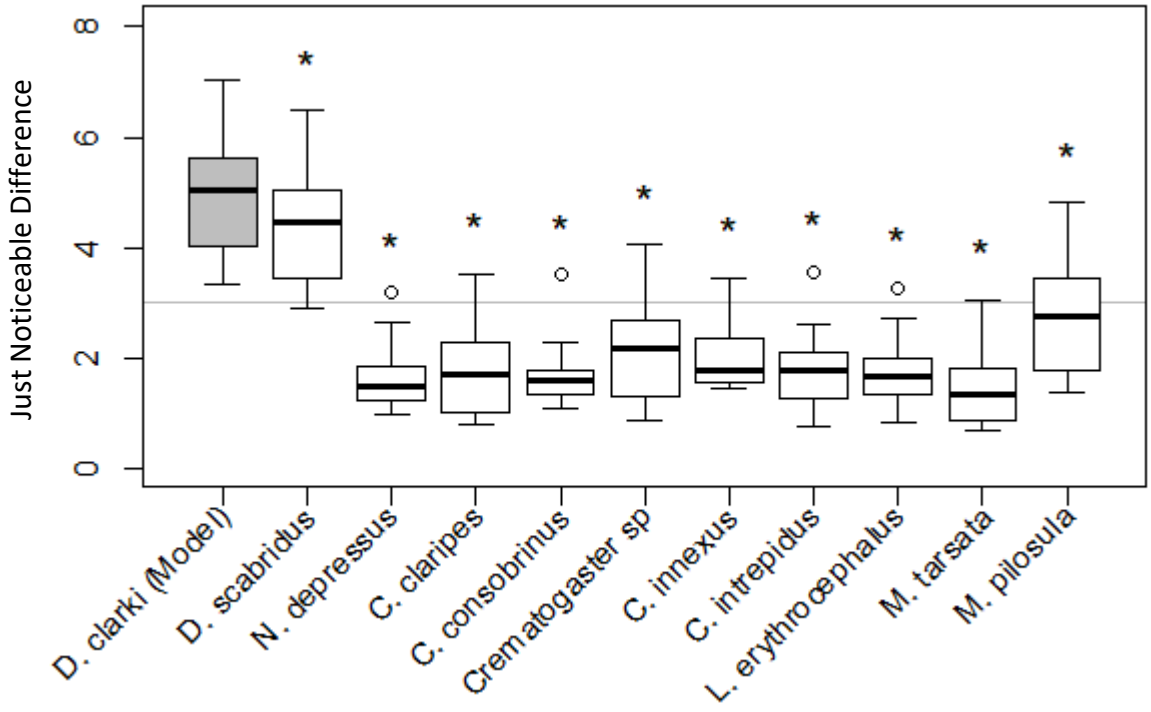

Figure 9. Boxplots comparing the abdomen colour of 3rd instar *E. rubrolimbata* to that of *D. clarki* (grey) and other species (white). JND calculated for a blue tit visual model. The horizontal line at 3 JND indicates the theoretical threshold below which colours are difficult to distinguish. Species with JND significantly different to *D. clarki* (lmm: CI does not include 0) are indicated with '*'.

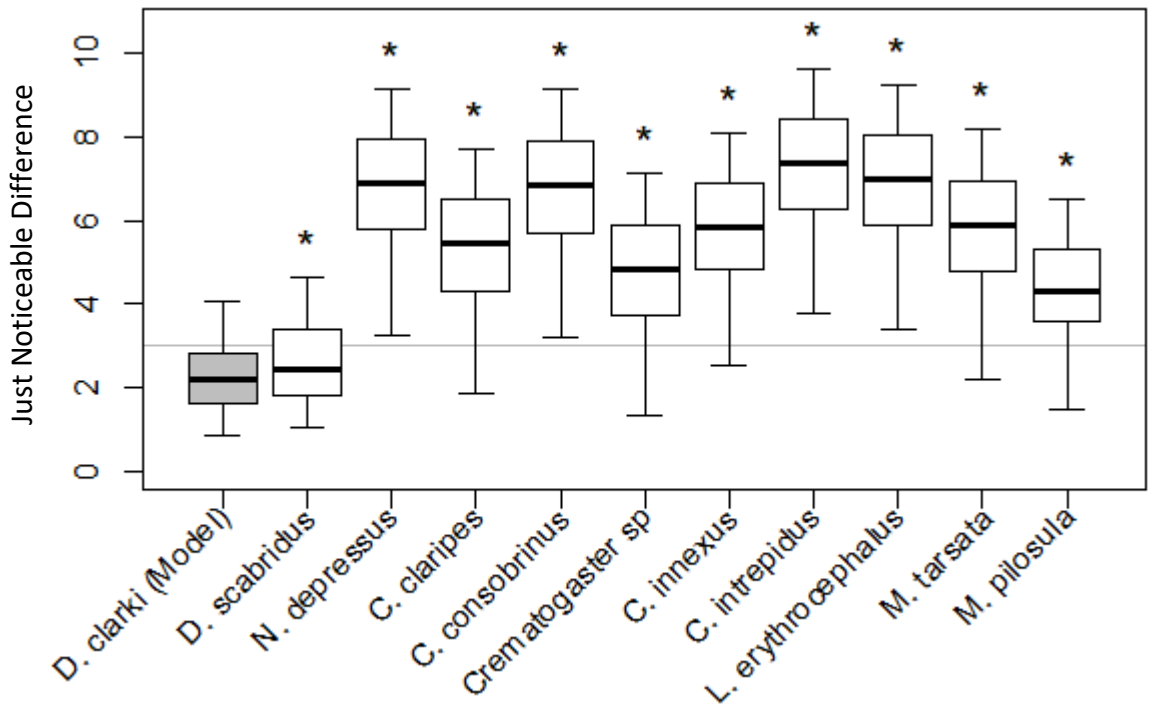

Figure 10. Boxplots comparing the abdomen colour of 4th instar *E. rubrolimbata* to that of *D. clarki* (grey) and other species (white). JND calculated for a blue tit visual model. The horizontal line at 3 JND indicates the theoretical threshold below which colours are difficult to distinguish. Species with JND significantly different to *D. clarki* (lmm: CI does not include 0) are indicated with '*'.

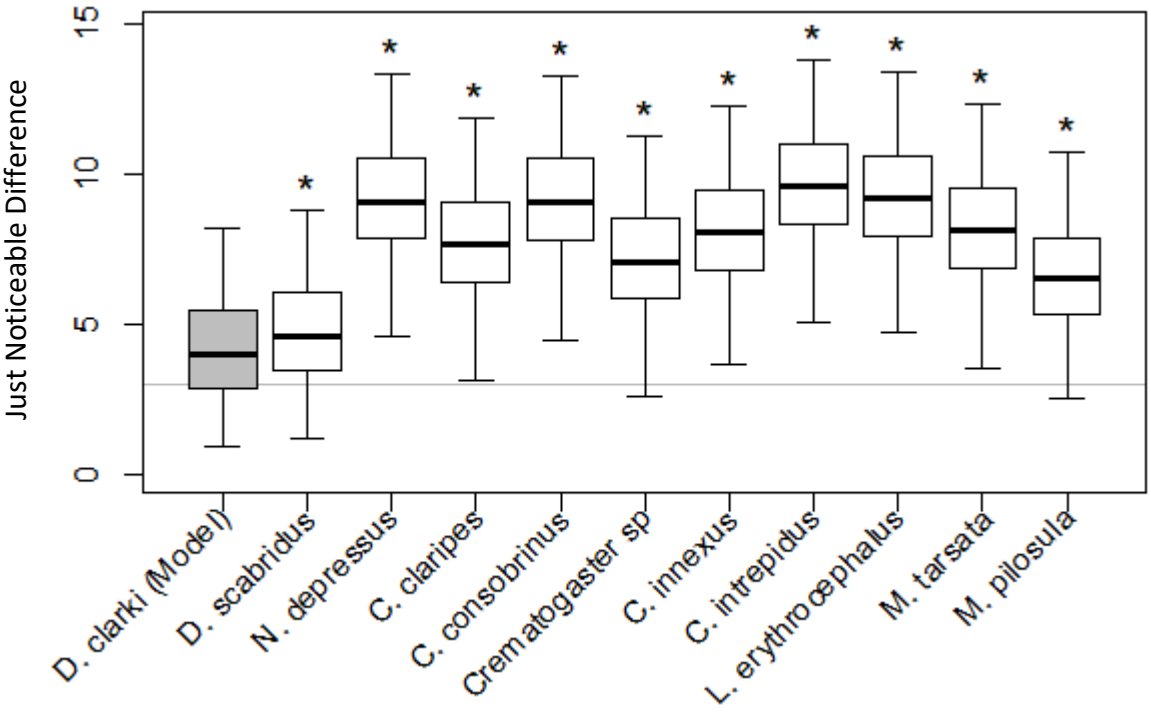

Figure 11. Boxplots comparing the abdomen colour of 5th instar *E. rubrolimbata* to that of *D. clarki* (grey) and other species (white). JND calculated for a blue tit visual model. The horizontal line at 3 JND indicates the theoretical threshold below which colours are difficult to distinguish. Species with JND significantly different to *D. clarki* (lmm: CI does not include 0) are indicated with '*'.

(c) Comparing *E. rubrolimbata* to the background

The perception of the body shape can be influenced by how body colour interacts with background colouration. Pale patches above the waistline of the hoppers extended to the edge of the body and intersect with the background (figure 2). This differs from the gold on the abdomen which is separated from the leafhoppers edge by a black outline around the abdomen. Based on JND, these pale waist patches are predicted to be harder to discriminate from pale bark than from dark bark by a factor exceeding 2 (table 1). The reverse is true for the leafhoppers black abdomen outline which had a lower JND on dark bark than on the pale bark (table 1). More significantly though, the edges at the pale patches are more difficult to discriminate from the pale bark than the edges of the abdomen's black outline. From a perception perspective, this potentially means that, on the pale bark, the pale patches are less defined and thus, less salient than the leafhoppers black outline.

Table 1. Colour comparisons between the two main background types of *E. rubrolimbata* and the leafhoppers pale waist patches, and the black outline around the abdomen. Mean ± SE of JND values calculated using the visual model of a blue tit.

Bark type	Pale bark (JND ± SE)	Dark bark (JND ± SE)
Pale Patches	4.85 ± 0.22	11.67 ± 0.25
Black outline	11.61 ± 0.14	4.53 ± 0.12

(d) Shape morphometrics

The first two principle component analyses accounted for over 89% of the variation (figure 12). The first axes described the overall 'stockiness' and the second axes reflected the shape of the abdomen relative to the constriction at the waist. MANOVA analyses incorporating 99% of the explained variation found that the body and abdomen shapes of all included species were significantly different (Body Shape: $F_{(19)} = 303.83$, $p < 0.001$: Abdomen Shape: $F_{(16)} = 124.16$, $p < 0.001$). This is not surprising as the analyses does not account for the visual system of the viewer and is much more sensitive to differences. Instead, relative differences between species are important for interpreting shape similarity. All instar body shapes, without consideration of how the colours interact with the background, cluster nearer the true bug *N. depressus* than any of the ants (figure 12). Euclidean distance measures indicate that all *E. rubrolimbata* resemble *N. depressus* more so than the ants when all PCA axes are taken into account (table 2). This is because the instars are much broader than the ants, which have a thinner mid-section.

Table 2. Mean Euclidean distances in body shape morphospace. Distances are between *E. rubrolimbata* instars and *Dolichoderus* ants, adult *E. rubrolimbata*, *N. depressus* and the nearest non-*Dolichoderus* ant species *I. calvus* (other ants in appendix 8). An (a) refers to whole body shape while a (b) refers to body shape following pale patch removal.

Species	Instars						
	1	2	3	4(a)	4 (b)	5(a)	5 (b)
Adult *E. rubrolimbata*	0.079	0.087	0.092	0.082	0.199	0.065	0.210
N. depressus	0.172	0.165	0.172	0.160	0.257	0.143	0.277
D. clarki	0.318	0.306	0.286	0.306	0.288	0.344	0.301
D. scabridus	0.316	0.302	0.280	0.301	0.273	0.341	0.289
I. calvus	0.296	0.289	0.271	0.291	0.316	0.329	0.330

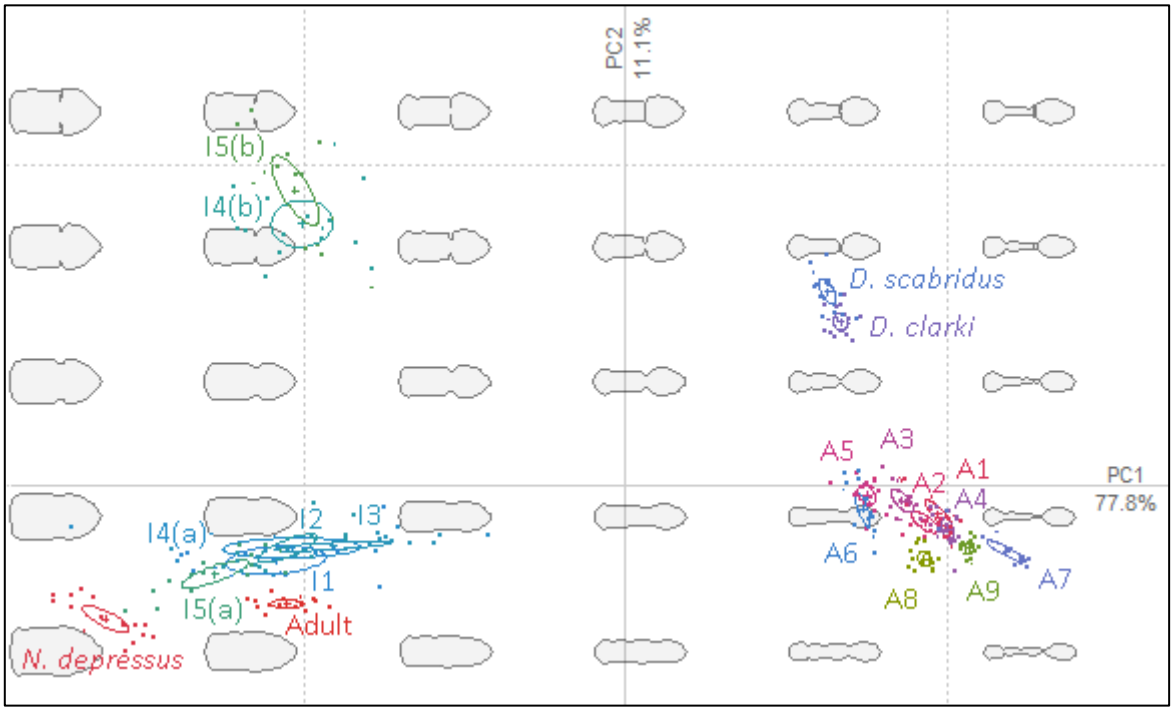

Figure 12. Principle component analyses generated using output from a 20 harmonic, Elliptical Fourier analysis on body shapes. Changes along axes in morphospace are indicated by the illustrations. The two axes which describe the greatest amount of variance in body shapes are shown. 'Adult' refers to adult *E. rubrolimbata*. I1, I2, I3, I4(a) and I5(a) refer to *E. rubrolimbata* instar stages 1-5. I4(b) and I5(b) refer to *E. rubrolimbata* instar stages 4 and 5 after pale patch removal. 'A#' refers to non-*Dolichoderus* ants. 'A1': *C. claripes*. 'A2': *C. consobrinus*. 'A3': *C. innexus*. 'A4': *C. intrepidus*. 'A5': *Crematogaster sp*. 'A6': *I. calvus*. 'A7': *L. erythrocephalus*. 'A8': *M. pilosula*. 'A9': *M. tarsata.*

When the pale patches of instars 4 and 5 are excluded, leafhopper body shape in morphospace shifts significantly closer to that of *D. clarki* (4th instars: $t_{(14)} = 11.187$, 95% CI = 0.0126 to 0.0186, $p < 0.001$; 5th instars: $t_{(14)} = 4.362$, 95% CI = 0.0195 to 0.0574, $p < 0.001$)(table 2). Based on the two PCA axes explaining the greatest amount of shape variation, this shift appears to be along the PC2 axis which encodes a distinct waist (figure 12). *Dolichoderus* species also appear to separate from the other ants along the PC2 axis due to their more distinct waist (figure 12).

When only the abdomen was considered which contains the gold colouration, 82.1% of the variation was explained by the first two PCA axes. The abdomen shape of 4th and 5th instars overlapped in morphospace with the two *Dolichoderus* species. They did not overlap with either *N. depressus* or the other ants (table 3, figure 13). This trend appears to be primarily due to the wider and less rounded anterior section of the abdomen as described by the second PCA axis (figure 13).

When only the shape of the gold signal itself was considered, both 4th and 5th instars moved significantly further away from *D. clarki's* abdomen shape in morphospace (4th instars: $t_{(14)} = -8.026$, 95% CI = -0.0831 to – 0.0480, $p < 0.001$; 5th instars: $t_{(14)} = -15.482$, 95% CI = -1.052 to -0.796, $p < 0.001$) (table 3, figure 13).

Table 3. Mean Euclidean distances in abdomen shape morphospace. Distances are between gold-bearing *E. rubrolimbata* instars and *Dolichoderus* ants, adult *E. rubrolimbata*, *N. depressus* and the nearest non-*Dolichoderus* ant species, I*. calvus* (other ants in appendix 9). An (a) refers to visible abdomen shape while a (b) refers to the shape of only the gold region.

Species	Instars			
	I4 (a)	I4 (b)	I5 (a)	I5 (b)
Adult *E. rubrolimbata*	0.282	0.295	0.293	0.369
N. depressus	0.157	0.176	0.192	0.200
D. clarki	0.040	0.113	0.068	0.193
D. scabridus	0.042	0.118	0.070	0.192
I. calvus	0.096	0.174	0.100	0.256

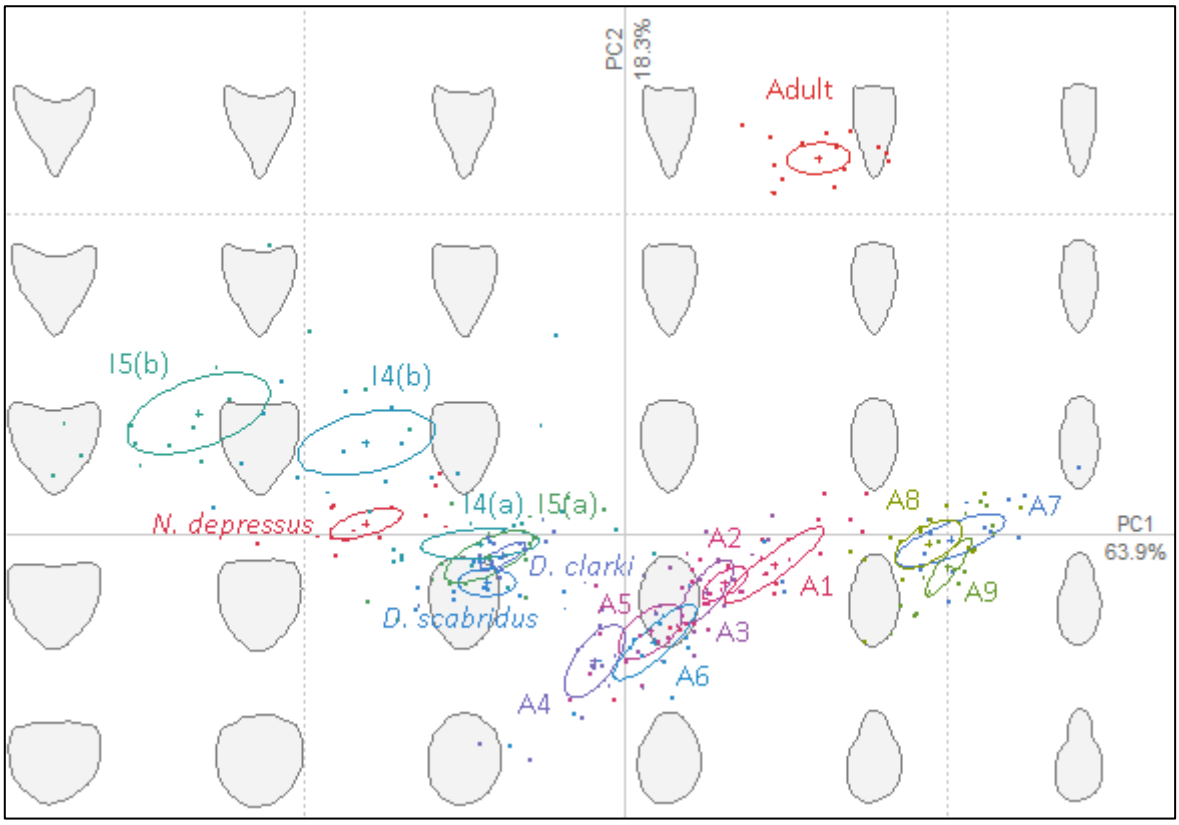

Figure 13. Principle component analyses generated using output from a 20 harmonic, Elliptical Fourier analysis on abdomen shapes. Changes along axes in morphospace are indicated by the illustrations. The two axes which describe the greatest amount of variance in body shapes are shown. 'Adult' refers to adult *E. rubrolimbata*. I1, I2, I3, I4(a) and I5(a) refer to *E. rubrolimbata* instar stages 1-5. I4(b) and I5(b) refer to *E. rubrolimbata* instar stages 4 and 5 after pale patch removal. 'A#' refers to non-*Dolichoderus* ants. 'A1': *C. claripes*. 'A2': *C. consobrinus*. 'A3': *C. innexus.* 'A4': *C. intrepidus*. 'A5': *Crematogaster sp*. 'A6': *I. calvus*. 'A7': *L. erythrocephalus*. 'A8': *M. pilosula*. 'A9': *M. tarsata.*

(e) Size morphometrics

The body length of the all black instars 1 to 3 were substantially smaller than all ant species except for *I. clavus* and *Crematogaster sp.* (figure 14). The adult body size varied significantly from all ants and none of its body range overlapped with that of the gold-bearing ants. In comparison to *D. clarki,* the suspected ant model, the adult was substantially larger at roughly 1.3 times the size. The gold-bearing 4th instar was significantly smaller than both *D. scabridus and D. clarki* ($p < 0.05$) (Stats: mean ± Se - 4th instar: n = 46, 4.853 ± 0.075 *cf D. clarki*: n = 30, 7.509 ± 0.041 - *D. scabridus*: n = 30, 6.235 ± 0.0412) but the upper range did overlap with that of *D. scabridus*. In comparison, the 5th instar body size encompassed the mean of both the *D. scabridus* and *D. clarki* with the mean closer to that of *D. clarki* than to *D. scabridus* (5th instar: n = 83, 6.905 ± 0.073). Although comparable, the body lengths were significantly different ($Z= -3.995$, $p < 0.001$) which probably

reflects the larger variation in leafhopper body sizes. In comparison to other ant species only *Camponotus claripes* (Z = -1.071, p = 0.283) and *Camponotus innexus* (Z = -5.336, p < 0.001) (figure 14 were comparable in size all other ants were substantially smaller or larger.

In terms of the entire abdomen area, only the gold-bearing instars had overlapping range sizes with the gold-bearing ants (figure 15). In fact, the 4th instar abdomen area was not significantly different from that of *D. scabridus* (Z = 0.227, p = 0.817) despite being smaller on average in body length. Abdomen area range of the 5th instars, like body length, encompassed both the mean of both *D. scabridus* and *D. clarki* and again was closer to *D. clarki* but still significantly different (Z = -4.29, p < 0.001). The abdomen area was not significantly different from *C. consobrinus* (Z = 1.663, p = 0.090), *C. intrepidus* (Z = -1.170, p = 0.257) and *M. pilosula* (Z = 1.081, p = 0.277) (figure 15). The range for the 5th instars was massive compared to the ants. When only the region of gold was considered both the 4th and 5th instars displayed significantly less gold than the *D. scabridus* and *D. clarki* respectively (p < 0.05) (figure 16). The gold region of adult *E. rubrolimbata* was significantly smaller than that of both gold-bearing *Dolichoderus* ants (p < 0.05). However, adults with gold in the 'T' form had gold size range overlap with *D. scabridus*.

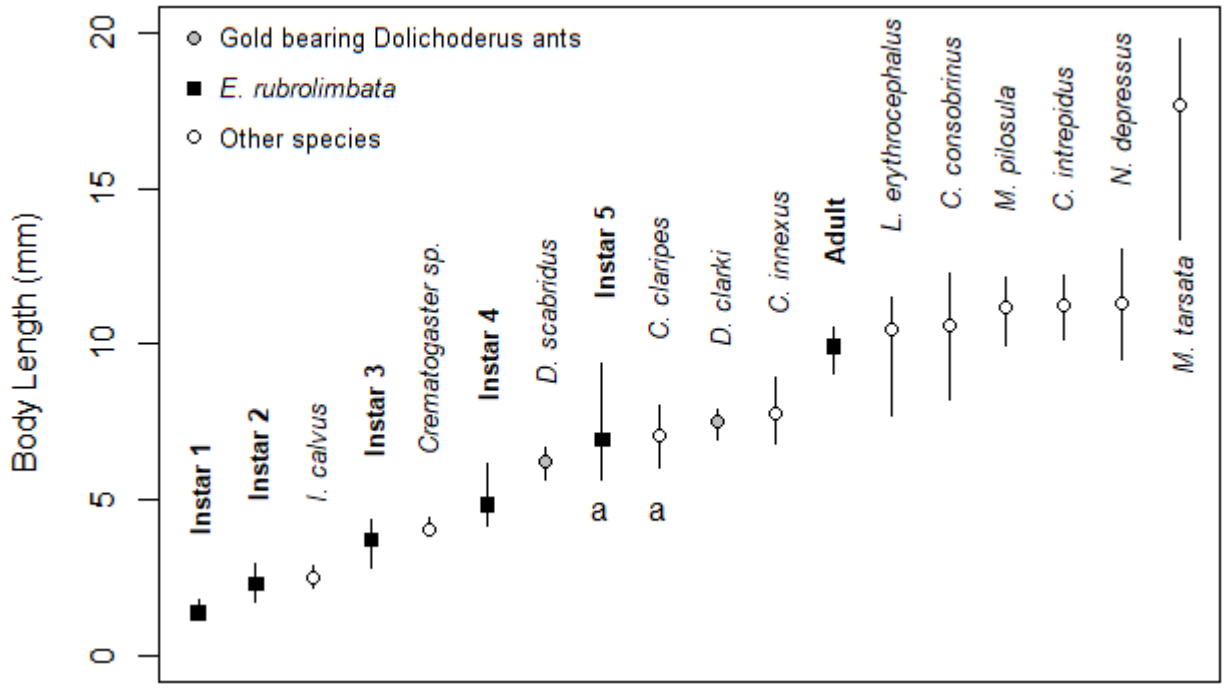

Figure 14. Ordered body lengths (mean and range) for *E. rubrolimbata*, *N. depressus* and ant species. 'Instar #' and 'Adult' relate to *E. rubrolimbata* life stages. Data points with the same letter underneath indicate non-significant differences from *E. rubrolimbata* (permutations test).

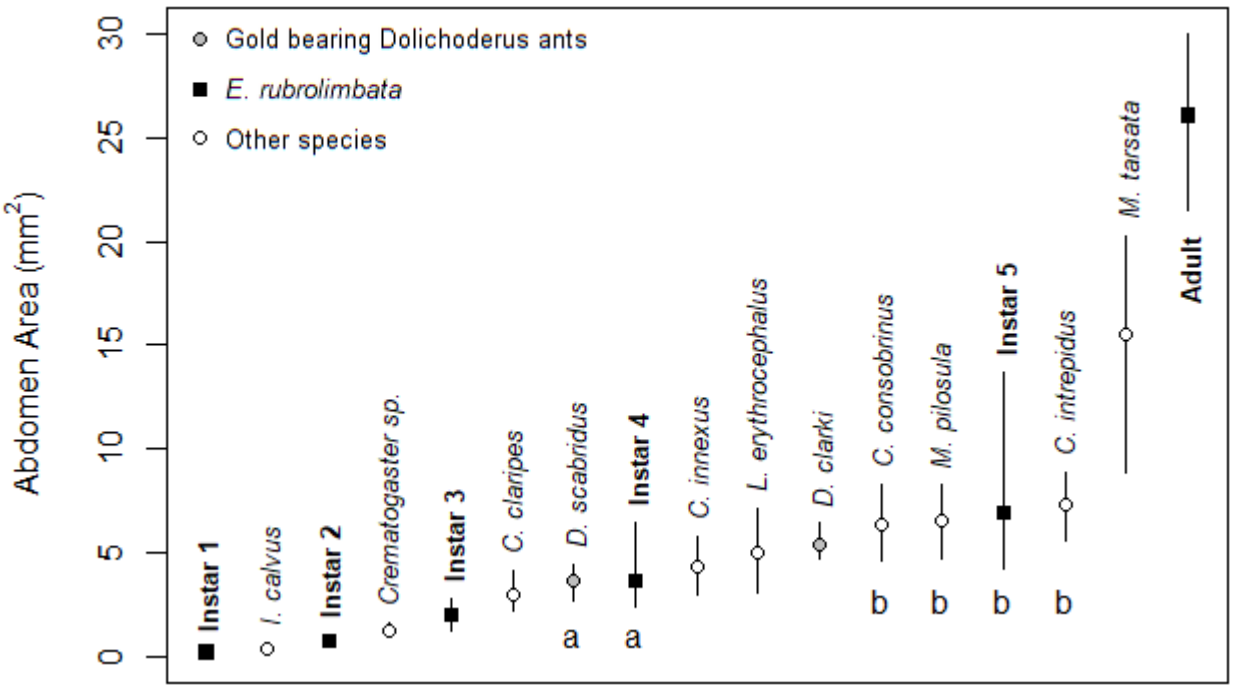

Figure 15. Ordered abdomen areas (mean and range) for *E. rubrolimbata* and ant species. *N. depressus* is excluded due to its much larger abdomen area. 'Instar #' and 'Adult' relate to *E. rubrolimbata* life stages. Data points with the same letter underneath indicate non-significant differences from *E. rubrolimbata* (permutations test).

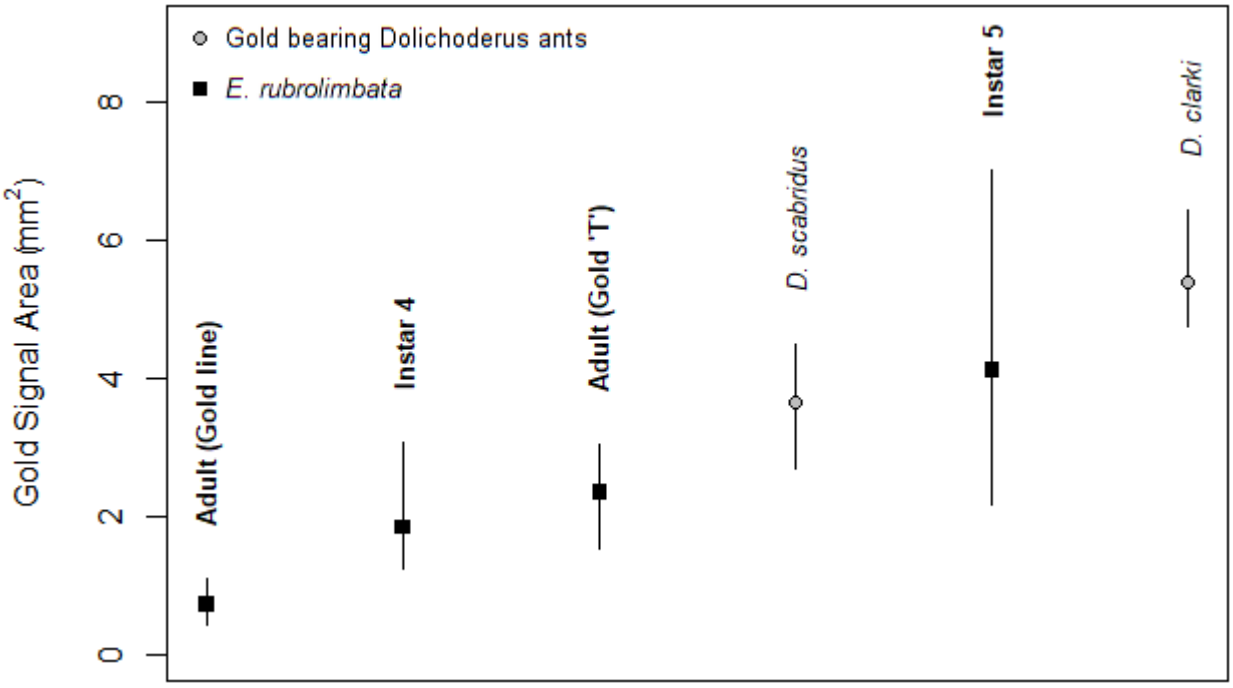

Figure 16. Ordered gold signal area (mean and range) for *E. rubrolimbata* and *Dolichoderus* ants. 'Instar #' relates to *E. rubrolimbata* life stages while 'Adult' relates to *E. rubrolimbata* adults. For *E. rubrolimbata* adults, the two different gold signal forms are included separately. Data points with the same letter underneath indicate non-significant differences (permutations test).

(f) Movement comparisons

I found that gold-bearing 4th instar *E. rubrolimbata* ran up the tree at absolute speeds statistically similar to 5th instars (t-test: $t_{(49)}$ = 0.277, 95% CI = -5.5082 to 7.2721, p = 0.783) (figure 17 A). Direction change was also significantly similar for the two gold bearing instars (t-test: $t_{(49)}$ = 0.358, 95% CI = -0.4138 to 0.5932, p = 0.722) (figure 19). For the 4th instars to move up the tree at the same absolute speed as the 5th instars they had to move significantly faster relative to their body length (4th instar: 12.01 ± 0.5 *cf* 5th instar: 8.54 ± 0.3 SE, t-test: $t_{(49)}$ = -6.3716, 95% CI = -4.5758 to -2.3815, p < 0.001) (figure 17 B). Regression models revealed no significant difference between the movement speed of 4th instar leafhoppers and that of *D. clarki* (t = -1.759, p = 0.160) or adult *E. rubrolimbata* (t = 0.015, p = 0.988). The 4th instars moved significantly faster than all other species (p < 0.05) (figure 18). In comparison, 5th instars moved at a significantly similar speed to the adult leafhoppers (t = -0.326, p = 0.744) but significant faster than all other species (p < 0.05) (figure 18). However, 5th instars move only marginally significantly faster than *D. clarki* (t = -2.264, p = 0.049). Both 4th and 5th instars displayed angle changes significantly similar to that of adult *E. rubrolimbata*, *D. clarki* and *M. pilosula* (p > 0.05). In comparison, *N. depressus* changed angle significantly less while *I. calvus* and the Crematogaster sp. change angle significantly more (p < 0.05) (figure 20).

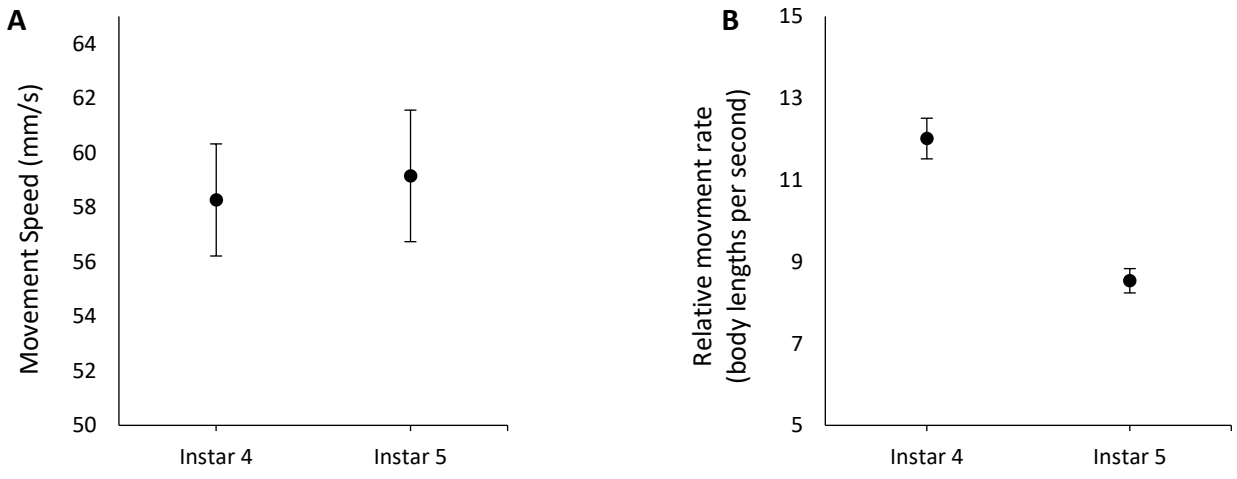

Figure 17. Movement comparisons (mean ± SE) between gold-bearing *E. rubrolimbata* instars 4 and 5 traveling up *E. viminalis* tree trunks. For movement speed (mm/s) (A) the two instar stages moved at the same speed. For relative movment speed (bodylengths/s) (B) 4th instars moved significantly faster relative to their body length.

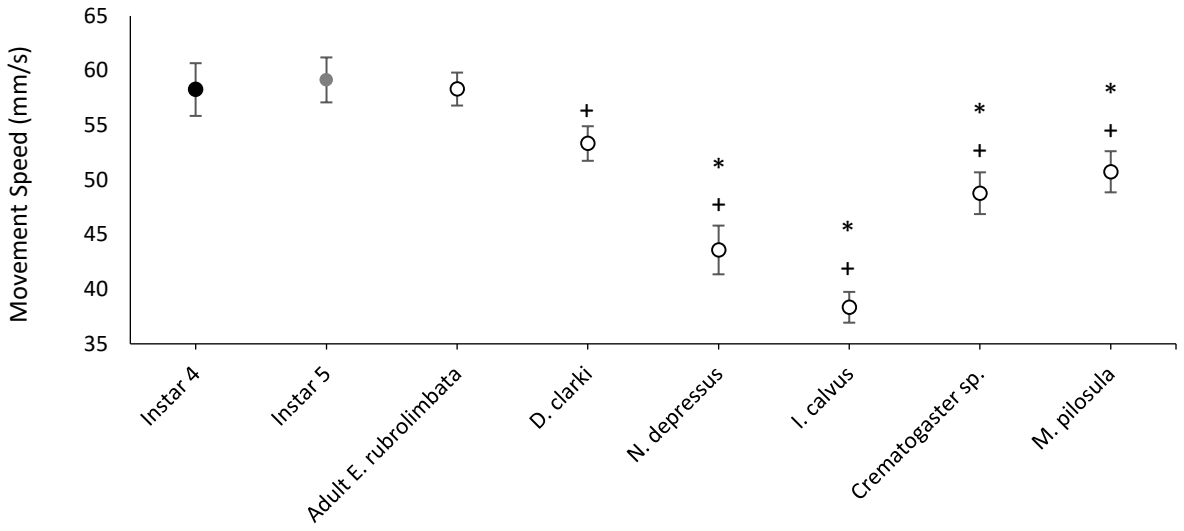

Figure 18. Movement speed (mm/s) (mean ± SE) of gold-bearing *E. rubrolimbata* instars 4 (black) and 5 (grey), adult *E. rubrolimbata*, *N. depresses* and ant species (white) traveling up tree trunks. Groups which moved at a significantly different speed to 4th instars are indicated with '*' while significant differences to 5th instars are indicated with '+'.

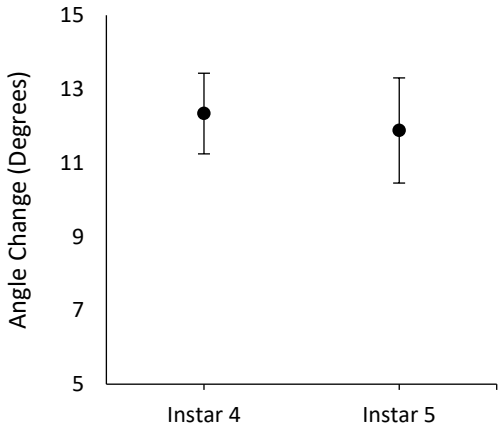

Figure 19. Angle change comparisons (mean ± SE) between gold-bearing *E. rubrolimbata* instars 4 and 5 traveling up *E. viminalis* tree trunks. The degree of turning was equivelent for the two instar stages moved at the same speed.

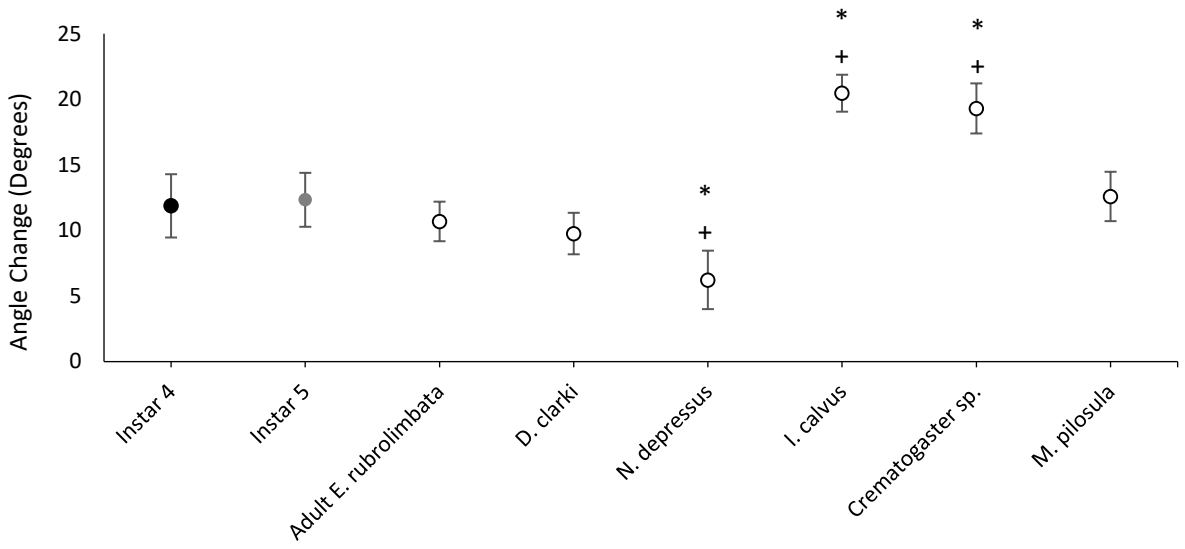

Figure 20. Angle change (mean ± SE) of gold-bearing *E. rubrolimbata* instars 4 (black) and 5 (grey), adult *E. rubrolimbata*, *N. depresses* and ant species (white) traveling up tree trunks. Groups with which exhibited significantly different amounts of angle change compared to 4th instars are indicated with '*' while significant differences to 5th instars are indicated with '+'.

4. Discussion

I investigated mimetic resemblance between juvenile *Eurymela rubrolimbata* leafhoppers and their attendant ants, *Dolichoderus clarki*. For this research, I took a multiple trait approach and assessed how visual cues changed with respect to developmental stage and environmental context. This revealed that *E. rubrolimbata* resemble *D. clarki* in colour and movement more so than the leafhoppers resemble other ants or the typical bug, *N. depressus*. Colour resemblance occurred in developmental stages that showed size range overlap with *D. clarki* or *D. scabridus*, another gold-bearing ant species. This suggests that colour characteristics evolved in reference to ant mimicry. Body shape resemblance is common in ant mimicry, but *E. rubrolimbata* did not resemble *D. clarki's* shape or that of other ant species. However, when considered in a natural context using the modelled perception of a predator, the resultant body shape shifted towards that of the leafhoppers ant associates. Hence, I conclude that *E. rubrolimbata* mimics the general ant gestalt of *D. clarki,* with mimetic accuracy varying between different traits. This outcome positions *E. rubrolimbata* as the only documented trophobiont to mimic its attendant ants.

Colour is typically the most salient visual cue that predators use to discriminate palatable and unpalatable prey [10, 115, 124]. Consequently, much focus in the mimicry literature has been placed on mimics resembling the colour and pattern of their model [21, 22, 125-128]. I found that,

with respect to an avian predator's visual sensitivities, *E. rubrolimbata's* gold and black colouration was similar to that of *D. clarki*. Of these two colours, gold is likely more salient as predators predominantly respond to bright signals [114, 124, 129]. Previous predator-prey trials, using members of the golden mimicry complex, have demonstrated that the coverage of gold has a positive relationship with survivability [16]. Thus, I presume that *E. rubrolimbata* displays gold for defensive purposes rather than for physiological benefits such as thermoregulation [130, 131]. While the colour of *E. rubrolimbata* juveniles and *D. clarki* was similar, colour mimicry was not always totally accurate.

Visual modelling indicates that the gold of 5th instar *E. rubrolimbata* can be distinguished from that of *D. clarki* but it is unknown whether this is biologically relevant. While the threshold of discrimination I applied has been used in previous studies, it may be overly conservative. When viewing prey, predators must deal with movement, varying lighting conditions and distance to their target [109]. Additionally, after consuming distasteful prey, avian predators have been identified to generalise colours, leading to increased levels of aversion towards similar hues [132, 133]. Thus, even though my results suggest discrimination is theoretically possible, this may not be reflected in a natural setting. Alternatively, features of colour production may facilitate marginally discriminable colouration.

To produce a golden signal, *E. rubrolimbata* use pigments while *D. clarki* possess hairs which generate a golden sheen [16]. Differences in colour production method have been identified as possible reasons for inaccurate colour mimicry [126] although in this system, *D. clarki's* sheen may degrade the importance of such differences. Objects with a sheen can shift in hue and brightness when viewed from different angles or under different illumination conditions [134-136]. Without being able to develop a strict discrimination rule set due to colour variability, predators may avoid a wider range of golden colours [126, 137]. Hence, discrimination between the gold of 5th instars and *D. clarki* may not occur. If this is the case it may also explain why 5th instars do not display more accurate colour mimicry despite the leafhoppers possessing the capability to do so as shown by the 4th instars. A lack of discrimination between colours does not confirm mimicry alone. Many animals exploit colours and patterns that infer unpalatability without resembling any particular model [10]. For ant mimicry, it is the placement of mimetic colouration and the size of instars which display it that is arguably more informative.

Patterns and colour distribution that resemble that of a model species are key features in mimetic displays [10, 138, 139]. For example, many butterflies form Batesian and Mullerian mimicry

complexes where mimics and models share not only the same colours but also colour patches in similar locations [126, 140, 141]. Regarding *E. rubrolimbata*, the juvenile's gold and black colouration directly aligns with that of *D. clarki*. In comparison, the non-mimetic adult leafhoppers possess gold on their pronotum. This firstly suggests the gold itself is important beyond the mimetic developmental stage; but secondly, it indicates that gold is not physiologically constrained to being displayed on the abdomen. Indeed, as signal strength increases with signal size [142], if gold was not being displayed for ant mimicry it may be expected on the pronotum and the abdomen to increase size and effect.

For mimicry to be successful, the mimic needs to be of comparable size to the model. This is evident in transformational mimics which switch to new models after outgrowing the original [18, 31, 143, 144]. As mimetic colouration is not ubiquitous across *E. rubrolimbata's* life stages it may be constrained to instars which are close enough in size to *D. clarki* for mimicry to be a successful defence strategy. Initially, this appears to be the case as only the two largest instars, which are closest in size to *D. clarki*, display gold colouration. However, while the 5th instars overlap *D. clarki's* size range in body length, abdomen area and signal size, the 4th instars do not. This indicates that mimetic accuracy likely varies between the two gold-bearing instars.

Mimicry may persist in 4th instar *E. rubrolimbata* despite size inaccuracy due to relaxed selection. In a study on hoverflies, Penny et al (2012)[145] found a positive relationship between hoverfly body size and mimetic accuracy to wasps. From this, the authors proposed that smaller hoverflies were lower value prey items and hence were sampled less, resulting in relaxed pressure to obtain more accurate mimicry. A comparable relationship may also be occurring in *E. rubrolimbata*. If 4th instars experience less sampling pressure due to their size, then even inaccurate size mimicry may provide them with protection from predators. However, the interaction between size based sampling pressure and mimetic accuracy requires further exploration as a more recent hoverfly study found no relationship between size and mimetic accuracy [146].

The smaller size of 4th instars may alternatively be facilitated by the presence of *D. scabridus* within the leafhoppers local environment. *Dolichoderus scabridus* possess a gold abdomen and are smaller than *D. clarki*. By being present in the leafhoppers environment, although not on the same tree, *D. scabridus* may widen the size range over which golden ant mimicry is effective. Indeed, body size of 4th instars consistently overlapped that of *D. scabridus.* Body size metrics of gold-bearing instars resembled that of several non-*Dolichoderus* ant species. However, given the

considerable colour differences between *E. rubrolimbata* and these ants, I propose that these similarities are coincidental rather than examples of deliberate size mimicry.

In addition to creating a strong mimetic similarity, colour can improve mimic – model resemblance in body shape. A characteristic ant shape is regularly observed in mimics [13, 18, 28, 147], particularly in spiders which have evolved constricted body regions [21]. In *E. rubrolimbata*, overall body shape does not resemble that of *D. clarki* or other ants, being much more like that of *N. depressus*. However, I also investigated the notion that ant shape may be generated by a colour illusion rather than physical modifications. This concept has been suggested for other ant mimics [13, 21] but has not been tested experimentally or explored considering a predator's visual model as I did here. Compared to previous camouflage research [105-107], the colour difference between *E. rubrolimbata's* black colouration and the dark bark of *E. viminalis* is considerably low. Hence, body shape is unlikely to be a salient trait for discrimination in this context. In comparison, the leafhopper's black colouration is distinct from the pale bark, allowing predators to perceive body shape [148, 149]. On the pale bark, the leafhoppers waist patches are much less distinct from the background than the rest of *E. rubrolimbata's* outline is. Hence, these patches may act as disruptive colouration [10, 148, 150], causing predators to perceive *E. rubrolimbata* as possessing a constricted waist. Shape analysis suggests that removal of these patches makes *E. rubrolimbata's* shape more comparable to that of *D. clarki* by shifting the leafhoppers along the PCA axis encoding a distinct waist. This axis also distinguishes *Dolichoderus* ants from the other ants, potentially highlighting the value for a constricted waist in *E. rubrolimbata's* mimicry display. Indeed, abdomen shape analysis indicates that *E. rubrolimbata* could possess a gold signal more closely resembling that of *D. clarki's* gold abdomen if the waist patches were not present and the entire abdomen was gold. This suggests that the waist patches are a particularly important feature. However, even after accounting for background interactions, *E. rubrolimbata* is an inaccurate shape mimic. This suggests body shape mimicry is not as important in *E. rubrolimbata's* mimicry system as it is in others.

Accurate body shape mimicry does not appear to be important for *E. rubrolimbata*, possibly due to salient colour features or movement. Body shape is likely a salient cue used by predators to identify ants as many ants do not possess distinct colouration. However, when colour and shape traits are available to distinguish prey items, predators have been identified to favour colour [115, 124]. By mimicking an ant with distinct aposematic colouration, *E. rubrolimbata* may be under reduced pressure to develop an ant-like shape. Indeed, spiders mimicking ants with distinct

colours have been found to lack the constricted body regions of other ant mimicking spiders [22]. However, examples also exist of ant mimics resembling both the vibrant colours and characteristic shape of their model [16]. An alternative explanation for *E. rubrolimbata's* low shape accuracy is the context in which shape is visible to predators.

The leafhoppers shape is likely to be most readily visible while traveling along the pale trunk, the region which facilitates a constricted waist illusion. When not moving along the trunk, *E. rubrolimbata* forms aggregations with other conspecifics and attendant ants, likely making shape difficult to interpret, particularly in the dark bark where the leafhoppers outline is less distinct from the background. However, while moving on the pale bark, shape may still be difficult to interpret due to the very act of moving. Indeed, movement has been proposed to reduce the ability of predators to accurately interpret shape characteristics [109, 151]. Hence, *E. rubrolimbata's* rapid movement on the pale trunk may reduce the need to develop more accurate shape mimicry.

Movement can influence mimicry beyond reducing the need for an accurate body shape as behavioural resemblance is regularly described in ant mimicking species [13, 22, 30, 152]. Manipulative experiments have shown the behavioural profile of an ant mimic is important for duping predators [29]. Gold-bearing instars moved at a speed comparable to *D. clarki*, faster than the larger bug *N. depressus* and the other ants assayed, suggesting movement mimicry. While 5th instars moved marginally faster than *D. clarki*, this slightly faster speed may serve to improve predator deterrence by portraying an ant which is more agitated and potentially aggressive [153, 154]. Interestingly, the 4th instars achieved *D. clarki*-like speed by moving faster relative to their body length than 5th instars, potentially indicating speed is mediated to better reflect that of *D. clarki*. However, further research is required to eliminate the possibility that 4th instars move at a faster relative rate due to their size range being optimal for *E. rubrolimbata* climbing up vertical surfaces [155]. To explore this, *E. rubrolimbata's* non-mimetic juvenile stages would need to be analysed. In addition, variation in movement speed between instar stages of other ant mimics and their non-mimetic relatives could be assessed.

In addition to differing movement speeds, insects exhibit varying movement patterns [156-158]. These movement patterns can be highly characteristic and may be used by predators when identifying potential prey [21, 125, 159, 160]. Indeed, despite all species examined in this study moving in a general upward direction, differences in movement patterns were identified.

Regarding *E. rubrolimbata*, the leafhopper instars exhibited comparable amounts of angle change to *D. clarki,* further reinforcing the notion of movement mimicry.

A limitation of this study is the sole reliance on visual modelling. Visual modelling is an important first step for gaining a better understanding of the methods and circumstances under which *E. rubrolimbata* mimics *D. clarki*. This approach has generated a range of clear questions to test with predator-prey assays. The most basic assay would involve varying the presence/absence of golden colouration. Similar studies have been conducted to evaluate the aposematic signal of ladybirds [161] and firebugs [133]. More intriguing assays would involve manipulating the size and location of *E. rubrolimbata's* gold colouration. By varying the size of the gold signal, the amount of leeway in signal size mimicry could be unravelled. Painting the abdomen of juveniles black and the pronotum gold, as seen in the adults, would confirm the value of *D. clarki*-like colour distribution. Finally, despite waist patches being reported in other species and reputed to create the illusion of a more ant-like shape [13, 23-26], this theory is yet to be experimentally tested, along with whether background context is important.

This is the first study to explore the mimetic relationship between a trophobiont and its attendant ant species, and the first research involving *E. rubrolimbata*. The study system provided an opportunity to introduce recently developed colour and shape analyses tools to the issue of ant mimicry. By taking a multiple trait approach and considering variation between instars and backgrounds, I have established the existence of ant mimicry in *E. rubrolimbata*. This raises a new question; Why does *E. rubrolimbata* display mimicry when very few other trophobiotic species do? As *E. rubrolimbata* has not been previously studied, precious little is known regarding the leafhoppers relationship with *D. clarki* or behaviours which may have led to mimicry being required alongside trophobiosis. Hence, further research into the leafhoppers ecology is required. Such research has the potential for improving our understanding regarding the factors that can lead to the development of mimicry as a defence tactic.

Chapter 2

Ant attended *Eurymela rubrolimbata* utilises mimicry to travel between spatially separated resources

1. Introduction

To avoid predation, a wide variety of invertebrates mimic ants, duping ant adverse predators into misclassify them as unpalatable prey [13, 152]. The success of this defence tactic is attributed to the formidable nature of ants as they are aggressive, cooperative and possess powerful weaponry such as strong jaws and painful stings [1]. Ant mimicry has been documented in over 2000 species across 11 invertebrate orders [13]. However, the prevalence of ant mimicry varies between orders, being rare in taxa such as katydids [162] and mantids [18] but much more common in spiders [21] and true bugs [163]. Some clades consist primarily of ant mimics, such as Myrmerachne spiders [21], while in other taxa, including Staphylinidae, the defence tactic has evolved on multiple occasions [164]. In some species, ant mimicry is transitionary, only being displayed at particular life stages. For example, Alydidae true bugs have mimetic instars and non-mimetic adults [159, 163]. The multiple independent origins and within species variation of ant mimicry means that many mimics have non-mimetic congeners or different life stages which rely on other defence strategies. The set of conditions that facilitate the adoption of ant mimicry, relative to these other defence strategies, is largely unknown. However, we may be able to gain an understanding by examining taxa in which ant mimicry is rare.

Ant attendance (trophobiosis) is another ant-based defensive strategy, although it is considerably distinct from ant mimicry. Ant attended species (trophobionts) recruit ants as 'bodyguards' by providing them with food resources. This tactic differs from ant mimicry as trophobionts rely on the physical presence of the ants to deter predators rather than visual deceit [34, 35, 39]. In comparison to ant mimicry, trophobiosis is only present in 3 invertebrate orders [13, 34]. This is likely due to the biological mechanisms required for trophobiosis, i.e. trophobionts must be able to provide ants with a nutritious food resource which could alternatively be used for their own growth and development. The majority of ant attended species are honeydew producing

hemipteran which are typically gregarious [34, 39, 48, 165]. This behaviour is potentially key to their success as aggregations provide a large and reliable source of honeydew, attracting and retaining more ant protectors [65, 87, 166-169] than lone trophobionts [170, 171]. This is in stark contrast to ant mimics which are predominantly solitary. Spiders, for example, are one of the largest groups of ant mimicking invertebrates and the majority are solitary species [21] with the exception of the group living ant mimic, *Myrmaraehne melanotarsa* [172]. In fact, many ant mimics actively avoid ants as they are often just as susceptible to ant attacks as non-mimics. Although, notable exceptions include ant mimicking *Zodarion* spiders which consume ants [173], and ant mimicking Staphylinidae which interact with army ants in migratory columns [147].

Eurymela rubrolimbata is an ant attended leafhopper species which bares resemblance to its ant partner, *Dolichoderus clarki*. In Chapter 1 I established that the leafhoppers resemble their hosts well enough to be considered ant mimics. This positions *E. rubrolimbata* as one of the very few ant mimicking trophobionts [69, 72] and the only trophobiont whose ant mimicry has been experimentally assessed. This makes *E. rubrolimbata* an ideal model for examining the conditions under which ant mimicry is adopted. I conducted an extensive imaged-based survey and found that most leafhoppers use crypsis, masquerade or aposematism for primary defence. There are no prior examples of ant mimicry in leafhoppers and only a few examples of mimicry in general, with leafhoppers resembling bees [174], wasps [175, 176] and ladybird pupae [177]. Hence, understanding why *E. rubrolimbata* requires mimicry, when other leafhoppers do not, may provide invaluable insight into the conditions under which ant mimicry is expected to arise.

In this study, I assessed the validity of two ecology-based hypotheses to address the question of why *E. rubrolimbata* has adopted both ant mimicry and ant attendance. My first hypothesis is that *E. rubrolimbata* is not an obligately ant attended species and so, relies on mimicry when dispersing onto trees free of *D. clarki* – the leafhopper's ant associate and model. This hypothesis implies that the ants will gain honeydew from the hoppers when available but the hoppers are more widely distributed locally than the ants. If this is the case, I predict the leafhoppers will be distributed by chance on the closest *D. clarki*-free tree (i.e. within the immediate environment) and/or on other trees with suitable resources for the leafhopper that are not visited by the ants. My second hypothesis is that *E. rubrolimbata's* key resources are spatially separated on individual trees and so the leafhopper relies on mimicry to move between ant protected aggregations of conspecifics. These resources are potentially the canopy, where the leafhoppers consume sap from supple growth, and exfoliating bark at the tree base which may offer refugia. Finally, this research

represents the first ecology based investigation of *E. rubrolimbata*. Records for this species are poor and its distribution is unknown as the leafhopper has only been documented in three locations including my field site [82]. For this reason, manipulative experiments were not conducted to test the relative strength of the ant-trophobiont relation.

2. Methods

(a) Study Site

All fieldwork was conducted at Duckmaloi crown reserve (-33.7131S, 149.971E) as detailed in Chapter 1. Surveys were conducted on *Eucalyptus viminalis*, which are the dominant species at the site. All assessments of insect presence were made at ground level as the tree canopy could not be accessed due to safety regulations. This was not an issue for *D. clarki* as the ants create trails up trees from ground based nests. For *E. rubrolimbata,* I assumed that the leafhoppers would access bark at the tree base regardless of whether *D. clarki* were present or not and thus, be detectable on all trees which hosted them. This assumption is based on two observations: 1) that *D. clarki* continue to enter the canopy after *E. rubrolimbata* have retreated to the bark and 2) that *D. clarki* do not dwell under the bark on trees without the leafhoppers. These observations suggest that *E. rubrolimbata* access the bark for reasons unrelated to the presence of the ants within the bark region.

(b) Surveying for *E. rubrolimbata*

I started with the *a priori* assumption that *E. rubrolimbata* is an obligate trophobiont of *D. clarki.* This assumption was based on ant association patterns in *E. rubrolimbata's* close relatives [34, 48, 90, 178] and *E. rubrolimbata* being regularly observed traveling along *D. clarki* foraging trails. I initiated an extensive ad hoc search between 0730 and 2030 to locate *E. viminalis* trees hosting *D. clarki*. This search involved systematically observing trees in the study area, starting at the sites eastern most boundary and working westward, mapping visited areas. Each tree was observed from all sides over 15 seconds. This time frame was deemed suitable as the ants are easy to locate due to their bright gold gaster and the extensive foraging trails they form on tree trunks. Trees hosting *D. clarki* were then surveyed for *E. rubrolimbata* either in the morning (0730 to 1030) or evening (1730 to 2030) as the leafhoppers were most active during these times. Surveys consisted of 30 seconds of actively searching below the bark followed by an additional 3 minutes of passive observation. For each tree, I recorded whether juvenile and adult *E. rubrolimbata* were present.

(c) Are *E. rubrolimbata* obligate associates of *D. clarki*?

I randomly selected 40 of the trees hosting juvenile *E. rubrolimbata*. For each of these focal trees, I surveyed two nearby *D. clarki*-free trees considered to have the greatest probability of hosting *E. rubrolimbata* if the hoppers were not obligate trophobionts. These trees were the nearest tree, which the leafhoppers may distribute onto by chance, and the nearest tree of comparable size. Tree size was used as a surrogate for growth stage as leafhoppers have previously been found to show growth stage preferences [89]. The trees surveyed had no *D. clarki* and had not been surveyed previously. Additionally, the nearest tree of comparable size to the focal tree was required to have a diameter at breast height (DBH) (measured at 1.3 metres) of within 50 mm of that of the focal tree. The DBH of each tree was measured using a Crafttech 100m open reel tape measure.

(d) Does *E. rubrolimbata* regularly and reliably use spatially separated resources?

To address this question, I determined whether *E. rubrolimbata* consistently moved between the canopy and bark and then attempted to gauge the importance of these resources by assessing their relationship with leafhopper presence. Firstly, I conducted 32 surveys of the direction leafhoppers moved when traveling along tree trunks. Of the trees previously identified to host *E. rubrolimbata* juveniles, 16 were monitored during both the morning (0730 to 1030) and the evening (1730 to 2030). Only trees with juveniles were chosen as they represent trees which support resident *E. rubrolimbata*. In comparison, adult *E. rubrolimbata* can fly between trees and hence may briefly be found on trees with unsuitable resources. During each observation period, I surveyed the tree trunk for 5 minutes. During these surveys I recorded the number of *E. rubrolimbata* individuals, the broad age class (juvenile or adult), and the direction they were walking, i.e. upwards towards the canopy or downwards towards the bark. To be included in the survey, leafhoppers had to cross an imagined line at eye level. Preliminary observations identified that the leafhopper's movement was strongly directional rather than wandering. Hence, the likelihood a leafhopper crossing the line more than once during the observation period was low.

To ascertain how important tree-based resources were to leafhopper presence, I measured 5 traits for 76 of the previously surveyed trees which hosted *D. clarki*. I treated the traits as surrogates of food availability and bark refugia. The characteristics I measured were canopy height, bare trunk height, DBH and the height of thick and thin bark. Canopy height and trunk DBH provided an indication of tree growth stage and hence potential food resources. Bark and trunk height corresponded to the availability of refugia and the distance between resources

respectively. Thick bark occurs at the base of *E. viminalis* and transitions into thin bark higher on the trunk. This distinction between bark types is important as the thin bark provides refuge opportunities due to the space behind it while thick bark is appressed to the trunk. As *E. rubrolimbata* travels along *D. clarki* foraging trails, I made bark height measurements at the point these ant trails crossed from one surface to the next. This was deemed more ecologically relevant than simply measuring the average height of the thick and thin bark. For example, the ants and leafhoppers exploit gaps between ribbons of thin bark, traveling over the bare trunk despite thin bark reaching much higher on other regions of the tree. Bare trunk height was measured from when *D. clarki* began walking along the trunk to the height of the first branch. Only branches with live foliage were considered while leaf-less branches and trunk forks were ignored. Canopy height was measured from the height of the first live branch to the crest of the tree. The height of thick bark, thin bark and DBH was measured using a tape measure while trunk height and canopy height where measured using a Haglof Vertex IV. The Vertex uses ultrasound to accurately measure the height of an object by triangulating between the hand-held device, the point of interest and an ultrasound emitter positioned at a known height. The emitter was positioned at 1.3 m up the tree trunk and the Vertex was calibrated to 10 m prior to data collection.

(e) Data analyses

All statistical analyses were conducted in the statistical software program 'R' version 3.3.2 [117] within 'R Studio' version 1.0.136 [118] using base packages. I used Fishers exact test to determine if adult and juvenile *E. rubrolimbata* frequented trees with *D. clarki* more so than neighbouring trees without the ants. To determine how time of day influences leafhopper movement direction I used a binomial logistic regression fitted with a logit link function. Adult and juvenile leafhoppers travelling up towards the canopy were scored as successes while hoppers travelling down towards the bark were scored as failures. The relationship between tree characteristics and leafhopper presence (1) and absence (0) was assessed using a binomial logistic regression with a logit link function. Prior to specifying a model, I assessed the degree to which the measured tree characteristics were correlated via a Pearson product-moment correlation. Tree characteristics were considered to be highly correlated if they had a Pearsons r greater than 0.8 and moderately correlated if above 0.5. I included all variables in models as none of the variables were highly correlated. After a model was created including all tree characteristics, a backwards stepwise procedure was used to eliminate variables whose removal did not increase the models Akaike

Information Criterion (AIC). Chi-square analysis was used to assess the significance of resulting models compared to the null model.

3. Results

(a) Presence of *E. rubrolimbata* relative to the presence of *D. clarki*

My initial surveys successfully located *E. rubrolimbata* on trees with *D. clarki* foraging trails. The ants were found on 89 trees of which 61% (n= 54) hosted *E. rubrolimbata*. Juvenile leafhoppers were found on 42% (n = 37) of the trees hosting *D. clarki* while adult leafhoppers were found on 55% (n = 49). In stark contrast, no juvenile leafhoppers were found on any of the *D. clarki*-free trees and only five trees had adult leafhoppers, a single individual in each case. For both *D. clarki*-free tree groups, adult leafhopper presence was significantly less than that on trees which hosted the ants ($P < 0.001$). Statistical tests were not performed for juvenile leafhoppers due to their absence on *D. clarki*-free trees.

(b) Movement of *E. rubrolimbata* between resources

The direction juvenile *E. rubrolimbata* moved between the bark and the canopy varied significantly between the morning and evening ($Z = -8.418$, 95% CI = -4.941 to -3.087, $P < 0.001$). A similar result was also found for the adult leafhoppers ($Z = -7.531$, 95% CI = -4.6531 to -2.7451, $P < 0.001$). I found that *E. rubrolimbata* more readily travelled down the tree trunk in the morning (Combined total: Up = 25, Down = 151) and upwards towards the canopy in the evening (Combined total: Up = 128, Down = 21) (figure 1).

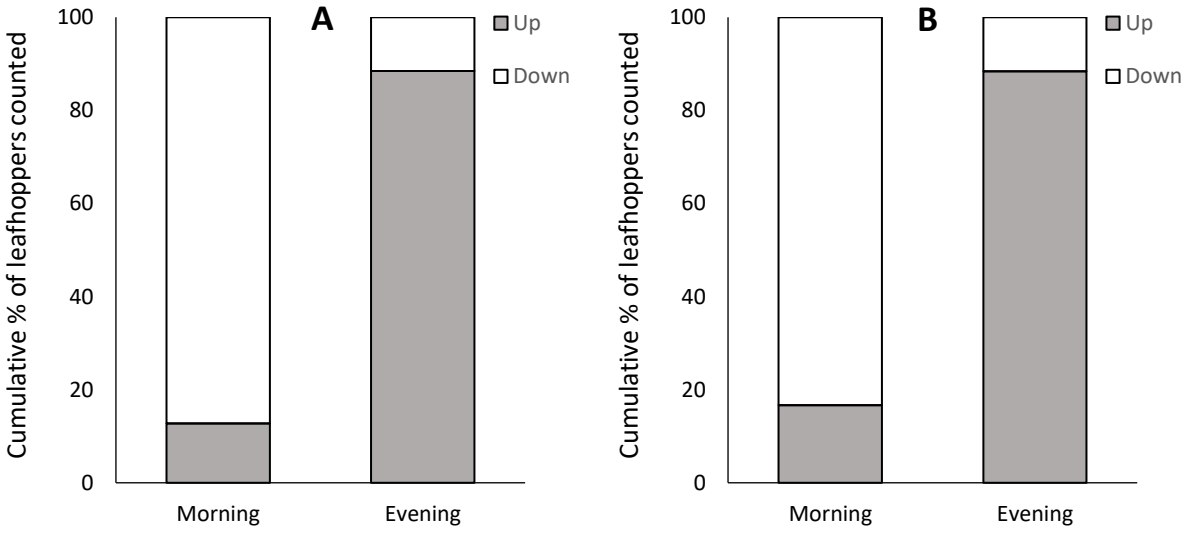

Figure 1. Stacked bar plots indicating the percentage of total juvenile (A) and adult (B) *E. rubrolimbata* moving towards the bark (down) or canopy (up) at Duckmaloi crown reserve during the morning (0730 to 1030) and evening (1730 to 2030).

(c) Presence of *E. rubrolimbata* relative to tree characteristics

For adult leafhoppers, none of the measured tree characteristics predicted presence as the full model was not significantly different from the null model ($\chi^2(2) = 5.0135$, $P = 0.0815$). In contrast, tree traits significantly influenced juvenile leafhopper presence ($\chi^2(2) = 12.4962$, $P = 0.0019$). The most parsimonious model based on AIC included two tree variables, thin bark height and trunk height. Of these two variables, only the thin bark height was significantly influential (Z = -2.988, 95% CI = -2.0942 to -0.4728, p = 0.0028). In particular, the likelihood of juveniles being found on a tree declined with an increase in thin bark height (figure 2A). A similar trend was found for trunk height although it was not significant (Z = -1.737, 95% CI = -0.5902 to 0.0259, p = 0.0823) (figure 2B). However, trunk height offered enough explanatory power for it to be retained in the model.

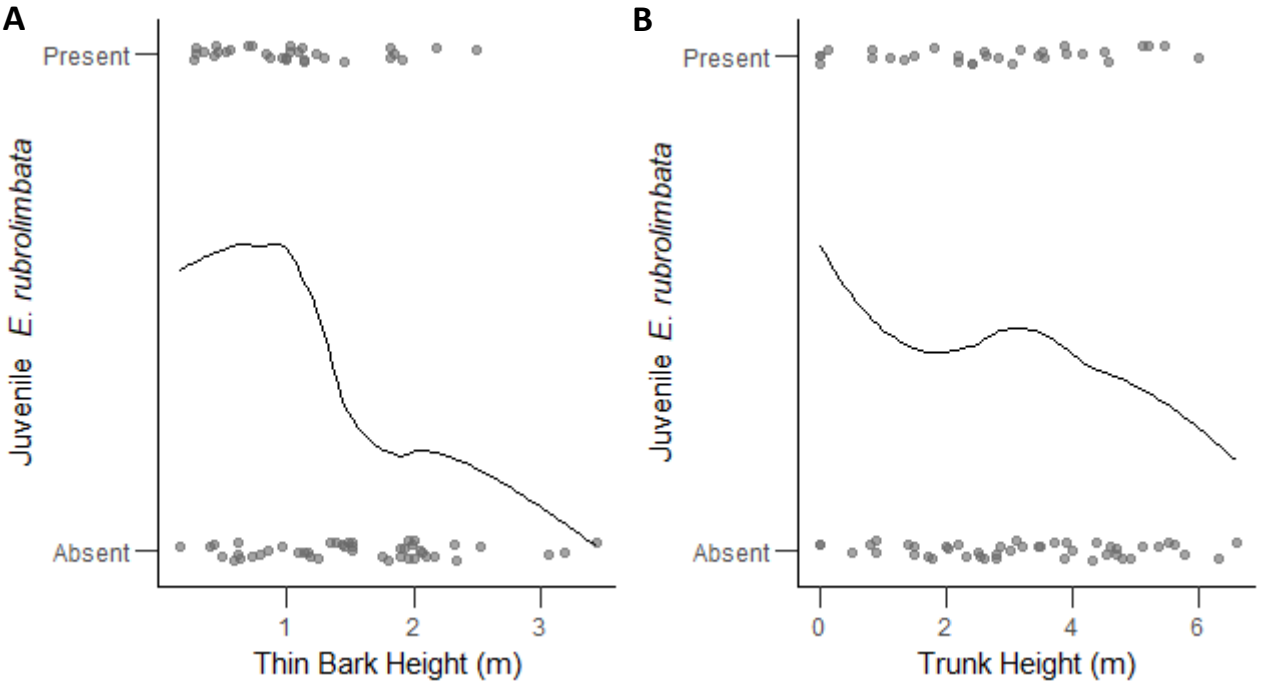

Figure 2. Binomial plots of juvenile *E. rubrolimbata* presence/absence depending on (A) thin bark height and (B) trunk height. Data have been scattered vertically and a Loess curve fitted to aid interpretation.

4. Discussion

I investigated two potential ecological hypotheses for why *E. rubrolimbata* exhibits mimicry in addition to ant attendance. The first hypothesis, that *E. rubrolimbata* are not obligate associates of *D. clarki,* was refuted as I found a strong association between the leafhoppers and the ants. In comparison, there was support for the hypothesis that mimicry evolved as a consequence of *E. rubrolimbata* relying on spatially separated resources. The leafhoppers consistently travelled along *D. clarki* foraging trails between the canopy and bark at the tree base. This behaviour required the leafhoppers to exit ant attended aggregations and to traverse an exposed tree trunk along *D. clarki* foraging trails. Behaviour which presumably degrades trophobiotic protection but aligns with mimicry.

My research indicates that leafhopper presence is directly linked to that of *D. clarki*, refuting the concept that mimicry is a consequence of a facultative relationship. This outcome corresponds with previous research that suggests all leafhoppers from *E. rubrolimbata's* tribe, Eurymelini, are ant attended [34, 48, 178]. Not all trees hosting *D. clarki*, hosted the leafhoppers and of those that did, the ants continued to enter the canopy even when *E. rubrolimbata* had retreated to the bark at the tree base. This suggests that the close relationship *E. rubrolimbata* has with *D. clarki* is not reciprocated by the ants. Instead, the ants appear to consume honeydew only when it is available to them, rather than making it a key component of their diet as in some other ant species [39, 179]. Although these findings indicate the leafhoppers have an obligate relationship with *D. clarki*, further manipulative experiments are required to fully understand the influence *D. clarki* have on populations of *E. rubrolimbata*. For example, *E. rubrolimbata* could be monitored following *D. clarki* exclusion to determine whether a population decline occurs as has been observed in other ant attended Eurymelids [61, 180, 181].

The daily movement patterns of *E. rubrolimbata* highlight the value of the spatially separated canopy and bark for the leafhoppers. The importance of the canopy is obvious as the leafhoppers consume sap from stems and leaves and my results suggest trees of a particular size are not preferred. In comparison, the value of the bark is less clear. The use of bark as refugia is well recognised for a range of fauna [182-186] including other leafhoppers [89]. Bark can provide a thermal buffer [186, 187] and hence, I suggest the bark provides a cool location for the

leafhoppers to reside during the heat of the day. I had anticipated that more thin bark to shelter behind would increase the likelihood of *E. rubrolimbata* being present. However, I found the reverse relationship. Compared to the smooth trunk of *E. viminalis*, bark at the tree base is a rough and complex surface that likely makes movement slow and tortuous. Hence, the leafhoppers may show preference for less thin bark to minimise their energy expenditure [188-190]. Alternatively, predators such as spiders may cause the observed result as a more complex bark environment means more habitat for invertebrates [191, 192]. This may also explain why adult *E. rubrolimbata* presence was not impacted. The adult leafhoppers possess hardened elytra, have a powerful jump to escape predators [83, 193] and are larger than juveniles which likely makes them more difficult to capture [194, 195]. To truly understand *E. rubrolimbata's* bark preferences, the distance the leafhoppers move over the bark before forming aggregations would need to be assessed, along with extended observations of bark zone predation.

By moving between spatially separated resources, *E. rubrolimbata* is periodically isolated from ants and aggregated conspecifics. This is at odds with other honeydew producing trophobionts which primarily remain in aggregations [34, 35, 39, 48, 165]. In contrast to an aggregated lifestyle, ant mimics are primarily solitary and do not require their ant models to cluster around them to deter threats [13, 152]. Based on this, it appears that mimicry and trophobiosis may exist in juxtaposition with trophobiosis occurring when organisms are surrounded by conspecifics and/or ants, and mimicry occurring in more solitary conditions. However, mimicry is not limited to species which have little contact with their models. Several species of mimetic spider run among their model's trails [22] and ant mimicking Staphylinidae inhabit the bivouacs of army ants [22, 51]. These exceptions suggest periodical isolation alone is not enough to drive the evolution of mimicry.

Instead, I propose that it is the need for extended periods of movement, irrespective of the length of isolation, that is key to the development of mimicry in *E. rubrolimbata*. Indeed, not only does *E. rubrolimbata* differ from other trophobionts by regularly abandoning aggregations, but it also differs in the amount of movement it engages in. Ant attended species typically exhibit sedentary lifestyles [34, 35] and have been described as "sluggish" compared to their non-ant attended relatives [86]. In comparison, ant mimicking species generally share the conspicuous and often rapid movement of their model [13, 21, 30, 125, 159] and have been recorded as being more active than their non-mimetic relatives [31]. For example, ant mimicking spiders are primarily active hunters, pursuing their prey rather than building webs [21]. Additionally, ant mimicking

Staphylinidae regularly run among migratory trails of army ants [147, 196]. As trophobiosis, and other defence strategies such as camouflage and masquerade, encourage a stationary lifestyle, ant mimicry may evolve to allow freedom of movement. Hence, movement requirements may be a predominate factor in the evolution of ant mimicry and possibly mimicry in general.

The movement of leafhoppers between resources was consistent among all growth stages but not all stages mimic *D. clarki*. For example, adult *E. rubrolimbata* possess black and white elytra, possibly for aposematic or disruptive purposes, and do not resemble ants. The adults were also occasionally seen on trees without *D. clarki* which potentially suggests the adults are not obligate trophobionts. However, unlike the juveniles, the adults can jump and fly which provides them with an alternative escape strategy that does not rely on mimicry [193]. These abilities allow for dispersal and hence, adult *E. rubrolimbata* likely use *D. clarki*-free trees as intermediary stops while dispersing. Indeed, the few adults on *D. clarki*-free trees remained stationary on the trunk rather than walking up to the canopy or down to the bark like those on trees hosting the ants. Unlike the older *E. rubrolimbata* instars, younger instars do not display mimetic colours (Chapter 1). These juveniles may not rely on mimicry due to their smaller size making them both more difficult to detect [197] and a less valuable food resources if they are detected [198].

The described relationship between the need to move and the adoption of either ant attendance or ant mimicry parallels the suggested dynamic between crypsis and aposematism. Theoretically, aposematism is expected to arise when the costs of maintaining crypsis outweigh the costs of maintaining aposematism [10, 199]. For example, a major cost of maintaining crypsis is constrained mobility [200]. This has associated opportunity costs such as limitations on foraging and locating mates [10]. In comparison, aposematism relies on conspicuous warnings and so allows animals to roam more freely. Likewise, trophobiosis is comparable to crypsis in that the leafhoppers remain stationary for protection. If the conditions are suitable then movement is not required. However, if circumstances are unfavourable, such as due to unsuitable temperature conditions, then movement is required which is not conducive to a trophobiotic lifestyle. The adoption of both trophobiosis and mimicry effectively allows the leafhoppers to be protected when they move and protected when they are feeding. Further investigation is required to better understand how movement relates to ant mimicry and ant attendance. Leafhopper ecology is understudied in Australia and indeed, other leafhopper species may share *E. rubrolimbata's* movement requirements. Thus, it would be pertinent to do a comparative phylogenetic analysis of

Eurymelids and their closest ancestors. In doing so the leafhoppers activity, ant associate and host tree could be mapped and explored in relation to relative ant-likeness.

In conclusion, I found *E. rubrolimbata* possesses a strong relationship with *D. clarki* and a need to access both the canopy and bark on a daily basis. This requirement results in the leafhopper exhibiting behaviour which is unusual for a trophobiotic species, i.e. regularly exiting ant attended aggregations and traveling across a large exposed surface. I propose that the need to travel between the resources facilitated the unusual development of both ant attendance and ant mimicry in *E. rubrolimbata*. This research is an initial step in understanding the factors that can drive the evolution of particular defence traits, in this case the evolution of mimicry. However, further ecological research is required on Australia's understudied leafhoppers to establish the validity of propositions I have made in this study.

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Appendix

Appendix 1. Transocular width for all five *E. rubrolimbata* instar stages and adults.

E. rubrolimbata Growth stage	Transocular width (mm)	
	Mean	Standard error
Instar 1	0.637	0.01
Instar 2	0.970	0.012
Instar 3	1.422	0.008
Instar 4	2.083	0.011
Instar 5	3.014	0.010
Adult	4.1	0.15

Appendix 2. Statistical output from linear mixed model for head colour comparisons between *D. clarki* and other species included in this study. *D. clarki* was set as the reference level to which all other species were compared. Confidence intervals which do not pass through 0 are bold and indicate a significant difference

Species	Estimate	Std. error	t value	Lower 95% CI	Upper 95% CI
Intercept (*D. clarki*)	0.3982	0.0320	12.4478	0.3358	0.4606
D. scabridus	-0.4256	0.0400	-10.6339	**-0.5036**	**-0.3475**
N. depressus	-0.0177	0.0400	-0.4429	-0.0958	0.0603
C. claripes	0.0740	0.0400	1.8495	-0.0040	0.1521
C. consobrinus	-0.1255	0.0400	-3.1354	**-0.2035**	**-0.0474**
Crematogaster sp.	0.0135	0.0400	0.3378	-0.0645	0.0916
C. innexus	0.1797	0.0400	4.4910	**0.1017**	**0.2578**
C. intrepidus	-0.0351	0.0400	-0.8764	-0.1131	0.0430
L. erythrocephalus	2.0400	0.0400	50.9719	**1.9619**	**2.1180**
M. tarsata	0.6212	0.0400	15.5218	**0.5431**	**0.6993**
M. pilosula	-0.1184	0.0400	-2.9583	**-0.1965**	**-0.0403**

Appendix 3. Statistical output from linear mixed model for thorax colour comparisons between *D. clarki* and other species included in this study. *D. clarki* was set as the reference level to which all other species were compared. Confidence intervals which do not pass through 0 are bold and indicate a significant difference

Species	Estimate	Std. error	t value	Lower 95% CI	Upper 95% CI
Intercept (*D. clarki*)	-0.1287	0.0557	-2.3083	**-0.2375**	**-0.0198**
D. scabridus	-0.0003	0.0611	-0.0051	-0.1196	0.1189
N. depressus	0.8224	0.0611	13.4515	**0.7032**	**0.9417**
C. claripes	2.5232	0.0611	41.2690	**2.4039**	**2.6424**
C. consobrinus	0.1418	0.0611	2.3193	**0.0226**	**0.2611**
Crematogaster sp.	1.8028	0.0611	29.4866	**1.6835**	**1.9220**
C. innexus	0.0396	0.0611	0.6472	-0.0797	0.1588
C. intrepidus	0.0589	0.0611	0.9639	-0.0603	0.1782
L. erythrocephalus	0.0592	0.0611	0.9683	-0.0600	0.1785
M. tarsata	1.2724	0.0611	20.8118	**1.1532**	**1.3917**
M. pilosula	0.2235	0.0611	3.6549	**0.1042**	**0.3427**

Appendix 4. Statistical output from linear mixed model for leg colour comparisons between *D. clarki* and other species included in this study. *D. clarki* was set as the reference level to which all other species were compared. Confidence intervals which do not pass through 0 are bold and indicate a significant difference

Species	Estimate	Std. error	t value	Lower 95% CI	Upper 95% CI
Intercept (*D. clarki*)	0.7917	0.0248	31.9216	0.7432	0.8402
D. scabridus	1.7677	0.0261	67.6142	**1.7167**	**1.8187**
N. depressus	0.4670	0.0261	17.8638	**0.4160**	**0.5180**
C. claripes	1.9495	0.0261	74.5675	**1.8985**	**2.0005**
C. consobrinus	2.1610	0.0261	82.6569	**2.1100**	**2.2120**
Crematogaster sp.	0.4073	0.0261	15.5778	**0.3563**	**0.4583**
C. innexus	1.5731	0.0261	60.1727	**1.5222**	**1.6241**
C. intrepidus	1.0872	0.0261	41.5836	**1.0362**	**1.1381**
L. erythrocephalus	-0.2879	0.0261	-11.0124	**-0.3389**	**-0.2369**
M. tarsata	-0.5265	0.0261	-20.1381	**-0.5775**	**-0.4755**
M. pilosula	-0.3355	0.0261	-12.8328	**-0.3865**	**-0.2845**

Appendix 5. Statistical output from linear mixed model for 3rd instar abdomen colour comparisons between *D. clarki* and other species included in this study. *D. clarki* was set as the reference level to which all other species were compared. Confidence intervals which do not pass through 0 are bold and indicate a significant difference.

Species	Estimate	Std. error	t value	Lower 95% CI	Upper 95% CI
Intercept (*D. clarki*)	1.5735	0.0558	28.2126	1.4648	1.6821
D. scabridus	-0.1252	0.0572	-2.1898	**-0.2361**	**-0.0144**
N. depressus	-1.1425	0.0572	-19.9755	**-1.2533**	**-1.0317**
C. claripes	-1.1232	0.0572	-19.6383	**-1.2340**	**-1.0124**
C. consobrinus	-1.1051	0.0572	-19.3209	**-1.2159**	**-0.9942**
Crematogaster sp.	-0.9034	0.0572	-15.7955	**-1.0143**	**-0.7926**
C. innexus	-0.8961	0.0572	-15.6667	**-1.0069**	**-0.7852**
C. intrepidus	-1.0752	0.0572	-18.7983	**-1.1860**	**-0.9643**
L. erythrocephalus	-1.0702	0.0572	-18.7109	**-1.1810**	**-0.9594**
M. tarsata	-1.2838	0.0572	-22.4450	**-1.3946**	**-1.1729**
M. pilosula	-0.6457	0.0572	-11.2893	**-0.7565**	**-0.5349**

Appendix 6. Statistical output from linear mixed model for 4th instar abdomen colour comparisons between *D. clarki* and other species included in this study. *D. clarki* was set as the reference level to which all other species were compared. Confidence intervals which do not pass through 0 are bold and indicate a significant difference.

Species	Estimate	Std. error	t value	Lower 95% CI	Upper 95% CI
Intercept (*D. clarki*)	0.7258	0.0474	15.2967	0.6324	0.8192
D. scabridus	0.1822	0.0272	6.6973	**0.1293**	**0.2350**
N. depressus	1.1452	0.0272	42.0997	**1.0923**	**1.1980**
C. claripes	0.8822	0.0272	32.4313	**0.8293**	**0.9350**
C. consobrinus	1.1362	0.0272	41.7691	**1.0833**	**1.1890**
Crematogaster sp.	0.7506	0.0272	27.5929	**0.6977**	**0.8034**
C. innexus	0.9775	0.0272	35.9346	**0.9246**	**1.0303**
C. intrepidus	1.2212	0.0272	44.8944	**1.1684**	**1.2740**
L. erythrocephalus	1.1645	0.0272	42.8089	**1.1116**	**1.2173**
M. tarsata	0.9700	0.0272	35.6581	**0.9171**	**1.0228**
M. pilosula	0.6756	0.0272	24.8357	**0.6227**	**0.7284**

Appendix 7. Statistical output from linear mixed model for 5th instar abdomen colour comparisons between *D. clarki* and other species included in this study. *D. clarki* was set as the reference level to which all other species were compared. Confidence intervals which do not pass through 0 are bold and indicate a significant difference.

Species	Estimate	Std. error	t value	Lower 95% CI	Upper 95% CI
Intercept (*D. clarki*)	1.2917	0.0351	36.7979	1.2226	1.3607
D. scabridus	0.1516	0.0161	9.4190	**0.1202**	**0.1830**
N. depressus	0.8829	0.0161	54.8647	**0.8515**	**0.9143**
C. claripes	0.6974	0.0161	43.3357	**0.6660**	**0.7288**
C. consobrinus	0.8791	0.0161	54.6268	**0.8477**	**0.9105**
Crematogaster sp.	0.6092	0.0161	37.8580	**0.5778**	**0.6406**
C. innexus	0.7539	0.0161	46.8446	**0.7225**	**0.7852**
C. intrepidus	0.9381	0.0161	58.2958	**0.9067**	**0.9695**
L. erythrocephalus	0.8943	0.0161	55.5743	**0.8629**	**0.9257**
M. tarsata	0.7616	0.0161	47.3287	**0.7303**	**0.7930**
M. pilosula	0.5274	0.0161	32.7698	**0.4960**	**0.5587**

Appendix 8. Mean Euclidean distances in body shape morphospace. Distances are between *E. rubrolimbata* instars and adult *E. rubrolimbata*, *N. depressus* and all other ant species. An (a) refers to whole body shape while a (b) refers to body shape following pale patch removal.

Species	Instars						
	I1	I2	I3	I4 (a)	I4 (b)	I5 (a)	I5 (b)
Adult *E. rubrolimbata*	0.079	0.087	0.092	0.082	0.199	0.065	0.210
N. depressus	0.172	0.165	0.172	0.160	0.257	0.143	0.277
C. claripes	0.334	0.324	0.303	0.326	0.349	0.362	0.361
C. consobrinus	0.325	0.317	0.297	0.318	0.343	0.354	0.355
C. innexus	0.316	0.307	0.287	0.308	0.331	0.347	0.346
C. intrepidus	0.337	0.328	0.308	0.329	0.356	0.366	0.368
Crematogaster sp.	0.301	0.293	0.275	0.295	0.317	0.334	0.333
D. clarki	0.318	0.306	0.286	0.306	0.287	0.343	0.301
D. scabridus	0.316	0.302	0.280	0.301	0.273	0.341	0.288
I. calvus	0.296	0.289	0.271	0.291	0.316	0.329	0.330
L. erythrocephalus	0.371	0.362	0.340	0.363	0.390	0.397	0.401
M. pilosula	0.325	0.317	0.297	0.317	0.351	0.352	0.363
M. tarsata	0.348	0.340	0.319	0.340	0.369	0.375	0.381

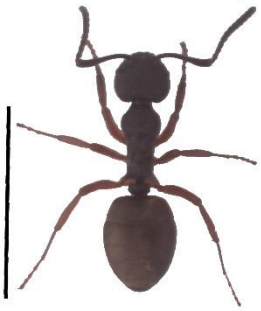
Appendix 9. Mean Euclidean distances in abdomen shape morphospace. Distances are between gold-bearing *E. rubrolimbata* instars and adult *E. rubrolimbata*, *N. depressus* and all other ant species. An (a) refers to visible abdomen shape while a (b) refers to the shape of only the gold region.

Species	Instars			
	I4 (a)	I4 (b)	I5 (a)	I5 (b)
Adult *E. rubrolimbata*	0.282	0.295	0.293	0.369
N. depressus	0.157	0.176	0.192	0.200
C. claripes	0.161	0.234	0.163	0.325
C. consobrinus	0.138	0.211	0.142	0.302
C. innexus	0.131	0.205	0.137	0.295
C. intrepidus	0.096	0.174	0.100	0.256
Crematogaster sp.	0.116	0.191	0.127	0.278
D. clarki	0.041	0.114	0.068	0.193
D. scabridus	0.043	0.118	0.070	0.192
I. calvus	0.119	0.197	0.129	0.282
L. erythrocephalus	0.261	0.324	0.262	0.415
M. pilosula	0.246	0.315	0.244	0.403
M. tarsata	0.261	0.332	0.257	0.417

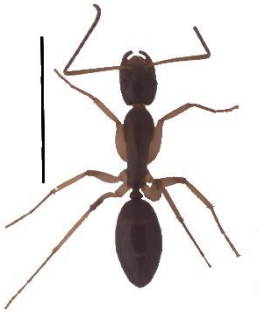
Appendix 10. Images of all ant species and the bug species included in the study. Scale bar = 5mm.

Notius depressus

Dolichoderus clarki

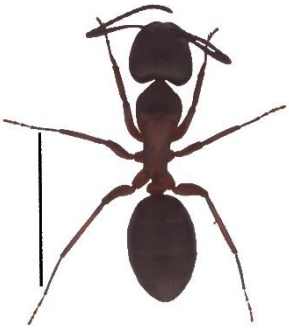


Dolichoderus scabridus

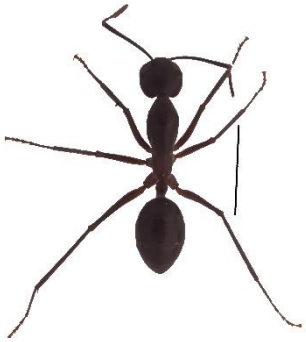


Camponotus claripes

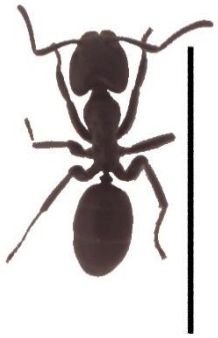
Camponotus consobrinus



Camponotus innexus

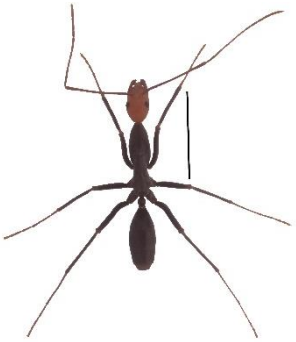


Camponotus intrepidus



Crematogaster sp.

Iridomyrmex calvus



Leptomyrmex erythrocephalus

Myrmecia pilosula

Myrmecia tarsata