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RESEARCH

Body size rather than reflectivity explains thermal constraints on colour variation in an aposematic jewel bug

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Abstract

Theory posits that warning signals should converge phenotypically to reinforce predator memory, yet many aposematic species show substantial variation in warning signals within and between populations. This could be explained by alternative selective pressures on phenotype beyond predation such as thermoregulation, but empirical tests are scarce and do not consider the full spectrum of sunlight. To examine whether trade-offs with thermoregulation could explain variation in warning colouration, we examined thermal properties of colour variants of the aposematic cotton harlequin bug (*Tectocoris diophthalmus*). This species shows striking variation in warning signals: it is sexually dichromatic, varies within sexes, and shows clinal variation in colour frequencies, with iridescent blue-green colouration prevalent in cooler climates and non-iridescent red–orange colouration prevalent in warmer climates. We quantified reflectivity between colour variants over the full solar spectrum and investigated the contribution of ultraviolet and visible (UV–visible, 300–700 nm) and near infrared (NIR, 700–1700 nm) light on heating. Differences in reflectivity of iridescent and non-iridescent colour patches were greater in NIR than UV–visible wavelengths but did not result in significant differences in heating. However, we found a tight link between body size and reflectivity, with smaller males having lower reflectivity and heating faster. Due to the strong correlation between body size and colouration, thermal constraints related to body size may help to explain the observed clinal variation in colour frequencies.

Keywords Aposematism · Variation · Thermoregulation · Colour · Reflectivity · Near-infrared

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Introduction

Aposematism is a visually striking anti-predator defence strategy where animals honestly advertise unprofitability using conspicuous colour patterns (Ruxton et al. 2004; Mappes et al. 2005). This strategy relies on predator learning (Stevens and Ruxton 2012), so theory predicts that aposematism is maintained by positive frequency-dependent selection where predators learn to avoid the most common phenotype (Endler et al. 1988; Sword 1999). These signals are expected to exhibit low variation to ensure consistency and reinforce predator learning and memory (Joron and Mallet 1998). However, in nature we often see substantial variation in warning signals observed between species, populations, or even individuals in the same populations (Briolat et al. 2019). Such widespread variation (Hegna et al. 2013; Gautier et al. 2018; Elias 2019; de Araujo Miles et al. 2023) might be explained by competing selection pressures (Caro et al. 2016; Cuthill et al. 2017) with optimal solutions depending on context. For instance, warning signal variation may arise from the need to target a diversity of predator species with distinct visual systems (Stevens 2007). Additionally, trade-offs with sexual signalling can explain variation between sexes and among populations experiencing different strengths of natural selection (Heinen-Kay et al. 2014). Individuals or populations occupying different microclimates or occurring along environmental clines may also face trade-offs related to physiology or camouflage, resulting in signal variation. Despite the widespread recognition that such trade-offs can shape variation in warning colouration, empirical examples are rare (Postema et al. 2022).

Most of the existing research on warning signal trade-offs comes from the well-studied aposematic wood tiger moth, in which competing selection pressures for thermoregulation, predation, and sexual signalling simultaneously act to maintain different frequencies of colour morphs between populations (Nokelainen et al. 2012; Gordon et al. 2015). More melanic individuals warm faster in cool climates, but face higher predation risk (Hegna et al. 2013). Females also prefer males with whiter, more UV-reflective colouration, but due to weaker aposematic signalling, these are more susceptible to bird predators compared to the yellow morph (Henze et al. 2018). Several other studies have investigated trade-offs between aposematism and thermoregulation by looking at correlations between colour and climate (Badejo et al. 2018; Lopez et al. 2021), and experiments in aposematic species such as the butterfly *Battus philenor* have shown that larval colour morphs can vary with ambient temperature and differ in thermal properties (Nice and Fordyce 2006). However, these studies generally only consider visible colour, which ignores the invisible parts of the solar spectrum that contribute to heat gain or loss. The ultraviolet–visible (UV–visible, 300–700 nm) and near-infrared (NIR, 700–2500 nm) wavelength ranges each account for approximately half the energy in direct sunlight (Stuart-Fox et al. 2017) and contribute substantially to radiative heating. Importantly, adaptive variation in NIR should primarily indicate selection for thermoregulation because these wavelengths cannot be perceived by animal visual systems and therefore are not directly constrained by selection for signalling (Stuart-Fox et al. 2017).

Here, we use the gregarious and aposematic cotton harlequin bug (*Tectocoris diophthalmus*, family Scutelleridae; also known as the hibiscus harlequin bug) to investigate the effect that variation in warning signals can have on thermoregulation, considering the full solar spectrum. These bugs are broadly distributed along the eastern coast of Australia and show a combination of structural iridescent dark blue-green and pigmented non-iridescent red–

orange colouration (Fabricant et al. 2013), the proportions of which are influenced by both environmental and genetic factors. Higher developmental temperatures and humidity are linked to lower proportions of iridescent blue-green (Fabricant et al. 2018), but iridescent colouration also shows negative heritability between sexes– brothers with high proportions of iridescence corresponded with sisters with low proportions of iridescence, and vice versa (Burdfield-Steel and Kemp 2021). Both colours contribute to aposematic signalling against bird predators (Fabricant et al. 2014; Fabricant and Smith 2014; Medina et al. 2020a) but despite being unpalatable and aposematic, the colours of the cotton harlequin bug vary markedly between individuals and populations (Cassis and Vanags 2006). Although there is overlap between the sexes, males tend to be smaller with a higher overall proportion of iridescence and redder non-iridescent patches, while females are larger with less iridescence and more orange non-iridescent patches. All this variation exists within a population, but frequencies vary geographically in a gradient of mostly red–orange non-iridescent to mostly blue-green iridescent from north to south (Ballard and Holdaway 1926; Fabricant et al. 2018).

The higher frequency of the darker, more iridescent individuals in the south may indicate thermoregulatory adaptation because all else being equal, darker colours absorb more solar radiation than lighter colours. Reflectivity, the fraction of solar energy reflected, can provide a mechanism for passive thermoregulation (Ospina-Rozo et al. 2022b). In this study, we examined the role of reflectivity in thermoregulation and its influence on warning signal variation in the cotton harlequin bug. We hypothesized that colour serves a thermoregulatory function and individuals with a higher proportion of iridescent (darker) colouration will warm faster, which may be advantageous in the cooler climates of the southern part of their range. To test this, we conducted experiments on 43 individuals presenting extensive variation in their warning signals to measure changes in heating due to direct radiation. We quantified reflectivity of the bugs over the full solar spectrum, and used a solar simulator that closely resembles the natural spectral power distribution of sunlight to test the contribution of UV–visible (300–700 nm) and NIR (700–1700 nm) light on heating. Our results provide insights into how thermal constraints may shape colour variation in aposematic species.

Methods

Insect collection and colony maintenance

We collected adult male and female cotton harlequin bugs (females = 82, males = 83) in February 2024. Our sampling sites were near Sydney, New South Wales, Australia, including: Sydney Park (33.91 S, 151.18 E), University of Sydney (33.89 S, 151.19 E), Kirribilli (33.84 S, 151.21 E), Narrabeen (33.72 S, 151.29 E), and Dee Why (33.75 S, 151.29 E). We collected adults by hand from known host plants (*Lagunaria patersonia, Hibiscus tiliaceus, and Brachychiton sp.*), ensuring that we obtained phenotypes ranging from mostly orange to mostly iridescent blue-green.

Once collected, we established the bugs on cuttings of *L. patersonia* in net cages located at the University of Melbourne, Australia. Plant cuttings were changed every two to three days and the room temperature was maintained at 30º C. Bugs were kept alive until they were used in heating experiments, which took place from early March to late April. We euthanised the bugs by placing them in a chamber adjacent to dry ice for 10 min. The bug was separated from the dry ice by a porous plastic barrier padded with tissues to increase the flow of carbon dioxide to the bug but reduce the effect of freezing by direct dry ice contact. Photographs, reflectance measurements, and heating experiments were conducted immediately after euthanasia.

Colour and reflectance measurements

To visualise variation in reflectance, we photographed the dorsal side of each bug using a modified Nikon D7200 digital SLR camera that captured images within three wavelength ranges: UV (320–400 nm), visible (400–700 nm), and NIR (700–1100 nm). Optical filters (Edmund Optics, Singapore, details in Supplementary Methods) were used to isolate wavelengths for each image, following the protocol outlined in a previous study (Munro et al. 2019). We used the images in the visible spectrum to measure body size and quantify the area of iridescent blue green and non-iridescent red orange. We scaled each image to the scale bar and normalised it to the 40% grey standard in the image using the micaToolbox plugin in ImageJ (Troscianko and Stevens 2015). We then calculated the total area of the bug and the proportion of iridescent patches using colour thresholding within ImageJ (Schneider et al. 2012).

We measured the total hemispherical reflectance for a subset of individuals (n = 11), which integrates reflectance over 180º and accounts for angle-dependent reflectance (encompassing both iridescence and specularity) (Ospina-Rozo et al. 2022a). We used an integrating sphere (Ocean Optics Inc.) with an inbuilt tungsten-halogen light source connected to two spectrophotometers (USB 2000 +; and NIRQuest; Ocean Optics Inc., Dunedin, FL, USA) via a bifurcated fibre optic. We measured reflectance of the spectral range of 400–2100 nm, incorporating around 92.5% of energy in sunlight. Measurements were calibrated against a 99% white reflectance standard and a 2% black reflectance standard and recorded using OceanView 1.6.7 software. We measured the bugs alive and immediately after euthanasia to validate that reflectance remained similar post-mortem (Fig. S1). Although our reflectance measurements do not include UV, our multispectral imaging showed that the bugs do not reflect UV and thus there is no difference in UV reflectance (Fig. 2a).

Calculating reflectivity

Reflectance as measured above is a wavelength specific measure (percentage reflected at each wavelength interval), whereas heating is a function of the overall proportion of incident light reflected over a specified wavelength range, which is termed reflectivity (Ospina-Rozo et al. 2022a). Specifically, reflectivity R is:

$$R = \frac{\int_i^n S\left(\lambda\right) I\left(\lambda\right) d\lambda}{\int_i^n I(\lambda) d\lambda}$$

where S is reflectance, I is irradiance, and λ is wavelength. The integrals are evaluated over the wavelength range from i to n, representing the lower and upper bounds of the wavelength range of interest, respectively. We calculated reflectivity values for three wavelength ranges: full spectrum (excluding UV, 400–1700 nm), visible (400–700 nm), and NIR

(700–1700 nm). We used the irradiance spectrum of the solar simulator (see next section; irradiance obtained from the manufacturer). To obtain the reflected radiation, we used the measured reflectance of colour patches in each of the three possible colour categories: iridescent, female non-iridescent, and male non-iridescent. We used these colour categories because on their dorsal surfaces, both male and female bugs have iridescent blue-green patches, but the non-iridescent patches are generally more red in males and more orange in females. Reflectance within each colour category (iridescent, female non-iridescent, and male non-iridescent) showed little variation, so we used mean reflectance (Fig. 1) to calculate reflectivity for each colour category. To calculate the total reflectivity of the individual bugs, we took the sum of the reflectivity of each colour category present on the bug (Fig. 1), multiplied by the proportion of the dorsal surface it occupied. The proportion of each patch was obtained from the multispectral images (Fig. 2a).

Heating experiments

We conducted heating experiments using a solar simulator (model: UHE-NS-E75; Sciencetech., London, Ontario, Canada), which approximates the spectral power distribution of natural sunlight and has an energy density of 660 W/m2 (0.66 Sun). To isolate the effect of radiation from the solar simulator and to minimise the effects of convection and conduction, we used a closed glass thermal chamber surrounded by flowing cool water (Fig. 3a). We controlled the water temperature using a recirculating chiller (ThermoScientific, Polar Series Accel 250 LC) to keep the ambient air temperature in the chamber constant at 20º C as measured by a thermocouple (ca. 5 mm TP-K01, K type, Center Technology Corp., Taiwan). Inside the chamber, the sample bug was placed dorsal side up on a transparent acrylic platform and with a thermocouple inserted in the posterior to record body tempera-

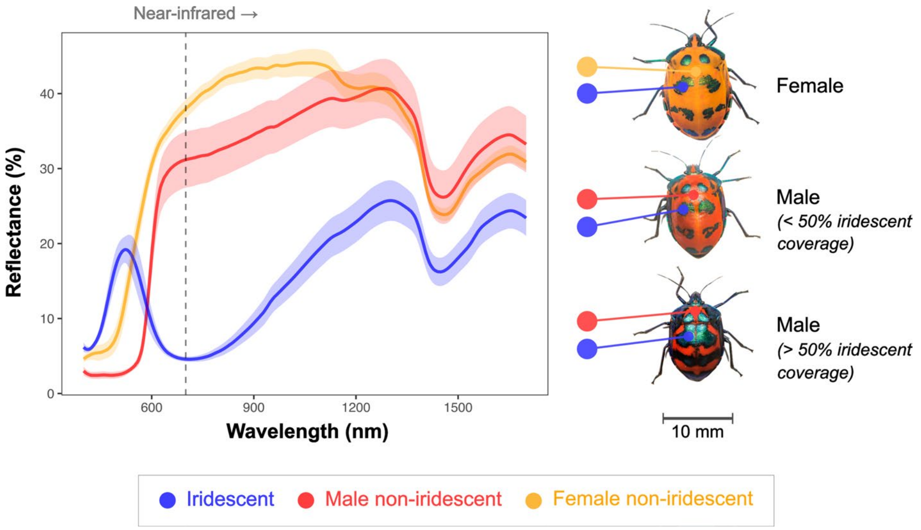

Fig. 1 Mean (solid line) and standard error of the mean (shaded area) of reflectance for each colour category. Reflectance measurements were taken from colour patches on the bugs, with examples shown on the right

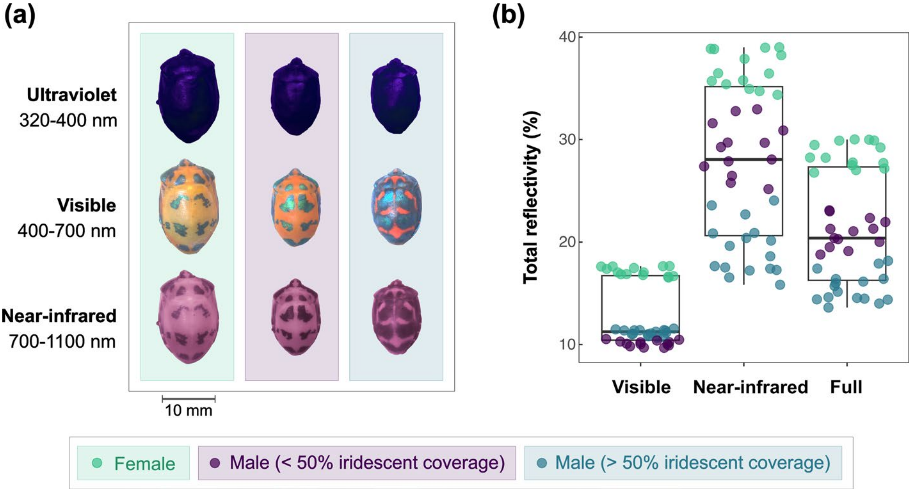

Fig. 2 (**a**) Female and two male variants of *Tectocoris diophthalmus*, photographed in different wavelengths: ultraviolet (UV, 320–400 nm), visible (400–700 nm), and near-infrared (NIR, 700–1100 nm). Images in the visible spectrum have been modified to improve aesthetics. Lighter colour indicates higher reflectance, with all bugs showing minimal reflectance in UV wavelengths. Females on average are larger than males. (**b**) Total reflectivity values for female and male bugs in visible (400–700 nm), near-infrared (700–1700 nm), and full (excluding UV, 400–1700 nm) wavelength ranges. Legend below is shared between panels

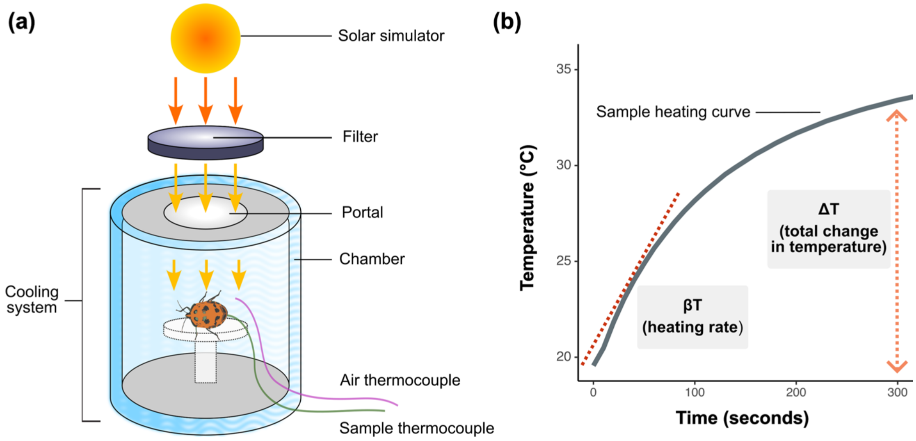

Fig. 3 (**a**) Closed glass thermal chamber to isolate the effect of radiative heat transfer. A solar simulator was used as an illumination source, and we used filters to isolate different wavelengths for each treatment. (**b**) Total change in temperature after 5 min (ΔT) and the maximum heating rate (βT), calculated for each bug, for every treatment (full, UV–visible, NIR)

ture. Temperature readings of both the air and the bug were taken every 10 s using a digital thermometer (Center 521, Center Technology Corp., Taiwan) (Fig. S2).

To understand the contribution of different parts of the solar spectrum (UV–visible and NIR), we ran each individual sample once for each heating treatment: full (300–1700 nm), NIR (700–1700 nm), and UV–visible (300–700 nm). To test the effect of the full solar spectrum, the solar simulator was positioned directly above the chamber which had a silicon glass window allowing transmission of all wavelengths. Then, we used optical filters (Edmund Optics, Singapore) over the silicon window to isolate the effect of radiation in different wavelength ranges, using a filter (item numbers 84760, 84,735) to allow transmission of only NIR and another filter (item numbers 84727, 49,095 [KG-5 heat absorbing glass]) to allow transmission of only UV–visible light. After placing the bug in the chamber, we allowed a 10 min period for the bug and air to achieve an equilibrium temperature of 20º C. We then opened the solar simulator shutter to begin the 10 min heating period. Although each heating trial ran for 10 min, all bugs reached a stable maximum temperature by 5 min; therefore, all heating estimates (see below) were calculated based on the first 5 min of the heating period.

Heating estimates

We extracted the final temperature after 5 min (ΔT) and heating rate (βT) for each bug for every treatment (Fig. 3b). We calculated the final temperature as the difference in temperature of the bug at the starting point and at 5 min (30 measurement cycles). The air temperature of the chamber was constant (Table S1). We calculated heating rate as the maximum change in temperature for the sample due to direct radiation over a 20 s period. This translates to the maximum slope of the heating curve in ºC/second (Fig. 3b), which is directly comparable between individuals, irrespective of the time taken to reach a steady state temperature.

Statistical analysis

All analyses were conducted in R 4.3.0 (R Core Team 2023). Quality checking of reflectance measures was completed using the *pavo* package (Maia et al. 2019), and data was visualised using ggplot2 (Wickham 2009). We used a multiple linear regression, implemented in the “lm” function in R, to investigate how reflectivity and body size affected temperature after 5 min (ΔT) and heating rate (βT).

Radiative heating is a physical consequence of a surface's reflectivity and the area exposed to light. To check that our setup could detect variation in heating rates, we used a linear regression to examine the relationship between heating and area-dependent reflectivity, which accounts for the reflectivity of absolute surface area of the bug. We calculated bug area-dependent reflectivity as the reflectivity of colour patches multiplied by their area– as opposed to the proportion– which means this is a measure of reflectivity that incorporates body size.

We then examined the relationship between the reflectivity of each individual (mean reflectivity of each colour patch multiplied by the proportion of that colour; *see Methods: Calculating reflectivity*) and body size, our two predictor variables. Body size was characterised by the area of the dorsal side of the bug in millimetres squared, and was square root transformed. Because these two variables were highly correlated in full and NIR (Spearman’s correlation: rho = 0.56, p = 1.1e-4* for full and NIR, rho = 0.20, p = 0.19 for visible), including reflectivity and body size as predictors of heating rates would lead to collinearity. To avoid this, we used a linear model to regress these variables and extract the residuals to represent reflectivity controlled for body size for each spectral band (full, NIR, UV–visible). We also used a linear model to confirm that the reflectivity residuals were no longer correlated with body size (Fig. S3).

The residual values represent a measure of reflectivity independent of body size; to test the effect of reflectivity controlled for size on heating, we ran separate models for all treatments (full, NIR, UV–visible). We used both ΔT and βT as response variables in separate models and used reflectivity (controlled for size residual values) and body size (square root transformed) as predictor variables.

Results

Reflectivity across all bugs ranged from 10 to 18% in UV–visible (imaging revealed the bugs do not reflect UV) wavelengths and 15% to 39% in NIR, revealing that most of the variation in bug reflectivity occurs in the NIR spectrum (Fig. 2b). This is partly attributed to greater variance in all colour categories in the NIR spectrum as compared to UV–visible: iridescent (NIR: 14.4%, UV–visible: 11.7%), female non-iridescent patches (NIR: 39.4%, UV–visible: 17.7%), and male non-iridescent (NIR: 34.7%, UV–visible: 9.5%). There were also differences in average reflectivity between males and females in the full (male: 18%, female: 28%), NIR (male: 24%, female: 37%), and visible (male: 11%, female: 17%) wavelengths (Fig. 2b and Fig. S5).

We found that area-dependent reflectivity (bug reflectivity multiplied by the absolute size of colour patches as opposed to proportion) significantly affected heating rate over the full spectrum ($R^2 = 0.15$, estimate $= -0.001$, $p = 0.01^*$) (Fig. S4b). NIR wavelengths contributed more strongly to this effect ($R^2 = 0.9$, estimate $= -0.001$, $p = 0.004^*$) than UV–visible wavelengths ($R^2 = 0.12$, estimate $= -0.001$, $p = 0.05^*$). NIR reflectivity also significantly affected the final temperature ($R^2 = 0.19$, estimate $= -0.04$, $p = 0.003^*$) (Table S2); however, there was no significant effect of UV–visible wavelengths ($R^2 = 0.06$, estimate $= -0.03$, $p = 0.12$) or full wavelengths (full: $R^2 = 0.07$, estimate $= -0.05$, $p = 0.10$; Fig. S4a).

In our dataset, reflectivity and body size were significantly correlated (Spearman's correlation: rho $= 0.56$, $p = 0.0001$ for full and NIR, rho $= 0.20$, $p = 0.19$ for visible). Confirming previous studies, we found that female body area (mean $= 159$ mm^2, SD $= 32$) was on average larger than males (mean $= 118$ mm^2, SD $= 23$); (Welch's Two Sample t-test: $t_{20} = 4.23$, $p = 0.0002$, Fig S4a). Within males, larger males had a lower proportion of blue-green iridescent colouration than smaller males (Spearman's correlation: rho $= -0.49$, $p = 0.007$). Furthermore, we also found that females had higher reflectivity than their similarly sized male counterparts (Fig. 4a). There are two main reasons for females having a higher reflectivity than males: 1) female non-iridescent patches are more orange rather than red, with orange having higher reflectivity, and 2) females tend to have a much lower proportion of blue-green iridescence than males.

Size-independent reflectivity (residual values extracted from the regression of body size and reflectivity) did not have a significant effect on final temperature or heating rate (Fig. 4b and Fig. 4c, respectively) in any of the three wavelength ranges. However, body size did have a strong effect (Table 1). Specifically, body size affected heating rate in both the full and NIR ranges, but not the UV–visible spectrum alone. Body size affected the final temperature only in the NIR spectrum. We obtained similar results when we ran the models

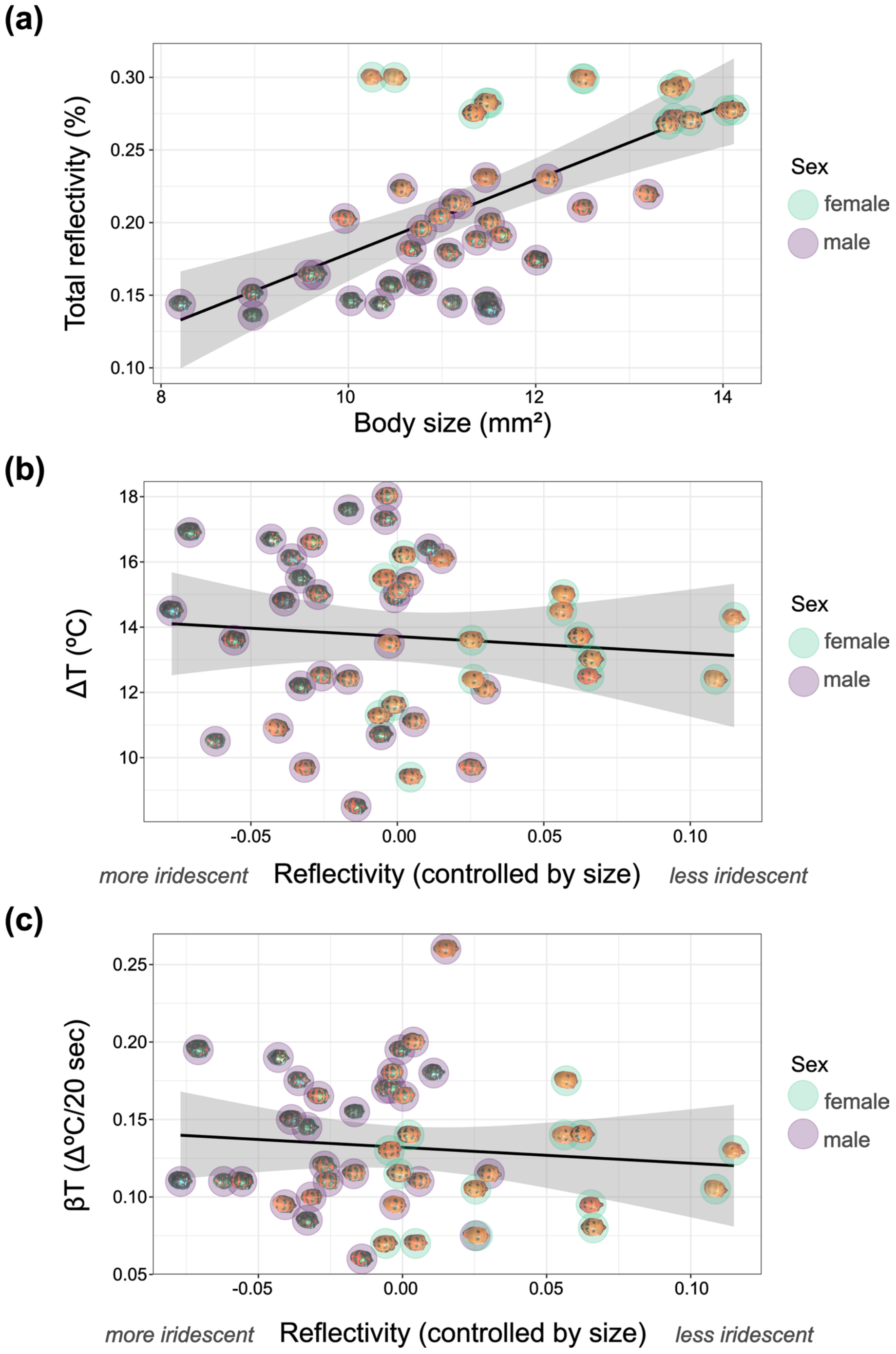

Fig. 4 (**a**) Reflectivity and body size show a strong association (R^2= 0.41, estimate =0.03, $p< 0.0001$), with females usually larger with higher reflectivity. (**b**) Reflectivity over the full irradiance spectrum does not show a significant effect on final temperature after 5 min (R^2= 0.1, estimate =−5.1, $p= 0.6$). (**c**) Reflectivity over the full irradiance spectrum does not show a significant effect on heating rate (R^2= 0.16, estimate =−0.10, $p= 0.49$)

Table 1 Summary of multiple linear regression models for ΔT and βT, run for full, NIR, and UV–visible wavelengths

Response	Spectral range	R^2	Parameter	Estimate	Std. error	t-value	p-value
Final temp. after 5 min	full	0.08	(Intercept)	19.10	3.02	6.32	< 0.001
(ΔT)			Reflectivity residuals (full)	−5.07	8.65	−0.59	0.56
			Body area (mm^2)	−0.47	0.26	−1.80	0.08
	NIR	0.20	(Intercept)	11.83	1.67	7.10	< 0.001
			Reflectivity residuals (NIR)	−6.38	3.46	−1.85	0.07
			Body area (mm^2)	−0.37	0.15	−2.57	0.01*
	UV–visible	0.06	(Intercept)	6.12	1.13	5.42	< 0.001
			Reflectivity residuals (visible)	−3.54	5.16	−0.69	0.50
			Body area (mm^2)	−0.14	0.10	−1.42	0.17
Heating rate	full	0.16	(Intercept)	0.27	0.05	5.23	< 0.001
(βT)			Reflectivity residuals (full)	−0.10	0.15	−0.69	0.49
			Body area (mm^2)	−0.01	0.01	−2.69	0.01*
	NIR	0.19	(Intercept)	0.15	0.03	5.21	< 0.001
			Reflectivity residuals (NIR)	−0.08	0.06	−1.30	0.201
			Body area (mm^2)	−0.01	0.00	−2.72	0.01*
	UV–visible	0.12	(Intercept)	0.08	0.02	4.34	< 0.001
			Reflectivity residuals (visible)	−0.10	0.08	−1.18	0.25
			Body area (mm^2)	−0.00	0.00	−1.69	0.10

with both factors together, or when we ran models separately with only reflectivity residuals or body size. Models with only body size showed a significant effect on heating rate in both the full and NIR spectra (R^2: full = 0.15, NIR = 0.15), while models with only reflectivity residuals showed no significant results. Full results for reflectivity residuals and body size are given in Table S3 and S4, respectively.

Discussion

We tested whether competing selection for thermal benefits could explain some of the striking variation in aposematic colouration within and between populations of the cotton harlequin bug, *Tectocoris diophthalmus*. Our results showed that individuals differ in their thermal properties, with larger, more red–orange bugs heating slower than smaller, more blue-green iridescent bugs. The absolute difference in reflectivity between colour patches was modest, with most variation in the NIR spectrum. Consequently, the effect on heating rate was driven more strongly by body size than reflectivity, and more strongly by differences in reflectivity in NIR than UV–visible wavelengths. Additionally, body size was the strongest driver of differences in final temperature, and this was only significant under

NIR illumination. However, body size and reflectivity were tightly linked: individuals with more iridescent coloration were smaller. The tight link between these two traits suggests that selection for smaller body sizes in cooler environments may constrain colour variation and result in the observed geographic cline in colour frequencies. Our study highlights the importance of testing wavelengths beyond the range of human vision particularly when studying thermoregulation, which operates independently of visual perception.

We tested the effect of heat transfer due to radiation, which is ultimately a function of the reflectivity and body size (Willmer and Unwin 1981; Walsberg 2015; Wang et al. 2021). We found differences in heating based on the absolute area of colour patches: smaller bugs with a higher proportion of iridescent blue heated faster and reached higher final temperatures. This result verified that we were able to detect differences in heating rate and final temperature between individuals using our experimental set-up. However, in cotton harlequin bugs, the effect of reflectivity is difficult to isolate from the effect of sex and body size because larger males often have higher reflectivity than smaller males, and females are larger than males and have a higher proportion of non-iridescence. We found that the residuals of reflectivity regressed against body size (i.e. size-independent reflectivity) explained very little of the variation in heating, with observed differences being driven primarily by body size. These results are consistent with previous studies examining the effect of body size and reflectivity on heating in insects (Umbers et al. 2013; Wang et al. 2023), where body size had a larger effect on heating rates than reflectivity.

Despite the evident colour variation between individuals with more or less iridescence, we found that overall differences in reflectivity were quite modest in all spectral bands, with variation in total reflectivity largely attributed to differences in NIR reflectivity. The maximum observed difference in reflectivity between individuals was 16.4%, driven primarily by a 23% variation in NIR reflectance (compared to 8% variation in UV–visible). While this variation alone did not significantly impact heating rates or final temperatures, there was a non-significant trend for NIR reflectivity influencing final temperature. Additionally, the greater variation in NIR reflectivity compared to UV–visible explained the observed differences in heating between the wavebands. Animal visual systems cannot detect NIR wavelengths, so often variation in NIR indicates selection for thermoregulation (Stuart-Fox et al. 2017). Previous studies providing evidence for NIR-modulated thermal benefits in ectotherms have typically revealed significantly greater variance in NIR than in UV–visible– more than 9 times higher in jewel beetles and 1.67 times higher in butterflies (Munro et al. 2019; Wang et al. 2021). Although cotton harlequin bugs show much more variation in NIR than UV–visible reflectance, this may be a coincidental consequence of the pigments and structures that absorb or reflect light in these wavelengths (Mielewczik et al. 2012), rather than being indicative of selection for thermoregulation. In cotton harlequin bugs, non-iridescent patches are formed by erythropterin pigment and iridescent patches are formed by epicuticular layers of melanin (Fabricant et al. 2013). Melanin has much higher absorption in the NIR than erythropterin (Wilts et al. 2011; Andrade and Carneiro 2021). Thus, mechanistic constraints, rather than selection for thermoregulation, may explain variation in NIR reflectance.

Our results showed that larger bugs have higher reflectivity, consistent with previous studies of this species (Ballard and Holdaway 1926; Fabricant et al. 2018; Medina et al. 2020b; Burdfield-Steel and Kemp 2021). This can be partly attributed to sex differences, as females are larger than males and tend to have higher reflectivity due to both a lower proportion of iridescence and higher reflectivity values of their non-iridescent patches (more orange compared to the redder males). Sex differences in body size in bugs are common and likely due to selection for fecundity (Cueva Del Castillo 2015). Differences in reflectivity for males and females could indicate that there are different selective pressures acting on males and females. In this species, females provide maternal care by guarding egg clutches for approximately 17 days (Giffney and Kemp 2014). During this period of limited mobility, females likely experience difference selective pressures to males. Still, the link between size and colour was present among males, and less iridescent males tended to be larger than their more iridescent counterparts. The proportion of iridescence, body size, and development time are all linked by strong genetic correlations and have a large heritable component (Burdfield-Steel and Kemp 2021); this is especially prevalent in males where there is a positive relationship between iridescent coverage and development time. Potential ecological reasons for the link between reflectivity and size, especially prominent within males, remain unclear.

The influence of radiative heat gain on thermoregulation is highly dependent on microclimate and specific conditions (Porter and Gates 1969; Gates 1980; Willmer and Unwin 1981), and so selection on reflectivity is expected to be strongest in environments where air temperature is low and there is very little wind (Umbers et al. 2013; Zverev et al. 2018; Britton and Davidowitz 2023). This effect is more pronounced in animals with a large surface area to volume ratio such as butterflies, which often bask to increase their body temperature when air temperature is low (Kingsolver and Moffat 1982; Kingsolver and Wiernasz 1991). However, cotton harlequin bugs are active in the summer and in warm environments. In such circumstances, convection and evaporative heat loss may have a greater influence on body temperature than reflectivity (Turner and Lombard 1990; Umbers et al. 2013). Additionally, cotton harlequin bugs can behaviourally regulate their temperature by basking or seeking shelter, moving vertically through plants to do so (Wilson et al. 1983). Since their host plants provide both shelter and food, there may be negligible trade-offs between feeding and thermoregulating in this species (Heinrich 1993; Lahondère et al. 2017).

Selection unrelated to thermoregulation may also maintain colour variation in the cotton harlequin bug. Although the classic view is that aposematism is under positive frequency-dependent selection by predators, it has become increasingly clear that this is an oversimplification (Skelhorn et al. 2016). Variation in warning signals may be sustained by multiple factors, including variation in unpalatability, predator learning and predator community composition (Endler and Mappes 2004; Ihalainen et al. 2007; Barnett et al. 2014; van den Berg et al. 2024). The cotton harlequin bug has both variable and modest chemical defences especially compared to other heteropterans (Schaefer 1972; Staddon et al. 1987), although the differences in toxicity do not seem to be explicitly linked with colour variation (Medina et al. 2020b). The bugs appear conspicuous to birds, which are sensitive to and avoid their chemical defences (Fabricant and Smith 2014). On the other hand, the red–orange colouration appears cryptic to mantids, which are undeterred by the chemical defences (Fabricant and Herberstein 2015). Hence, the bugs may be employing different antipredator strategies with different predators, and these strategies may be linked to body size. Evidence suggests that with increasing latitude, arthropod predation decreases while bird predation increases (Zvereva et al. 2019), which could drive clinal variation in bug colouration. Our results suggest that although selection for thermoregulation is not the primary driver of colour variation, thermal constraints could contribute to this clinal variation because smaller, more

iridescent individuals, which are prevalent in cooler climates, heat faster. Overall, our study highlights the complexity of factors influencing colour pattern variation and the value of considering both visible and NIR wavelengths when examining the potential contribution of selection for thermoregulation.

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Declarations

Conflicts of Interest The authors declare no competing interests.

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Code availability Data and code are available on Github at: https://github.com/keilinooi/tectocoris_analysis

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