

Contents lists available at [SciVerse ScienceDirect](#)

Journal of Insect Physiology

journal homepage: [www.elsevier.com/locate/jinsphys](http://www.elsevier.com/locate/jinsphys)

## Colour in insect thermoregulation: Empirical and theoretical tests in the colour-changing grasshopper, *Kosciuscola tristis*

K.D.L. Umbers\*, M.E. Herberstein, J.S. Madin

Department of Biological Sciences, Macquarie University, NSW 2109, Australia

### ARTICLE INFO

#### Article history:

Received 13 August 2012

Received in revised form 17 October 2012

Accepted 17 October 2012

Available online xxxx

#### Keywords:

Alpine

Colour change

Heat budget

Grasshopper

*Kosciuscola tristis*

Temperature

### SUMMARY

Body colours can result in different internal body temperatures, but evidence for the biological significance of colour-induced temperature differences is inconsistent. We investigated the relationship between body colour and temperature in a model insect species that rapidly changes colour. We used an empirical approach and constructed a heat budget model to quantify whether a colour change from black to turquoise has a role in thermoregulation for the chameleon grasshopper (*Kosciuscola tristis*). Our study shows that colour change in *K. tristis* provides relatively small temperature differences that vary greatly with wind speed (0.55 °C at ms<sup>-1</sup> to 0.05 °C at 10 ms<sup>-1</sup>). The biological significance of this difference is unclear and we discuss the requirement for more studies that directly test hypotheses regarding the fitness effects of colour in manipulating body temperature.

© 2012 Elsevier Ltd. All rights reserved.

### 1. Introduction

Solar energy is arguably the most important factor in the ecology of insects (Digby, 1955; May, 1979; Casey, 1981; Chappell and Whitman, 1990). On average, around half of all solar energy that reaches the earth's surface is in wavelengths spanning the animal visual range (350 nm (ultraviolet) – 700 nm (red)). The differential reflectance of light over this range is perceived as colour and is directly related to the amount of solar energy an object, in this case an insect, absorbs or reflects at different wavelengths. Therefore an insect's colour may be important in determining its body temperature, which may affect every phase of its life, from development to reproduction (Casey, 1981; Chappell and Whitman, 1990; Punzalan et al., 2008). As ectotherms, insects mostly utilise solar radiation to regulate heat gain via behavioural (e.g., shuttling from sun to shade, and posturing) and/or physiological means (e.g., Slifer's patches – thin areas of cuticle for cooling) (Slifer, 1953a,b; Casey, 1981; Makings and Saeed, 1989). For some insects, body temperature may influence time spent foraging and mating (Whitman, 1988), and even their external colour, indicating a potential link between temperature and colour (Key and Day, 1954a,b; Veron, 1974; Joern, 1981; Umbers, 2011).

The optical principle that low-reflectance colours absorb more energy than high-reflectance colours has been readily applied to insect thermal ecology (May, 1979). The evolution of colour as a

thermoregulatory adaptation is possible only if the resulting differences in temperature produce a fitness advantage. If the temperature difference between colour morphs is too small to alter body temperature in a selectively meaningful way, we may conclude that an organism's colour evolved for other functions or no function at all (e.g., detection avoidance or signaling [Muggleton, 1978; Evans and Schmidt, 1990]).

Several studies have attempted to determine the role of colour in thermoregulation by comparing the temperature excess (degrees above ambient) of differently coloured insects under solar or artificial radiation (Digby, 1955). Theoretical studies have highlighted the importance of radiation and convection in predicting insect temperature (Parry, 1951). Empirical studies however, have described vastly disparate patterns. The temperature differences reported for insects of varying colours range from colour having no effect (0 °C) to substantial effects (up to 12 °C) on body temperature (Table 1). Some of this variation is due to the very different methodologies applied. For example, some studies used natural variation in colour while in others, insects were painted; some studies exposed insects to laboratory light, others to natural sunlight. Some studies measured temperature of live insects and others of dead insects. Furthermore, prior to the availability of spectrophotometers, the quantification of colour was largely subjective.

Using spectrophotometry to quantify colour allows objective, comparable measurement independent of visual systems. Furthermore, the percentage reflectance of colour can be directly converted to energy (Watts) to determine how much heat enters the

\* Corresponding author.

E-mail address: [kate.umbers@mq.edu.au](mailto:kate.umbers@mq.edu.au) (K.D.L. Umbers).

**Table 1**

Summary of experiments that have directly tested for a role of colour in thermoregulation of insects. Light colour and Dark colour refer to the polymorphism being tested. Polymorphism denotes whether the colour morphs are naturally occurring or artificially created. If the latter, then what substances were used for the manipulation. Largest reported temperature differences are Digby's temperature excess of the dark morph minus that of the light morph. Claim for a difference denotes whether the authors interpreted that the temperature difference they observed would have a biologically important effect on the insect. Animal activity is included as metabolic heat gain is more important in some systems than others and certain activities (notably flying) will greatly impact of an insect's temperature excess. Where thermocouples were placed to measure temperature, whether the experiment was conducted in the lab or the field, whether the animal was dead or alive and what kind of lighting was used are all important factors to consider when comparing results across these studies because each one can greatly affect the outcome and thus should be considered in interpreting the results.

Order	Species	Dark colour	Light colour	Polymorphism	Largest reported temperature difference (dark colour – light colour) (°C)	Claim for a difference?	Animal's activity	Temperature measurement technique (part measured, if described)	Lab or field	Dead or alive	Light source	Spectrum emitted	Reference
Diptera	<i>Mesembrina meridiana</i>	Black	Black	Painted with an aqueous solution of lamp-black	−0.5	n	Rest	Implanted thermocouple (thorax)	Lab	Live	2 kW projector bulb 0.5 cal/cm <sup>2</sup> /min plus 2 cm N/20 CuCl solution	Washington Sunshine	Digby (1955)
Hymenoptera	<i>Eristalis tenax</i>	Black	Brown	Black artists oil paint	−0.3	n	Rest	Implanted thermocouple	Lab	Live	2 kW projector bulb 0.5 cal/cm <sup>2</sup> /min plus 2 cm N/20 CuCl solution	Washington Sunshine	Digby (1955)
Diptera	<i>Sarcophaga camaria</i>	Black	Dull blue black	Black artists oil paint	−0.3	n	Rest	Implanted thermocouple (thorax)	Lab	Live	2 kW projector bulb 0.5 cal/cm <sup>2</sup> /min plus 2 cm N/20 CuCl solution	Washington Sunshine	Digby (1955)
Diptera	<i>Phormia caerulea</i>	Black	Metallic blue black	Painted with an aqueous solution of lamp-black	−0.2	n	Rest	Implanted thermocouple (thorax)	Lab	Live	2 kW projector bulb 0.5 cal/cm <sup>2</sup> /min plus 2 cm N/20 CuCl solution	Washington Sunshine	Digby (1955)
Diptera	<i>Lucilia caesar</i>	Black	Metallic green	Painted with an aqueous solution of lamp-black	0	n	Rest	Implanted thermocouple (thorax)	Lab	Live	2 kW projector bulb 0.5 cal/cm <sup>2</sup> /min plus 2 cm N/20 CuCl solution	Washington Sunshine	Digby (1955)
Orthoptera	<i>Schistocerca gregaria</i>	Red	Green	Natural	0.1	n	Rest	Implanted thermocouple (thorax)	Field	Live	Sunlight	Full	Stower and Griffiths (1966)
Coleoptera	<i>Eleodes armata</i>	Black	White	White paint	0.1	N/A	Rest	Internal (abdomen)	Field	Live	Sunlight	Full	Hadley (1970)
Odonata	<i>Austrolestes annulosus</i>	Black	Blue	Natural colour change	0.23	n	Rest	Implanted thermocouple	Lab	Live	Wotan 24 V 250 W quartz-iodine lamp with 2 cm KSu/KCu solution 65 mW/cm <sup>2</sup>	Not described	Veron (1974)
Orthoptera	<i>Tetrix subulata</i>	Black	White	Natural colour morphs	0.42	y	Rest	Internal abdomen	Lab	Live	ELUKE model 52 K/j lamp	Not described	Forsman (1997)
Diptera	<i>Calliphora erythrocephala</i>	Black	Dull blue-black	Black artists oil paint	0.44	n	Rest	Implanted thermocouple (thorax)	Lab	Live	2 kW projector bulb 0.5 cal/cm <sup>2</sup> /min plus 2 cm N/20 CuCl solution	Washington Sunshine	Digby (1955)
Orthoptera	<i>Carausius morosus</i>	Black	Green	Painted with an aqueous solution of lamp-black	0.66	n	Rest	Implanted thermocouple (thorax)	Lab	Live	2 kW projector bulb 0.5 cal/cm <sup>2</sup> /min plus 2 cm N/20 CuCl solution	Washington Sunshine	Anderson et al. (1979)
Dictyoptera	<i>Periplaneta americana</i>	Black	Brown	Painted with an aqueous solution of lamp-black	0.8	n	Rest	Implanted thermocouple (thorax)	Lab	Live	2 kW projector bulb 0.5 cal/cm <sup>2</sup> /min plus 2 cm N/20 CuCl solution	Washington Sunshine	Anderson et al. (1979)
Hymenoptera	<i>Vespa vulgaris</i>	Black	Yellow and black	Painted with an aqueous solution of	1.1	n	Rest	Implanted thermocouple (abdomen)	Lab	Live	2 kW projector bulb 0.5 cal/cm <sup>2</sup> /min plus 2 cm N/20 CuCl	Washington Sunshine	Anderson et al. (1979)

Table 1 (continued)

Order	Species	Dark colour	Light colour	Polymorphism	Largest reported temperature difference (dark colour – light colour) (°C)	Claim for a difference?	Animal's activity	Temperature measurement technique (part measured, if described)	Lab or field	Dead or alive	Light source	Spectrum emitted	Reference
Dictyoptera	<i>Periplaneta americana</i>	Black	Brown	lamp-black Black artists oil paint	1.32	n	Rest	Implanted thermocouple (thorax)	Lab	Live	solution 2 kW projector bulb 0.5 cal/cm <sup>2</sup> /min plus 2 cm N/20 CuCl solution	Washington Sunshine	Anderson et al. (1979)
Coleoptera	<i>Coccinella septempunctata</i>	Large spots	Small spots	Natural (size of spots)	1.5	y	Rest	Thermocouple microprobe under elytron	Lab	Live	Halogen lamp	Not described	Stewart and Dixon (1989)
Orthoptera	<i>Melanoplus differentialis</i>	Black	Yellow	Natural	1.6	n	Rest	Implanted thermocouple (meta thoracic sternum)	Lab	Live	Sunlight	Full	Pepper and Hastings (1952)
Coleoptera	<i>Adalia bipunctata</i>	Two black spots on orange	Orange	Natural (number of spots)	1.9	y	Rest	Thermocouple microprobe under elytron	Lab	Live	Halogen lamp	Not described	Stewart and Dixon (1989)
Hemiptera	<i>Drepanosiphum platanooides</i>	Melanic	Non-melanic	Seasonal	2.1	y	Rest	Implanted thermocouple (anus)	Lab	Live	100 W tungsten lamp (ambient temp 6 °C)	Not described	Dixon (1972)
Lepidoptera	<i>Colias eurytheme</i>	Black	Orange	Seasonal	2.5	y	After flying	Bead thermistor (thoracic dorsum)	Both	Live	Sunlight and flood lamps	Full spectrum and not described	Watt (1969)
Orthoptera	<i>Schistocerca gregaria</i>	Black	Dull yellow green	Painted with an aqueous solution of lamp-black	3.3	n	Rest	Implanted thermocouple	Lab	Live	2 kW projector bulb 0.5 cal/cm <sup>2</sup> /min plus 2 cm N/20 CuCl solution	Washington Sunshine	Anderson et al. (1979)
Orthoptera	<i>Brachystola magna</i>	Black	Tan	Both painted	3.7	y	N/A	Implanted thermocouple (thorax)	Field	Dead	Sunlight	Full spectrum	Joern (1981)
Orthoptera	<i>Carausius morosus</i>	Black	Green	Painted with an aqueous solution of lamp-black	4.4	n	Rest	Implanted thermocouple (thorax)	Lab	Live	2 kW projector bulb 0.5 cal/cm <sup>2</sup> /min plus 2 cm N/20 CuCl solution	Washington Sunshine	Anderson et al. (1979)
Orthoptera	<i>Calliptamus coelestriensis</i>	Chocolate (rarer)	Buff	Natural fixed	5	y	Rest	Thermocouple (sub elytral space)	Field	Live	Sunlight	Full spectrum	Buxton (1924)
Coleoptera	<i>Coccinella septempunctata</i>	Black	Orange	Nail polish (black and clear)	6	y	Rest	Thermocouple microprobe (under elytron)	Lab	Live	Halogen lamp	Not described	Stewart and Dixon (1989)
Coleoptera	<i>Onymacris brincki</i>	Black	White	Sister species	6	y	Rest	Internal (thoracic and subelytral cavity)	Field	Live	Sunlight (1.1 – 1.2 cal/cm <sup>2</sup> /min)	Full spectrum	Edney (1971)
Orthoptera	<i>O. rugatipennis</i> <i>Brachystola magna</i>	( <i>O. rug.</i> ) Tan	( <i>O. bri.</i> ) White	Both painted	8.3	y	N/A	Implanted thermocouple (thorax)	Field	Dead	Sunlight	Full spectrum	Joern (1981)
Orthoptera	<i>Brachystola magna</i>	Black	White	Both painted	12	y	N/A	Implanted thermocouple (thorax)	Field	Dead	Sunlight	Full spectrum	Digby (1955)
Hemiptera	<i>Phymata americana</i>	Black	Yellow	Both painted	0.3	y	N/A	Implanted thermocouple (thorax)	Lab	Dead	60 w lamp	Not described	Punzalan et al. (2008)

(continued on next page)

Table 1 (continued)

Order	Species	Dark colour	Light colour	Polymorphism	Largest reported temperature difference (dark colour – light colour) (°C)	Claim for a difference?	Animal's activity	Temperature measurement technique (part measured, if described)	Lab or field	Dead or alive	Light source	Spectrum emitted	Reference
Lepidoptera	<i>Colias nastes</i>	Melanin	Less melanin	Natural variation in number of dark coloured scales	4	y	N/A	Implanted thermocouple	Field	Dead	Sunlight	Full spectrum	Roland (1982)
Lepidoptera	<i>Pterid species</i>	More melanin	Less melanin	Natural variation in number of dark coloured scales	2	y	Rest	Implanted thermocouple	Lab	Paralysed	Sunlight	Full spectrum	Kingsolver (1987)
Lepidoptera	<i>Parnassius phoebus</i>	More melanin	Less melanin	Natural variation in number of dark coloured scales	significantly different	n	Rest	Implanted thermocouple	Lab	Dead	Sunlight	Full spectrum	Guppy (1986)
Lepidoptera	<i>Adalia bipunctata</i>	2 spots	6 spots	Natural variation in number of different morphs	2.1	y	Hibernating	Glued thermocouple	Lab	Dead	60 W tungsten lamp		Brakefield and Willmer (1985)

body. Spectrophotometry has typically only included reflectance across the visual part of the electromagnetic spectrum (UV to red). Less often, researchers have turned their attention to the role of the near-infrared (700–2500 nm), which can significantly influence insect body temperature (Porter and Kearney, 2009).

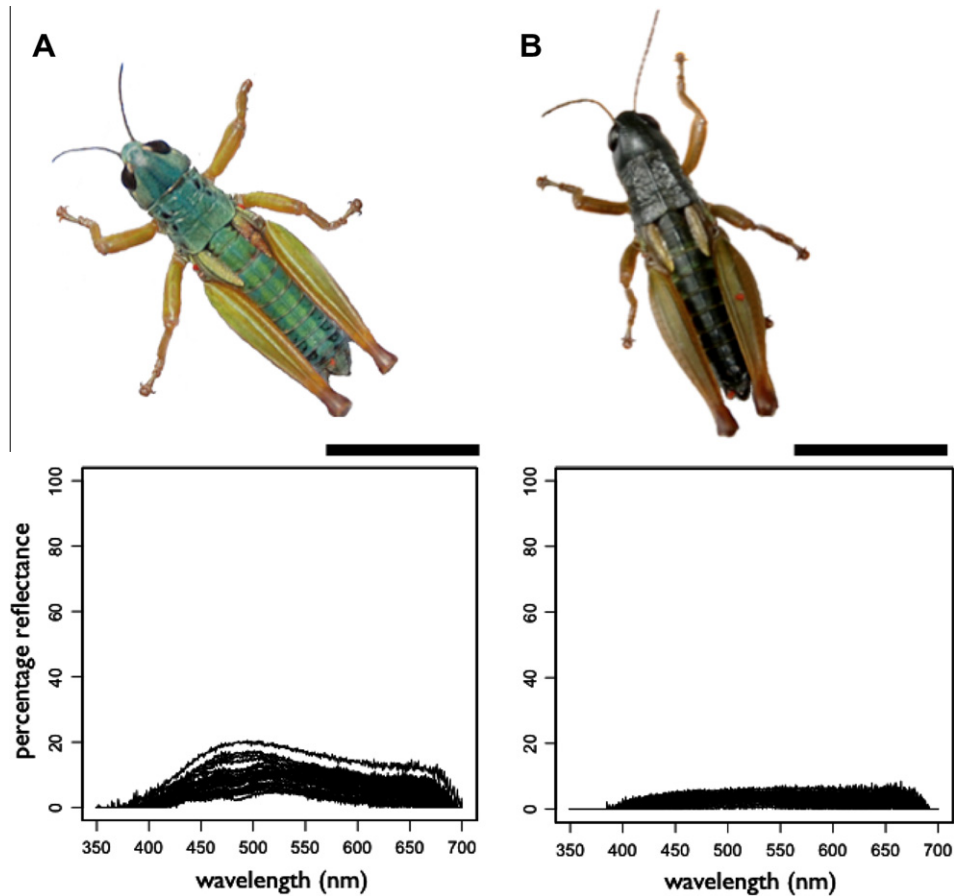
Our aim was to investigate the role of colour in thermoregulation in a system where we could control for many of the variables mentioned above. The male chameleon grasshopper (*Kosciuscola tristis*) exhibits rapid reversible colour change (Key and Day, 1954a,b; Umbers, 2011). The head, thorax and abdomen change colour from black to bright turquoise as a function of ambient temperature (black under 10 °C, turquoise over 25 °C, intermediate colours at temperatures between 10 and 25 °C).

Upon the discovery of colour change in this species, Key and Day (1954b) proposed that it has a thermoregulatory function: the black phase allows rapid heating while the turquoise phase prevents overheating. Such a strategy may allow males to forage and seek mates through the hottest part of the day without having to seek shade to avoid overheating (Heinrich, 1996). *Kosciuscola tristis* is an ideal model with which to test the idea that colour can mediate thermoregulation, because we can compare the rate of heating for different colour phases. So, while Key and Day's (1954b) thermoregulation hypothesis seems intuitive, we addressed it in this study with two questions: first, do black and turquoise grasshoppers heat up at different rates? And second, do turquoise grasshoppers have a lower maximum temperature than black ones? We predicted that the turquoise phase would result in lower body temperatures than the black phase while the black phase would allow for more rapid heating than the turquoise phase. To investigate the thermodynamic consequences of colour change more thoroughly, we developed a heat budget model to estimate body temperature over a range of colours and environmental conditions.

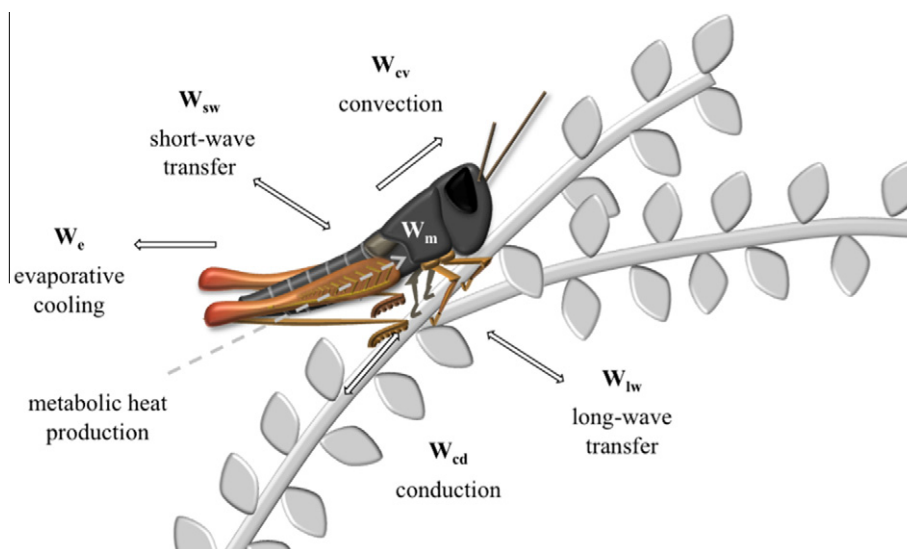
## 2. Materials and methods

### 2.1. Natural history of *Kosciuscola tristis*

The chameleon grasshopper (*K. tristis*) (Sjöstedt, 1933) is a medium sized short-horned grasshopper (Acrididae: Orthoptera) endemic to the alpine and sub-alpine habitat of southeastern Australia. In this region, the grasshopper is superabundant from December to May, although this varies greatly both annually and spatially. Chameleon grasshoppers have been observed to hatch in mid spring, thought this also varies considerably from year to year (Green, pers. comm. and Umbers, pers. obs.). Mating begins as adults eclose (make their final moult); all males and most females are adult by early February (late summer). Egg laying occurs from late summer onward (Umbers et al., 2012). By the end of May/beginning of June, *K. tristis* are rare and most of the remaining individuals are dead or killed by the first blizzards that sweep the alpine slopes (Umbers pers. obs.). Being alpine, average ambient temperatures during the summer can exceed 25 °C in the early afternoon and then fall below freezing at night. In early autumn (March), for example, the average daily temperature range in this region fluctuates between –6 and 25 °C (Bureau of Meteorology 2010). Chameleon grasshoppers tend to favour open grassy patches that are surrounded by short subalpine shrubs. Males and females are active at the same times of day (Umbers, pers. obs.), and thus at similar ambient temperatures, and we have never observed a day so hot to render chameleon grasshoppers inactive (>25 field trips). Chameleon grasshoppers exhibit sexual size and colour dimorphism where females are larger (0.29 ± 0.05 g,  $n = 17$  pronotum length 4.26 ± 0.73 mm,  $n = 224$ ) and have a more fixed dark green or brown colour and males are



**Fig. 1.** Two male chameleon grasshoppers one above 25 °C (A), and one below 10 °C (B) (scale bar: 1 cm); graphs show some representative spectral reflectance across 350–700 nm of the two colour phases of male *K. tristis* as percentage reflectance when compared to a white standard.



**Fig. 2.** Schematic diagram of factors that influence the internal temperature of a *K. tristis* grasshopper. See Eq. (2) in text and Table 2 for definitions.

smaller ( $0.22 \pm 0.04$  g,  $n = 151$ , pronotum length  $3.55 \pm 0.37$  mm,  $n = 435$ ) and exhibit rapid reversible colour change from black to turquoise (Fig. 1). Colour change is faster from black to turquoise (around 30 min on average) than the reverse (around 5 h on average) (Umbers, 2011). Both sexes are flightless and the eggs overwinter in subnivean soil (Green and Osborne, 1994).

## 2.2. Empirical measurements of grasshopper heating

Male *K. tristis* grasshoppers were collected at an altitude of 1939 m along the Dead Horse Gap walking track, south of Thredbo Village on Alpine Way, NSW ( $36^{\circ} 30' 14.05''$  S  $148^{\circ} 16' 36.7''$  E) in February of 2009. Fourteen grasshoppers were kept at 4 °C overnight to

ensure they had reached maximum black colouration and then divided at random into two treatment groups (one died, leaving 13). Grasshoppers in the 'black' treatment group ( $n = 6$ ) were maintained at 4 °C in a cool room. Grasshoppers in the turquoise treatment group ( $n = 7$ ) were allowed to heat in the sun (turning turquoise) for at least 4 h before being transferred to the cool room. Because it takes around 5 h to change from turquoise to black, grasshoppers that were snap chilled to 4 °C retained their turquoise colour whilst being cooled (Umbers, 2011). From each group, an individual was chosen at random, its spectral reflectance measured as in Umbers (2011), and one of two thermocouple probes (Digital Thermometer 206–3738 with 22 gauge K-type thermocouple chromel–alumel, RS Components Ltd. Corby, Northants, UK) was inserted perpendicular to the body between the segments under the posterior end of the pronotum piercing the inter-segmental membrane and the other left uncovered to record ambient temperature. We did not notice any bleeding that may have aided in evaporative cooling, but if this did occur it occurred the same way in all grasshoppers across treatments. The individual was then taken outside and held in the sun in a mesh bag made of wide gauze with the dorsal surface perpendicular to the sun and with researchers' fingers more than 3 cm away from the grasshopper at all times. The experiments were conducted between 10:30 am and 2:30 pm on cloudless, windless days with similar air temperatures (average temperature during experiments  $\pm$ SD = 30.08  $\pm$  1.13, range: 28–33 °C). The temperature of both probes was recorded at 30 s intervals for 10 min, which was long enough for the body temperature to reach equilibrium (Umbers, 2011). This procedure was repeated for each individual.

Grasshopper body temperature (measured by thermocouple) exponentially approached an asymptote usually in around 5 min. To account for any differences in air temperature among trials, each body temperature time series ( $T_g$ ) was divided by the trial's mean ambient air temperature ( $T_a$ ). To capture the heating rate  $k$  and the asymptotic body temperature  $s$  (relative to air temperature), an exponential model as a function of time  $t$  was fitted to the data:

$$\frac{T_g}{T_a} = a - e^{-k(t-s)} \quad (1)$$

$a$  is the model intercept (ca. constant). Welch's two sample  $t$ -tests were used to assess differences in the maximum temperature and heating rates of turquoise and black phases.

### 2.3. Heat budget model

We developed a heat budget model for *K. tristis* males to compare internal body temperature at different colour phases at

equilibrium with the environment. Similar models have been built for other species, such as limpets and beetles (Henwood, 1975; Denny and Harley, 2006), and the underlying theory is described in detail elsewhere (Gates, 1980). Six components were considered when constructing the model for *K. tristis* (Fig. 2).

The total energy budget of any organism can be described as:

$$W_{lw} \pm W_{sw} \pm W_{cd} \pm W_{cv} \pm W_e \pm W_m = W_{stored} \quad (2)$$

where  $W$  represents the rate at which heat energy enters or leaves an organism (measured in Watts), including long wave flux ( $lw$ ), short wave flux ( $sw$ ), conduction ( $cd$ ), convection ( $cv$ ), evaporation ( $e$ ), and metabolic heat production ( $m$ ), respectively (Fig. 2). We assume that *K. tristis* grasshoppers act as inert objects with small thermal mass and high thermal inertia, and so  $W_{stored} = 0$ . We excluded metabolic heat, evaporation, and conduction from the model for *K. tristis*, given that it is generally accepted that heat production via metabolism and heat lost via evapotranspiration are over two orders of magnitude smaller than other factors like convection for small insects like grasshoppers (Digby, 1955; Church, 1960; Lovelidge, 1968; Anderson et al., 1979; Stevenson, 1985; Chappell and Whitman, 1990). Also, although found in other Acridid species, *K. tristis* has never been observed holding its abdomen to the substrate (Umbers, obs.), and as such conduction may not be an important component in the loss or gain of heat. Eq. (2) then becomes:

$$W_{lw} \pm W_{sw} \pm W_{cv} = 0 \quad (3)$$

The rate at which heat is absorbed from solar radiation (short-wave energy flux) was modeled as:

$$W_{sw} = A_p \alpha_{sw} I_{sw} \quad (4)$$

where  $A_p$  is the planar area of the grasshopper,  $I_{sw}$  is solar irradiance (ca. 1300 W m<sup>-2</sup>), and  $\alpha_{sw}$  is the fraction of solar irradiance absorbed by grasshopper integument, which may depend on colour. Short-wave flux is also dependent on sun angle, cloud cover and time of the day, but these variables are maximised to be conservative (i.e., we assume the grasshopper is exposed maximally to sunlight). The projected area of the grasshopper ( $A_p$ ) is the average of the dorsal surface of the head, pronotum and abdomen measured of five male *K. tristis* chosen at random. The fraction of solar irradiance absorbed by a grasshopper was calculated by integrating the maximum potential irradiance (given by Planck's law) and the proportion grasshopper absorbance (1 – reflectance,  $p$ ) as a function of wavelength ( $\lambda$ ):

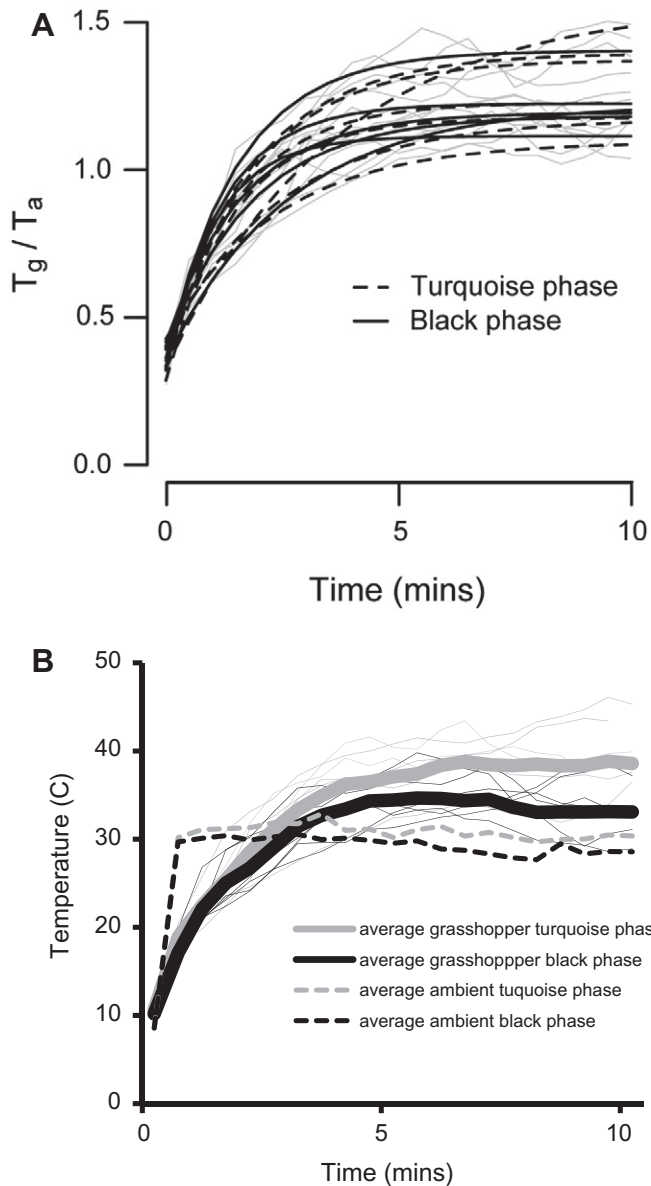
$$\alpha_{sw} = \int_{\lambda_1}^{\lambda_2} I(\lambda)p(\lambda)d\lambda \quad (5)$$

Planck's law as a function of wavelength is given by:

**Table 2**

Definition of symbols used in the model, the equation in which it is first encountered and the equation in which it is calculated and values of constants.

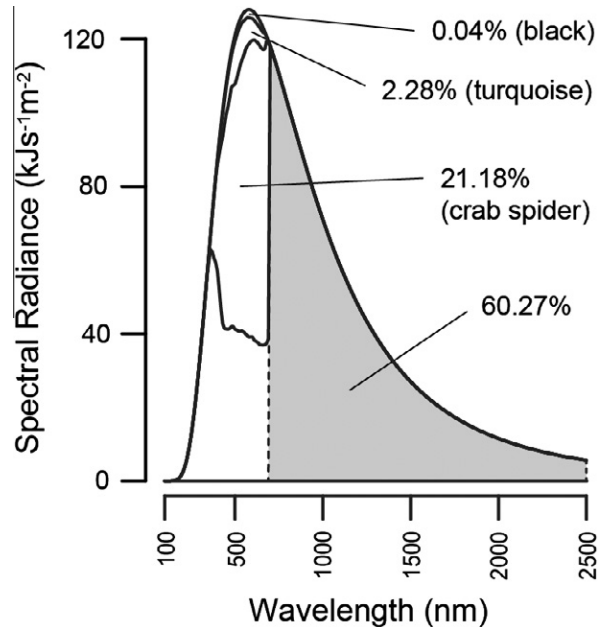
Symbol	Definition	Units	Estimate	Equation
$A_s$	Surface area of grasshopper	m <sup>2</sup>	$2.38 \times 10^{-4}$	(7), (8)
$A_p$	Planar area of grasshopper	m <sup>2</sup>	$5.5 \times 10^{-5}$	(4), (6)
$\alpha_{sw}$	Short wave absorptivity	Proportion	1 (black phase), 0.988 (blue phase), 0.718 (white crab spider)	(4), (5)
$c$	Speed of light	m s <sup>-2</sup>	$2.998 \times 10^8$	(6)
$\epsilon_{lw,a}$	Long wave emissivity of air	Dimensionless	0.97	(7)
$\epsilon_{lw,g}$	Long wave emissivity of grasshopper	Dimensionless	0.97	(7)
$h$	Planck's constant	J s	$6.62606896 \times 10^{-34}$	(6)
$hc$	Heat transfer coefficient	W m <sup>-2</sup> K <sup>-1</sup>	Eq. (8)	(8)
$I_{sw}$	Solar irradiance	W m <sup>-2</sup>	1300	(4)
$m$	Grasshopper body mass	g	0.2	(9)
$\sigma$	Stefan–Boltzmann constant	W m <sup>-2</sup> K <sup>-4</sup>	$1.3806504 \times 10^{-23}$	(6), (7)
$T$	Surface temperature of sun	K	5000	(6)
$T_a$	Air temperature range	K	273–323 (0–50 °C)	(7), (8)
$T_g$	Grasshopper body temperature	K	Estimated variable	(7), (8)
$u$	Wind speed range	m s <sup>-1</sup>	0.1–100	(9)
$V_s$	Portion of $A_g$ that sees sky	m <sup>2</sup>	$5.95 \times 10^{-5}$	(7)



**Fig. 3.** Heating curves of grasshoppers in turquoise and black phases. (A) Heating curves of black phase grasshoppers ( $n = 6$ ) and turquoise phase ( $n = 7$ ) grasshoppers for ten minutes of exposure to the sun with an initial body temperature of  $5\text{ }^{\circ}\text{C}$  (grey curves) and best-fit exponential model (black and dashed curves). Vertical axis represents grasshopper body temperature standardized by ambient temperature. (B) individual (fine solid lines) and average (heavy solid lines) heating curves for grasshoppers in the turquoise (grey lines) and black (black lines) phases. Ambient temperature is included in dashed lines.

$$I(\lambda) = \frac{2hc^2}{\lambda^5} \frac{1}{e^{\frac{hc}{\lambda T}} - 1} \quad (6)$$

where  $h$  is Planck's constant,  $c$  is the speed of light,  $s$  is the Stefan-Boltzmann constant, and  $T$  is the surface temperature of the sun (Table 2). The grasshopper absorbance function was derived empirically by measuring the average reflectance spectra of the same five male grasshoppers in the bright turquoise (hot) condition and then in the black (cold) condition using an Ocean Optics 2000 spectrophotometer (USB2000 with PX-2 light source Ocean Optics Inc., Dunedin, USA). By keeping them at  $4\text{ }^{\circ}\text{C}$  overnight, we ensured grasshoppers were at their darkest colour prior to measurement. Bright turquoise grasshoppers had been maintained at  $30\text{ }^{\circ}\text{C}$  for 5 h. Colour was measured as in Umbers (2011). Integration of Eq.



**Fig. 4.** Median short-wave reflectance of incoming solar radiation over the visual spectrum. The proportions indicated on the graph refer to the amount of the total solar energy reflected for the various spectra modeled: backphase grasshopper reflectance (0.04%), turquoise phase grasshopper reflectance (2.28%) and crab spider reflectance (21.18%). Grey region illustrates the unmeasured near-infrared region.

(5) was calculated iteratively from  $\lambda_1 = 100\text{ nm}$  to  $\lambda_2 = 2500\text{ nm}$  based on the spectral increments outputted by the spectrophotometer ( $\sim 0.35\text{ nm}$ ) using the software package R (R Core Development Team 2009). Due to logistical and equipment constraints, we could not empirically measure grasshopper reflectance above  $700\text{ nm}$  (near-infrared). The spectral range measured adequately quantified differences in visible colour between the two body-colour phases. However, how unmeasured longer wavelengths might differentially influence body temperatures in the two colour morphs is unclear. Nonetheless, we modelled several conservative scenarios in order to understand the potential influence of near-infrared reflectance on our model conclusions (see discussion of scenarios, below).

Long-wave energy flux, the rate at which energy is radiated between a grasshopper and the sky, was modeled similarly to Denny and Harley (2006):

$$W_{lw} = A_s V_s \epsilon_{lw,g} \sigma (\epsilon_{lw,a} T_a^4 - T_g^4) \quad (7)$$

where  $A_s$  is the surface area of the grasshopper,  $V_s$  is the proportion of that area that is exposed directly to the sky,  $\epsilon_{lw,g}$  is the long-wave emissivity of the grasshopper's integument,  $\epsilon_{lw,a}$  is the long-wave emissivity of the air,  $\sigma$  is the Stefan-Boltzmann constant, and  $T_g$  and  $T_a$  are the grasshopper and air temperatures, respectively. *K. tristis* have not been observed to expose their lateral surface to the sun as seen in other Acrididae (Chappell and Whitman, 1990). The long-wave equation assumes that there is equal radiative exchange between the grasshopper and the ground (i.e., they have approximately the same temperature and emissivity) at equilibrium. We also assumed that a grasshopper's surface area exposed to the sky is not behaviourally altered.

The rate at which a grasshopper gains or loses heat convectively (i.e., with air) was also modeled similarly to Denny and Harley (2006):

$$W_{cv} = h_c A_s (T_a - T_g) \quad (8)$$

where  $h_c$  is the heat transfer coefficient,  $A_s$  is the surface area of the grasshopper in contact with the air (equal to  $A_g$ ), and  $T_a - T_g$  is the

difference between air and grasshopper body temperatures. We use the empirically-derived equation for the heat transfer coefficient from Lactin and Johnson (1998) for grasshoppers, which is a function of wind speed  $u$  ( $\text{m s}^{-1}$ ) and body mass  $m$  (g):

$$h_c = e^{2.679 - 0.37981 \ln m + 0.5659 \ln u} \quad (9)$$

The minimum of speed  $u$  is bounded by  $0.1 \text{ m s}^{-1}$ , due to surface micro-layer heat convection adjacent to grasshopper integument during still wind conditions (Lactin and Johnson, 1998). Five adult grasshoppers were weighed resulting in a mean body mass of approximately  $m = 0.2 \text{ g}$ .

Grasshopper body temperature was modeled across a continuum of air temperature, wind speed and near-infrared reflectance scenarios. For a given combination, grasshopper temperature was estimated by finding the best-fit body temperature  $T_b$  using the *optimise* function in the software package R (R Core Development Team 2009). We modeled the difference in body temperatures for the two grasshopper colour phases for a broad range of environmental scenarios (i.e.,  $0.1\text{--}10 \text{ m s}^{-1}$  wind speeds, and  $0\text{--}45 \text{ }^\circ\text{C}$  ambient air temperatures). To understand the influence of the unmeasured near-infrared reflectance, we selected the environmental scenario with the greatest temperature difference between phases (i.e.,  $0.1 \text{ m s}^{-1}$  wind speed,  $0 \text{ }^\circ\text{C}$ ) and then re-calculated temperature differences when allowing each phase to range independently from 0 to 100% reflectance in this spectral range. We ran two additional scenarios to investigate the conditions under which grasshopper colour might more strongly influence body temperature. First, we sourced empirically measured spectra of highly a reflective arthropod with a similar surface area to volume ratio, the crab spider (*Thomisus spectabilis*: Thomisidae) and substituted its reflectance spectrum to the turquoise grasshopper phase. Second, we allowed a bright grasshopper to reflect 100% of the incoming solar radiation across the whole visual range (up to  $700 \text{ nm}$ ).

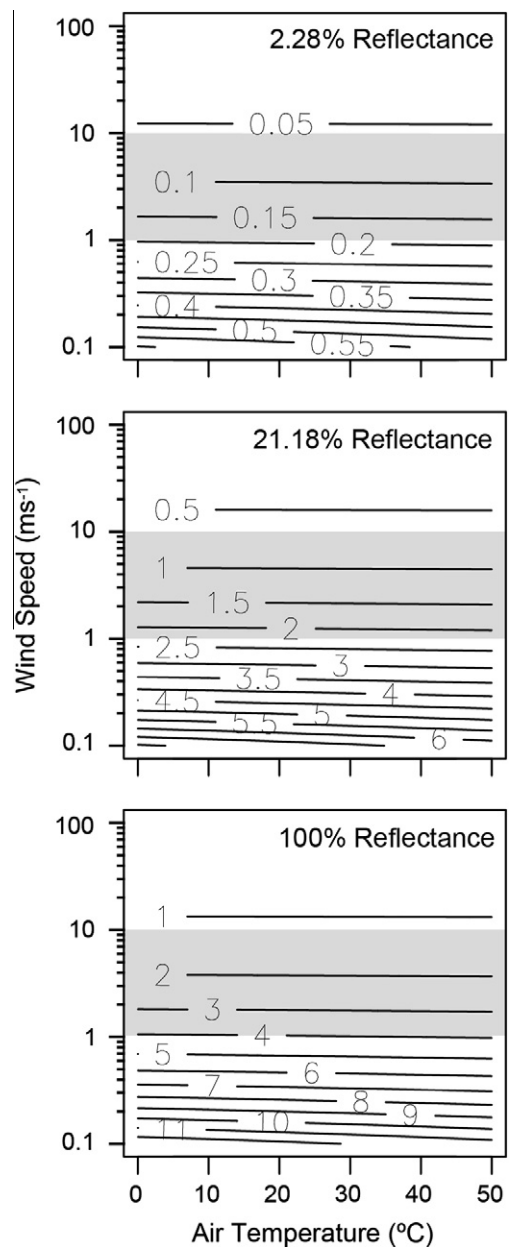
### 3. Results

#### 3.1. Empirical measurements

Based on internal temperature data measured with thermocouples, cold black and cold turquoise grasshoppers reached statistically indistinguishable asymptotic body temperatures in the sun, with mean body to air temperature ratios  $s = 1.29$  for the turquoise phase and  $s = 1.22$  for the black phase ( $t_{9,09} = -0.85$ ,  $p = 0.42$ ,  $n = 6$ ) – the black phase was not significantly higher than turquoise in asymptotic temperature. The rate at which grasshoppers in different colour phases heated up was also statistically indistinguishable, with a mean heating rate of  $k = 0.51$  for the turquoise phase and  $k = 0.65$  for the black phase ( $t_{9,09} = 1.51$ ,  $p = 0.16$ ) (Fig. 3a and b).

#### 3.2. Heat budget model

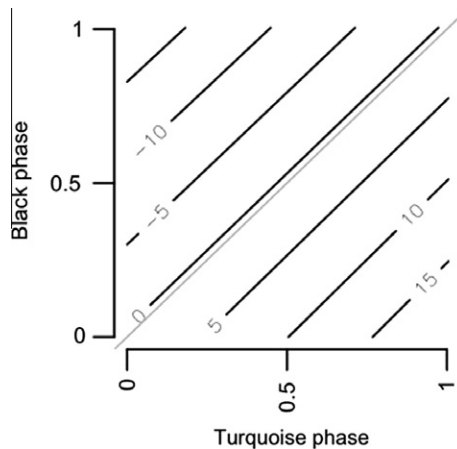
The average empirically measured reflectance spectrum of *K. tristis* in the turquoise phase reflected 2.28% of incoming radiation between wavelengths of 350 and  $700 \text{ nm}$ . Black phase grasshoppers absorbed almost all the incoming radiation (0.04% reflectance) (Fig. 4). Subsequently, the heat budget model showed that differences between the black and turquoise phases are small even when considering a wide range of wind speeds and ambient air temperatures (Fig. 5). The greatest temperature difference between phases was approximately  $0.55 \text{ }^\circ\text{C}$ , which occurred when wind speed, and thus convective heat loss, was  $0.1 \text{ m s}^{-1}$  (Fig. 5A). As wind speed increased (from  $1$  to  $10 \text{ m s}^{-1}$ ) the effect of colour on internal temperature is reduced and the differences in body temperature for different colours ranged between  $0.05$  and  $0.18 \text{ }^\circ\text{C}$



**Fig. 5.** Modeled differences in body temperatures between the black grasshopper phase and (A) the turquoise phase, (B) bright white crab spider, and (C) 100% visual spectrum reflectance at equilibrium. In this model, we assume that both phases reflect the same amount of near-infrared radiation. Therefore, differences are independent of the amount of the near-infrared spectrum reflected. We also assume wind speed  $> 0.1 \text{ m s}^{-1}$  because of convection at the body surface. Grey bands represent the range of typical wind conditions for the study location. Numbers within solid lines represent internal grasshopper body temperature at various wind speeds and ambient temperatures. Lines depicting differences at low wind speeds decline slightly with increasing air temperature because ambient temperature has a stronger effect when wind is low.

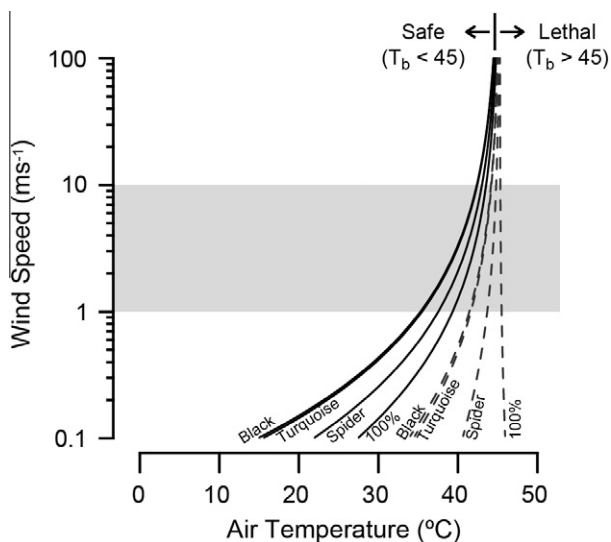
(Fig. 5A). Assuming that the two grasshopper colour phases reflect the unmeasured near-infrared irradiance similarly (although further empirical tests are required to determine if this is the case), the proportion of reflectance across this range had a non-detectable effect on temperature differences between phases (Fig. 6). Where reflectance was considered as that of the crab spider (*T. spectabilis*) and 100% across the whole visible range (100% reflectance), differences in body temperature between phases increased to  $0.70\text{--}2 \text{ }^\circ\text{C}$  and  $1\text{--}4 \text{ }^\circ\text{C}$ , respectively (Figs. 5B and C). Finally, when assuming that the upper limit of non-lethal body





**Fig. 6.** The difference in body temperature (contours; °C) between the turquoise and black grasshopper colour phases at different proportions of near infrared reflectance (a maximum of 60.27% of total reflectance; Fig. 5). The grey line represents a scenario where near-infrared reflection is the same for both phases and illustrates a consistent difference of ca. +0.6 °C for the black phase.

temperature in this grasshopper was approximately 45 °C (May, 1979; Chappell and Whitman, 1990), the model estimated turquoise and black grasshoppers to be approximately equally vulnerable to reaching lethal temperatures (over 45 °C) for all combinations of environmental conditions (Fig. 6); although, where these lethal thresholds occurred for both phases depended on the amount of near-infrared reflected and whether it was different for different colours (Fig. 7).



**Fig. 7.** Survival threshold curves at thermal equilibrium showing the influence of visual plus near-infrared reflectance and environmental conditions on lethal body temperature. Solid curves represent no near-infrared reflectance and dashed curves represent 100% near-infrared reflectance. Labelled curves represent different scenarios: black and turquoise phase grasshoppers; the bright white crab spider; and the 100% reflectance of the visible wavelengths. After equilibrium, grasshoppers to the right of a curve would have body temperatures greater than 45 °C for the given ambient conditions. The modeled threshold for the black vs. turquoise phases are on the whole indistinguishable regardless of near-infrared scenario. Grey band represents the typical range of wind conditions at the study location.

#### 4. Discussion

We found no clear support for Key and Day's (1954a,b) hypothesis that the black and turquoise colour phases in the chameleon grasshopper have a substantial thermoregulatory function. Our empirical data showed no clear difference in the rate of heating between black and turquoise colour phases (Fig. 3). Instead, the heating curves show variation independent of colour phase, suggesting that small fluctuations in air temperature and wind speed during our controlled experiments were more important in grasshopper body temperature than colour phase (Fig. 3). Our theoretical model indicates that the maximum temperature difference between the two colour phases on a still day is 0.55 °C (Fig. 5A). This difference becomes smaller with increasing wind speed. It is unknown whether a 0.55 °C difference in body temperature as a result of colour change is adaptively significant in the chameleon grasshopper. Since only males change colour, any fitness gain must be sex specific. The sexually dimorphic nature of colour change in *K. tristis* may instead suggest that males advertise some measure of quality to females or their fighting abilities to other males (Umbers et al., 2012; Umbers et al., submitted for publication). Like *K. tristis*, the damselfly *Austrolestes annulosus* also changes colour in response to temperature. In *A. annulosus*, Veron (1974) investigated the role of colour change in thermoregulation and found a similar result to ours: when in the turquoise phase, damselflies were 0.23 °C cooler than when black. Veron concluded that colour phase had little influence over body temperature, but did not test for potential fitness consequences of the difference.

Can our study generalise about the role of colour in insect thermoregulation? Our heat budget model, while parameterised for the chameleon grasshopper, is describing thermodynamic processes that should apply to insects of similar shape, size, physiology and behaviour. The model indicates that colour has the greatest thermoregulatory effect in such insects when its colour is extremely bright (reflects strongly across most of the electromagnetic spectrum), air temperature is low, and when there is very little or no wind. Our model was developed to estimate body temperatures of animals with the major part of their surface area surrounding their tagmata (body segments), resulting in a relatively low surface area to volume ratio. Our model would need to be specifically adapted for insects with a high surface area to volume ratio, such as flat arthropods or those with broad, thin appendages (e.g., butterflies). In natural field scenarios, our experimental and model data indicate that factors such as wind speed, sun patchiness, humidity and altitude may swamp any temperature differences based on colour in insects even when reflectance is high (Fig. 5). The reflectance of the white crab spider (*T. spectabilis*) results in a body temperature between 0.75 and 2.25 °C cooler than a hypothetical black morph, which does not exist in this species. In a hypothetical wind-free environment, the white morph would be up to 6 °C cooler (Fig. 5B). Six degrees may be important for an insect or spider, allowing the brightest morphs to remain in the sun (e.g., on top of a flower hunting), rather than having to shuttle into the shade (e.g., underside of the petals) to avoid reaching lethal thermal limits. This reinforces the importance of wind speed and microhabitat choice in understanding the likely magnitude of temperature and fitness consequences of colouration.

"Hotter is better" (Kingsolver and Huey 2008, p. 251) and "darker is hotter," but the question that researchers have rarely attempted to address is: does colour cause a temperature advantage that is significantly better? The answer depends on whether the temperature difference, no matter how small, results in a fitness benefit. If we are to understand exactly which temperature differences, and hence colour differences are important to insects, then we must directly measure the fitness consequences.

We propose three non-exclusive approaches that may be important in future studies on the function of insect colour in thermoregulation. First, a multi-taxon analysis of reflectance across a broad range of insects (Willmer and Unwin, 1981) will help determine if the broad patterns of colour and body temperature fit colour-mediated thermoregulation predictions. Second, targeting insects that reversibly change colour may control for crucial factors compared to insects with static colour. Finally, it is important to consider alternative explanations when considering the function of colours in insects rather than thermoregulation in isolation.

### Acknowledgments

Thanks to Chris Harley for helpful discussion regarding the development of the heat budget model, Bernd Heinrich, Nemanja Jovanovic, Graham Marshall and Aaron Harmer for helpful discussion of optics theory, James O'Hanlon and Michelle Vines for assistance in the laboratory, Felipe Gawryszewski for crab spider reflectance spectra, Gregory Sword for the loan of a thermal imaging camera and Michael Kearney, Joel Kingsolver, Darrell Kemp, Mark Westoby, Daniel Falster, Judith Bronstein, Michael Angilletta, Gregory Sword, Devi Stuart-Fox, Lisa Schwanz, Hanna Kokko and several anonymous reviewers for constructive feedback on earlier versions of this manuscript. We acknowledge funding from Macquarie University's Research Excellence Scholarship.

### References

- Anderson, R.V., Tracy, C.R., Abramsky, Z., 1979. Habitat selection in two species of short-horned grasshoppers: the role of thermal and hydric stresses. *Oecologia* 38, 359–374.
- Brakefield, P.M., Willmer, P.G., 1985. The basis of thermal melanism in the ladybird *Adalia bipunctata* differences in the reflectance and thermal properties between the morphs. *Heredity* 54, 9–14.
- Buxton, P.A., 1924. Heat, moisture and animal life in deserts. *Proceedings of the Royal Society B* 96, 123–131.
- Casey, T.M., 1981. Behavioral mechanisms of thermoregulation. John Wiley and Sons, Toronto.
- Chappell, M.A., Whitman, D.W., 1990. Grasshopper thermoregulation. In: *Biology of Grasshoppers*. John Wiley and Sons, New York, pp. 143–172.
- Church, N.S., 1960. Heat loss and the body temperatures of flying insects. II. Heat conduction within the body and its loss by radiation and convection. *Journal of Experimental Biology* 37, 186–212.
- Denny, M.W., Harley, C.D.G., 2006. Hot limpets: predicting body temperature in a conductance-mediated thermal system. *Journal of Experimental Biology* 209, 2409–2419.
- Digby, P.S.B., 1955. Factors affecting the temperature excess of insects in sunshine. *Journal of Experimental Biology* 32, 279–298.
- Dixon, A.F.G., 1972. Control and significance of the seasonal development of color forms in the sycamore aphid *Drepanosiphum platanoides*. *Journal of Animal Ecology* 41, 689–697.
- Edney, E.B., 1971. The body temperature of Tenebrionid beetles in the Namib Desert of Southern Africa. *Journal of Experimental Biology* 55, 253–272.
- Evans, D.L., Schmidt, J.O. (Eds.), 1990. *Insect defenses: adaptive mechanisms and strategies of prey and predators*. SUNY Press, Albany, New York.
- Forsman, A., 1997. Thermal capacity of different colour morphs in the pygmy grasshopper *Tetrix subulata*. *Annales Zoologici Fennici* 34, 145–149.
- Gates, D.M., 1980. *Biophysical Ecology*. Dover Publications, New York.
- Green, K., Osborne, M.J., 1994. *Wildlife of the Australian snow-country*. Reed Books, Chatswood.
- Guppy, C.S., 1986. The adaptive significance of alpine melanism in the butterfly *Parnassius phoebus* Lepidoptera: Papilionidae. *Oecologia* 70, 205–213.
- Hadley, N.F., 1970. Micrometeorology and energy exchange in two desert arthropods. *Oecologia* 51, 434–444.
- Heinrich, B., 1996. *The Thermal Warriors: Strategies of Insect Survival*. Harvard University Press.
- Henwood, K., 1975. A field tested thermoregulation model for two diurnal Namib Desert South-West Africa Tenebrionid beetles. *Ecology* 56, 1329–1342.
- Joern, A., 1981. Importance of behavior and coloration in the control of body temperature by *Brachystola magna* Orthoptera: Acrididae. *Acrida* 10, 117–130.
- Key, K.H.L., Day, M.F., 1954a. The physiological mechanism of colour change in the grasshopper, *Kosciuscola tristis* Sjöst. (Orthoptera: Acrididae). *Australian Journal of Zoology* 2, 340–363.
- Key, K.H.L., Day, M.F., 1954b. A temperature-controlled physiological colour response in the grasshopper, *Kosciuscola tristis* Sjöst. (Orthoptera: Acrididae). *Australian Journal of Zoology* 2, 309–339.
- Kingsolver, J.G., 1987. Predation, thermoregulation and wing color in Pierid butterflies. *Oecologia* 73, 301–306.
- Kingsolver, J.G., Huey, R.B., 2008. Size, temperature, and fitness: three rules. *Evolutionary Ecology Research* 10, 251–268.
- Lactin, D.J., Johnson, D.L., 1998. Convective heat loss and change in body temperature of grasshopper and locust nymphs: relative importance of wind speed, insect size and insect orientation. *Journal of Thermal Biology* 23, 5–13.
- Loveridge, J.P., 1968. The control of water loss in *Locusta migratoria migratorioides*. I. Cuticular water loss. *Journal of Experimental Biology* 49, 1–13.
- Makings, P., Saeed, F.N., 1989. Influence of Slifer's patches on survival and behavior of locusts *Locusta migratoria migratorioides* at a high temperature. *Journal of Insect Physiology* 35, 363–368.
- May, M.L., 1979. Insect thermoregulation. *Annual Review of Entomology* 24, 313–349.
- Muggleton, J., 1978. Selection against the melanic morphs of *Adalia bipunctata* two-spot ladybird: a review and some new data. *Heredity* 40, 269–280.
- Parry, D., 1951. Factors determining the temperature of terrestrial arthropods in sunlight. *Journal of Experimental Biology* 28, 445–462.
- Pepper, J.H., Hastings, E., 1952. The effects of solar radiation on grasshopper temperatures and activities. *Ecology* 33, 96–103.
- Porter, W.P., Kearney, M., 2009. Colloquium papers: size, shape, and the thermal niche of endotherms. *Proceedings of the National Academy of Sciences* 106, 19666–19672.
- Punzalan, D., Rodd, F.H., Rowe, L., 2008. Contemporary sexual selection on sexually dimorphic traits in the ambush bug *Phymata americana*. *Behavioral Ecology* 19, 860–870.
- Roland, J., 1982. Melanism and diel activity of alpine *Colias* Lepidoptera: Pieridae. *Oecologia* 53, 214–221.
- Sjöstedt, Y., 1933. Neue australische acrididen. *Arkiv för Zoology* 26A, 1–9.
- Slifer, E.H., 1953a. The pattern of specialized heat-sensitive areas on the surface of the body of Acrididae (Orthoptera). II. The females. *Transactions of the American Entomological Society* 79, 69–97.
- Slifer, E.H., 1953b. The pattern of specialized heat-sensitive areas on the surface of the body of Acrididae (Orthoptera). I. The males. *Transactions of the American Entomological Society* 79, 37–68.
- Stevenson, R.D., 1985. Body size and limits to the daily range of body temperature in terrestrial ectotherms. *American Naturalist* 125, 102–117.
- Stewart, L.A., Dixon, A.F.G., 1989. Why big species of ladybird beetles are not melanic. *Functional Ecology* 3, 165–171.
- Stower, W.J., Griffiths, J.F., 1966. The body temperature of the desert locust *Schistocerca gregaria*. *Entomologia Experimentalis et Applicata* 9, 127–178.
- Umbers, K.D.L., 2011. Turn the temperature to turquoise: cues for colour change in the male chameleon grasshopper (*Kosciuscola tristis*) (Orthoptera: Acrididae). *Journal of Insect Physiology* 57, 1198–1204.
- Umbers, K.D.L., Tatarnic, N.J., Holwell, G.I., Herberstein, M.E., in press. Ferocious fighting in a grasshopper. *PLoS ONE*. <http://dx.doi.org/10.1371/journal.pone.0049600>.
- Umbers, K.D.L., Tatarnic, N.J., Holwell, G.I., Herberstein, M.E., submitted for publication. Bright turquoise as an intraspecific signal in the chameleon grasshopper (*Kosciuscola tristis*). *Behavioural Ecology and Sociobiology*.
- Veron, J.E.N., 1974. The role of physiological colour change in the thermoregulation of *Austrolestes annulosus* (Selys) (Odonata). *Australian Journal of Zoology* 22, 457–469.
- Watt, W.B., 1969. Adaptive significance of pigment polymorphisms in *Colias* butterflies Part 2: thermoregulation and photoperiodically controlled melanin variation in *Colias eurytheme*. *Proceedings of the National Academy of Sciences of the United States of America* 63, 767–774.
- Whitman, D.W., 1988. Function and evolution of thermoregulation in the desert grasshopper *Taeniopoda eques*. *Journal of Animal Ecology* 57, 369–383.
- Willmer, P.G., Unwin, D.M., 1981. Field analysis of insect heat budgets reflectance, size and heating rates. *Oecologia* 50, 250–255.