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Turn the temperature to turquoise: Cues for colour change in the male chameleon grasshopper (*Kosciuscola tristis*) (Orthoptera: Acrididae)

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ABSTRACT

Rapid, reversible colour change is unusual in animals, but is a feature of male chameleon grasshoppers (*Kosciuscola tristis*). Understanding what triggers this colour change is paramount to developing hypotheses explaining its evolutionary significance. In a series of manipulative experiments the author quantified the effects of temperature, and time of day, as well as internal body temperature, on the colour of male *K. tristis*. The results suggest that male chameleon grasshoppers change colour primarily in response to temperature and that the rate of colour change varies considerably, with the change from black to turquoise occurring up to 10 times faster than the reverse. Body temperature changed quickly (within 10 min) in response to changes in ambient temperature, but colour change did not match this speed and thus colour is decoupled from internal temperature. This indicates that male colour change is driven primarily by ambient temperature but that their colour does not necessarily reflect current internal temperature. I propose several functional hypotheses for male colour change in *K. tristis*.

1. Introduction

Fast, reversible colour change in animals is an unusual trait, that can be influenced by many different mechanisms and have a variety of functions. Colour change may be used in communication within or between species, or be a consequence of physiological change triggered by internal, rather than external cues (Fitzstephens and Getty, 2000). The functions, mechanisms and cues involved in colour change are inextricably linked, and these must be independently manipulated if we are to fully understand the evolution of colour change.

Colour change in some species may have a specific function and associated fitness benefit (ultimate cause). For example, it may function as a signal in mate attraction, an aggressive anti-predator display or function to deter rivals. Colour change for aggressive display occurs in the Panamanian Tortoise Beetle (*Charidotella egregia*), which changes from gold to red when disturbed (Vigneron et al., 2007). In the giant cuttlefish (*Sepia apama*), spectacular colour and pattern change displays are important in signalling gender and have been implicated in the evolution of alternative mating strategies. Some small males display as a female to sneak past dominant males in order to acquire mates (Hanlon et al., 2005). Colour change may also reduce the likelihood of detection through active mimicry or crypsis. When exposed to increased predation risk, fiddler crabs (*Uca vomeris*) change colour from blue and white to a cryptic mottled brown, which allows them to blend in with the mottled brown substrate of their mud-flat homes (Hemmi et al., 2006). How quickly these colour changes can occur is dependent upon the proximate mechanisms in place.

The mechanisms of colour change (proximate causes) are the physiological processes that shift an animal's hue. Colour change may be unidirectional throughout the life of an individual, or reversible over minutes, days or seasons (Fox and Ververs, 1960). An animal's colours may change through many mechanisms including: the sequestration of pigments acquired from its environment, the physiological construction or deconstruction of pigments, via nanoscale sphere migration, or by changing the angle and positioning of multi layer reflectors or any other structures or chemicals that interfere with light (Vigneron et al., 2007; Hill and Montgomerie, 1994; Vigneron and Simonis, 2010).

Proximate mechanisms of colour change can generally be assigned to one of two categories, morphological colour change or physiological colour change (Bradbury and Vehrencamp, 1998). Physiological colour change can occur very quickly, over hours or even milliseconds (Fox and Ververs, 1960). Such physiological changes are often the result of small structures moving in or on an organism's integument, or via the dilation of apertures that change the way light is reflected (Hanlon and Messenger, 1996; Hadley and Goldman, 1969). Only a handful of insects can change their body colour quickly. These include the laboratory stick insect (*Carausius morosus*), which changes colour via both morphological and physiological pathways in response to a combination of temperature and circadian rhythm (Prestwich, 1985). When disturbed,

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the Panamanian tortoise beetle (*C. egregia*) changes colour by minute reflectors, whose hue are altered by absorbing liquid and then quickly expelling it (Vigneron et al., 2007). Similarly the longhorn beetle (*Tmesisternus isabellae*) changes colour when wet via swelling of the space between microscopic grating structures in its integument (Liu et al., 2009). In some genera of Australian damselflies, males turn blue via the stratification and migration of intracellular granules (e.g. whitewater rockmasters, *Diphlebia lestoides* Veron, 1974; Prum et al., 2004). While the mechanisms of colour change for some species are well documented, the cues that trigger these processes are often unclear. Colour change may occur in response to several different cues, which could be biotic, abiotic, or a combination of both (Boughman, 2007; Stuart-Fox et al., 2008).

Cues for colour change are events or conditions that stimulate an individual to change its colour. While cues are sometimes internal, such as the release of hormones at sexual maturation, they can also come from external stimuli such as encounters with conspecifics, competitors, predators or prey (Hanlon and Messenger, 1996; Stuart-Fox et al., 2008). Colours may also be changed in response to substrate hue or patterning, which in turn may be driven by abiotic factors such as changing seasons, precipitation patterns, light intensity or time of day (e.g. Veron, 1974).

Male chameleon grasshoppers (*K. tristis*; Orthoptera: Acrididae; Sjösted 1933) can change their body colour over several minutes (Key and Day, 1954a,b). Grasshoppers are black when ambient temperatures are below 15 °C, and when the ambient temperature reaches 25 °C the head, pronotum and abdomen turn turquoise (Key and Day, 1954a,b; Fig. 1). Under laboratory conditions, Key and Day (1954b) showed that *K. tristis*' colour change has clear switch points. At 10 °C grasshoppers begin to change from black to turquoise and are of intermediate colouration at 15 °C. The colour change continues rapidly until 25 °C when it slows, and is complete at 30 °C. Key and Day also showed that colour change is independent of grasshopper density, unlike the much slower and irreversible colour changes observed in some other Acrididae (e.g. Sword et al., 2000). Further, colour change in *K. tristis* is not under any centralized neural or hormonal control. This has been demonstrated by exposing different body segments of living males to disparate temperature regimes: an intact, living grasshopper can have a hot, turquoise head, and a cold black body (or the reverse) (Key and Day, 1954b). Freshly killed grasshoppers and excised fragments of exoskeleton will retain the ability to change colour several hours post mortem (Key and Day, 1954b). Key and Day did not suggest a mechanism for the colour change but proposed that it may function in thermoregulation.

Filshie et al. (1975) proposed that colour change in male *K. tristis* is caused by the migration of intracellular granules immediately below the cuticular surface (they did not assay females). In the black (cold) phase, large and small granules are haphazardly interspersed throughout the cell (Filshie et al., 1975). In the turquoise (warm) phase the small, highly reflective granules – a few hundred nanometres in diameter – concentrate distally in cells just below the exoskeleton. Consequently, the small and large granules become stratified, the former over the latter. When the grasshopper becomes cold, the granules disperse throughout the cell once more. This mechanism is strikingly similar to those described for some colour-changing damselflies (Veron, 1974). Despite the unusual nature of this colour change, its proposed mechanism and its unknown functional significance, Filshie et al.'s (1975) work was the last on the chameleon grasshopper until the present study.

Previous studies on the chameleon grasshopper did not attempt to distinguish between the possible roles of temperature and time of day in explaining the resulting colour change. Additionally, in the absence of modern spectrophotometric technology they could not quantify the colour change accurately. Spectral measurements allow objective and accurate quantification of colour, and are important for understanding the evolution of colour traits. In this study I quantified the role of temperature and time of day in the chameleon grasshopper's colour change through a suite of manipulative experiments. I tested whether time of day with external cues (sunlight), either independently or in concert with temperature, caused grasshopper colour to change. I then removed

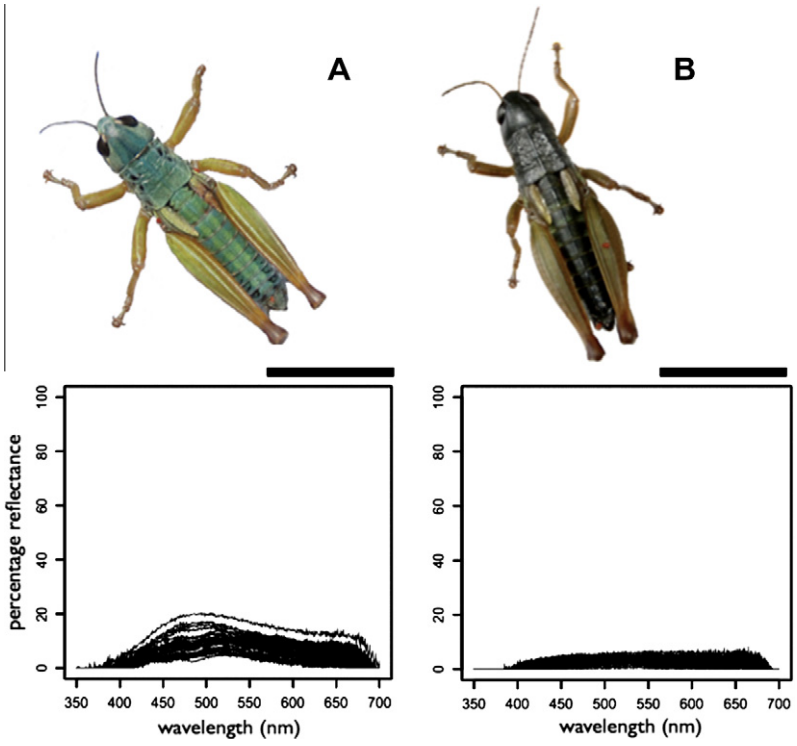

Fig. 1. An example of male *Kosciuscola tristis* colouration and spectra when over 25 °C (a) and under 10 °C (b).

environmental cues of time of day (sunlight) and I retested the effects of time of day and temperature. I also quantified the speed at which grasshoppers change colour in both directions (black to turquoise and turquoise to black) as well as the speed at which their body temperature reached equilibrium with ambient temperature. Finally, I synthesised these data to generate several functional hypotheses for the role of this colour change.

2. Materials and methods

This study was designed to experimentally quantify the effects of temperature, and time of day on colour change in male chameleon grasshoppers, using a four-pronged manipulative approach: (1) the effect of temperature and time of day with environmental cues present on grasshopper reflectance, (2) temperature and time of day without environmental cues present on grasshopper reflectance, (3) the speed of colour change, and (4) how quickly internal body temperature changes in response to ambient temperature.

2.1. Collection and pretreatment

Adult male *K. tristis* were collected at 1939 m altitude along the Dead Horse Gap walking track south of Thredbo Village on Alpine Way, NSW, Australia (36° 30 14.0 S 148° 16 36.7 E). Twelve new grasshoppers were collected in the afternoons of February 24th, 26th and 28th, 2008, the days immediately before each of the treatment replicates were conducted. Grasshoppers were returned to Thredbo Youth Hostel, Thredbo Village, where they were placed outside in individual enclosures (80 cm^3) after sunset. With the falling evening temperature grasshoppers returned to their black colour overnight. Grasshoppers were kept outside under these conditions for at least 12 h until their first spectral reading (at 05:00). Initial readings were always taken before sunrise.

2.2. Temperature and time of day experiments

For temperature and the time of day with cues experiments, the pretreatment procedure above was applied before randomly allocating grasshoppers to treatments. Grasshoppers were placed individually in experimental enclosures (30 cm × 30 cm × 15 cm), which were either clear plastic boxes with gauze panels in sides and top (temperature and cues present) or lightproof containers with loose screw-down lids for ventilation (square black plastic bottles 15 m × 15 cm × 10 cm) (temperature and cues absent). Each enclosure for the temperature and cues experiment was furnished with a thermometer and a 30 cm long piece of local vegetation (from the same large subalpine shrub – give species or a more detailed description) for substrate. This vegetation was not a food plant for the grasshoppers and they were never observed eating it. Grasshopper's enclosures were sprayed with water regularly throughout the experiment.

2.2.1. Temperature and time of day with environmental cues

After the pretreatment procedure, four grasshoppers were allocated to one of two treatments, hot or cold ($n = 8$). To manipulate temperature, cold treatments were exposed to a prevailing southerly wind while hot treatments were sheltered. Measurements of grasshopper colour and enclosure temperature were repeated five times over 14 h, at 5 am, 10 am, 2 pm, 5 pm and 9 pm. The colour of the dorsal surface of each grasshopper's pronotum and abdomen were measured five times at each time step (see Section 2.3), while the temperature of the enclosure was measured with an ethanol thermometer. This experiment was repeated over three days with a total of six grasshoppers per treatment ($n = 24$).

2.2.2. Temperature and time of day and without environmental cues

After collection and pretreatment, two grasshoppers were allocated to one of two treatment groups: hot or cold. These experiments were conducted in lightproof enclosures placed in a refrigerator or next to a gas heater for 29 hours (described above). Grasshopper colour and enclosure temperature were recorded six times over 29 h, at 05:00, 10:00, 14:00, 17:00, 21:00 on Day One and 09:00 on Day Two. This experiment was repeated over three days with a total of six grasshoppers per treatment ($n = 12$).

2.3. Reflectance spectra measurement

All colour measurements were taken using an Ocean Optics spectrophotometer (USB2000, Ocean Optics Inc., Dunedin, USA). A fibre optic cable (Ocean Optics Inc., Dunedin, USA) with incorporated light source (PX-2 light source, Ocean Optics Inc., Dunedin, USA) was connected to the spectrophotometer and when taking measurements was held at 45°. All samples were measured against a white (WS-1 Diffuse Reflectance Standard Ocean Optics Inc., Dunedin, USA; >98% reflectance from 250 to 1500 nm) and a black (black velvet; 0% reflectance) standard. This spectrometer is accurate between 350 and 700 nm and bins reflectance measurements at 0.33 nm intervals on average. We therefore measured reflectance over 350–700 nm. At each time step the colour of the dorsal surface of the pronotum and the dorsal surface of the abdomen was measured five times and averaged. For each measurement grasshoppers were placed on a bed of black velvet to reduce non-specific reflectance.

2.4. Reflectance spectra analysis

We calculated the reflectance of individuals as a proportion. Using R Software (ver. 2.8.1) we first integrated the reflectance curve of each spectra (five per part, per grasshopper, per time step) and then subtracted this from a reflectance curve at 100% reflectance from 350 to 700 nm. We then calculated the proportion reflectance for the average of five spectra for the pronotum and abdomen of each grasshopper at each time step as a representative value for the reflectance of the colour of the respective grasshopper body parts (pronotum and abdomen).

2.5. Speed of colour change

In this experiment I asked how long colour change from (1) black to turquoise and (2) from turquoise to black, took to complete. This question quantified how long it took for male grasshoppers to change colour from black to turquoise and *vice versa*. I exposed previously chilled grasshoppers (at 4 °C) to 35 °C, and grasshoppers previously kept at 42–10 °C degrees (Table 1). We ensured that the temperature differentials were very similar (31 and 32 °C), working within constrains of available equipment.

Table 1
Experimental design for speed of colour change experiment showing the number of experimental and control grasshoppers used in each replicate and the temperatures for each treatment.

Treatment (repeated four times)	Number of experimental grasshoppers	Number of control grasshoppers	Total (n)
Cold to hot (chamber temp.: 35 °C)	5 grasshoppers from 4 °C	1 grasshopper from 42 °C	20 (+4 controls)
Hot to cold (chamber temp.:10 °C)	5 grasshoppers from 42 °C	1 grasshopper from 4 °C	20 (+4 controls)

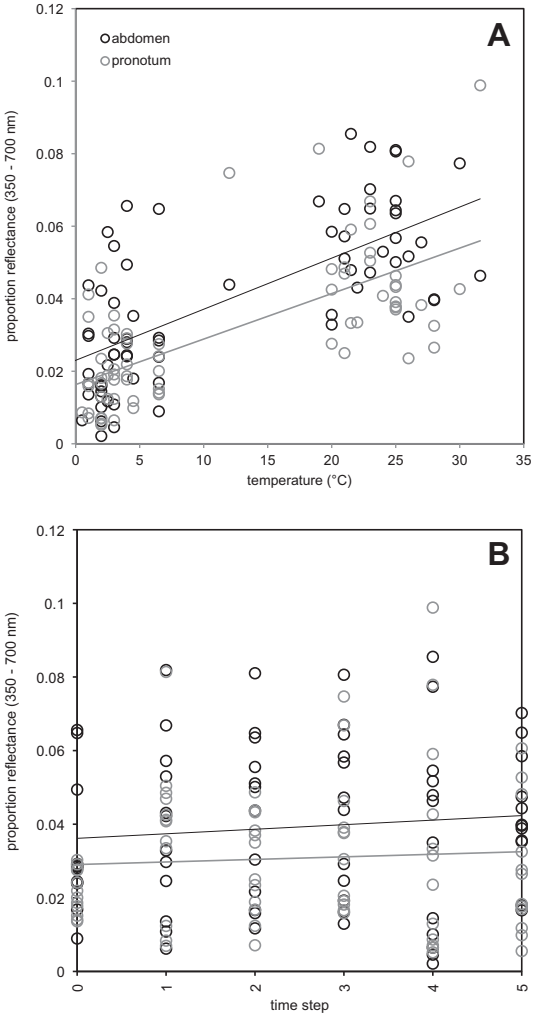

Fig. 2. The proportion of 100% reflectance (white) reflected by grasshoppers in relation to (a) temperature and (b) time of day, without environmental cues. Time steps are: 0 – 05:00, 1 – 09:00, 2 – 14:00, 3 – 17:00, 4 – 21:00 on Day One and 5 – 09:00 on Day Two.

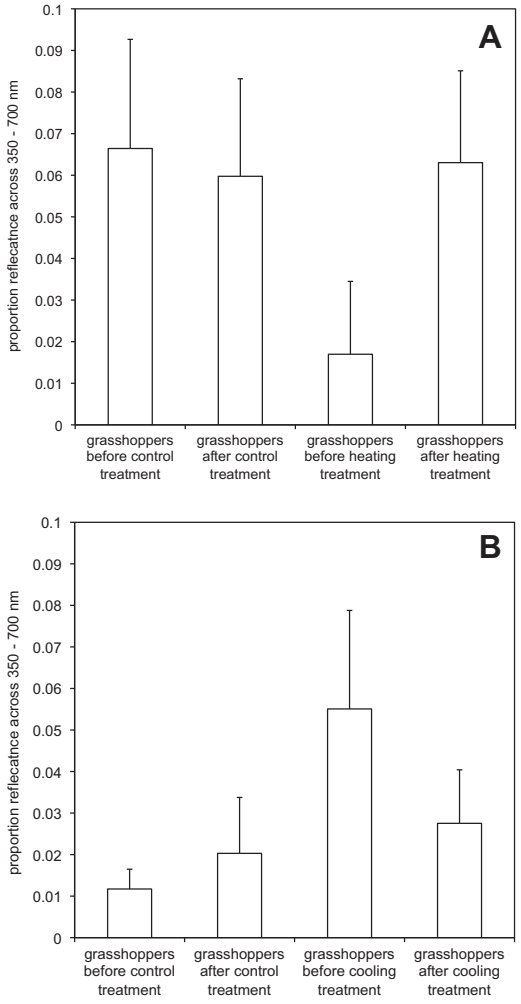

Fig. 3. Average ± SD grasshopper reflectance before and after treatment compared with control grasshoppers. In the heating experiment (a) grasshoppers rapidly matched the brightness of the control where as in the cooling experiment (b), grasshoppers took much longer even to approach a similar colour to the control.

Key and Day (1954b) showed that the absolute temperature, rather than temperature differential, is the effecter of colour change. The total time elapsed until they reached the turquoise or black of their control colour was subsequently recorded.

I collected a further 48 male chameleon grasshoppers from Dead Horse Gap, Thredbo Village, NSW, Australia, in 2008. Upon returning to the laboratory at Macquarie University, Sydney, Australia, I placed half of them in a temperature controlled cabinet at 42 °C and the other half in a cool room at 4 °C. Grasshoppers were contained in small nylon mesh enclosures, the latter at 4 °C over night, while the former were left at 42 °C for at least five hours (leaving grasshoppers at 42 °C for any longer resulted in premature death). A third, purpose-built temperature-controlled chamber integrated with a laboratory cooler, was maintained at 35 °C when running the heating treatment and 10 °C during the cooling treatment. As controls I used cold black animals in the cooling treatment and hot turquoise animals in the heating treatment. The control animals' colours did not change markedly over the course of the experiment and were used as standards for comparison with test animals.

For each replicate of the heating treatment, ten grasshoppers were taken from 4 °C (experimental) and one from 42 °C (control) and placed in the experimental chamber at 35 °C in individual gauze

Table 2
The average ± SD proportion of light reflected compared to 100% reflectance (white) by grasshopper's pronotum before and after treatments. The heating control treatment experienced high temperature before the experiment, while the grasshoppers in the experimental treatment experienced low temperature before the experiment and both were then transferred to 35 °C (Table 1). The cooling control was kept cold before the treatment and the experimental animals were kept hot before both being transferred to 10 °C (Table 1). In the heating treatment, the control remained bright throughout the observation period, while the experimental animals become much brighter when heated. In the cooling treatment the control was dark and remained dark throughout the experiment whereas the experimental animals became darker while cooling down.

	Cooling		Heating	
	Control	Experimental	Control	Experimental
Before treatment	0.015 ± 0.005	0.055 ± 0.024	0.066 ± 0.026	0.017 ± 0.018
After treatment	0.020 ± 0.014	0.028 ± 0.013	0.060 ± 0.024	0.063 ± 0.022

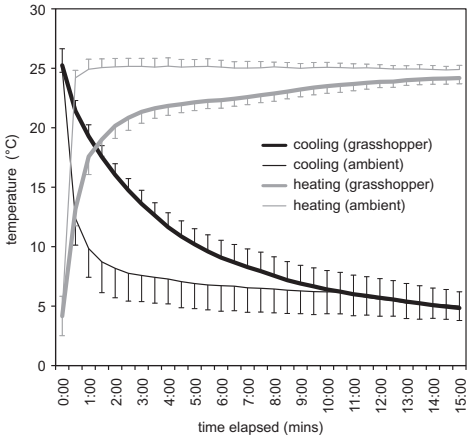

Fig. 4. The average (±SD) heating and cooling rates of grasshoppers when moved from 4 to 25 °C and *vice versa*. Ambient temperatures were measured by an exposed thermocouple probe while grasshopper temperatures were measured by a thermocouple probe implanted under the pronotum. Cooled grasshoppers' internal temperatures reached that of the ambient in around 10 min and the heated grasshoppers' internal temperatures approached within 1 °C of the ambient temperature in 15 min.

enclosures. Grasshoppers were measured approximately every five minutes with the spectrophotometer and compared with the control. A grasshopper's colour was always measured within one minute of removing it from the experimental chamber. This procedure was continued until each of the experimental grasshoppers had reached a similar colour to the control grasshopper. To compare their colour, I assessed spectral reflectance curves by eye. At this point, the amount of time the grasshopper had been in the temperature-controlled chamber was recorded. The colour of grasshoppers was measured using the procedure described above (see Section 2.3). This was repeated a further three times with new individuals (total $n = 20$). The same procedure was used for the cooling treatment (total $n = 20$), with the temperature controlled chamber maintained at 10 °C (Table 1).

2.6. Internal body temperature change

I compared the speed of colour change to changes in internal body temperature and ambient temperature, using a digital thermometer with two thermocouples (Digital Thermometer 206-3738 with 22 gauge K-type thermocouple chromel–alumel RS Components Ltd. Corby, Northants, UK). The end of one thermocouple was inserted under the posterior margin of the grasshopper's pronotum, parallel with its dorsal surface. The other was exposed to the ambient air temperature. Grasshoppers were first subjected to a heating or a cooling treatment and then the treatments were reversed. For the heating treatment, grasshoppers were placed in the refrigerator until their body temperature equilibrated with the ambient temperature (between 4 and 8 °C). They were then removed from the refrigerator into 25 °C, the ambient temperature in the laboratory. I immediately recorded the temperature change for both thermocouples every 30 s for the first 15 min out of the refrigerator. For the cooling treatment, the same grasshopper (now with a body temperature of 25 °C) and control thermocouple were returned to the fridge, and both thermocouple temperatures were recorded for the first 15 min ($n = 5$).

2.7. Statistical analysis

I fit a mixed model to analyse the effect of the treatments from both the time of day and temperature on grasshopper colour change. Grasshopper proportion reflectance was the response variable, individual was set as a randomized effect to account for repeated measures, and the model included the effects of time of day, temperature and time of day × temperature. Day was not included in the analyses as an effect because the variation between days (i.e. temperature) was directly accounted for in the analysis. All analyses were executed in JMP Ver. 5.0.1.2. Software (SAS Institute Inc.).

3. Results

3.1. Grasshopper reflectance with temperature and time of day with environmental cues

Grasshopper abdomen reflectance was not explained by either factor or their interaction (temperature: $F_{1, 21} = 1.98$, $p = 0.16$; time of day: $F_{3, 21} = 1.48$, $p = 0.23$; temperature × time of day: $F_{3, 21} = 0.78$, $p = 0.51$). For pronotum reflectance there was a significant positive linear effect of time of day ($F_{3, 21} = 3.19$, $p = 0.03$) but no effect of temperature ($F_{1, 21} = 1.22$, $p = 0.27$) and no interaction ($F_{3, 21} = 0.39$, $p = 0.76$).

3.2. Grasshopper reflectance with temperature and time of day without environmental cues

Without other environmental cues, temperature explained 72.4% of the variation in reflectance for abdomen ($F_{1, 11} = 23.05$, $R^2 = 0.72$, $p < 0.0001$) and 68.9% of the variation in pronotum reflectance ($F_{4, 11} = 17.94$, $R^2 = 0.69$, $p < 0.0001$) (Fig 2a) but there was no significant relationship with time of day (abdomen: $F_{4, 11} = 1.17$, $p = 0.34$; pronotum: $F_{4, 11} = 1.59$, $p = 0.20$) (Fig 2b). The interaction between temperature and time of day was not significant (abdomen: $F_{4, 11} = 0.37$, $p = 0.83$; pronotum: $F_{4, 11} = 1.48$, $p = 0.23$). Regardless of time of day, grasshoppers that were maintained at 4 °C remained black in colour and grasshoppers at 35 °C were always turquoise (Fig. 2).

3.3. Speed of colour change

Grasshoppers changed from black to turquoise quicker than the reverse (Table 2). In the heating treatment (Fig. 3a), black grasshoppers took 38.43 ± 21.02 min to reach the same reflectance as their control. For turquoise grasshoppers to approach the same colour as the dark control (cooling treatment (Fig. 3b)) it took 392.55 ± 21.91 min.

3.4. Internal body temperature change

The body temperature of grasshoppers reached that of the ambient temperature in less than 15 min. This occurred regardless of whether their body temperature was rising or falling (Fig. 4).

4. Discussion

My study confirms temperature as the primary cue for colour change in *K. tristis* males. There was no relationship between grasshopper reflectance and time of day when time of day cues were removed (Fig 2b). However, I cannot rule out that time of day has an impact on colouration when these cues are present as it is in other systems (e.g. Filadelfi et al., 2005). These data show a strong trend that support findings by Key and Day (1954a) that there is a difference in the speed at which colour change is completed. Colour change in male *K. tristis* occurs faster in one direction than the other, with males turning from black to turquoise much more quickly than from turquoise to

black. The rate at which this colour change takes place is decoupled from the rate of internal body temperature change. Taken together, these results suggest that grasshopper reflectance is driven by temperature but is not directly indicative of body temperature.

Temperature as the primary driver of rapid colour change has not been recorded in any other organism. Colour change of the laboratory stick insect *C. morosus*, and several Odonata species is not exclusively driven by temperature. *C. morosus* changes colour in response to temperature from dark to pale, but the return to dark is under centrally coordinated neural control. Similarly, in the damselfly *Austrolestes annulosus*, colour change from black to turquoise is autonomous and occurs in response to temperature (Veron, 1973), however, colour change from turquoise to black, although triggered by temperature, is not autonomous but centrally controlled, as in *C. morosus*. At temperatures of less than 12 °C a 'darkening factor', which returns *A. annulosus* to its dark phase, is released by nervous tissue associated with the epidermal cell layer in the posterior end of the abdomen (Veron, 1976). The 'darkening factor' then moves forward into the pronotum and head. The pronotum cannot turn dark without being attached to the abdomen and, in turn, the head cannot change in colour if it is not attached to an intact pronotum and abdomen. In contrast, there is no evidence of centrally controlled colour change in either direction for *K. tristis*. Unlike *A. annulosus*, the integument from any tagma of the body changes to turquoise and return to black under control of temperature both *in vivo* and *in vitro* (Key and Day, 1954b).

My study also suggests that the disparity between the speed of colour change in either direction cannot be explained by a lag in body temperature because grasshoppers matched ambient temperature in under 10 min whether warming or cooling. Therefore, grasshoppers are unlikely to display a colour by actively maintaining a warmer or cooler body temperature. While the disparity in time to change colour for damselflies might be explained by the different mechanisms driving colour change in each direction, there is no evidence to suggest a similar explanation for *K. tristis*. Perhaps, as with other physiological processes, the cold temperature associated with the change to black also slows the movement of intracellular granules, while the warm temperatures in the turquoise phase, allows their movement to be more rapid (Filshie et al., 1975). Other hypotheses, with particular focus on the 'back to black' change are required to adequately explain this difference.

When environmental cues (sunlight) were available they may have had an effect on grasshopper reflectance. This may suggest that when available, grasshoppers use sunlight as a cue for colour change. In the closest analogue to *K. tristis* colour change, *A. annulosus*, Veron (1976) found that time of day rather than light intensity was important in achieving their turquoise phase. Damselflies remained blue for 24 h when the temperature did not fall below 15 °C (Veron, 1976). In the dark phase however, damselflies remained dark until midnight, but from then until sunrise they did not maintain their dark colour (Veron, 1976). Veron (1976) suggested that this was not a response to increasing ambient light (because it occurred before sunrise) but was controlled by circadian rhythm. The present study cannot exclude the possibility of a period of resistance to dark colouration in *K. tristis* because colouration was not quantified after 9 pm and before 10 am. However, *K. tristis* is not active around midnight when it burrows into the base of plants (Green and Osborne, 1994) and is unlikely to be active until after sunrise because of low ambient temperatures in their alpine habitat. Still, given the striking similarities between *K. tristis*' and *A. annulosus*' colour change, further studies on the nature of colour change at night may be warranted if the mechanism is to be fully understood.

5. Conclusion

The switch to turquoise colouration is the most unusual aspect of this colour change trait. Key and Day (1954a) suggested that the colour change of *K. tristis* could function in thermoregulation. They hypothesized that in their cold alpine habitat, grasshoppers could have the advantage of dark colouration in the morning to heat up rapidly but then change to a paler colour to avoid overheating during the day. There are however, many black coloured insects that presumably face this problem, yet this colour change is an extremely rare trait. Nevertheless, Key and Day argued that colour change might allow grasshoppers to continue foraging and mating through the hottest part of the day rather than having to shuttle from sun to shade the way permanently black insects must. Similarly, Veron (1974) suggested that perhaps a dark colour in early morning was advantageous for damselflies to gain heat rapidly for flight but found no such advantage, recording a temperature difference of just 0.23 °C greater in damselflies in the dark phase than the blue. Although this hypothesis has not yet been tested directly for *K. tristis*, the present study shows that grasshopper body temperature rises so quickly to the ambient, that the animal is likely to heat up before the colour change has taken place.

Temperature is confirmed here as the mechanism for colour change. This colour change is likely to be the product of stratification of reflective nanospheres as suggested by Filshie et al. (1975). Further work should directly test whether such stratification results in thermal benefits for the brightest males. Also, given that only males exhibit this colour change and the substantial variation in colour between males at a given temperature, other explanations should also be considered, such as a role in inter- or intraspecific communication.

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