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Bright turquoise as an intraspecific signal in the chameleon grasshopper (*Kosciuscola tristis*)

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Abstract Bright colours often communicate important information between conspecifics. In sexually dichromatic species where males exhibit bright colours, two hypotheses are often invoked to explain the function of the colour. First, if a male's bright colour contains information about his quality, females may prefer brighter males. Equally, male colour may reliably provide other males with information about fighting ability or resource holding potential. In such circumstances, brighter males may win altercations and/or males may use rival colour to assess their likelihood of winning an interaction. In the chameleon grasshopper (*Kosciuscola tristis*), males but not females turn bright turquoise when their body temperature exceeds 25 °C. In this study, we tested whether the turquoise phase of colour change has a signaling role in inter- and intrasexual contexts. We predicted that females would prefer bright turquoise males over dull males, but found no evidence from several choice experiments to support this hypothesis. We also predicted that brighter males would win more fights than duller males. Whilst we did not find that brighter males won more fights in staged experiments, we found that the brightness of males who chose to enter fights was significantly correlated with their opponents' brightness. Our results suggest that the brightness of males' turquoise phase may provide competitors with important information about their rival's fighting ability.

Keywords Colour change · Brightness · Alpine · Male competition · Female choice · Sexual signal

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Introduction

Many animals can rapidly change colour (physiological colour change), and two broad categories of non-mutually exclusive hypotheses explain why this occurs: the different phases of colour change may (1) have a physiological function or (2) operate as a cue in communication (Stuart-Fox and Moussalli 2008a). Physiological hypotheses suggest that changing colour from one phase to another may facilitate thermoregulation (Umbers et al. 2012a; Henwood 1975; May 1979; Forsman 1997; Bosi et al. 2008) and/or protect from ultraviolet wavelengths (Herring 1965). More often, however, authors have investigated the role of various phases of colour change as cues in inter- and intraspecific interactions especially lizards and frogs (Sword and Simpson 2000; Velando et al. 2006; Ries et al. 2008; Stuart-Fox and Moussalli 2008b; Hettyey et al. 2009).

In undergoing colour change, animals may change their hue, brightness and chroma. At equilibrium, these changes constitute separate phases of colour change. In intraspecific interaction, phases of colour change may be important cues to potential competitors (intrasexual) and/or to potential mates (intersexual), and evidence for both these functions is emerging. For example, colour change phases as intraspecific cues have been demonstrated in several species of cephalopod (Norman et al. 1999; Boal et al. 2004; Palmer et al. 2006; Langridge 2006; Adamo et al. 2006). Also, in some

chameleons, different phases of colour change have a role in sexual communication (Cuadrado 1998a, b; Cuadrado 2000; Kelso and Verrell 2002; Stuart-Fox and Moussalli 2008b; Stuart-Fox et al. 2008). For example, female veiled chameleons (*Chameleo calyptratus*) use different colour patterns to communicate their sexual receptivity (Kelso and Verrell 2002). The function of colour phases in insects has received comparatively little attention.

The ability of some insects to rapidly change colour is taxonomically widespread, having been reported for example, in beetles (Coleoptera; Vigneron et al. 2007); walking sticks (Phasmotodea), damselflies and dragonflies (Odonata; O'Farrell 1964; Veron 1973, 1974; Veron et al. 1974; Conrad and Pritchard 1989; Prum et al. 1994); and grasshoppers (Orthoptera; Key and Day 1954a, b; Filshie et al. 1975; Umbers 2011). Across these taxa, colour change can produce black, turquoise, gold, red, green and brown phases. Whilst some of the mechanisms responsible for rapid reversible colour change in insects have been investigated (O'Farrell 1964; Veron 1973; Veron et al. 1974; Filshie et al. 1975; Berthold 1980; Tichy and Loftus 1987; Vigneron et al. 2007), but the functions of these phases remain largely unknown.

In the chameleon grasshopper, *Kosciuscola tristis*, males rapidly change colour from black to turquoise with increasing temperature, returning to black when they cool down (Key and Day 1954b; Umbers 2011). Its relationship with temperature has lead to the hypothesis that this colour change functions in thermoregulation (Key and Day 1954a). However, although controlled by temperature, it is unclear whether colour change in this species provides any thermoregulatory benefit (Umbers et al. 2012a). Alternatively, the sex-specific expression of the turquoise colour phase in chameleon grasshoppers suggests that it may be important in signalling. Therefore, the aim of this study was to test whether the male chameleon grasshopper's turquoise phase is an intraspecific signal that provides information about male quality to females (intersexual signal) or to other males (intrasexual signal).

As an intersexual signal, variations in male colour could inform females about the various aspects of male quality, such as competitive ability or relative proficiency in successfully elevating their internal temperature, which may be challenging in an alpine environment. Alternatively, if females have a preexisting bias for attraction to turquoise, males may exploit it by converging on this sensory target (Endler 1992). Regardless of the precise nature of the information communicated, if the turquoise phase functions as an intersexual signal, we predict that females will prefer males that have a more pronounced (brighter) turquoise colour phase.

Equally, *K. tristis*' turquoise phase may be an important intrasexual signal. Unlike other grasshoppers (Otte 1970), male *K. tristis* engage in fierce physical combat over access to females (Umbers et al. 2012b). Under this scenario, the brightness of a male's turquoise phase may be an honest indicator of his fighting ability and act to deter challengers (Bonduriansky 2007; Simmons and Tomkins 1996). Thus, if this hypothesis is true, we predict that males with brighter turquoise phases are superior in physical combat compared to males that are less bright. Furthermore, consistent with what is found in most other male–male combat systems (Maynard Smith and Parker 1976; e.g. Knowlton and Keller 1982), fights should only escalate between males that are matched in fighting ability and, in this case, brightness of the turquoise phase.

Methods

Kosciuscola tristis is found across the alpine peaks of the Australian Alps from Mt. Kosciuszko, New South Wales, to Mounts Hotham, Buffalo and Baw Baw in Victoria, Australia. Australia's alpine region is low altitude compared to other alpine regions, but experiences relatively extreme temperatures and stochasticity characteristic of alpine regions worldwide. A small, semelparous (overwinter as eggs), flightless acridid grasshopper, the chameleon grasshopper is hyperabundant at the height of its season (March and April) and becomes active at temperatures above freezing (0 °C; Umbers personal observation). As ectotherms, grasshoppers must modify their behaviour in order to maintain the optimum body temperatures (Casey 1981; Heinrich 1993). In the mating season, between 10 A.M. and 3 P.M. *K. tristis* body temperatures are on average 11.1 (±2.8) degrees above ambient, at around 23.0 °C (±4.5 °C; Mahoney et al., unpublished data).

Colour change in male chameleon grasshoppers is rapid and temperature-dependent. Each morning males need to bask for their colour change to take place: black males require 38.34±21.02 min at 30 °C to turn their brightest turquoise (Umbers 2011). The colour change from black to turquoise appears to be independent of hormonal drivers (M.F. Day, personal communication). Although colour change is driven by temperature, there is measurable variation in colour between males at any given temperature (Umbers 2011).

Kosciuscola tristis males engage in fierce fighting as they compete for access to females and, in doing so, inflict damage on each other (Umbers et al. 2012b). Fighting generally occurs between the late morning and late afternoon, during the warmest part of the day and on sheltered patches of bare earth that receive direct sun (Tatarnic, personal observation). On cold days or if the weather turns cold suddenly, the sites—usually filled with fighting grasshoppers—are abandoned and empty.

Grasshoppers for this study were collected from Dead Horse Gap (36°30′14.0 S, 148°16′36.7 E), south of Thredbo NSW at 1,939 m altitude in the summers of 2009 and 2010. Females and males were kept separate for at least 24 h prior to experiments. These grasshoppers have short life spans and, being adapted to an alpine habitat, prove difficult to maintain

in captivity. Thus, our sample sizes are modest given the species' abundance in the field. To ensure that prolonged time in captivity did not alter their colouration, we ran all experiments within five days of field collection. Experiments were conducted in natural sunlight or under a full-spectrum lamp (J.B. Lighting Products 1,000-W metal halide) to ensure all colours were reflected as they are in nature.

Prior to our behavioural experiments (intra- and intersexual signalling), we measured the weight and brightness of all males to investigate the relationship between male colour and body size as it relates to fighting success. To quantify grasshopper colour, we measured the brightness (sum of the excitation values using *Locusta migratoria* opsin lambda max in ultraviolet, blue and green; Briscoe and Chittka 2001); chroma (the three ratios of ultraviolet/blue, ultraviolet/green and blue/green); and hue (wavelength at which the spectral reflectance curve peaked the excitation of the opsin sensitivities used). Each male's pronotum was measured three times and the average used. We measured colour using an Ocean Optics spectrometer as in Umbers (2011).

Turquoise phase as an intersexual signal

Dichotomous choice tests We ran three different iterations of dichotomous choice tests to assess whether females preferred turquoise males to black males. Firstly, (a), we gave females a choice between turquoise (hot) and black (cold) males, controlling for male size (by weight matching) but not for activity level (temperature). Second, (b), we gave females the choice between two painted live males (one painted turquoise and one painted black) to represent different colour phases, controlling for temperature and activity level. Third, (c), females were given the choice between weight-matched pairs of males at the same temperature (30 °C), relying on individual variation in brightness of turquoise colour phase. Whilst we endeavoured to maintain consistent sample sizes across experiments, limitations in maintaining grasshoppers in the laboratory meant that this was not always possible.

All experiments were carried out in the same type of arenas (40×20×30 cm plastic boxes with mesh sides). Males were tethered by cotton thread tied around the pronotum between the fore legs and middle legs, with the other end affixed to a blade of sedge poked through the lid of a vial of water. Both males were placed at the same end of the arena with their tethers short enough to prevent them from interacting with each other whilst still allowing them to walk a little and to posture naturally. Females were introduced at the opposite end of the enclosure to the males.

(a) Weight matched turquoise (hot) and black (cold) males

Sixteen females were presented with the choice to approach one of two weight-matched males. Thirty-two males were matched for weight (paired *t* test: *n*=

16, $t_{15}=-1.22$, $p=0.24$). One male from each pair was then randomly assigned to either the turquoise (hot) or black (cold) treatment. Males in the black (cold) treatment were kept at 4 °C in a cold room. Males in the turquoise (hot) treatment were kept at room temperature overnight and then warmed to 30 °C for at least 1 h prior to the choice test. Turquoise (hot) and black (cold) males were placed on alternating sides of the arena for each replicate. Males were tethered to blades of sedge to restrict their movement, but allow a natural posture and limited walking. The ambient temperature in the arena was maintained at approximately 30 °C, and females were placed into the arena at the same time as the males to ensure they saw the males at their greatest contrast. For the first 30 min - whilst male colour was maxmially different - we recorded (1) which male the female approached first and (2) how many times the female approached each male.

(b) Painted live males

Fifty-six live male grasshoppers were painted turquoise or black (with non-toxic acrylic paint) and prepared as above. Paints were compared using spectrophotometry to the real grasshopper colour, matched as close as possible and administered to randomly chosen males. After the males had been in the arena for 10 min, a female was introduced to the opposite end and allowed 30 min to approach either male. We recorded how many times the female (1) interacted with either male and (2) approached either male. We did not notice any difference in the behaviours of painted males compared to males in other experiments, and males painted with black paint behaved no differently from males painted with turquoise paint. This is likely to be because males were tethered to blades of sedge and only able to sit still or walk a few centimetres.

(c) Weight-matched males with natural colour variation at 30 °C

Twenty females were presented with the choice of approaching two size-matched, tethered males. Males were paired by weight (paired *t* test: *n*=20, $t_{19}=0.82$, $p=0.42$) and thus haphazardly paired with respect to brightness. Males were maintained for more than 3 h at 30 °C and their colour had equilibrated. Brightness of males was variable in both groups (left males' brightness: range=0.22–0.97, *n*=20; right males' brightness: range=0.10–0.98, *n*=19 — one male died before brightness was measured). The choice arena was maintained at 30 °C. Females were introduced immediately after the tethered males (tethered to blades of sedge and unable to move around the arena, but allowed to posture naturally) were placed in the arena to ensure they saw males at their brightest. Females were allowed up to 1 h to make a choice. We recorded (1) which male

the female approached first and (2) how many times the female approached each male.

We used binomial probability tests (Vassar Stats) to determine whether females chose to approach the turquoise male first and whether females preferred to approach the turquoise males overall. We then used a generalised linear model with an ordinal logistic fit to determine whether there was an effect of experimental iteration (a, b or c) using JMP (ver. 2.0). After finding no effect of experimental iteration, we pooled the data and used a further binomial probability test to see whether females showed an overall preference for brighter coloured males. Finally, we conducted a post hoc power test to ensure that our sample size was sufficient to detect a medium level effect (0.3; Cohen 1988; Thomas and Juanes 1996; Buchner et al. 1997). Mate choice effect sizes often have medium to large effect sizes, though can be variable (Møller and Alatalo 1999; Ekblom et al. 2004).

Turquoise phase as an intrasexual signal

Does male turquoise phase brightness predict success in accessing females? To determine whether turquoise brightness predicts male mating success, we conducted trials in which five males competed for one female. On day 1, grasshoppers were collected, with males and females separated and housed on potted sedges (*Carex apressa*) enclosed in large mesh bags and sprayed periodically with ample water. On day 2, all males ($n=116$) still on their plant were placed in a small room maintained at 30 °C for 3 h. This ensured that all males had reached their maximum brightness, which varies among males at any given temperature (Umbers 2011). We measured male colour and weight and marked each individual with a numbered, coloured bee tag (Pender's Bee Supplies). Males were then returned to their all-male enclosure overnight. Females ($n=32$) were also weighed, marked with a bee tag and returned to their all-female enclosure overnight. On day 3, we conducted the trials.

The mating arenas were constructed using a plastic box with mesh sides and no lid ($40 \times 30 \times 20$ cm). Inside each, we placed an ethanol thermometer and two types of common substrate vegetation collected from the field: snow grass (*Poa hiemata*) as a matted substrate (20×40 cm) and a spray of alpine grevillea approximately 30 cm long (*Grevillia australis*). Arenas were maintained at 30 °C to ensure males were at their maximum brightness for the duration of the experiment. By keeping all males at the same temperature, we eliminated the influence of ambient temperature on fighting ability. Five males were randomly selected out of the all-male enclosure and placed in an arena together. We used five males to ensure an intensely competitive environment. This number of males was within the range observed fighting over a female

in nature, with groups ranging from one to six males (Umbers et al. 2012b). Some males were used in more than one trial (which we account for statistically), but the same combination of five males was never used more than once. Males were allowed to acclimatise for 10 min before the single adult female was introduced into the centre of the arena. In previous experiments, we have found that males do not fight in the absence of females (Umbers et al unpublished data) and thus did not include observations from before the female was introduced. We allowed 30 min for a copulation to begin; if it did not, the trial was abandoned. However, when copulation did occur, we continued the trial until copulation ceased. The trial was run for a further 10 min; if no further mating took place, the trial ended. If, within those 10 min, a second male began to mate with a female, the trial continued until the second copulation was completed. We then recorded the outcomes of interactions between the mating grasshopper and his challengers (Umbers et al. 2012b). The mating male was deemed the winner of an interaction if he stayed on the female after being mounted by a challenger. Conversely, the challenger was deemed the winner if he successfully removed the mating male.

Does male colour predict the outcomes of fights? During the experiments, males directly challenged the mating males, but also fought among themselves. As above, the mating male was deemed the winner of an interaction if he stayed on the female after being mounted by a challenger. Conversely, the challenger was deemed the winner if he successfully removed the mating male. In addition, when interactions occurred between two males, neither of which were mating with the female, we used scored the winner as the male that mounted the other (Umbers et al. 2012b). Using the Bradley–Terry model in R (Turner and Firth 2010), we ranked all the grasshoppers that entered into altercations based on the number of fights they won and lost and to whom. This method includes the importance of beating higher ranked males when putting the participants in order. The Bradley–Terry analysis also allows for the repeated use of males because it incorporates the number of times an outcome occurred. It also assumes that all males initially had a chance of interacting with each other (before they were randomly allocated to replicates). We constructed the Bradley–Terry input file, which is a list of all possible combinations of two-on-two encounters in both possible configurations (a beats b and b beats a), a total of 6,162 possible interactions. We then scored those interactions: 1 for occurred, 0 for did not occur. When challengers won by usurping a mating male, their victories were subjectively counted as two wins because this was a rarer and arguably much more difficult task than a mating male simply maintaining his position (for which they scored one win). Scoring these two means of winning in this way therefore

incorporated a clear asymmetry between what was required for a male to win as a defender and as a challenger. Once the males were ranked, we used multiple regression (JMP) to test whether brightness or weight explained male success.

Results

Turquoise as an intersexual signal

Dichotomous choice tests Overall (pooling data from all choice experiments), females made no discernable choice based on colour: only around 70 % of females approached a male; the rest remained at a distance from both males throughout the trials. Those females that did make a choice did not display any left or right turn bias in any of the experiments (bionomial tests: all $p > 0.24$). Brightness measurements of 20 pairs of males showed no difference in brightness between males that were presented on the right-hand side of the arena and those presented on the left (average difference in brightness, 0.088 (left−right); paired t test: $t_{18} = 0.14$, $p = 0.89$, $n = 19$—one male died before colour was measured; see "Methods"; average difference in male weight, −0.0016 (left−right); $t_{19} = -0.82$, $p = 0.42$, $n = 20$).

(a) Weight-matched turquoise (hot) and black (cold) males

Both males were somewhat active during the trial, without any obvious difference between the activity levels of hot and cold males (this is likely to be because these are alpine grasshoppers and used to cold temperatures). Of the 16 females tested, five made no choice. Females that did choose ($n = 11$) showed no preference for turquoise (hot) males in their first choices (binomial test: $n = 11$, $k = 5$, $p = 0.77$). Only one female made a second approach (to the other male); all others approached just one male for the duration of the trial.

(b) Painted live males

Sixteen females out of 28 did not make an approach during their trial. The 12 remaining females showed no significant preference for turquoise males, with 4 out of 12 females approaching the turquoise male first (binomial test: $n = 12$, $k = 4$, $p = 0.12$). During these trials, females did not interact with the second male.

(c) Weight matched males with natural colour variation at 30 °C

Out of the 20 females tested, two did not make a choice. Of the females that did choose, they showed no statistical preference either on first

approach (binomial test: $n = 18$, $k = 9$, $p = 1.0$) or total number of approaches to the brighter male (Wilcoxon signed-rank test: $n = 18$, $z = -0.45$, $p = 0.65$). There was also no difference in the weight and brightness between the approached and not-approached males (average brightness difference, −0.0011 (loser−winner); paired t test: $t_{17} = -0.02$, $p = 0.99$, $n = 18$; average difference in weight, −0.0004 (loser−winner); paired t test: $t_{17} = -0.19$, $p = 0.85$, $n = 18$).

Overall, there was no difference in female choice between experimental iteration (a, b or c) of the choice tests ($\chi^2_{1,2} = 1.94$, $p = 0.40$); pooling the data increased our power to detect an effect. Overall, females chose randomly with respect to male colour, with no indication that they prefer brighter males (binomial probability test: $n = 45$, $k = 22$, $p = 1.0$). A post hoc power analysis for an exact, generic binomial test indicated that our sample size of 45 gave us sufficient power to detect a medium level effect (0.3) if one was present (α err prob$ = 0.05$, lower critical $n = 15$, upper critical $n = 30$, power$ = 0.99$, $1 - \beta = 0.04$).

Turquoise as an intrasexual signal

Do either male brightness or weight predict success in mounting females? In 29 of the 32 replicates, a male began mating with the female shortly after she was introduced into the arena (average latency to mating, 13.45 min$\pm$19.88, $n = 29$). The average copulation duration of the first mating was 101.96 min ($\pm$37.43 min, $n = 29$). The duration of second copulations (when they occurred) was much shorter (40.32$\pm$34.67 min, $n = 5$). In 27 of the 29 trials in which mating occurred, we observed antagonistic interactions between males (who challenged one at a time) as they vied for the position atop the female ($n = 63$ interactions). In these interactions, there were two possible outcomes: a challenger unsuccessfully attempted to remove a mating male from atop a female ($n = 49$; winner=stays on, loser=unsuccessful usurper) or a challenger successfully removed a mating male ($n = 14$; winner=successful usurper, loser=male that was removed from female). We analysed these groups separately and together.

Where challengers were unsuccessful at removing a mating male, we found no difference in brightness between winners and losers (brightness winner$\pm$SD$ = 0.4\pm0.16$, brightness loser $\pm$SD$ = 0.37\pm0.15$; paired t test: $t_{48} = 1.2$, $p = 0.24$). We did find that winners were significantly heavier that losers (weight winner$\pm$SD$ = 0.26\pm0.05$, weight loser$\pm$SD$ = 0.23\pm0.04$; paired t test: $t_{48} = 2.93$, $p = 0.005$; Pearson's correlation: $r = 0.07$, $p = 0.65$, $n = 49$; Fig. 1). Also, our data revealed a significant correlation between the brightness of males in

interactions where challengers unsuccessfully attempted to usurp guarding males (brightness winner±SD=0.4±0.16, brightness loser±SD=0.37±0.15; paired t test: $t_{48}=1.2$, $p=0.24$; Pearson's correlation between the brightness of the winner and the brightness of the loser: $r=0.44$, $p=0.001$, $n=49$; Fig. 2). However, there was no correlation between the weight of winners and losers (weight winner±SD=0.26±0.05, weight loser±SD=0.23±0.04; Pearson's correlation: $r=0.07$, $p=0.65$, $n=49$). In interactions where males successfully removed a mating male ($n=14$), we found no difference or correlation between brightness or weight (brightness winner±SD=0.37±0.22, brightness loser±SD=0.35±0.12; paired t test: $t_{13}=0.79$, $p=0.44$; Pearson's correlation: $r=0.38$, $p=0.18$; weight winner±SD=0.23±0.03, weight loser±SD=0.24±0.05; paired t test: $t_{13}=0.40$, $p=0.70$; Pearson's correlation: $r=0.11$, $p=0.73$, $n=14$).

Taking all the winners and losers together ($n=63$ interactions), we found that winners were heavier than losers (average weight winner±SD=0.26±0.23 g, average weight loser±SD=0.24±0.04 g; paired t test: $t_{124}=2.71$, $p<0.01$), but winners were not brighter than losers (average brightness winner±SD=0.44±0.20, average brightness loser±SD=0.40±0.17; paired t test: $t_{124}=1.17$, $p=0.24$). We also found that the brightness of winners and losers was correlated (Pearson's $r=0.45$, $p<0.001$), but that the weight of winners and losers was not correlated (Pearson's $r=0.06$, $p=0.63$).

Does brightness or weight predict male fighting success? Antagonistic interactions did not only occur between mating males and their challengers but also between two non-mating males. In order to rank male fighting success, we included the 63 interactions where a mating male and a challenger fought, plus we included 196 interactions that occurred between non-mating males. All together, we observed 259 antagonistic interactions between 74 males. Male fighting success was ranked using the Bradley–Terry
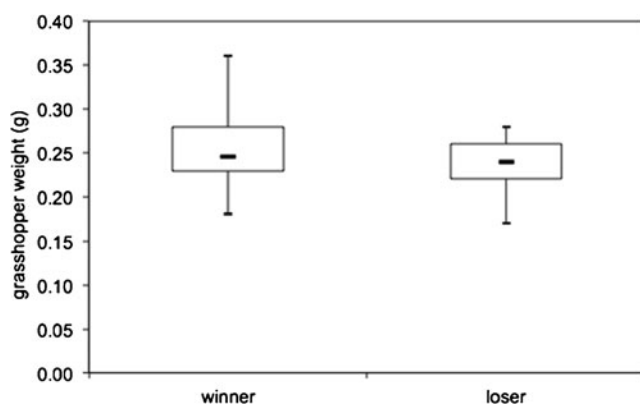

Fig. 1 There is a significant weight difference between winners and losers in competitive interactions, where winners are on average heavier than losers
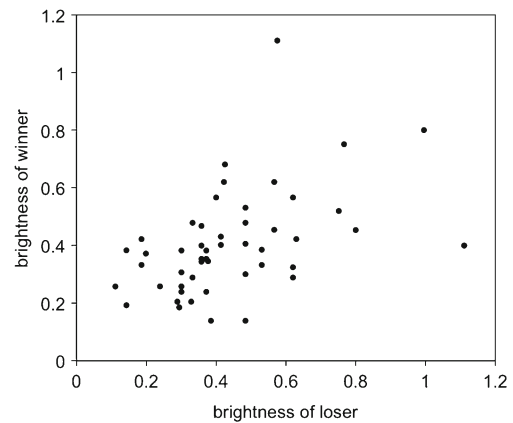

Fig. 2 In competitive interactions, the brightness of the winners is correlated with that of the losers, perhaps indicating a tendency for interactions between rivals more closely matched in brightness to escalate

model (which accounts for males being involved in multiple interactions), and we used rank-order correlation to assess whether body size or colour correlated with rank. Our model was not significant ($n=74$, $r=0.04$, $p=0.77$), and neither weight nor brightness showed a significant correlation with fighting success rank (all $F<3.11$, all $p>0.08$).

Discussion

The aims of our study were to investigate whether male *K. tristis'* turquoise colour phase functions as a signal either from males to females (intersexual) or between males (intrasexual). We predicted that if males use bright turquoise to advertise their quality, females would base mate choice preferences on variation in the brightness of that signal. Contrary to our predictions, however, our data show no support for this hypothesis and strongly suggest that females have no preference for approaching or interacting with brighter males over duller males. This lack of preference is probably not due to limitations in the visual capacity of females as most insect visual systems studied so far report the presence of trichromatic or tetrachromatic vision (Briscoe and Chittka 2001). Additionally, the differences between the chameleon grasshopper's colour phases are not a shift in hue but a change in brightness, which can be detected using achromatic vision alone (Osorio and Vorobyev 2005).

Even though it is tempting to assume that female choice is linked to male colour traits, especially in dichromatic species, there are well-established examples in galliforme birds where female choice is not correlated with the quality of male ornaments (Ligon and Zwartjes 1995; Hagelin and Ligon 2001). In addition, the lack of evidence for female preference towards bright males may make sense

considering that brightness in *K. tristis* males does not correlate with any variables that are traditionally associated with male quality, such as measures of body size (Umbers et al. 2012b).

Our data indicate that many females (30 %) made no choice when presented with males, despite having had no access to any males for up to several days prior to testing. This is unlikely to be a result of lack of female receptivity because in the male competition experiments where males were not tethered, females readily mated (within a few minutes of males being introduced). Whilst the lack of female participation may be a laboratory artefact, it may also be indicative of natural behaviour. In the field, males fight fiercely over females, who suffer collateral damage (Umbers et al. 2012b). Also, males eclose to adulthood before females and ride on subadult females, suggesting that scramble competition may lead to female harassment in this species. Taken together, these observations may explain the reluctance of females to approach males and choose between them. Here, we have focused on brightness as a potential subject of precopulatory selection, but we cannot rule out the possibility that females use brightness as a cue that influences postcopulatory selection.

Male chameleon grasshoppers engage in fierce fights: in the field and under experimental conditions, they readily compete for the opportunity to mate with a female (Umbers et al. 2012b). Our hypothesis that brighter turquoise males win fights is not supported by our data. Although male brightness was not different between winners and losers, brightness may still be important in male–male combat because the brightness of winners and losers was significantly correlated. This is also true for the brightness of males fighting in the wild (Umbers et al. 2012b). We suggest that the correlation between rival male colouration in the chameleon grasshopper may indicate that males challenge others with similar brightness to their own; that is, antagonistic interactions only escalate to physical fights if they cannot be easily resolved using other forms of communication (e.g. cues that indicate fighting ability, in this case brightness of colour). Thus, we predict that highly asymmetric pairs of males do not engage in fights as the inferior male retreats, and we only see escalated fights when males are matched in signals that convey fighting ability. Whilst only males closely matched in brightness engage in fights, brightness itself was not a good indicator of the likelihood of winning. Brightness may, however, be used as an indication of the profitability of entering a fight, and once an interaction has escalated, other factors such as weight or mandible size may be more important in affecting the outcome (Umbers et al. 2012b). To be a useful indicator of fighting ability, brightness should represent something about the quality of the individual (Maynard Smith and Parker 1976; Hurd 1997; Arnott and Elwood 2008). Colouration

may be indicative of basking ability, in this case the ability to raise one's body temperature above 25 °C. We predict that basking ability should be selectively advantageous in an alpine habitat as finding warm microhabitats is a potentially difficult task, but is necessary for metabolic processes including fighting ability. The story is more complex, however, given that once turquoise, male body temperature can be far lower than 25 °C as the change to black takes up to 5 h (Umbers 2011). Further work is required to understand what limits the production of their turquoise colour if we are to elucidate whether it conveys information about male quality. For example, the maximum brightness a male can achieve may be influenced by the presence of parasites, hydration or age.

As predicted by game theory, escalation between closely matched competitors is a well-documented phenomenon (Clutton-Brock and Albon 1979; Hammerstein and Parker 1982; Jennions and Backwell 1996; Taylor et al. 2001). In circumstances of mutual assessment, this requires that competitors have the ability to assess their own chance of success as well as that of their rival. In at least one grasshopper species, self-colour assessment has been shown (Gillis 1982). It is therefore possible that male chameleon grasshoppers can assess their own colour (Gillis 1982; or, indirectly, correlates of their colour) relative to that of their rival (Hauber and Sherman 2003; Dijkstra et al. 2005). Males may then use this information in their decision whether to escalate an interaction. Alternatively, grasshoppers may assess their own fighting ability (self-assessment) through past experience and make decisions based on their inherent quality rather than through comparison with a rival (Taylor and Elwood 2003). Our study suggests that males are paying attention to each other's brightness when deciding whether or not to enter antagonistic interactions, even though we found no evidence that brighter males win competitions. Thus, the decision on whether or not to enter a fight may be based on characteristics different from those that determine the outcomes of fights. Future studies should test this idea in the chameleon grasshopper by comparing the likelihood at which males matched in brightness escalate fights compared to poorly matched males.

In some of our experiments, weight was significantly different between winners and losers. In interactions where challengers tried but failed to remove a mating male from atop a female, we found that the mating males were on average heavier. This result is intuitive in that it should be more difficult for a challenger to usurp a larger and heavier male, and this pattern is commonly reported in the literature (Jennings et al. 2004; Elias et al. 2008). However, weight advantages may be overridden by other factors such as prior experience or the value of a resource that we have not addressed in this study (Wells 1988; Kasumovic et al. 2009).

In light of the evidence we present here, we tentatively suggest that turquoise brightness in male chameleon

grasshoppers does not function as a precopulatory intersexual signal. It may, however, function as an intrasexual signal, with the prediction that brightness correlates with male contest escalation in some contexts. Since male–male competition is fierce and costly in *K. tristis*, it is plausible that colouration is used as an indicator of fighting ability and that only males with closely matched signals engage in escalated combat. Finally, if this trait is indeed an important cue, the question arises: why is it not perpetually 'on'? Understanding why a potentially important intraspecific signal is environmentally determined rather than continuously expressed remains one of the challenges of elucidating the biology of this unique grasshopper. Future research into the colour change mechanism and the factors that limit it may yield vital clues in explaining the evolution of this trait.

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