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ARTICLE

Mate guarding and male mate choice in the chameleon grasshopper *Kosciuscola tristis* (Orthoptera: Acrididae)

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Abstract In the wild, male chameleon grasshoppers (*Kosciuscola tristis*) are frequently observed mounted on the back of females even when not in copula, and will fight off other usurping males. If this behaviour is mate guarding and reflects investment in male mate choice, then we expect males to preferably guard females based on reliable cues of quality. Cues for female quality likely include female size and egg development that together may indicate fecundity. We investigated male mate choice in the field expressed as mate-guarding preference, by comparing size and egg development in guarded and unguarded females. We found no difference between guarded and unguarded females in measures of fecundity or body size. The majority of females sampled did not contain any viable eggs. This finding suggests that male *K. tristis* indiscriminately guard females in a scramble mating system.

Keywords Sexual selection · Mating behaviour · Alpine biology · Sperm competition

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Introduction

Mate guarding, where males establish a close association with a female either before or after mating, is a common and effective strategy to avoid sperm competition (Alcock 1994; Simmons 2001). It can occur where paternity benefits outweigh the costs involved including time (e.g. Jarrige et al. 2016), energy (e.g. Elias et al. 2014), lost mating opportunities with other females (Parker 1974) and challenges from other males (e.g. Umbers et al. 2012). Pre-copulatory mate guarding allows males to secure future mating opportunities yet presents a risk as it requires investment before copulation has occurred. As such, it is expected to occur where paternity is driven by first male sperm precedence (Parker 1974; Grafen and Ridley 2004) or where it can reduce the likelihood of female re-mating (e.g. Elias et al. 2014). By guarding mates post-copula, males reduce the likelihood of female re-mating and potentially achieve paternity through last male sperm precedence (Parker 1974). Given that mate guarding can involve 'opportunity costs', males should be selective as to which females they choose to guard (Parker 1974; Bonduriansky 2001).

Male mate choice manifests in many forms including selective courting, male–male competition (display or combat), variable allocation of sperm, and mate guarding (Parker 1974; Amundsen and Forsgren 2001; Bonduriansky 2001; Reinhold et al. 2002; Edward and Chapman 2011). It is expected to occur in systems where costs of mate search or assessment are low, female variability is high, and male investment in reproduction is high (Bonduriansky 2001). Mate choice by males has been less represented than female choice in the literature, but has attracted significant interest in recent decades (e.g. Bonduriansky 2001; Gaskett et al. 2004; Preston et al. 2005; Barry and Kokko 2010; Dougherty and Shuker 2014). While studies of male mate choice are becoming more common, such choice tests are typically conducted in laboratory environments. Field studies also offer valuable insights and may yield different results from laboratory studies (Dougherty and Shuker 2014); For example, laboratory experiments with house finches (*Carpodacus mexicanus*) found that males selected for females with bright plumage, but subsequent field studies revealed a strong, and previously undetected, role of female age in male mate choice (Hill 1993).

Here, we investigate mate guarding in the chameleon grasshopper (*Kosciuscola tristis*) (Orthoptera: Acrididae) in the field. Chameleon grasshoppers are endemic to alpine regions of south eastern Australia (Umbers 2011; Tatarnic et al. 2013; Slatyer et al. 2014) and exhibit characteristics common to other insect groups where male mate choice occurs. Males and females occur in high densities, so costs of mate search should be low. Male investment in reproduction appears to be high, as males are often observed guarding females by mounting on their backs for extended periods of time without copulation (K.D.L.U. personal observation). Furthermore, males guarding females are challenged for their position by other males, resulting in ferocious and damaging contests (Umbers et al. 2012).

Whether this apparent guarding behaviour represents pre-copulatory or post-copulatory guarding is unknown. The mating system of *K. tristis* has not received significant research attention, and whether males are selective in their choice of females, or whether sperm precedence patterns occur, is also unknown. To test whether males exhibit preferences for females in the wild, we compared the traits of females that were guarded by males with those of females that were not guarded. As guarding a female represents a significant investment by males, we hypothesised that there would be a difference between guarded females and unguarded females and that this difference may ultimately confer a fitness advantage to the males. If males are preferentially guarding more fecund females, then we predict that guarded females would be larger (Bonduriansky 2001; Edward and Chapman 2011) and have greater egg mass than unguarded females. Alternatively, if males gain fertilisation success through first male precedence (Simmons et al. 1994), males may preferentially guard non-gravid or lighter females, as this may indicate early stages of egg development and a lower chance that the female has already mated (Butlin et al. 1987; Bonduriansky 2001).

Materials and methods

Study species

Adult *K. tristis* emerge in late January, and egg laying typically begins in late February. Adults die with the first winter snowfall (June), and eggs overwinter in the soil (Green and Osborne 1994; Umbers et al. 2013). Solo males, solo females, and pairs where males are guarding females are all regularly observed in the field. Females are almost always guarded by a male when they oviposit (Umbers et al. 2012). Males remain mounted during female oviposition, and additional males surround the pair and attack the mounted male, whilst nearby non-ovipositing females appear to be ignored (N.J.T. personal observation).

Data collection

Sexually mature female *K. tristis* ($n = 129$) were collected from Dead Horse Gap (36°31.032′ S, 148°15.897′ E) near Thredbo, NSW (9 am–3 pm, 3 April and 11 May 2012). When collected, females were either mounted by a male ($n = 65$) or not ($n = 64$). Females were not collected if they were mating or ovipositing at the time of sampling. All females were frozen within four hours of collection and kept at −20 °C until dissection. Because many specimens were missing one hind leg at the time of collection (commonly observed in the field), all specimens with two legs were weighed (JS-VG20 digital scale, Jennings Scale Company, Phoenix, USA) with one randomly chosen hind leg removed. We measured size (pronotum length and width) using digital callipers (Sontax Australia). To investigate whether males may be responding to female mass relative to her body size, we calculated an index of body mass as the residual values of the linear relationship between pronotum length and body weight (herein referred to as body mass index). Females were dissected and classed as either gravid or non-gravid. The eggs were counted and removed, and weighed for total egg mass.

We were unable to collect data on the males that were guarding the females, as upon approaching mounted pairs, males would frequently jump off the backs of females into nearby vegetation. We were then unable to identify these males amongst others, as *K. tristis* are abundant and occur in high densities, making tracking individual males by eye impossible.

Data analysis

Data were initially explored using factorial binomial models to test for differences between guarded and unguarded females. However, this resulted in significance levels that were difficult to interpret and likely to be type I errors. We manually inspected the data to remove variables with high levels of co-linearity, and attempted to use forward and backward stepwise methods to build a model. This did not remove issues with apparent false positives, nor did it improve models based on Akaike information criterion (AIC) values. These issues, and a relatively low sample size for multiple logistic regression methods, meant that a factorial model approach was deemed inappropriate. Rather, we employed a conservative approach and present here a series of separate univariate binomial analyses using significance threshold values corrected for multiple comparisons ($P < 0.008$). We tested whether the weight, body size (pronotum length and width), body mass index, number of eggs, and total egg mass of females were predictors of whether females were guarded or unguarded. All statistical analyses were carried out using R version 3.3.0 (R Development Core Team 2011).

To assess whether these female characteristics could potentially serve as cues of fecundity, we used zero-inflated Poisson regression analyses to assess whether fecundity (number of eggs) correlated with female weight, body mass index, pronotum length, and pronotum width. We used the pscl package (Zeileis et al. 2008) and calculated pseudo-R^2 values as in McFadden (1973).

Results

The majority of collected females were non-gravid (78 out of 129). Of the guarded females, 63% were non-gravid, and of the unguarded females, 56% were non-gravid. We found no evidence for differences between guarded and unguarded females in terms of body weight, body mass index, pronotum length, pronotum width, number of eggs, or total egg mass (Table 1; Fig. 1).

There was no evidence for a correlation between number of eggs and body mass index, or body size measured by pronotum width, or pronotum length (Table 2; Fig. 2). There was a positive correlation between number of eggs and total weight of females (Table 2; Fig. 2).

Discussion

Male chameleon grasshoppers invest in mate guarding and engage in costly fights to gain access to females, even in the presence of unguarded females. As such, we predicted that males would exhibit preferences for the females into which they invest time and energy. However, we found no evidence that males select females based on fecundity, as the presence, number and mass of eggs in females did not differ significantly between guarded and unguarded females (Fig. 1). In fact, the majority of guarded females were non-gravid. This is not to suggest that males preferred non-gravid females, but is more likely due to the fact that non-gravid females were more common overall.

Table 1 Univariate binomial regression models comparing guarded and unguarded female characteristics assuming a significance level of $P = 0.008$

Predictor variable	Z_{127}	P
Weight (g)	−1.040	0.299
Pronotum length (mm)	0.130	0.897
Pronotum width (mm)	2.188	0.029
Body mass index	0.428	0.668
Number of eggs	0.965	0.334
Total egg mass (g)	−0.134	0.893

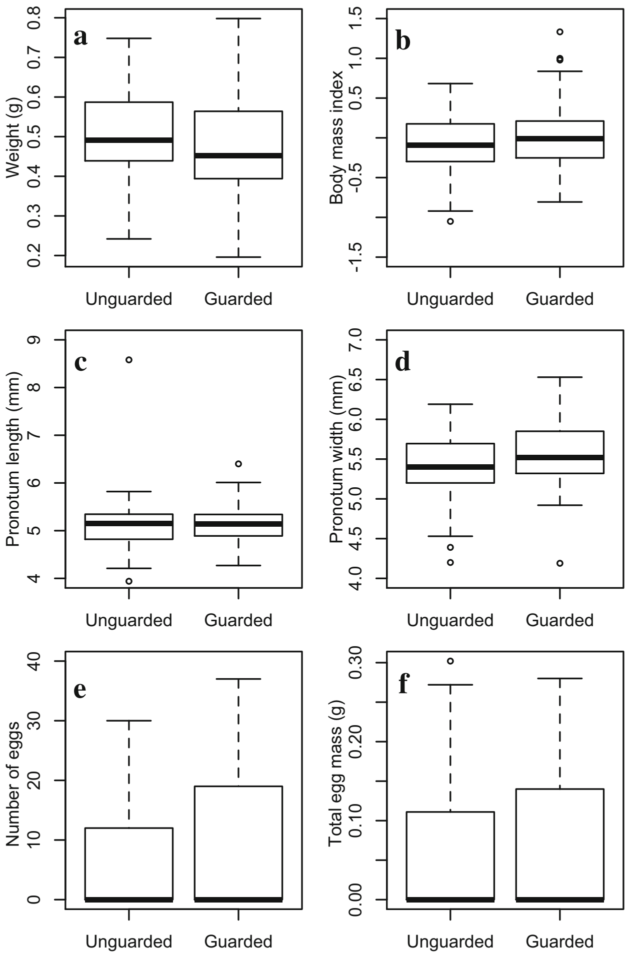

Fig. 1 Comparison of female **a** weight, **b** body mass index, **c** pronotum length, **d** pronotum width, **e** egg number, and **f** total egg mass, between guarded and unguarded females

Table 2 Zero-inflated Poisson regression models investigating the relationship between female fecundity (number of eggs) and female morphology

Variable	Z_4	Y	R^2	P
Pronotum length (mm)	−1.008	−3.801	0.004	0.314
Pronotum width (mm)	−0.387	−0.173	0.016	0.698
Weight (g)	−5.200	−12.09	0.230	**<0.001**
Body mass index	−0.057	−0.946	0.002	0.344

Bold type indicates significance at $p < 0.05$

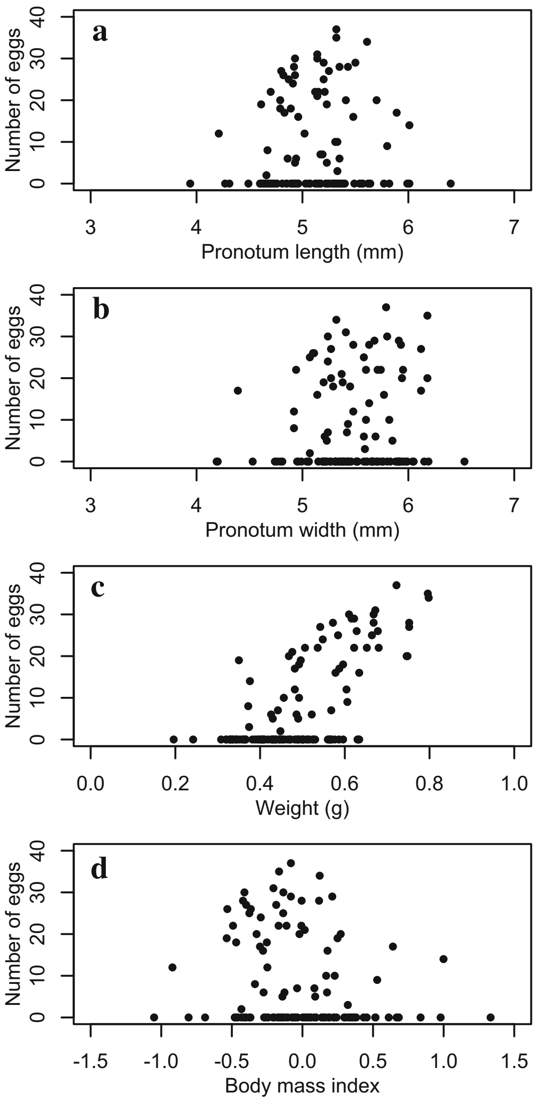

Fig. 2 Relationship between female fecundity and **a** pronotum length, **b** pronotum width, **c** weight and **d** body mass index

Additionally, we did not find any evidence for males preferring to guard heavier or lighter females, which could indicate selection for either fecundity or first male sperm preference, respectively.

There were no differences in the overall size or relative mass (body mass index) of females that were guarded or unguarded. Pronotum size is fixed throughout the adult life stage, and whilst it may correlate with a female's maximum potential fecundity, it is unlikely to serve as a reliable indicator of fecundity at a single point in time. Whether pronotum size influences the ability of males to stay attached when guarding requires further investigation.

Male *K. tristis* were frequently found guarding non-gravid females, and are known to fight over ovipositing females at a time when they are unable to affect the paternity of the clutch being laid (Umbers et al. 2012). Pre-copulatory guarding has been reported in a number of insect species (e.g. Estrada et al. 2010; Kureck et al. 2011) and is commonly associated with first male sperm precedence (Simmons 2001). Furthermore, mating is known to induce egg development and oviposition in numerous orthopteran species via the transfer of compounds from the male accessory gland (e.g. Pickford et al. 1969; Lange and Loughton 1985; Murtaugh and Denlinger 1985). Male *K. tristis* may then be able to secure paternity of future clutches by securing the earliest copulation with post-ovipositional females, thereby gaining first male sperm precedence and stimulating further egg development. The possibility of pre-copulatory guarding is further evidenced by observations of adult males mounted on penultimate-instar females early in the mating season (K.D.L.U. personal observation).

The lack of male preference for fecundity in *K. tristis* suggests that their mating system functions as a scramble competition in which males indiscriminately mount females. This finding is in apparent contrast to accounts of costly fights between males for access to particular females over others (Umbers et al. 2012). A lack of female variation, and/or the inability of males to detect cues related to fecundity, may prohibit males from being able to exercise mate choice. Female weight correlated with female fecundity (Fig. 2), however males may be unable to detect or distinguish this cue. There still exists a possibility that mate choice occurs after guarding, and that guarding reflects a form of mate assessment, in which case we will not necessarily observe preferences at this stage (Bonduriansky 2001); rather, we expect males to exert choice subsequently by only initiating copulation with high-quality females. Further supplemental manipulative experiments and paternity analysis will assist in understanding the mechanisms underpinning mate guarding and fertilisation success in *K. tristis*.

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Compliance with ethical standards

The authors declare no conflicts of interest. All animals in this study were collected under permit from the NSW National Parks and Wildlife Service and were studied in adherence with Australian ethical research legislation.

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