

Male mate choice in the chameleon grasshopper (*Kosciuscola tristis*)

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Abstract

In many species, males can increase their fitness by mating with the highest quality females. Female quality can be indicated by cues, such as body size, age and mating status. In the alpine grasshopper *Kosciuscola tristis*, males can be found riding on sub-adult females early in the season, and as the season progresses, males engage in fights over ovipositing females. These observations suggest that males may be competing for females that are either unmated (early season) or sperm-depleted (late season). We thus hypothesised that male *K. tristis* may be choosy in relation to female mating status, and specifically, we predicted that males prefer females that are unmated. We conducted behavioural experiments in which males were given the choice of two females, one mated and one unmated. Contrary to our prediction, males did not mate preferentially with unmated females. However, copulation duration with unmated females was, on average, 24 times the length of copulation with mated females. While female *K. tristis* can reject mates, we did not observe any evidence of overt female choice during our trials. Females may gain additional benefits from mating multiply and may therefore not readily reject males. While our experiment cannot definitively disentangle female from male control over copulation duration, we suggest that males choose to invest more time *in copula* with unmated females, perhaps for paternity assurance, and that male mate assessment occurs during copulation rather than beforehand.

KEYWORDS

alpine region, Australia, female mating status, mate choice

1 | INTRODUCTION

Males can maximise fitness by mating preferentially with high-quality females. Males show a preference for phenotypes that are indicative of high female fecundity or that are associated with a reduced risk of sperm competition (Bonduriansky, 2001). Female quality can be related to body size (Blanckenhorn, 2005; Wiernasz, 1995), age (Polak, Starmer, & Barker, 1998), low parasite loads (Watson, 1993), mating status (Simmons, 2001; Simmons, Llorens, Schinzig, Hosken, & Craig, 1994) or a combination thereof. Because female body size can be strongly correlated with fecundity, it is common for males to prefer larger females (Barbosa, 2011; Bonduriansky, 2001; Byrne &

Rice, 2006; Jarrige, Greenfield, & Goubault, 2013; Win, Kojima, & Ishikawa, 2015), although there are some exceptions (e.g., Wearing-Wilde, 1996; Chenoweth, Petfield, Doughty, & Blows, 2007). In addition, many males prefer younger over older females (bush-crickets, Simmons et al., 1994; fruit flies, Polak et al., 1998; Shelly, Edu, & Pahio, 2012; moths, Win et al., 2015) and unparasitised over infected females (e.g., *Drosophila*, Wittman & Fedorka, 2015). In systems where there is high first-male sperm precedence, males are expected to reject already mated females because of the low probability of paternity (Simmons et al., 1994; Bonduriansky, 2001; Thomas, 2011; Lehmann, 2012; Gaskett, Herberstein, Downes, & Elgar, 2004; Schneider, Zimmer, Gatz, & Sauerland, 2016). Females

can also exhibit behavioural changes once mated and can become avoidant or aggressive towards males (Bauer, Samietz, & Berger, 2005; Hartmann & Loher, 1999; Herberstein, Schneider, & Elgar, 2002; Judge, Tran, & Gwynne, 2010; Martel, Damiens, & Boivin, 2008).

Males may assess female traits prior to, or during, copulation. In precopulatory choice, males may exhibit preference for a particular female, or they may vary the intensity with which they perform courtship or copulation among females (Bonduriansky, 2001). For example, in the long-tailed dance fly (*Rhaphomyia longicauda*) males visually assess females prior to copulation and show a preference for larger females (Funk & Tallamy, 2000). Instead, males may try to outperform other males in courtship (e.g., bowerbirds, Borgia & Coleman, 2000), they may engage in costly physical fights against each other for specific females (e.g., bighorn sheep, Hogg, 1984; grasshoppers, Umbers, Tataric, Holwell, & Herberstein, 2012; Umbers, Tataric, Holwell, & Herberstein, 2013), or they may guard females that are about to become sexually receptive (e.g., crustaceans, Jormalainen, 1998; beetles, Chaudhary, Mishra, & Omkar, & Tregenza, T., 2017).

During copulation, males can vary the amount or quality of resources that they provide to females of different quality (Bonduriansky, 2001). The resources males contribute to females can include the amount or quality of ejaculate transfer or of the nuptial gift, copulation duration or mate guarding (Abraham, Vera, Pérez-Staples, & Foster, 2015; Eberhard, Krebs, & Clutton-Brock, 1996; Pitnick & Brown, 2000). Males cannot produce limitless numbers of sperm (Wedell, Gage, & Parker, 2002), and ejaculate production generates nontrivial costs (Dewsbury, 1982); therefore, some males choose to use sperm strategically. For example, because larger females mate more frequently, males of the Australian bush cricket *Kawanaphila nartee* typically reduce the ejaculate size allocated to larger females (Simmons & Kvarnemo, 1997; Wedell et al., 2002). In addition, males of many species prolong genital contact far beyond the time needed to transfer sperm (Alcock, 1994; Jivoff, 1997). In blue crabs (*Callinectes sapidus*), long postcopulatory associations allowed the male's ejaculate to harden, creating a plug that limits the size of the ejaculate of subsequent males. Among grasshoppers, a female's mating status can influence male mate choice. In the migratory grasshopper, *Melanoplus sanguinipes*, males show a preference for unmated females, apparently due to strong first-male sperm precedence (Chapman & Joern, 1990; Pickford & Gillott, 1972). By being choosy, males can increase the probability of paternity and reduce the risk of sperm competition (Simmons, 2001). Despite these theoretical predictions, male mate choice is rarely tested experimentally.

In chameleon grasshoppers (*Kosciuscola tristis*), males engage in fierce fights over access to females, a rare behaviour among grasshoppers. Fights involve mandible displays, biting, kicking and grappling, particularly at the end of the breeding season (Muschett, Umbers, & Herberstein, 2017; Umbers et al., 2012; Umbers, Tataric, et al., 2013). In opposition, early in the season, there is little to no aggression among males. At this time, males are found riding on adult females, presumably mate guarding,

and on subadult females perhaps to ensure they are the first to mate with her once she emerges as an adult (Mahoney, Tataric, O'Hanlon, & Umbers, 2017; Muschett et al., 2017). *Kosciuscola tristis* are protandrous, adult females emerge a few weeks after the males and are quickly mated (Dearn, 1977; Green & Osborne, 1981; Umbers, Herberstein, & Madin, 2013; Umbers, Tataric, et al., 2013; Umbers and Muschett, pers. obs.). Females readily mate with multiple males, sometimes within minutes of a previous mating (Muschett et al., 2017), suggesting there is a risk of sperm competition in this species and thus potentially selection on males to avoid it, or traits that improve male competitiveness such as differential sperm allocation. While female *K. tristis* do not exhibit precopulatory mate choice regarding male size or colour (Umbers, Herberstein, et al., 2013; Umbers, Tataric, et al., 2013), they can avoid copulation by hopping away and/or by kicking and grappling with males (Muschett et al., 2017). This avoidance suggests that females exhibit some preference for mates but the traits females favour are not clear. In the event of copulation, mating duration can be significantly reduced in instances where females kick or grapple with males (Muschett et al., 2017). In addition to the effects of mating behaviour on aggression, female quality varies across the breeding season. Early-season females vary considerably in the number of mature oocytes, but as the season progresses, most females have mature oocytes (Muschett et al., 2017).

In this study, we aimed to test the hypothesis that male chameleon grasshoppers may be choosy in relation to female mating status. Considering the variation in female quality and the potential costs of mating for males, we predicted that males would not mate indiscriminately. Further, because *K. tristis* is protandrous and recently emerged adult females are quickly mated (Umbers and Muschett, pers. obs.), we also predicted male chameleon grasshoppers would show a preference for unmated females, thereby reducing the risk of sperm competition.

2 | METHODS

2.1 | Study species

Chameleon grasshoppers *K. tristis* (Acrididae: Oxyinae) are endemic to the highest elevations of the Australian Alpine region in south-east Australia. It is small, flightless and semelparous insect; adults have a single reproductive season during their lifetime, and generations do not overlap (Green & Osborne, 1981; Umbers et al., 2012; Umbers, Herberstein, et al., 2013; Umbers, Tataric, et al., 2013). Although there is some variation between years (Umbers, pers. obs.; Green, pers. comm.), nymphs begin to emerge in late October and most adults have emerged by late January and early February. The mating season lasts through to early May. The adults begin to die with the first snowfalls, and the population overwinters as eggs (Umbers, Herberstein, et al., 2013; Umbers, Tataric, et al., 2013).

TABLE 1 Comparison of mean \pm SD age (days), mean femur length (mm), mean weight (mg), number of mature eggs and number of parasites between “unmated” and “mated” *Kosciuscola tristis* females used in male mate choice experiments

	Unmated	Mated	Statistics	<i>p</i>
Mean age (days) ^a	7 \pm 3.1	7.2 \pm 2.1	0.54	0.46
Mean size (femur length, mm) ^a	14.62 \pm 0.61	14.74 \pm 0.82	0.69	0.41
Mean weight difference (mg) ^{a,b}	11 \pm 20.5	43.8 \pm 36.8	6.88	0.008
Mature eggs	<i>n</i> = 0	<i>n</i> = 0	–	–
Parasite (Nematoda, Diptera) ^c	<i>n</i> = 1	<i>n</i> = 1	–	0.99

Notes. *p* values in bold are statistically significant.

^aKruskal–Wallis H_1 . ^bMean weight difference refers to the difference between a female's original weight at time of emergence and weight on experiment day. ^cFisher's exact test.

2.2 | Specimen collection and rearing conditions

We collected adult males and late-instar female *K. tristis* nymphs from Dead Horse Gap trail (36°50'21.0"S, 148°27'85.3"E) in Thredbo, Kosciuszko National Park, NSW, from 23 January to 10 February 2015. Males and females were housed separately as isolation provides adult males the opportunity to recover from previous mating experiences (Adamo & Hoy, 1995). Males were kept in medium-sized mesh enclosures (20 \times 20 \times 20 cm), while female nymphs were kept in large mesh enclosures (69 \times 69 \times 122 cm). All enclosures were kept in Thredbo Village (1,375 m) under natural light and temperature conditions with a mixture of potted sedge (*Carex* sp.) and snow grass (*Poa* sp.) and were sprayed with ample water three times a day. The female enclosure was checked daily for individuals that had emerged as adults.

As adult females emerged, they were temporarily marked with nontoxic paint (Uni-POSCA™) to avoid damage to the recently emerged female's soft body and were randomly assigned to an “unmated” or “mated” treatment groups. Adult females were weighed to the nearest 0.1 mg within 24 hr of emergence using a Jscale™ (Phoenix Arizona, USA) before being moved to a separate enclosure, where they were kept in identical conditions as described above. There were no significant differences in weight between females assigned to the “unmated” and “mated” treatments before experiments (Kruskal–Wallis H_1 = 2.16, *p* = 0.14). Two days after the adult moult, the temporary mark was replaced with a bee tag (Penders Beekeeping Supplies™) glued to the pronotum. On the same day, we placed females assigned to the “mated” group into individual mating arenas consisting of a small mesh cage (18 \times 25 cm) with a single randomly selected male from the all-male enclosure and the pair was allowed to copulate. The enclosure was checked every 10 min until mating ended. Mated females were returned to the “mated female” enclosure, and males were placed in a –20°C freezer for 24 hr and preserved in 70% ethanol. Each male only mated once to avoid pseudoreplication. Mated females were kept isolated for an additional 24–48 hr before the mate choice experiments.

2.3 | Male mate choice experiment

All females used in the experiments (mated and unmated) were between 5 and 7 days old (days since eclosion). Adult males were

kept isolated from females for 72 hr prior to experiments. Males exhibit the shortest latency to mate at the beginning of the breeding season (Muschett et al., 2017), and as such, trials were run early in the season, from 28 January to 15 February, to ensure the shortest latency to mate. On the day of the experiment, we arbitrarily chose one female from the mated group and one from the unmated group and weighed them again before placing them in an experimental arena. The arena consisted of a plastic box with mesh sides (40 \times 30 \times 20 cm). To limit the distance females could travel during the experiment, and to ensure the more receptive female did not approach a male before he had made a choice, females were individually tethered and secured into place in the experimental arena. Females were tethered using a 5-cm-long string that was either tied loosely around the pronotum (between the first and second pair of legs) or glued to the pronotum, behind the bee tag. The string did not impede the females' body or limb movements and allowed for a full range of motion of all appendages. The string was then attached to the sides of the arena with adhesive tape. The position of the unmated and mated females within the arena was alternated in each trial to avoid left/right bias by males. A male was haphazardly chosen from the all-male enclosure and placed approximately 15 cm from the tethered females. A separate observer, who was blind to the females' mated status, recorded all interactions for 15 min. If the male made no apparent choice within the initial 15-min period, he was removed and replaced with a different male. All individuals were used only once.

We collected ca. 170 late-instar female nymphs, of which 42 emerged into adults. However, nine adult females died before the experiments, two were used in a preliminary trial, before conditions were standardised, and the last female could not be paired as no other adults emerged. Therefore from an initial collection of 170 females, our final sample size was *N* = 30 females (15 unmated and 15 mated) for a total of *N* = 15 trials. To create the mated group, females were placed in a small enclosure (18 \times 25 cm) with a single male 24 to 48 hr before being used in a trial and was allowed to mate. These males were removed and placed in a freezer before being preserved in 70% ethanol, and mated females were returned to the “mated female” enclosure. There were no significant differences in age (days since emerging) or size (femur length) between unmated and mated

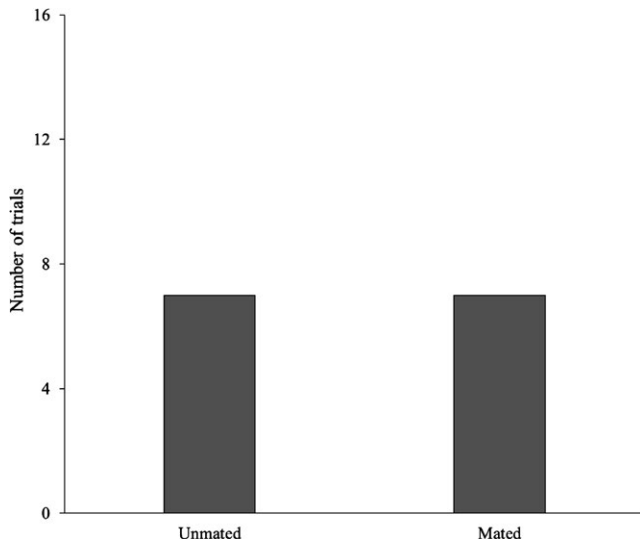


FIGURE 1 Number of times a *Kosciuscola tristis* male chose a female based on her mating status: unmated or mated

females (Table 1). Females' original weight at time of emergence and weight on experiment day varied significantly between unmated and mated females. Mated females were heavier than unmated females (Table 1).

Like most other grasshopper species, *K. tristis* does not have an obvious courtship phase (Otte & Museum of Zoology, University of Michigan, 1970). A male was therefore considered to have made a choice if he hopped on a female and either attempted to mate (curving abdomen beneath female's and probing, with the epiphallus visible) or successfully copulated with the female (attaching genitalia). We also recorded any changes in initial choice, that is, (a) mounting a female but hopping off without attempting to mate, (b) attempting to mate, but hopping off without mating, and (c) hopping on and/or attempting to mate with the second female after attempting to mate or successfully mating with the first female chosen. Previous experiments on *K. tristis* have shown that copulation duration can be either short (<10 min) or long (>120 min; Muschett et al., 2017; Umbers, Herberstein, et al., 2013; Umbers, Tataric, et al., 2013). During our trials if copulation lasted more than 30 min, the mating pair was collected and placed in a separate mesh enclosure (18 × 25 cm) that was checked every 10 min until mating ended. If mating lasted less than 30 min, the experiment continued for a further 10 min to assess whether the male attempted to mate again. Because female *K. tristis* can be aggressive towards males, either by kicking or grappling (Muschett et al., 2017), observers also recorded any behavioural interactions. Between experiments, arenas were cleaned with 70% ethanol to eliminate any chemical cues. During experiments, ambient temperature was maintained between 22 and 25°C.

2.4 | Size measurements and dissections

At the end of the experiment, individuals were placed in a -20°C freezer for 24 hr and then preserved in 70% ethanol. To determine

whether there was a difference in size between the unmated and mated females, we measured femur length using Vertex™ Vernier digital callipers (England, UK) to the nearest 0.01 mm. To determine female fecundity, the number of mature ovarioles was counted. Females were dissected dorsally by making an incision from the epiproct to the pronotum following the medial carina, exposing the reproductive system. In grasshoppers, the ovaries are paired and consist of tube-shaped ovarioles attached to two lateral oviducts with the largest and more developed eggs located at the distal end (Chapman, 2013; Uvarov, 1966). Females were also thoroughly examined to assess infection by nematodes or Diptera larvae (Umbers et al., 2015). We carried out dissections under a stereoscope at 10× magnification (Olympus SZ40, Olympus, Japan).

2.5 | Data analysis

All analyses were carried out in R v2.15.1 (R Development Core Team, 2010). Data are presented as mean ± SD unless stated otherwise. Due to small sample size, we used a Kruskal–Wallis test based on 9,999 Monte Carlo resamplings to evaluate the differences between the mated and unmated groups. For this analysis, we used the R package “coin” (Hothorn, Bretz, & Westfall, 2008). To compare the presence of parasites between unmated and mated females, we used a Fisher's exact test. Because the sample size was small ($N = 15$), we used Hedge's g to assess the effect size (Hedges, 1981).

3 | RESULTS

3.1 | Male mate choice experiments

During the male mate choice trials, we used $N = 15$ males. These males were wild-caught, kept isolated from the females for at least 72 hr prior to experiments, and were not the males used to create the “mated” group. Males made a clear choice in 14 of 15 trials. Of those, mating occurred in 13 trials. Mating duration varied from 4 to 187 min (67.8 ± 80.7 min, $N = 13$). Males chose at random with respect to female mated status (Kruskal–Wallis $H_1 = 0.0769$, $p = 0.782$; Figure 1). However, mating duration varied significantly between unmated and mated females: Copulation duration with unmated females was longer (unmated females $\bar{x} = 153.8$ min $\pm SD = 45.2$; mated females $\bar{x} = 6.4$ min, $\pm SD = 3.7$) (Kruskal–Wallis $H_1 = 8.1053$, $p = 0.0025$, $g_{\text{Hedge}} = 5.1$; Figure 2). There were no instances where males changed their initial choice. During the trials where a male made a choice but did not mate ($N = 1$) and where mating lasted less than 30 min ($N = 7$), males did not make a second choice. During the experiments, both females were somewhat active but with no discernible differences in behaviour between unmated and mated females. Neither of the females kicked or grappled with males in any trial. Mated and unmated females had no mature eggs in the ovariole (Table 1), and parasites were rare (Table 1).

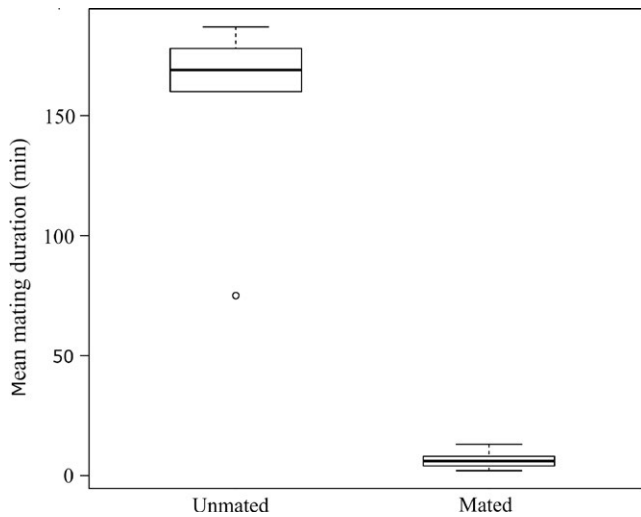


FIGURE 2 Mean mating duration of *Kosciuscola tristis* males with mated and unmated females. Data are presented in box plots, depicting the median value (solid horizontal line), 25th and 75th percentile (box outline), 90th percentile (whiskers) and outliers (open circle)

4 | DISCUSSION

We predicted that male *K. tristis* would prefer to mate with unmated over mated females to avoid potential sperm competition. Contrary to our prediction, we found that males did not display explicit precopulatory choice and readily initiated copulation with either unmated or mated females. In keeping with our prediction, however, copulation duration was clearly different between the treatments with males mating with unmated females for much longer than with mated females. There are several explanations as to why males did not exhibit precopulatory choice including that males do not have a reliable precopulatory cue on which to base mate choice or that there is no selection on males to be choosy. The marked difference in copulation duration between mated and unmated females could be attributed to a previous male leaving a physical barrier such as a sperm plug which requires the initiation of copulation to be detected (Herberstein, Wignall, Nessler, Harmer, & Schneider, 2012; López-León, Cabrero, Pardo, Viseras, & Camacho, 1993; Parker, 1970; Zimmer, Schneider, & Herberstein, 2014). In addition, long copulation duration with a previously unmated female might be a form of paternity assurance, for example, mate guarding (Alcock, 1994; Allard, Gobin, & Billen, 2007; Svård & Wiklund, 1988). At last, the greater value that unmated females may offer could be limited. However, evidence suggests that unmated females offer increased fitness in other Orthoptera, such as *Melanopus sanguipes* (Acrididae), *Decticus verrucivorus* and *Requena verticalis* (Tettigoniidae) (Bonduriansky, 2001).

4.1 | Precopulatory male mate choice

Female *K. tristis* may not have an externally detectable trait that indicates their mating status or males may lack a mechanism by

which to recognise one. For males to utilise a precopulatory trait that indicates mated status, mating would need to induce a change in females that reduces receptivity, their ability to remate (Gillott, 2003; Hartmann & Loher, 1999) and/or that makes females less attractive to males (Carazo, Sanchez, Font, & Desfilis, 2004; Simmons, Alcock, & Reeder, 2003). It is common in insects that males cannot distinguish between females with different mated status (De Freitas, Mendonça, Nascimento, & Sant'Ana, 2004; Hammack, 1986). For example, some species show no reduction in receptivity in females following copulation (Grant, Fowler, & Chapman, 2002; Mossinson & Yuval, 2003). In *K. tristis*, copulation duration was different when males mated with mated or unmated females. This suggests the presence of a detectable cue of female mated status that seems only to become obvious once genital coupling takes place. In an interesting manner, females in the “mated” treatment increased in weight over just a few days and that might be due to rapid egg production triggered by mating as seen in other insects (Arnqvist & Nilsson, 2000; Xu & Wang, 2011). If males could detect differences in female weight or girth, we would expect them to assess females either visually prior to mounting or upon hopping on them to mate. However, our results indicate that if males detected differences in weight or size they did not respond to them because: First, males hopped on females irrespective of mated status, and second, males engaged genitalia irrespective of mated status. Although we cannot conclusively rule out precopulatory male assessment, it seems internal cues influenced copulation duration rather than any precopula assessment of female size.

While our sample size is small ($N = 15$), our effect size was comparatively large ($g_{\text{Hedge}} = 5.1$); thus, our sample size is unlikely to have interfered with our ability to detect a lack of precopulatory choice by males. It is possible that males can detect the difference between mated and unmated females precopula, but in the absence of any selective advantage for males to be choosy, males are unlikely to exercise a choice (see also Elgar et al., 2003). A male's share of paternity may be similar when copulating with an unmated female or a mated female, especially if there is rapid remating and equal sperm mixing (Reinhardt, 2000; Scolari et al., 2014; Simmons, 2001). At last, even if there is a fitness advantage to mating with an unmated female over a mated one, increased alternative mating opportunities during the short *K. tristis* mating season (~70 days) may erode any benefits from mating selectively (Muschett et al., 2017; Umbers, Herberstein, et al., 2013; Umbers, Tatarnic, et al., 2013).

4.2 | Female control

Mate rejection is common in *K. tristis*. In previous studies, we have found that females reject males in up to 30% of trials and show aggression in up to 10% of trials (Muschett et al., 2017). Previous observations have also revealed evidence of females rejecting males by kicking and grappling with the male (Muschett et al., 2017). However, we did not observe female mate rejection in our experiments. So, while our results do not allow us to rule out cryptic female choice in explaining the disparity in copulation duration

(Simmons, 2001), this species' typical female mate choice behaviour was absent. While it is possible that females are using a cryptic mate-rejection strategy that has not previously been seen in this species, our interpretation of our data coupled with our experience with this system suggests that males are exerting a preference for unmated females via increasing copulation time (Muschett et al., 2017).

4.3 | Postcopulatory choice

Despite the lack of overt precopulatory mate choice in our experiments, it appears that males distinguished between mated and unmated females during copulation. Copulation with unmated females lasted up to 18 times the duration of copulation with mated females. One possible explanation is that males may have encountered a physical barrier or obstruction, such as a genital plug, during copulation with a mated female. Substances transferred along with male ejaculate can form a physical barrier that prevents females from remating or leads to diminished sperm transfer during a subsequent mating (Parker, 1970). In some spider species, for example, males are unable to determine whether a female has a plug during courtship but fail to successfully copulate when they insert their genitalia (Zimmer et al., 2014). However, genital plugs can be temporary and can either be ejected during oviposition or simply not persist for long periods (Herberstein et al., 2012; López-León et al., 1993). Instead, males can determine female mating status during copulation based on differences in body size. During our trials, mated females were significantly heavier than unmated females which could give rise to detectable differences in girth when males grip females *in copula*. At last, there may be strong first-male sperm precedence, and once males detect the female is mated, based on an unknown cue, they may terminate copulation if there is little fitness benefit in persisting (Simmons, 2001).

Prolonged copulations can also be a form of mate guarding (Alcock, 1994; Allard et al., 2007; Svård & Wiklund, 1988) where genital contact continues without sperm transfer. Therefore, when mating with an already mated female, males may be transferring sperm during the short copulation but do not invest in mate guarding. In contrast, prolonged copulations could be a way of maximising sperm transfer, and a strategic allocation of resources (Dickinson, 1986; Parker, Simmons, & Kirk, 1990), and long copulations may enhance a male's competitive advantage by increasing sperm numbers (Parker, 1982, 1984, 1993). However, we do not know whether those short copulations with mated females entailed sperm transfer. When copulation duration was less than 30 min (i.e., copulations with mated females), males had the opportunity to mate with the second (unmated) female. There were no instances where males made a second attempt, suggesting that *K. tristis* males may have transferred sperm even during short copulations, and entered a refractory period. Because sperm and ejaculate production generally entail high costs, males of many species enter a refractory period after mating, where they show decreased receptivity to females while they replenish

sperm reserves (Dewsbury, 1982; Hettyey, Vági, Hévizi, & Török, 2009; Radhakrishnan & Taylor, 2008). At last, it is likely that the prevalence of male choice varies throughout the breeding season. Muschett et al. (2017) determined male *K. tristis* become more aggressive towards each other at the end of the breeding season, as opportunities to mate dwindle. An assessment of the seasonal variability of this behaviour would provide much-needed insight into the mechanisms of male choice in this species.

4.4 | Parasite load and female fertility

Besides their mated status, females from the two treatment groups may have been different in other ways known to affect male choice, for example, parasite load, and sexual maturity (Bonduriansky, 2001). Infection rate by parasites in *Kosciuscola* grasshoppers is generally low (Umbers et al., 2015), and only two females from our trials were infected with parasites—one each from the mated and unmated groups.

While neither group of females had mature ovarioles, we are confident females were sexually mature at the time of our experiments for several reasons. As in many insects, female sexual maturity in Orthoptera varies significantly between species, from a few hours to a few days postemergence (Tregenza, Buckley, Pritchard, & Butlin, 2000), while in *Catasigerpes occidentalis*, *Miomantis paykullii*, *Sphodromantis lineola* and *Tenodera supertitiosa* mantids, females reach sexual maturity within 48 hr of emerging (Maxwell, 1999). In our experiments, females were all at least 5 days old. In addition, *K. tristis* males are often seen riding late-instar nymphs but have never been observed *in copula* with subadult females, while recently emerged *K. tristis* females are quickly mated (Dearn, 1977; Green & Osborne, 1981; Umbers, Herberstein, et al., 2013; Umbers, Tatarnic, et al., 2013; Mahoney et al., 2017, Umbers and Muschett pers. obs.). At last, due to the high elevations of its alpine habitat, *K. tristis* has a very short breeding season (~70 days), suggesting that selection would favour rapid female maturation. Taken together, these factors suggest females reach sexual maturity within a very short time of emerging.

4.5 | Conclusions

Male *K. tristis* appear to exert some level of mate choice in the form of copulation duration. While female *K. tristis* can also be selective through aggressive behaviour, their selectivity does not preclude male selectivity. In our experiment, we saw no typical evidence of female choice (mate rejection via grappling and kicking), but cannot rule out cryptic female choice, nor the existence of a sperm plug from a previous male. In this species, it seems a precopulatory mechanism to determine mating status has not evolved and males require physical contact (mounting and genital coupling) in order to detect cues on which to base their choice. We suggest histological study of female reproductive organs postcopula would provide an interesting avenue for future research and help tease apart potential alternate mechanisms.

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