Aggressive behaviour in the skyhoppers of the Australian Alps

Giselle E. Muschett1,2 · Christina J. Painting3 · Marie E. Herberstein2 · Kate D. L. Umbers4,5

Received: 26 April 2021 / Accepted: 1 May 2022 / Published online: 25 May 2022
© The Author(s) 2022

Abstract

The evolution of male-male aggression is of interest because at its extreme it can be very energetically costly, leave males vulnerable to predators, and give rise to weaponry such as exaggerated traits. In grasshoppers (Acrididae), one group stands out as exceptionally aggressive, the skyhoppers (Kosciuscola) in which males bite, kick, mandible flare, and wrestle each other for access to females or when females are laying eggs. In this study we asked whether there is variation in aggressive behaviour among four skyhopper species and aimed to determine whether the traits used in fighting bear signatures of sexual selection in their size, variability, and allometric scaling. We found clear differences in the numbers and types of aggressive behaviours among species. Kosciuscola tristis and K. usitatus were the most aggressive, K. cognatus was the least aggressive, and K. tristis was the only species that performed the ‘mandible flare’ behaviour. Mandible size was larger among the three species that showed aggressive behaviour, all except K. cognatus, and was negatively allometric for all species possibly suggesting a functional size constraint. Pronotum size was different among most species and K. tristis’ pronotum was the largest and borderline positively allometric perhaps suggesting that pronotum size is related to aggressive behaviour but the nature of that relationship remains obscured. Our study suggests that further work investigates skyhoppers’ aggressive behaviour and how it varies with ecology, and paves the way for establishing them as a model system in the evolution of aggressive behaviour.

Keywords Orthoptera · Fighting · Combat · Elevation · Mountain · Allometry
Introduction

Partaking in combative behaviour can be very costly in terms of energy expenditure (Taylor and Elwood 2003), increased risk of injury or death from opponent (Parker 1974; Bean and Cook 2001), and increased risk of predation (Jakobsson et al. 1995). As such, conflict resolution without physical contact is common and can take the form of visual, acoustic, and/or ritualized displays (Arnott and Elwood 2008; Scott et al. 2010). For example, in house crickets (Acheta domestica), male aggressive songs correlate both with body size and ability to win fights (Brown et al. 2006), masked birch caterpillars (Drepana arcuata) use anal scraping, mandible scraping, and mandible drumming to successfully deter conspecific rivals (Scott et al. 2010) and in blue moon butterflies Hypolimnas bolina, males compete for territory with aerial stunts, (Kemp 2000). Nevertheless, aggression that escalates to physical combat does occur and is most likely when opponents are evenly matched in size or ability (or resource holding potential (Lindström 1992)), the resource under dispute is extremely valuable (Mathiron et al. 2018), or one opponent has nothing left to lose (terminal investment) (Andrade and Kasumovic 2005).

Behavioural traits can be precursors to the evolution of extreme morphological structures (Blomberg et al. 2003). In many species, combat has evolutionary consequences for male morphology (Andersson 1982; Emlen 2008). Intense competition can drive the evolution of sexual size dimorphism where males have larger body size relative to females, common in mammals and taken to an extreme in Southern elephant seals Mirounga leonina (Parker 1974; Haley 1994). In most arthropods, females are the larger sex (Teder and Tammaru 2005), and departures from that pattern provide exciting examples of the selective power of male-male competition. For example, male New Zealand giraffe weevils Lasioclyrhynchus barbicornis can be much larger than females and engage in frequent and extreme fighting behaviour (Painting et al. 2014). However, large body size is among the least spectacular ways in which male morphology is influenced by selection–males in many species carry weapons, traits that are often much larger than would be predicted by the size of other body parts, referred to as positively allometric (Emlen 2008). Weapons of male arthropods can be novel structures such as horns (Emlen 1997) and apophyses (Buzatto et al. 2014), or modifications of existing body parts such as enlarged legs (Buzatto et al. 2011; Fea and Holwell 2018), rostra (Painting and Holwell 2014) and mandibles (Knell et al. 2004). Larger males may have disproportionately larger weapons (Kodric-Brown and Brown 1984), but there is considerable variation in allometric patterns among species (Bonduriansky 2007).

Understanding the mechanisms by which aggression has evolved can be informed by comparative analyses that allow an assessment of behavioural, morphological, or environmental characteristics (Bean and Cook 2001; Blatchford et al. 2011), and may reveal the role of adaptive differentiation (Foster 1999; Clark et al. 2015). Using a comparative species approach Bertram et al. (2011) showed that, in general, burrowing cricket (Gryllus) species are more aggressive than non-burrowing species. For example, in G assimilis crickets fights between males frequently escalate to grapples, whereas fights among G. veletis males seldom escalate (Bertram et al. 2011). In a phylogenetic comparative analysis, Vander Linden and Dumont (2019) showed that the morphology of neck bones in ungulate mammals can be predicted by the type of fighting behaviour in which they engage. Comparative approaches thus provide broad context for understanding the evolution of aggression.
Environmental factors can exert pressures on species and drive the evolution of extreme behaviours like costly fighting. The cold temperatures and short seasons of high-mountain environments typically feature periods of extensive snow cover and dormant life with intervening periods of snowmelt, warmth and abundant, busy life. For mountain insects, this means delayed emergence, rapid development, small body size, and short, intense breeding seasons (Hodkinson 2005; Dillon et al. 2006). The limited number of development days (from egg to adult) imposes strong selection pressure on individuals (Mathies and Andrews 1995; Badyaev and Ghalambor 2001). Condensed timeframes at high elevations mean that fundamental resources such as temperature days (number of days that reach a temperature warm enough for species to be active), and sexually mature females are rarer resources than at low elevations, setting up a gradient of selection pressure (Eikenaar et al. 2012).

Among Orthoptera, crickets (Gryllidae) have been used extensively to study male-male combat (Hack 1997; Hofmann and Schilberger 2001), however aggressive behaviour is extremely rare among other Orthoptera such as grasshoppers (Acrididae) (Otte 1970; Greenfield and Minckley 1993; Umbers et al. 2012, 2013). The thermocolour skyhopper or ‘chameleon’ skyhopper, Kosciuscola tristis, presents an interesting exception, and males frequently engage in fierce fights over access to females (Umbers et al. 2012, 2013; Muschett et al. 2017, 2018). These fights involve males flaring their mandibles, biting, kicking, and grappling with opponents, often leading to injury (Umbers et al. 2012) (Fig. 1). Male combat in this species raises questions relating to the evolution and maintenance of aggression in an otherwise relatively non-combative taxon (Otte 1970).

The aim of our study was to compare aggressive behaviour of four species of skyhoppers (Kosciuscola) which are endemic to the Australian Alps (Fig. 2). Three features make this group particularly interesting for investigating the patterns of aggression. Firstly, on mainland Australia, Kosciuscola grasshoppers are distributed in discrete but overlapping elevational bands from approximately 800 m to 2200 m above sea level (Rehn 1957; Umbers et al. 2021). Secondly, Kosciuscola are semelparous, restricted to a single, short alpine season (Dearn 1977; Green and Osborne 1994). And thirdly, one of the species, Kosciuscola tristis, is highly aggressive despite little to no evidence of aggression in other grasshoppers (Greenfield and Minckley 1993; Umbers et al. 2012.

Fig. 1 Biting a and mandible flaring b in male Kosciuscola trisits tristis males from Thredbo while a female lays eggs. Photos: Kate Umbers
We addressed two questions, (1) Does aggression vary among the species? and (2) Are morphological traits related to aggressive behaviour?

For question one, we predicted that there would be differences among the species based on differences in body size and ecology. Specifically, we expected that (1) *K. tristis* would be the most aggressive species based on preliminary field observations, that it would have a relatively large body size and that its distribution would be concentrated at high elevation where its breeding season is relatively short, (2) that *K. usitatus* would be less aggressive than *K. tristis* because its distribution includes lower elevations and thus has a less restricted season, (3) that *K. cuneatus* being sister to *K. tristis* but at lower elevation would be similar to *K. usitatus* and that (4) *K. cognatus* being noticeably smaller and at the lowest elevation might be the least aggressive.

Fig. 2 Skyhoppers of the Australian Alps: a female *K. tristis*, b male *K. tristis*, c distribution map of *K. tristis* sensu lato (s.l.), d female *K. usitatus*, e male *K. usitatus*, f distribution map of *K. usitatus* s.l., g female *K. cuneatus*, h male *K. cuneatus*, i distribution map of *K. cuneatus* s.l., j female *K. cognatus*, k male *K. cognatus*, l distribution map of *K. cognatus* s.l.. Distribution maps show the distributions of the Kosciuscola as previously described by Rehn (1957), different colour points represent new clades as described by Umbers et al (2021), upper white line represents Australian Capital Territory border, lower white line represents New South Wales/Victorian state border, black arrows indicate sampling site for this study.
For question two, we also predicted that we would see differences in morphological traits among the species that correlate with their aggressiveness. In the field we have observed *Kosciuscola* using mandibles in display and to bite as well as, grappling and mounting each other during fights, and predicted that mandibles and pronota might show morphological shifts in the more aggressive species compared to the less aggressive species. Specifically, we predicted that traits under selection would be larger, more variable, and show positive allometry in species that are more aggressive.

**Methods**

**Study species**

On mainland Australia, skyhoppers are found from southeast New South Wales (NSW) and Australian Capital Territory (ACT) into the highlands of Victoria (Vic), including Australia’s highest peak Mt. Kosciuszko (Fig. 2) (Rehn 1957; Tatarnic et al. 2013; Umbers et al. 2021). They are small, flightless members of the Acrididae, and generally inhabit open grassy areas in montane, subalpine and alpine habitats feeding on a wide variety of grasses. In our study we compared the antagonistic behaviour of four mainland *Kosciuscola* species (*K. tristis, K. cuneatus, K. usitatus, and K. cognatus*), from near-sympatric populations in the southern NSW Alps (Umbers et al. 2021). In southern NSW, *K. tristis* is normally found at the highest elevations from approximately 1500 m to over 2200 m. *Kosciuscola usitatus* is also found at the highest elevations but extends to elevations as low as 1400 m. *Kosciuscola tristis* is more abundant at higher elevations than *K. usitatus* (Key and Day 1954; Rehn 1957; Slatyer et al. 2014; Umbers et al. 2021). Where they occur in sympathy, *K. tristis* and *K. usitatus* inhabit grassy open shrublands and grasslands near alpine creeks and bogs. *Kosciuscola cognatus* occupies grasslands and shrubs found at middle elevations approximately 1000–1850 m, while *K. cuneatus* is commonly found in the grassy understory of subalpine forests from approximately 1100–1650 m (Rehn 1957; Dearn 1977; Slatyer et al. 2014; Umbers et al. 2021). The phylogenetic relationships of this group have recently been re-described and cryptic species have been identified, however it is clear that the individuals in this study are all from their same respective species because the sampling was undertaken at single sites (Fig. 2) (Umbers et al. 2021). We cross reference the clade names here to Umbers et al (2021) in anticipation of new species being described. *Kosciuscola tristis* (*Sth NSW Clade*) and *K. usitatus* (*ACT & NSW Clade*) were collected from Dead Horse Gap Trail, Thredbo, NSW. *Kosciuscola cuneatus* (*Sth NSW Clade*) was collected ~8 km SW of Thredbo, while *K. cognatus* (probably *K. cognatus* (*NSW & Vic Clade*) from (Umbers et al. 2021)) was collected from Guthega, NSW (approximately 15 km NE of Thredbo) (Table 1). *Kosciuscola cognatus* in this current publication is probably an undescribed species (Umbers et al. 2021).

**Specimen collection and husbandry**

To collect all four species, we visited three sites located in Kosciuszko National Park all within 10 kms of each other on the ’main range’. All *K. cuneatus* individuals were collected near Thredbo, all *K. cognatus* individuals were collected at Guthega, and both *K. usitatus* and *K. tristis* were collected at Dead Horse Gap (Table 1). We carried out collections on two occasions in the Autumn of 2015: from 20 to 22 March and from 9 to 13 April.
Grasshoppers of each species were kept in mesh enclosures (69 cm × 69 cm × 122 cm), two enclosures per species, each with males and females kept separately for at least 72 h prior to experiments, outdoors at accommodation in Thredbo. Separating males and females ensured that they had not mated prior to the trial; males typically do not fight in the absence of a mating pair or a female laying eggs (Umbers et al. 2012). All enclosures were kept under natural light and temperature conditions with a mixture of potted sedge (Carex sp.) and snow grass (Poa sp.) and sprayed with water three times a day.

Measurements of aggressive behaviour

Following Umbers et al. (2013), arenas consisted of a plastic box (40 × 30 × 20 cm) with mesh sides. Common vegetation collected from the field, mainly dead snow grass (Poa hiemata) and alpine grevillea (Grevillea australis), served as a substrate to line the bottom of each arena. To minimize the effects of temperature, all arenas were maintained between 22 and 26 °C. On the day of the experiment, five males of one species were arbitrarily selected from their enclosure and placed in the arena. Once in the arena males were marked with non-toxic paint pen (Uni-POSCA™) on the femur and/or pronotum, allowing for several colour and placement combinations to ensure we could identify individuals. After a 5 min acclimatisation period, a single adult female of the same species was introduced, and individuals were allowed to acclimatise for a further 5 min. If mating occurred we counted the number and duration (in min) of each copulation. We also compared the number of male-male aggressive interactions that occurred before the start of the first mating event to the number that occurred after the first mating. Each trial was run for 60 min and the behaviour of each individual was recorded via continuous observation using a set of shorthand codes to allow rapid note taking for each individually marked male. At the end of each trial males and females were placed in a freezer for 24 h and preserved in 70% ethanol. Arenas were emptied of substrate and cleaned with 70% ethanol to minimise the potential effect of chemical cues on subsequent trials.

From observations in previous studies (Umbers et al. 2012, 2013; Muschett et al. 2017) we summarised the behavioural repertoire of Kosciuscola grasshoppers and defined eight aggressive behaviours (Table 2). Male-male aggressive interactions in Kosciuscola frequently escalated through a sequence of behaviours and interactions including a display (e.g. mandible flare in K. tristis), sparring (where individuals touch one another and often strike out with their front femurs), or a male jumping on top of another male (Table 2).
Table 2  Male grasshopper aggressive behaviours in aggression trials adapted for *Kosciuscola* from (Hofmann and Schildberger 2001)

| Behaviour     | Description                                                                 | Intensity Score |
|---------------|-----------------------------------------------------------------------------|-----------------|
| Sparring      | Individuals touch one another with their front quarters and often strike out with front and medial legs | 1               |
| Femur tipping | Alternate, quick raising and lowering of the femora                          | 1               |
| Jump on       | Male jumps on another male, aligning himself with the anterior–posterior axis of opponent, perhaps sizing up against him | 2               |
| Jump on pair  | Male jumps on the male on the mating pair, aligning himself with the anterior–posterior axis of opponent, perhaps sizing up against him | 2               |
| Bite          | Pinches opponent with mandibles, typically lasting for several seconds        | 3               |
| Kick          | Quick movement of either or both hind legs, tibia rushes backwards and/or upwards | 3               |
| Mandible flare| Male raises himself on forelegs and arching the back and raising the head, hyperextends the mandibles, while shaking its head shaking side to side, wigging and flattening antennae laterally. We note that this behaviour is more energetic than ‘mandible spread’ and is thus ranked the same intensity as ‘bite’ | 3               |
| Grapple       | Repeatedly pushes or pulls opponent mainly with front and mid legs, hind legs/tibia may also be used | 4               |
Not all behaviours were equally aggressive, for example mandible flares may be indicative of fighting ability (Hofmann and Schildberger 2001), while grappling may entail greatest energy expenditure and risk of injury (Hack 1997). During trials we recorded the total number of the different aggressive behaviours and calculated ‘weighted aggression scores’ by multiplying the number of times the behaviour was observed by its intensity score (Table 2).

**Measures of mandible, pronotum and femur length**

We compared the size, variance and allometry of body size traits to determine whether they correlate with aggressive behaviour. We used vernier callipers (Vertex™, England, UK) to measure femur and pronotum length to the nearest 0.01 mm under a stereoscope at 10× magnification (Olympus SZ40, Olympus, Japan). Pronotum length was measured because the head movements during mandible flaring involve musculature that extends into the pronotum. To improve accuracy, the average of two measurements was taken. We used femur length as a proxy for body size in all species. From our field observations we see that biting and mandible flaring are the most conspicuous and potentially energetically costly aggressive behaviours in this group which are likely to include pronotum musculature because of the movement of the head. As such, even though it may be more common, we cannot justify the use of pronotum as a ‘neutral’ measure of body size. The legs play a less significant role in aggressive behaviour and our analyses show they have relatively low variability and do not contribute significantly to the variation in aggressive behaviour (results not presented). To include a measure of condition in our analyses we used mass divided by femur length as an index of body condition. We also analysed the relationships using the geometric mean of the traits as the predictor variable in comparison to the femur as the predictor variable (Table S5) Because Kosciuscola seem to use their mandibles both in an aggressive display (mandible flare) and as a weapon (biting rivals), we compared mandible lengths, coefficients of variation and allometry among species. Mandibles were dissected out under a stereoscope at 4× magnification (Olympus SZ40, Olympus, Japan), photographed at the same magnification and orientation, and their overall length was measured using ImageJ® (Schneider et al. 2012). Mandible length was measured from the ventral view that shows the melanised ‘teeth’. Length was taken anterior-posteriorly from tuft of bristles at the anterior to the furthest point in the melanised region. Data on mandible length were available for N=319 males; mandible measures were excluded where damage to the mandible rendered an accurate measurement impossible. Unfortunately, a box of preserved material was lost, however, there was no systematic bias in the size of individual lost. The missing data are roughly 50% from K. tristis, 20% from K. usitatus and K. cognatus and 10% from K. cuneatus. Despite the loss of some individuals our sample size is sufficient for the morphometric analysis.

**Data analysis**

**Does aggressive behaviour vary among species?**

To compare aggressive behaviour and the effect of trait size and body condition among species, we used a generalised linear mixed model (GLMM) with the lme4 package (Bates et al. 2015) with ‘weighted aggression score’ as the response variable and ‘species’, ‘mandible length’, ‘pronotum length’ and ‘body condition’ as fixed effects and
accounted for potential effects of different trials by including ‘trial’ as a random effect. We used the `emmeans` function from the `lsmeans` package (Lenth et al. 2020) to conduct post hoc pairwise comparisons between species with Tukey-adjusted p-values.

Do body size traits vary among species in size, variability or allometry?

We compared the trait size means using two-way analysis of variation for mandible length and pronotum length independently. We conducted post hoc pairwise comparisons among species using Tukey’s Honestly Significant Difference with the TukeyHSD function in the `stats` package v3.6.2. We calculated the coefficients of variation by dividing the standard deviation by the mean for each measurement (Painting et al. 2014). Coefficients of variation among species were then compared using z-tests (Zar 1999). To determine whether there were any differences among species in the allometric relationship between trait and body size we generated descriptions of the slopes for log mandible v log femur for each species and log pronotum v log femur for each species by fitting an ordinary least squares (OLS) linear regression model and then retrieved slopes, and upper and lower confidence limits with the function `lstrends` in the package `emmeans` (Lenth et al. 2020). Whether allometric slopes should be calculated using OLS or reduced major axis regression has been the subject of debate. OLS regression methods are often criticised for underestimating slope estimates because variables used on the x axis are measured with error (Warton et al. 2006). However, when the data are asymmetric (i.e. the resulting slope estimates will vary depending on which trait is placed on the x and y axis), and when measurement error is minimal, then OLS is recommended (Smith 2009; Kilmer and Rodríguez 2017). We then conducted pairwise comparisons of the slopes with the function ‘pairs’ from `emmeans` (Lenth et al. 2020). All analyses were preformed using R v4.0.0 (R Core Team 2020).

Results

Description of aggression during behavioural trials

Aggression between males was observed in 66 out of the 78 trials we conducted (85%). Among the four species, *K. tristis* had the greatest number of aggressive interactions, with some form of aggression occurring in every trial (N = 24/24, 100%). In *K. usitatus* aggression occurred in most trials (N = 17/18, 94%), in *K. cuneatus* aggression occurred in three quarters of the trials (N = 13/17, 76%) and *K. cognatus* displayed aggression in two-thirds of the trials (N = 12/19, 63%). The types of behaviours performed were similar among species except for mandible flaring which was only ever observed in *K. tristis* (Fig. 1). Of the total number of aggressive behaviours recorded for all four species (N = 646), males jumping on other males was the most common, followed by femur tipping, while bites were the least common (Fig. 3). Mandible flares made up approximately one quarter of all observed aggressive behaviours in *K. tristis* (N = 311). During the trials *K. cognatus* did not mate however, *K. cuneatus* mated in 29% of trials, *K. usitatus* in 44% of trials and *K. tristis* mated in 75% of trials. Female *K. tristis* frequently
mated with multiple males \(N=7, 38\%\) of trials), while neither *K. cuneatus* nor *K. usitatus* females mated with multiple males.

**Does aggressive behaviour vary among species?**

In our final model species, pronotum length, and mandible length all had significant effects on weighted aggression score, while body condition (mass / femur length) did not (Table 3). Tukey post-hoc comparisons showed *K. cognatus*’ aggression scores were lower than all the other species, and *K. cuneatus*’ scores were lower than *K. usitatus* and *K. tristis*. However, there was no significant difference in aggression scores between *K. usitatus* and *K. tristis* (Fig. 4, Table S1). This final model had an AIC score of 2207 compared to an AIC score of 2209 for our global model which, included all the variables in our final model except body condition. In place of body condition we included mass, femur length and their interaction none of which made a significant or

| Predictors                      | Estimate | SE    | z-value | p value |
|---------------------------------|----------|-------|---------|---------|
| Intercept (*K. cognatus*)       | −5.083   | 0.715 | −7.113  | <0.001  |
| *K. cuneatus*                   | 1.043    | 0.150 | 6.818   | <0.001  |
| *K. tristis*                    | 1.491    | 0.151 | 9.856   | <0.001  |
| *K. usitatus*                   | 1.757    | 0.147 | 11.962  | <0.001  |
| Mandible length                 | 1.928    | 0.580 | 3.323   | <0.001  |
| Pronotum length                 | 2.235    | 0.649 | 3.443   | <0.001  |
| Body condition (mass/femur)     | <0.001   | 0.015 | 0.002   | 0.998   |

Estimate is the effect size and direction, SE is standard error, the test statistics are the z-values and significant p-values are in bold (<0.05). The random effect ‘trial’ had a variance of \(0.186 \pm 0.432\)
large contribution to the model and all the contributions of all the other effects remained unchanged.

**Do body size traits vary among species in size, variability or allometry?**

Among males of each of the four species, we compared the mean lengths of three traits: mandible, pronotum, and femur (Figure S1, Table S2). Post hoc pairwise comparisons showed significant differences among some species in the mean log lengths of the three traits (Table S3). Mean mandible length was smaller in *K. cognatus* than in the other three species, while mean pronotum length was larger in *K. tristis* than in the other three species (Table S3). Mean femur length was smaller in *K. cognatus* than in *K. cuneatus* and *K. usitatus*, but no different from *K. tristis* (Table S3).

Similarly, there were some significant differences in the coefficients of variation (CV) for each trait among species (Figure S1, Table S3). There was greater variance in mandible length in *K. tristis* compared to *K. cuneatus* but no significant differences among other species. *Kosciuscola tristis* also had greater variation in their pronotum than *K. cuneatus* and *K. cognatus*, while pronotum length was largest in *K. tristis* and *K. usitatus* (Figure S1, Table S3). Both *K. tristis* and *K. cognatus* had more variable femur lengths than *K. cognatus* (Figure S1, Table S3).

Finally, we asked whether the allometric relationships differed among species when using femur length as a proxy for body size against mandible length and pronotum length, the two traits we hypothesized were under sexual selection. Neither trait was positively allometric for any species (Fig. 5, Tables S4, S6a-l). For mandible length, all species showed negative allometry (Fig. 5, Table S4a). Isometry was the dominant pattern for pronotum length, though in *K. tristis* pronotum length was borderline positively allometric. In a linear model, both species and femur length had significant effects on mandible length, and there was no effect of the interaction term (Fig. 5a, Table S5). Similarly, both species and femur length have a significant effect on pronotum length, and here the interaction term was significant suggesting that the relationship between femur and pronotum is different among species (Fig. 5b, Table S5). Pairwise comparisons showed no significant
differences between species in mandible-femur allometry. Similar results were returned for pronotum-femur allometry save for a single marginal p value when comparing *K. tristis*’ slope to *K. cuneatus* (Table S3).

**Discussion**

The first aim of this study was to quantify and compare aggressive behaviour in four species of skyhoppers (*Kosciuscola*). We found clear variation in species’ aggression both in the number and type of behaviours performed. The thermocolour skyhoppers (*K. tristis*) and the lightning-bolt skyhoppers (*K. usitatus*) performed the most aggressive behaviours and *K. tristis* was the only species to perform mandible flaring, potentially implicating species or ecological differences as sources of variation in aggressive behaviour. The second aim was to determine whether body size traits predict aggressive behaviour in skyhoppers. We found that across species aggressive behaviours increased with mandible length and pronotum length indicating that individuals in which these traits were larger were more likely to be aggressive. We did not, however, find an effect of body condition (mass / femur length) on aggressive behaviour. Finally, we aimed to
describe the allometric patterns in traits used in aggressive encounters and therefore suspected to be under selection. We found that mandible length is negatively allometric for all species, while pronotum length is essentially isometric for all species, with *K. tristis* being borderline positively allometric, suggesting that conflicting selective pressures act on the allometric relationships in skyhoppers. More broadly this study lends support to behaviour traits being precursors to morphological traits (Blomberg et al. 2003; Vidal-García et al. 2020).

**Variation in aggressive behaviour among skyhoppers**

In our behavioural experiments, we found that *K. tristis* and *K. usitatus* are similarly aggressive, *K. cuneatus* is less aggressive, and *K. cognatus* display very few aggressive behaviours. We propose several ideas to explain this pattern: (1) that there are fundamental species differences, (2) that there is a seasonal effect on mating that underpins aggressive behaviour, and (3) that there is an elevational effect on aggressiveness.

The kinds and amounts of aggressive behaviours that skyhoppers perform varied among species. It is possible that the differences we have observed among the skyhoppers are simply a result of their genetic isolation from each other over the past 1–2 million years (Umbers et al. 2021). Examples of phylogenetic comparative work on behaviour are rare but available evidence shows that behavioural traits are more labile than morphological traits and phylogenetic signal in behaviours is typically low (Blomberg et al. 2003; Ligon et al. 2018; Vidal-García et al. 2020). Not so, however, in macaques. Thierry et al. (2000, 2008) and Balasubramaniam et al. (2012) show strong phylogenetic signal in most of the conflict resolution and aggression traits they measured across nine species. If the patterns of aggressive behaviour we observed in our experiments is driven primarily by phylogeny, however, they would probably be different than what we observed. For instance, *K. tristis* and *K. usitatus* are not each other’s closest relatives (Umbers et al. 2021) and yet they show similarly strong aggressive behaviours compared to their sister taxa (*K. cuneatus* is sister to *K. tristis* and *K. cognatus* is sister to *K. usitatus*) (Umbers et al. 2021). However, our data cannot rule out phylogenetic signal in aggressive behaviour in this group, and we suggest that a larger scale comparative approach now that 14 species are recognised in the genus is an exciting future avenue.

There are fundamental differences in the ecology of these species that may explain the variation in their aggressive behaviour. Elevation influences many morphological (Campbell and Dearn 1980) and life history characters (Dearn 1977). If their elevational distributions translate into different phenologies (Nufio and Buckley 2019) species may have been at different stages in their lifecycles when our experiment was conducted. For example, species at high elevation can be in the middle of their mating season while the mating season of species at lower elevation may have ended. Our observations of aggressive behaviours in the wild have primarily been of *K. tristis* during egg laying and during mating in captivity which happens in late Summer and Autumn (Umbers et al. 2012). If mating during the trials triggered aggressive behaviour it is possible that phenological effects influenced our results, but the evidence is not strong. The greatest proportion of trials with mating was in *K. tristis* (75%), whereas mating occurred in ~45% of trials in *K. usitatus* but these two species showed similar levels of aggressive behaviour. If aggressive behaviours were tightly linked to mating we would have expected to see the most aggressive behaviour from *K. tristis*, which is not the case. Nevertheless, standardising analyses to include only
trials in which mating occurred might be an important aim for future work. In addition, in this study we also assumed that the species responded similarly to our captive conditions. We note that species specific ecologies may contradict that assumption and suggest that future work includes field observations to quantify phenological patterns among different species and their aggressive behaviour in the wild.

One striking similarity in the two most aggressive species, *K. tristis* and *K. usitatus*, is that they are both found in, and were sampled from, very high elevation habitats. Because both are from high elevation but are not each other’s closest relatives (Umbers et al. 2021), we hypothesise that aggression may be under positive selection at high elevation. There is some evidence of high-elevation species being the more aggressive in Australian alpine lizards (Senior et al. 2021). At the highest elevations of the Australian Alps, sympatric with the skyhoppers, the Guthega skink *Liopholis guthega* replaces the mountain rock skink *L. montana*, with the latter found at lower elevations. Senior et al (2021) showed that *L. guthega* is more aggressive toward conspecific and heterospecific model lizards than *L. montana*. To test the hypothesis that elevational distribution correlates with aggressive behaviour in skyhoppers, measures of the aggressive behaviour of high and low elevation populations of all 14 putative species (Umbers et al. 2021) would be required – no mean feat.

Unique and rare behavioural traits are important targets for conservation however they are rarely considered (Caro and Sherman 2012). *Kosciuscola tristis* is the only species in our study to use mandible flaring during contests a behaviour that was surprisingly absent from all other species tested. However, behavioural uniqueness within a clade is not uncommon. A comparative study on crocodilians showed that most species have stereotypical agonistic behaviours (Brien et al. 2013) and that the ‘inflated tail sweep’ is only present in the New Guinea Crocodile (*Crocodyllus novaeguineae*). The precise rarity of the mandible flaring behaviour among the skyhoppers is currently unknown, but there is some tantalising further evidence—casual observations of a close relative of *K. tristis*, *K.t. restrictus*, which is found at very high elevations on Mt Buffalo, Victoria, reveals mandible flaring as well (Umbers, Tatarnic, Muschett and Song Pers. Obs). As more congeners are examined, we will gain a fuller picture of the repertoires of the skyhoppers and the extent of their behavioural diversity.

**Allometric relationships**

Although we found differences in mandible length among species (Fig. 3), there were no differences among species in the allometry, i.e. slope of the relationship, between mandible and femur (Table S3, 5, Fig. 5); mandibles were negatively allometric with respect to femur length for all skyhopper species tested. That mandible length tracks body size similarly for all species was a surprising result given that *K. tristis* seem to use their mandibles differently to the other species, potentially as signals of resource holding potential (Umbers et al. 2013), as weapons in combat (Umbers et al. 2012), or both (Rico-Guevara and Hurme 2019). One possible explanation for the lack of difference is that mandible length is constrained by the anatomical requirement to fit inside the head cavity (Knell et al. 2004). In addition, or alternatively, mandible size may be constrained to maintain their primary function of feeding (Rico-Guevara and Hurme 2019). Traits that have important functions outside of agonistic integrations show similar patterns of constraint in other species. For example, in cave-dwelling wētā (*Pachyrhamma waitomoensis*) leg length
scales isometrically with body size despite males with longer legs being more likely to win contests and suggesting that selective forces like mobility constrain leg length (Fea and Holwell 2018).

It is possible that our results show no difference in allometry between species because our measure of mandible length did not capture the variation under selection. Mandibles showed significant wear across their width in many individuals so we measured length as it was unaffected by that wear. It is possible, however, that mandible width, particular parts of the mandibles such as the ‘teeth’, or the degree of melanisation are under selection. For example, in the fly Drosophila subobscura, wing shape but not size responds to variation in thermal environment (Santos et al. 2004). Future studies that consider including a more nuanced assessment of mandible morphology such as geometric morphometrics (Klingenberg 2011), bite force (Herrel et al. 1999), and the inclusion of females as a comparator (Bonduriansky 2006) in the study would build on our approach.

There was a significant difference among species in pronotum length with K. tristis’ pronotum longer than all other species (Tables S3, S4, and Fig. 5). Comparing allometric slopes, our results show a significant interaction between femur and species indicating differences among species (Table 4) and Kosciuscola tristis’ slope stands out as steeper than the rest of the species’ (Fig. 5). Pairwise comparisons revealed a borderline significant difference (p = 0.05) between K. tristis and K. cuneatus’ slopes (Table S4). The relationships between pronotum and femur length were essentially isometric for all species though it is worth noting that K. tristis’ pronotum~femur slope was borderline positively allometric with the lower confidence limit very close to 1 (0.96) and the upper confidence limit well above 1 (1.28) and the highest for all species (Table S4). Taken together, our results hint at a difference in selection on pronotum length in K. tristis relative to other species. Why might pronotum size be under positive selection in K. tristis? When displaying their mandibles and fighting off other males by biting (Umbers et al. 2012), K. tristis flex their heads backward and from side-to-side movements that could benefit from a higher volume of musculature in the pronotum, analogous to co-opted flight muscles (Wipfler et al. 2015). However, further evidence is required to support that conclusion.

Finally, the relationship between body size and elevation is complex in insects and from a vertebrate-centric view Bergmann’s Rule may be naïvely applied (Hodkinson 2005; Dillon et al. 2006). For example, some mountain insects including K. usitatus and K. cognatus show a distinct negative correlation between body size and elevation (Campbell and Dearn 1980; Dillon et al. 2006; Yadav et al. 2020), while K. tristis body size is uniform with respect to elevation (Yadav et al. 2020). Selection for larger than expected body size in K. tristis may indicate that elevational gradients influence allometric relationships in this species too (Dillon et al. 2006). Future work should compare the allometric slopes of K. tristis across elevation with those of other skyhoppers.

Future directions

Our study shows variability in aggressive behaviour among closely related species but minimal evidence for positive allometric scaling of the traits involved. In the skyhoppers, this may be explained by behavioural traits being more labile than morphological ones, positive allometry of morphological traits may be constrained by primary function, or ecological correlates like elevation (unaccounted for here) that have an important effect on body size. Our study provides solid ground for a range of future experiments on the evolution
of aggressive behaviour within ecological constraints. Our results indicate that thorough field-based observations across the recently recognised diversity in this genus and phylogenetic comparative work (Umbers et al. 2021), the skyhoppers will continue to develop as an important model system to understand the evolution of aggressive behaviour.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10682-022-10185-6.

Acknowledgements The authors thank Rose Lownds and Justin McNab for dissecting, photographing and measuring grasshoppers and, Narkis Moralis, Katherine McClellan, Madeline Sanders, Lachlan Byatt, Marlis Dumke, and Hywel Barker for assistance in the field. We also thank the Australia and Pacific Science Foundation (APSF 09-7), the Australian Biodiversity Resource Study (RF211-29), Thredbo Sports, Macquarie University, and Western Sydney University for funding and support. We acknowledge the Dharug nation as the traditional custodians of the land on which Macquarie University and Western Sydney University now stand, and the Ngarigo nation on whose land the field work was undertaken and that their sovereignty was never ceded. We strongly support equity, diversity and inclusion in science (Rößler et al. 2020). The authors come from different countries (Australia, Austria, New Zealand, Panama) and represent different career stages (from postdoc to Professor). Our gender balance is biased towards women and one of the authors self-identifies as a member of the LGBTQI+ community. This study has spanned the pregnancies and births of five babies of three of the authors and we wish to acknowledge the women that have come before us that fought for and won maternity leave and related entitlements.

Author contributions The study conception and design, material preparation and data collection were carried out by Giselle Muschett, Marie Herberstein and Kate Umbers. Analysis was carried out by Kate Umbers and Christina Painting. The manuscript was written by Kate Umbers and Giselle Muschett with input from both other authors who have read and approved the final manuscript.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions. This study was funded by the Australian Biological Resources Study RF211-29, Australia and Pacific Science Foundation (APSF 09–7), Thredbo Sports, Macquarie University and Western Sydney University.

Data availability Supplementary file.

Code availability Supplementary file.

Declarations

Conflict of interest All authors certify that they have no conflicts of interest or relevant financial interests to declare that are relevant to the content of this article.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Andersson M (1982) Female choice selects for extreme tail length in a widowbird. Nature 299: 1–3.
Andrade MCB, Kasumovic MM (2005) Terminal investment strategies and male mate choice: extreme tests of Bateman1. Integr Comp Biol 45:838–847. https://doi.org/10.1093/icb/45.5.838
Arnott G, Elwood RW (2008) Information gathering and decision making about resource value in animal contests. Anim Behav 76:529–542. https://doi.org/10.1016/j.anbehav.2008.04.019
Hack M (1997) Assessment strategies in the contests of male crickets, Acheta domestica (L.). Anim Behav 53:733–747. https://doi.org/10.1006/anbe.1996.0310
Haley MP (1994) Resource-holding power asymmetries, the prior residence effect, and reproductive payoffs in male northern elephant seal fights. Behav Ecol Sociobiol 34:427–434. https://doi.org/10.1007/BF00167334
Herrel A, Spithoven L, Damme RV, Vree FD (1999) Sexual dimorphism of head size in Gallotia galloti: testing the niche divergence hypothesis by functional analyses. Funct Ecol 13:289–297. https://doi.org/10.1046/j.1365-2435.1999.00305.x
Hodkinson ID (2005) Terrestrial insects along elevation gradients: species and community responses to altitude. Biol Rev 80:489–513. https://doi.org/10.1017/S1464793105006767
Hofmann HA, Schildberger K (2001) Assessment of strength and willingness to fight during aggressive encounters in crickets. Anim Behav 62:337–348. https://doi.org/10.1006/anbe.2001.1746
Jakobsson S, Brick O, Kullberg C (1995) Escalated fighting behaviour incurs increased predation risk. Anim Behav 49:235–239
Kemp DJ (2000) Contest behavior in territorial male butterflies: does size matter? Behav Ecol 11:591–596. https://doi.org/10.1093/beheco/11.6.591
Key KHL, Day MF (1954) A temperature-controlled physiological colour response in the grasshopper, Kosciuscola tristis Sjøst. (Orthoptera: Acrididae). Aust J Zool 2:309–339
Kilmer JT, Rodriguez RL (2017) Ordinary least squares regression is indicated for studies of allometry. J Evol Biol 30:4–12. https://doi.org/10.1111/jeb.12986
Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. Mol Ecol Resour 11:353–357. https://doi.org/10.1111/j.1755-0998.2010.02924.x
Knell RJ, Pomfret JC, Tomkins JL (2004) The limits of elaboration: curve allometries reveal the constraints on mandible size in stag beetles. Proc R Soc Lond B 271:523–528. https://doi.org/10.1098/rspb.2003.2641
Kodric-Brown A, Brown JH (1984) Truth in advertising: the kinds of traits favored by sexual selection. Am Nat 124:309–323
Lenth R, Singmann H, Love J, et al (2020) emmeans: Estimated Marginal Means, aka Least-Squares Means. Version 1.4.8URL https://CRAN.R-project.org/package=emmeans
Ligon RA, Diaz CD, Morano JL et al (2018) Evolution of correlated complexity in the radically different courtship signals of birds-of-paradise. PLoS Biol 16:e2006962. https://doi.org/10.1371/journal.pbio.2006962
Lindström K (1992) The effect of resource holding potential, nest size and information about resource quality on the outcome of intruder-owner conflicts in the sand goby. Behav Ecol Sociobiol 30:53–58
Mathies T, Andrews RM (1995) Thermal and reproductive biology of high and low elevation populations of the lizard Sceloporus scalaris: implications for the evolution of viviparity. Oecologia 104:101–111
Mathirown AGE, Pottier P, Goubault M (2018) Let the most motivated win: resource value components affect contest outcome in a parasitoid wasp. Behav Ecol 29:1088–1095. https://doi.org/10.1093/beheco/ary084
Muschett G, Umbers KDL, Herberstein ME (2017) Within-season variability of fighting behaviour in an Australian alpine grasshopper. PLoS ONE 12:e0171697. https://doi.org/10.1371/journal.pone.0171697
Muschett G, Umbers KDL, Herberstein ME (2018) Male mate choice in the chameleon grasshopper (Kosciuscosa tristis). Ethology 124:751–759. https://doi.org/10.1111/eth.12809
Nufio CR, Buckley LB (2019) Grasshopper phenological responses to climate conditions, variability, and change. Ecosphere 10:e02866. https://doi.org/10.1002/ecs2.2866
Otte D (1970) A comparative study of communicative behavior in grasshoppers. Misc Publ Museum Zool Univ Michigan 141:1–168
Painting CJ, Holwell GI (2014) Exaggerated rostra as weapons and the competitive assessment strategy of male giraffe weevils. Behav Ecol 25:1223–1232. https://doi.org/10.1093/beheco/aru119
Painting CJ, Buckley TR, Holwell GI (2014) Male-biased sexual size dimorphism and sex ratio in the New Zealand Giraffe Weevil, Lasthynchus barbaricornis (Fabricius, 1775) (Coleoptera: Brentidae). Austral Entomol 53:317–327. https://doi.org/10.1111/aen.12080
Parker GA (1974) Assessment strategy and the evolution of fighting behaviour. J Theor Biol 47:223–243. https://doi.org/10.1016/0022-5193(74)90111-8
R Core Team (2020) A language and environment for statistical computing. Version 1.13. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
Rehn JAG (1957) The grasshoppers and locusts (Acridoidea) of Australia. Family Acrididae: Subfamily Cyrtacanthacridinae tribes Oxyini. Spathosternini. and Praunulini. CSIRO, Melbourne
Rico-Guevara A, Hurme KJ (2019) Intrasexually selected weapons. Biol Rev 94:60–101. https://doi.org/10.1111/brv.12436
