# The loss of nuptial gifts in sclerosomatid Opiliones coincides with increased behavioural sexual conflict

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**Abstract:**

Nuptial gifts serve to increase donor fitness through a variety of pathways independent of their effect on the recipient. Some nuptial gifts deliver benefits similar to those of antagonistic male behaviours: functioning to secure additional copulations, increase sperm transfer or storage, or increase paternity share. These commonalities may result in evolutionary transitions between solicitous and coercive strategies, wherein behavioural sexual conflict could function to secure mates in lieu of nuptial gifts. In temperate leiobunine harvesters (Arachnida: Opiliones), nuptial gifts have been repeatedly lost, resulting in two primary mating syndromes: an ancestral, sacculate state in which males endogenously produce high-investment nuptial gifts and females lack pregenital barriers, and a derived, nonsacculate state in which females have pregenital barriers and males produce significantly reduced, low-investment nuptial gifts. In this study, we investigated whether behavioural sexual conflict compensates for reduced nuptial gifts in nonsacculate harvesters by comparing the intensity of pre-, peri-, and postcopulatory behavioural sexual conflict between the nonsacculate species *Leiobunum vittatum* and *L. euserratipalpe* and the sacculate species *L. aldrichi* and *L. bracchiolum*. We additionally sought to establish an automated behavioural analysis pipeline by developing analogues for metrics traditionally scored manually. Our results revealed significantly higher sexual conflict behaviour in nonsacculate species, indicating that the loss and reduction of pre- and pericopulatory nuptial gifts may contribute to increased behavioural antagonism. Sexual conflict behaviour also differed significantly between *L. vittatum* and *L. euserratipalpe*, indicating there are multiple suites of antagonistic behaviours. Together, these results suggest that multiple behavioural strategies may be effective substitutes for nuptial gifts in leiobunine Opiliones, although the mechanisms through which male fitness is increased requires further research.

**Highlights:**

- Temperate Opiliones have repeatedly evolved reduced nuptial gift investment
- Reduced-gift (nonsacculate) species possess morphology indicative of antagonism
- We tested whether sexual conflict is higher in nonsacculate versus sacculate species
- Nonsacculates displayed significantly higher behavioural antagonism than sacculates
- Nonsacculate species differed in their suites of antagonistic behaviours

**Keywords:**

- Antagonism
- Mate guarding
- Mating behaviour
- Nuptial gift
- Opiliones
- Sexual conflict

# Introduction

## Nuptial gift background

Animals employ a multitude of tactics to increase their fitness during mating interactions, including direct intrasexual competition, mate choice, elaborate signals, and physical or chemical coercion (Buchinger & Li 2023; Davranoglue et al. 2023; Edward & Chapman 2011; Órfão et al. 2023; Rosvall 2011; Schaefer & Ruxton 2015; Shuker & Kvarnemo 2021). Of these myriad strategies, nuptial gifts are among the most unique in their almost altruistic appearance. Nuptial gifts are any materials, beyond gametes, which are provided by a donor to a recipient before or during copulation in order to increase the donor's fitness (Gwynne 2008; Lewis & South 2012; Lewis et al. 2014). These gifts might be consumed orally or received internally during copulation and can range from exogenous materials (such as captured prey wrapped in silk) to endogenous materials (such as proteinaceous fluids) (Gao et al. 2019; Lewis & South 2012). The fitness consequences to the recipient remains intentionally unspecified under this definition, and extensive research shows nuptial gifts can range from being highly beneficial (Albo et al. 2017; Brown & Barry 2016; Gwynne 2008; Lehmann & Lehmann 2016; O'Hara & Brown 2021; Toft & Albo 2015; Voigt et al. 2005; Welke & Schneider 2011) to neutral (Martínez Villar et al. 2021) to highly costly (Arnqvist & Rowe 2005; Sakaluk et al. 2019; Sirot et al. 2015; Wolfner 2002).

Predicting these consequences, however, can be difficult due to the highly variant nature of many nuptial gifts, particularly those that are endogenously produced (Kahn et al. 2018; Lewis & South 2012; Wedell 1994). Even when accounting for various sources and delivery mechanisms, nuptial gift quality can be highly variable both within and between individuals. Gifts may fluctuate with environmental conditions, rearing conditions, donor nutrition, or competition intensity (Fox et al. 2006; Ghislandi et al. 2018; Jarrige et al. 2015; Křemenová et al. 2021; McPherson et al. 2022; Rapkin et al. 2016). Recipient traits can further influence nuptial gift

quality, as some male crickets can alter their gift contents in response to the age of the female receiving it (Jarrige et al. 2015).

While the nature and mode of delivery might prove insufficient to predict the fitness consequences of nuptial gifts, a theoretical approach based on the mechanistic origins may provide sufficient framework to make inferences. Donor fitness benefits have long been debated with little conclusive support for a single hypothesis, potentially due to the primary candidates (the mating effort and parental investment hypotheses) being treated as mutually exclusive (Arnqvist & Rowe 2005; Gwynne 2008; Lewis & South 2012; Lewis et al. 2014; Sakaluk 2000; Thornhill 1976; Vahed 1998, 2007). Lewis and South (2012) present a revised approach to describing nuptial gift benefits, suggesting eight non-exclusive benefits (increasing mate attraction, copulation success, insemination success, sperm transfer, sperm storage, paternity share, female fecundity, or offspring fitness) through which donor fitness may be increased. By resolving the non-independence of the mating effort and parental investment hypotheses, this episodic framework can reduce potentially ambiguous interpretations of nuptial gift experiments.

Within this framework, researchers can investigate the ultimate function of a nuptial gift by using species that have trade-offs between gifts and alternative fitness-enhancing reproductive strategies (Lewis & South 2012). Behavioural antagonism can serve many of these functions, often increasing copulation and insemination success, sperm storage and transfer, and paternity share (Lewis & South 2012; Gwynne 2008; Arnqvist & Rowe 2005). In 2014, Vahed et al. demonstrated the efficacy of this approach, providing convincing evidence that prolonged clasping time can function as a substitute for nuptial gifts. This has subsequently been shown in other orthopterans, suggesting that these nuptial gifts function in some capacity to increase sperm transfer because increased clasping time would be unlikely to directly increase any other aspect of the eight potential nuptial gift fitness consequences (Gwynne et al. 2008; Lehmann et al. 2016; Vahed et al. 2014).

Deleterious nuptial gifts are conspicuous symptoms of sexual conflict and therefore the subject of much research, yet the presence of beneficial nuptial gifts does not necessarily signal an interaction lacking conflict (Arnqvist & Rowe 2005; Gwynne 2008; Lewis & South 2012; Sirot et al. 2015). Mating interactions can be extremely costly to females – whether due to direct physical or physiological harm from males, resistance costs, or simply energy expenditure and predation risks – each mate encounter can yield grave fitness consequences and can reduce overall population-level fitness (Arnqvist & Rowe 2005; Boulton et al. 2018; Kokko & Jennions 2014; Lehtonen et al. 2012; Sentenská et al. 2020; Tatarnic et al. 2014). Regardless of the female costs, nuptial gifts are typically an energy-intensive reproductive strategy for males. Nuptial gift production can increase male mortality (Małek et al. 2019; Perry & Tse 2013) and reduce male courtship quality (del Castillo & Gwynne 2007), and even simply carrying nuptial gifts can significantly increase male energy expenditure (Prokop & Okrouhlik 2021). Both endogenous fluid and exogenous prey have high production demands which often leads to selective pressures to maximise the gift efficiency and efficacy through various strategies, including deceiving females or plastically altering gifts in response to female quality (Albo et al. 2019; Beyer et al. 2021; Ghislandi et al. 2018; Martínez-Villar et al. 2023; Pandulli-Alonso et al. 2021; Solano-Brenes et al. 2021; Stålhandske 2002).

Energetic investment into one reproductive tactic at the expense of another trait is common throughout arthropods (del Sol et al. 2021; Fry 2006; Körner et al. 2017; Okada & Miyatake 2009; Sentenská et al. 2020; Simmons et al. 2017; Yamane et al. 2010). While precopulatory intrasexual contest traits are frequently subject to these trade-offs, trade-offs involving sperm competition or nuptial gifts are potentially just as numerous, albeit not as readily apparent (del Castillo & Gwynne 2007; Kelly 2008; Lehmann et al. 2016; Miller et al. 2019; Moczek & Nijhout 2003; Simmons & Emlen 2006; Yamane et al. 2010). The trade-offs between gift quality and female manipulation, however, whether through deception or physical coercion, would be

particularly evident when measuring sperm transfer or storage, paternity share, or insemination success (Gao et al. 2019; Lewis & South 2012).

Trade-offs between nuptial gifts and other strategies have been increasingly documented across arthropod species (del Castillo & Gwynne 2007; South et al. 2011). This is particularly evident in Corydalidae ("dobsonflies"), a family notable for species with traumatic mating strategies, elaborate male weapons, and/or large nuptial gifts (Liu et al. 2015; Tong et al. 2021). Of the four major dobsonfly lineages, two retained large, ancestral nuptial gifts and two experienced a single loss before divergence. In these giftless lineages, dobsonflies have subsequently independently evolved enhanced clasping mandibles twice (Liu et al. 2015). Across Corydalidae, no documented species has both large nuptial gifts and enhanced mandibles, indicating a trade-off between solicitous and antagonistic strategies that might suggest a relaxation of male competition for mates (Liu et al. 2015). Interestingly, *Neopanorpa* dobsonflies display additional trade-offs, as some males have lost nuptial gifts in favour of coercing females to mate through clasping (Tong & Hua 2019). *Leiobunum* Opiliones show a highly similar pattern of ancestral nuptial gifts and derived conflict traits, although to date, behavioural studies that directly compare multiple species are lacking.

## Study Species

North American sclerosomatid harvesters (Opiliones: Sclerosomatidae), also known as "daddy-longlegs", form a widespread yet understudied family of arachnids with a mating system ideal for studying the role of nuptial gifts in sexual conflict (reproductive biology reviewed in Machado & Burns 2023). Burns and colleagues have described repeated correlated evolution of both female and male reproductive morphology, revealing two primary reproductive syndromes (Burns et al. 2012, 2013): 1) An ancestral, sacculate state in which males have terminal nuptial gift sacs on their penises and females do not have pregenital barriers; and 2) a derived, nonsacculate state in which males do not have terminal nuptial gift sacs on their penises and females have pregenital barriers. Both sacculate and nonsacculate *Leiobunum* harvesters

produce nuptial gifts to some degree, although the presence of terminal nuptial gift sacs increases the quantity transferred to females during mating interactions and allows for precopulatory transfer (Machado & Burns 2023; Machado & Macías-Ordóñez 2007). Sacculate females receive nuptial gift fluid before and during copulation, while nonsacculate females can only receive it during copulation (Figure 1). In addition to these genital morphologies, sacculate and nonsacculate species also differ in genital biomechanics (Burns & Shultz 2015, 2016), somatic morphology (Burns et al. 2013; Machado & Burns 2023), and nuptial gift biochemistry (Kahn et al. 2018). Nonsacculate species tend to have more robust genital biomechanics, specialised clasping structures, and less nutritious nuptial gifts, while sacculate species tend to have less robust genital biomechanics, unspecialized clasping structures, and higher-value nuptial gifts (Burns et al. 2013; Burns & Shultz 2015, 2016; Kahn et al. 2018; Machado & Burns 2023).

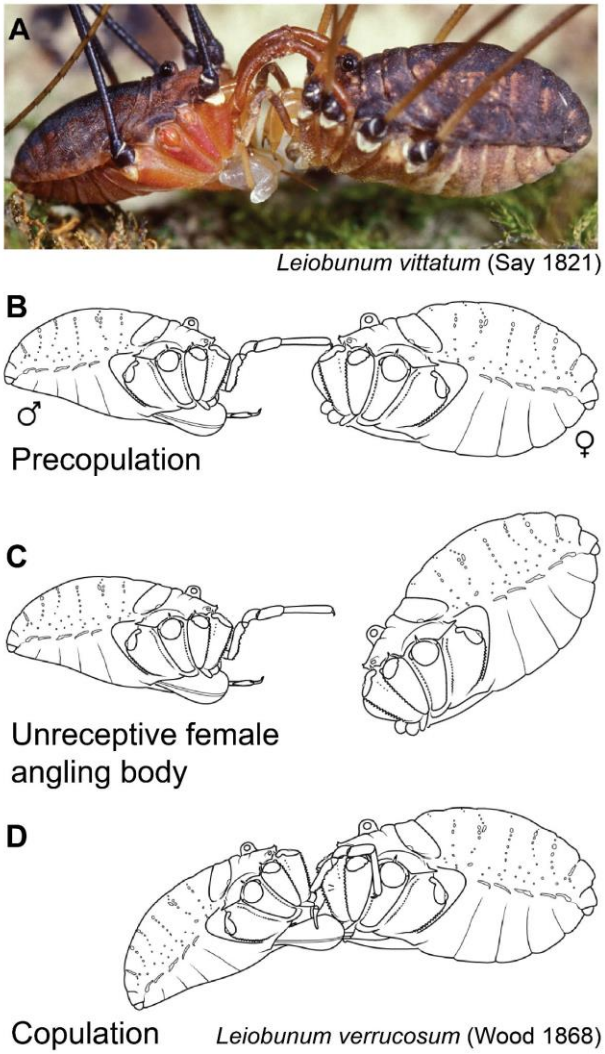


**Figure 1:** Primary mating phases and body orientations of *Leiobunum spp.* **A)** Male (left) *L. vittatum* clasping female (right) precopulation. Photo courtesy of Joe Warfel. **B)** Precopulatory phase, during which consumption of precopulatory nuptial gift (when present in the species) occurs. **C)** Precopulatory posture of an unreceptive female. Female's anterior is angled downwards to prevent male access to the ventrally located genital operculum. **D)** Copulatory phase, during which consumption of pericopulatory nuptial gift occurs. **B-D)** Images are semi-diagrammatic and the legs are not pictured to increase clarity (*L. verrucosum*, adapted from Figure 1 in Burns et al. 2013).

Although there is morphological and chemical evidence of a shift towards increased conflict, comparative behavioural evidence in the genus is limited. Fowler-Finn and colleagues (2014, 2018, 2019) have thoroughly documented the mating behaviour of the nonsacculate *L. vittatum* as well as several other species, but no direct, comparative, behavioural work between sacculate and nonsacculate species has been conducted. The multiple independent transitions to a derived, antagonistic state in these harvesters provide an ideal system for studying the role of sexual conflict in the loss of nuptial gifts and how other behavioural or morphological changes may compensate for this loss. In this study, we tested whether nonsacculate leiobunine species displayed significantly higher levels of behavioural sexual conflict than sacculate *Leiobunum* species. We performed mating trials in two sacculate and two nonsacculate *Leiobunum* species and compared the intensity and frequency of behavioural sexual conflict to search for differences between the two reproductive syndromes.

# Methods

## Study Species

Leiobunine harvesters have a broad, holarctic distribution and require extensive taxonomic revision (Hedin et al. 2012), however, the “late-season” (defined as overwintering as eggs) leiobunines of eastern North America form a well-supported monophyletic group (Burns et al. 2012). We therefore restricted our study species to this clade, within which there are three major phylogenetically-supported groups of harvesters. From these we selected one representative species per group, as well as one species which was not a member of any major group (Table 1).

| Species | Species Group | Penis Morphology | Male Pedipalp Morphology | Nuptial Gift Investment | Conflict Predictions |
|---|---|---|---|---|---|
| *L. euserratipalpe* | *calcar* | Nonsacculate | Unmodified | Low | High |
| *L. vittatum* | *vittatum* | Nonsacculate | Specialized for clasping | Low | High |
| *L. bracchiolum* | *politum* | Sacculate | Unmodified | High | Low |
| *L. aldrichi* | - | Sacculate | Unmodified | High | Low |

**Table 1:** Morphological and biochemical phenotypes of the four species in this study, as well as overall behaviour expected in trials (Burns et al. 2012; Kahn et al. 2018; Shultz 2018). High conflict would be indicated by reduced latency to make a clasping attempt, elevated contact duration, elevated time within one leg span, elevated clasping duration, elevated guarding duration, elevated female body angle, reduced association distance, and reduced pursuit angle. Low conflict would be indicated by the inverse of these metrics.

In sacculate species, males first use their pedipalps to clasp females face-to-face by the coxae of leg II (Figure 1A and D). The male then extends its penis to the female's mouth, allowing the female to consume the endogenously-produced precopulatory nuptial gift that accumulates in the terminal sacs after being produced by accessory glands (Figure 1B). The male redirects its penis to the female's genital operculum, which the female then opens to allow intromission. At this stage, the female consumes the endogenously-produced, pericopulatory nuptial gift directly from the male's papilla as its mouth aligns with male's haematodocha (Figure 1D; Burns et al. 2012, 2013, Machado & Macías-Ordóñez 2007). In nonsacculate species, this behavioural sequence proceeds much the same, save for two notable differences: 1) the precopulatory nuptial

gift does not occur, and 2) the male may use its penis to pry the female genital operculum open should the female resist copulation (Burns et al. 2012, 2013).

## Study Populations

We collected harvesters as juveniles in the spring and summer of 2021, 2022, and 2023. The exact timing and location of collection depended upon the species, as each species matures at different times, and some are restricted in their geographic range across central Maryland. *Leiobunum vittatum* were collected in June and July of 2021 at Rockburn Branch Park (39.219471, -76.761198) and the University of Maryland, Baltimore County (39.247397, -76.710568); *L. aldrichi* were collected in June and July of 2021 at Sugarloaf Mountain (39.261630, -77.395560) and Plummer's Island (39.261630, -77.395560); and *L. euserratipalpe* were collected in April and May of 2022 at Schooley Mill Park (39.166325, -76.961830); and *L. bracchiolum* were initially collected in May and June of 2021 at Rockburn Branch park. Due to a low rate of successful clasps, additional *L. bracchiolum* were collected in May and June of 2023 at the same location to ensure sufficient data for analyses which required successful clasps.

Following collection, we separated harvesters by species and sex and housed them communally at 24° C, 80% humidity, and 12:12 h light:dark periods in 37 cm x 22 cm x 24.5 cm plastic terraria. Each terrarium contained ~ 2 cm of Eco Earth coconut fibre substrate (Zoo Med), petri dishes with TetraMin Select tropical flakes fish food (Tetra), moist cotton balls, and leaves, sticks, and bark gathered from the collection locations for shelter. All terraria were moistened daily and checked for mould, and all were cleaned weekly. Upon maturation, we maintained harvesters in similar conditions until mating trials were complete, at which point we housed them communally by sex until natural senescence.

## Behaviour

Behavioural trials consisted of pairing male and female conspecifics and recording mating behaviour. We conducted all behavioural trials in a 29.21 cm x 29.21 cm acrylic arena lined with white cardstock to reduce reflections and allow for easy cleaning. The arena was housed in a room kept at 24º C and illuminated with white light (leiobunine Opiliones have poor vision and lighting is unlikely to impact mating behaviour (Machado & Macías-Ordóñez 2007; Shultz & Pinto-da-Rocha 2007) and we video recorded interactions dorsally and laterally with Microsoft LifeCam Studio cameras. Following the methods used in several studies by Fowler-Finn and colleagues (2014, 2018, 2019), we conducted trials as follows: We first sterilised the arena with 95% ethanol before placing a male and female harvester in the arena. Initially, harvesters were isolated for 5 minutes under transparent plastic containers to allow acclimation to the arena without contact with one another. Following acclimation, we removed the containers and the trial began. Trials lasted for either 20-minutes or until the mating interaction ended. Interactions were considered concluded when contact was broken between individuals and the male made no attempt to chase the female. If the interaction had not ended by the 60-minute mark, the trial was concluded.

We aimed to complete 20 trials per species, though the exact number per species varied due to random senescence and exclusions due to inactivity (Appendix Table A1). Trials were included in analyses if the male made an attempt to hook its pedipalps around the female's coxae within the first 20 minutes. If no mating attempt was made at this point, we removed the harvesters from the arena, excluded them from future trials, and did not include the trial in analyses. The majority of males (86.36%) made an attempt to clasp within the first 20 minutes, meaning only 12 of 88 potential trials were excluded due to inactivity. The majority of interactions (86.67%) had concluded before the imposed 60-minute limit, with only 11 of 75 trials artificially ended. Interestingly, seven of these 11 trials were with *L. euserratipalpe* pairs.

## Video Analyses

In order to construct a scoring protocol to reduce the time spent manually scoring videos, we elected to use the automated tracking program TRex, built by Walter and Couzin (2021). The computing requirements to execute TRex are significantly lower than many machine learning based programs, and the training and tracking stages were completed on standard consumer-grade laptops equipped with stock GPUs (NVIDIA GeForce RTX3060; NVIDIA GeForce GTX 1650). Some behaviours, particularly those occurring on the ventral side of the harvesters, cannot be reliably scored with TRex, yet the manual scoring portion of our protocol was nonetheless reduced. The legs of harvesters presented a challenge to TRex during our video processing – 16 relatively identical, extremely thin legs which overlap frequently during mating and locomotion created significant visual interference and prevented accurate tracking. Due to this we elected to use a background filter value in order to exclude the legs from tracking, leaving behind only the body centroids.

To eliminate minor errors inherent to automated tracking, we averaged the per-frame TRex centroid coordinates over 30-frame sections to create per-second body centroid coordinates. From these coordinates, we calculated several metrics: distance between individuals, total time individuals were within one male leg span (measured using the longest leg length specific to the male in each trial), and total clasping duration (Table 2). We additionally explored a method to assess the degree to which males followed females between first contact and first mating attempt. We used the node coordinates to first calculate male and female trajectory vectors and magnitudes before using these to derive the angle between vectors. This metric describes the “pursuit angle” between male and female movement; values closer to 0 degrees indicate the individuals were moving in the exact same direction, which we would expect if the male were chasing the female along parallel vectors (Table 2). Conversely, values closer to 180 degrees indicate the individuals were moving in the exact opposite direction.

| Metric | Definition |
|---|---|
| AC trial | Any trial in which the male attempted to clasp the female. |
| SC trial | Any trial in which the male successfully clasped the female. |
| Restraint | Male attempts to restrain the female by grabbing her legs, body, or pedipalps with his pedipalps or legs. |
| Struggle | Female attempts to escape male restraint or flees from male contact. |
| Attempted clasp | Male attempts to hook his pedipalps behind the female's leg II coxae. |
| Successful clasp | Male successfully hooks his pedipalps behind the female's leg II coxae. |
| Guarding | Any instance of contact greater than five seconds post-successful clasp. Subsequent clasps were scored as clasps. |
| Pre-attempt contact | Any contact between individuals before a successful clasp has occurred. |
| Clasping time | Time during which the male maintains a clasp with his pedipalps hooked behind the female's leg II coxae. |
| Guarding time | Total time of all non-clasp contact instances lasting greater than five seconds post-successful clasp. |
| Time within 1 leg span | Time during which the distance between male and female bodies was less than the length of the male's longest leg. |
| Distance | Distance between the female and male body centroid post-initial contact. |
| Pursuit angle | Angle formed by the male and female velocity vectors post-initial contact. |
| Female body angle | Maximum angle between the female's genital operculum and the substrate during a clasping attempt or success. |
| Latency to attempt | Time between the initial contact between individuals and the first clasping attempt. |

**Table 2:** Behavioural sexual conflict metrics recorded during all trials. Rationale for each metric is noted in text with supporting citations.

To ensure TRex performed accurately and matched manual tracking expectations, we additionally scored all trials with JWatcher (Blumstein & Daniel 2007). For all attempted clasp trials (AC trials), we scored the total time the harvesters were in contact and the latency between first contact and first mating attempt (Table 2). For successful clasp trials (SC trials), we additionally scored the time between first mating attempt and first successful clasp, the total time the individuals were in contact, the total time the male clasped the female as a proxy for intromission time (as per Fowler-Finn et al. 2014, 2018, 2019), and the total time the male guarded the female post-copulation (Table 2). In leiobunine harvesters, mate guarding consists of the male keeping in constant contact with at least one leg grasping the female's leg; because they primarily rely on tactile sensing using antenniform legs and their vision is particularly poorly-developed, this contact is likely required (Machado & Macías-Ordóñez 2007; Shultz & Pinto-da-Rocha 2007; Fowler-Finn et al. 2018, 2019). We considered post-copulatory contact lasting greater than 5 seconds to constitute mate guarding (Fowler-Finn et al. 2014, 2018, 2019).

Finally, in all AC trials, we used the image analysis program FIJI-ImageJ (Schindelin et al. 2012) to measure the maximum angle the female's genital operculum formed with the substrate of the arena (Figure 1C). Due to the degree to which some mating pairs moved during their interactions, we could not effectively automate this metric to obtain a continuous measure of the angle, thus we elected to use the maximum angle reached during a clasp attempt (Table 2).

## Statistical Analyses

Due to our selected study species being closely related, we first tested for phylogenetic signal across all our behavioural sexual conflict metrics. Previous studies have not found evidence for signal among morphological traits (Burns & Shultz 2015); and significant evidence suggests that behavioural traits are among the least likely to display patterns of phylogenetic signal because of their lability (Blomberg et al. 2003). We imported our behavioural data to R (v4.3.1; R Core Team 2023) and calculated Pagel's lambda (Pagel 1999) using the *phytools* package (Revell 2012). We used a pruned version (consisting of our four study species) of a maximum

clade credibility tree constructed from mitochondrial and nuclear sequences by Burns et al 2013. We found extremely low levels of phylogenetic signal (Likelihood ratio test versus Brownian motion: All $P > 0.6$; Appendix Table A2) and after consideration with previous evidence (Burns & Shultz 2016, 2015; Blomberg et al. 2003) proceeded with frequentist statistical analyses.

We then evaluated normality using the Shapiro-Wilk test and followed this with Kruskal-Wallis tests for nonparametric data to determine whether the individuals used in our study differed significantly between species in the number of sensory legs and total legs. Because Opiliones can autotomize their legs, severing them to escape potential predators (Gnaspini & Hara 2007), not all individuals in the study had the same number of total legs or sensory legs. We incorporated this variability by constructing multiple linear regression models with behavioural sexual conflict metrics as response variables (Table 2) and several leg metrics (number of male legs, number of male sensory legs, number of female legs, and number of female sensory legs) as predictor variables. The results of these multiple linear regression models allowed us to determine whether the loss of legs impacted behavioural sexual conflict.

For all behavioural sexual conflict metrics, we used the Shapiro-Wilk test for normality and found at least one species in all but one metric to be non-normally distributed. Due to this, we used the Kruskal-Wallis test to compare the four species for all metrics except the average distance between individuals. For both AC and SC trials this metric was normally distributed after a logarithmic transformation, and so these data were analysed with a one-way ANOVA after transformation. We followed any significant groupwise comparisons with planned pairwise comparisons using Dunn’s multiple comparisons test or Šídák's multiple comparisons test, where appropriate, between sacculate and nonsacculate species (*L. bracchiolum x L. vittatum*; *L. bracchiolum x L. euserratipalpe*; *L. aldrichi x L. vittatum*; and *L. aldrichi x L. euserratipalpe*). Following inspection of several metrics (pursuit angle, attempt latency, clasping duration, and maximum female body angle), we decided to compare the two nonsacculate species (*L. vittatum*

and *L. euserratipalpe*) to one another with post-hoc Dunn's tests, corrected for multiple comparisons.

We concluded our statistical analyses with multiple logistic regression models to detect which behavioural sexual conflict metrics best predict clasping success. We built models with success/failure to secure a clasp as the response variable and the conflict metrics as the predictor variables. We constructed a total-species model using all data, a sacculate-species model using only *L. bracchiolum* and *L. aldrichi* data, and species models using species-level data. We could not build nonsacculate-species or species-level models for *L. vittatum* and *L. euserratipalpe* due to each species having only one failed clasp attempt, thus leading to perfect separation in our models.

## Ethical Note

We collected *L. vittatum*, *L. euserratipalpe*, *L. aldrichi*, and *L. bracchiolum* from populations throughout their natural range in central Maryland. The Washington Biologists' Field Club provided collection permission for Plummer's Island, while collection permits were not required for any other location. All source populations used for collections were well-established, with the species in question being clearly abundant and unlikely to be affected by our collections. The harvesters were then held in the lab following ASAB/ABS guidelines for the treatment of animals used in behavioural research. Harvesters were reared collectively whenever possible in accordance with their gregarious behaviour in the field. Behavioural trials were generally not harmful to individuals – females experienced harassment from males during trials, but it was likely far less frequent than harassment experienced in natural conditions. Following trials, individuals were maintained in the laboratory until natural senescence, typically within one to two months.

# Results

## Overall Contact and Association Metrics

We found one significant difference when comparing the number of legs between species; female *L. bracchiolum* had significantly more total, but not sensory, legs when compared to female *L. aldrichi*. Neither males nor any other comparison between females yielded significant differences. Of our multiple linear regression models, the model with the response variable of clasping duration indicated that the number of legs significantly impacted this metric, with the number of male legs, the number of male sensory legs, and the number of female sensory legs each being significant predictor variables (Appendix Table A3). For all metrics save clasping duration, our multiple linear regression models did not provide any evidence that leg loss impacted behavioural sexual conflict. Because none of the significant predictor variables differed between species for our data, we can conclude that it is unlikely leg number variation impacted our data.

We found significant differences between species for all contact and association metrics, although the specific relationships varied. We measured the degree of contact between male and female harvesters during AC and SC trials using several metrics: total time in contact scored (JWatcher), total time within one male leg span (TRex), and distance between individuals (TRex). Overall, females and males of nonsacculate species (*L. vittatum* and *L. euserratipalpe*) were in contact significantly longer and associated significantly more closely than females and males of sacculate species (*L. bracchiolum* and *L. aldrichi*; Figure 2).

We used JWatcher to score trials manually for total contact time. In AC trials, nonsacculate males and females were in contact for significantly longer than sacculate males and females. In these trials, median contact time was between three and four times longer in nonsacculate species than in sacculate species (Figure 2A). When comparing only trials in which males successfully clasped the females, both nonsacculate species retained significantly longer contact

time when compared to *L. aldrichi*, however, *L. bracchiolum* was not significantly different (Figure 2B).

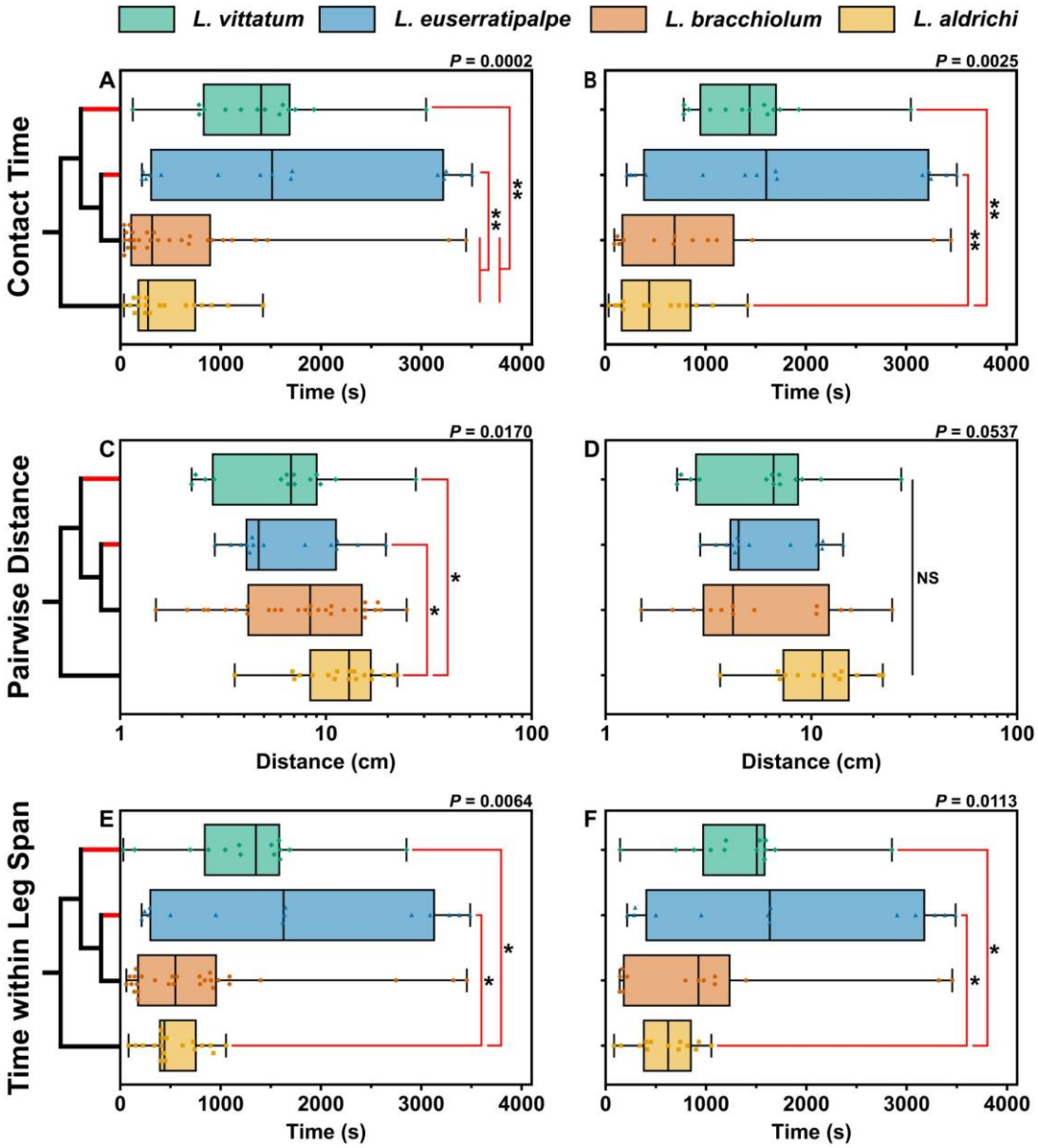


**Figure 2:** Association metrics between male and female pairs. Boxes represent medians plotted with IQR, symbols represent individual trial values, and whiskers correspond to the minimum and maximum trial values. The results from group comparison tests are shown in the upper right-hand corners, while significant results from post-hoc multiple comparisons tests are noted by red brackets with asterisks (* = $P < 0.05$; ** = $P < 0.01$). The simplified phylogeny indicates

reproductive morphology; red tips indicate nonsacculate species and black tips represent sacculate species (phylogeny adapted from Burns et al. 2013). **A)** Total contact time in seconds between males and females during AC trials. **B)** Total contact time in seconds between males and females during SC trials. **C)** Average distance in centimetres between male and female pairs during AC trials. **D)** Average distance in centimetres between male and female pairs during SC trials. **E)** Total time in seconds the female was within one leg span of the male during AC trials. **F)** Total time in seconds the female was within one leg span of the male during SC trials.

Using automated tracking with TRex, we found that in both AC and SC trials males were within one leg span of females significantly more often in nonsacculate species than in the nonsacculate *L. aldrichi* (Figure 2C & D). This matched the contact patterns found in the SC trials manual scoring (Figure 2D), however, for AC trials we failed to find significance comparing *L. bracchiolum* to nonsacculates. This discrepancy in contact time is likely due to using the body centroids for position in TRex – we only considered females to be within the male's leg span if the female's body centroid was within the given male leg span, meaning leg-leg contact may occur without being scored. During AC trials, nonsacculate females and males were also significantly closer to one another when compared to *L. aldrichi*, though this pattern did not hold for SC trials (Figure 2E & F). The average distance between nonsacculates during AC trials (*L. vittatum* 6.77 cm [IQR 2.79 - 9.11 cm]; *L. euserratipalpe* = 4.71 [IQR 4.07 - 11.31 cm]) was less than their respective maximum male leg lengths, while for sacculates (*L. aldrichi* = 12.96 cm [IQR 8.31 - 16.68 cm]; *L. bracchiolum* = 8.39 cm [IQR 4.15 - 15.10 cm]) the median distance was greater than their respective maximum male leg lengths.

## Precopulatory Phase

Across all species, we found significant differences in the latency between first contact and first clasping attempt (Figure 3). *Leiobunum euserratipalpe* median attempt latency was 6.0 s (IQR 3.0 - 10.0 s), significantly lower than *L. vittatum* (76.5 s, IQR 20.75 - 605.3 s) and *L. aldrichi*

(128.0 s, IQR 7.25 - 462.0 s), but was not significantly different from that of *L. bracchiolum* (18.0 s, IQR 4.0 - 327.5 s). All species displayed marked variation in latency to attempt a clasp except *L. euserratipalpe*, which had a standard deviation of 10.72 seconds (*L. bracchiolum* SD = 230.7 s; *L. aldrichi* SD = 363.5 s; *L. vittatum* SD = 330.4 s). A minority of male *L. aldrichi*, *L. bracchiolum*, and *L. vittatum* exhibited latencies exceeding 500 s, in steep contrast to *L. euserratipalpe*. The longest attempt latency for *L. euserratipalpe* was just 41 s, with all but three males making an attempt within 10 s of their initial contact with a female (Figure 3). We additionally compared the latency to attempt in *L. bracchiolum* and *L. aldrichi* trials with failed attempts to SC trials and found no significant differences in time (*L. bracchiolum* Mann-Whitney test $P = 0.3319$; N = 13, 15; U = 76; *L. aldrichi* Mann-Whitney test $P = 0.2003$; N = 5, 13; U = 19). There were insufficient failed attempts in the nonsacculate species (one each) to test for any differences. We also measured the latency between first attempt and first success, finding overall significance comparing sacculate species to nonsacculate species. However, despite the sacculate species each having longer latencies than the nonsacculate species, we failed to find any pairwise differences with our Dunn's multiple comparisons tests.

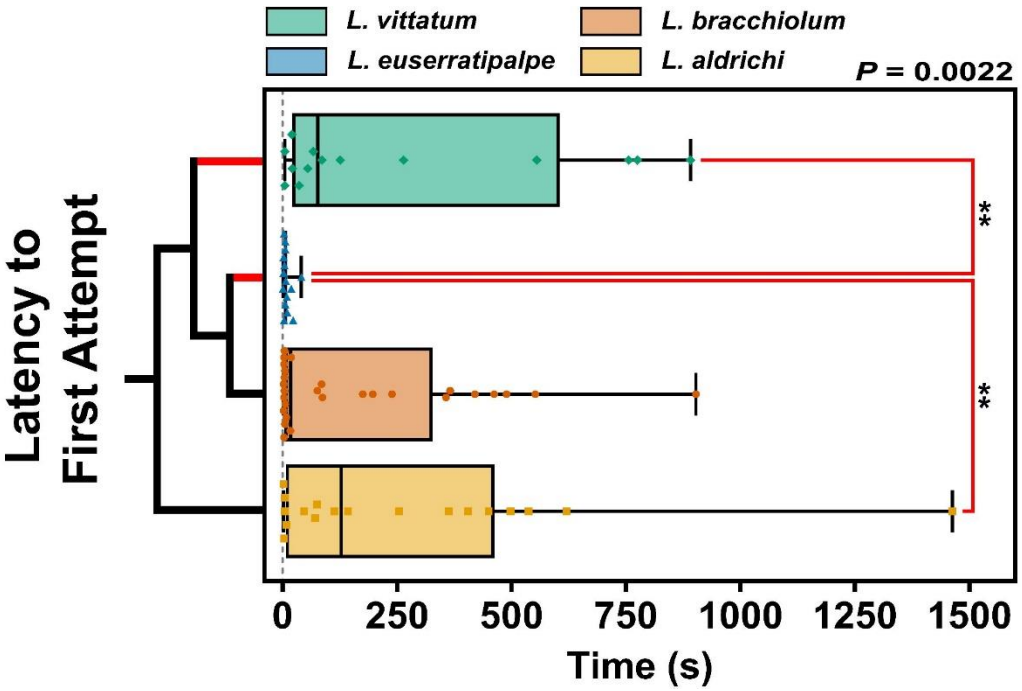

**Figure 3:** Latency in seconds between first contact and first clasp attempt during SC trials. Boxes represent medians plotted with IQR, symbols represent within-trial values, and whiskers correspond to the minimum and maximum trial values. The result from a Kruskal-Wallis test is shown in the upper right-hand corner, while significant results from Dunn's multiple comparisons tests are noted by red brackets with asterisks (** = $P < 0.01$). The simplified phylogeny indicates reproductive morphology; red tips indicate nonsacculate species and black tips represent sacculate species (phylogeny adapted from Burns et al. 2013).

Finally, we found significant differences in pursuit angle between several species for AC and SC trials. In AC trials, the median pursuit angle for *L. vittatum* (82.64 degrees, IQR 82.05 - 85.45 degrees) was significantly smaller than that of *L. bracchiolum* (87.75 degrees, IQR 85.79 - 88.86 degrees) (Figure 4A). *Leiobunum vittatum* also had a significantly smaller angle than that of *L. euserratipalpe* (88.71 degrees, IQR 85.67 - 89.92 degrees) despite both being nonsacculate. Though SC trials the *L. vittatum* pursuit angle (82.30 degrees, IQR 82.00 - 84.66 degrees) was similar to both sacculate species, it remained significantly smaller than *L. euserratipalpe* (89.04 degrees, IQR 85.53 - 89.89 degrees) (Figure 4B).

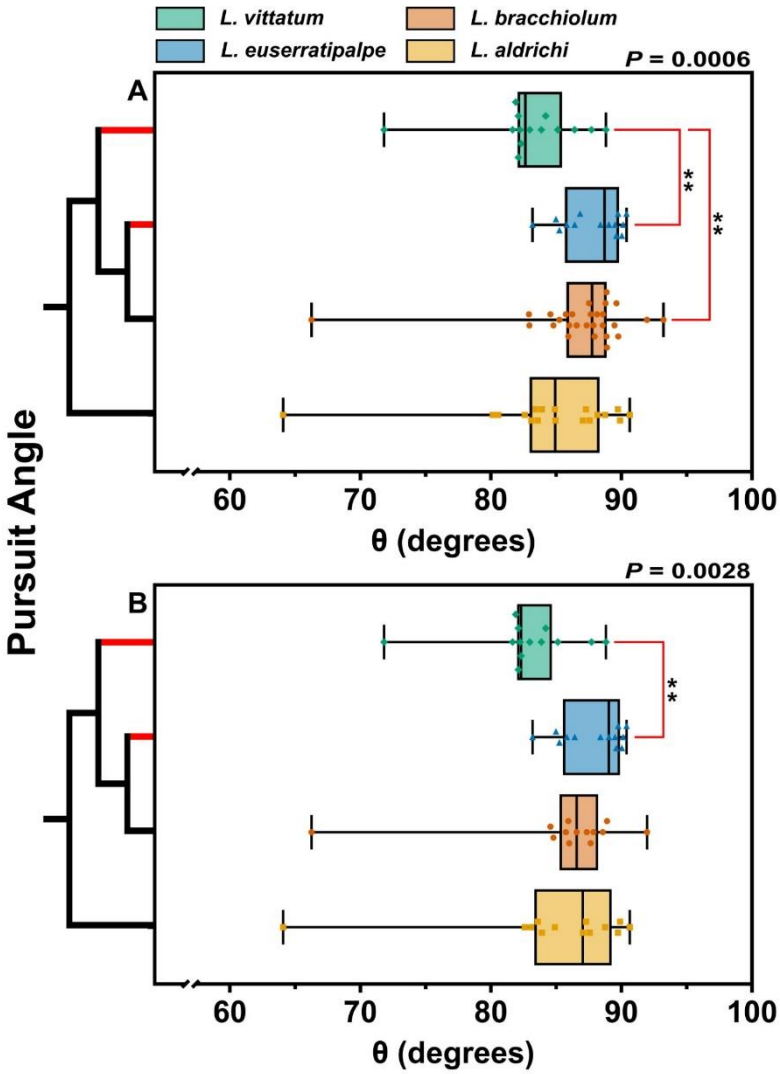


**Figure 4:** Angle between male and female trajectories between first contact and first clasp attempt. Boxes represent medians plotted with IQR, symbols represent within-trial means, and whiskers correspond to the minimum and maximum trial means. The simplified phylogeny indicates reproductive morphology; red tips indicate nonsacculate species and black tips represent sacculate species (phylogeny adapted from Burns et al. 2013). The results from Kruskal-Wallis tests are shown in the upper right-hand corner, while significant results from Dunn's multiple comparisons tests are noted by red brackets with asterisks (** = $P < 0.01$). **A)** AC trials. **B)** SC trials.

Copulatory Phase

We found significant differences when comparing the overall clasping duration of each species using a Kruskal-Wallis test, although there were no clear patterns between sacculate and nonsacculate species (Figure 5). During SC trials *L. vittatum* males clasped females significantly longer than either sacculate species, with a median time of 676.8 s (IQR 310.6 - 1271.0 s) compared to 118.7 s (IQR 98.47 - 179.1 s) for *L. bracchiolum* and 260.5 s (IQR 17.11 - 662.8 s) for *L. aldrichi* (Figure 5). Interestingly it appeared that *L. euserratipalpe* clasped far less than expected, and an unplanned post-hoc test comparing the species to *L. vittatum* revealed that *L. euserratipalpe* clasped for significantly less time than its nonsacculate counterpart (Figure 5). The median clasping duration for *L. euserratipalpe*, 155.7 s (IQR 87.22 - 370.7 s), was more aligned with the clasping durations of sacculate species, and was approximately half that of *L. aldrichi*, contrary to our predictions.

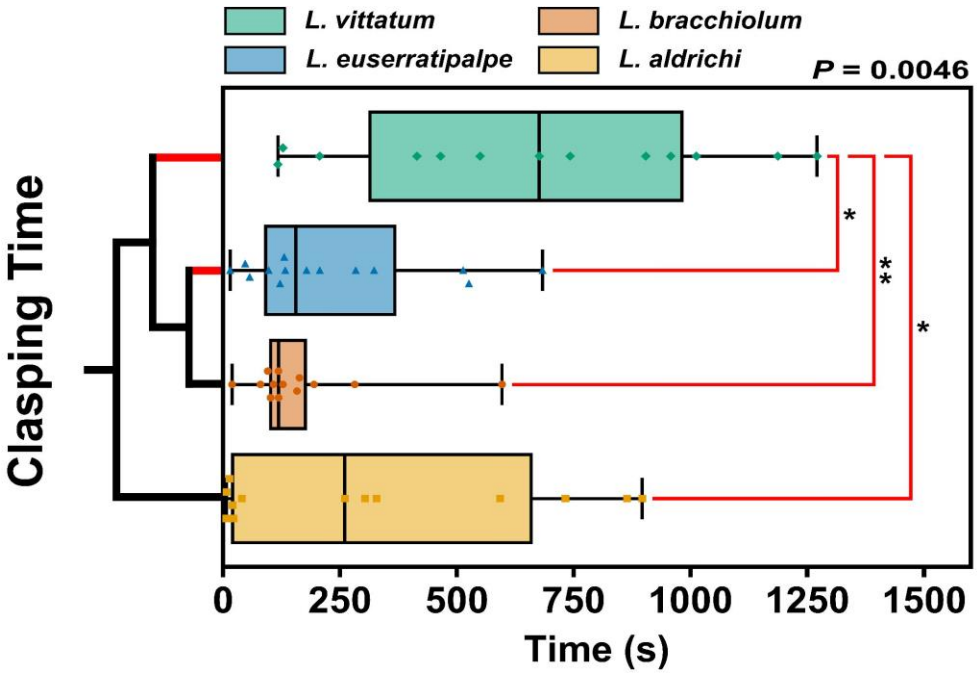


**Figure 5:** Time in seconds that males clasping females during trials with successful clasps. Boxes represent medians plotted with IQR, symbols represent within-trial values, and whiskers correspond to the minimum and maximum trial values. The result from a Kruskal-Wallis test is

shown in the upper right-hand corner, while significant results from Dunn's multiple comparisons tests are noted by red brackets with asterisks (* = $P < 0.05$; ** = $P < 0.01$). The simplified phylogeny indicates reproductive morphology; red tips indicate nonsacculate species and black tips represent sacculate species (phylogeny adapted from Burns et al. 2013).

We additionally found significant differences when comparing female body angle (Figure 1C) among all species, but these analyses resulted in similarly nebulous results when comparing sacculate and nonsacculate species. *Leiobunum vittatum* was again significantly different from its nonsacculate counterpart, *L. euserratipalpe*, and the sacculate *L. aldrichi*, but it was not significantly different from *L. bracchiolum*. In all trials with attempted clasps, *L. vittatum* females angled their bodies to a median of 46.39 degrees (IQR 33.54 - 90.0 degrees) with four females reaching a full 90 degrees perpendicular to the substrate (Figure 6A). *Leiobunum euserratipalpe* females displayed a median angle of 0 degrees + (IQR 0 - 39.54 degrees) and rarely angled their bodies, with only six of 15 females doing so and only one female reaching 90 degrees. While *L. bracchiolum* and *L. aldrichi* females angled their bodies more often than *L. euserratipalpe* females, no females reached 90 degrees, and their median angles were 28.98 degrees (IQR 0 - 49.35 degrees) and 25.5 degrees + (IQR 0 - 42.84 degrees) respectively. Surprisingly, the relationships between *L. vittatum* and the sacculate species reversed in SC trials (Figure 6B). The median angle formed by *L. vittatum* females was 47.26 degrees (IQR 39.31 - 90.0 degrees) compared to 4.725 (IQR 0.0 - 49.94 degrees) for *L. bracchiolum* and 27.61 degrees (IQR 19.13 - 45.00 degrees) for *L. aldrichi*, and *L. bracchiolum* was the only species in which the median angle was lower in SC trials than in AC trials (Figure 6A & B).

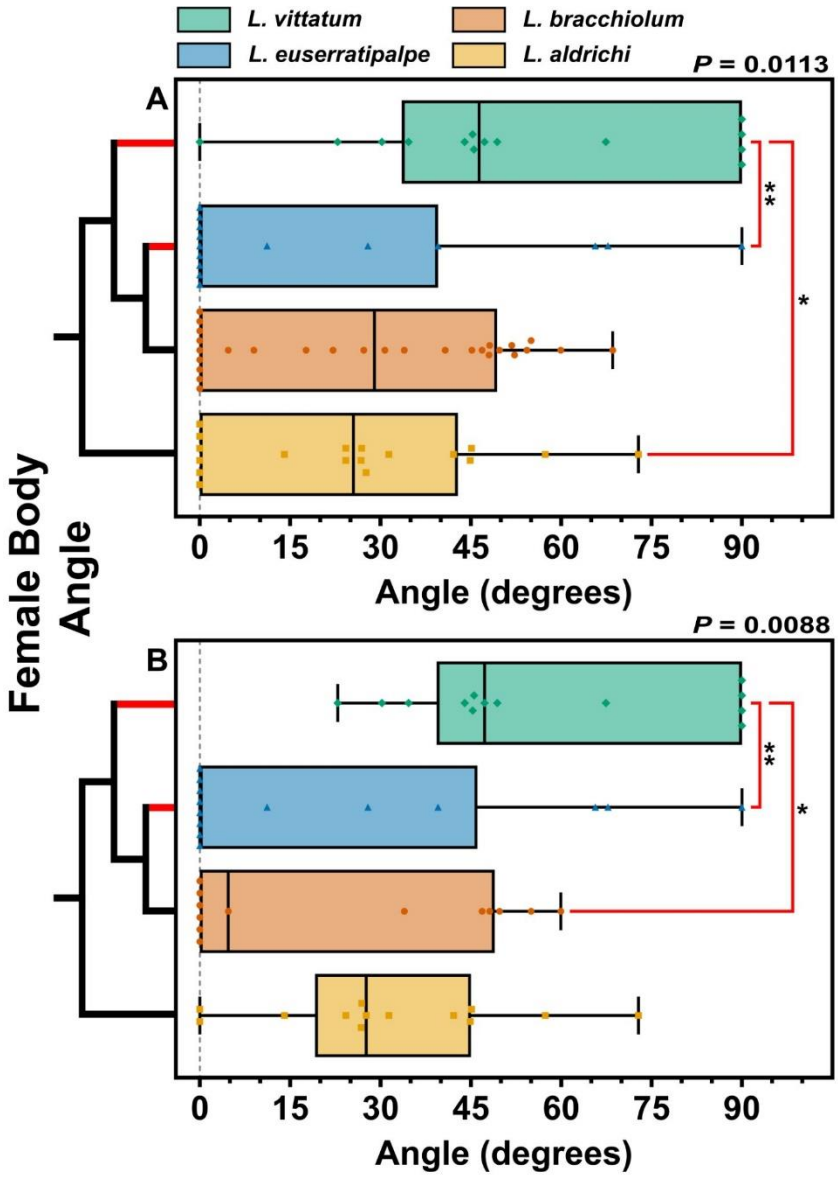


**Figure 6:** Maximum angle females formed between genital operculum and substrate. Boxes represent medians plotted with IQR, symbols represent within-trial values, and whiskers correspond to the minimum and maximum trial values. The simplified phylogeny indicates reproductive morphology; red tips indicate nonsacculate species and black tips represent sacculate species (phylogeny adapted from Burns et al. 2013). The results from Kruskal-Wallis tests are shown in the upper right-hand corner, while significant results from Dunn's multiple comparisons tests are noted by red brackets with asterisks (* = $P < 0.05$; ** = $P < 0.01$). **A)** AC trials. **B)** SC trials.

The contact time (between first touch and first clasp attempt) and the total time within one leg span were significant predictors of clasping success for both the total-species full model and the total-species reduced model (Table 3). Though the Akaike Information Criterion (AICc, corrected for small sample size) supports the reduced model, McFadden's pseudo-$R^2$ suggests the total-species model should be favoured (Table 3). Species-level models for *L. bracchiolum* and *L. aldrichi* were non-significant, while nonsacculate, *L. vittatum*, and *L. euserratipalpe* models were not possible due to few failed clasping attempts.

| **Model/Factor** | **AICc** | **Pseudo-$R^2$** | **Estimate** | **SE** | **\|Z\|** | ***P*-value** |
|---|---|---|---|---|---|---|
| *Total-species full model* | 73.66 | 0.323 | - | - | - | - |
| Intercept | - | - | 4.652 | 8.225 | 0.566 | 0.5717 |
| Initial latency | - | - | -0.001 | 0.001 | 0.880 | 0.3790 |
| **Pre-attempt contact time** | - | - | **-0.004** | **0.001** | **3.015** | **0.0026** |
| Average distance | - | - | 0.138 | 0.086 | 1.613 | 0.1067 |
| **Time within 1 leg span** | - | - | **0.003** | **0.001** | **2.765** | **0.0057** |
| Female body angle | - | - | 0.024 | 0.015 | 1.610 | 0.1073 |
| Pursuit angle | - | - | -0.082 | 0.095 | 0.865 | 0.3868 |
| *Total-species reduced model* | 71.60 | 0.245 | - | - | - | - |
| Intercept | - | - | 0.025 | 0.427 | 0.059 | 0.9526 |
| **Pre-attempt contact time** | - | - | **-0.003** | **0.001** | **2.935** | **0.0033** |
| **Time within 1 leg span** | - | - | **0.002** | **0.001** | **3.077** | **0.0021** |

**Table 3:** Multiple logistic regression modelling of clasping success with behavioural sexual conflict metrics as predictor variables for the total-species full model and the total-species reduced model. Predictor variables were iteratively removed beginning with the variable with the greatest *P*-value until no variables with $P > 0.1$ remained. Significant predictor variables are in bold.

Postcopulatory

Finally, when analysing the duration of mate guarding – the primary indicator of postcopulatory conflict in leiobunine harvesters – we found significant differences across species (Figure 7). Nonetheless, there were few pairwise significant differences, potentially due to significant intraspecific variation in all species except *L. aldrichi*. *Leiobunum aldrichi* males guarded for a median time of just 59.0 s + (IQR 0 - 270.5 s), while *L. bracchiolum* and *L. vittatum* males guarded for 474.0 s (IQR 0 - 857.0 s) and 351 s (IQR 18.0 - 1145.0 s), respectively (Figure 7). *Leiobunum euserratipalpe* males guarded females for significantly longer than *L. aldrichi* males did, with its median guarding times being 1209.0 s (IQR 209.8 - 2919.0 s) (Figure 7). Although male *L. euserratipalpe* tended to guard females for extended periods, they did not always do so – five males guarded for less than 320.0 s, and two of these did not guard at all. Due to its broad distribution of guarding duration, we did not have sufficient statistical power to resolve any potential differences between *L. euserratipalpe*, *L. vittatum*, and *L. bracchiolum*, and further study may allow the resolution of these differences.

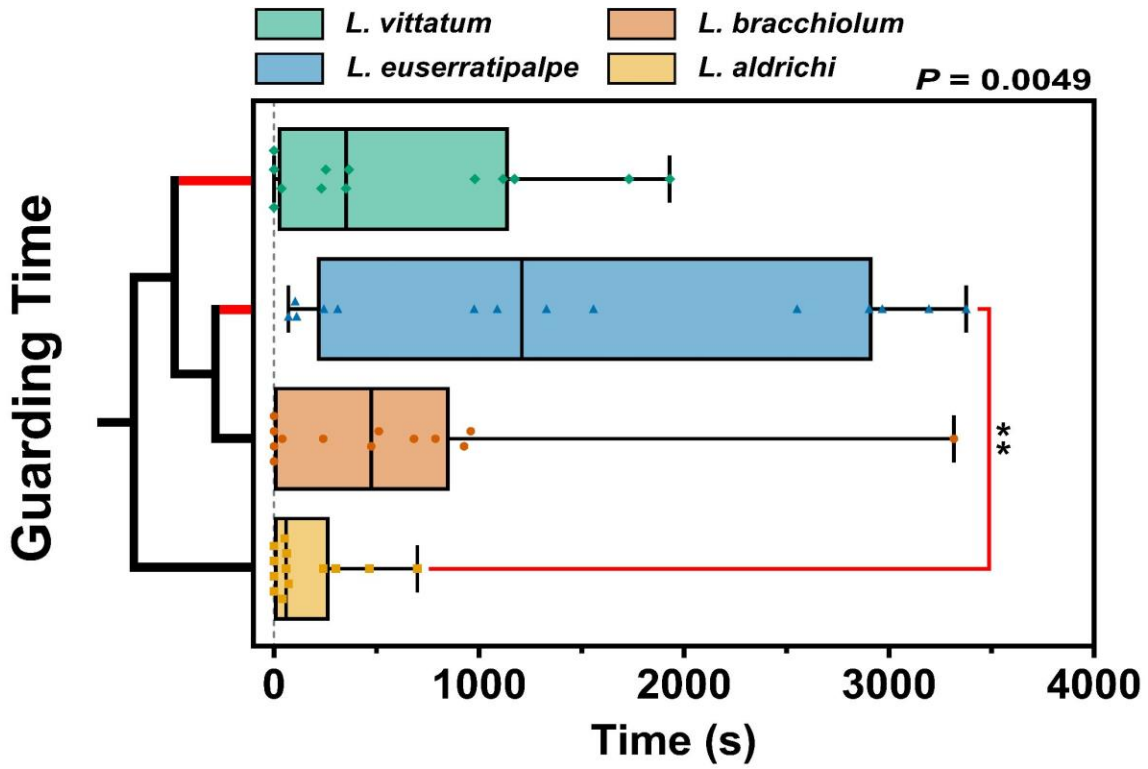

**Figure 7:** Time in seconds that males guarded females during SC trials. Boxes represent medians plotted with IQR, symbols represent within-trial values, and whiskers correspond to the minimum and maximum trial values. The result from a Kruskal-Wallis test is shown in the upper right-hand corner, while significant results from Dunn's multiple comparisons tests are noted by red brackets with asterisks (** = $P < 0.01$). The simplified phylogeny indicates reproductive morphology; red tips indicate nonsacculate species and black tips represent sacculate species (phylogeny adapted from Burns et al. 2013).

# Discussion

## Behavioural comparisons between sacculate and nonsacculate species

Our results reveal that the nonsacculate harvesters *L. vittatum* and *L. euserratipalpe* display significantly higher levels of behavioural sexual conflict than the sacculate species *L. aldrichi* and *L. bracchiolum*. This variation in mating behaviour corresponds to the penis morphology and reproductive syndromes of each species; *L. vittatum* and *L. euserratipalpe* lack precopulatory nuptial gifts and possess antagonistic morphology, while *L. aldrichi* and *L. bracchiolum* give precopulatory nuptial gifts and lack antagonistic morphology (Burns et al. 2013). Across all mating trials, nonsacculate males repeatedly displayed elevated levels of coercive behaviour through significantly longer median association times and by being significantly closer to females throughout trials (Figure 2). Male *L. euserratipalpe* attempted mating clasps significantly faster upon first touch than male *L. aldrichi* and *L. vittatum* (Figure 3), while male *L. vittatum* pursued females more vigorously and female *L. vittatum* also displayed higher levels of resistance behaviour (Figures 4 & 6). Hallmark conflict behaviours, mate clasping and mate guarding, were not found to be elevated across both nonsacculate species, with instead *L. vittatum* displaying extended clasping behaviour (Figure 5) and *L. euserratipalpe* displaying extended guarding behaviour (Figure 7). Altogether these data indicate that nonsacculate

harvesters resort to antagonistic behaviours to compensate for the loss of precopulatory nuptial gifts and the reduction of pericopulatory nuptial gifts.

With these results we provide the first direct behavioural comparison between sacculate and nonsacculate leiobunine harvesters. Despite extensive evidence of a shift from a solicitous suite of traits to an antagonistic suite, the role of behaviour had not yet been investigated with respect to sexual conflict in this system. Previous studies have revealed various losses of solicitous traits such as penile sacs, precopulatory nuptial gifts, and high essential amino acid content in pericopulatory nuptial gifts (Burns et al. 2013; Kahn et al. 2018). Meanwhile, these and other studies have also shown gains of antagonistic traits, such as enhanced penile prying force, female reproductive barriers, and modified clasping structures (Burns et al. 2013; Burns & Shultz 2015, 2016; Karachiwalla et al. 2020). While these changes arose independently across leiobunine harvesters, many have reached convergent states. Our results indicate that these morphological and biochemical changes have been accompanied by marked behavioural changes as well.

Leiobunine harvesters primarily rely on tactile senses; sweeping and tapping their antenniform second pair of legs to investigate their surroundings (Shultz & Pinto-da-Rocha 2007; Willemart & Hebets 2012). Therefore, males keeping the female within their leg span is likely vital to facilitating control during a mating interaction, and nonsacculate species maintained this control over females for two to three times longer than sacculate species. The distance between individuals might be indicative of sexual conflict for a variety of reasons which would require further experiments – across mammals, reptiles, fish, and arthropods, many studies have shown cohabitation with males or proximity to males can each impact female fitness negatively through harassment (Arnqvist & Rowe 2005;  Krupa et al. 1990; Kunz et al. 2021; Pilastro et al. 2003; Plath et al. 2003; Rönn et al. 2006; Schlupp et al. 2001; Schütz & Taborsky 2005; Shine et al. 2005). In particular, even if copulation does not occur, close association with males can lower female fitness, whether due to direct stress from male activity or from selecting low-quality refuge

instead of a high-quality habitat with males as an avoidance strategy (Bokides et al. 2012; Iglesias-Carrasco et al. 2019). An increase in overall association time might serve several functions originally fulfilled by nuptial gifts, with copulation success and paternity share potentially benefiting through increased opportunity to clasp and reduced competition from other males. Furthermore, the exceptionally low latency between first contact and first attempt in *L. euserratipalpe* might be driven by a variety of factors, although many which might normally influence latency are controlled in this experiment, such as female age or sexual history, male starvation, and the presence of male rivals (Burke & Bonduriansky 2018; Dmitriew & Blanckenhorn 2012; Fowler et al. 2022). Low latency has previously been associated with both high conflict (Blanckenhorn et al. 2020; Skwierzyńska & Plesnar-Bielak 2018), and in concert with the elevated guarding duration in *L. euserratipalpe* this low latency likely indicates a species with high conflict.

## Clasping, guarding, and conflict

Clasping time is a classic indicator of sexual conflict found in myriad arthropod and vertebrate systems (Arnqvist & Rowe 2005; Johns et al. 2009; Rueda-Solano et al. 2022; Sigvardt et al. 2017). Indeed, males of some species, including those in this study, are entirely unable to ensure copulation when unable to clasp the female, providing significant motivation for unreceptive females to prevent clasping (Machado & Macías-Ordóñez et al. 2007; Myers et al. 2016). Extended clasping can function as a multifaceted advantage for males, the primary explanatory hypotheses being the Ejaculate Transfer (ET) hypothesis (Edvardsson & Canal 2006; Mazzi et al. 2009; Simmons 2002) and the Extended Mate Guarding (EMG) hypothesis (Alcock 1994; Mazzi et al. 2009). In the ET hypothesis, males extend clasping time to maximise the removal of sperm from previous males and the transfer of ejaculate materials such as sperm, seminal fluids, and mating plugs (Arnqvist & Rowe 2005; Edward et al. 2015; Mazzi et al. 2009; Macías-Ordóñez et al. 2010; Simmons 2002; Townsend et al. 2019; Vahed et al. 2014). In the EMG hypothesis, males instead maximise clasping time as a function of mate guarding in order

to reduce the potential for female remating (Mazzi et al. 2009; Zatz et al. 2011). In both these nonexclusive hypotheses, the ultimate result may be quite similar to those achieved by nuptial gifts (Lewis & South 2012). Mating plugs, sperm removal, and clasping as mate guarding have all been observed in tropical harvesters (Machado & Burns 2023; Macías-Ordóñez et al. 2010; Townsend et al. 2015, 2019; Zatz et al. 2011), and so the behavioural conflict over clasping time in these North American harvesters might be attributable to either hypothesis or some combination of the two.

To circumvent male clasping, female harvesters angle their body face-down into the substrate to block access to their genital operculum, potentially making it more difficult for males to maintain their clasp (Figure 1C; Fowler-Finn et al. 2014, 2018, 2019; Machado & Macías-Ordóñez 2007). If females orient their bodies in this manner, males cannot access the genital operculum and therefore cannot initiate intromission (Figure 1C). Many arthropod species have evolved morphological anti-clasping adaptations (Arnqvist & Rowe 1995, 2005; Karlsson Green et al. 2013) similarly, body angling might be considered a concordant behavioural anti-clasping adaptation – actions to move the genitals away from males are common among arthropods with high levels of sexual conflict (Akinyemi et al. 2021; Burke et al. 2015; Golov et al. 2018; Machado & Macías-Ordóñez 2007; Maroni et al. 2023; Zweerus et al. 2021)

Alternatively, there is some evidence that extended clasping time in *L. vittatum* may be attributable to female antagonism rather than male, which would simultaneously serve to explain the discrepancy between clasping times in the two nonsacculate species. First suggested by Fowler-Finn et al. (2014), female *L. vittatum* may clasp the male's penis using the genital operculum, allowing the female to receive more nuptial gift than is optimal for the male to provide. Once clasping has been achieved and intromission has begun, male *L. vittatum* occasionally appear to struggle to end copulation (Fowler-Finn et al. 2014). This phenomenon is supported by genital morphology; female *L. vittatum* have well-developed opercular musculature and sclerotization, in stark contrast to the male's relatively fragile penis (Burns & Shultz 2016). Burns

and Shultz (2016) additionally cite reports of males with broken penises in the field, an injury consistent with struggles to disengage females. Evidence from crickets and spiders supports the notion that female aggression can negatively correlate with nuptial gift quality and quantity (Kuriwada & Kasuya 2012; Toft & Albo 2016), and in *L. vittatum* this manipulation by females might be the direct result of the deterioration of nuptial gift quality and the loss of a precopulatory nuptial gift (Khan et al. 2018; Machado & Burns 2023; Machado & Macías-Ordóñez 2007). Because females of nonsacculate species can only receive nuptial gift fluid during intromission, rather than both before and during, there may be a tangible benefit to females remaining in copula as long as possible. It may be that this acts as a cost-mitigating strategy for females, although it seems unlikely that females would prefer this as opposed to simply avoiding the encounter altogether due to the elevated levels of precopulatory resistance from females. The potential transition from male pre-copulatory antagonism to female copulatory antagonism would provide a fascinating dichotomy of sexual conflict deserving further research.

Mate guarding is another strong indicator of sexual conflict commonly found in arthropods (Alcock 1994; Arnqvist & Rowe 2005; Simmons 2002). Mate guarding may allow males additional control over female mating decisions by discouraging remating, preventing sperm removal by rival males, and reducing sperm competition – all factors which might alternatively (or additionally) be influenced by nuptial gifts (Alcock 1994; Arnqvist & Rowe 2005; Elias et al. 2014; Frankino & Sakaluk 1994; Gór et al. 2023; Hagg et al. 2024; Lewis & South 2012; Machado & Burns 2023; Sakaluk 1991; Simmons 2002). Sperm competition and cryptic female choice are distinct possibilities in leiobunine harvesters – all females have a sperm-storing spermatheca, while some species have a complex, multi-chambered spermatheca potentially affording the female control over sperm fate (Karachiwalla et al. 2020). These potential confounding factors would only further incentivize males to guard females and prevent remating, as suggested by both models and experimental evidence (Bateman et al. 2001; Del Matto et al. 2021; Gór et al. 2023; Harts & Kokko 2013). Many male Opiliones, including *Leiobunum* species, engage in extended mate guarding

which typically lasts until oviposition (Fowler-Finn et al. 2014, 2018, 2019; Machado & Burns 2023, Macías-Ordóñez 1997; Wijnhoven 2011; Zatz et al. 2011). This is likely an extremely costly behaviour for males because these harvesters often aggregate in high density, leading to elevated levels of male-male competition (Macías-Ordóñez 1997). Indeed, the scant field studies of *Leiobunum* mating behaviour have observed male *Leiobunum spp.* defending ovipositing females from upwards of 6 rival males, although evidence for late-season male sex-ratio biases and the dearth of field studies likely leads to this being an underestimate (Bacon, R. & Brown, T. A., pers. observ; Macías-Ordóñez 1997; Wijnhoven 2011). Our study did not find *L. vittatum* guarding time to be significantly longer than sacculate species, but a key missing factor may be the absence of competitors. In several studies, male *L. vittatum* were found to be most aggressive when in the presence of both males and females during their reproductive period (Macías-Ordóñez 1997). While this aggression was often male-directed, it remains possible that the absence of male competitors resulted in less guarding than expected.

## Multiple antagonistic strategies

The *calcar* species-group and *vittatum* species-group have convergently evolved similar nuptial gift chemistries and penis morphologies (Burns et al. 2013; Kahn et al. 2018), yet the groups vary significantly in sexual and somatic morphology and biomechanics. Burns and Shultz (2015, 2016) detected significant biomechanical differences between the major nonsacculate groups, finding that *calcar* species-group penises are significantly more resistant to flexural strain than the *vittatum* species-group, which more closely resembles a transitional state between antagonistic and solicitous penile structures. Female genital operculum morphology differs in reproductive barrier structure between groups as well (Burns et al. 2013; Ingianni et al. 2011). Despite females of both groups possessing a pregenital barrier formed through sclerotization of the operculum and sternum, the mechanism through which these structures engage varies between groups (Burns et al. 2013; Shultz 2018). *Leiobunum euserratipalpe* does not have sexually dimorphic palpal morphology, while *L. vittatum* does (Ingianni et al. 2011; Shultz 2018).

This variation in somatic morphology correlates with the significant differences in clasping duration revealed in this study – *L. vittatum* males clasp longer than *L. euserratipalpe* and possess clasping structures indicative of this. *Leiobunum euserratipalpe* males, lacking enhanced palpal clasping structures, do not have particularly long clasping durations and instead guard females for extended periods postcopulation.

These variable suites of antagonistic traits indicate the evolutionary forces driving the transition to antagonism may differ between the *calcar* and *vittatum* species-groups, and further research investigating these mechanisms is needed. There are many temperate species within the Opiliones family Sclerosomatidae with documented transitions towards antagonistic morphology, but to date no tropical species with these transitions have been reported (Burns et al. 2013; Burns & Tsurusaki 2016; Machado & Burns 2023; Martens 2020). While there is a dearth of tropical sexual conflict research overall, neotropical Opiliones have been fairly well studied, albeit with a focus on non-Sclerosomatidae species (Bacon et al. 2023; Burns & Machado 2023; Pinto-da-Rocha et al. 2007). This conspicuous lack of antagonistic structures in tropical Sclerosomatidae might indicate that harsher climes drive the transition towards antagonism in temperate sclerosomatids.

## Conclusions

Our results also provide a framework for expedited video analysis of sexual conflict behaviour in harvesters through a combination of automated tracking and manual scoring which could be applied to a wide range of arthropod behavioural studies. In particular, a frame-by-frame measurement of the distance between two individuals allows for a true average over the duration of the trial; manual scoring would only allow estimates through periodic measurements of the distance. Not all behaviours can be easily automated – tracking leg movement remains difficult due to species-specific factors, and ventrally-occurring behaviours require manual scoring due to frequent positional shifts by harvesters when interacting. Regardless, manual scoring time was significantly reduced, and new metrics were available through our hybrid approach.

This study provides a critical framework for further sexual conflict research in this group of leiobunine Opiliones. By establishing a protocol for rapidly processing videos, future work using mating trials will be analysed significantly faster and will require far less time spent manually scoring videos in real time. Together, our data suggests that nonsacculate harvesters resort to antagonistic behaviours in lieu of nuptial gifts to increase copulation success, insemination success, sperm transfer, sperm storage, and paternity share. A significant area that remains unclear is the effect of reproductive phenology on sexual conflict. North American leiobunine harvesters have significant variation in the timing of major life events such as maturation, oviposition, and senescence, as well as in the length of the reproductive period (Curtis & Machado 2007; Machado & Macías-Ordóñez 2007). Additionally, male-male competition and female antagonism are further factors which likely contribute to the evolution and maintenance of male antagonism in leiobunine Opiliones. How these myriad factors interact and impact sexual conflict is not well established, and the advent of a streamlined video processing protocol combined with evidence of significant variation in behavioural sexual conflict in leiobunines provides the perfect opportunity for further behavioural research.

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## Appendix

| Metric | Trial Type | Normal? | Number of Trials | | | | |
|---|---|---|---|---|---|---|---|
| | | | Total | VI | EU | BR | AL |
| Latency to 1st Attempt | Attempts | No | 75 | 14 | 15 | 28 | 18 |
| Pursuit Angle | Attempts | No | 74 | 14 | 14 | 28 | 18 |
| | Successes | No | 52 | 13 | 13 | 13 | 13 |
| Contact Time | Attempts | No | 75 | 14 | 15 | 28 | 18 |
| | Successes | No | 53 | 13 | 14 | 13 | 13 |
| Pre-Attempt Contact Time | Attempts | No | 75 | 14 | 15 | 28 | 18 |
| | Successes | No | 53 | 13 | 14 | 13 | 13 |
| Time within 1 Leg Span | Attempts | No | 74 | 14 | 14 | 28 | 18 |
| | Successes | No | 52 | 13 | 13 | 13 | 13 |
| Average Distance | Attempts | Log | 74 | 14 | 14 | 28 | 18 |
| | Successes | Log | 52 | 13 | 13 | 13 | 13 |
| Maximum Female Body Angle | Attempts | No | 75 | 14 | 15 | 28 | 18 |
| | Successes | No | 53 | 13 | 14 | 13 | 13 |
| Clasping Time | Successes | No | 53 | 13 | 14 | 13 | 13 |
| Guarding Time | Successes | No | 53 | 13 | 14 | 13 | 13 |

**Table A1:** Behavioural sexual conflict metrics recorded during this study (species codes: *Leiobunum vittatum*: VI; *L. euserratipalpe*: EU; *L. bracchiolum*: BR; *L. aldrichi*: AL). Shapiro-Wilk test for normality results are shown for all metrics save average distance, which was log-normal based on a Shapiro-Wilk test for lognormality. Sample sizes for each metric follow. Note that for pursuit angle, time within one leg span, and average distance, one *L. euserratipalpe* trial could not be used due to video corruption.

| Metric | Trial Type | Pagel's λ | logL(λ) | LR(λ=0) | *P*-value |
|---|---|---|---|---|---|
| Latency to 1st Attempt | Attempts | 6.61e-05 | -29.451 | -6.88e-05 | 1 |
| Pursuit Angle | Attempts | 6.61e-05 | -9.263 | -2.32e-05 | 1 |
| | Successes | 6.61e-05 | -9.831 | -3.37e-05 | 1 |
| Contact Time | Attempts | 6.61e-05 | -30.302 | -1.02e-04 | 1 |
| | Successes | 0.999 | -28.626 | 0.187 | 0.6658 |
| Pre-Attempt Contact Time | Attempts | 6.61e-05 | -25.851 | -5.73e-05 | 1 |
| | Successes | 6.61e-05 | -24.774 | -2.57e-05 | 1 |
| Time within 1 Leg Span | Attempts | 6.61e-05 | -29.705 | -1e-04 | 1 |
| | Successes | 0.999 | -28.167 | 0.189 | 0.6634 |
| Average Distance | Attempts | 6.61e-05 | -6.717 | -9.68e-05 | 1 |
| | Successes | 0.999 | -6.788 | 0.229 | 0.6325 |
| Maximum Female Body Angle | Attempts | 0.999 | -19.163 | 0.057 | 0.8111 |
| | Successes | 6.61e-05 | -18.764 | -6.09e-05 | 1 |
| Clasping Time | Successes | 6.61e-05 | -28.053 | -1.97e-05 | 1 |
| Guarding Time | Successes | 6.61e-05 | -30.947 | -7.55e-06 | 1 |

**Table A2:** Degree of phylogenetic signal λ within the various behavioural sexual conflict metrics. Pagel's lambda (Pagel 1999) was calculated using the phytools package (Revell 2012). We used a pruned version (consisting of our four study species) of a maximum clade credibility tree constructed from mitochondrial and nuclear sequences by Burns et al (2013). These models were then compared to Brownian motion predictions with a likelihood ratio test to produce our *P*-values.

| Model/Factor | *F* (*df*) | Estimate | SE | \|t\| | *P*-value |
|---|---|---|---|---|---|
| ***Total-species model*** | 3.672 (4, 48) | - | - | - | **0.0109** |
| **Intercept** | - | **1378** | **453.6** | **3.038** | **0.0038** |
| **# male legs** | - | **-290.4** | **81.68** | **3.556** | **0.0009** |
| # female legs | - | 92.59 | 72.23 | 1.282 | 0.2061 |
| **# male sensory legs** | - | **489.2** | **155.6** | **3.143** | **0.0029** |
| **# female sensory legs** | - | **-257.7** | **114.7** | **2.247** | **0.0292** |
| *L. vittatum model* | 1.186 (4, 8) | - | - | - | 0.3867 |
| *L. euserratipalpe model* | 1.272 (4, 8) | - | - | - | 0.3493 |
| *L. bracchiolum model* | 0.795 (4, 8) | - | - | - | 0.5607 |
| *L. aldrichi model* | 0.239 (4, 8) | - | - | - | 0.9087 |

**Table A3:** Multiple linear regression of clasping duration with leg number metrics as predictor variables for the total-species model and species-level models. Significant predictor variables are in bold; no predictor variables were dropped from modelling.

## References


Akinyemi, A. O., Subramanian, S., Mfuti, D. K., Pope, T. W., Tamiru, A., & Kirk, W. D. J. (2021). Mating behaviour, mate choice and female resistance in the bean flower thrips (*Megalurothrips sjostedti*). *Scientific Reports*, *11*(1), 14504. https://doi.org/10.1038/s41598-021-93891-5

Albo, M. J., Franco-Trecu, V., Wojciechowski, F. J., Toft, S., & Bilde, T. (2019). Maintenance of deceptive gifts in a natural spider population: Ecological and

demographic factors. *Behavioral Ecology*, *30*(4), 993–1000. https://doi.org/10.1093/beheco/arz040

Albo, M. J., Macías-Hernández, N., Bilde, T., & Toft, S. (2017). Mutual benefit from exploitation of female foraging motivation may account for the early evolution of gifts in spiders. *Animal Behaviour*, *129*, 9–14. https://doi.org/10.1016/j.anbehav.2017.05.001

Alcock, J. (1994). Postinsemination Associations Between Males and Females in Insects: The Mate-Guarding Hypothesis. *Annual Review of Entomology*, *39*, 1–21.

Arnqvist, G., & Rowe, L. (1995). Sexual conflict and arms races between the sexes: A morphological adaptation for control of mating in a female insect. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *261*(1360), 123–127. https://doi.org/10.1098/rspb.1995.0126

Arnqvist, G., & Rowe, L. (2005). *Sexual Conflict*. Princeton University Press.

Bacon, R., Washington, D., Johnson, M.-K., & Burns, M. (2023). The Geography of Sexual Conflict: A Synthetic Review. *The American Naturalist*, *201*(3), 429–441. https://doi.org/10.1086/722797

Bateman, P. W., Gilson, L. N., & Ferguson, J. W. H. (2001). Investment in Mate Guarding May Compensate for Constraints on Ejaculate Production in the Cricket *Gryllodes sigillatus*. *Ethology*, *107*(12), 1087–1098. https://doi.org/10.1046/j.1439-0310.2001.00756.x

Beyer, M., Mangliers, J., & Tuni, C. (2021). Silk-borne chemicals of spider nuptial gifts elicit female gift acceptance. *Biology Letters*, *17*(11), 20210386. https://doi.org/10.1098/rsbl.2021.0386

Blanckenhorn, W. U., Baur, J., Busso, J. P., Giesen, A., Gourgoulianni, N., Van Koppenhagen, N., Roy, J., Schäfer, M. A., Wegmann, A., & Rohner, P. T. (2020). Sexual size dimorphism is associated with reproductive life history trait differentiation in coexisting sepsid flies. *Oikos*, *129*(8), 1152–1162. https://doi.org/10.1111/oik.07036

Blomberg, S. P., Garland, T., & Ives, A. R. (2003). Testing for Phylogenetic Signal in Comparative Data: Behavioral Traits are More Labile. *Evolution*, *57*(4), 717–745. https://doi.org/10.1111/j.0014-3820.2003.tb00285.x

Blumstein, D. T., & Daniel, J. C. (2007a). *Quantifying behavior the JWatcher way*. Sinauer Associates.

Bokides, D., Lou, Y., & Hamilton, I. M. (2012). A model of sexual selection and female use of refuge in a coercive mating system. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1741), 3209–3216. https://doi.org/10.1098/rspb.2012.0246

Boulton, R. A., Zuk, M., & Shuker, D. M. (2018). An Inconvenient Truth: The Unconsidered Benefits of Convenience Polyandry. *Trends in Ecology & Evolution*, *33*(12), 904–915. https://doi.org/10.1016/j.tree.2018.10.002

Brown, W. D., & Barry, K. L. (2016). Sexual cannibalism increases male material investment in offspring: Quantifying terminal reproductive effort in a praying mantis. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1833), 20160656. https://doi.org/10.1098/rspb.2016.0656

Buchinger, T. J., & Li, W. (2023). Chemical communication and its role in sexual selection across Animalia. *Communications Biology*, *6*(1), 1178. https://doi.org/10.1038/s42003-023-05572-w

Burke, N. W., & Bonduriansky, R. (2018). The fitness effects of delayed switching to sex in a facultatively asexual insect. *Ecology and Evolution*, *8*(5), 2698–2711. https://doi.org/10.1002/ece3.3895

Burke, N. W., Crean, A. J., & Bonduriansky, R. (2015). The role of sexual conflict in the evolution of facultative parthenogenesis: A study on the spiny leaf stick insect. *Animal Behaviour*, *101*, 117–127. https://doi.org/10.1016/j.anbehav.2014.12.017

Burns, M., Hedin, M., & Shultz, J. W. (2012). Molecular phylogeny of the leiobunine harvestmen of eastern North America (Opiliones: Sclerosomatidae: Leiobuninae).

*Molecular Phylogenetics and Evolution*, *63*(2), 291–298. https://doi.org/10.1016/j.ympev.2011.12.025

Burns, M. M., Hedin, M., & Shultz, J. W. (2013). Comparative Analyses of Reproductive Structures in Harvestmen (Opiliones) Reveal Multiple Transitions from Courtship to Precopulatory Antagonism. *PLoS ONE*, *8*(6), e66767. https://doi.org/10.1371/journal.pone.0066767

Burns, M., & Shultz, J. W. (2015). Biomechanical Diversity of Mating Structures among Harvestmen Species Is Consistent with a Spectrum of Precopulatory Strategies. *PLOS ONE*, *10*(9), e0137181. https://doi.org/10.1371/journal.pone.0137181

Burns, M., & Shultz, J. W. (2016). Mechanical properties of male genitalia in *Leiobunum* harvestmen (Opiliones: Sclerosomatidae). *Journal of Arachnology*, *44*(2), 199–209. https://doi.org/10.1636/JoA-S-15-010

Burns, M., & Tsurusaki, N. (2016). Male Reproductive Morphology Across Latitudinal Clines and Under Long-Term Female Sex-Ratio Bias. *Integrative and Comparative Biology*, *56*(4), 715–727. https://doi.org/10.1093/icb/icw017

Curtis, D. J., & Machado, G. (2007). Ecology. In R. Pinto da Rocha, G. Machado, & G. Giribet (Eds.), *Harvestmen: The Biology of Opiliones* (pp. 280–308). Harvard University Press.

Davranoglou, L., Taylor, G. K., & Mortimer, B. (2023). Sexual selection and predation drive the repeated evolution of stridulation in Heteroptera and other arthropods. *Biological Reviews*, *98*(3), 942–981. https://doi.org/10.1111/brv.12938

del Castillo, R. C., & Gwynne, D. T. (2007). Increase in song frequency decreases spermatophore size: Correlative evidence of a macroevolutionary trade- off in katydids (Orthoptera: Tettigoniidae). *Journal of Evolutionary Biology*, *20*(3), 1028–1036. https://doi.org/10.1111/j.1420-9101.2006.01298.x

Del Matto, L., De Macedo Rego, R., & Santos, E. (2021). Mate-guarding duration is mainly influenced by the risk of sperm competition and not by female quality in a golden orb-weaver spider. *PeerJ*, *9*, e12310. https://doi.org/10.32942/OSF.IO/2U8TC

del Sol, J. F., Hongo, Y., Boisseau, R. P., Berman, G. H., Allen, C. E., & Emlen, Douglas. J. (2021). Population differences in the strength of sexual selection match relative weapon size in the Japanese rhinoceros beetle, *Trypoxylus dichotomus* (Coleoptera: Scarabaeidae). *Evolution*, *75*(2), 394–413. https://doi.org/10.1111/evo.14101

Dmitriew, C., & Blanckenhorn, W. U. (2012). The Role of Sexual Selection and Conflict in Mediating Among-Population Variation in Mating Strategies and Sexually Dimorphic Traits in *Sepsis punctum*. *PLoS ONE*, *7*(12), e49511. https://doi.org/10.1371/journal.pone.0049511

Edvardsson, M., & Canal, D. (2006). The effects of copulation duration in the bruchid beetle *Callosobruchus maculatus*. *Behavioral Ecology*, *17*(3), 430–434. https://doi.org/10.1093/beheco/arj045

Edward, D. A., & Chapman, T. (2011). The evolution and significance of male mate choice. *Trends in Ecology & Evolution*, *26*(12), 647–654. https://doi.org/10.1016/j.tree.2011.07.012

Edward, D. A., Stockley, P., & Hosken, D. J. (2015). Sexual Conflict and Sperm Competition. *Cold Spring Harbor Perspectives in Biology*, *7*(4), a017707. https://doi.org/10.1101/cshperspect.a017707

Elias, D. O., Sivalinghem, S., Mason, A. C., Andrade, M. C. B., & Kasumovic, M. M. (2014). Mate-guarding courtship behaviour: Tactics in a changing world. *Animal Behaviour*, *97*, 25–33. https://doi.org/10.1016/j.anbehav.2014.08.007

Fowler, E. K., Leigh, S., Bretman, A., & Chapman, T. (2022). Plastic responses of males and females interact to determine mating behavior. *Evolution*, *76*(9), 2116–2129. https://doi.org/10.1111/evo.14568

Fowler-Finn, K., Boyer, S., Ikagawa, R., Jeffries, T., Kahn, P., Larsen, E., Lee, D., & Smeester, M. (2018). Variation in Mating Dynamics across Five Species of Leiobunine Harvestmen (Arachnida: Opliones). *Biology*, *7*(2), 36. https://doi.org/10.3390/biology7020036

Fowler-Finn, K., Boyer, S. L., Ikagawa, R., Jeffries, T., Kahn, P. C., Larsen, E. M., Lee, D., Sasson, D., & Smeester, M. (2019). Qualitative and quantitative comparisons of mating behaviour across multiple populations and six species of leiobunine harvestmen (Arachnida: Opiliones). *Behaviour*, *156*(3–4), 363–390. https://doi.org/10.1163/1568539X-00003545

Fowler-Finn, K. D., Triana, E., & Miller, O. G. (2014). Mating in the harvestman *Leiobunum vittatum* (Arachnida: Opiliones): from premating struggles to solicitous tactile engagement. *Behaviour*, *151*(12–13), 1663–1686. https://doi.org/10.1163/1568539X-00003209

Fox, C. W., Stillwell, R. C., Wallin, W. G., & Hitchcock, L. J. (2006). Temperature and host species affect nuptial gift size in a seed- feeding beetle. *Functional Ecology*, *20*(6), 1003–1011. https://doi.org/10.1111/j.1365-2435.2006.01197.x

Frankino, A. W., & Sakaluk, S. K. (1994). Post copulatory mate guarding delays promiscuous mating by female decorated crickets. *Animal Behaviour*, *48*, 1479–1481. https://doi.org/10.1006/anbe.1994.1389

Fry, C. L. (2006). Juvenile hormone mediates a trade- off between primary and secondary sexual traits in stalk- eyed flies. *Evolution & Development*, *8*(2), 191–201. https://doi.org/10.1111/j.1525-142X.2006.00089.x

Gao, Q., Turnell, B. R., Hua, B., & Shaw, K. L. (2019). The effect of nuptial gift number on fertilization success in a Hawaiian swordtail cricket. *Behavioral Ecology and Sociobiology*, *73*(7), 92. https://doi.org/10.1007/s00265-019-2705-9

Ghislandi, P. G., Pekár, S., Matzke, M., Schulte- Döinghaus, S., Bilde, T., & Tuni, C. (2018). Resource availability, mating opportunity and sexual selection intensity influence the expression of male alternative reproductive tactics. *Journal of Evolutionary Biology*, *31*(7), 1035–1046. https://doi.org/10.1111/jeb.13284

Gnaspini, P., & Hara, M. R. (2007). Defense Mechanisms. In R. Pinto da Rocha, G. Machado, & G. Giribet (Eds.), *Harvestmen: The Biology of Opiliones* (pp. 374–399). Harvard University Press.

Golov, Y., Harari, A., Rillich, J., & Ayali, A. (2018). Precopulatory behavior and sexual conflict in the desert locust. *PeerJ*, *6*, e4356. https://doi.org/10.7717/peerj.4356

Gór, Á., Lang, Z., Pásztor, K., Szigeti, V., Vajna, F., & Kis, J. (2023). Mate- guarding success depends on male investment in a butterfly. *Ecology and Evolution*, *13*(9), e10533. https://doi.org/10.1002/ece3.10533

Gwynne, D. T. (2008). Sexual Conflict over Nuptial Gifts in Insects. *Annual Review of Entomology*, *53*(1), 83–101. https://doi.org/10.1146/annurev.ento.53.103106.093423

Hagg, L., LaMere, C. J., & Zuk, M. (2024). Postcopulatory song as a mate-guarding tactic in the Pacific field cricket, *Teleogryllus oceanicus*. *Animal Behaviour*, *207*, 201–207. https://doi.org/10.1016/j.anbehav.2023.11.001

Harts, A. M. F., & Kokko, H. (2013). Understanding Promiscuity: When is Seeking Additional Mates Better than Guarding an Already Found One?: Mate-Guarding, Monogamy, and Promiscuity. *Evolution*, *67*(10), 2838–2848. https://doi.org/10.1111/evo.12163

Hedin, M., Tsurusaki, N., Macías-Ordóñez, R., & Shultz, J. W. (2012). Molecular systematics of sclerosomatid harvestmen (Opiliones, Phalangioidea, Sclerosomatidae): Geography is better than taxonomy in predicting phylogeny. *Molecular Phylogenetics and Evolution*, *62*(1), 224–236. https://doi.org/10.1016/j.ympev.2011.09.017

Iglesias-Carrasco, M., Fox, R. J., Vega-Trejo, R., Jennions, M. D., & Head, M. L. (2019). An experimental test for body size-dependent effects of male harassment and an elevated copulation rate on female lifetime fecundity and offspring performance. *Journal of Evolutionary Biology*, *32*(11), 1262–1273. https://doi.org/10.1111/jeb.13526

Ingianni, E. A., McGhee, C. R., & Shultz, J. W. (2011). Taxonomy of the *Leiobunum calcar* species-group (Opiliones: Sclerosomatidae: Leiobuninae). *Journal of Arachnology*, *39*(3), 454–481. https://doi.org/10.1636/Ha11-42.1

Jarrige, A., Body, M., Giron, D., Greenfield, M. D., & Goubault, M. (2015). Amino acid composition of the bushcricket spermatophore and the function of courtship feeding: Variable composition suggests a dynamic role of the nuptial gift. *Physiology & Behavior*, *151*, 463–468. https://doi.org/10.1016/j.physbeh.2015.08.009

Johns, J. L., Roberts, J. A., Clark, D. L., & Uetz, G. W. (2009). Love bites: Male fang use during coercive mating in wolf spiders. *Behavioral Ecology and Sociobiology*, *64*(1), 13–18. https://doi.org/10.1007/s00265-009-0812-8

Kahn, P. C., Cao, D. D., Burns, M., & Boyer, S. L. (2018). Nuptial gift chemistry reveals convergent evolution correlated with antagonism in mating systems of harvestmen (Arachnida, Opiliones). *Ecology and Evolution*, *8*(14), 7103–7110. https://doi.org/10.1002/ece3.4232

Karachiwalla, Z., deCarvahlo, T., & Burns, M. (2023). Spermathecal Variation in Temperate Opiliones. *Integrative And Comparative Biology*, *63*(1), 188–197. https://doi.org/10.1093/icb/icaa120

Karlsson Green, K., Kovalev, A., Svensson, E. I., & Gorb, S. N. (2013). Male clasping ability, female polymorphism and sexual conflict: Fine-scale elytral morphology as a sexually antagonistic adaptation in female diving beetles. *Journal of The Royal Society Interface*, *10*(86), 20130409. https://doi.org/10.1098/rsif.2013.0409

Kelly, C. D. (2008). Sperm investment in relation to weapon size in a male trimorphic insect? *Behavioral Ecology*, *19*(5), 1018–1024. https://doi.org/10.1093/beheco/arn058

Kokko, H., & Jennions, M. D. (2014). The Relationship between Sexual Selection and Sexual Conflict. *Cold Spring Harbor Perspectives in Biology*, *6*(9), a017517–a017517. https://doi.org/10.1101/cshperspect.a017517

Körner, M., Vogelweith, F., Foitzik, S., & Meunier, J. (2017). Condition-Dependent Trade-Off Between Weapon Size and Immunity in Males of the European Earwig. *Scientific Reports*, *7*(1), 7988. https://doi.org/10.1038/s41598-017-08339-6

Křemenová, J., Bartonička, T., Balvín, O., Massino, C., Reinhardt, K., Sasínková, M., Weig, A. R., & Otti, O. (2021). Male diet affects female fitness and sperm competition in human- and bat-associated lineages of the common bedbug, *Cimex lectularius*. *Scientific Reports*, *11*(1), 15538. https://doi.org/10.1038/s41598-021-94622-6

Krupa, J. J., Leopold, W. R., & Sih, A. (1990). Avoidance of Male Giant Water Striders By Females. *Behaviour*, *115*(3–4), 247–253. https://doi.org/10.1163/156853990X00590

Kunz, J. A., Duvot, G. J., Van Noordwijk, M. A., Willems, E. P., Townsend, M., Mardianah, N., Utami Atmoko, S. S., Vogel, E. R., Nugraha, T. P., Heistermann, M., Agil, M., Weingrill, T., & Van Schaik, C. P. (2021). The cost of associating with males for Bornean and Sumatran female orangutans: A hidden form of sexual conflict? *Behavioral Ecology and Sociobiology*, *75*(1), 6. https://doi.org/10.1007/s00265-020-02948-4

Kuriwada, T., & Kasuya, E. (2012). Nuptial gifts protect male bell crickets from female aggressive behavior. *Behavioral Ecology*, *23*(2), 302–306. https://doi.org/10.1093/beheco/arr186

Lehmann, G. U. C., Heller, K.-G., & Mai, R. (2016). Prolonged copulations as an alternative to male nuptial gift investment in the bushcricket *Letana inflata* (Orthoptera:

Tettigoniidae). *Journal of Ethology*, *34*(2), 191–194. https://doi.org/10.1007/s10164-016-0457-9

Lehmann, G. U. C., & Lehmann, A. W. (2016). Material benefit of mating: The bushcricket spermatophylax as a fast uptake nuptial gift. *Animal Behaviour*, *112*, 267–271. https://doi.org/10.1016/j.anbehav.2015.12.022

Lehtonen, J., Jennions, M. D., & Kokko, H. (2012). The many costs of sex. *Trends in Ecology & Evolution*, *27*(3), 172–178. https://doi.org/10.1016/j.tree.2011.09.016

Lewis, S. M., Vahed, K., Koene, J. M., Engqvist, L., Bussière, L. F., Perry, J. C., Gwynne, D., & Lehmann, G. U. C. (2014). Emerging issues in the evolution of animal nuptial gifts. *Biology Letters*, *10*(7), 20140336. https://doi.org/10.1098/rsbl.2014.0336

Lewis, S., & South, A. (2012). The Evolution of Animal Nuptial Gifts. In H. J. Brockmann, T. J. Roper, M. Naguib, J. C. Mitani, & L. W. Simmons (Eds.), *Advances in the Study of Behavior* (Vol. 44, pp. 53–97). Elsevier. https://doi.org/10.1016/B978-0-12-394288-3.00002-2

Liu, X., Hayashi, F., Lavine, L. C., & Yang, D. (2015). Is diversification in male reproductive traits driven by evolutionary trade-offs between weapons and nuptial gifts? *Proceedings of the Royal Society B: Biological Sciences*, *282*(1807), 20150247. https://doi.org/10.1098/rspb.2015.0247

Machado, G., & Burns, M. (2023). Reproductive biology of harvestmen (Arachnida: Opiliones): a review of a rapidly evolving research field. *Current Zoology*, *zoac102*, 1–21. https://doi.org/10.1093/cz/zoac102

Machado, G., & Macías-Ordóñez, R. (2007). Reproduction. In R. Pinto da Rocha, G. Machado, & G. Giribet (Eds.), *Harvestmen: The Biology of Opiliones* (pp. 414–454). Harvard University Press.

Macías-Ordóñez, R. (1997). *The mating system of* Leiobunum vittatum *(Say 1891) (Arachnida: Opiliones: Palpitores): Resource defense polygyny in the striped harvestman* (Doctoral dissertation). Bethlehem, PA: Lehigh University.

Macías-Ordóñez, R., Machado, G., Pérez-González, A., & Shultz, J. W. (2010). Genitalic evolution in Opiliones. In *The Evolution of Primary Sexual Characters in Animals* (p. 537). Oxford University Press.

Małek, D. K., Dańko, M. J., & Czarnoleski, M. (2019). Does seed size mediate sex-specific reproduction costs in the *Callosobruchus maculatus* bean beetle? *PLOS ONE*, *14*(12), e0225967. https://doi.org/10.1371/journal.pone.0225967

Maroni, P. J., Bryant, K. A., & Tatarnic, N. J. (2023). Female genital concealment and a corresponding male clasping apparatus in Australian ripple bugs (Hemiptera: Veliidae). *Arthropod Structure & Development*, *74*, 101254. https://doi.org/10.1016/j.asd.2023.101254

Martens, J. (2020). A harvestman with elaborate palpal pliers, *Thunbergia gretae n. gen. n. sp.* from China (Opiliones: Sclerosomatidae: Gagrellinae). *Zoologischer Anzeiger*, *287*, 160–166. https://doi.org/10.1016/j.jcz.2020.03.010

Martínez Villar, M., Germil, M., Pavón-Peláez, C., Tomasco, I. H., Bilde, T., Toft, S., & Albo, M. J. (2023). Lack of Female Preference for Nuptial Gifts May Have Led to Loss of the Male Sexual Trait. *Evolutionary Biology*, *50*(3), 318–331. https://doi.org/10.1007/s11692-023-09606-3

Martínez Villar, M., Trillo, M. C., & Albo, M. J. (2021). Ineffective nuptial gifts suggest female emancipation from sensory exploitation. *Behavioral Ecology and Sociobiology*, *75*(3), 61. https://doi.org/10.1007/s00265-021-02994-6

Mazzi, D., Kesäniemi, J., Hoikkala, A., & Klappert, K. (2009). Sexual conflict over the duration of copulation in *Drosophila montana*: Why is longer better? *BMC Evolutionary Biology*, *9*(1), 132. https://doi.org/10.1186/1471-2148-9-132

McPherson, S., Wada-Katsumata, A., Hatano, E., Silverman, J., & Schal, C. (2022). Nutritional condition affects tergal gland secretion and courtship success of male cockroaches. *PLOS ONE*, *17*(8), e0271344. https://doi.org/10.1371/journal.pone.0271344

Miller, C. W., Joseph, P. N., Kilner, R. M., & Emberts, Z. (2019). A weapons–testes trade-off in males is amplified in female traits. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1908), 20190906. https://doi.org/10.1098/rspb.2019.0906

Moczek, A. P., & Nijhout, H. F. (2004). Trade- offs during the Development of Primary and Secondary Sexual Traits in a Horned Beetle. *The American Naturalist*, *163*(2), 184–191. https://doi.org/10.1086/381741

Myers, S. S., Buckley, T. R., & Holwell, G. I. (2016). Male genital claspers influence female mate acceptance in the stick insect *Clitarchus hookeri*. *Behavioral Ecology and Sociobiology*, *70*(9), 1547–1556. https://doi.org/10.1007/s00265-016-2163-6

O'Hara, M. K., & Brown, W. D. (2021). Sexual Cannibalism Increases Female Egg Production in the Chinese Praying Mantid (*Tenodera sinensis*). *Journal of Insect Behavior*, *34*(3), 127–135. https://doi.org/10.1007/s10905-021-09776-y

Okada, K., & Miyatake, T. (2009). Genetic correlations between weapons, body shape and fighting behaviour in the horned beetle *Gnatocerus cornutus*. *Animal Behaviour*, *77*(5), 1057–1065. https://doi.org/10.1016/j.anbehav.2009.01.008

Órfão, I., Carvalho, C., Rodrigues, I., Ascensão, L., Pedaccini, M., Vicente, L., Barbosa, M., & Varela, S. A. M. (2023). The role of intrasexual competition on the evolution of male-male courtship display: A systematic review. *PeerJ*, *10*, e14638. https://doi.org/10.7717/peerj.14638

Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*, *401*(6756), 877–884. https://doi.org/10.1038/44766

Pandulli- Alonso, I., Tomasco, I. H., & Albo, M. J. (2022). The handsome liar: Male spiders offering worthless gifts can benefit increasing mating duration. *Ethology*, *128*(3), 215–222. https://doi.org/10.1111/eth.13258

Perry, J. C., & Tse, C. T. (2013). Extreme Costs of Mating for Male Two-Spot Ladybird Beetles. *PLoS ONE*, *8*(12), e81934. https://doi.org/10.1371/journal.pone.0081934

Pilastro, A., Benetton, S., & Bisazza, A. (2003). Female aggregation and male competition reduce costs of sexual harassment in the mosquitofish *Gambusia holbrooki*. *Animal Behaviour*, *65*(6), 1161–1167. https://doi.org/10.1006/anbe.2003.2118

Pinto da Rocha, R., Machado, G., & Giribet, G. (2007). *Harvestmen: The biology of Opiliones*. Harvard University Press.

Plath, M., Parzefall, J., & Schlupp, I. (2003). The role of sexual harassment in cave and surface dwelling populations of the Atlantic molly, *Poecilia mexicana* (Poeciliidae, Teleostei). *Behavioral Ecology and Sociobiology*, *54*(3), 303–309. https://doi.org/10.1007/s00265-003-0625-0

Prokop, P., & Okrouhlík, J. (2021). Metabolic cost of holding nuptial food gifts for male spiders. *Ecological Entomology*, *46*(3), 684–690. https://doi.org/10.1111/een.13008

Rapkin, J., Jensen, K., Lane, S. M., House, C. M., Sakaluk, S. K., & Hunt, J. (2016). Macronutrient intake regulates sexual conflict in decorated crickets. *Journal of Evolutionary Biology*, *29*(2), 395–406. https://doi.org/10.1111/jeb.12794

Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, *3*(2), 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Rönn, J., Katvala, M., & Arnqvist, G. (2006). The costs of mating and egg production in *Callosobruchus* seed beetles. *Animal Behaviour*, *72*(2), 335–342. https://doi.org/10.1016/j.anbehav.2005.10.024

Rosvall, K. A. (2011). Intrasexual competition in females: Evidence for sexual selection? *Behavioral Ecology*, *22*(6), 1131–1140. https://doi.org/10.1093/beheco/arr106

Rueda-Solano, L. A., Vargas-Salinas, F., Pérez-González, J. L., Sánchez-Ferreira, A., Ramírez-Guerra, A., Navas, C. A., & Crawford, A. J. (2022). Mate-guarding behaviour in anurans: Intrasexual selection and the evolution of prolonged amplexus in the harlequin toad *Atelopus laetissimus*. *Animal Behaviour*, *185*, 127–142. https://doi.org/10.1016/j.anbehav.2021.12.003

Sakaluk, S. K. (1991). Post-copulatory mate guarding in decorated crickets. *Animal Behaviour*, *41*(2), 207–216. https://doi.org/10.1016/S0003-3472(05)80472-5

Sakaluk, S. K. (2000). Sensory exploitation as an evolutionary origin to nuptial food gifts in insects. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1441), 339–343. https://doi.org/10.1098/rspb.2000.1006

Sakaluk, S. K., Duffield, K. R., Rapkin, J., Sadd, B. M., & Hunt, J. (2019). The troublesome gift: The spermatophylax as a purveyor of sexual conflict and coercion in crickets. In M. Naguib, L. Barrett, S. D. Healy, J. Podos, L. W. Simmons, & M. Zuk (Eds.), *Advances in the Study of Behavior* (Vol. 51, pp. 1–30). Elsevier. https://doi.org/10.1016/bs.asb.2018.12.001

Schaefer, H. M., & Ruxton, G. D. (2015). Signal Diversity, Sexual Selection, and Speciation. *Annual Review of Ecology, Evolution, and Systematics*, *46*(1), 573–592. https://doi.org/10.1146/annurev-ecolsys-112414-054158

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676–682. https://doi.org/10.1038/nmeth.2019

Schlupp, I., McKnab, R., & Ryan, M. (2001). Sexual Harassment as a Cost for Molly Females: Bigger Males Cost Less. *Behaviour*, *138*(2), 277–286. https://doi.org/10.1163/15685390151074438

Schütz, D., & Taborsky, M. (2005). Mate Choice and Sexual Conflict in the Size Dimorphic Water Spider *Argyroneta aquatica* (Araneae, Argyronetidae). *Journal of Arachnology*, *33*(3), 767–775. https://doi.org/10.1636/S03-56.1

Sentenská, L., Šedo, O., & Pekár, S. (2020). Biting and binding: An exclusive coercive mating strategy of males in a philodromid spider. *Animal Behaviour*, *168*, 59–68. https://doi.org/10.1016/j.anbehav.2020.08.001

Shine, R., Wall, M., Langkilde, T., & Mason, R. T. (2005). Do Female Garter Snakes Evade Males to Avoid Harassment or to Enhance Mate Quality? *The American Naturalist*, *165*(6), 660–668. https://doi.org/10.1086/429591

Shuker, D. M., & Kvarnemo, C. (2021). The definition of sexual selection. *Behavioral Ecology*, *32*(5), 781–794. https://doi.org/10.1093/beheco/arab055

Shultz, J. W. (2018). A Guide to the Identification of the Harvestmen (Arachnida: Opiliones) of Maryland. *Northeastern Naturalist*, *25*(1), 21–49. https://doi.org/10.1656/045.025.0102

Shultz, J. W., & Pinto-da-Rocha, R. (2007). Morphology and Functional Anatomy. In R. Pinto da Rocha, G. Machado, & G. Giribet (Eds.), *Harvestmen: The Biology of Opiliones* (pp. 14–61). Harvard University Press.

Sigvardt, Z. M. S., Rogers, D. C., & Olesen, J. (2017). Functional morphology of amplexus (clasping) in spinicaudatan clam shrimps (Crustacea, Branchiopoda) and its evolution in bivalved branchiopods: A video-based analysis. *Journal of Morphology*, *278*(4), 523–546. https://doi.org/10.1002/jmor.20649

Simmons, L. W. (2002). *Sperm Competition and Its Evolutionary Consequences in the Insects*. Princeton University Press. https://doi.org/10.1515/9780691207032

Simmons, L. W., & Emlen, D. J. (2006). Evolutionary trade-off between weapons and testes. *Proceedings of the National Academy of Sciences*, *103*(44), 16346–16351. https://doi.org/10.1073/pnas.0603474103

Simmons, L. W., Lüpold, S., & Fitzpatrick, J. L. (2017). Evolutionary Trade-Off between Secondary Sexual Traits and Ejaculates. *Trends in Ecology & Evolution*, *32*(12), 964–976. https://doi.org/10.1016/j.tree.2017.09.011

Sirot, L. K., Wong, A., Chapman, T., & Wolfner, M. F. (2015). Sexual Conflict and Seminal Fluid Proteins: A Dynamic Landscape of Sexual Interactions. *Cold Spring Harbor Perspectives in Biology*, *7*(2), a017533. https://doi.org/10.1101/cshperspect.a017533

Skwierzyńska, A. M., & Plesnar- Bielak, A. (2018). Proximate mechanisms of the differences in reproductive success of males bearing different alleles of Pgdh – a gene involved in a sexual conflict in bulb mite. *Journal of Evolutionary Biology*, *31*(5), 657–664. https://doi.org/10.1111/jeb.13250

Solano-Brenes, D., Costa-Schmidt, L. E., Albo, M. J., & Machado, G. (2021). Differential allocation in a gift-giving spider: Males adjust their reproductive investment in response to female condition. *BMC Ecology and Evolution*, *21*(1), 140. https://doi.org/10.1186/s12862-021-01870-1

South, A., Stanger-Hall, K., Jeng, M.-L., & Lewis, S. M. (2011). Correlated Evolution of Female Neoteny and Flightlessness with Male Spermatophore Production in Fireflies (Coleoptera: Lampyridae): Female Flight Linked to Male Spermatophore Production in Fireflies. *Evolution*, *65*(4), 1099–1113. https://doi.org/10.1111/j.1558-5646.2010.01199.x

Stålhandske, S. (2002). Nuptial gifts of male spiders function as sensory traps. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1494), 905–908. https://doi.org/10.1098/rspb.2001.1917

Tatarnic, N. J., Cassis, G., & Siva-Jothy, M. T. (2014). Traumatic Insemination in Terrestrial Arthropods. *Annual Review of Entomology*, *59*(1), 245–261. https://doi.org/10.1146/annurev-ento-011613-162111

Thornhill, R. (1976). Sexual Selection and Nuptial Feeding Behavior in *Bittacus apicalis* (Insecta: Mecoptera). *The American Naturalist*, *110*(974), 529–548. https://doi.org/10.1086/283089

Toft, S., & Albo, M. J. (2015). Optimal numbers of matings: The conditional balance between benefits and costs of mating for females of a nuptial gift- giving spider. *Journal of Evolutionary Biology*, *28*(2), 457–467. https://doi.org/10.1111/jeb.12581

Toft, S., & Albo, M. J. (2016). The shield effect: Nuptial gifts protect males against pre-copulatory sexual cannibalism. *Biology Letters*, *12*(5), 20151082. https://doi.org/10.1098/rsbl.2015.1082

Tong, X., & Hua, B.-Z. (2019). Nuptial feeding and genital coupling of *Neopanorpa* scorpionflies (Insecta: Mecoptera: Panorpidae) with notal organs of various lengths. *Contributions to Zoology*, *88*(4), 498–512. https://doi.org/10.1163/18759866-20191409

Tong, X., Wang, P.-Y., Jia, M.-Z., Thornhill, R., & Hua, B.-Z. (2021). Traumatic mating increases anchorage of mating male and reduces female remating duration and fecundity in a scorpionfly species. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1952), 20210235. https://doi.org/10.1098/rspb.2021.0235

Townsend, V. R., Bertram, M. S., & Milne, M. A. (2015). Variation in ovipositor morphology among laniatorean harvestmen (Arachnida: Opiliones). *Zoomorphology*, *134*(3), 487–497. https://doi.org/10.1007/s00435-015-0269-4

Townsend, V. R., Pérez-González, A., & Proud, D. N. (2019). Putative mating plugs of harvestmen (Opiliones, Laniatores). *Zoologischer Anzeiger*, *278*, 101–109. https://doi.org/10.1016/j.jcz.2018.12.005

Vahed, K. (1998). The function of nuptial feeding in insects: A review of empirical studies. *Biological Reviews of the Cambridge Philosophical Society*, *73*(1), 43–78. https://doi.org/10.1017/S0006323197005112

Vahed, K. (2007). All that Glisters is not Gold: Sensory Bias, Sexual Conflict and Nuptial Feeding in Insects and Spiders. *Ethology*, *113*(2), 105–127. https://doi.org/10.1111/j.1439-0310.2006.01312.x

Vahed, K., Gilbert, J. D. J., Weissman, D. B., & Barrientos-Lozano, L. (2014). Functional Equivalence of Grasping Cerci and Nuptial Food Gifts in Promoting Ejaculate Transfer in Katydids: Nuptial Gifts and Long Copulation are Analogous. *Evolution*, *68*(7), 2052–2065. https://doi.org/10.1111/evo.12421

Voigt, C. C., Michener, R., & Kunz, T. H. (2005). The Energetics of Trading Nuptial Gifts for Copulations in Katydids. *Physiological and Biochemical Zoology*, *78*(3), 417–423. https://doi.org/10.1086/430224

Walter, T., & Couzin, I. D. (2021). TRex, a fast multi-animal tracking system with markerless identification, and 2D estimation of posture and visual fields. *eLife*, *10*, e64000. https://doi.org/10.7554/eLife.64000

Wedell, N. (1994). Variation in nuptial gift quality in bush crickets (Orthoptera: Tettigoniidae). *Behavioral Ecology*, *5*(4), 418–425. https://doi.org/10.1093/beheco/5.4.418

Welke, K. W., & Schneider, J. M. (2012). Sexual cannibalism benefits offspring survival. *Animal Behaviour*, *83*(1), 201–207. https://doi.org/10.1016/j.anbehav.2011.10.027

Wijnhoven, H. (2011). Notes on the biology of the unidentified invasive harvestman *Leiobunum sp.* (Arachnida: Opiliones). *Arachnologische Mitteilungen*, *41*, 17–30. https://doi.org/10.5431/aramit4103

Willemart, R. H., & Hebets, E. A. (2012). Sexual Differences in the Behavior of the Harvestman *Leiobunum vittatum* (Opiliones, Sclerosomatidae) Towards Conspecific

Cues. *Journal of Insect Behavior*, *25*(1), 12–23. https://doi.org/10.1007/s10905-011-9268-6

Wolfner, M. F. (2002). The gifts that keep on giving: Physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity*, *88*(2), 85–93. https://doi.org/10.1038/sj.hdy.6800017

Yamane, T., Okada, K., Nakayama, S., & Miyatake, T. (2010). Dispersal and ejaculatory strategies associated with exaggeration of weapon in an armed beetle. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1688), 1705–1710. https://doi.org/10.1098/rspb.2009.2017

Zatz, C., Werneck, R. M., Macías-Ordóñez, R., & Machado, G. (2011). Alternative mating tactics in dimorphic males of the harvestman *Longiperna concolor* (Arachnida: Opiliones). *Behavioral Ecology and Sociobiology*, *65*(5), 995–1005. https://doi.org/10.1007/s00265-010-1103-0

Zweerus, N. L., Van Wijk, M., Schal, C., & Groot, A. T. (2021). Experimental evidence for female mate choice in a noctuid moth. *Animal Behaviour*, *179*, 1–13. https://doi.org/10.1016/j.anbehav.2021.06.022