Mating in the harvestman *Leiobunum vittatum* (Arachnida: Opiliones): from premating struggles to solicitous tactile engagement

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Abstract

When mating interactions are influenced by multiple sources of selection, they may involve multiple stages of mate assessment. At each stage, a different set of morphological and behavioural traits may be important in determining the outcome of the interaction. Here, we test the potential for multiple sources of selection to shape mating interactions in *Leiobunum vittatum* harvestmen, commonly known as 'daddy longlegs'. We provide a qualitative and quantitative study of mating interactions, and investigate the influence of multiple morphological traits on each of several distinct stages of their mating interactions. Mating interactions start with a struggle between males and females during which the male attempts to secure the females in a mating embrace. Success at this stage depends on the length of the male's clasping pedipalps: those with shorter pedipalps (and thus greater mechanical advantage) were more successful. Male size relative to the female determines how quickly males achieve this embrace. Mating interactions then proceed to tactile exchanges between males and females, indicating the potential for mutual mate choice and/or peri- and post-copulatory selection. We found no morphological predictors of the timing of these later stages of the mating interactions, and suggest that the exchange of a nuptial gift is important for the dynamics of these stages. Overall, our results highlight *L. vittatum* as a potentially highly informative group for studying how traits involved in mating are shaped by the interaction of selection across multiple stages in mating interactions.

1. Introduction

The culmination of mating often involves a complex series of behavioural exchanges centred around female assessment of potential mates (Andersson, 1994; Gibson & Langen, 1996). These behavioural exchanges may be influenced by a number of different sources of selection, including: conflict over mating (Arnqvist & Rowe, 2005; Parker, 2006), pre-copulatory choice (Andersson & Simmons, 2006), peri- and postcopulatory choice (Eberhard, 1985, 1996) and sperm competition (Parker, 1970; Simmons, 2001), in addition to other mechanisms of intra- and inter-sexual selection (Andersson, 1994). It is possible for more than one of these sources of selection to act to shape mating interactions in a single species (Brennan & Prum, 2012). When this occurs, selection from one source may reinforce that from another — for example, intrasexual selection in the form of male–male competition for access to mates, and intersexual conflict in the form of coercing females into mating may both favor large male body size and strength (Clutton-Brock & Parker, 1995). Selection from different sources may also act on different sets of male traits — for example, intrasexual selection resulting from male–male competition for access to mates may favour large body size and large weapons, but intrasexual selection resulting from sperm competition may favour large testes size (Preston et al., 2003).

When multiple sources of selection shape morphology and behaviour of mating interactions, they may do so at different stages during an interaction (e.g., during pre-, peri- and post-copulatory stages) (Andersson & Simmons, 2006; Kvarnemo & Simmons, 2013). For example, consider a situation in which mating is costly. The initial stage when pairs first come into contact — and a female has not been able to assess the male (and, thus, male and female interests are most likely to be divergent) — may be determined by behaviour and morphology that tests the strength of males and females (Alexander et al., 1997). In contrast, the outcome of peri- and post-copulatory stages may be determined by behaviour and morphology involved in more detailed assessment (Alexander et al., 1997) — for example, nuptial gift characteristics and other traits favoured by cryptic female choice (Eberhard, 1996).

By examining the determinants and dynamics of different stages in the mate selection process, we can test the hypothesis that multiple sources of selection operate to shape mating. Here, we test whether: (i) distinct stages of mating exist, and these stages exhibit qualitatively distinct behavioural dynamics; and (ii) at each stage, different morphological or behavioural traits

determine the outcome of an interaction in terms of the success or timing of that stage. To examine these questions, we study a species of eastern North American harvestmen, commonly known as 'daddy longlegs' (*Leiobunum vittatum*, Arachnida: Opiliones: Sclerosomatidae: Leiobuninae). Harvestmen in the leiobunum clade provide an excellent system for studying the evolution of behaviour and morphology involved in mating. Morphological variation in genitalia suggests a high diversity in mating behaviour across the group (Macías-Ordóñez et al., 2010; Burns et al., 2013). In particular, species vary in the presence/absence of specialized sacs at the distal portion of the penis that function in the delivery of nuptial gifts: the ancestral state of this morphological trait is the presence of sacs (McGhee, 1970; Burns et al., 2012, 2013). The loss of these sacs has occurred at 4+ independent points in the Leiobunine clade (Burns et al., 2012, 2013). In two of the species groups in which sacs have been lost — *calcar* and *vittatum*, the latter of which includes our focal species — male pedipalps are highly specialized for clasping the female during mating (Burns et al., 2012, 2013). These morphological changes have occurred in concert with shifts in mating behaviour from more choice-based to more conflict-based interactions (Burns et al., 2013).

Currently, our understanding of mating behaviour in temperate harvestmen is fairly limited; specifically, comprehensive quantitative descriptions and illustrations are lacking (but see Willemart et al., 2006; Wijnhoven, 2011; Zatz et al., 2011). However, what we do know about mating in leiobunine in particular suggests the presence of several distinct stages that occur during mating interactions (Macías-Ordóñez, 1997; Machado & Macías-Ordóñez, 2007). Additionally, the presence of both clasping pedipalps and nuptial gift glands in *L. vittatum* suggests the possibility for interesting mating dynamics, and the potential involvement of multiple independent morphological traits in the mate selection process. Thus, the goals of our study are two-fold. The first is to provide detailed description of mating behaviour to provide a framework for future research in temperate harvestmen. The second is to test the above-outlined hypothesis that multiple sources of selection operate to shape mating.

We first describe mating interactions in *L. vitattum* by: (i) identifying and defining key behaviours in a behavioural ethogram, and providing detailed illustrations of some of these key behaviours; (ii) quantifying transitions from one behaviour to another in a flow diagram; and (iii) identifying major stages throughout the course of a mating interaction. We determine if the different

stages of the mating interactions have qualitatively different behavioural dynamics — specifically, whether females show resistance in earlier stages. Finally, we identify morphological determinants of the outcome of different stages of the mating interactions, with particular interest in whether traits important in earlier stages are likely to be related to male size or strength.

2. Materials and methods

2.1. Study organisms

We collected Harvestmen in later summer of 2010 and 2013 along a section of the Wisconsin River in Milwaukee, Wisconsin (approximate location: 43°4′28″N, 87°53′31″W). This area is a moist, riparian environment, with vegetation consisting of trees, shrubs and grasses. We collected animals from low-lying leaves (approx. <1.5 m) of bushes and small trees directly off the path and brought them to the laboratory where we housed them in plastic deli dish containers (11 cm diameter × 8 cm depth). We pierced the lids of the containers to provide air flow, cut a small flap into the lid for minimal disturbance during feeding, and provided a mesh surface on the side wall of the dishes on which the animals could climb. We maintained a 12 h/12 h light/dark cycle and provided water ad libitum through a water-filled tube with a cotton wick plug placed in the containers. In 2010, we maintained specimens for 1–2 weeks in the lab, feeding them each a dead cricket and a small apple cube each week. In 2013, individuals were immediately fed after collection and maintained for one day prior to testing. During collections, we observed three independent pairs of individuals mating in the field.

2.2. Mating trials

Prior to a trial, we placed females in an acetate barrier (2 cm in diameter) in the centre of a mating enclosure (approx. 31 cm in diameter) to acclimate for 2 min (Figure 1). We introduced males to the arena and allowed them to acclimate for a further 2 min. The trial began when we released females and allowed them to interact freely with the male. In 2010 ($N = 15$ trials) we placed a leaf in the centre of the enclosure prior to the trial start, which was removed after the male successfully secured the female. Removal of the leaf did not appear to disturb the mating pair. In 2013 ($N = 19$ trials), we found that the leaf was not necessary for mating interactions, and it was excluded. We recorded all trials with two Sony HDR-HC5 HDV HandyCams simulta-

31 cm
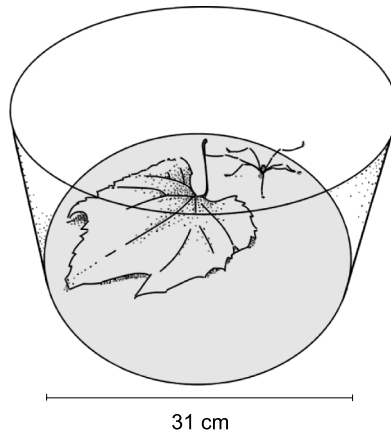
Figure 1. To-scale diagram of the experimental set-up for mating trials between male and female *L. vittatum*.

neously: one positioned perpendicular to the substrate and focused to record the fine details of male/female behaviour, and one positioned directly above the arena to capture general movements around the enclosure. We cleaned the mating arena with Ethanol in between trials to remove any potential chemical cues. Finally, we weighed individuals after the conclusion of each trial, and preserved them in 70% EtOH for further morphological analyses (see below).

2.3. Description of mating interactions

To construct the ethogram and behavioural flow diagram, we analysed the videos from the 2010 for behaviour consistently displayed across trials. We described each behaviour in an ethogram and then quantified the number of occurrences, duration, and the temporal position of each over the course of the interaction. The angles of the cameras allowed us to observe the vast majority of movements by the interacting individuals.

We categorized trials into 'unsuccessful' or 'successful', with successful trials being those in which the male secured the female in a mating embrace (Figures 2 and 3B). The embrace is indicated by the male wrapping his legs III around the female legs II and hooking his pedipalps behind her leg II coxae without being dislodged (Figures 2 and 3). In only one trial did the male not attempt to mate, and this trial was excluded from subsequent analyses. For all successful trials ($N = 11$ of 15 trials), we constructed a behavioural transition matrix. We summarized this matrix in a behavioural
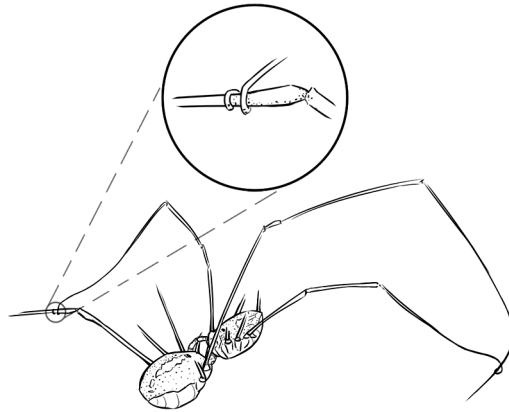
Figure 2. Drawing of a mating embrace between a female (bottom left) and male (upper right) *L. vittatum*. The male typically starts by wrapping his third pair of legs on the femur of the female's second pair of legs. Here, he is shown wrapping at the distal portion of her leg II. As the interaction continued, the female attempted to pull her leg free from his grasp. The inset shows a more detailed view of the leg wrapping behaviour.

flow diagram to visually represent common sequences of behaviour that occur over the course of a mating interaction. We classified mating behaviour into four stages: pre-copulatory embrace, pre-intromission, intromission and post-intromission. Termination of the trial happened when the male and female disengaged from the mating embrace.

2.4. Morphological determinants of mating success

For 2010 and 2013 trials, we tested which morphological features of males and females predicted male success in securing a female in a mating embrace. In addition to weighing each individual after the conclusion of a trial, we measured the cephalothorax width of preserved males and females, and the length of the femur of the male's right pedipalp (an important male trait used to secure the female in the embrace; Figure 3). Some individuals were excluded from these measurements because they were used in subsequent experiment and either died before the end of the season or incurred other damage that reduced measurement accuracy. We placed individuals in a stereotyped position secured with insect pins, and photographed the dorsal surface of their bodies using a USB 2.0 camera Moticam 2500 (Motic China Group) mounted on a dissecting scope and connected to an iMac computer. Using these pictures, we measured the cephalothorax width — a standard size measurement used in opilionids and other arachnids (Apontes & Brown,
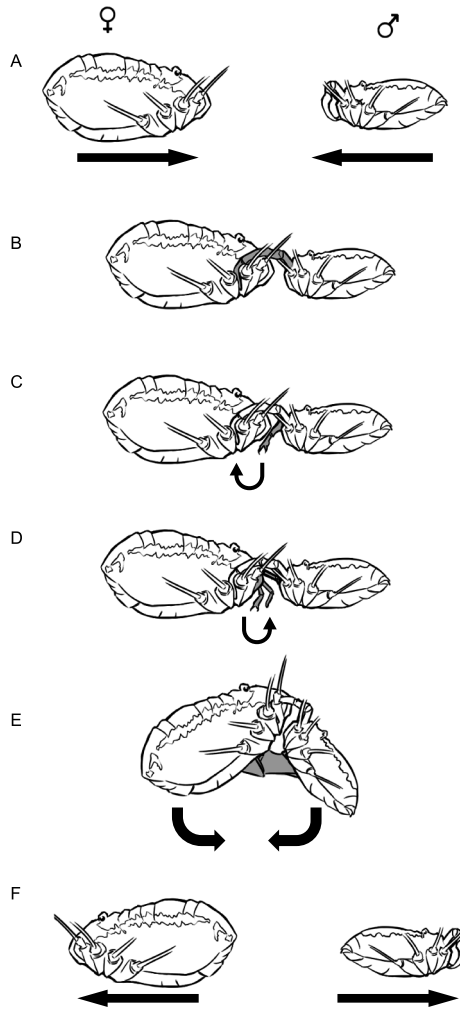
Figure 3. Visual summary of a typical mating encounter between male and female *L. vittatum*. (A) First contact occurs when males/females bump into a partner; (B) male coxal hooking using the pedipalps; (C) males extend their chelicerae; (D) females respond with pedipalpal tapping and cheliceral grasping; (E) intromission; (F) termination of the mating interaction.

2005; Willemart et al., 2006; Hebets et al., 2008). We then derived body size from a principal components analysis of weight and cephalothorax width for males and females separately. For both males and females, only the first principal component had an eigenvector greater than 1 (see Results), and so this first PC was used as a measure of body size in subsequent analyses. For

all males, we removed the right pedipalp, secured it in a stereotyped position, and photographed them as described above for cephalothorax width. We then measured the longest part of the femur of the pedipalp. We used a Pearson product–moment correlation to determine if male pedipalp length was correlated to male body size.

In order to determine if trial outcome varied with any morphological trait, we constructed a logistic regression with trial outcome as the dependent variable. The independent variables were male body size, female body size, and male pedipalp length. We additionally included interaction terms among all variables, but most were not significant and were removed from the final model. Pedipalp length relative to body size provided similar results as absolute pedipalp length, and so we used for the latter in all analyses.

2.5. *Morphology and stages of mating*

We then tested whether different morphological traits vary in their importance in mating interactions across different stages of assessment (2010 and 2013 trials). We partitioned mating interactions into the following components: (i) first contact to first mating attempt, (ii) first mating attempt to resolution of the attempt (either rejection of the male, or successful achievement of mating embrace), (iii) embrace to first intromission, (iv) first intromission to trial end, and (v) embrace to trial end (Figure 4). The first two components were measured for successful and unsuccessful trials; the last three components were relevant for only successful males (Figure 4).

For (i) contact to attempt and (ii) attempt to success/rejection, we constructed linear mixed models with log-transformed times as the dependent variables. The independent variables were trial outcome, morphological trait of interest (male pedipalp length, male size, female size, difference between
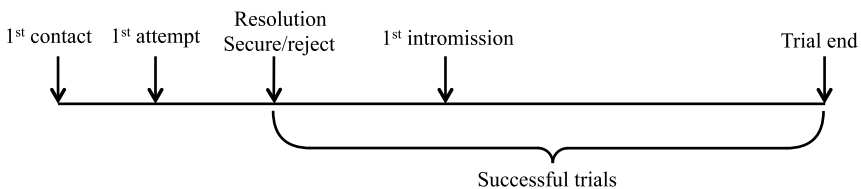
Figure 4. Timeline of a typical mating interaction between male and female *L. vitttatum* showing first contact, first male attempt, resolution, and for successful trials first intromission and termination of the interaction (trial end).

male size and female size), and the interaction between trial outcome and morphological trait. A significant interaction term indicates that the relationship between morphology and timing differs depending on whether the male was ultimately successful in securing the female in a mating embrace or not. We note that general lever mechanics dictate that because shorter pedipalps have a shorter lever arm, they would be capable of applying more force. It is also important to note that because the PC1 describing size for males was derived independently than PC1 describing size for females, the difference between male size (PC1) and female size (PC1) is in relative terms not absolute terms. However, larger positive numbers indicates the male is nearly equal or larger than the female in absolute terms (and larger negative numbers indicates he is smaller in absolute terms).

For (iii) embrace to first intromission, (iv) first intromission to trial end, and (v) embrace to trial end, we analysed the timing of each part in relation to male and female morphological traits as above for successful trials.

2.6. Statistical approach

All statistical analyses described above (see Sections 2.4 and 2.5) were conducted in JMP 7.0.1 (SAS Institute) using a significance level of alpha = 0.05. Year was not significant in any of the statistical analyses, and so was excluded from final models. All values are reported as means $\pm$ SE unless otherwise noted.

3. Results

3.1. Study organisms — field notes

Individuals were primarily found in the vegetation. Of the three mating pairs observed, two mated high in the vegetation (2 m off the ground), and one on the forest floor. In all cases, no other individuals were observed within two or more feet of the mating pair, and the female left the area upon termination of the mating interaction. The males remained in place and their legs were slightly curled, potentially from a lack of hydrostatic pressure.

3.2. Description of mating interactions

In general, the basic progression of behaviour matches that of previous qualitative descriptions (Machado & Macías-Ordóñez, 2007). We described a total of 14 major behaviours within our ethogram (Table 1) and the behavioural transition matrix identified 22 transitions between behaviours that

Table 1.
Ethogram providing descriptions of all observed behaviours that were conserved across all trials.

Behaviour	Category	Description
First contact	Pre-copulatory embrace	Physical contact between the male and female, often appears to be by chance.
Legs wrapping	Pre-intromission Throughout interaction	The male wraps the prehensile tarsi of his legs around the female's legs.
Coxal hooking	Pre-intromission Throughout interaction	The male hooks his pedipalps behind the female's legs II coxae, securing the pair in a face-to-face mating embrace.
Fleeing/struggling	Pre-copulatory embrace Pre-intromission resistance	Female flees males by moving in an opposing direction and/or male and female struggle against each other, as if either female or male were attempting to end the interaction pre-maturely.
Bobbing	Pre-intromission Resistance	The female bobs up and down violently, as if trying to remove the male from her.
Prying	Pre-copulatory embrace Pre-intromission resistance	The female forces her anterior end into the ground, as if trying to pry the male off of her or block access to her coxae.
Leg-tangling	Pre-copulatory embrace resistance	The female draws the femurs of her legs up vertically, blocking the male easy access to her coxae.
Cheliceral extensions	Pre-intromission	The male extends both chelicerae simultaneously, opening his pincers as they are extended furthest from his body. Occurs in series of repetitions.
Pedipalp tapping/ cheliceral grasping	Pre-intromission Intromission Post-intromission	The female taps the male's left and right sides with her pedipalps, and alternatively extends her chelicerae, opening her pincers as they are extended furthest from her body.
Male genital extension	Pre-intromission Intromission Post-intromission	The male opens his genital operculum and inflates his hematodocha, with the stylus-like penis being pushed forward.
Female contact with male genitalia	Pre-intromission Intromission Post-intromission	The female makes physical contact with the male's genitalia with either her pedipalps or her chelicerae.
Intromission	Intromission	The male inserts his penis into the female. This includes genital and oral insertions.

Table 1.
(Continued.)

Behaviour	Category	Description
Female pedipalpal/ cheliceral grooming	Pre-intromission Post-intromission	Grooming of female pedipalps and chelicerae by the mouthparts of a female.
Termination	Post-intromission	The male or female ends the mating interaction by breaking the embrace.

Behaviours were categorized as occurring during the pre-copulatory embrace struggle (before males hook female coxae), pre-intromission (after males hook female coxae but before first intromission), intromission, or post-intromission (as occurring after one intromission and before the next intromission or end of mating interaction), and female resistance (denying the male access to female coxae or to end a mating interaction).

occurred consistently across trials (Figures 4 and 5). Behaviour included in the matrix and diagram (identified with italics below) is described as follows.

3.2.1. Pre-copulatory embrace behaviour

Individuals made first contact by apparently bumping into each other at random, as there was no discernable observed effort by males to seek females, or vice versa. Typically within 10 s of contacting the females (69% of trials, $N = 16$), males made an initial attempt to mate by engaging the female in a face-to-face position and vigorously attempting to secure her in a mating embrace. These attempts involved leg wrapping, during which males wrapped the prehensile portion of their leg tarsi 1–3 times around a female's femurs or tibiae (Figure 2). Males most commonly attempted to wrap female legs II and legs III. Males then attempted coxal hooking by hooking the distal portion of their pedipalps around the female's legs II coxa (Figure 3B). After males succeeded in securing the female in a mating embrace, the pair would remain in face-to-face position for the remainder of the mating ritual. The point along the female leg at which males wrap varied across the duration of a trial, with his legs slipping to distal locations on the female's legs until they lose their grip/slip off the end of the female legs II during mating. When this occurred, they gently flick their released leg, and rewrap when the leg contacts the female leg II.

3.2.2. Female resistance to male attempts at the mating embrace

During first attempts by the male, some females physically resisted males (56%, $N = 16$), while other females offered no resistance (44%, $N = 16$).
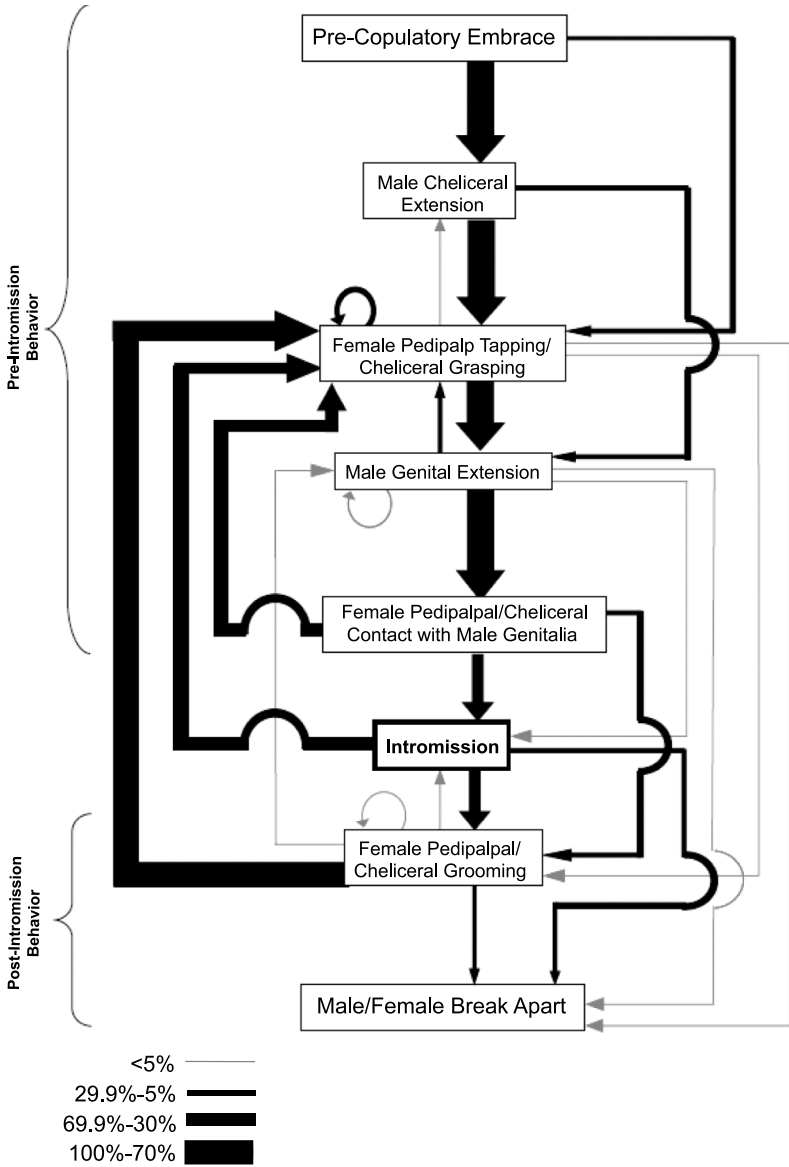
Figure 5. Flowchart of the behavioural transitions during a mating interaction between male and female *L. vittatum.* Arrowheads point towards stop state behaviours, and flow from start state behaviours. In some instances, the pair would pause for a period of >10 s, then resume the same behaviour, indicated by looping arrows. The thickness of lines refers to the percentage of the total number of the start state behaviour that resulted in the stop state behaviour the arrow points towards.

Of males that experienced female resistance, a little over half were capable of subduing females and successfully mating (56% trials in which a female resisted = 9). When males did not experience female resistance, they were more likely to be successful in copulating with females (71%, trials in which female did not resist = 7). Those that were unsuccessful despite a lack of female resistance did not appear to attempt to secure the female. Males were unable to overcome female resistance during their first attempt and were unsuccessful in any additional attempts (between 1–5 additional attempts) with only one exception across all trials.

Females resistance takes four forms: (1) Females struggle and flee by actively struggling against male legs wrapping and coxal hooking attempts and then darting away from the male (65% of 37 occurrences of resistance across trials); (2) Females may pry their coxae from the males hooked pedipalps by forcing the anterior end of her body into the ground (22% of 37 occurrences of resistance across trials); (3) If the male pedipalpal hooking is loosened or he only has one pedipalp hooked to her coxae, a female can block access to her coxae by drawing her femurs vertically (11% of 37 occurrences of resistance across trials); finally, (4) Females can bob up and down violently as if trying to throw the male off during coxal hooking (2.7% of 37 occurrences of resistance across trials).

3.2.3. Pre-intromission behaviour

During the pre-intromission period, a pair engaged in a mating embrace is often very mobile within the mating arena. Of the 11 males that eventually copulated during a trial, 82% engaged in cheliceral extension after securing the female in a mating embrace. A male extends his two chelicerae synchronously and opens the distal pincers as the tips of the chelicerae near the female's body. Cheliceral extensions occur in bouts of 1–13 individual extensions, and males display 1–4 bouts over the course of the mating interaction.

Bouts of male cheliceral extensions most often ended when females responded by pedipalpal tapping and cheliceral grasping, though in some rare but notable cases males immediately extended their penis before females exhibited this behaviour. Female pedipalpal tapping and cheliceral grasping involved a female tapping the male with her pedipalps and rapidly alternately extending her left and right chelicerae towards the male, appearing as though she were grasping. Similar to male cheliceral extension, the female opens the distal pincers of her chelicerae when they are extended farthest from her body and closest to the male's body.

3.2.4. Intromission

During female pedipalpal tapping and cheliceral extension we observed male genital extension, where a male opens his genital operculum and inflates the hematodocha. A male extends his penis 5–132 times (mean = 33.6 ± 37.6 times) during a mating interaction. The first genital extension in a trial never resulted in successful insertion into the female genital opening, the first insertion occurred during male genital extensions 2–14 (mean = 5.9 ± 3.9 times; successful trials, $N = 10$).

Following eversion, females contact the male genitalia by touching the hematodocha with her pedipalps or chelicerae. As the female contacts the penis, she ceases pedipalpal tapping and cheliceral grasping, and places both her pedipalps on the distal portion of the male's hematodocha, just behind two protrusions that adorn the right and left sides. During intromission, males and females bend their bodies such that their ventral sides are facing closer together to facilitate genital insertion ($<180°$; Figure 3), a typical position for many mating harvestmen (Machado & Macías-Ordóñez, 2007). We recorded two instances during intromission where a droplet had formed on the pair near the male genitalia, though we were unable to determine the source of the liquid. Because the female's oral cavity is located in close proximity to her genital opening, it was impossible to determine if insertions of the male penis were oral or genital. Oral insertion in leiobunine harvestmen was originally described in Shultz (2005), and is likely associated with the transfer of a nuptial gift (Macías-Ordóñez et al., 2010; Burns et al., 2013). Insertions normally lasted anywhere from roughly 3 s to approx. 180 s (mean = 19.2 ± 40.3 s; total insertions, $N = 139$).

3.2.5. Post-intromission behaviour

Following intromission and/or contact with the male hematodocha, a female typically engaged in cheliceral and pedipalpal grooming. This behaviour was characterized by her rubbing the chelicerae and pedipalps against her labrum. Immediately following cheliceral and pedipalpal grooming, females most frequently returned to bouts of pedipalpal tapping and cheliceral grasping, with male genital extensions and insertions occurring in between. Males may occasionally return to bouts of cheliceral extension during periods when the female is quiescent. Thus the pair can loop through the same progression of behaviours many times, resulting in multiple intromissions over the course of the mating period (Figure 5). Due to this looping effect mating interactions can vary greatly in length between trials (from 10 to 80 min).

At the conclusion of a mating interaction, the male dislodged his pedipalps from the female's coxa II, resulting in termination of the mating interaction. It is difficult to discern whether termination is male- or female-driven: in some of the trials where pairs did not move as quickly, the male separated from the female by pulling himself upwards, while the female remained still ($N = 4$). In another trial, the female bent downwards and pulled her body away from down while the male remained still ($N = 1$). Separation also appeared to sometimes result from female struggles similar to those in the pre-embrace stage ($N = 3$). Two additional separations occurred off-camera and could not be analysed.

3.3. Morphological determinants of mating success

On average, females were heavier than males and had wider cephalothoraxes (Table 2). For males, 91.5% of the total variation in weight and cephalothorax width was explained by the first principal component (eigenvector $= 1.8$), and pedipalp femur length did not correlate with body size ($r = 0.21$, $p = 0.33$). In females, 62.7% of variation in body size was explained by the first principal component (eigenvector 1.25). Males that successfully achieved the mating embrace had shorter pedipalps than unsuccessful males (Table 3; Figure 6). Success also varied in response to an interaction between male size and female size (Table 3).

3.4. Morphology and timing of stages of mate assessment

The time to the resolution of the first stage of a mating interaction, from first contact to either a successful mating embrace or rejection of the male, averaged 95 ± 24 s. Time to resolution did not differ between unsuccessful versus successful trials (successful mean time $= 90 \pm 32$ s, $N = 17$; unsuccessful mean time $= 103 \pm 39$ s, $N = 12$; $F_{1,27} = 0.07$, $p = 0.81$).

Unsuccessful males more quickly attempted to mate when the female was larger; successful males did not change the time to their first attempt in

Table 2.
Cephalothorax width and body weight of male and female *L. vittatum* used in mating trials.

| Morphological trait	Males	Females
Weight (mg)	4.4 ± 0.1 ($N = 29$)	7.6 ± 0.3 ($N = 29$)
Cephalothorax width (mm)	2.96 ± 0.02 ($N = 25$)	3.09 ± 0.02 ($N = 26$)
Pedipalp femur length (mm)	1.94 ± 0.03 ($N = 24$)	

Table 3.
Morphological determinants of the outcome of male–female mating interactions in *L. vittatum*.

Morphological factor	df	χ^2	p
Male pedipalp femur (mm)	1	15.9	<0.0001*
Male size (PC1)	1	2.1	0.1506
Female size (PC1)	1	3.3	0.0711
Male size (PC1) × female size (PC1)	1	11.4	0.0007*

   * Significant ($p < 0.05$).

response to female size (Table 4). No other morphological factors determined the timing of first contact to first attempt ($p > 0.1$ for all analyses).

   Unsuccessful males that were larger compared to females were able to delay rejection longer (Table 5; Figure 7). Successful males that were larger compared to females more quickly achieved a mating embrace (Table 5; Figure 7). For successful trials, no morphological traits predicted the timing to progress to any further stage after the embrace was achieved, or the total trial length ($p > 0.1$ for all analyses).
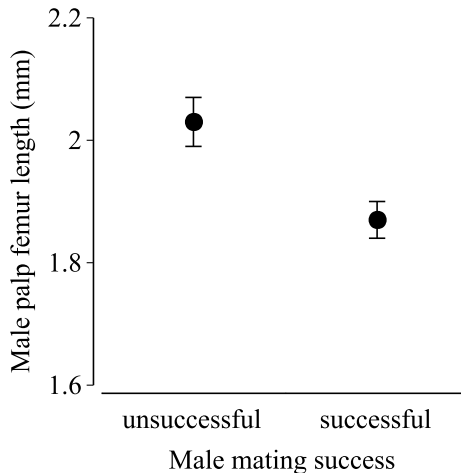
Figure 6. Difference in the size of male pedipalpal femur length of unsuccessful (male rejected) versus successful (male secures the mating embrace) mating trials between male–female pairs of *L. vittatum*. Shorter pedipalps provide greater mechanical advantage as per general lever mechanics ($F_i L_i = F_o L_o$).

Table 4.
Time elapsed between the first contact between a male and female *L. vittatum* to the male's first mating attempt as a function of whether the male was ultimately successful and variation in female size.

| Morphological factor	df	F	p
Whole model	3, 22	4.3	0.0156*
Trial outcome	1	4.6	0.0430*
Female size (PC1)	1	0.6	0.4500
Trial outcome × female size (PC1)	1	7.1	0.0141*

* Significant ($p < 0.05$).

4. Discussion

Mating interactions in *L. vittatum* occur through a complex but stereotyped series of stages. One of the most distinct characteristics of mating is the maintenance of physical contact throughout an interaction achieved by the male hooking his pedipalps behind the coxae of the female legs II, and wrapping his legs III around her legs II. The dynamics of each stage are qualitatively distinct, starting with pre-mating struggles and progressing to solicitous, tactile engagement between males and females. Each stage also influenced by a different set of morphological traits — male size relative to female size influenced the duration of the precopulatory struggle, the length of male pedipalps influenced the likelihood of the male successfully achieving the mating embrace, and none of the morphological traits measured influenced the peri- and post-copulatory stages of mating. We suggest that the influence of different morphological traits on mating dynamics at

Table 5.
Time elapsed between a *L. vittatum* male's first attempt to secure a female in a mating embrace and the resolution of that attempt according to the difference in male and female size, as well as whether the male was accepted or rejected (trial outcome).

Morphological factor	df	F	p
Whole model	3, 18	4.2	0.0211*
Trial outcome	1	0.005	0.9430*
Male size (PC1) − female size (PC1)	1	1	0.3278
Trial outcome × male size (PC1) − female size (PC1)	1	9.3	0.0070*

* Significant ($p < 0.05$).
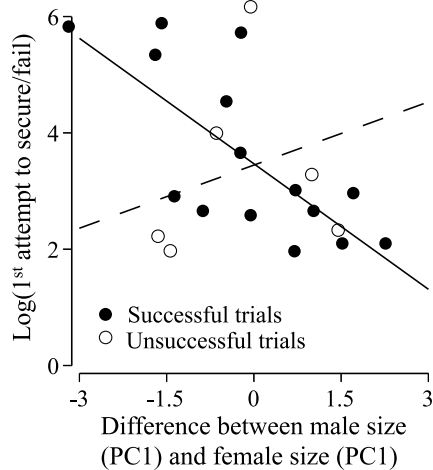
Figure 7. Length of the premating struggle between male and female pairs of *L. vittatum* in relation to the difference in the PC1 describing male size and the PC1 describing female size. Filled circles with solid lines represent the relationship between the length of a struggle in successful trials (time from first attempt to securing a female), and open circles with dashed lines represent the length of a struggle for unsuccessful trials (time from first attempt to rejection by a female).

each stage during an interaction suggests the operation of multiple sources of selection in shaping mating dynamics in *L. vittatum*.

Physical struggles and female resistance ensue upon first contact of a male and female, as would be predicted if females had little time to assess males and mating was costly (Alexander et al., 1997). Larger males appear to have more control over the struggle: they secured females more quickly in successful trials, and delayed rejection in unsuccessful trials. Because coxal hooking with the pedipalps depends upon a specific orientation of the male relative to the female, a size advantage may allow males to achieve this positioning even when females are actively resisting. However, the final outcome of the struggle depended on the length of the male pedipalp: those with shorter pedipalps (and therefore greater mechanical advantage) were more successful in securing the female in a mating embrace. The question of why not all males have short pedipalps is an interesting one that could be addressed by examining the function of these appendages in other contexts. For example, male–male competition has been important in shaping sexually-dimorphic appendages in a neotropical harvestman (Willemart et al., 2009).

Male *L. vittatum* possess pedipalps specialized for clasping — the evolution of these clasping pedipalps in the leiobunine clade is associated with a suite of morphological and behavioural traits consistent with a conflict-based mating syndrome (Burns et al., 2013). Males use these structures to secure the female in a mating embrace that may serve a similar function as physical coupling that restricts female mobility in other taxa like scorpionflies (Zhong & Hua, 2013), and water striders (Rowe et al., 2006). This embrace could function to: (i) restrict the female's use of her legs II, which are primarily used as sensory organs, (ii) maintain contact with a female during struggles as legs II are the longest and appear to be the hardest to extract from a male's grip (Fowler-Finn, personal observation), and (iii) allow a male to stabilize himself to gain more control over mating.

Measures of the costs and benefits of premating struggles can help determine if they represent tests of male strength under a model of mate choice, or female resistance to mating under a model of intersexual conflict (Arnqvist, 1992; Chapman et al., 2003; Cordero & Eberhard, 2003; Eberhard & Cordero, 2003; Kokko et al., 2003; Brennan & Prum, 2012). However, male persistence with unwilling females under either scenario can result in several costs for females. These include higher risk of predation (Magnhagen, 1991), negative effects on foraging (Magurran & Seghers, 1994; Rowe et al., 1994), and energy expenditure (Watson et al., 1998). One population of *L. vittatum* in Pennsylvania displays resource-defence polygyny and mate-guarding (Macías-Ordóñez, 1997, 2000; Machado & Macías-Ordóñez, 2007). In this population, females are often forced to abandon oviposition sites (a limiting resource) when resisting unwanted mating attempts (Macías-Ordóñez, 1997). Consistent with descriptions of a population in Michigan (Edgar, 1971), we also observed females in the field fleeing the site of mating after termination, but did not find that mating occurred near suitable oviposition sites.

After the mating embrace is achieved, the interaction proceeds to a pre-intromission stage during which females tap and grasp at the male genitalia with their pedipalps and chelicerae. These bouts are often followed by male penile extension, suggesting either a stimulatory role of tapping and/or male mate assessment. It would not be surprising if male mate assessment may occur in this species: males invest in the production of nuptial gifts and mating interactions can be quite lengthy (Machado & Macías-Ordóñez, 2007), both

predictors of male mate choice in insects (Bonduriansky, 2001). Further-more, mutual mate choice has been suggested in other species of harvestmen (Mora, 1990; Requena & Machado, 2014). Intromission requires the male and female to bend at an angle such that their ventral sides face towards each other. Being larger, females likely have some control over this angle and therefore male access to the genital opercula, as well as the length of copu-lation (also suggested to be the case in a Neotropical harvestman: Requena & Machado, 2014). Females also directly contact the male genitalia with her pedipalps and chelicerae — particularly towards the base of the hematadocal sac as he inflates it during genital extensions — giving the appearance of soliciting or guiding males in genital insertion (Edgar, 1971). None of the traits that we measured influenced the timing of this stage. However, mating in leobunine harvestmen involves the exchange of a nuptial gift produced by specialized glands at the base of the male penis (Burns et al., 2012, 2013). It is likely copulation is mediated at least to some degree by the exchange of a nuptial gift, as seen in other arachnids (Stålhandske, 2001; Costa-Schmidt et al., 2008).

In general, nuptial gifts can function as mating investment or paternal in-vestment for males (Thornhill, 1976; Parker & Simmons, 1989; Simmons, 1990). Given the prevalence of touching and tasting that we observed, and the highly tactile and chemical nature of harvestmen in general (Pinto-da-Rocha et al., 2007), we suggest these exchanges may be important in determin-ing dynamics at these stages. The direct benefits nuptial gifts can provide for females (reviewed in Gwynne, 2008) could explain why some trials in-volved lengthy interactions and multiple intromissions (also described by Machado & Macías-Ordóñez, 2007), and add incentive for some females to accept mating advances without resistance. Future work into nuptial gifts and other traits that are targeted by selection at the peri- and post-copulatory stages will be important, as female *L. vittatum* mate multiply (Macías-Ordóñez, 1997; Fowler-Finn, personal observation), and post-copulatory se-lection likely plays an important role in dictating male fitness.

Finally, termination occurs when either the female or male attempts to bend their body away from the other to release the male pedipalps from the female legs II coxae. In some cases, males appeared to be struggling against the female to end copulation. While males may exploit females with gifts by providing false advertisement of nuptial gift quality or quantity (Gwynne, 2008), we suggest that in *L. vittatum*, male struggles to end copulation may

indicate female exploitation for further gift-giving even when the males no longer gain additional benefits.

A shift from initial premating struggles to later to tactile exchanges between males and females during mating indicate the simultaneous influence of multiple sources of selection on mating in *L. vittatum*. We suggest that both intersexual conflict and mate choice operate, and that the simultaneous operation of both sources of selection could be more common than previously considered (reviewed in Brennan & Prum, 2012). This line of questioning warrants further investigation in a range of taxa, particularly because both intersexual conflict and mate choice are important factors influencing the evolution of male and female reproductive traits (Kirkpatrick, 1987; Holland & Rice, 1998; Kokko et al., 2002, 2006) and diversification (Arnqvist et al., 2000; Kraaijeveld et al., 2011). We posit that *L. vittatum* provides an excellent system to begin to tease apart the roles of intersexual conflict and mate choice in shaping mating dynamics to further our understanding of the evolution and ecology of mating systems.

Acknowledgements

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