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Courtship performance as function of body condition in an 'ancient' form of sperm transfer

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Abstract

In most animal species, body condition has a fundamental role in fitness. In males, sexual selection generally favors larger body size or greater weight. This may result in males with better condition performing more vigorous courtships, and biasing female preferences. The effects of body condition on mating performance have been extensively studied in different animal groups. Among arachnids, scorpions are an interesting group for evaluating the effects of these sexual traits on mating performance, since they exhibit an ancient mode of indirect sperm transfer. Scorpion males deposit a single spermatophore on the soil to transfer the sperm to the females, and therefore, the production of spermatophores involves a high cost for them. In this study, we use the scorpion *Bothriurus bonariensis* as a model to evaluate different patterns of sexual behavior as a function of the body condition of both males and females. We found that males with a better body condition performed the mating dance stage more quickly than males with a lower condition. In addition, males performed the sexual sting behavior for a longer time with females in a better condition. Our results suggest that a better condition provides a mating advantage to males and represents an indicator of courtship performance. Given that female quality is usually correlated with fecundity, males mating with females with a better body condition probably have higher reproductive success.

Keywords

Body condition; mating; scorpions; sexual selection

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Introduction

According to sexual selection theory, the principal processes by which sexual traits evolve are male–male competition and mate selection, i.e., individuals of one sex actively choosing individuals of the opposite sex (Miller & Svensson, 2014; West-Eberhard, 2014). These processes affect the mating success of males and females by the selection of particular sexual traits (Benelli et al., 2016). A greater mating success could be the result of many characteristics, including body condition or size, mating performance, competitive ability, and the benefits provided directly or indirectly to females. In males, sexual selection generally favors larger body size, greater weight or better body condition (Andersson, 1994). This may result in males with a better quality being more vigorous in courting, thus biasing female preferences (Thornhill & Alcock, 1983; Blaul & Ruther, 2012). For males, courtship and mating represent the major energy costs of reproduction (Wedell et al., 2002; Kotiaho & Simmons, 2003) and it is expected that they indirectly provide the female with information about their own condition (Rek, 2012). The body condition is usually highly related to the individual's size and weight. Individuals in higher condition can better face this reproductive effort than smaller and lighter individuals by increasing their energy expenditure (Kasumovic et al., 2009; Muller et al., 2016). However, males may also increase their fitness by selectively allocating their mating efforts, either to energy use during courtship or the production of sperm (Bonduriansky, 2001; Wedell et al., 2002).

The effects of body condition on mating performance and success have been extensively studied in different animal groups. In species of damselflies, for example, the male condition is important in the male's ability to win territorial contests, and possibly influences the male mating success (Marden & Waage, 1990). There are studies that suggest that fecundity is positively related with the body condition (Cotton et al., 2006). In mantid species, cannibalistic females improve their body condition and subsequently increase their fecundity, producing heavier egg cases (Barry et al., 2008). Among arachnids, wolf spiders in the genus *Schizocosa* Chamberlin, 1904 show elaborate sexual traits, often associated with noticeable courtship movements (Stratton, 2005; Framenau & Hebets, 2007). Males with a low condition mate for a longer time, but the female's choice in these species favors males with better quality even when these males present shorter copulations (Rypstra et al., 2003; Persons & Uetz, 2005). In this context, scorpions are a fascinating group for evaluating the effects of sexual traits on mating performance, since they exhibit an interesting mode of paired-indirect sperm transfer (Polis & Sissom, 1990). In this system, males transfer the sperm to the female trough a chitinous spermatophore deposited on the soil (Francke, 1979). Therefore, the production of spermatophores involves a cost for them (Proctor, 1992; Zia et al., 2000).

Mating in scorpions consist of a complex and ritualized courtship followed by a sperm transfer phase. Scorpions have been considered one of the most basal groups of arachnids and they show an ancient mode of paired-indirect sperm transfer (Weygoldt & Paulus, 1979; Polis & Sissom, 1990; Proctor, 1998; Benton, 2001; Peretti,

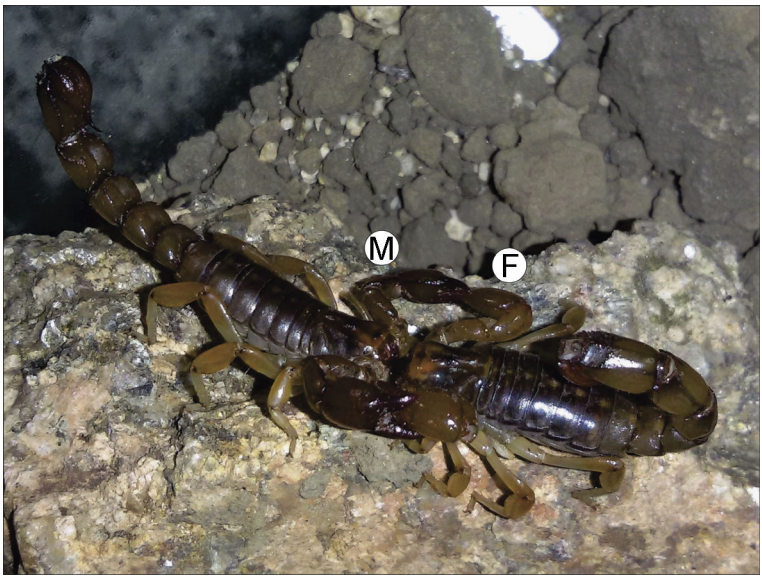

Figure 1. Mating pair of *Bothriurus bonariensis* during the mating dance in the courship. Abbreviations: F, female; M, male.

2003; Regier et al., 2010; but see Sharma et al., 2014, 2015). Although most of the studies have evaluated courtship in scorpions from the female choice perspective (Polis & Sissom, 1990; Nobile & Johns, 2005; Peretti & Carrera, 2005), we cannot rule out the existence of mutual choice. Classically, the male grasps the female with his pedipalps and begins the typical mating dance or *promenade à deux* (fig. 1). During courtship, males perform a series of behavioral patterns (e.g., kissing, rubbing with legs, rubbing with telson, sexual sting, etc.) to increase the female's receptivity and reach sperm transfer (Peretti, 1997; Peretti & Carrera, 2005; Olivero et al., 2015), in which the male supports its metasoma on the substrate and pushes the female back. The spermatophore bends slightly under the female's weight, allowing sperm to enter the female's reproductive tract (Polis & Sissom, 1990; Benton, 2001). Interruptions of mating are not rare in scorpions. In some cases, males fail to hold the female's chelae during courtship and therefore the couple separates. Also, there are cases where the substrate is not appropriate for spermatophore deposition or the aperture mechanism fails, causing sperm transfer not to occur (Peretti, 2000). There is evidence indicating that some body traits are correlated with mating success in some scorpion's species. For example, larger individuals are better at facing the risk of predation or male–male competition for obtaining territory (McCormick & Polis, 1990; Myers, 2001). In some scorpions, males with larger chelae are more successful in mating (Benton, 1991). Also, studies in *Centruroides vittatus* (Say, 1821) have demonstrated that larger males perform the mating dance in less time than smaller males. However, larger males spent more time on the kissing behavior, during which sperm transfer occurs (Nobile & Johns, 2005).

Evidence in other taxa indicates that body weight or size are important predictors of the male's mating success (Proulx et al., 2002; De Luca, 2015). However, little is known about the effect of body condition on mating success in scorpions. It is currently unknown whether the male's body condition is an important trait for female choice, or if the duration of courtship or any behaviors are influenced by the condition of males or females. In this study, we analyze these issues using the bothriurid scorpion *Bothriurus bonariensis* (C.L. Koch, 1842) as a model species. Our aim is to examine the duration of courtship and behaviors that occur before sperm transfer (sexual sting, rubbing with telson, etc.) as a function of male and female body size, weight and condition. We expect that males of *B. bonariensis* with better body condition should allocate more energy to mating functions, performing more vigorous courtship behaviors and achieving greater receptivity in females. Second, the female's mate preferences could be costlier since they reject a great proportion of male's encounters or they need to sample more males before mating. Therefore, we expect that females in better body condition are more demanding for the same cost of the mate preference than females of poorer body condition (Cotton et al., 2006).

Material and methods

Collection and rearing

We collected adult males and females of *B. bonariensis* in Central Argentina (Mendiolaza, Córdoba; 31°15′42.1″ S, 64°16′51.0″ W; 563 m a.s.l.) from November to December in 2009–2011. The individuals were collected at the beginning of the reproductive season to ensure their sexual receptivity. Afterward, we selected virgin females in the laboratory, verifying the absence of a genital plug within the genital atrium (Mattoni & Peretti, 2004; Olivero, 2014). Specimens were kept individually in plastic containers (8 cm high × 10 cm diameter) and were fed with mealworm larvae of *Tenebrio* sp. once a week. Each container included one moistened cotton ball to provide humidity. The temperature varied from 28 to 32°C during the reproductive period. We kept all the animals under an inverted 12:12 h light:dark cycle.

Behavioral analyses

Randomly selected males ($n = 13$) and virgin females ($n = 13$) were exposed to perform courtship and sperm transfer. Each individual was used only once for testing. The couple was placed in a mating arena (W × L × H, 18 × 30 × 30 cm) with soil as substrate, and stones and pieces of tree bark from the capture site. We observed 13 successful mating trials (i.e., complete mating sequence: the sperm transfer was completed, and the female was inseminated) during three mating seasons. We recorded the mating sequences and the behavior associated (reorientation, mating dance, pull and approximation, rubbing with telson and sexual sting – Peretti,

1993; Olivero et al., 2017) with a digital video camera (SONY DCR-TRV 351) equipped with +4 × x close-up lenses and night shot function, because the matings occurred in darkness. The observations were analyzed with the JWatcher 1.0 software (Blumstein et al., 2000). Before exposure, each specimen was weighted with a digital balance (Pioneer PA114, Ohaus, Parsippany, NJ, USA). After exposure, the body size of males and females was taken by measurements of carapace length. We used a stereomicroscope with an ocular micrometer (SMZ1500, Nikon, Tokyo, Japan), equipped with a digital camera (Nikon Sight DS-Fi1). Digital pictures were analyzed with the ImageJ measuring software (Schneider et al., 2012).

Statistical analyses

We performed statistical analyses with R v.3.3.2 (R Core Team, 2017). Normality and homogeneity of variance assumptions were assessed graphically and analytically. The explanatory variables used as indicators of quality of individuals were body size, body weight and body condition of males and females. The body condition was obtained through the Residuals index (R_i), which uses the residuals from an ordinary least squares regression of body weight against one or more length measurements (Jakob et al., 1996; Schulte-Hostedde et al., 2001, 2005; Ardia, 2005), in our case the carapace length. The response variables were the duration of courtship (from the moment when the male grasps the female with his pedipalps until spermatophore deposition) and duration of the behavioral patterns (reorientation, mating dance, pull and approximation, sexual sting and rubbing with telson, see table 1). We correlated the size, weight and body condition using a Pearson correlation to assess the collinearity among the covariates. We used model selection with the likelihood ratio test (LRT) to compare the goodness of fit of the possible statistical models (Johnson & Omland, 2004).

Results

The carapace length and body weight were collinear variables for both males and females. According to the model selection, the variable that better explained the response variables was body condition, and therefore we limited our analysis to the effects of body condition on courtship behavior to conserve statistical power. Males weighed an average of 0.58 ± 0.08 g the day of the mating trial, and females averaged 0.63 ± 0.14 g. The average of carapace length (body size) was 5.49 ± 0.30 mm for males and 5.27 ± 0.47 mm for females. During the courtship stage, all the analyzed couples performed the behavioral units described for the species (table 1). The duration of courtship averaged 40.61 ± 21.01 min (fig. 2). There was no relationship between the body condition of males or females and the duration of courtship. Regarding behavioral units, males with a better condition performed the mating dance in less time that males with low condition (fig. 3, table 2). Also,

Table 1.
Behavioral patterns of bothriurid scorpions.

Behavioral unit	Description	Executed by
Grasping	The male grasps any part of the female's body with his pedipalps.	Male
Reorientation	With the female grasped, the male rotates his body until they both stand facing the chelicera, in a position ready to begin mating.	Male and female
Pedipalp grip	The male grasps the female's pedipalps with his pedipalps.	Male and female
Mating dance	The motile stage of courtship, during which the male grasps the female's pedipalps and then leads her as the pair moves together.	Male and female
Pull and approximation	The male pulls slightly at the female and approaches her, reaching her mouth region. Then he moves away and repeats the sequence.	Male and female
Rubbing with telson	The male spreads the chemical product of the telson gland, rubbing the dorsal side of the telson on the female body.	Male
Sexual sting	The male uses its sting to puncture the female's body, usually at the mesosome pleura.	Male
Kissing	The couple draws together and touch chelicerae as the spermatophore enters the female's genital opening and releases its contents.	Male and female
Pull	The male pulls the female and forces her to walk when she is not willing to move.	Male

Description of main behavioral patterns of the courtship of bothriurid scorpions. The terms and definitions are based on scorpion literature for the Bothriuridae family (see Peretti, 1993, 1995; Peretti et al., 2000; Carrera et al., 2009).

we found a positive relationship between the female body condition and the sexual sting behavior (table 2, supplementary video S1). There was no relationship between other courtship behaviors and the body condition of males or females.

Discussion

The results showed that, although the body condition of males or females did not influence the total duration of courtship, it influenced the duration of some courtship behaviors. Female preference for males with better quality has been documented in many animal groups, including insects and arachnids (Markow & Ricker, 1991; Pitnick & Markow, 1994; Benton, 2001). Studies suggest that body condition is positively related to mating success (e.g., Uetz et al., 2002 in spiders; Aluja et al., 2008 in tephritids). Also, it has been demonstrated that females are expected to choose attractive males to obtain more attractive offspring (Wilcockson et al., 1995; Kotiaho et al., 1996). It is probable that there is a signal exchange between male and female during the courtship, and males can intensify their behavioral vigor

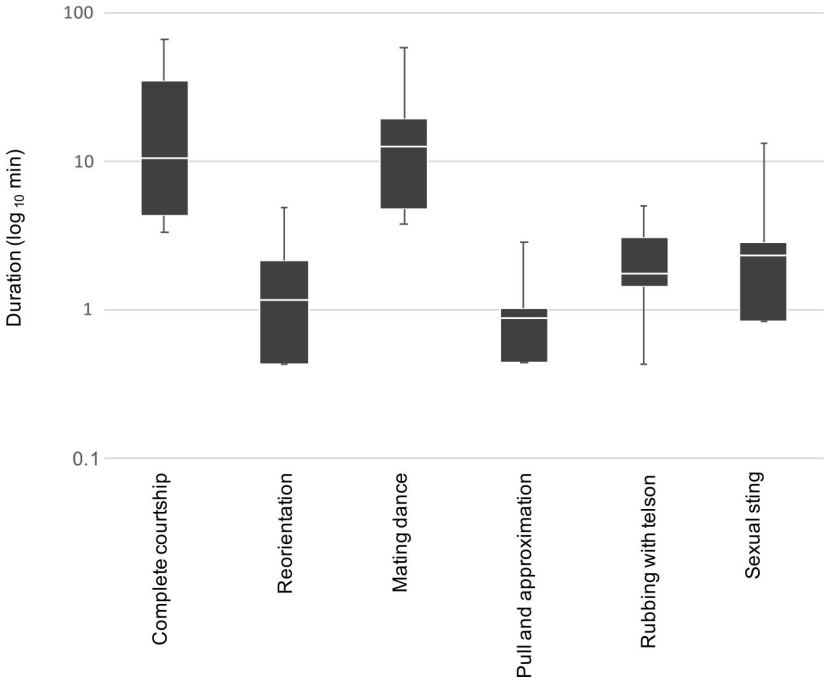

Figure 2. Box plot of duration (min.) of complete courtship and behavioral patterns of *Bothriurus bonariensis*.

upon receiving information about female receptivity (Johnstone, 1997). Scorpions, for example, have a complex sensory system in which the mechanical signals are dominant (Root, 1990). There is also evidence for chemical sensitivity (Gaffin & Brownell, 1992, 2001; Melville et al., 2003; Olivero et al., 2015). The close body interaction between the sexes during courtship could give information about the individual's quality and be an important feature in mate choice.

We found that males of *B. bonariensis* with better body condition performed faster mating dances than males with a poor condition. This can be the result of two possibilities. On the one hand, males with a poor condition may have a shorter lifetime and fewer opportunities to locate additional females to mate because they have a lower ability to escape from predators (Kotiaho et al., 1999; Hu & Morse, 2004; Morse & Hu, 2004). Adult males of scorpions are often vagrant during the mating season, and this characteristic predisposes them to a higher incidence of predation or starvation (Polis & Farley, 1979). For this reason, the low chance of poor condition males to mate with an additional female may result in higher investment and a longer duration of the mating dance with the current female (Wilder & Rypstra, 2007). In this way, males with a poor condition could ensure the mating by allocating more energy to the current courtship and maintaining the female's receptivity to reach sperm transfer. In addition, females could be more attracted and show more willingness to mate with better quality males. Mating tests performed on pairs of

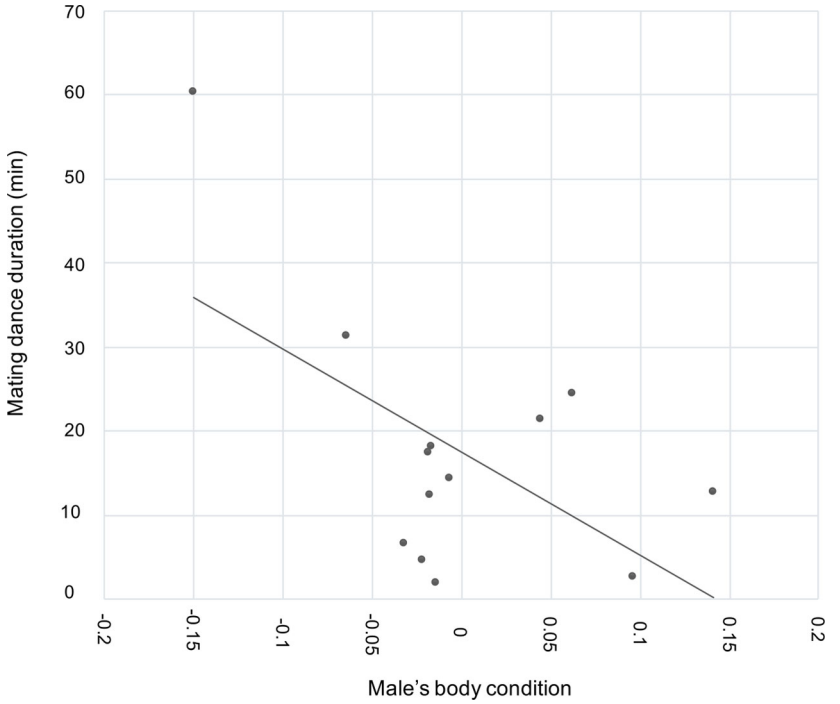

Figure 3. Relationship between mating dance duration (min.) and the body condition of males of *Bothriurus bonariensis*. See table 2 for statistics.

Table 2.
Summary of linear models results.

Unit	Estimate	Adjusted R^2	T value	P value
Courtship	−133.667	0.145	−1.744	0.109
Reorientation	−9.948	0.037	8.324	0.260
Mating dance	−122.259	0.272	−2.341	0.039*
Pull and approximation	0.843	−0.092	0.266	0.796
Rubbing with telson	−2.496	−0.073	−0.428	0.677
Sexual sting	−5.894	−0.073	−0.431	0.675
Courtship	−9.705	−0.084	−0.271	0.791
Reorientation	−3.410	−0.004	−0.975	0.352
Mating dance	−29.895	0.035	−1.200	0.255
Pull and approximation	1.177	−0.013	0.923	0.378
Rubbing with telson	2.174	−0.012	0.925	0.375
Sexual sting	10.945	0.272	2.344	0.038*

Linear models of the duration of courtship and behavioral patterns as a function of the body condition of males and females of *Bothriurus bonariensis*. *, $P < 0.05$.

this species indicate, for example, that males in high condition show less latency to begin the courtship than males in low condition (Olivero, 2014). Furthermore, previous studies have demonstrated that larger males of *B. bonariensis* show better sexual performance and transfer a higher ejaculate volume in the spermatophore (Vrech, Oviedo-Diego, Olivero & Peretti, unpublished), which could indicate that these males display a good quality. Hence, males of this species with a low body condition should probably court females for a longer time to achieve sperm transfer.

On the other hand, in other scorpion species, it has been shown that large males may be better equipped to push and pull females during the mating dance, achieving a shorter duration of this mating stage (Nobile & Johns, 2005). In the present study, the male body condition of *B. bonariensis* may influence the ease and speed with which males can carry the female during the mating dance until he finds a proper place for spermatophore deposition. However, females of *B. bonariensis* are generally larger than males, and therefore the duration of this behavior probably also depends on the female's willingness to mate. As seen in other scorpion species, females may be assessing the male size, and larger males may spend less time during the mating dance because females are more receptive to them (Nobile & Johns, 2005). In *B. bonariensis*, faster mating dances would indicate greater female receptivity to males with better body condition.

The female body condition was positively related to the duration of the sexual sting. The mate choice theory predicts that males prefer females with high condition, because female body size or condition is usually correlated with fecundity (Bonduriansky, 2001; Dosen & Montgomerie, 2004; Barry et al., 2010; Kahn et al., 2013; Jones et al., 2014). Therefore, sexual selection would favor male mate choice if they mate with higher quality females because they have higher fecundity, and this could mean greater reproductive success for these males (Bonduriansky, 2001).

During the sexual sting behavior, the male inserts the aculeus almost completely into the female mesosome pleura. This sexual behavior can last from 1 to 10 minutes or more in *B. bonariensis* and the female remains motionless with no apparent resistance (Peretti, 1993; Olivero, 2014). The function of this sexual behavior is still unknown and currently discussed (Francke, 1979; Toscano-Gadea, 2010). It has been suggested that the sexual sting might appease the female during courtship and diminish the chances of mating interruption (Polis & Sissom, 1990; Peretti, 2013). However, whether the injection of venom components during the stinging really occurs is a key topic that remains unclear (Peretti, 2013). Inceoglu et al. (2003) suggested that, in the buthid scorpion *Parabuthus transvalicus* (Purcell, 1899), males could inoculate a prevenom (usually used for immobilizing the prey) to "drug" the female during the sexual sting and reduce her aggressiveness. Nevertheless, sexual sting behavior has never been observed in buthid scorpions (Polis, 1990) and appears to be absent during mating of *P. transvalicus* (https://www.youtube.com/watch?v=NppNKeH8VaM; https://www.youtube.com/watch?v=gPC_NJYF-4o; https://www.youtube.com/watch?v=H5mgFnvwIDw). In addition, previous studies in bothriurid scorpions showed that the male just places

the stinger on the female's body without piercing the cuticle (Carrera, 2008; Toscano-Gadea, 2010). Coercive mating behaviors are usually absent in scorpion species (Benton, 1992, 1993, 2001; Peretti, 1997, 2001, 2003). Instead, males increase their stimulatory behaviors, adopting different behavioral sequences according to the female response (Peretti & Carrera, 2005; Olivero et al., 2015). In this context, we think that the sexual sting behavior probably has the function of appeasing the female. Considering that larger females should have a higher reproductive value for males (Reinhold et al., 2002; Engqvist & Sauer, 2003), performing a longer sexual sting may correlate with increased female receptivity and higher mating success (Jiao & Zhu, 2010).

In conclusion, this study describes the relationship between male and female body condition and the characteristics of courtship and mating, highlighting the role of different courtship behaviors. Our results suggest that sexual selection is acting on courtship in *B. bonariensis*. There may be indirect fitness benefits for males and females mating with individuals of the opposite sex with a better body condition. Further studies on male fecundity and multiple paternity would help clarify these issues.

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