Sexual behavior of *Metaltella iheringi* (Keyserling, 1891) (Araneae: Desidae): sexual patterns, female quiescence and comparisons with other spiders

Débora A. Abregú[1,2], **Catalina Simian**[1,2], **Camilo I. Mattoni**[1,2] and **Alfredo V. Peretti**[1,2]:   [1]Universidad Nacional de Córdoba, Facultad de Ciencias Exactas, Físicas y Naturales, Departamento de Diversidad Biológica y Ecología, Córdoba, Argentina; E-mail address: debo_abregu@yahoo.com.ar; [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Diversidad y Ecología Animal (IDEA), Laboratorio de Biología Reproductiva y Evolución, Córdoba, Argentina.

Abstract.   There are few works on the reproductive behavior of species of cribellate spiders. Even more scarce are studies of the reproductive behavior of representatives of the Desidae, such as the genus *Metaltella* Mello-Leitão, 1931. In this paper, we describe for the first time the reproductive behavior of *Metaltella iheringi* (Keyserling, 1891), a species that is characterized by complex genitalia in both sexes. We determined frequencies and durations of the behaviors in the different phases. Thirty virgin males were exposed to virgin females and the behaviors performed by each sex, as well as their frequencies, were recorded. Three phases were identified: pre-copulatory, copulatory, and post-copulatory. The most frequent behaviors were chelicera-palp rubbing, abdomen vibration and web-stretching by the male, and body-shaking and leg-tapping on the web by the female. When the male grabbed the female, she typically fell into a state of quiescence (the female remained motionless with legs in a semi-flexed position) and she remained so even after the male ended the copulation. Mating was characterized by low aggression by the female and a low incidence of cannibalism. In the post-copulatory phase, the male performed frequent behaviors such as abdomen vibration, sperm induction, and post-copulatory cohabitation. We discuss the possible implications of these behaviors in a pre- and post-copulatory sexual selection context. We also provide information that serves as a basis for future studies to understand the mechanisms involved in these behaviors.

Keywords: Courtship, copulation, female quiescence, sperm induction

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Sexual selection can affect pre-copulatory sexual behavior through mechanisms of male-male competition and mate choice, and during and after copulation by mechanisms of sperm competition and cryptic female choice (Parker 1970; Eberhard 2004; Peretti & Aisenberg 2015). These processes have been studied in arthropods, but mainly in insects (Thornhill & Alcock 2013; Shuker & Simmons 2014). Within the arthropods, spiders have been proposed as good models for the study of these evolutionary mechanisms (e.g., sperm competition and cryptic female choice), due to their particular morphological and behavioral characteristics (Elgar 1998; Eberhard 2004; Huber 2005; Gaskett 2007). Although these studies have been conducted in various families, e.g., in Linyphiidae (Helsdingen 1965); Zoropsidae (Barrantes 2008), Lycosidae (Vaccaro et al. 2010; González et al. 2013; Toscano-Gadea & Costa 2016), Dictynidae (Starr 1988), Araneidae (Christenson et al. 1985; Bukowski & Christenson 1997), and Pholcidae (Huber & Eberhard 1997; Hutton & Rypstra 2016), there are groups where the male and female reproductive behavior is still poorly known. One of these groups is the family Desidae, which comprises 60 genera and 296 species (World Spider Catalog 2020). Except for a few cosmopolitan species, its distribution is restricted to Australia, Africa and South America (Wheeler et al. 2017; World Spider Catalog 2020). It contains ecribellate species of wandering habits and cribellate web-builder spiders (Griswold et al. 2005; Foelix 2011; Joel et al. 2015). Studies on sexual mating are few and have been reported for an Australian spider *Phryganoporus candidus* (L. Koch, 1872) (Downes 1994) and for a cosmopolitan spider *Badumna longinqua* (L. Koch, 1867) (Costa 1993).

In Argentina, this family is represented by the invasive spider *B. longinqua* and endemic genera *Calacadia* Exline, 1960 and *Metaltella* Mello-Leitão, 1931 (Leech & Steiner 1992; Vetter & Visscher 1994; Sackman 2004; Pompozzi et al. 2013; World Spider Catalog 2020). In the genus *Metaltella*, some morphological (Edwards 2005; Griswold et al. 2005), ecological (Costa & Simó 2014), and preliminary behavioral studies (Escalante & Aisenberg 2015) have been performed only in *Metaltella simoni* (Keyserling, 1878).

Another species of the genus is *M. iheringi* (Keyserling, 1891), that has a distribution in Argentina and Brazil (World Spider Catalog 2020). This species inhabits humid places under stones and logs and constructs a cribellate web that is similar to that built by *M. simoni* (Griswold et al. 2005; DA Abregú, pers. obs.). Females present long copulatory ducts that correspond to a long embolus of the male (Griswold et al. 2005; DA Abegú, MA Izquierdo, AV Peretti, pers. obs.).

Our objective in this work is to describe the sexual behavior of *M. iheringi*. We analyze qualitatively and quantitatively the different mating phases and their behavioral patterns. This information will help to understand the reproductive behavior of the species and will also contribute significantly to the knowledge of the family within a context of sexual selection.

METHODS

Collecting and rearing.—Males and female juveniles of *M. iheringi* were collected during October–March 2017 and 2018 in the day turning rocks in the localities of El Cóndor (31° 39′ 59.435″ S, 64° 50′ 57.304″ W) and Copina (31° 34′ 32.98″ S,

64° 39′ 40.647″ W), Córdoba, Argentina. The individuals were taken to the Laboratory of Reproductive Biology and Evolution (IDEA, CONICET-FCEFN, UNC). They were kept in Petri dishes (9.5 cm in diameter and 1.5 cm high), with small bush branches placed to favor the construction of the web and a piece of cotton embedded in water. All specimens were fed with adults of *Drosophila melanogaster* and larvae of *Tenebrio molitor* twice a week and were observed daily until they grew to adulthood. Males and females at least 10 days past their last molt were used in the trials to ensure that the genitalia were sclerotized (Papke et al. 2001; Baruffaldi & Costa 2010; González et al. 2013). The room temperature and humidity were 26 ± 2.41 °C and 50 ± 7.18 %, respectively, and the spiders were exposed to a 12:12 h photoperiod of light:darkness.

Behavioral observations and analysis.—Virgin males ($n = 30$) and females ($n = 30$) were paired to observe courtship, copulation and post-copulation. The trials were conducted between October and March 2017/2018. Plastic boxes (10 cm in diameter, height = 11 cm) with sand as substrate and branches were used to assist in the building of the web. The female was placed in the cage at least 72 hr before the trials to allow the building of the web.

Each male was randomly selected and carefully placed on the female's web at the beginning of each experiment. Trials in which courtship did not begin within 30 minutes or copulation did not start within 60 minutes after the male was placed on the web were discarded. Interactions were filmed during the day (artificial light) with a Sony DCR-SR85 HD camera and analyzed with the JWatcher program (Blumstein et al. 2000). The duration of the mating phases, the behavior patterns in each one, as well as their frequency of occurrence among the couples were recorded. The frequency of transition from one behavior pattern to another was used for the elaboration of a flow chart. Within the copulation phase, palpal insertions and ejaculations were counted. The ejaculations were indirectly estimated by the erection of the spines of the last pair of legs of the male and/or the expansion of the hematodocha (Dolejs et al. 2010; Foelix 2011; González et al. 2013; Toscano-Gadea & Costa 2016). We recorded the number of completed matings, as well as the number of female attacks or cannibalism of the male.

RESULTS

Of the 30 trials carried out, 20 successful mating sequences were observed. Of the remaining 10 in which the male courted but did not mate, two resulted in pre-copulatory cannibalism by the female. This cannibalism was performed after the male courted, approached the female and touched her with his forelegs. An additional case of cannibalism was also observed during copulation, where the attack occurred after several failed palpal insertions.

Complete matings had an average total duration of 158.08 ± 57.01 min (range: 97.69–270.66 min). A total of 18 behavioral patterns were recorded (see descriptions in Table 1). Three phases were distinguished: Pre-copulatory (18.75 ± 15.17, range 2.35–63.13 min), copulatory (89.37 ± 41.65, range 48.46–218.25 min) and post-copulatory phase (58.13 ± 39.46, range 22.49–173.18 min). The durations and frequencies of the behavioral patterns in each of the phases are shown in

Table 2. The transition probabilities between the main behaviors are given in Fig. 1.

Pre-copulatory phase.—It includes from the first occurrence of courtship behavioral patterns to the first male insertion. The first male behaviors can be web-stretching, abdominal vibration, chelicera-palp rubbing or cheliceral opening (Table 1, Fig. 1). The duration of the courtship and the frequency of the behaviors during this phase were highly variable among the mating sequences. In 90% of the cases, the male was the first to perform some of the behaviors involved in courtship. In the remaining cases, it was the female who first performed few and infrequent behaviors (abdominal vibration or body-shaking). The pre-copulatory phase can be separated into two parts: the first includes the first occurrence of courtship behavioral patterns (e.g., web-stretching, abdominal vibration, chelicera-palp rubbing) to the male grappling behavior. The second part includes from the moment when the male grapples the female (male grappling) to the first palpal insertion. In the grappling, the male positions himself over the female in the same or opposite orientation and grabs her with all his legs (see Supplementary Video S2, online at https://doi.org/10.1636/JoA-S-20-045.s2). After that the female enters a quiescent state (see below).

The most frequent behavior performed by the male in the first stage was the rubbing movement of the chelicerae together with the pedipalps ('chelicera-palp rubbing') (100% of the cases) (378 ± 245 times), which consists of taking the tip of the palps between the chelicerae and opening and closing them repeatedly (see Supplementary Video S1, online at https://doi.org/10.1636/JoA-S-20-045.s1). This behavior was preceded and followed by any of the male's and female's behaviors present in the courtship with the same frequency (Fig. 1). The other frequent male behaviors were web-stretching (77 ± 123 times), abdominal vibration (115 ± 83 times), and cheliceral male opening (17 ± 36 times) (see frequency of occurrence in Table 2). The web-stretching, in which the male used all his legs to stretch the web (Supplementary Video S1), was often accompanied and alternated by cheliceral opening or abdominal vibration with a high transition frequency (Table 1, Fig. 1). In 35% of the trials, both sexes performed mutual cheliceral touching. The female responded to these behavioral patterns (chelicera-palp rubbing and abdominal vibration) mainly by doing body-shaking (25 ± 29 times, 35% of the pairs) and leg-tapping on the web (4 ± 6 times, 30% of the pairs) (Table 2, Fig. 1). Behaviors rarely observed were abdominal vibration (12 ± 1) and palp-tapping on the web (5 ± 3 times) performed by the female, and web-testing (5 ± 6 times) (Table 1) and leg-tapping on the web (5 ± 3 times) by the male.

In the second stage, when the grappling started and the male grabbed the female, she entered a quiescence state where she remained immobile with her legs semi-flexed (Supplementary Video S2). Before grappling occured, the male slowly approached the female by touching her with the forelegs and performing chelicera-palp rubbing. Both sexes could initiate the approach for the grappling.

During the quiescent state, the female was able to perform small displacements (produced by the movement of her legs) but did not exhibit any defined behavior. Forty percent of the males performed palp-tapping on the female, abdominal vibration, or chelicera-palp rubbing while grappling her. In

Table 1.—Description of the behaviors performed by males and females of *Metaltella iheringi* during different phases of the courtship and sperm transfer.

Behavior	Description	Phase
Chelicera-palp rubbing (CPR) ♂	Opening and closing of chelicerae with a tip of the palp between them.	Pre-copulatory, copulatory and post-copulatory
Abdominal vibration (V) ♀ ♂	Rapid sagittal shaking of the abdomen during mating. In the female it only occurs in courtship.	Pre-copulatory, copulatory and post-copulatory
Body-shaking (BS) ♀	Short, vigorous jumps.	Pre-copulatory
Palp-tapping on the web (PTw) ♂ ♀	Alternate up-and-down movements of palp on the web.	Pre-copulatory and post-copulatory
Mutual cheliceral touching (CT) ♀ ♂	Opening of chelicerae (basal segments and fangs) and mutual touching with them.	Pre-copulatory
Web-stretching (WS) ♂	Pull the web by bringing all the legs slightly towards the centre of the body. Sometimes with alternate opening and closing of chelicerae	Pre-copulatory
Web testing (WT) ♂	Alternate up-and-down movements of palp on the web and then takes them to the chelicerae	Precopulatory, copulatory and post-copulatory
Cheliceral opening (CO) ♀ ♂	Exposure of the opened chelicerae to opposite sex.	Precopulatory, copulatory and post-copulatory
Grappling (G) ♂	The male positions himself in the same or opposite direction on the female, grabbing her with all his legs. No insertion occurs but other behaviors do, such as abdominal vibration.	Pre-copulatory and copulatory
Palp-tapping on the female (PTf) ♂	Alternate up-and-down movements of palp on the female.	Pre-copulatory and copulatory
Female testing (FT) ♂	Alternate up-and-down movements of palp on the female and then takes them to the chelicerae.	Pre-copulatory
Leg-tapping on the web ♀	Alternate up-and-down movements of legs on the web	Pre-copulatory and post-copulatory
Copulation positioning ♀ ♂	The male is positioned beside the female, but in opposite way, both in ventral position.	
Palpal insertion ♂	Introduction of the embolus of one palpus into the genital opening. During the insertions there is an expansion of the hematodocha which corresponds to the erection of the hind-leg spines (possible ejaculation).	
Silk-laying (SL) ♂	The male deposits silk threads on the female's web.	Pre-copulatory and post-copulatory
Leg-rubbing (LR) ♀	Rubbing between second, third and fourth pair of legs (II and III), (III and IV),	Post-copulatory
Clap between palps (SHP) ♂	Quick shock of palps with each other.	Post-copulatory
Sperm induction ♂	Building a sperm-web over the female's web and releasing a drop of sperm from the male's genital opening over the sperm-web and taking it with the palps.	Post-copulatory

the remaining (60% pairs), the males did not display any sexual behaviors and ended the grab directly (Fig. 1). The duration of the grappling was variable between the different pairs (0.616 ± 0.39 min). One pair performed grappling twice, while the remaining pairs did it only once. Once the grappling finished, the male performed other behaviors such as chelicera-palp rubbing (39 ± 37 times), abdominal male vibration (5 ± 4 times), palp-tapping on the female (6 ± 5 times), and web-testing (6 ± 7 times) (see frequency of occurrence in Table 2, Fig. 1). The male did not start insertions without grappling and grappling was always performed prior to copulation.

Copulatory phase.—It was the period between the first male insertion to the last insertion, when the genitalia were decoupled. In the copulation position the male was positioned next to the female, facing in the opposite direction, both with the ventral side facing up (Fig. 2, Supplementary Video S3, online at https://doi.org/10.1636/JoA-S-20-045.s3). Males performed on average 3 ± 2 total insertions, alternately or consecutively with each palp, and several ejaculations in each

one (left: 126 ± 116; right: 105 ± 80). In all trials, the male exhibited sexual behaviors between insertions. In 75% of the matings, the male performed chelicera-palp rubbing (87 ± 61 times), and less frequently abdominal vibration (27 ± 43 times) and palp-tapping on the female (8 ± 6 times) (see frequency of occurrence in Table 2). On one occasion, the male performed chelicera-palp rubbing with the free and inserted palp alternately, but without removing it from the female's epigynum. The female also periodically opened and closed the chelicerae (25% of the pairs), but in most cases remained quiescent. The grappling also occurred in this phase in almost half (45%) of the pairs, where the male grabbed the female again (17.94 ± 19.85 seconds) before performing the next insertion. In most of the cases, the last insertion was terminated by the male (94% of the pairs). In some cases (26%), the male seemed to have difficulties removing the palp from the genital opening, as he performed several jerks before withdrawing his palp. When the female ended the copulation or insertion, she started to move away from the male while the

Table 2.—Number of times (mean ± SD), duration (in minutes, average ± SD) and frequency of occurrence (Freq.) of the behaviors in the different phases of mating in *Metaltella iheringi*.

Phase and behaviors	Average	Freq.	Phase and behaviors	Average	Freq.
1. PRE-COPULATORY PHASE Total duration: 18.75 ± 15.17					
First part			Second Part		
Chelicera-palp rubbing ♂	378 ± 245	1	Grappling	0.616 ± 0.39 min.	1
Cheliceral opening ♂	17 ± 36	0.85	Chelicera-palp rubbing ♂	39 ± 37	0.9
Mutual cheliceral touching	1	0.35	Palp-tapping on the female ♂	6 ± 5	0.25
Abdominal vibration ♂	115 ± 83	0.4	Web-testing ♂	6 ± 7	0.1
Abdominal vibration ♀	12 ± 1	0.15	Abdominal vibration ♂	5 ± 4	0.25
Body-shaking ♀	25 ± 29	0.35			
Leg-tapping on the web ♂	5 ± 3	0.3			
Palp-tapping on the web ♀	5 ± 3	0.15			
Leg-tapping on the web ♀	4 ± 6	0.3			
Web-testing ♂	5 ± 6	0.2			
Web-stretching ♂	77 ± 123	0.5			
2. COPULATORY PHASE Total duration: 89.37 ± 41.65 min.			**3. POST- COPULATORY PHASE** Total duration: 58.13 ± 39.46 min.		
Left insertion duration	33.15 ± 25.30 min.		Chelicera-palp rubbing ♂	171 ± 217	0.89
Left ejaculation	126 ± 116		Abdominal vibration ♂	78 ± 101	0.88
Right insertion duration	24.10 ± 19.90 min.		Silk-laying ♂	110 ± 75	1
Right ejaculation	105 ± 80		Web-testing ♂	11 ± 11	0.77
Total ejaculation	322 ± 198		Sperm induction duration ♂	19.54 ± 6.78 min.	0.95
Grappling inter-insertion ♂	2 ± 2	0.45	Time to induction	45.21 ± 39.63 min.	
Inter-insertion courtship duration	2.93 ± 2.24 min.	1	Palp-tapping on the web ♂	28 ± 20	0.88
Chelicera-palp rubbing ♂	87±61	0.75	Grooming ♂	13 ± 11	1
Abdominal vibration ♂	27 ± 43	0.2	Leg-rubbing ♀	3 ± 4	0.11
Palp-tapping on the female ♂	8 ± 6	0.2	Clap between palps ♂	3 ± 2	0.22
			Palp-tapping on the web ♀	4 ± 5	0.27
			Leg-tapping on the web ♀	41 ± 72	0.22
			Cheliceral opening ♂	7 ± 4	0.5

palp was still inserted into the genital opening. This resulted in the male pulling the palp strongly, which ended in an abrupt disengagement of the genitalia. After that, the male did not re-approach the female and the female did not return to quiescence.

Post-copulatory phase.—This phase begins when the male ends the last insertion and the genitalia are decoupled, until the male completes the sperm induction (see below). After insertion, most males performed three principal behaviors: abdominal vibration (78 ± 101 times), silk-laying (see below) (110 ± 75 times), and/or chelicera-palp rubbing (171 ± 217 times) (Tables 1, 2). Other behaviors with a high frequency of occurrence in both sexes were web-testing (11 ± 11 times) and palp-tapping on the web (28 ± 20 times) (Tables 1, 2, Fig. 1). A particular and persistent behavior of the male was silk-laying on the female's web. This behavior was alternated mainly with abdominal vibration and palp-tapping on the web (see Fig. 1). At that time, the female remained quiescent or performed body displacement and/or behaviors such as palp-tapping (4 ± 5 times) or leg-tapping (41 ± 72 times) on the web and rubbing (3 ± 4 times) between the second, third, and fourth pairs of legs. Male behaviors such as clap between palps (3 ± 2 times) and cheliceral opening (7 ± 4 times) were less frequent (See description of behavior and frequency of occurrence in Tables 1, 2)

One of the events observed in this phase was sperm induction on the female's web (19.54 ± 6.78 min), where 95% of the males constructed a triangular sperm-web while sometimes displaying abdominal vibration and/or chelicera-palp rubbing. Then the male deposited a drop of sperm from

the genital opening onto the sperm web and performed repeated tapping movements of the palps over the sperm-drop. In this instance, the female was not quiescent and always remained close to the male without performing any particular behavior. However, in four instances, she touched the male or his sperm-web with her forelegs, interrupting the sperm induction process. On only one occasion, the male was observed to perform the induction behaviors, including the movement of palps, but without the release of the sperm-drop.

In the post-copulatory phase, the male remained close to the female after copulation and during and after sperm induction. It was recorded in some cases (5 of the 20 analyzed) that between the end of copulation and sperm induction, the couple remained together and stood so for several minutes (4.23 ± 3.90 min). Re-mating was observed only once, 4 hours after the sperm induction.

One typical behavior that occurred until copulation was over was the quiescence of the female. Most of the females (0.68 frequency) remained in this state for several minutes (5.1 ± 4.45 min) (Supplementary Video S4, online at https://doi.org/10.1636/JoA-S-20-045.s4), while the male exhibited behaviors such as silk-laying and abdominal vibration. The females showed a low level of aggression during the whole phase. There was no occurrence of post-copulatory cannibalism. The only case of sexual cannibalism was observed during copulation, where the male repeatedly attempted to find a position to perform insertions that were unsuccessful while the female was quiescent. Then the female suddenly began to move and attacked the male.
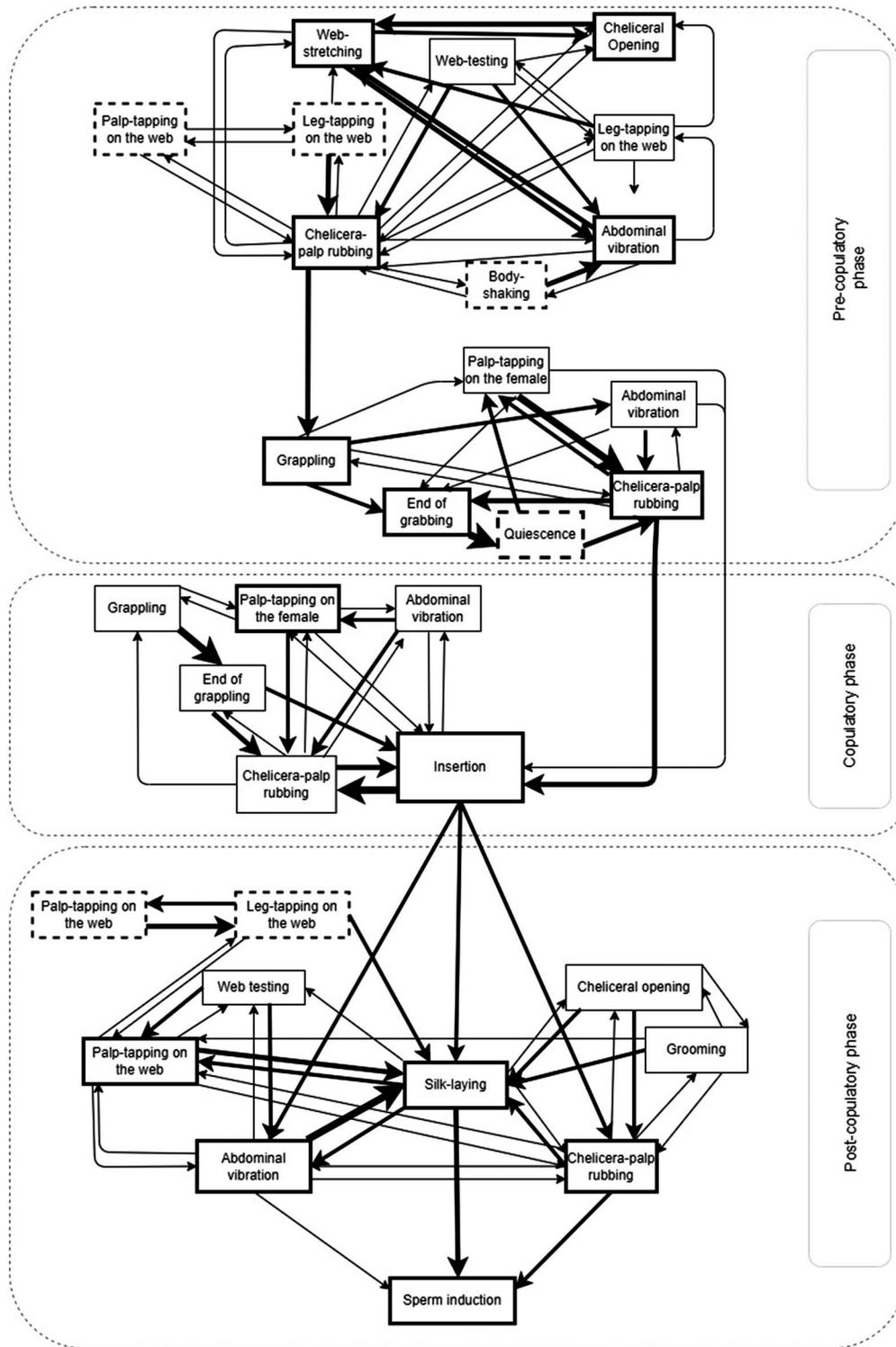
Figure 1.—Main behavioral patterns performed during the different phases of mating behavior in *Metaltella iheringi*. Arrow width represents the transition frequency of behavioral acts in time. The behaviors performed by the females are marked with dotted lines. The width of the box line indicates a higher frequency of occurrence. Behaviors that occurred fewer than ten times were not included in this diagram.

DISCUSSION

The courtship in *M. iheringi* was very variable both in the behaviors produced by both sexes and in the durations and frequencies of the behaviors. The main behavior (and one of the first to appear and that is present in all stages), is the chelicera-palp rubbing. This behavior has also been observed in other species of the genus (*M. simoni* and *Metaltella* sp.; AV Peretti, DA Abregú, unpub. data). The movements produced

Figure 2.—Copulation position of *Metaltella iheringi*. Ventral view, showing both sexes below the web. The male (left) is positioned laterally and opposite to the female (right), inserting his left palp into the left female genital opening. The arrow shows the swollen hematodocha, which indicates an ejaculation.

during this behavior are different from the grooming behaviors reported in several species of spiders such as *Latrodectus hasseltii* Thorell, 1870, *Tengella radiata* (Kulczyński, 1909) or *Schizocosa bilineata* (Emerton, 1885) (Andrade & Banta 2002; Barrantes 2008; Vaccaro et al. 2010), or the chewing-like behavior reported for *B. longinqua* or *Schizocosa malitiosa* (Tullgren, 1905) during copulation (Costa 1993; Estramil & Costa 2007). *Metaltella iheringi* presents a file of cuticular ridges on the chelicera (AV Peretti, DA Abregú, unpub. data) (typical structure to produce stridulation) (Dutto et al. 2011). The palps have hard hairs on the cymbium and a rugosity on the conductor that could play an important role in stridulation, but this is not clear yet. Thus, the male could stridulate during the chelicera-palp rubbing behavior. As with many insects, spiders can generate sounds (Foelix 2011). Stridulation is a type of acoustic communication in which vibration and sound is produced by friction between two rigid structures (a scraper of cuticular ridges and a file) present in different parts of the body (Legendre 1963). These stridulatory organs have been recorded in different groups of spiders (Legendre 1963; Hinton & Wilson 1970; Edwards 1981; Huber 1995; Dutto et al. 2011). Stridulatory structures were observed in the chelicerae of some pholcids, such as *Physocyclus globosus* (Taczanowski, 1874) (Peretti et al. 2006) and *Holocnemus pluchei* (Scopoli, 1763) (Dutto et al. 2011), where both sexes have been found to generate sound for communication in a sexual context. Future SEM analyses of chelicerae

and palps, as well as high-frequency recordings during chelicera-palp rubbing behavior, will help to understand the role of these traits in *M. iheringi* courtship.

Another common male courtship behavior in *M. iheringi* is web-stretching. Similar behaviors have been reported for other web spiders such as the lycosid *Aglaoctenus lagotis* (Holmberg, 1876) (González et al. 2013), and *Metaltella* sp. (DA Abregú, pers. obs.). This behavior, together with the abdominal vibration and chelicera-palp rubbing, or leg-tapping, could produce a vibratory communication through the web. This type of communication was also suggested for some members of the family Dictynidae, where abdominal vibration and leg extension and flexion, or leg-tapping on the web, were observed during courtship (Jackson 1979).

The female of *M. iheringi* carried out infrequent and non-repetitive behaviors. When both are close, the female may remain still or perform leg-tapping or palp-tapping movements on the web, body-shaking, or abdomen vibration. Studies of Desidae have not detailed the activity of the female during courtship (Costa 1993; Downes 1994), but behaviors such as abdominal vibration, and leg and palp-tapping, have been observed by some members of the close family Dictynidae (e.g., *Dictyna calcarata* Banks, 1904; Jackson 1979). In *M. iherigi*, vision seems not to be important in courtship. We suggest that sexual communication during this phase could involve vibratory signals transmitted through the female's web, produced by percussion (through male and

female palp and leg-tapping or abdominal vibration) and perhaps by stridulation (through chelicera-palp rubbing). This type of communication involving seismic and acoustic channels may be useful for nocturnal and/or low vision species (Jackson 1979).

The quiescence of the female is very clear after grappling and is a crucial state for the start of copulation. Female quiescence has been recorded in other groups of spiders such as in the families Homalonychidae (Alvarado-Castro & Jiménez 2011), Agelenidae (Becker et al. 2005; Xiao et al. 2015) and Lycosidae (Aisenberg & Costa 2005; Brown 2006; Dolejs et al. 2010). This quiescence of the female can occur in response to male pheromones, as in *Agelenopsis aperta* (Gertsch, 1934) (Becker et al. 2005) or to different male behaviors, for example, grabbing the female's leg with its chelicerae (Bennet et al. 1987; Xiao et al. 2015). As in *M. iheringi,* the female can change her quiescent position by, for example, flexing some or all her legs towards the body (Homalonychidae: Alvarado-Castro & Jiménez 2011, Ageleni-dae: Fraser 1987; Xiao et al. 2015), or extending them (Lycosidae: Aisenberg & Costa 2005; Brown 2006). Becker et al. (2005) showed that the male of *A. aperta* could induce immobility by short-distance pheromones spread with the help of the palps or by contact with the female. There are several hypotheses about the reasons for the male inducing female quiescence, and they vary among different groups of spiders and authors (Gering 1953; Coyle & O'Shields 1990; Berendonk 2003; Becker et al. 2005). However, there are no studies that have tested them. In the present study, it is also the female that approaches the male to begin the grappling. Also, we observed that female aggression is low in this phase. Therefore, we believe that this phenomenon does not occur to avoid the attack or cannibalism of aggressive females during copulation, as proposed for *A. aperta* (Becker et al. 2005).

Quiescence behavior could be reflecting a possible mate choice (Coyle 1985; Coyle & O'Shields 1990), whereby the female would allow the male to perform copulatory behavior (e.g., mounting, insertion, ejaculation) in a more efficient manner. For example, as in other species of the genus *Metaltella*, the embolus in *M. iheringi* is extremely long (D Abregu, M Izquierdo, A Peretti, unpub. data). Thus, total stillness, such as that characterizing quiescence, may help the insertion and removal of the embolus to be effective. Future studies focused on this phenomenon may help to elucidate these questions.

Regarding the copulation phase of *M. iheringi,* this shows a variant of the position II described by Foelix (2011) for example, in the family Linyphiidae, where the male is positioned opposite to the female, but with both being suspended upside down from the web. A similar position was observed for *M. simoni* (AV Peretti, DA Abregú, unpub. data) and *B. longiqua* (Costa 1993). The copulatory phase, characterized by few and prolonged alternating insertions of both palps and several hematodochal expansions per inser-tion, was similar in *M. simoni* (AV Peretti, DA Abregú, unpub. datas). However, it was different for *B. longiqua* where the males performed many brief insertions (Costa 1993). The courtship between insertions (that is, dismounting the female) has been observed within the family Dictynidae (Jackson 1979; Starr 1988). In some species of this family (e.g., *Dictyna volucripes,* Keyserling, 1881), like *M. iheringi,* a lack of aggression and passivity of the female during copulation has

been observed. As a result, Starr (1988) suggested that these behaviors between insertions would not have an inhibitory function on female aggression. Other functions of male copulation behaviors have been indicated as copulatory courtship and cryptic female choice (Eberhard 1996). In contrast to other species such as *A. lagotis*, *Allocosa senex* (Mello-Leitão, 1945) and *Pavocosa gallopavo* (Mello-Leitão, 1941), where the female is mobile during copulation (González et al. 2013; Garcia Diaz et al. 2015; Toscano-Gadea & Costa 2016), *M. iheringi* is quiescent. However, the possibility of female movement or ending any insertion or copulation is present, even without cannibalism events. As suggested by Coyle & O'Shields (1990), the female's quiescence during copulation may challenge the male's ability to copulate by monitoring his performance.

With regards to the post-copulatory phase of *M. iheringi*, this is characterized by a marked activity of the male and low aggression of the female. The main male behavior is silk-laying on the female's web, often accompanied by abdominal vibration. This behavior is usually observed in diverse groups of spiders during courtship such as in the families Dictynidae (Jackson 1979), Filistatidae (Barrantes & Ramirez 2013), Theridiidae (Knoflach 2004) and Agelenidae (Galasso 2012). However, few studies have focused on the persistence of this behavior after copulation (e.g., *Porrhothele antipodiana* (Walck-enaer, 1837) (Jackson & Pollard 1990). It has been suggested that the silk placed by the males may have components that transmit chemical information to the opposite sex, these may decrease the attractiveness of the female's web and/or dissuade rival males (Jackson & Pollard 1990; Yáñez et al. 1999; Scott et al. 2018). In *M. iheringi,* males lay silk over the entire surface of the female web until they perform sperm induction. We cannot precisely say the function of this behavior, but along with post-copulatory cohabitation these could be mechanisms to reduce the risk of losing paternity due to polyandry (Scott et al. 2018). However, further studies are needed to support this potential function of male silk in a post-copulatory context.

The presence of post-copulatory short-term cohabitation, where the male remains close to the female with a lack of aggression by her, has also been observed within the family in *Cambridgea foliata* (L. Koch, 1872) (Walker & Holwell 2018) and *B. longinqua* (Costa 1993), and the family Dictynidae (Bristowe 1971; Gertsch 1979; Jackson 1979; Starr 1988). As suggested for *C. foliata* (Walker & Holwell 2018) and *D. volucripes* (Starr 1988), the post-copulatory cohabitation in *M. iheringi* could present a form of mate-guarding (Jackson 1977; Starr 1988). In mate-guarding, the male ensures that insemination of the female is completed without expulsión of sperm and could also be a strategy to prevent the female from mating with other males and ensure paternity. Future studies where an additional male is introduced during this cohabita-tion period may help to clarify this question.

Nearly all of the males performed a complete sperm induction after copulation, although the time in which they did so was variable. Sperm induction after copulation has also been observed in *B. longinqua* (Desidae, Costa 1993), and in *Brigittea civica* (Lucas, 1850) (Dictynidae, Billaudelle 1957), and in some species of Theridiidae of the genus *Achaearanea* Strand, 1929 (Knoflach 2004) and *Anelosimus* Simon, 1891 (Knoflach 2004; Buffet & Viera 2016). In the present work, we

observed a case of re-mating with the same female. Re-mating with the same female after sperm induction has also been observed, for example, in *Anelosimus vierae* Agnarsson, 2012 (Theridiidae) (Buffet & Viera 2016).

An interesting aspect of *M. iheringi* is that during sperm induction, the female remains close to the male, and in some cases she even touches him while taking up the sperm-drop. It is an unusual phenomenon when compared with other species. Furthermore, one male performed the induction behavior without the deposition of the sperm-drop. As proposed by Scott et al. (2018), it is unknown whether the sperm web has another role than to charge palps to fertilize the female. But according to the above, we cannot exclude the possibility that it has an additional function, perhaps testing the female in some way, and/or cryptic female choice (Scott et al. 2018).

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SUPPLEMENTAL MATERIALS

Supplemental Video S1—*Metaltella iheringi* courtship, showing the male performing cheliceral palp rubbing and web-stretching. Online at https://doi.org/10.1636/JoA-S-20-045.s1

Supplemental Video S2—*Metaltella iheringi* grappling, showing the male opposite to the female, grabbing her with their legs. Online at https://doi.org/10.1636/JoA-S-20-045.s2

Supplemental Video S3—*Metaltella iheringi* copulation, showing the male inserting his right palp into the right female genitalia opening. The swollen hematodocha can be seen, which indicates an ejaculation, corresponding to the erection of the hind leg's spine. The swollen hematodocha is indicated by the red arrow. Online at https://doi.org/10.1636/JoA-S-20-045.s3

Supplemental Video S4—*Metaltella iheringi* post-copulatory phase, showing the ending of the left insertion (finish copulation) and the female remaining in a quiescent state. After copulation, the male performs palp-tapping over the web. Online at https://doi.org/10.1636/JoA-S-20-045.s4

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