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Sexual behavior, cannibalism, and mating plugs as sticky traps in the orb weaver spider *Leucauge argyra* (Tetragnathidae)

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Abstract Unpublished field observations in *Leucauge argyra*, a tropical orb weaver spider, suggest the occurrence of conspicuous mating plugs that could reduce or prevent remating attempts. Otherwise, the sexual behavior of this species remains unknown. The aims of this study were to describe the courtship behavior and copulation in *L. argyra* and investigate mating plug formation in this species. Fourteen virgin females and 12 plugged females were exposed to up to three males and checked for mating plug formation. Of the 12 virgins that copulated, nine produced plugs (five immediately after copulation), and the five plugged females that copulated produced another mating plug immediately after copulation. We did not detect the transfer of any male substance during copulation but observed a whitish liquid emerging from female genital ducts. Plug formation was positively associated with male twanging during courtship. One virgin and four plugged females cannibalized males. In seven trials with virgins and in three trials with plugged females, the male's palp adhered to a substance that emerged from female genital ducts and spread on her genital plate. The male had to struggle energetically to free his glued palp; two of these males were cannibalized while trying to release their palps. Females

seem to determine copulation duration by altering the timing of mating plug formation and through sexual cannibalism. This is the first case reported of a mating plug as a sticky trap for males.

Keywords Genital plug · Plug removal · Sexual cannibalism · Sexual selection

Introduction

Mating plugs are widespread in various animal taxa, from insects and arachnids to reptiles, birds, and mammals (Drummond 1984; Andrade 1996; Gomiendo et al. 1998; Knoflach 1998; Wigby and Chapman 2004; Simmons 2001; Mattoni and Peretti 2004; Aisenberg and Eberhard 2009; Leonard and Córdoba-Aguilar 2010; Uhl et al. 2010). These plugs are composed by sperm, glandular products, parts of spermatophores or male genitalia, and in some cases the male's entire body that remains attached to female genitalia (Uhl et al. 2010). Mating plugs are thought to function primarily to prevent or minimize female future mating attempts and consequently, favor the paternity of the copulating male. However, plugs could also have other functions presumably beneficial to females as prevent sperm desiccation or minimize sperm loss (Boorman and Parker 1976; Huber 1995, 2005; Simmons 2001; Uhl et al. 2010).

Though most mating plugs consist of parts of male genitalia or substances transferred by males, in some cases they are formed by a mixture of female and male transferred substances or exclusively by female substances (Méndez 2004; Uhl et al. 2010). In the orb-weaving spider *Leucauge mariana* (Keyserling 1881) (Tetragnathidae), substances transferred by both sexes are necessary to compose an efficient mating plug (Eberhard and Huber

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1998; Méndez 2004; Aisenberg and Eberhard 2009). Larger numbers of short male genital insertions and of rhythmic pushes of the male's front legs against female's legs during copulation are associated with a higher probability of female supplying her substance and efficient plug formation in this species (Aisenberg and Eberhard 2009). This was the first description of female participation in mating plug formation as a possible mechanism of cryptic female choice based on female selection on the basis of male copulatory courtship.

The genus *Leucauge* White 1841 comprises more than 100 spider species, but sexual behavior has been studied in only few species (Eberhard and Huber 1998). Male copulatory courtship seems to be widespread in this genus (Eberhard 1994; Castro 1995; Eberhard and Huber 1998) and mating plugs have been observed at least in three species: *L. mariana*, *Leucauge venusta* (Walckenaer 1841) and *Leucauge argyra* (Walckenaer 1841) (Castro 1995; Eberhard and Huber 1998). Cheliceral claspings between sexes prior to copulation also occurs in these three *Leucauge* species, though it is absent or varies (males seize female chelicerae) in other species of Tetragnathidae (Eberhard 1994; Castro 1995; Eberhard and Huber 1998; Aisenberg and Eberhard 2009; Aisenberg 2009). Spiders of this genus are thus interesting for studies of sperm competition, female choice, and the evolution of mating strategies.

L. argyra is an orb-weaving spider that constructs approximately horizontal orb webs in lowland wet areas in Costa Rica (Eberhard 2001). In some sites, this species is extremely abundant year round and during the rainy season males are frequently observed guarding penultimate female close to molt (W.G. Eberhard, G. Barrantes and A. Aisenberg, unpublished data). Unpublished field observations show that females of this species have conspicuous mating plugs largely covering their epigyna; their sexual behavior, however, is unknown. The objectives of this study were to describe courtship and copulation in *L. argyra* and investigate mating plug formation in this species. Additionally, we tested whether plug production is related to female and/or male sexual behavior and tried to elucidate the effectiveness of genital plugs in preventing female rematings.

Materials and methods

We collected 14 penultimate females, 12 adult females with genital plugs that covered entirely their epigynum, and 35 adult males from September to December 2009 in plantations of African oil palm near Parrita, Puntarenas Province, Costa Rica (09°30' N, 84°10' W; elevation 10 m). Penultimate females were recognized in the field by being accompanied by an adult male in their webs, as occurs

with *L. mariana* (Eberhard et al. 1993). We obtained virgin adult females by allowing penultimate females to molt in captivity. Each penultimate female was maintained in a cylindrical glass jar (5–10 cm diameter and 8 cm height) covered with a thin mesh lid and was monitored everyday to record molting occurrence. Each jar was provided with a piece of wet cotton and two small branches with leaves.

After molting to maturity, each virgin female ($n=14$) and each female collected at the field with a complete genital plug ($n=12$) was placed on a glass terrarium of 30 cm length, 16 cm width, and 20 cm height. We attached a cardboard horizontal frame near the top as substrate for web construction and placed a small plant inside the terrarium to provide humidity and an additional substrate for construction of the web. Spiders were offered a prey three times a week: a *Tenebrio molitor* larvae (Tenebrionidae) or an adult fly (Sarcophagidae or Calliphoridae). The plants were sprayed everyday with water.

Between 1 and 5 days after the female molted to maturity or 5 days after an adult female with a genital plug had been captured, each female was exposed to one male that was placed on the border of the orb web. If the female did not mate after 30 min of male courtship, she was exposed to a second male within 1 h and then to a third male if the second was not accepted. Females that rejected three consecutive males were discarded. If spiders courted but did not copulate, we ended the trial when one spider abandoned the terrarium, when both spiders remained motionless for 15 min, or when the female attacked and injured the male or cannibalized him. For determining periods of experimentation, we followed previous studies on *L. mariana* (Aisenberg 2009; Aisenberg and Eberhard 2009) and preliminary observations on the study species.

Individuals were used once and were randomly assigned to each trial. The temperature during the trials averaged $24.44 \pm 1.46^\circ\text{C}$, range: 21–26. The arena was illuminated with a fiber optic lamp and all trials were recorded with a SONY DCR TRV50 digital video camera (SONY, San Diego, CA, USA) equipped with +4 close-up lenses. Immediately after copulation, each female was observed and photographed under a Wild model M3Z dissecting microscope (Wild Company, New York, USA), to check for mating plug occurrence, and was checked again 24 h later. All plugged females were inspected before the expositions and after copulation to check if mating plugs were intact or presented slits. Partial plugs were those mating plugs that covered only part of female epigynum, in contrast to complete plugs that covered both inseminatory duct orifices of the epigynum. We checked male palps after copulation under the dissecting microscope looking for evidence of secretion. We measured carapace width as an estimator of body size in spiders (Eberhard et al. 1998; Moya-Laraño and Cabeza 2003) and tibia length as an estimator of leg length

(Foellmer and Fairbairn 2004) under a dissecting microscope. We calculated the sexual size dimorphism index (male carapace width/female carapace width) to examine its effect on mating acceptance and plug formation.

We analyzed courtship and copulatory behavior of females and males using Jwatcher program (Blumstein et al. 2000). Detailed analyses of courtship and copulatory behavior provided information for a full description of the sexual behavior of this species and for examining the relationship between courtship and copulatory behaviors, mating acceptance, and plug occurrence. The definitions of courtship and copulatory behaviors follow Robinson and Robinson (1980) and Eberhard and Huber (1998). We recorded the number and duration of courtship and copulatory behavioral units performed by females and males, and compared the results between virgin and plugged females. We also counted the number of hematodochal inflations per insertion, characteristic associated with sperm transfer in spiders (Foelix 1996). To investigate the relation between courtship and copulatory behaviors and plug occurrence, we used only data from virgin females because all plugged females produced plugs

after mating. For the comparisons between females, we included courtship and copulatory behaviors that had a total sample size of at least $n=5$ for each category. We considered that a plug was formed when the epigynum was partially or totally covered by a hard whitish substance. We compared the frequencies of copulation, plug formation, and cannibalism frequencies with chi-square test for independent samples and Fisher's exact probability test. The statistical analyses were performed with Past Palaeontological Statistics, version 1.18 (Hammer et al. 2003), NCSS 2001 (Copyright 2000 Jerry Hintze), and WINPEPI (Abramson 2004).

Results

Description of sexual behavior and plug puncturing behavior

We identified six courtship and five copulatory behaviors (Tables 1 and 2). Three of these behaviors (jerk the web, bursts of twanging, and tap the web) were performed by

Table 1 Descriptions of courtship behavior of *L. argyra*, with their corresponding frequency and durations (median±quartile)

Courtship behavior	Description	Frequency	Duration (s)
Jerk the web	Facing the other sex, the female or male flexes legs I and II strongly and quickly without releasing the silk line	Female jerk, virgins, 5 ($n=1$) plugged ^a Male jerk, virgins, 3.0±6.0 ($n=7$); plugged, 4.5±1 ($n=2$); $U=4.5$, $p=0.90$	Female jerk, virgins, 13 ($n=1$) plugged ^a Male jerk, virgins, 16.2±21.3 ($n=7$); plugged, 63.3±84.0 ($n=2$) $U=2$, $p=0.22$
Bursts of palpal rubbing	Alternate and circular movements of the palps, during which right and left male cymbium hooks contact or brush against each other	Virgins, 24.0±21.0 ($n=12$); plugged, 47±53 ($n=5$); $U=23$, $p=0.64$	Virgins, 111.5±91.1 ($n=12$); plugged, 288.1±279.6 ($n=5$); $U=25$, $p=0.83$
Bursts of twanging (silk thread tension and release)	The female or male folds legs III ventrally and rapidly tensioning the silk line that connects the two individuals and then the tension is suddenly released	Female twanging, virgins: 2.0±48 ($n=12$); plugged, 5.0±3 ($n=5$); $U=15$, $p=0.17$ Male twanging, virgins, 49.0±40 ($n=12$); plugged, 56.0±91 ($n=5$); $U=25$, $p=0.83$	Female twanging, virgins, 6.3±53.1 ($n=12$); plugged, 32.1±19.9 ($n=5$); $U=17$, $p=0.37$ Male twanging: virgins, 220.8±105.5 ($n=12$); plugged 377.7±550.8 ($n=5$); $U=21$, $p=0.51$
Tap the female	The male taps the female on the dorsum of the carapace or on her legs I or II with his anterior leg tarsi or metatarsi	Virgins, 4.0±7.0 ($n=5$); plugged, 2.0±3.0 ($n=5$); $U=7.5$, $p=0.58$	Virgins, 84.9±128.9 ($n=5$); plugged, 3.3±6.6 ($n=5$); $U=1$, $p=0.12$
Tap the web	The female or the male performs quick taps with the forelegs on the silk line that connects both individuals	Female tapping, virgins, 8.0±6.0 ($n=9$); plugged, 16.0±18.0 ($n=5$); $U=14$, $p=0.28$ Male tapping, virgins, 15.0±11.0 ($n=12$); plugged, 5.0±23.0 ($n=5$); $U=19.5$, $p=0.40$	Female tapping, virgins, 21.4±82 ($n=9$); plugged, 60.7±195.7 ($n=5$); $U=18$, $p=0.16$ Male tapping, virgins, 54.5±105.9 ($n=5$); plugged, 26.1±190.1 ($n=5$); $U=24$, $p=0.74$
Foreleg rubbing	The male rubs legs I and II against each other, after contacting the female web	Virgins, 2.0±2.0 ($n=5$); plugged, 4.5±2.5 ($n=4$) $U=6.5$, $p=0.45$	Virgins, 9.4±7.0 ($n=5$); plugged, 24.9±22.9 ($n=4$); $U=9$, $p=0.03$

We distinguish between trials with virgin and plugged females and show the results of the statistical comparisons between both groups (Mann–Whitney U test). Sample sizes are shown in parentheses

^a Insufficient sample size for performing statistical comparisons

Table 2 Descriptions of copulatory behaviors of *L. argyra*, with their corresponding frequency and duration (median \pm quartile)

Copulatory behavior	Description	Frequency	Duration (s)
Long insertions	Multiple hematodochal inflations after a single insertion.	Virgins, 3.0 ± 1.0 ($n=12$); plugged, 2.0 ± 3.0 ($n=5$); $U=26$, $p=0.86$	Virgins, 343.6 ± 173.2 ($n=12$); Plugged, 235.2 ± 286.7 ($n=5$); $U=16$, $p=0.22$
Inflations	Hematodochal expansions	Virgins, 208.0 ± 139.0 ($n=12$); plugged, 119.0 ± 83.0 ($n=5$); $U=13.5$, $p=0.12$	The duration coincides with long insertions duration
Flubs	Failed insertion attempts. The hematodochae inflates but the embolus scrapes the epigynum without engaging it as in a successful insertion, or briefly engaging it at an inappropriate site	Virgins: 2.0 ± 1.0 ($n=5$); plugged, 5.0 ± 11.0 ($n=4$); $U=0.5$, $p=0.04$	Virgins, 7.9 ± 2.7 ($n=5$); plugged, 6.65 ± 15.3 ($n=4$); $U=7$, $p=0.99$
Palpal displacement	The female pushes the male's palp away from her epigynum using her legs III	Virgins, 2.0 ± 2.0 ($n=9$); plugged, 8.5 ± 12.0 ($n=5$); $U=0.5$, $p=0.01$	Virgins, 2.5 ± 0.9 ($n=9$); plugged, 10.1 ± 34.1 ($n=5$); $U=8.5$, $p=0.02$
Hug the male	The female embraces or hugs the male's body or legs with her own legs, while chelicerae remain locked and the male tries to escape. This behavior often ends with the female wrapping the male body with silk, similar to prey-wrapping behavior	Virgins, $n=10$; plugged, $n=5$	–
Palpal stuck	The male's palp gets stuck to substances on female genital plug and the male tries to withdraw it using the other palp	Virgins, $n=7$; plugged, $n=3$	–

We distinguish between trials with virgin and plugged females and show the results of the statistical comparisons between both groups (Mann–Whitney U test). Sample sizes are shown in parentheses. The behaviors hug the male and palpal stuck occurred only once per copulation so we report only the number of copulations in which they occurred

both females and males. Frequency and duration of these behaviors are shown in Table 1. Male and female courtship occurred in all the trials, both with virgin ($n=32$) and plugged females ($n=15$). Courtship duration that ended with copulation did not differ significantly between virgins (median \pm quartile, 783.0 ± 530.3 s) and plugged females ($1,054.6 \pm 1,701.6$ s; $U=20$, $n_1=12$, $n_2=5$, $p=0.44$). The courtship behaviors that occurred with highest frequency were bursts of male palpal rubbing, bursts of male twanging, and tapping the web by both females and males. These behaviors also had the longest durations, along with male tapping the female (Table 1). Both frequency and duration of courtship and copulation behaviors varied widely in virgin and plugged females. Male foreleg rubbing showed longer duration with plugged females compared to virgins (see Table 1), but other courtship behaviors did not differ between virgin and plugged females.

We did not find an effect of age of adult female on mating acceptance (age: median \pm quartile, 2.0 ± 3.0 days, range: 1–5; $U=8$, $n_1=12$, $n_2=2$, $p=0.65$). Copulations occurred more frequently with virgins than with plugged females (Fisher's exact test: $p=0.03$). In 12 of 14 virgin females and in all plugged females ($n=12$), both sexes locked their chelicerae together prior to copulation, as is typical for tetragnathids. The female closed her fangs on male's chelicerae, rather than the opposite. In all virgin females that locked their chelicerae, courtship ended with copulation. However, though all plugged females locked

chelicerae and adopted the mating posture, and males always tried to insert their palps, only five of these females mated. Males mating with plugged females did not perform any behavior indicating plug extraction but they just punctured the genital plug with the embolus of the palp and made a slit to allow the insertion to occur. After puncturing, long insertions and hematodocha inflations occurred. Considering the total number of expositions with plugged females, puncturing the mating plug was more frequent when the plugged female was larger (median \pm quartile; female size when plug was formed, 2.2 ± 0.7 mm; female size when plug was not formed, 1.8 ± 0.3 mm; $U=3$, $n_1=5$, $n_2=7$, $p=0.02$), but did not vary with male body size (median \pm quartile; male size when plug was formed, 1.6 ± 0.3 mm; male size when plug was not formed: 1.8 ± 0.2 mm; $U=19.5$, $n_1=5$, $n_2=7$, $p=0.95$), or with sexual size dimorphism index (median \pm quartile; index when plug was formed: 0.8 ± 0.1 ; index when plug was not formed, 0.9 ± 0.1 ; $U=7$, $n_1=5$, $n_2=7$, $p=0.10$).

Copulation started when the male inserted the conductor of one of his palps onto one of the epigynum orifices, the hematodocha of the corresponding palp inflated and deflated with the cymbium hook positioned in the opposite epigynum aperture (see Fig. 1 for details on male genitalia). Copulation duration did not differ between virgin (median \pm quartile, 649 ± 898 s) and plugged females (297 ± 230 s), possibly due to their high variances ($U=20$, $n_1=12$, $n_2=5$, $p=0.42$). The copulation consisted on long insertions with

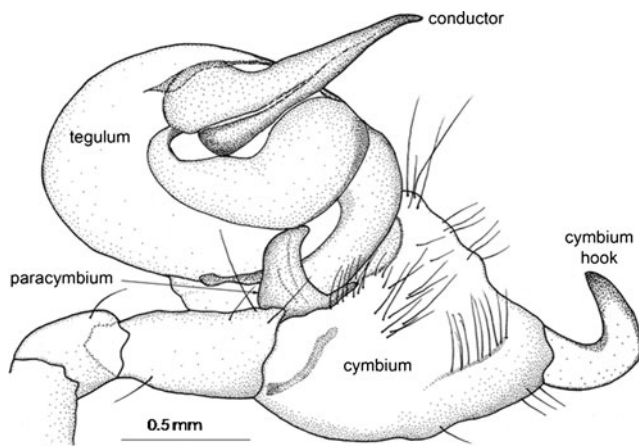


Fig. 1 Drawing of ventral view of left male palpal organ in *L. argyra* showing the conductor where the embolus projects during insemination and the cymbium hook that is placed simultaneously in the opposite aperture of the female epigynum

multiple hematodochal inflations (Table 2). Considering copulations with virgins and plugged females altogether, males performed an average of 1.5 ± 0.7 palpal insertions with the right palp and 1.3 ± 0.5 with the left palp. Only in two cases, the males performed a single palpal insertion. The frequency and duration of long insertions and hematodochal inflations did not differ between virgin and plugged females (Table 2). However, the number of flubs and the number and duration of palpal displacements were higher in plugged females.

In ten copulations with virgins and in all those of plugged females, copulation ended with the female embracing male legs and sometimes wrapping them with silk, with chelicerae still locked and with the male trying to escape (Table 2). Sexual cannibalism on males occurred in one case with a virgin and four cases with plugged females; cannibalism was more frequent with plugged females (Fisher's exact test, $p=0.04$). In all cases of cannibalism, both the female and the male had performed courtship and the female had locked the male's chelicerae. In the cases of cannibalism with plugged females, none of the males had punctured the mating plug before the attack. In one case with a virgin female, the fatal attack occurred post insertion, close to the end of copulation. Sexual size dimorphism index was not related to mating acceptance (virgins, $U=48$, $n_1=10$, $n_2=12$, $p=0.44$; previously plugged, $U=16.5$, $n_1=7$, $n_2=5$, $p=0.93$), or with the occurrence of cannibalism ($U=9$, $n_1=8$, $n_2=4$, $p=0.26$).

Genital plug occurrence in relation with male performance

Of the 12 virgins that copulated, nine produced genital plugs (five were produced immediately after copulation, four were observed 24 h later). Of the plugged females that copulated, all of them produced an additional copulatory

plug immediately after copulation. We did not observe the transfer of a whitish substance by males during copulation, as occurs in *L. mariana* (Eberhard and Huber 1998; Aisenberg and Eberhard 2009). However, we observed a whitish liquid emerging from the openings of the female genital ducts during copulation, during or after male insemination.

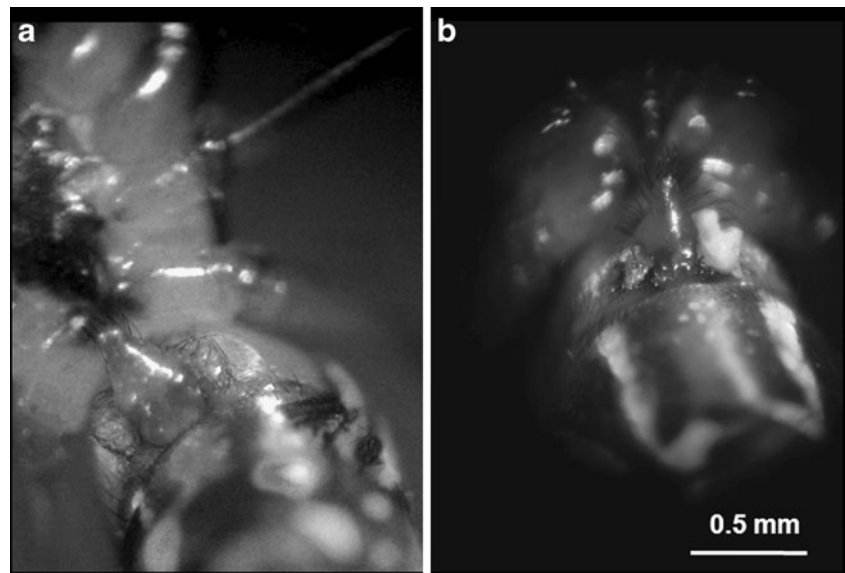
In seven trials with virgins and in three trials with plugged females, the male palp adhered to a whitish sticky substance that had extended on the female genital plate (Table 2) and the male had to struggle quite energetically to free his palp. In two cases, one with a virgin and one with a plugged female, the male's struggle to free his palp ended with the female cannibalizing him. Of the seven cases in which the male's palp adhered to the substance that emerged from the female genitalia during mating, in five occasions, the mating plug was formed immediately after copulation. However, in two cases, the substance had disappeared by the end of the copulation, but the plug was present 24 h later. Copulatory plugs appeared to solidify in a few seconds and covered totally or partially the female genital openings (Fig. 2). Virgin females produced four partial and five total plugs, but plugged females produced mating plugs that covered the entire epigynum in all cases. The two copulations with a single palpal insertion resulted in plug formation. Formation of total or partial plugs was not associated with the number of palpal insertions performed during copulation ($U=8$, $n_1=4$, $n_2=5$, $p=0.99$).

We did not find a significant effect of most courtship and copulatory behaviors on mating plug formation (Table 3). The only exception was that plug formation occurred more frequently when males had a significantly higher number of bursts of twanging during courtship (Table 3). Copulation duration or female age had no effect on plug formation (copulation duration, $U=4$, $n_1=9$, $n_2=3$, $p=0.13$; female age, $U=4.5$, $n_1=9$, $n_2=3$, $p=0.06$).

Discussion

Sexual encounters involve substantial risks for *L. argyra* males. Cannibalism was more frequent when males were exposed to plugged females compared to virgins. In several trials (with both virgin and plugged females), the male's palp adhered to mating plug substances that emerged from the female's epigynum while the palp was still inserted. In two of these cases, the male was cannibalized by the female while he was trying to pull his palp free from the sticky substance. The substance that emerged from the female epigynum which later formed the copulatory plug could appear after few palpal insertions, after multiple insertions, immediately after

Fig. 2 Epigynum of a female of *L. argyra*: **a** without copulatory plug (left), **b** with copulatory plug covering the two genital openings, one with a small and the other one with a large plug (right)



copulation, or during the next 24 h. By initiating plug formation at different times during copulation, females might determine copulation duration and affect the possibilities of remating. Hence, this behavior of *L.*

argyra resembles that of other species in which females attack or cannibalize males and thus define the duration of insemination and copulation (Elgar et al. 2000; Schneider and Elgar 2001; Fromhage et al. 2003). As

Table 3 Frequency and duration of courtship and copulatory behaviors in *L. argyra* (median \pm quartile) in virgins, distinguishing between trials in which plug formation occurred and did not occur

Courtship behavior	Plug was formed	Plug was not formed	Statistics
Male jerk the web	Occurrences, 3.5 ± 6.0 ($n=6$) Duration, 13.3 ± 18.8 ($n=6$)	Frequency, 2.0 ($n=1$) Duration, 33.8 ($n=1$)	Frequency ^a Duration ^a
Bursts of palpal rubbing	Occurrences, 32.0 ± 43.4 ($n=9$) Duration, 200.4 ± 71.7 ($n=3$)	Occurrences, 17.0 ± 3.2 ($n=9$) Duration, 133.9 ± 115.6 ($n=3$)	Frequency, $U=4.5$, $p=0.14$ Duration, $U=8$, $p=0.49$
Female bursts of twanging	Occurrences, 3.5 ± 4.0 ($n=9$) Duration, 41.9 ± 74.0 ($n=9$)	Occurrences, 1.0 ± 1.0 ($n=3$) Duration, 4.7 ± 4.5 ($n=3$)	Frequency, $U=4.5$, $p=0.17$ Duration, $U=5$, $p=0.54$
Male bursts of twanging	Occurrences, 83.4 ± 63.0 ($n=9$) Duration, 337.1 ± 182.2 ($n=9$)	Occurrences, 16.7 ± 13.0 ($n=3$) Duration, 140.1 ± 173.4 ($n=3$)	Frequency, $U=1$, $p=0.03$ Duration, $U=4$, $p=0.14$
Female tap the web	Occurrences, 9.5 ± 3.0 ($n=6$) Duration, 65.4 ± 81.6 ($n=6$)	Occurrences, 3.0 ± 1.0 ($n=3$) Duration, 11.7 ± 13.6 ($n=3$)	Frequency, $U=0$, $p=0.02$ Duration, $U=2$, $p=0.09$
Male tap the web	Occurrences, 12.0 ± 7.0 ($n=9$) Duration, 80.1 ± 121.1 ($n=9$)	Occurrences, 8.0 ± 4.0 ($n=3$) Duration, 22.5 ± 74.2 ($n=3$)	Frequency, $U=4.5$, $p=0.14$ Duration, $U=5$, $p=0.19$
Copulatory behavior			
Long insertions	Occurrences, 2.5 ± 1.5 ($n=9$) Duration, 313.0 ± 246.5 ($n=9$)	Occurrences, 3 \pm 3 ($n=3$) Duration, 393.0 ± 100.0 ($n=3$)	Frequency, $U=7$, $p=0.31$ Duration, $U=10$, $p=0.77$
Inflations	Occurrences, 203.5 ± 129.5 ($n=9$) Duration, coincides with long insertions duration	Occurrences, 230.0 ± 72.0 ($n=3$) Duration, coincides with long insertions duration	Frequency, $U=7$, $p=0.38$ Duration, –
Flubs	Occurrences, 2.0 ± 1.0 ($n=4$) Duration, 7.4 ± 9.1 ($n=4$)	Occurrences, 2 ($n=1$) Duration, 7.94 ($n=1$)	Frequency ^a Duration ^a
Palpal displacements	Occurrences, 2.0 ± 2.0 ($n=9$) Duration, 2.5 ± 0.9 ($n=9$)	Occurrences, – Duration, –	Frequency ^a Duration ^a

We provide sample sizes in parentheses and the results of the statistical comparisons between both groups (Mann–Whitney U test)

^a Insufficient sample size to perform statistical comparisons

far as we know, this is the first observation of a mating plug that functions as a sticky mortal trap for males. In addition, most copulations ended with females trying to seize or wrap the males as preys while they were trying to escape. This female aggression is apparently not an artifact of captivity, as two courtships and copulations in the field also involved apparently highly aggressive behavior by the female (WG Eberhard, unpublished data), and three additional adult females were observed feeding on adult males at the wild (G Barrantes, unpublished data).

The association between male twanging during courtship and plug formation, the high levels of sexual cannibalism, and the initiation of plug formation at different stages during mating (with the consequent risks for males of getting trapped), suggest that male sexual behavior could be under selection through cryptic female choice (Eberhard 1991, 1996). Females could exert extreme mate choice (Elgar and Nash 1988; Schneider and Elgar 2005) and decide to cannibalize or mate based on male twanging and/or plug-puncturing performance. On the other hand, as a consequence of the high mating risks, males could also be selective when making mating decisions, as has been reported for other spider species (Herberstein et al. 2002; Rypstra et al. 2003; Gaskett et al. 2004; Andrade and Kasumovic 2005; Stoltz et al. 2007; Baruffaldi and Costa 2009; Pruitt and Riechert 2009; Schulte et al. 2010). Males mated more frequently with virgin females and they were more successful in perforating plugs of larger females. This could be a result of higher mating effort towards females with characteristics that in spiders are associated with higher fecundity expectations (Uhl et al. 2005). However, it is difficult to determine if mating behavior in *L. argyra* is under female or male control, or if both play a role in this context.

We did not find evidence of male transfer of components of the mating plug as occurs in *L. mariana* (Eberhard and Huber 1998; Méndez 2004; Aisenberg and Eberhard 2009). In addition, copulation in *L. argyra* did not include short palpal insertions, and these insertions in *L. mariana* are associated with male transfer of plug substances (Eberhard and Huber 1998; Méndez 2004). Preliminary observations indicate that the mating plugs in *L. argyra* do not include encapsulated or decapsulated sperm and appear to be composed exclusively of female substances (A. Aisenberg, G. Barrantes, W.G. Eberhard, unpublished data). However, male transference of sperm associated compounds needed for mating plug formation could have been undetected by our observations, so new studies are needed to address these topics.

In general, courtship behavior did not differ between virgin and plugged females with the exceptions of a higher frequency of foreleg rubbing, and a higher frequency and

duration of palpal displacements in plugged females. Palpal displacements are female movements performed with legs III intended to remove male palps away from the epigynum. So, the higher frequencies and duration of palpal displacements in plugged females agree with a reduction in sexual receptivity in these females. A reduction in sexual receptivity in mated females has been described for other spider species (Helsdingen 1965; Jackson 1980; Elgar and Bathgate 1996; Andrade and Banta 2002; Schäfer and Uhl 2005; Aisenberg and Costa 2005). However, as occurs in the tetragnathid *Glenognatha emertoni* (Danielson-François 2006), in *L. argyra* copulations of virgin and plugged females did not differ in mating occurrences, total number of palpal insertions, or copulation duration. The higher number of flubs during copulations of plugged females could be the result of males trying to remove the female's mating plug, hypothesis that requires further observations.

Plugged females performed courtship behaviors, adopted mating posture, and locked chelicerae in all the cases, but only in 41.6 % of these cases mating occurred. The reduced frequency of successful copulations in plugged females indicates that mating plugs in *L. argyra* probably function as barriers that reduce, at least during a certain period, other males' mating access. Males did not remove copulatory plugs as has been described for other spiders, in which males spend long periods of time extracting the genital plug (Jackson 1980; Masumoto 1993; Uhl et al. 2010). Copulation occurred with plugged females when males succeeded in making a slit through the mating plug (apparently with the conductor), while holding firmly to the female chelicerae. The fact that males were more efficient in puncturing the copulatory plugs of larger females could imply that larger females are preferred by males, that larger females have more fragile plugs, or that these females are more willing to accept rematings. This last possibility has been reported for several taxa (Masumoto 1993; Gage 1998; Bergström et al. 2002; Schäfer and Uhl 2005; Aisenberg et al. 2009; Aisenberg 2009). We did not document, however, whether sperm transfer really occurred in the copulations we observed though they showed similar behavioral patterns to those of virgin females. Sperm counts will help answer these questions.

Finally, *Leucauge* is a very promising genus for studies on sperm competition, female choice, and the evolution of sexual strategies. Detailed studies on the behavior looking for similarities and differences in sexual strategies, genital morphology and ecology between the species, and considering variables such as male and female age and reproductive history, will help disentangle the pressures driving female participation in mating plug formation and male adaptations to overcome sperm competition in this spider genus.

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