

Female cooperation in plug formation in a spider: effects of male copulatory courtship

Anita Aisenberg^a and William G. Eberhard^b

^aLaboratorio de Etología, Ecología y Evolución, Instituto de Investigaciones Biológicas Clemente Estable, Avenida Italia 3318 CP 11600, Montevideo, Uruguay and ^bSmithsonian Tropical Research Institute, and Escuela de Biología, Universidad de Costa Rica, Ciudad Universitaria, Costa Rica

One of the strongest indications that cryptic female choice is an evolutionary phenomenon of general importance is the widespread existence of male courtship behavior during copulation. It has been presumed that such copulatory courtship functions to induce female reproductive behavior that favors the male's reproductive interests, but this function has seldom been demonstrated. Here, we examine the possible effects of male copulatory courtship in the spider *Leucauge mariana* (Tetragnathidae) on whether a female will aid the male in forming a copulatory plug, thus reducing the chances that future mates will be able to inseminate her. Greater numbers of 2 behavior patterns by the male during copulation, rhythmic pushing on the female's legs with his front legs, and repeated short insertions with his genitalia were associated with increased chances that the female would cooperate in plug formation. This confirms that these behavior patterns do indeed function as courtship. *Key words*: copulatory courtship, copulatory plug, cryptic female choice, sexual selection. [*Behav Ecol*]

Sexual selection on male traits can continue even after copulation has begun, by direct interactions between males and their gametes (sperm competition) and by cryptic female choice (CFC). The conditions necessary for CFC to occur include the following: 1) the female must mate at least occasionally with more than one male, 2) differences in her behavioral or physiological responses to the male must bias his chances of paternity, and 3) differences in these responses must be correlated with particular behavioral or morphological traits of the male (Thornhill 1983; Eberhard 1996). There are more than 20 possible mechanisms by which females can theoretically produce such biases (Eberhard 1996) but few have been checked as possible causes of CFC. Female modulation of copulatory plug formation has never to our knowledge been studied as a possible mechanism of CFC.

Some types of male behavior during copulation have been termed "copulatory courtship" because they have no obvious role in sperm transfer per se, are repeated during and between copulations, and appear designed to stimulate the female (Eberhard 1991, 1994). Copulatory courtship behavior is thought to result from sexual selection by CFC and to function to induce the female to respond in ways that increase the male's chances of paternity (Eberhard 1994, 1996). Copulatory courtship occurs in many species (Eberhard 1991, 1994, 1996) and has been thought to constitute some of the best evidence of the widespread importance of CFC. The function of copulatory courtship has been tested only a few times, however (Humphries 1967; Arnqvist and Danielsson 1999; Edvardsson and Arnqvist 2000; Ortiz 2003; Tallamy et al. 2003; King and Fischer 2005; Cuatrecasas and Cordero 2006; Barbosa F, unpublished data).

One female reproductive process that might be influenced by male copulatory courtship is her cooperation in the formation of a copulatory plug. This study concerns factors related to the formation of copulatory plugs in the spider *Leucauge*

mariana (Tetragnathidae). Males of this species perform at least 8 types of nongenital behavior that appear to constitute copulatory courtship (Eberhard and Huber 1998). In some copulations, the female adds a clear liquid to the small blobs of white paste deposited on her genital plate (epigynum) by the male's genitalia (palps) near the openings of her insemination ducts; the mixture of male and female products forms a smooth, white copulatory plug (Eberhard and Huber 1998). Both the male-produced blobs of white paste and the finished white plugs contain sperm (Méndez 2004). In other copulations, the female does not add liquid, and the blobs of white paste end up being incidentally removed during the repeated insertion and withdrawal movements of his genitalia; in these cases, no plug is formed (Eberhard and Huber 1998). Plugs are rigid and adhere firmly to the epigynum. Successful copulations that fail to result in plugs are apparently common in the field, as 82.8% of 64 females collected with no plugs nevertheless had sperm in their spermathecae (Méndez 2004).

Some plugs are effective in preventing subsequent males from copulating. Of 29 males that attempted to copulate with an equal number of plugged females, only 34.5% succeeded in inserting at least one palp into an insemination duct; in each successful pair, penetration only occurred after the plug was broken or displaced during previous insertion attempts with the palps (Méndez 2004). Males use both scraping and prying movements of their palps in attempts to displace plugs.

Another type of covering seen on some female epigyna was a thin transparent sheet. Transparent sheets are fragile and are easily broken and displaced by males; transparent sheets are apparently produced by the female, as at least some females with these sheets lack sperm (Méndez 2004). Males often visit nonvirgin females in the field (Eberhard et al. 1993), and nonvirgin females mate readily in captivity, so females in nature are probably polyandrous. Females are able to control termination of copulation because just before the male begins to insert his palps, the female grasps his chelicerae with hers, locking the couple together; the female can interrupt copulation at any time by opening her fangs, and such separations sometimes occur (Eberhard and Huber 1998).

This study tested whether female cooperation in plug formation was correlated with any male behavioral and/or

Address correspondence to A. Aisenberg. E-mail: aisenber@iibce.edu.uy.

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morphological traits and thus whether the female might be exercising CFC on such male traits. Copulatory courtship by the male was thought to be an especially likely candidate and was checked carefully because of its otherwise paradoxical nature (why would the male court if copulation has already been achieved?). There are numerous other female reproductive processes that copulatory courtship might function to elicit (Eberhard 1996), so there was no strong prediction that any particular male trait should correlate with female cooperation in plug formation. We expected, however, that male traits would have positive rather than negative effects on female cooperation.

METHODS

Females were captured in the field as penultimate nymphs ready to molt (recognized by being accompanied by an adult male; Eberhard et al. 1993) and allowed to molt to virgin adults in captivity. Spiders were individually housed in cylindrical glass jars 5.5 cm in diameter and 12.5 high, with a small branch as support and a small wad of wet cotton. On days 0–5 after molting to maturity, each female was placed on silk lines that spanned a 22-cm diameter plastic plate with an adult male collected that same day and illuminated with a fiber optic lamp. If the female did not mate after being courted, she was presented with another male within 30 min and then with a third male if she still did not copulate. If a female rejected 3 males, she was discarded. On day 2 after mating with her first male, each female that lacked a plug was placed with another series of up to 3 males until she mated. Males were only used for a single copulation. The temperature averaged 20.21 ± 0.42 °C during the trials (range: 19–21).

Immediately after copulation, each female was observed and photographed as she rested on lines under a dissecting microscope to check for the presence of objects covering part or all of her epigynum (hereafter “presence of a plug”); she was checked again approximately 24 h later. We measured the width of the cephalothorax (a single sclerite whose size is unaltered by preservation in 70% alcohol) using a micrometer in the ocular of a dissecting microscope; specimens were later preserved in 70% ethyl alcohol. Female age is expressed below in number of days after the final molt. Voucher specimens (labeled as such) were deposited in the Museo de Zoología, Escuela de Biología, Universidad de Costa Rica.

Courtship and copulation behavior were recorded from the beginning of each interaction in an oblique frontal view with a SONY DCR TRV50 digital video camera equipped with +7 close-up lenses. Male behavior patterns prior to initiation of palpal insertion attempts were recorded from videotapes using “J Watcher” software (Blumstein et al. 2000) and were classified as in Eberhard and Huber (1998) (Table 1). Precopulatory behavior included jerk web, rock, vibrate abdomen, bicycle palps, twang or strum line with legs III, tap the web, and tap female with legs. As with most spiders, copulation in *L. mariana* involves repeated insertions (and insertion attempts) with both palps, one at a time. Insertion of palpal sclerites is accomplished by inflation of membranous areas (hematodochae). Copulation behavior included the following types of genital (palpal) movements: short insertion in the epigynum (only a single hematodochal inflation); long insertion in the epigynum (multiple hematodochal inflations during the insertion); long insertion with large inflations of the basal hematodocha; failed attempts to insert, in which the palp contacted but failed to snag on the female epigynum (“flubs with contact”); and failed attempts to insert in which the palp failed to contact the epigynum (“flubs without contact”). Nongenital movements that also occurred during copulation included bursts of abdomen vibration and bursts of

Table 1

Male precopulatory and copulatory courtship behavior patterns in the present study, following Eberhard and Huber (1998)

Male courtship behavior prior to copulation	Male courtship behavior during copulation	
	Genital	Nongenital
Jerk the web	Long insertions	Gently push the female's legs
Rock the body	Short insertions	Rock the body
Vibrate the abdomen	Flubs	Tap the female
Bicycle with palps		
Twang the line with legs III		
Tap the web		
Tap the female		

rhythmic gentle pushing of the female's legs with the male's legs (causing the female's legs to cede slightly but never to change their grips on the web; pushing occurred during both long and short palpal insertions).

Analyses were performed with Past Palaeontological Statistics, version 1.18 (Hammer et al. 2003) and NCSS 2001 (Copyright 2000 Jerry Hintze). Normally distributed variables (Shapiro–Wilk test) that showed homogeneity of variances (Levene test) were analyzed with Student's *t*-tests for independent samples. Other variables were analyzed with nonparametric Mann–Whitney *U* tests. We compared frequencies with chi-square tests for independent samples and Fisher's Exact probability tests or Mc Nemar tests for dependent variables. We performed logistic regressions and multivariate regression analyses. Data were log transformed before the analyses when appropriate.

RESULTS

Of 43 virgin females that copulated, 19 formed white copulatory plugs (7 observed immediately after copulation, 12 others 24 h later); 8 of those without plugs had transparent sheets. Of the 24 once-mated females lacking plugs, 18 mated a second time (of these, 5 had transparent sheets after the first copulation). Ten of these copulations resulted in plug formation (one seen immediately after copulation, 9 seen 24 h later); none had transparent sheets. First and second copulations did not differ with respect to the presence of white or transparent plugs (Mc Nemar test, $\chi^2 = 3.78$, degrees of freedom [df] = 1, $P = 0.06$). All copulations were apparently terminated by the female, when she released her hold on the male's chelicerae.

Variables not associated with plugs

Logistic regressions showed that in neither the first nor the second copulation was male size, female size, male size/female size, or female age as an adult (Table 2) related to the likelihood that a plug would be formed ($\chi^2 = 6.57$ and 2.68; df = 4 and 4; $P = 0.16$ and 0.61 for first and second copulations, respectively).

None of the precopulatory male courtship behavior patterns (Table 3) showed any correlation with the presence of a plug. Neither the number of bursts of behavior nor the rate at which bursts of behavior occurred was related to the presence of a plug (multiple logistic analyses including the 7 behavior patterns listed above gave $\chi^2 = 5.64$, df = 7, $P = 0.58$ for numbers and $\chi^2 = 5.14$, df = 8, $P = 0.74$ for rates in first copulations; $\chi^2 = 4.82$, df = 8, $P = 0.31$ and $\chi^2 = 13.1$, df = 8,

Table 2
The mean widths (in mm) \pm standard deviation of the carapaces of females and males that participated in copulations which did and did not result in copulatory plug formation

	First copulation		Second copulation	
	No plug	Plug	No plug	Plug
Female carapace width (mm)	1.66 \pm 0.16	1.72 \pm 0.19	1.80 \pm 0.20	1.63 \pm 0.16
Male carapace width (mm)	1.44 \pm 0.15	1.46 \pm 0.15	1.48 \pm 0.17	1.41 \pm 0.20
Male carapace width/female carapace width (mm)	0.86 \pm 0.14	0.88 \pm 0.15	0.84 \pm 0.14	0.82 \pm 0.23
Female age as an adult (days)	2.00 \pm 3.00	2.00 \pm 2.00	3.00 \pm 2.50	2.50 \pm 2.00

$P = 0.11$ for numbers and rates, respectively in second copulations). Similarly, there was no significant relation between the presence of a plug and the total duration of any of the courtship behavior patterns prior to copulation (multiple logistic analysis, $\chi^2 = 4.82$, $df = 7$, $P = 0.31$ for first copulations; $\chi^2 = 11.7$, $df = 7$, $P = 0.11$ for second copulations).

Behavioral variables associated with plugs

Two of the several male behavior patterns during copulation (Table 4) had strong associations with the presence of a plug (Table 5). For genital behavior, multiple logistic analysis of the presence of a plug in first copulations versus the number of long insertions, short insertions, and the total number of flubs (with and without contact with the female's epigynum) showed a highly significant positive relationship (first column in Table 5, A). This relation was due to a strong relationship with short insertions but not with long insertions or flubs. An alternative, bivariate analysis also showed an association with short insertions (Figure 1). The rates of these same variables (number/s) in first copulations (second column in Table 5, A)

showed a weaker relation to the presence of a plug. This relation was due to long insertions and flubs but not short insertions. The relative durations of these palpal movements were not related to plug presence (third column in Table 5, A). Similar analyses of numbers and rates in second copulations revealed no significant effects (Table 5, A).

With respect to nongenital copulatory courtship, a logistic regression analysis of first copulations that combined the numbers of several behavior patterns, including rocks, taps, pushes, and bursts of pushing showed a highly significant association; this association was due to pushes and bursts of pushing but not to rocks or taps (first column in Table 5, B). A similar set of relationships occurred in second copulations (rocks were not common enough to analyze). In addition, the total time spent performing nongenital copulatory courtship behavior was also associated positively with the presence of a plug (third column in Table 5, B) in both first and second copulations, in both cases because of an association with pushes. Leg pushes occurred during short and long insertions of the pedipalps, but the rhythm of leg pushes was different from that of palpal movements, so leg pushing was not just an incidental by-product of palpal movements.

Because there was a significant association between the number of pushes and the number of short insertions/copulation in first copulations ($R = 0.53$, $P = 0.001$) but not in second copulations ($R = 0.47$, $P = 0.15$), we performed a further multiple logistic regression to check whether the associations of these 2 variables with plug formation were independent of each other (Table 5, C). In both first and second copulations, the number of short intromissions and pushes showed significant, independent associations with the presence of a plug. Both the rate and the total duration of pushing also correlated with the formation of a plug (second and third columns of Table 5, C).

DISCUSSION

Leucauge mariana fulfills the criteria for CFC: 1) females in the field are apparently polyandrous, 2) behavior performed by the female after copulation begins has variable effects on the male's chances of reproduction (female participation that is necessary for plug formation is inconsistent and plug

Table 3
Numbers, durations, and rates of precopulatory male courtship patterns (medians \pm quartiles; the number of pairs in which each behavior occurred is in parentheses)

	Number		Duration (s)		Rate (n/s)	
	No plug	Plug	No plug	Plug	No plug	Plug
Prior to first copulation						
Jerk the web	1.00 \pm 4.00 ($n = 3$)	3.00 \pm 4.00 ($n = 2$)	6.00 \pm 12.00	31.00 \pm 56.00	0.33 \pm 0.17	0.22 \pm 0.25
Rock	7.00 \pm 10.00 ($n = 7$)	3.50 \pm 4.00 ($n = 10$)	46.00 \pm 81.00	10.55 \pm 17.00	0.15 \pm 0.17	0.32 \pm 0.33
Vibrate abdomen	5.00 \pm 4.00 ($n = 4$)	3.50 \pm 1.00 ($n = 2$)	3.00 \pm 5.00	68.50 \pm 129.00	0.78 \pm 1.47	0.39 \pm 0.72
Bicycle with palps	4.50 \pm 10.00 ($n = 8$)	3.00 \pm 6.50 ($n = 8$)	45.5 \pm 91.50	14.00 \pm 132.50	0.09 \pm 0.30	0.17 \pm 0.51
Twang	3.00 \pm 5.00 ($n = 21$)	4.00 \pm 7.00 ($n = 17$)	7.00 \pm 21.00	13.00 \pm 29.50	0.43 \pm 0.47	0.48 \pm 0.34
Taps on the web	8.00 \pm 9.50 ($n = 24$)	14.00 \pm 16.00 ($n = 19$)	15.00 \pm 29.50	31.00 \pm 49.00	0.50 \pm 0.36	0.45 \pm 0.40
Taps on the female	9.00 \pm 11.00 ($n = 23$)	9.00 \pm 7.00 ($n = 19$)	17.00 \pm 24.50	26.00 \pm 32.20	0.53 \pm 0.31	0.41 \pm 0.22
Prior to second copulation						
Jerk the web	6 ($n = 1$)	1.00 ($n = 1$)	20.00	1.00	0.30	1.00
Rock	2.00 \pm 3.00 ($n = 2$)	4.00 \pm 3.00 ($n = 5$)	5.00 \pm 16.00	23.00 \pm 23.00	0.50 \pm 0.69	0.43 \pm 0.81
Vibrate abdomen	1 ($n = 1$)	$\times \times \times$	3	$\times \times \times$	0.33	$\times \times \times$
Bicycle with palps	1.00 \pm 3.00 ($n = 3$)	3.00 \pm 4.00 ($n = 5$)	10.00 \pm 9.00	31.00 \pm 3.00	0.05 \pm 0.13	0.09 \pm 0.13
Twang	5.00 \pm 1.00 ($n = 5$)	4.00 \pm 4.00 ($n = 9$)	10.00 \pm 18.00	11.00 \pm 37.00	0.15 \pm 0.51	0.29 \pm 0.72
Taps on the web	10.00 \pm 12.00 ($n = 7$)	14.00 \pm 11.00 ($n = 10$)	34.00 \pm 34.00	32.00 \pm 92.00	0.24 \pm 0.25	0.27 \pm 0.32
Taps on the female	7.00 \pm 8.50 ($n = 8$)	10.00 \pm 7.00 ($n = 10$)	13.50 \pm 27.00	21.50 \pm 30.00	0.11 \pm 0.41	0.39 \pm 0.24

Table 4
Occurrence and duration of copulatory patterns (medians \pm quartiles; the number of cases that showed each behavior is in parentheses)

	Number		Duration (s)		Rate (n/s)	
	No plug	Plug	No plug	Plug	No plug	Plug
First copulation						
Short insertions	8.00 \pm 31.00 (<i>n</i> = 15)	71.00 \pm 52.00 (<i>n</i> = 18)	52.00 \pm 166.00	138.00 \pm 247.00	0.27 \pm 0.24	0.39 \pm 0.27
Long insertions	4.00 \pm 4.00 (<i>n</i> = 25)	6.00 \pm 4.00 (<i>n</i> = 18)	267.00 \pm 334.00	364.50 \pm 392.00	0.02 \pm 0.03	0.01 \pm 0.02
Flubs	9.50 \pm 37.00 (<i>n</i> = 20)	28.00 \pm 17.00 (<i>n</i> = 17)	41.00 \pm 67.50	44.00 \pm 39.00	0.34 \pm 0.21	0.48 \pm 0.20
Pushes	4.00 \pm 5.00 (<i>n</i> = 20)	35.00 \pm 23.00 (<i>n</i> = 17)	5.63 \pm 10.80	19.92 \pm 68.47	0.90 \pm 1.80	1.35 \pm 0.87
Bursts of pushes	2.00 \pm 3.00 (<i>n</i> = 20)	7.00 \pm 10.00 (<i>n</i> = 17)	5.63 \pm 10.80	19.92 \pm 68.47	0.61 \pm 0.61	0.32 \pm 0.28
Rocks	1.00 \pm 2.00 (<i>n</i> = 3)	2.00 \pm 17.00 (<i>n</i> = 3)	1.97 \pm 8.33	4.06 \pm 53.08	0.51 \pm 1.03	0.49 \pm 1.55
Taps on the female	×××	19.00 (<i>n</i> = 1)	×××	5.61	×××	3.39
Second copulation						
Short insertions	44.00 \pm 94.00 (<i>n</i> = 7)	141.50 \pm 212.00 (<i>n</i> = 10)	128.00 \pm 303.00	384.50 \pm 533.00	0.32 \pm 0.48	0.35 \pm 0.21
Long insertions	4.00 \pm 3.00 (<i>n</i> = 6)	2.00 \pm 2.00 (<i>n</i> = 9)	146.50 \pm 235.00	114.00 \pm 258.00	0.03 \pm 0.05	0.03 \pm 0.09
Flubs	47.00 \pm 168.00 (<i>n</i> = 7)	33.50 \pm 72.00 (<i>n</i> = 10)	112.00 \pm 258.00	82.00 \pm 146.00	0.42 \pm 1.14	0.68 \pm 0.31
Pushes	2.50 \pm 4.00 (<i>n</i> = 4)	33.00 \pm 35.00 (<i>n</i> = 9)	5.33 \pm 6.56	28.83 \pm 26.77	0.54 \pm 0.08	1.37 \pm 1.09
Bursts of pushes	2.00 \pm 3.00 (<i>n</i> = 4)	13.00 \pm 13.00 (<i>n</i> = 9)	5.33 \pm 6.56	28.83 \pm 26.77	0.41 \pm 0.20	0.32 \pm 0.17
Rocks	×××	×××	×××	×××	×××	×××
Taps on the female	1 (<i>n</i> = 1)	13.00 \pm 24.00 (<i>n</i> = 2)	1.90	24.00 \pm 44.00	0.53	0.52 \pm 0.04

The crosses (×××) indicate no values for that category.

formation affects the chances of sperm competition due to insemination by future males), and 3) the likelihood that the female will perform behavior that favors the male is affected by male traits (both genital and nongenital behavior during copulation). More pushing behavior and more short genital insertions by male *L. mariana* are associated with a greater probability that the female will add liquid to the paste deposited by the male and thus permit formation of a copulatory plug. Thus these behavior patterns appear to function to elicit favorable female responses, as has previously been supposed to be the case for copulatory courtship behavior (Eberhard 1991, 1994, 1996).

An alternative to CFC is sexually antagonistic coevolution (SAC) between males and females (Arnqvist and Rowe 2005). The 2 hypotheses differ in the reproductive payoffs that females are thought to gain from biasing male paternity (Chapman et al. 2003). The payoffs to female *L. mariana* from biasing plug formation are not known, so this difference between SAC and CFC cannot be evaluated confidently. However, one version of SAC, which emphasizes physical coercion of the female by the male (Alexander et al. 1997; Arnqvist and Rowe 2002a, 2002b), seems unlikely to apply in *L. mariana* because neither the pushes with the male's front legs nor the short insertions of his genitalia physically coerce the female. In addition, females showed no sign of resistance to these male behavior patterns, despite the fact that they can easily interrupt undesired coercion by breaking away from males at any time during copulation or by pushing the male's palp away from the epigynum with their tarsi (Eberhard and Huber 1998; Méndez 2004). A sensory trap version of SAC (Arnqvist 2006), which supposes that female cooperation with the male is not in her own reproductive best interests, but persists because the male uses a sensory trap, also seems unlikely (though it cannot be eliminated) because it depends on females having been unable to free themselves from these traps (Eberhard 2009). For instance, a female change in response threshold or context-specific changes in female responsiveness could free the female from maladaptive oversensitivity to male stimulation.

Larger numbers of short palpal insertions and greater amounts of time dedicated to these insertions were associated with increased chances of production of a copulatory plug. Short insertions are also associated, however, with deposition

of the male component of plug, so might seem possible that the production of plugs was associated with deposition of the male portion of plug material, rather than with the insertion behavior itself. An active female role is clear, however, because the smooth-surfaced objects we counted as plugs could not have been produced without the addition of female-derived liquid.

To our knowledge, this is the first demonstration of female participation in copulatory plug formation as a possible CFC mechanism and also the first demonstration of a correlation between the pattern of intromission behavior in a spider and a female response that may exercise CFC. This study thus confirms previous hypotheses regarding such correlations (Eberhard 1996; Stratton et al. 1996; Eberhard and Huber 1998). The possibility that palpal insertions might be under sexual selection as courtship signals was mentioned previously in studies of other spiders, based on divergence in closely related species (Eberhard 1996; Stratton et al. 1996; Huber 1998; Knoflach 1998). Another suggestion that palpal insertion patterns may be associated with possible CFC comes from the association of irregular patterns of insertion in a wolf spider with reduced suppression of subsequent female resistance to further copulations (González and Costa 2008). Intromission patterns are also known to influence female reproductive decisions in rodents and primates (Diamond 1970; Dewsbury 1972, 1988; Dixson 1998). In 2 species of pholcid spiders, the number and rate of squeezes with the palps (rather than insertions and withdrawals) affect paternity (Schäfer and Uhl 2002; Peretti et al. 2006); in one and perhaps both of these species, the effects are probably due to female rather than male sperm manipulation (Peretti A, Eberhard WG, unpublished data). Leg pushing during copulation occurs in 2 of 3 other species of *Leucauge* that have been observed (Eberhard 1994), but the descriptions are not sufficiently detailed to permit interspecific comparisons.

We did not determine whether the copulatory plugs we observed affected either the intromission attempts or the fertilization success of future mates. Males displaced the plugs of about 65% of females collected in the field (Méndez 2004), so probably only some of the plugs we observed would have been effective obstacles to mating. There are no data on sperm precedence in this species. Males show a preference for mating with virgin females (Eberhard et al. 1993), suggesting

Table 5

Results of multiple logistic regression analyses of relations between the presence of a white copulatory plug and (A) genital and (B) nongenital behavior of the male during first and second copulations; (C) presents tests of the independence of the effects of the numbers of pushes and of short insertions (“global analysis” refers to the result of a multiple regression including all the variables listed immediately below)

	Number (<i>n</i> /copulation) (χ^2 , <i>df</i> , <i>P</i>)	Rate (<i>n</i> / <i>s</i>) (χ^2 , <i>df</i> , <i>P</i>)	Duration (s unit/total s) (χ^2 , <i>df</i> , <i>P</i>)
A. Genital behavior during copulation			
First copulation			
Global analysis	6.2, 3, <0.0001	9.7, 3, 0.02	0.3, 3, 0.61
Long insertions	0.7, 1, 0.71	4.4, 1, 0.04	0.7, 1, 0.70
Short insertions	14.7, 1, <0.0001	0.05, 1, 0.83	1.1, 1, 0.26
Flubs	0.002, 1, <0.98	5.7, 1, 0.02	0.2, 1, 0.63
Second copulation			
Global analysis	1.2, 3, 0.28	3.3, 3, 0.35	0.3, 3, 0.63
Long insertions	0.7, 1, 0.64	0.7, 3, 0.39	0.01, 1, 0.92
Short insertions	1.6, 1, 0.20	0.01, 1, 0.91	0.59, 1, 0.44
Flubs	0.2, 1, 0.65	0.60, 1, 0.44	0.85, 1, 0.36
B. Nongenital behavior during copulation			
First copulation			
Global analysis	22.4, 3, <0.0001	2.8, 2, 0.25	12.5, 4, 0.006
Pushes	20.3, 1, <0.0001	0.02, 1, 0.90	12.5, 1, <0.0001
Bursts of pushing	12.7, 1, <0.0001	1.2, 1, 0.27	3.4, 1, 0.06
Rocks	2.4, 1, 0.12	— ^a	1.8, 1, 0.18
Taps	1.7, 1, 0.20	— ^a	1.3, 1, 0.25
Second copulation			
Global analysis	14.6, 3, 0.002	5.1, 2, 0.08	15.6, 3, 0.001
Pushes	20.3, 1, <0.0001	4.7, 1, 0.03	9.9, 1, 0.002
Bursts of pushing	8.7, 1, 0.003	0.02, 1, 0.88	8.1, 1, 0.004
Rocks	— ^a	— ^a	— ^a
Taps	1.2, 1, 0.28	— ^a	1.2, 1, 0.28
C. Short insertions and pushing behavior			
First copulation			
Global analysis	28.3, 2, <0.0001	112.8, 2, 0.002	13.3, 2, 0.003
Pushes	20.3, 1, 0.01	12.2, 1, 0.001	12.2, 1, 0.005
Short insertions	14.7, 1, <0.0001	2.6, 1, 0.11	1.41, 1, 0.26
Second copulation			
Global analysis	12.9, 2, 0.001	11.5, 2, 0.003	10.1, 2, 0.01
Pushes	12.6, 1, <0.0001	11.4, 1, 0.001	9.9, 1, 0.002
Short insertions	1.64, 1, 0.02	0.01, 1, 0.91	0.6, 1, 0.44

^a Many values were missing because of 0 values (and thus nonexistent quotients), so calculations were omitted.

that the first male to mate with a female probably tends to fertilize a substantial fraction of her eggs. However, the production of copulatory plugs, the ability of males to remove plugs (Méndez 2004), and the presence of a male genital trait (the hooked conductor) apparently specialized to remove

such plugs (Méndez V, Eberhard W, unpublished data) suggest that first male precedence is not absolute. Thus, the failure to induce the female to help form a plug probably reduces a male's expected reproduction.

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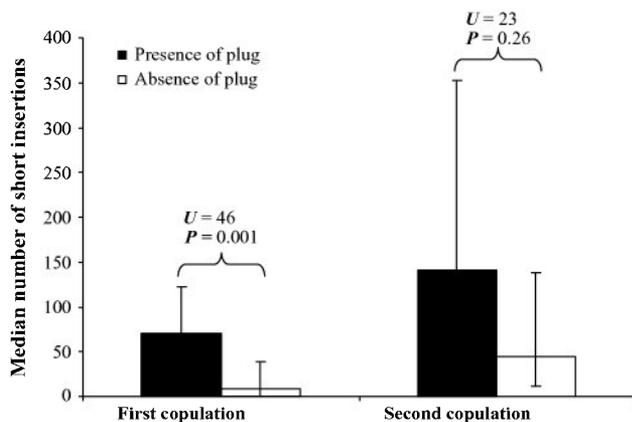


Figure 1
Number of short insertions in copulations with (black) and without (white) plugs. Data are presented as medians and quartiles, and results of the comparisons are shown with the *U* coefficient and corresponding probability value (Mann–Whitney *U* test).

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