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Tuning in to the male: evidence contradicting sexually antagonistic coevolution models of sexual selection in *Leucauge mariana* (Araneae Tetragnathidae)

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Male courtship signals often stimulate female sense organs whose sensitivities originally evolved under natural selection. In classic female choice models of sexual selection, females can benefit from increasing their sensitivity and responsiveness to male stimuli; but in sexually antagonistic models, increased female sensitivity or responsiveness to males could increase the costs that females are thought to suffer from male stimulation. We tested these contrasting expectations in a previously discovered sexually dimorphic trait in the spider *Leucauge mariana*: 12 elongate setae on the female's sternum that are deflected by movements of the male's cheliceral fangs during copulation. By removing these setae, we tested whether this stimulation induces female responses that favor the male's chances of paternity. We found that these stimuli induced females to add a product crucial to the formation of copulatory plugs. These results do not fit expectations from sexually antagonistic coevolution models of sexual selection. This conclusion resembles the heretofore unappreciated implications of specialized female sense organs in other species, and of the temporary increases in female sensitivities and responsiveness to male stimuli during the breeding season in female fish and frogs.

KEY WORDS: female choice, sensory blinding, tactile stimulation, male copulatory courtship, copulatory plug.

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INTRODUCTION

Sexual selection on males often results in the evolution of male courtship signals that exploit pre-existing female traits that originally arose under natural selection; these include both the female's sensory abilities (determined by properties of her sense organs), and her physiological and behavioral responses to these types of stimuli (determined by properties of her central nervous system) (e.g., Ryan 1990; Andersson 1994; Christy 1995). Classic sexual selection by female choice that occurs either prior to copulation or during and following initiation of copulation (cryptic female choice or CFC) can favor both superior male signalers, and females that are better able to sense and to respond to stimuli from the male in ways that favor the male's paternity (Andersson 1994; Eberhard 1996). One mechanism by which a female can improve her ability to make advantageous choices among males is to increase her sensitivity to the stimuli produced by those males best able to trigger favorable female responses. Classic sexual selection by female choice is thus compatible with the existence of specialized female abilities to sense and then to respond positively to male courtship stimuli.

In contrast, the alternate sexually antagonistic coevolution (SAC) models of sexual selection propose that male-induced responses in the female reduce the female's reproduction, lowering the number of her surviving offspring (Holland & Rice 1998; Chapman et al. 2003; Arnqvist & Rowe 2005; Arnqvist 2006). Take, for instance, a male genital structure that induces the female to discard sperm from previous males by pressing against a receptor in her oviduct that senses each egg as it descends, and induces her spermatheca to move sperm into her oviduct (where the male can then remove them) (Córdoba-Aguilar 1999). This female response to the male's stimulation could be disadvantageous for the female, depriving her of the chance to have more genetically diverse offspring (Arnqvist 2006). Selection under SAC would tend to favor reductions rather than increases in the female's sensitivity and/or in her responsiveness to these male courtship stimuli. Some female sensitivity and responsiveness to the male stimuli could be maintained, however, by selection favoring egg fertilization in the context of normal oviposition, thus placing the female in a "sensory trap" (Christy 1995; Córdoba-Aguilar 1999). The receptor in the female's oviduct, and the connections in her nervous system that result in her responding to stimulation of this receptor by releasing sperm, are likely to persist due to the advantage of responding to stimulation of this receptor in order to fertilize eggs as they descend. Only the minimum sensitivity to guarantee fertilization would be expected, however, not increases in sensitivity; as noted by Arnqvist (2006) "females that are more responsive to male exploitation may suffer direct costs from their sensory bias".

In many species in which the female sense organs that perceive male stimuli are sight or hearing, which are crucial in other contexts such as foraging or defense, the contrasting expectations between CFC and SAC models regarding increases in female sensitivity and responsiveness to male stimuli are difficult to test by experimentally reducing female sensitivities. Male stimulation of the female via her sense of touch is different. The models' predictions regarding female sensitivity and responsiveness to tactile stimuli can be easier to evaluate experimentally when the relevant female sense organs are restricted to the particular portions of her body that the male touches, and when these sense organs have only minor effects on other important female processes such as foraging or defense. This study involves such a test of tactile stimulation in a spider.

In spiders, as in many animals, tactile non-genitalic stimulation during copulation is inevitable because the male contacts specific sites on the female's body during copulation. Searches for the female receptors involved in eliciting responses to copulation can thus be concentrated in these areas of contact (Eberhard 1985, 1996). Natural selection will often favor both female sensory responses to male stimuli that are associated with copulation, and also female behavioral or physiological responses to this stimulation, such as sperm transport, sperm storage, egg maturation and ovulation, oviposition, and lack of receptivity to further matings (Eberhard 1996). These naturally selected female responses to copulation stimuli are likely to also be beneficial to the male's chances of paternity (e.g., to increase sperm transport, to increase her tendency to oviposit, to increase her resistance to future males). A female may thus be "preadapted" by natural selection to be exploited by males that are competing via sexual selection to father her offspring (Eberhard 1985, 1996).

Comparisons between male and female morphology can be used to deduce the likelihood that particular female sense organs function as receptors for tactile stimuli from the male. For instance, if the female has denser or otherwise more elaborate tactile sense organs in an area that is contacted by the male compared with other similar parts of her own body or with the same part on the male's body, she is likely to be especially able to perceive male tactile stimulation. Female sensitivity is especially feasible to study in spiders and other arthropods, because they have easily observable sensory structures such as setae and other specialized sense organs on the outer surface of the body (Barth 2002). Evidence from comparative morphology is especially useful when tactile stimuli from the particular portion of the female's body that the male touches appear to not to have special selective significance in any other context. Previous studies that have used the numbers, sizes and locations of female tactile sense organs as guides to indicate female perception of male tactile stimuli on the female pronotum and oviduct of damselflies (Robertson & Paterson 1982; Córdoba-Aguilar 1999; Barnard & Masly 2018), the wings of female sepsid flies (Eberhard 2001, 2003), the internal female genitalia of katydids (Wulff et al. 2015, 2018), and the female chelicerae and sternum of a spider (Aisenberg et al. 2015a).

Confident deductions concerning sexual selection cannot be made, however, from such studies of comparative sensory morphology unless they are accompanied by demonstrations that females respond to male stimulation of the female sense organs by altering their reproductive behavior, and that these responses are likely to increase the male's chances of paternity. Demonstrations can involve experimental alteration of male contact structures (e.g., Loibl 1958; Robertson & Paterson 1982 on damselflies), or masking of female receptors; the latter technique is particularly powerful because it controls for possible changes in male behavior that may result from manipulation of his contact organ. Female sensitivities and responses to tactile stimulation from species-specific male structures have been demonstrated by experimental masking of female receptors in tsetse flies, a bush cricket, a sepsid fly, and a spider (Eberhard 2001, 2003; Briceño & Eberhard 2009a, 2009b; Aisenberg et al. 2015a; Wulff et al. 2018).

Are these types of female responses to stimulation of sexually dimorphic sense organs exceptional, or are they common, as would be expected under the CFC hypothesis (Eberhard 1996)? The present study is an experimental test in another case, the spider *Leucauge mariana*. The female possesses hundreds or perhaps thousands of setae that cover her entire body surface. Among these setae is a small set of elongate setae on the anterior margin of her sternum (Fig. 1a-b) that are proportionally longer

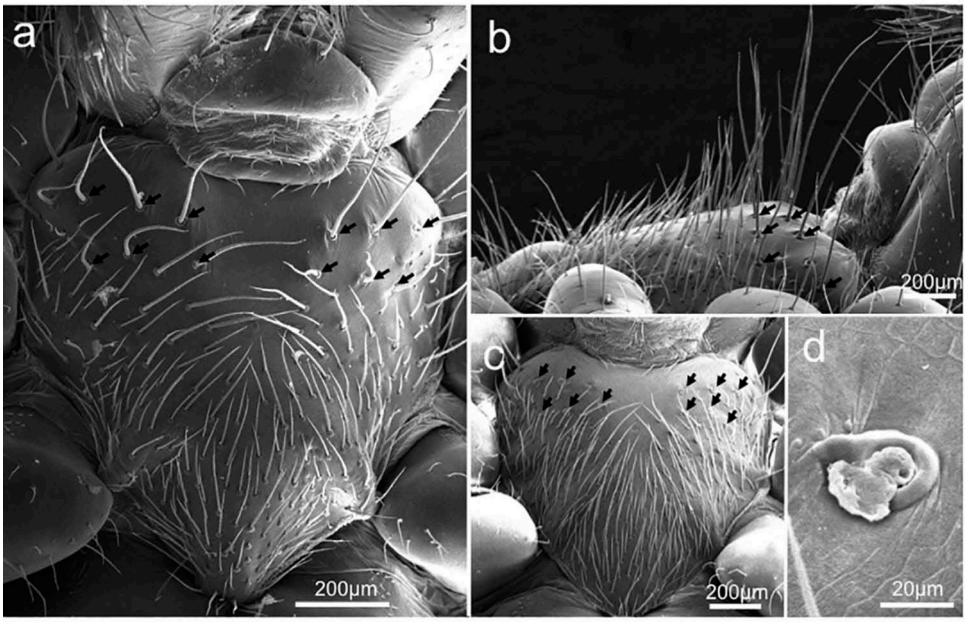

Fig. 1. — The elongate setae (arrows point to their bases) on the anterior margin of the sternum of a control female of *Leucauge mariana* are seen in ventral (a) and lateral (b) view. The empty sockets left on an experimental female when these setae were removed are shown in (c) and (d).

than the corresponding setae at the same site in males; during copulation, males contacted these elongate female setae with rhythmic movements of their fangs (Aisenberg et al. 2015a).

We used details of the normal mating behavior of *L mariana* to test predictions of CFC and SAC. Copulation typically includes multiple insertions of the male's palps while the female clasps the male's chelicerae. Palpal insertions never occur if the female has opened her chelicerae and ceases to clasp the male, though occasionally she releases and then re-clasps him and he makes further palpal insertions. Copulatory plugs are formed on the female's genitalia (epigynum) following some but not all copulations (Eberhard & Huber 1998), and increased male courtship during copulation (greater numbers of rhythmic pushes on the female's legs) is associated with increased chances that the female would cooperate in plug formation (Aisenberg & Eberhard 2009). The male and female both contribute materials to the plug; no plugs can be formed without the female contribution, which is a clear liquid in which the small masses of the white sticky paste deposited by the male are dispersed to cover the epigynum surface (Eberhard & Huber 1998). Without the female liquid contribution, the male's small masses are removed from the epigynum during subsequent palpal insertions. Copulatory plugs sometimes (but not always) mechanically prevent palpal insertions by subsequent males (Méndez & Eberhard 2014).

If stimulation of the female's modified sternal setae by the male's fang movements induces a female response that favors the male's reproductive interests, then the increased sensitivity to stimuli from the male fang movements that results from the extra length of these setae in females would have an important implication for sexual

selection theory: the specialization of these setae to increase the female's sensitivity to male stimulation would be compatible with classic female choice hypotheses, but it would not fit expectations of the SAC hypothesis. To date, however, it is not known whether male contact with these sternal setae is sensed by the female, or whether such contact affects any aspect of the female's reproductive behavior. This study tested these possibilities. We expected that if the elongate female setae are involved in classic sexual selection by female choice, females would be sensitive to male stimulation of the setae, and that they would respond to it by altering their reproduction in ways favoring the male's chances of paternity. On the other hand, under sexually antagonistic male-female coevolution (SAC) hypotheses, in which females are thought to coevolve with males so as to reduce their responses to male stimulation because responding to male stimuli reduces the female's reproduction, the elongate female setae would not be expected to trigger female reproductive responses that favor the male's reproductive interests. We also discuss other data indicating that an alternative hypothesis, selection for species isolation, is not involved.

MATERIALS AND METHODS

Natural history background

The sexual biology of *L. mariana* was reviewed by Aisenberg et al. (2015b), and is summarized briefly here. Males wait on the webs of penultimate instar females that are about to molt, and mate with them soon after they molt; they also mate with older mature females. Females mate multiple times. Mating typically lasts 10–15 min. After having courted the female at a distance, vibrating the web and tapping the female with stylized leg and body movements (Aisenberg & Eberhard 2009), the male brings his chelicerae close to those of the female. When receptive, the female turns toward the male, bends her abdomen ventrally, and opens her fangs and clasps the basal segment of each male chelicera for several minutes while the male introduces his palps repeatedly into her external genitalia (epigynum) (Eberhard & Huber 1998). Palpal insertions never occur if the female releases her clasp on the male's chelicerae. Occasionally, however, a female releases and then re-clasps the male and he then performs further palpal insertions. The male often opens and closes his cheliceral fangs rhythmically while the female is clasping his chelicerae; when opened, his fangs sometimes deflect the tips of elongate setae on the anterior margin of her sternum (Aisenberg et al. 2015a, 2015b). Spider setae are generally innervated in the base (Barth 2002), so these deflections probably result in tactile stimuli being perceived by the female.

A copulatory plug that contains material from both the male and the female is deposited on the female's paired genital openings during the final stages or immediately following some (but not all) copulations (Eberhard & Huber 1998; Aisenberg & Eberhard 2009). Males mating with plugged females sometimes succeed and sometimes fail in attempts to penetrate or pry off copulatory plugs and insert their palps (Méndez & Eberhard 2014).

Collecting and housing

Virgin females were raised from subadults collected in the field while resting on webs accompanied by males in the Reserva Biológica Leonel Oviedo and the Instalaciones Deportivas of the Universidad de Costa Rica, San José Province, Costa Rica (9º54´N, 84º03´W; elevation 1200 m), from 18 June 2015 to 13 January 2016. We housed subadult and mature females individually in plastic cups with a small branch and wet cotton, and checked the subadults once daily for molting to adulthood.

Experimental design

Mature males were collected in the field on the day of the trial, and were paired randomly during the day with females. Females were first exposed to a male 2 to 6 days after they molted to maturity. To test the effects of male stimulation of the female's dimorphic sternal setae on female behavior, we created two experimental groups: a male with a female whose 12 long sternal setae (the six long setae in the middle of the first row plus the six on the middle of the second row) had been removed (treatment) (N = 19); and a male with an unmodified female under similar conditions (N = 18) (control) (Fig. 1). One day before a trial, we immobilized the female between two blocks of foam rubber for 5–10 min under a dissecting microscope; in the treatment group we used a fine forceps to pluck the 12 long setae (six on the left and six on the right with respect to her longitudinal axis) on the anterior portion of her sternum (Fig. 1c-d). Only tiny amounts of hemolymph were lost as a result (Fig. 1d), and we saw no other sign of injury or obvious impairment in treated females. We immobilized control females in the same way for similar lengths of time, but we did not remove any setae.

On the day of the trial, we placed each female on a newly-built, field-collected orb web of a mature *L. mariana* female that was attached with masking tape to the borders of a plastic plate 22 cm in diameter and 2 cm deep, as in previous studies of mating (Aisenberg 2009; Aisenberg & Eberhard 2009; Méndez & Eberhard 2014; Aisenberg et al. 2015a). We illuminated the test arena during the sexual encounters with a LED lamp. After 5–10 min, when the female was at or close to the hub, we gently placed a mature male on a radius, at least 6 cm from the female.

We ended a trial if any of the following occurred: one of the spiders abandoned the web; 30 min passed during which the male courted but there was no cheliceral clasp; the male remained motionless for more than 15 min after at least one cheliceral clasp had occurred. We used each male for only a single trial. If the female did not mate, we presented one or two additional males to her on the same day. We checked the female's epigynum under the dissecting microscope for mating plug material immediately after each mating. We checked her again for a mating plug 24 hr after her first mating, and then exposed her to a second male. If she mated again, we also checked her epigynum for a mating plug immediately after this second mating ended, and 24 hr later. We deposited voucher specimens in the Museo de Zoología, Escuela de Biología, Universidad de Costa Rica.

Data recording

We made two video recordings of all first matings of females. Details such as male cheliceral movements and attempts to insert the palps were recorded with a Dino-Eye Eyepiece digital color camera (Model AM423X) attached to the ocular of a Wild Model M3Z dissecting microscope (Wild Company, New York, USA). Spiders copulated with their ventral sides approximately upward, so these details were usually visible. Simultaneously, a SONY HDR SR11 digital video camera (SONY, San Diego, CA, USA) equipped with + 4 close-up SONY lenses recorded more general movements such as leg movements and palpal insertions.

We used the video recordings to register the numbers and durations of several aspects of female and male mating behavior (Table 1); most of these behaviors were chosen on the basis of their having been shown to have significant effects on the outcomes of sexual interactions in previous studies of this species (Aisenberg et al. 2015b). Data on all variables were taken blind, without knowledge of whether the female was in the control or experimental group. We followed the terminology used in previous studies (Eberhard & Huber 1998; Aisenberg & Eberhard 2009). The initiation of pre-copulatory courtship was defined as the moment when the male first shook the web (strong, abrupt flexions performed by his anterior legs without releasing the silk lines). Pre-copulatory courtship was defined as ending when the female first clasped the male's chelicerae. An "interruption" of mating occurred when the female released her cheliceral clasp and the two animals pulled apart, but subsequently engaged in another cheliceral clasp. We distinguished male-initiated interruptions (the male began struggling before the clasp ended) from female-initiated interruptions (the female moved first to end the cheliceral clasp) when it was possible to

Table 1.

Predictor and response variables included in the MCMC glmm models.

Variable	Definition	Type
Treatment	Control or experimental	Predictor
Mean duration long palp insertions	Total duration/*N* periods	Predictor
Rate long palp insertions	*N* long insertions/total duration	Predictor
Mean duration short palp insertions	Total duration/*N* periods	Predictor
Rate of short palp insertions	*N* short insertions/total duration	Predictor
Mean mating duration	Duration of engagements/*N* engagements	Predictor
Courtship duration	Time from beginning of first engagement to the end of the last	Predictor
Plug formation	Plug formation 24 hr after copulation	Response
Immediate plug formation	Plug formation right after copulation	Response
Re-mate	Second mating after 24 hr	Response
Interruptions by female	*N* copulation interruptions by female	Response
Proportion of interruptions by female	*N* copulation interruptions by female/total *N* of interruptions	Response

judge these details. All interruptions required participation by the female (she had to open her fangs to release her clasp of the male's chelicerae); but male participation was not necessary, and sometimes the male remained immobile while the female pulled away from him. The duration of mating was measured from the beginning of the first cheliceral clasp to the end of the last clasp. As in previous studies (Eberhard & Huber 1998; Aisenberg & Eberhard 2009), we defined long palpal insertions as those in which there were multiple hematodochal inflations during the insertion, and short insertions as having only a single inflation.

Statistical analysis

We used Markov chain Monte Carlo Model (MCMCglmm) analyses of the effects of several behavioral variables on three binomial response variables (plug formation immediately following copulation, plug formation 24 hr after copulation, and female remating) and two discrete numerical, Poisson distributed variables (number of interruptions by the female, proportion of the interruptions initiated by the female) (Tables 2–4). We performed two sets of analyses regarding plug formation. In the most conservative "single-variable" set, we included only the experimental treatment variable as a predictor; this represented the most conservative test of the effects of the treatment, as it did not compensate for the effects of any other predictor variables. In the second "multi-variable" set of analyses, we attempted to find the multi-variable model that best explained the variation in plug formation. We included in these models only those predictor variables for which it seemed possible that their duration or frequency could affect the response variables. For instance, to test the effects on "immediate plug formation" we only included those variables that occurred prior to this event. We included palp behavior such as insertions, because the palps deposit plug material; and we included both the duration and the rate of palpal insertions because these two variables were not correlated with each other. We performed separate tests of whether the relative duration of male cheliceral fang movements during copulation was affected by the female condition (experimental vs control); we did not include this variable in the models because the data were incomplete (the male's cheliceral fangs were not

Table 2.

Effect of group treatment and behavioral variables in MCMC models on (A) the number of interruptions of copulations initiated by the female, and on (B) the proportion of interruptions of copulations that were initiated by females: female-initiated/(female-initiated + male-initiated).

Factor	Posterior mean	Confidence interval	*P*MCMC
A - Number of interruptions (treatment and behavioral variables)			
Intercept	0.75	– 4.20::4.52	0.674
Experimental control	– 0.41	– 2.06::0.76	0.518
Mean duration long insertions	0.00	– 0.01::0.02	0.609
Rate long insertions	– 0.13	– 3.00::2.75	0.946
Mean duration short insertions	– 0.01	– 0.03:: 0.01	0.401
Rate short insertions	– 1.33	– 5.19::2.16	0.416
Mean mating duration	– 0.01	– 0.02::– 0.00	< 0.001
Courtship duration	0.00	– 0.00::0.00	0.081
B - Proportion of interruptions (treatment and behavioral variables)			
Intercept	2.18	1.53::2.83	< 0.001
Experimental-control	– 0.215	– 0.52::– 0.11	0.216
Mean duration long insertions	0.00	– 0.00::0.00	0.612
Rate long insertions	– 0.02	– 0.48::0.45	0.912
Mean duration short insertions	– 0.00	– 0.00::0.00	0.648
Rate short insertions	– 0.32	– 0.87::0.33	0.314
Mean mating duration	– 0.00	– 0.00::0.00	0.764
Courtship duration	– 0.00	–0.00::0.00	0.158

always in good focus or at the right angle to observe their movements). We used the library MCMCglmm in R statistical language, version 3.0.3 (R Development Core Team 2013) for all analyses.

RESULTS

Male fang movements

During copulation males often opened their cheliceral fangs repeatedly; in some videos it was possible to see that some fang movements deflected the long setae on the anterior portion of the female's sternum, as was reported previously in this species (Aisenberg et al. 2015a). Some fang movements occurred when the male's fangs were out of range of the female's setae; it was clear that the extra length of the female sternal setae increased the proportion of male fang movements that deflected them. Males paired with control and experimental females spent similar proportions of the time opening their fangs this way (compared with the total time during which it was possible to see the cheliceral fangs with sufficient clarity): the average proportion of time spent moving their fangs in males paired with control females was 0.81; in males paired with treatment females it was 0.82 (P = 0.92).

Table 3.

Effects in MCMC models of group treatment and behavioral variables on plug formation (A and B) and in MCMC models of plug formation with treatment only included (C and D). Analyses A and C concern plugs observed immediately after copulation; B and D concern plugs observed 24-hr after copulation.

Factor	Posterior mean	Confidence interval	*P*MCMC
A - Immediately after copulation (treatment and behavioral variables)			
Intercept	26.51	17.86::34.94	< 0.001
Experimental-control	– 21.82	– 27.98::– 14.15	< 0.001
Rate long insertions	– 6.92	– 13.91::– 0.75	0.028
B - 24 hr after copulation (treatment and behavioral variables)			
Intercept	36.71	16.58::60.45	< 0.001
Experimental-control	– 23.69	– 34.65::– 13.74	< 0.001
Mean duration long insertions	– 0.21	– 0.32::– 0.12	< 0.001
Rate long insertions	–11.64	– 22.13::– 1.74	0.028
Rate short insertions	37.06	15.53::65.5	< 0.001
Mean mating duration	0.049	0.02:: 0.08	< 0.001
Courtship duration	– 0.01	– 0.03::– 0.00	0.008
C - Immediately after copulation (treatment only)			
Intercept	9.474	2.84:: 19.83	< 0.001
Experimental-control	– 9.10	– 19.41::– 2.04	< 0.001
D - 24 hr after copulation (treatment only)			
Intercept	3.76	1.62::6.46	< 0.001
Experimental-control	– 3.08	– 5.7::– 0.29	0.004

Table 4.

(A) Effect of group treatment and behavioral variables in MCMC models of female re-mating 24 hr after the first copulation including all variables (A) and including treatment only (B).

Factor	Posterior mean	Confidence interval	*P*MCMC
A - Treatment and behavioral variables			
Intercept	– 0.49	– 0.10::– 0.32	0.032
Experimental-control	1.09	– 0.69::2.94	0.240
Mean duration long insertions	0.01	– 0.00::0.04	0.136
Rate long insertions	2.75	– 0.27::6.97	0.086
Courtship duration	0.00	– 0.00::0.00	0.076
B - Treatment only			
Intercept	0.30	– 0.76::1.52	0.628
Experimental-control	0.37	– 1.35::1.95	0.654

Copulation interruption

Interruption of the copulation was sometimes initiated by the male, and sometimes by the female; some copulations were interrupted several times. Neither the number of interruptions initiated by the female (Control: 16, Treatment: 17; Table 2A) nor the proportion of interruptions that were initiated by the female (female-initiated interruptions/male + female-initiated interruptions) (Control: 0.24, Treatment: 0.30; Table 2B) differed between control and experimental females. The mean duration of mating correlated negatively with the number of interruptions initiated by females, independent of the treatment group (Table 2A). Other behavioral traits did not have significant effects on the numbers or the proportions of interruptions initiated by the female.

Copulatory plugs

Copulatory plugs were observed immediately after copulation significantly less frequently with experimental females than with control females in both multi-variable and single-variable analyses (Tables 3A, C, 4; Fig. 2). Females whose partners performed lower rates of long insertions had a somewhat higher probability of producing plugs immediately after copulation independently of the treatment (Table 3A). The difference between control and experimental females was independent of the effect of other factors (Table 3C).

Copulatory plugs were observed 24 hr after copulation significantly less frequently with experimental females than with control females in both the multi-variable and single-variable analyses (Table 3B, D; Fig. 2). Plugs were also more common when the males had shorter periods of long insertions (Table 3B), shorter courtship and mating durations (Table 3B), when the rates of short insertions were higher (Table 3B), and when the periods of engagement were longer (Table 3B). The difference between control and experimental females was independent of the effect of other factors (Table 3D).

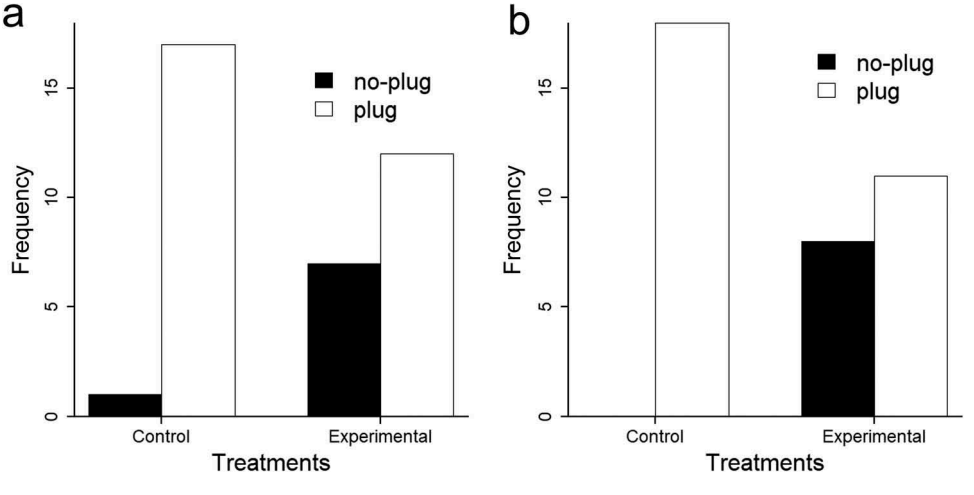

Fig. 2. — Frequency of which plugs were present immediately after copulation (a) and 24-hr later (b) in control and experimental females of *Leucauge mariana*.

Re-mating

Re-mating did not differ between control and experimental females in multi- or single variable analyses (Table 4A-B). More than half of both the experimental ($N = 12$) and the control females ($N = 10$) re-mated (clasped the male's chelicerae and allowed pedipalp contact with her epigynum) when courted by a different male 24 hr after the first copulation; none of the behavioral variables correlated with re-mating probabilities (Table 4A).

DISCUSSION

A female *L. mariana* spider undoubtedly senses stimuli from the male from both web vibrations and direct body contact prior to clasping him, and she then receives more tactile stimuli from several parts of her body while she clasps him. The present study showed that despite this storm of stimuli, the absence of sensations from just a few setae near the anterior edge of her sternum (12 among hundreds or perhaps thousands of setae on her body) was enough to sharply reduce the likelihood that she would form a copulatory plug. The fact that removal of these sexually dimorphic, elongate sternal setae altered her behavior supports the hypothesis that the function of their elongation is to increase her sensitivity to stimuli from the male during copulation. As noted above, a sense organ specialized for this sort of function is compatible with classic female choice models of sexual selection. The stimulation occurs during rather than preceding copulation, so this selection in *L. mariana* could involve cryptic female choice rather than pre-copulatory choice. By sensing the male's fang movements and then responding in a way that improves his chances of paternity (forming a copulatory plug), the female could gain through having sons particularly likely to move their fangs. She could also benefit from depositing a copulatory plug by filtering future mates on the basis of their abilities to remove plugs; this hypothesis could explain why females were receptive to remating despite having plugs.

One limitation of our study is that the pattern of sperm precedence is not known in this species. There would be no chance of any type of post-copulatory sexual selection (CFC or SAC) if second males always sired either 0% or 100% of the female's offspring. In such cases, the tendency of females to contribute to plug formation that we have documented here would be reproductively irrelevant for the male. Neither of these possibilities is in accord, however, with other data on the natural history and behavior of *L. mariana*. The behavior of males indicates that the last male to mate with a female does not obtain 100% paternity: males in the field seek out, accompany, and fight over immature, penultimate females to be the first to mate with them (Eberhard et al. 1993); and males leave almost immediately after copulating with newly molted females (Eberhard et al. 1993; W. Eberhard unpublished data). The opposite possibility, 100% paternity for the first male to mate with the female, is also unlikely: males mate readily with previously mated females in captivity (Méndez & Eberhard 2014) and in the field (A. Aisenberg & W. Eberhard unpublished data); and copulatory plugs sometimes fail to impede subsequent intromission attempts (Méndez & Eberhard 2014). In addition, the fact that males deposit plug material during copulation (Eberhard & Huber 1998) also suggests at least some paternity for subsequent males, because selection would cease to favor plugs if they were always completely effective in preventing subsequent inseminations (Parker 1984). The plugs of *L. mariana* could benefit males even though they are only partially effective in impeding sperm transfer by future males; they might also be favored by any of the other

possible advantages of copulatory plugs in spiders (Huber 2005; Uhl et al. 2010; Kuntner et al. 2012).

One possible alternative to the CFC hypothesis just described is that the elongate setae are part of a species isolation mechanism used by females to avoid insemination by males of other species. We believe that this function is less likely, because stimulation of these setae occurs only at an advanced stage in male-female interactions, after the elaborate pre-copulatory courtship behavior; this behavior differs in each of the three species of *Leucauge* that have been observed (*L. mariana*, *L. argyra*, and *L. venusta*, Aisenberg et al. 2015b). Several types of active female cooperation behavior, including turning to face the male, opening her chelicerae, lowering her body and spreading her legs, and bending her abdomen ventrally, all follow this courtship and precede the male fang movements, and must occur for copulation to occur (Aisenberg et al. 2015b). Postponing species discrimination until this late stage, after sperm transfer has already begun and cross-fertilization could occur, instead of earlier (prior to sperm transfer) would be disadvantageous for the female. No data are available regarding male cheliceral movements during copulation in other species of *Leucauge*.

A second alternative hypothesis, that sexual selection due to sexually antagonistic coevolution (SAC) has played an important role in the evolution of *L. mariana* sexual behavior, was tested more directly by our data and rejected. As noted above, female organs that are specialized to increase her sensitivity to male manipulative stimuli are not expected in SAC models. The female is thought to evolve to either reduce her sensitivity or her responsiveness to male stimuli, rather than to facilitate the reception of stimuli from the male, because her responses to the male stimuli alter her reproductive processes in ways that are disadvantageous to her (Holland & Rice 1998; Arnqvist & Rowe 2005). In other words, the fact that sternal setae have evolved to be longer in the females of *L. mariana* and that they trigger female reproductive responses that favor the male's reproduction indicates that sensing the stimuli from the male's cheliceral fangs was not disadvantageous for the female, contrary to SAC arguments (Holland & Rice 1998; Rosenthal & Servedio 1999). The fact that improved female sensitivity evolved in these spiders indicates that, on balance, the ability to sense male cheliceral movements was instead advantageous for females.

This logic also applies in a heretofore unappreciated way to other morphologically specialized female sensory structures that increase the female's ability to sense male stimuli, such as for instance the abundant tactile sense organs, including Ruffini's corpuscles, Meissner corpuscles, Pacinian corpuscles in the external genitalia and the distal anterior vaginal wall of female *H. sapiens* (Cold & Taylor 1999; Rogers 2001; Komisaruk et al. 2011). Other mammal species show similar enervation of female genitalia (Martin-Alguacil et al. 2018). Physiological changes that increase the female's ability to sense male courtship stimuli during the mating season have also been documented in other species, and these have similar implications for SAC. Temporary increases in female sensitivity to male signals that occur during the mating season were discussed by Maney and Pinaud (2011): "a growing literature supports the hypothesis that in many species, from fish to birds to humans, steroids may alter sensory processing, thus affecting the way sensory signals are perceived". Several of these steroid-induced alterations potentiate female reception of stimuli from males. Females of the Tanganyikan ciclid *Astatotilapia burtoni* were 2 to 5 times more sensitive to low frequency sounds (in the spectral range of male courtship sounds) when they were in the breeding phase, as contrasted with the subsequent mouth-brooding, parental phase (Maruska et al. 2012). In the plainfin midshipman fish *Porichthys notatus*, the auditory sensitivity of

females to the frequencies of conspecific male songs peaked during the breeding season (when plasma concentrations of testosterone or 17ß-estradiol were higher), and disappeared at other times of the year (Sisneros 2009). In mice, olfactory sensitivity to stimuli from male pheromones was periodically eliminated (due to increased plasma concentrations of progesterone) when the female was not ovulating (Dey et al. 2015). Increases in female sensitivities to male stimuli also occurred during breeding periods in other fish (Maruska & Tricas 2011) and in green frogs (Miranda & Wilczynski 2009).

Both the especially dense female genital mechanoreceptors and the temporal coordination between a female's readiness to breed and temporary increases in her sensitivity to male signals in these other species resemble our findings in *L. mariana*, in that they all represent mechanisms that improve female abilities to sense male courtship stimuli. Such mechanisms that potentiate male stimuli fit classic female choice (and species isolation) models; but they but they do not fit easily with the expected female resistance that characterizes sexually antagonistic coevolution models of sexual selection (Holland & Rice 1998; Arnqvist & Rowe 2005).

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