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Removal of genital plugs and insemination by males with normal and experimentally modified palps in *Leucauge mariana* (Araneae: Tetragnathidae)

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Abstract. Both males and females of the spider *Leucauge mariana* (Taczanowski 1881) contribute material to the plugs that often occlude the genital openings of females in the field. Males were sometimes unable to remove or penetrate these plugs, but overcame others using three different mechanical mechanisms: snag the plug and pull it off; break and penetrate through it; and break its adhesion to the epigynum by injecting material under it. They used their genitalia to accomplish these tasks, despite the fact that the genital bulb lacks muscles and innervation, thus limiting the male's ability to guide genital movements precisely. The effects of two male genital structures, the conductor tip and the conductor hook on sperm transfer and genital plug removal were tested by direct observations of their morphology and behavior, and by experimental removal of structures from one but not the other palp of the same male. Removal of the conductor tip reduced sperm transfer, while removal of both the hook and the conductor reduced plug removal. A preliminary characterization of palp movements and their sequences did not reveal any behavior that seemed especially designed for removing plugs, as opposed to inseminating the female.

Keywords: Copulatory plugs, genitalic function, cryptic female choice, plug removal

Genital plugs in female genitalia occur in many animals, and are generally formed from male seminal products or parts of the male's own genitalia (Smith 1984; Birkhead & Møller 1998; Simmons 2000; Uhl et al. 2010). Some plugs prevent subsequent males from gaining access to the female's reproductive tract, and plugs are often included in lists of sperm competition devices of males (Parker 1970; Thornhill & Alcock 1983; Smith 1984; Birkhead & Møller 1998; Simmons 2000). Active female participation in making plugs occurs, however, in some spiders (Knoflach 1998; Uhl et al. 2010; Aisenberg & Barrantes 2011) and insects (Markow & Ankney 1988; Hosken et al. 2009).

In several groups, plugs do not consistently exclude subsequent males (reviewed in Eberhard 1996; Uhl et al. 2010), and males of some species remove at least some copulatory plugs from the female (Milligan 1979; Masumoto 1993; Eberhard 1996; Knoflach 1997). The male's genitalia often seem to be active during the process of plug removal, but details of the mechanisms by which plugs are removed have been little studied. Most data involve only extrapolations from the probable mechanical properties of male genital structures. For instance, penile spines in microtine rodents and eversion movements of the hemipenes in lizards have been hypothesized to function to remove plugs (Milligan 1979; In den Bosch 1994), but direct observations and experimental evidence are lacking. The thin pointed shape of the distal portion of the aedeagus of a papilionid butterfly has been hypothesized to allow the male to tunnel through or to slip past soft, recently formed or small plugs (Matsumoto & Suzuki 1992). The male of the linyphiid spider *Dubiaranea* (?) apparently dissolves plugs *in situ*, perhaps with liquid from either his mouth or his palps, and he then removes the pieces with undetermined portions of his palps (Eberhard 1996). Male *Agelena limbata* Thorell 1897 spiders also use unspecified portions of their palps to pry plugs from the female (Masumoto 1993). To our knowledge, no male morphological structure has ever been demonstrated experimentally to be specialized for plug removal.

Given the selective importance to males of gaining access to internal female genitalia, it seems likely that male structures specialized for plug removal exist. Male genitalia seem particularly likely to have plug removal structures, as they probably often contact plugs. Plug removal devices could evolve under sexual selection by male-male competition (sperm competition), female choice (if females influence plug deposition, the necessity for plug removal, or the effectiveness of removal attempts), male-female conflict (if the female's best interests involve maintaining a plug), or combinations of these factors (e.g. Wiley & Posten 1996; Arnqvist & Rowe 2005; Eberhard 2010).

The present study documents female effects on plug deposition and removal, and a male genital structure whose form, mechanical properties and behavior suggest that it represents an adaptation to remove plugs in the tetragnathid spider *Leucauge mariana* (Taczanowski 1881), a member of the large cosmopolitan genus *Leucauge* White 1841 (>150 species; Platnick 2013) that is abundant in early second growth and secondary forest in the Central Valle (San José Province) of Costa Rica. Copulation and sperm transfer have been studied in detail in this species (Eberhard et al. 1993; Eberhard & Huber 1998a; Méndez 2002; Aisenberg 2009; Aisenberg & Eberhard 2009; Barrantes et al. 2013), but nearly exclusively in virgin females.

As in other spiders (Eberhard & Huber 2010), the sperm of *L. mariana* are encapsulated when they are transferred from the male's palp to the female's internal spermathecae in a viscous liquid matrix (Figs. 1, 2c). Once inside the female, the sperm emerge from their capsules (Eberhard & Huber 1998a), as in the related *Nephila clavipes* (Linnaeus 1767) (Brown 1985). Sperm precedence patterns are not known in *L.*

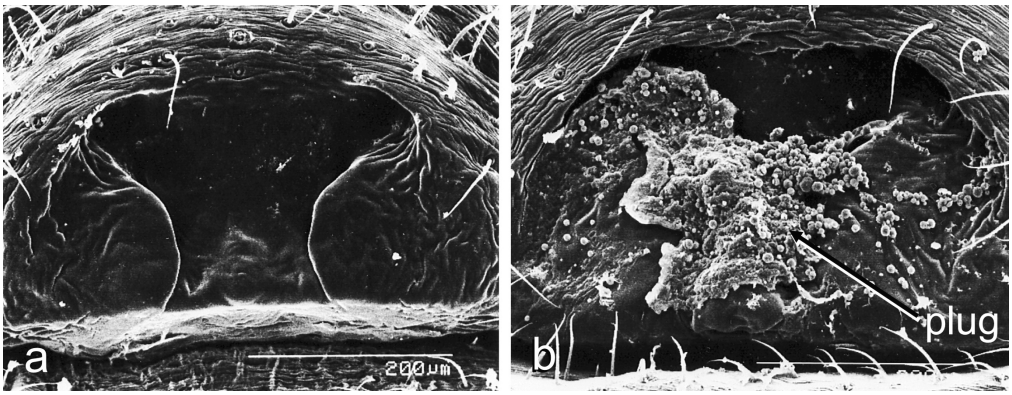

Figure 1.—Epigynum without a plug (left) and with a partial, asymmetrical plug (right).

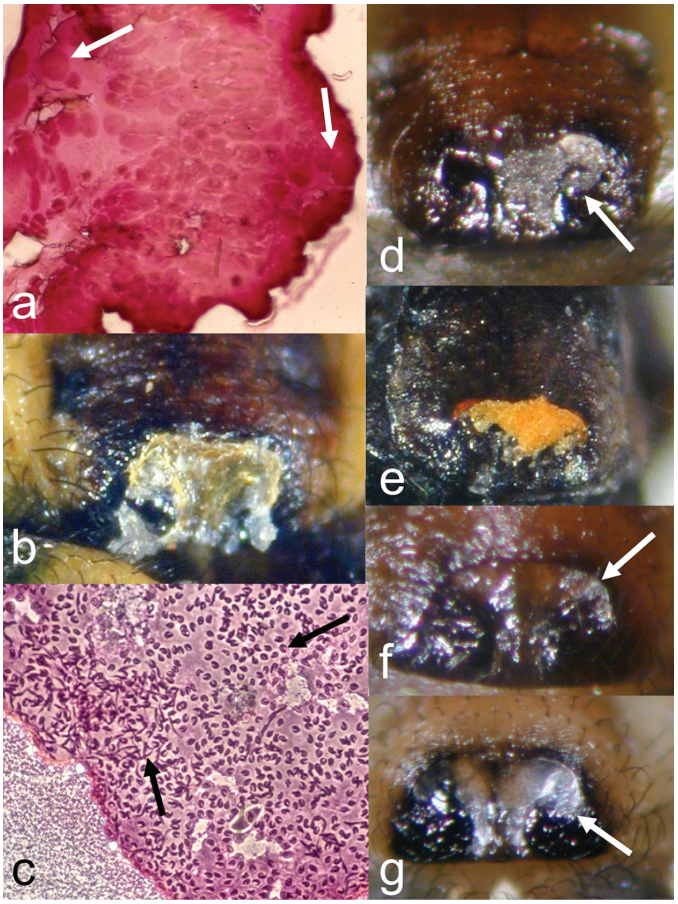

Figure 2.—Ventral and microscopic views of plugs of *L. mariana*. a) a yellow plug containing spheres (arrows) but no sperm; b) a large mixed white and yellow plug overflowing the central cavity, with an irregular surface; c) contents of a plug stained with acetocarmine which contained both encapsulated sperm (right arrow) and decapsulated sperm (left arrow); d) a white plug covering the lower portions of one side of the epigynum (arrow indicates a portion of the epigynal curved ridge that was not covered; e) a yellow-orange plug with a granular surface; f) a small yellowish plug with a smooth surface at the anterior corner of the left side of the central cavity (arrow); g) a white plug with a smooth surface that covers most of the central cavity.

mariana, but the fact that males in the field occur preferentially with penultimate instar females rather than mature females (Eberhard et al. 1993), indicates that the first male to mate with a female often sires at least some of her offspring. On the other hand, the following combination of observations indicates that first male sperm precedence is not complete: males mate with non-virgin females both in the field and in captivity (Méndez 2002; W. Eberhard unpub. obs.); distinctive behavior of the male's genitalia results in deposition of one component of the plug during the latter stages of copulation (Eberhard & Huber 1998a); females in some cases add a second component to the plug (Eberhard & Huber 1998a; Aisenberg 2009; Aisenberg & Eberhard 2009); and males push and scrape at some plugs with their genitalia without dislodging them, but dislodge others and then apparently succeed in inserting their genitalia in the female (Méndez 2002; the present study). Mixed first and last male paternity has been observed in the related genus *Tetragnatha* Latreille 1841 (Danielson-François & Bukowski 2005).

The female's epigynum, where all male insertion, plugging, and unplugging attempts occur, is a sclerotized plate on the ventral surface of her abdomen, with a central cavity that is bounded anteriorly by an overhanging wall (Fig. 1); access to the entrance of each of the two insemination ducts, which lead to the two spermathecae, is through slits at the base of the rounded lateral wall of the central cavity. Plugs consist of masses that vary in size, shape, consistency and texture that are located at variable sites on the epigynum (Figs. 1b, 2b, d–g) (Méndez 2002).

During copulation, the palps are extended, and contact the female's abdomen in alternation. The subapical cymbium of the palp (Fig. 3) is first placed on a featureless region of the ventral surface of the female's abdomen just anterior to her epigynum. Then the basal hematodocha inflates ("primary inflation"), causing the distal bulb to rotate so that its terminal portion, which includes the intromittent embolus and the tip and hook of the conductor sclerite, moves ventrally away from the cymbium and then dorsally toward the entrance of the insemination duct on the female's epigynum. If the entrance is unobstructed and the palp is correctly aligned, the conductor hook sweeps antero-laterally across the female's epigynum until it is arrested by the anterior wall, and the basal hematodocha then swells further (a "secondary inflation"), causing further rotation that drives the conductor tip and the

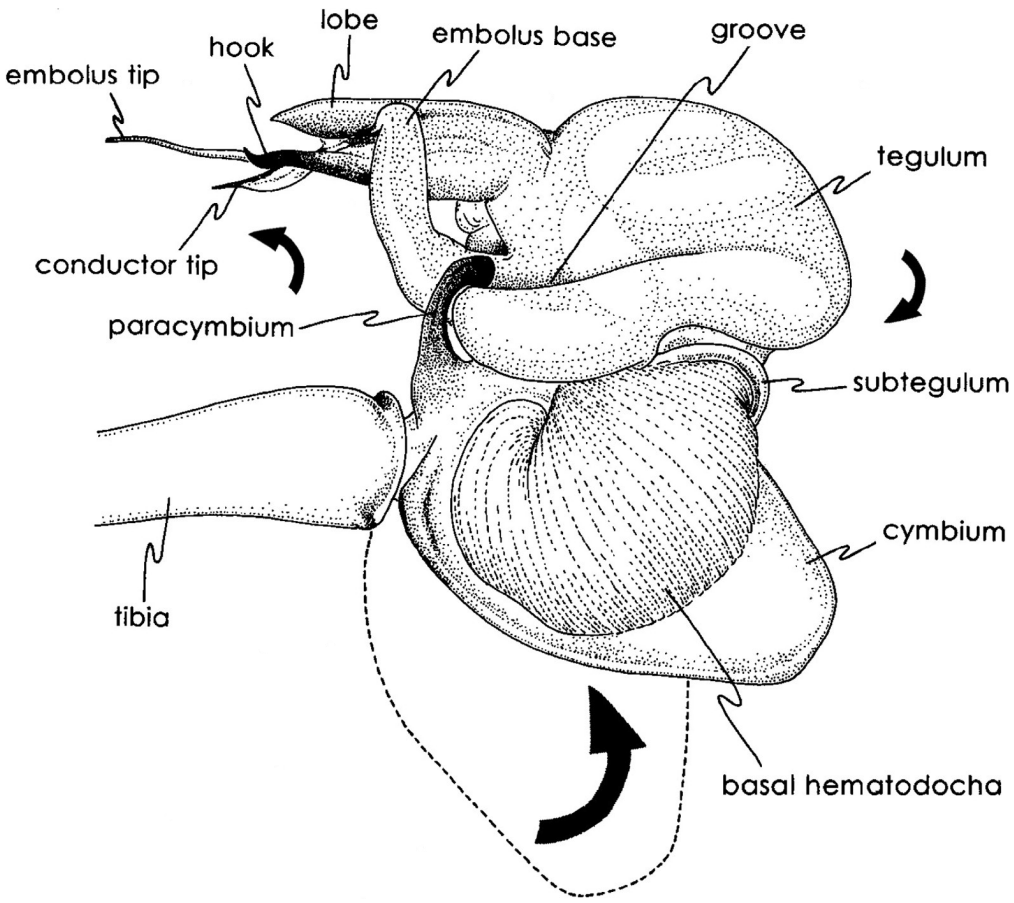

Figure 3.—Movements (small arrows) of embolus base and tegulum that resulted from inflation of the basal hematodocha (partially collapsed in this preparation; the approximate position of the cymbium in life is indicated by the dotted lines). The tegulum rotated against the paracymbium, whose tip slid along the groove in the tegulum as the expansion of the basal hematodocha drove the embolus distally from the conductor (from Eberhard & Huber 1998a).

embolus into the insemination duct (an "insertion") (Eberhard & Huber 1998a). Substantial force is applied to the female during primary and secondary inflations, sometimes displacing her entire abdomen laterally.

Two types of palpal insertion occur in copulations with virgin females (Eberhard & Huber 1998a). "Long" insertions (when sperm transfer probably occurs, at least in copulations with virgin females) last on the order of 1 min. Repeated secondary inflations of the basal hematodocha alternate with brief collapses; each inflation drives the embolus tip into the insemination duct. "Short" insertions last on the order of 1s and involve only a single secondary inflation, and both the embolus and conductor are then pulled away from the epigynum when the basal hematodocha collapses. Short insertions usually occur in bouts, and later in copulation. A small mass of white material emerges from the tip of the embolus and is deposited on the surface of the epigynum during many short insertions. Many apparent insertion attempts fail (44% in copulations with virgin females; Eberhard & Huber 1998a), when the conductor tip and or the hook snag the epigynum only momentarily or miss it completely during a primary inflation ("flubs" in the terminology of Watson 1991). On average, copulation with virgin females lasted 17.3 ± 6.1 min; there were 3.5 ± 2.0 long insertions, averaging about 108–120 s in duration, and 6.2 ± 5.2 bouts of short insertions with a mean of 14.6 ± 7.0 inflations per bout. Copulations with unplugged non-virgin females were shorter (9.9 ± 13.3 min), and had fewer long insertions (0.2 ± 0.6).

It is important to keep in mind that insertion attempts by male *L. mariana* are "blind" in two senses. The male's eyes are on his dorsal side, so he cannot possibly see his palps, copulatory plugs, or the female's genitalia during copulation. In addition, his palpal bulb is not innervated (Eberhard & Huber 1998b, 2010), so he has no direct sensory feedback from the bulbal structures (conductor tip, hook, embolus) that contact the female's genitalia. Movements of bulb sclerites are produced by changes in internal pressure and expansion of hematodochal membranes, rather than by contractions of muscles. The only sensory feedback that may be available to the male is from more basal structures such as his cymbium, which is innervated and has abundant setae on its surface that contact the female's abdomen during intromission attempts, or other segments of his palp.

METHODS

Spiders were readily induced to copulate ventral side upward under a dissecting microscope, where details of the

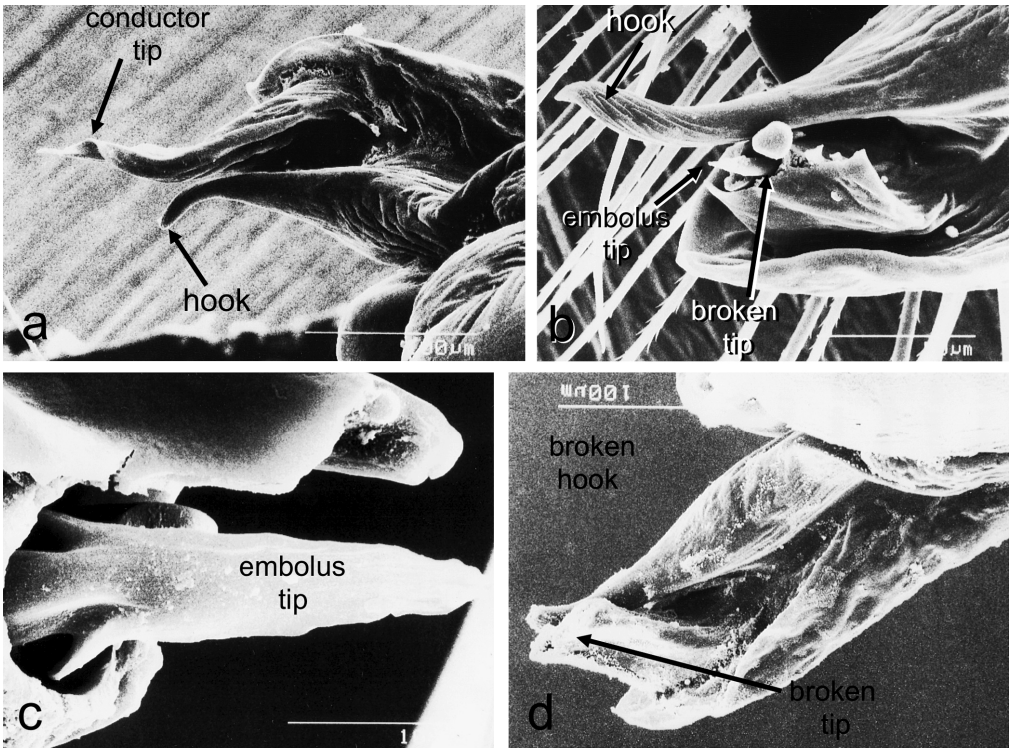

Figure 4.—SEM images of the distal portions of the male palpal bulb of *L. mariana*. a) intact palp; b both the hook and the conductor tip removed; c only the tip of the conductor removed; d) close-up of embolus tip at the site where the conductor tip was cut off.

behavior of the male's palps and their mechanical interactions with the female's epigynum were observed and recorded. An orb of a mature female in the field was mounted on the raised edges of a plastic plate about 30 cm in diameter, and the female to be observed and then the male were induced to climb onto the web. The plate was then placed under a microscope. We captured each mature male in the field the day he was observed. All spiders were collected on the campus of the Universidad de Costa Rica in San Pedro de Montes de Oca, San José Province, Costa Rica (el. 1100 m).

Each palp introduces sperm into only one of the female's two spermathecae, so paired tests were possible to test for effects of modifying one of the male's palps but not the other on plug removal, sperm transfer, and palp behavior (see Discussion for limits on details of the replications). We modified the palps of some males experimentally by first clamping the male gently between the foam-rubber covered tips of a fine forceps with one palp exposed, then cutting palpal sclerites with a fine scissors under a dissecting microscope (Fig. 4). We made two types of cut: both the conductor tip and the conductor hook of one palp were cut near the tip of the conductor lobe (Fig. 4d); or the conductor tip was cut leaving the hook intact (Fig. 4b). The tip of the male's intromittent organ (the embolus) (Fig. 4c) was enclosed in a slot in the conductor tip, basal to the tip of the lobe; it was thus not affected by cuts at the level of the hook, and little affected by more basal cuts. No fluid was seen to leak from these injuries, either when the cuts were made or subsequently during copulation. Incidental contact with the sclerites during these operations revealed that the tip of the conductor was flexible and bent easily when contacted; the hook, in contrast, was more rigid and bent little if at all.

We left the male's other palp intact as a control. Thus, in contrast to other well-known tests of the effects of experimental modifications of male morphology on female responses (e.g. Andersson 1982; Møller 1988; Basolo 1990), we controlled at least partially for the possibility that modification of the male's morphology affected him in additional ways (e.g., his courtship behavior) that could affect his reproductive success. The asymmetric nature of some plugs (e.g., Fig. 1b) meant, however, that the conditions encountered by the male's two palps were not always identical (see Discussion). The plugs in all plugged females that were mated to males with modified palps were white and apparently hard. Nearly all operated males were observed copulating with only one female; one male was observed with one female with a plug and another female that was virgin.

We checked insemination success in matings with virgin females by dissecting the epigynum and the spermathecae from the female, placing the pair of spermathecae on a microscope slide in a drop of saline and squashing them under a coverslip. The areas of the separate sperm masses that were expelled from each of the membranous first spermathecal chamber (where sperm are deposited by the male; Eberhard & Huber 1998a) were compared for the spermatheca that corresponded to the experimentally modified palp versus the spermatheca that was inseminated by the control palp. While the pressure of the squash with the cover slip was not standardized, the two spermathecae were squashed simultaneously and with enough pressure to extrude whatever sperm

they contained, so meaningful comparisons of their contents could be made.

Sperm from the male genitalia, complete plugs, and white masses that we collected from the male's palp on the tip of a fine needle without allowing the material to contact the epigynum were mounted on microscope slides and stained with acetocarmine, a DNA stain that colored the sperm nuclei bright red while leaving the other material relatively transparent (Fig. 2c). We assessed plug consistency by gently poking and prying at plugs on the epigyna of live spiders with a small needle under a dissecting microscope.

We captured plugged females in the field, and obtained virgin females by allowing field-captured penultimate instar females to molt in isolation in captivity. We recorded copulation behavior using a Sanyo VDC-2950 video camera that was attached to a dissecting microscope and focused tightly on the female's epigynum, so that its width occupied about 75% of the width of the screen. Male palp behavior was classified in video recordings as follows: flub with a brief snag on plug or epigynum; flub without a snag; reposition cymbium on female abdomen; secondary inflation without insertion (of at least the conductor – see below); secondary inflation with insertion; palp immobile (motionless for > 1 s); and withdraw palp from abdomen (usually to change palps).

RESULTS

Origin and composition of genital plugs.—Genital plugs on the epigyna of mature field-collected females varied in size, color, surface texture, site, and contents (Fig. 2). Yellowish plugs (Fig. 2e) were rare (2.5% of 200 females checked in January 2007), and often lacked sperm (56.7% of 33), but sometimes contained spheres (Fig. 2a) (39.4% of 33). All broke easily into flakes when poked with a needle. Silvery-white plugs (Fig. 2b, d, f, g), in contrast, all contained sperm (100% of 57) (Fig. 2c), never contained spheres (0% of 57), were hard, did not break into flakes when poked (although they occasionally broke into large chunks), and adhered more tightly to the epigynum than did yellowish plugs. Some field-collected genital plugs were heterogeneous, possibly the result of the mixture of new plug material and partially dislodged previous plugs; mixing of this sort occurred in matings in captivity. Plugs that were not disturbed by subsequent matings were long-lasting. Each of ten wild-caught females that were kept isolated from males for 22 days in captivity had the same type of plug at the end that she had had when captured.

All sperm inside the palpal bulbs of two males were encapsulated (right arrow in Fig. 2c). The small masses of white material deposited by the male on the epigynum and collected directly from the palps also contained abundant sperm that were almost exclusively encapsulated (all sperm in ten masses were encapsulated; all but a single sperm among many sperm in one other mass were encapsulated). No spheres were present in the material collected directly from the palps or the white masses.

We confirmed previous suggestions that females contribute material to plugs (Eberhard & Huber 1998a; Aisenberg 2009; Aisenberg & Eberhard 2009) in three ways. Direct observations of copulating pairs under the dissecting microscope showed, in a few cases in which visibility was good, that liquid welled up into the atrium from inside the female's insemination duct during copulation, replicating previous observations (Eberhard & Huber 1998a). This liquid appeared to cause the white masses from the male to dissolve or disperse, forming a silvery-white or transparent plug. In two cases, a plug that was composed of both new material and parts of a previous plug that was not completely dislodged apparently hardened rapidly and blocked further insertion attempts; but more often the male easily penetrated the apparently liquid plug repeatedly during copulation. Some females may have also added liquid soon after copulation ended and the spiders separated, as the material on the epigynum generally acquired a more liquid appearance following the end of copulation. When no liquid emerged from the interior of the female during copulation, as was common in copulations with virgin females in captivity (Eberhard & Huber 1998a; Aisenberg & Eberhard 2009), the male removed nearly all or (more often) all of the white masses that he deposited; the small masses adhered to his palps during subsequent insertions, and were withdrawn adhering to them and then fell or were lost.

Plug composition gave a second indication of active female participation in the formation of both yellow and white plugs. Of 57 white plugs, 18 contained multiple decapsulated sperm (left arrow in Fig. 2c). Because sperm in the spermatheca become decapsulated following insemination (Eberhard & Huber 1998a), while all or nearly all of the sperm in the male's genitalia prior to copulation and in the white mass that he deposited on the female epigynum were encapsulated (above), the abundant decapsulated sperm in these plugs suggest that the plugs contained material from the female, probably from her spermatheca. Yellow plugs, on the other hand, are probably often produced by only the female; 56.7% of 33 contained no sperm at all. In contrast, all material we collected from male palps, as well as material seen in sections of the distal portions of the sperm ducts inside intact palps (Eberhard & Huber 1998a) contained numerous sperm (all of which were encapsulated).

Finally, the sites of some small plugs that did not cover the entire central cavity were consistent with female contributions. They were along the sides of the cavity or at its anterior-lateral corners, and covered the lower rather than the more salient portions of the epigynum (Fig. 2, d f, g); these are sites where liquid ejected from the insemination ducts would be expected to first accumulate. This evidence does not clarify which sex produced the plug substance because male contributions could not be ruled out, but they are compatible with female participation.

Copulations that fail to result in plugs may be common in the field. Of 64 females collected with no plugs, 82.8% nevertheless had sperm in their spermathecae. Plug removal by the female with her legs could not be ruled out in these cases, but only infrequent removal is seen in captive females (above) so this is probably not the sole explanation. Field populations of *L. mariana* showed strong seasonal peaks of abundance, and unplugged females were more common in the field early in population peaks than later (Méndez 2002).

Plug removal.—Intact males attempting to copulate with a female with a white plug were only sometimes (68% of 28 pairings) able to dislodge it enough to allow insertion of the conductor into at least the outer portion of the insemination duct on at least one side of the epigynum (''plug removal''

hereafter) (in these and other "insertions" described below, direct determination of whether deeper penetration by the embolus occurred was not possible, because the tip of the conductor was out of sight). Plugs were dislodged by the palps in three different ways. In each case removal occurred after the palp had "snagged" against the plug (its movement was interrupted at least briefly by contact with the plug). In 21 pairs, the mechanism of removal was determined: pulling or prying the plug away as a single piece from the epigynum (14%); breaking the plug and then either prying away the pieces or penetrating past them (33%); and injecting material under the plug and then pulling it off as a unit (53%). In pulling a plug off as a unit, the conductor tip or hook scraped across the surface of the epigynum, snagged the plug, and then pulled or pried it free. No material emerged from the palp during these movements. In perforating or inserting the tip of his palp through a crack in the plug, the male apparently drove the conductor tip toward or into the insemination duct. Some broken pieces of these plugs were pulled from the epigynum during subsequent inflations. In removing a plug by injecting material under it, the conductor tip and probably the hook (it was not possible to resolve this detail in direct observations) penetrated through the plug, but did not appear to enter the insemination duct. The palp ejected material that accumulated between the plug and the surface of the epigynum and broke the plug free from the epigynum; it was then pulled away during subsequent inflations. We did not discern differences in the movements of the male's genitalia that seemed to be specially designed to utilize these different mechanisms.

In some cases, when the plug consisted of more than one mass or was broken into pieces but not all the pieces were removed, the male nevertheless succeeded in inserting one or both of his palps into at least the entrance of the female's insemination ducts. In some video sequences it was clear that the conductor tip was bent back sharply as it scraped across the surface of the plug, suggesting that the more rigid hook was more effective than the conductor tip in applying force to the plug. In all copulations in which a plug was removed the male subsequently deposited new plug material.

The basic movements of the palp before and after the plug was dislodged were compared in ten intact males that were paired with females with white plugs. Cymbium placement, and primary and secondary basal hematodochal expansions that swung the conductor tip and hook across the epigynum were at least qualitatively similar before and after the plug was dislodged.

Effects of experimental modifications on plug removal and sperm transfer.—The frequency of plug removal was only barely significantly reduced when both the hook and the conductor tip of one palp were removed compared with intact males (41% of 17 pairs) ($P = 0.04$ with one-tailed χ^2); there was no significant reduction when only the conductor tip was removed (52% of 21 pairs ($P = 0.27$ with χ^2). Comparisons between the modified and unmodified palps of the same male gave more dramatic differences in some respects. Of seven cases in which a plug was broken by a male that had lost both hook and conductor tip, all breaks were produced by the intact rather than the modified palp ($\chi^2 = 7.0$, df = 1, $P = .008$); in contrast, of 20 cases in which the plug was broken when the male had lost only the conductor tip, half were produced by the intact palp and half by the modified palp. Of five cases in which a plug was removed as a unit from both sides of the epigynum at once in experiments in which both the hook and the conductor tip were removed, the trend was in the expected direction: the intact palp removed the plug in four of them ($\chi^2 = 1.8$, df = 1, one-tailed $P = 0.09$). Summing the two modification experiments, the plug was dislodged as a unit by the intact palp in seven of eight cases ($\chi^2 = 4.50$, df = 1, one tailed $P = 0.017$).

In contrast, both modified and control palps were effective once a plug was broken. When the plug was broken and at least one piece was removed, the intact palp removed a piece of the plug on its side of the epigynum in eight cases and the modified palp in seven. The frequency with which a palp snagged the plug at least once was not altered (59% for palp lacking both the hook and the conductor tip, 76% for palp lacking only the conductor tip, 71% for the intact palp).

Insemination of virgin females was reduced when the palps were modified. The spermatheca on the side into which the intact palp was inserted (the "control" spermatheca) was full in all 19 females that were dissected after being mated to males with both conductor tip and hook removed, while the "experimental" spermatheca (into which the modified palp was inserted) was uninflated and apparently empty of sperm in 53% of these females ($\chi^2 = 13.6$, df = 1, $P = 0.0002$, comparing empty and non-empty spermathecae). The control spermatheca was more full than the experimental in 17 (90%) of these females ($\chi^2 = 13.5$, df = 1, $P = 0.00024$). Corresponding data when only the conductor tip was removed were 11 of 11 control spermathecae full, and 64% of the experimental spermathecae not inflated ($\chi^2 = 10.3$, df = 1, $P = 0.0014$, comparing empty and non-empty spermathecae). The control spemathecae contained a greater amount of sperm than the experimental spermatheca in nine of 11 (82%) cases ($\chi^2 = 4.45$, df = 1, $P = 0.035$). The differences in the frequency of uninflated spermathcae between the two experimental treatments with respect to the control spermathecae were not significant ($P = 0.71$ with a two-tailed Fisher Exact Test).

The total durations of attempts to intromit (including both primary and secondary inflations) in 39 matings with modified males were not significantly shorter than in 29 matings with intact males ($P = 0.39$ with Wilcoxon/Kruskal Wallis Rank Sums Test). The total numbers of primary inflations (with and without subsequent secondary inflations) of control and modified palp were nearly equal in 37 copulations (2056 inflations by control palps, 2098 by modified palps; respective means = 69.4 ± 59.3 and 69.3 ± 58.5; $P = 0.92$ with Mann-Whitney U Test). The proportion of flubs in which control and modified palps snagged at least briefly on the plug or the epigynum also did not differ (respective means = 55 ± 34% and 58 ± 34%; $P = 0.99$ with Mann-Whitney U Test).

The female pushed the male's palp away from her genital opening with her legs in two pairs in which the male lacked both conductor tip and hook, but also pushed the male's palp away in two matings with intact males; in one additional case, the female pushed the plug material out of her epigynum with her leg.

DISCUSSION

Some genital plugs impeded subsequent mating attempts, and such exclusion presumably benefits the male that made

the plug. Females also participated actively in the formation of successful plugs, so they presumably also benefit, but their benefits are less clear. One possible female benefit is biasing the paternity of her offspring in favor of males with certain traits (cryptic female choice). By helping some males but not others to form a plug, the female could favor paternity for subsequent males better able to remove plugs. Other female behaviors, such as pushing the male's palp or plug material from her epigynum with her tarsus, may also influence paternity. It is not known in most of these cases, however, whether these cooperative or resistant processes of the female are biased toward males with certain traits. An exception is the association between larger numbers and durations of bursts of one type of male copulatory courtship (gentle pushing with his legs on those of the female) and a greater frequency of plug production (Aisenberg & Eberhard 2009). Thus cryptic choice involving plug production and removal is feasible, but so far strong support has been demonstrated only with respect to male leg pushing.

Females also apparently occasionally formed some epigynal plugs without male participation. These yellowish and orange plugs crumbled easily when poked with a pin, and it seems very unlikely that they could exclude forceful intromission attempts by subsequent males. Presumably they have some other, as yet undetermined function.

Despite the limited mobility of genital sclerites in the male palpal bulb and their inability to provide the male with sensory feedback, male *L. mariana* frequently penetrated or dislodged even hard, firmly-attached epigynal plugs. They were also able to insert their genitalia at least in the entrance of the insemination duct, even when the contours of the epigynal surface were substantially altered by remaining pieces of plugs. The male's ability to adjust to striking variations in female morphology contrasts strongly with the tight mechanical fit between male and female morphology that is typical of many other spiders (Gering 1953; Grasshoff 1973; Huber 1995; Eberhard & Huber 1998b, 2010). The relative simplicity of the morphology of the male genitalia of *Leucauge* and other tetragnathids is apparently derived (Griswold et al. 1998); perhaps this simplicity (especially of the relatively small fraction of the *Leucauge* palp that physically contacts the female) increases this ability to adjust. Similar flexibility, in the form of an ability to inseminate both sides of the female with a single palp, has been demonstrated in two other, distantly related spiders (Costa et al. 2000; Knoflach & van Harten 2000), one which also has a very simple palp design. Tetragnathid spiders have changed the sides of the female that are inseminated by the male palps (Huber & Senglet 1997), also suggesting flexibility at some point in their evolutionary history.

Male genital movements in a species like *L. mariana* may be under two types of selection—to couple mechanically with the female genitalia in order to inseminate (and perhaps stimulate) her, and to remove plugs that impede such coupling. Nevertheless, male *L. mariana* used the same or similar basic genitalic movements in copulations with plugged and unplugged females. The relative frequencies of different types of palp movement changed, but it was uncertain whether these changes were simply consequences of greater difficulty in mechanically engaging the palp with the epigynum when it was plugged, or the changes in male behavioral tactics were designed to remove plugs. Our behavioral categorizations were only general, however, and more detailed observations might reveal differences. It is at least possible that a male could sense the presence of a plug. The more frequent withdrawal of the palpal bulb following a flub seen by Eberhard & Huber (1998a) suggests that a male obtains enough sensory feedback from his palps to sense whether mechanical coupling has occurred. Males of some other spiders appear to use their palps to search for the female's genitalic openings (Huber 1995), also implying some sensory feedback.

The conductor hook may be especially important for plug removal. Its rigidity combined with its hooked design probably improves its ability to snag and pull or pry off plugs, and perhaps also to perforate them. The results of copulations when the hook was experimentally removed, however, showed only a weak trend toward less frequent plug removal. The plugs in *L. mariana* vary in many ways, however, that could affect removal, including composition, size, the portion of the epigynum that is covered, left-right asymmetry, and the roughness of the outer surface; none of these traits was standardized in these experiments. Thus even in comparisons between the intact and modified palps of the same male, the experimental results can at best be only suggestive. Our ability to determine whether it was the modified or unmodified palp that originally dislodged the plug may also have been imperfect. Many plugs consisted of a mass of material that extended to both sides of the epigynum, and it was not always possible to eliminate the possibility that a minor, difficult to perceive preliminary dislodgement with one palp could have led to a subsequent removal by the other. In sum, the intramale differences observed in plug removal by intact and modified palps are compatible with the hypothesis that the hook functions to remove plugs, but are not conclusive.

The flexibility of the conductor tip makes it poorly designed to remove plugs by hooking and prying, but well designed to slip along the curved external wall of the epigynum and of the insemination duct. We speculate that it may facilitate deeper intromission by the embolus, slipping between the plug and the epigynum wall to inject material below the plug, allowing the male to dislodge the plug as a unit. This facilitation of embolus insertion could explain its positive effects on sperm transfer documented here. Our experimental modifications of palpal morphology were crude, however, and cannot illuminate the functional significance of details of their forms.

Details of the forms of both hooks and conductor tips vary interspecifically in *Leucauge*. Hooks that are similar in shape to that of *L. mariana* occur in *L. venusta* (Walckenaer 1841) (Levi 1980), *L. wulingensis* Song & Zhu 1992 (Song & Zhu 1992), and *L. argentata* (O.P.-Cambridge 1869) (Chrysanthus 1975). In contrast, the hooks have quite different forms in *L. decorata* (Blackwall 1864) (Chrysanthus 1975; Tanikawa 1990) and *L. tessellata* (Thorell 1887) (= *termistica*) (Song & Zhu 1992), while conductor hooks are missing in still others, such as *Opadometa* (= *Leucauge*) *grata* (Guérin 1838) (Chrysanthus 1963), *L. (=Plesiometa) argyra* (Walckenaer 1841) (Barrantes et al. 2013), and possibly *Tylorida* (= *Leucauge*) *mornensis* (Benoit 1978) (Benoit 1978). Epigynal plugs occur in at least one of the species (*L. argyra*) in which the conductor hook is missing (Barrantes et al. 2013). The genus *Leucauge* has

apparently never been revised, and no phylogeny is available which could clarify the order in which different forms and functions for the hook and conductor tip evolved. It seems likely that the hook was favored by sexual selection, but the data do not permit discrimination among possible (non-exclusive) types of selection such as sperm competition, cryptic female choice, or sexually antagonistic coevolution.

This is to our knowledge the first experimental demonstration of effects on plug removal for any particular male genitalic structure, and also the first demonstration of multiple functions for genitalic structures and the behavior patterns which they execute. The evolutionary interactions between male and female genitalia in *Leucauge* are obviously complex and merit further study.

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