

Chapter 4

Post-copulatory Sexual Selection in Two Tropical Orb-weaving *Leucauge* Spiders

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Abstract This chapter focuses on descriptive and experimental studies of the sexual biology of two spider species, *Leucauge mariana* and *Leucauge argyra*. We examine general questions related to female effects on paternity by taking advantage of several unusual traits: direct female participation in forming copulatory plugs and physical clasping by the female rather than the male during copulation in both species; and derived traits in the genitalia of males and females and occasional female cannibalism of conspecific males by trapping the male's genitalia in adhesive copulatory plugs in *L. argyra*. These derived traits, combined with other aspects of sexual interactions that are typical of spiders in general, such as complete, easy female avoidance of unwanted sexual advances of males, copulatory courtship by males, and imperfect male ability to remove copulatory plugs from the female's genitalia, constitute a rich mixture from which clear lessons can be drawn. Post-copulatory sexual selection probably acts on male genitalia and their behavior in both species. Two derived male genitalic clasping and clamping devices *L. argyra* may be associated with the extremely aggressive female behavior. One genital structure in male *L. mariana* may have evolved under sexual selection by sperm competition to remove copulatory plugs from female genitalia. In contrast to expectations from theory based on male–female conflict, the female genitalia of *L. argyra* has a derived trait that selectively facilitates rather than impedes male genital clamping. In contrast to

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lock-and-key expectations, another prominent, derived female genital structure in *L. argyra* does not mesh with any male structure during copulation; it may instead function in sensing male genital movements. Additional non-genital male traits associated with copulation are also probably under post-copulatory sexual selection in both species. Experimental manipulations of male chelicerae and of possible female receptors of stimuli from the male chelicerae demonstrated that stimulation from secondary sexual modifications of the male chelicerae influences several female reproductive processes in *L. mariana*, including receptivity to remating, female interruption of copulation, and copulatory plug formation. Additionally, two male copulatory courtship behavioral patterns correlate with greater plug formation in *L. mariana*, and one pre-copulatory courtship behavioral pattern in males of *L. argyra* correlates with plug formation, suggesting additional possible male effects on post-copulatory female reproductive processes. Other male sexual behavior correlates with the timing of female emission of plug material that may affect a male's chances of surviving the encounter. In sum, female *Leucauge* have powerful effects on whether copulation occurs, when it will end, whether a mating plug will be formed, and whether the male will survive the encounter. Several genital and non-genital male traits likely evolved under a mix of post-copulatory sexual selection (sperm competition and probably cryptic female choice [CFC]) to elicit favorable modulation of these female effects.

4.1 Introduction

Sexual selection by cryptic female choice (CFC), as described in Chap. 1, is feasible in many different animal groups (Eberhard 1996). Whether or not CFC is actually widespread in nature, however, is yet to be determined. The kinds of data needed to test for CFC and to discriminate it from other post-copulatory processes such as sexually antagonistic coevolution (SAC), sperm competition, and species isolation are only available in a limited number of animal groups (see Chap. 1). The present chapter summarizes descriptive and experimental studies that have expanded the taxonomic range of CFC studies to include two species in the large, widespread tetragnathid spider genus *Leucauge*, *L. mariana*, and *Leucauge argyra* (Fig. 4.1). The first section includes background information that provides a context in which to evaluate the significance of the results of the behavioral correlations and experimental manipulations that are described in the second section.

Studies of *Leucauge* sexual biology illustrate how concentrating attention on a particular group can reveal multiple mechanisms of post-copulatory sexual selection acting on multiple male characters. Questions related to female control of paternity are especially susceptible to study in *Leucauge*, due to several unusual traits (Eberhard and Huber 1998a; Aisenberg and Eberhard 2009; Aisenberg and Barrantes 2011): Females participate directly in forming copulatory plugs; the female physically clasps the male during copulation rather than vice versa; there are several major derived genital traits in the males and females of *L. argyra*; and females occasionally cannibalize conspecific males in this species by trapping the male's genitalia in adhesive copulatory plugs. These derived traits are

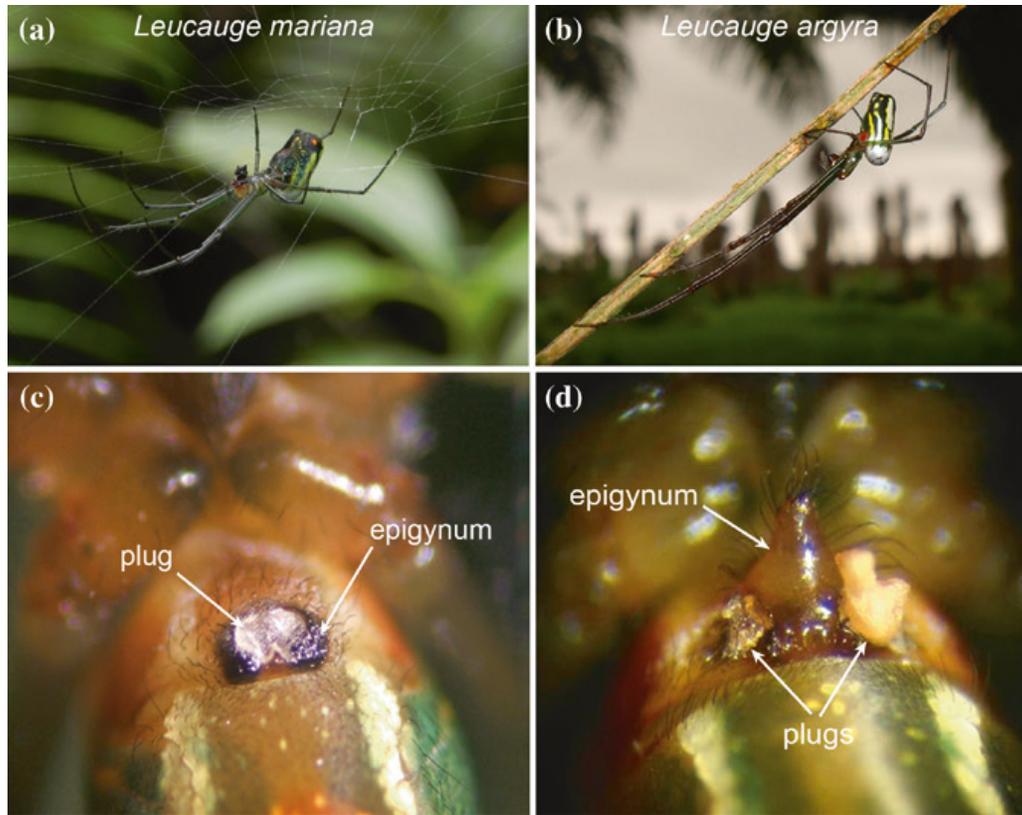


Fig. 4.1 **a** *Leucauge mariana*; **b** *Leucauge argyra*; **c**, **d** mating plugs on the genitalia of field-collected *Leucauge mariana* (left) and *L. argyra* (right)

accompanied by some other aspects of sexual interactions that are typical of spiders in general, including easy and effective female avoidance of unwanted sexual advances of males; copulatory courtship by males (Huber 1998, 2005); transfer of immobile, encapsulated sperm; and a male ability to remove some but not all copulatory plugs from female genitalia (e.g., Masumoto 1993). We will give general background information that is important to interpret observations of reproductive behavior, then focus on several different topics that were examined in different studies, and close with a general discussion.

4.2 Background on *Leucauge* Sexual Biology

Members of this large genus of 150 or more species are medium-sized orb weavers (approximately 40–100 mg) which build more or less horizontal webs. To date, published detailed descriptions of the sexual behavior are available for only two species, *L. mariana* and *L. argyra*; there are also brief observations of *Leucauge regnyi* by Alayón (1979), and unpublished observations of *Leucauge venusta* by Castro (1995). These species were chosen not because they were known to be interesting with respect to CFC, but for the more practical reason that they are

both very common in easily accessible habitats and copulate readily in captivity where their genitalia can be observed under a dissecting microscope.

Both are very abundant in early second-growth vegetation at some sites in Costa Rica. Population densities of *L. mariana* in the Valle Central are highly seasonal (Méndez 2002); those of *L. argyra*, which occur in a habitat with more highly seasonal rainfall along the Pacific coast of Costa Rica, nevertheless seem to be more stable (careful counts have not been made, however). In the past, both species presumably lived in natural second growth ‘vegetation’, such as along the edges of creeks and rivers, landslides, and tree-fall gaps. The localized but persistent nature of second-growth sites along water courses in the past may have led to locally dense populations. This is important, because the chances that multiple males would find and attempt to mate with any given female probably increase with greater population density.

The longevity of mature males (henceforth “males”) has never been measured in the field, but there are two indications that they may survive for up to several weeks; field-captured males (of undetermined ages) generally survive for a week or more in captivity; and males in the field evict medium-sized nymphs from their orbs for several hours in the field and use it for prey capture (often, when a male is found at the hub of an orb, there is an immobile nymph on a peripheral frame or anchor line). In *L. mariana*, males also sometimes ingest silk from the webs of mature females (WE unpub.). Long-term survival of males would increase the density of males and thus the chances that a female will be courted by multiple males during her lifetime. Incidental observations in the field and in captivity suggest that females also live for several weeks. Females probably lay multiple clutches of eggs in nature, because females which laid eggs in captivity subsequently built orbs.

Several other aspects of the sexual biology of *Leucauge* in the field also provide perspectives for the observations that are described below. Males of both *L. mariana* (Eberhard et al. 1993; Eberhard and Huber 1998; Méndez 2002) and *L. argyra* (A. Aisenberg, G. Barrantes, and W. Eberhard, unpub.) have two different mating strategies. Males often occupy the small tangle web that is built by a penultimate female nymph just prior to molting (males were never found in similar webs built by penultimate male nymphs, which are of similar size). Any female nymph found with an accompanying male invariably molts within 1–2 days, and the male then mates with her in the first hours after she has molted. Males also occur on the orb webs of mature females (henceforth “females”), and court and mate with them there. Males fight with each other on the webs of both adult and penultimate females (Méndez 2002; A. Aisenberg, G. Barrantes, W. Eberhard, unpub.). Aggressive male defense of both penultimate and mature females suggests that both the first male to mate with a female and also later males achieve some paternity.

Insemination (at least in virgin *L. mariana* females) occurs early rather than late during the approximately 15-min copulation (Eberhard and Huber 1998a). The male intromittent structure, the embolus, is a thin, hollow, hairlike sclerite. The sperm in both species are stored in the female in the large, soft-walled “chamber I” of the spermatheca, which is connected with additional, complex, hard-walled chambers deeper in the female’s body that differ greatly in form in *L. mariana* and *L. argyra* (Eberhard and Huber 1998a; Triana and Quesada, in prep.). Measurements of the

lengths of the portion of the male embolus that can be everted and of the insemination duct of the female in *L. mariana* showed that the embolus probably reaches into the lumen of chamber I during copulation (Eberhard and Huber 1998a).

Two details of the sexual morphology and behavior of these species are especially important in the context of possible post-copulatory sexual selection (we will use “post” in the usual sense in such discussions, of any event occurring after the beginning of a copulation) and merit special attention: mating pairs are held together with a cheliceral clasp, in which the female grasps the male, rather than vice versa; and females in the field often have “plugs” of material adhering to their genitalia on or near the openings to their insemination ducts (Fig. 4.1).

4.3 Pre-copulatory Behavior

Male *L. mariana* court females with diverse behaviors, most of which probably produce vibrations in the web that are perceived at a distance by the female (we will use “courtship” to refer to behavior that was repeated both within and between pairs, that obviously resulted in stimuli being received by the other spider, and that had no obvious mechanical function in bringing and keeping the spiders together; the term “copulation” is used to include the period during a cheliceral clasp, including but not restricted to genitalic contact; the term “insertion” designates the entrance of the embolus and conductor into the mouth of the insemination duct on the epigynum). Males performed at least seven types of courtship behavior (Eberhard and Huber 1998a; Aisenberg 2009; Aisenberg and Eberhard 2009): jerks, rocking, abdomen bobbing, palp rubbing, twanging, line tapping, and tapping the female. None of these types of courtship physically coerces the female in any way.

Sexually receptive females of *L. mariana* gave four behavioral responses: (1) Turn to face males: Sometimes they turned only after the males had performed repeated bouts of courtship behavior; males never responded to females that were facing in some other direction by moving to place themselves in front of females. All orientation movements, when they occurred, were performed by females; (2) Open chelicerae: Females often repeatedly opened and closed their chelicerae (both the basal segments and the fangs) prior to linking with males; presumably, these were intention or exploratory movements associated with cheliceral clasping. Occasionally, when females had not opened their fangs, males made repeated small lunging movements nearby, in an apparent attempt to induce females to open their fangs; (3) Assume mating position: Just prior to copulation, females lowered their body and spread their anterior legs, thus making space for males to approach them close enough for cheliceral clasp and insertion of their palps; and (4) Bend abdomen ventrally: Ventral flexion of females’ abdomen was probably critical, at least when males were small with respect to females. In two cases, females returned their abdomen to the more typical orientation, while males were attempting to insert their palps, and in one of these pairs, the male was then unable to reach her epigynum with his palp.

In pre-copulatory courtship in *L. argyra*, the males perform jerks, palpal rubbing, twangs, tap lines, and tap the female, as well as an additional behavior, fore-leg rubbing (Aisenberg and Barrantes 2011). All the courtship behavioral patterns were usually performed while the male was on the same line or lines on which the female was resting, and could thus convey vibratory stimuli to her.

The important point, with respect to possible CFC as well as the species isolation and male–female conflict hypotheses (see below), is that a female *Leucauge* has received a rich set of stimuli from the male before he ever contacts her; and she has had ample opportunity to reject his advances by failing to respond in any of several ways. The fact that females must be persuaded to perform four different behaviors for copulation to occur suggests that pre-copulatory male courtship

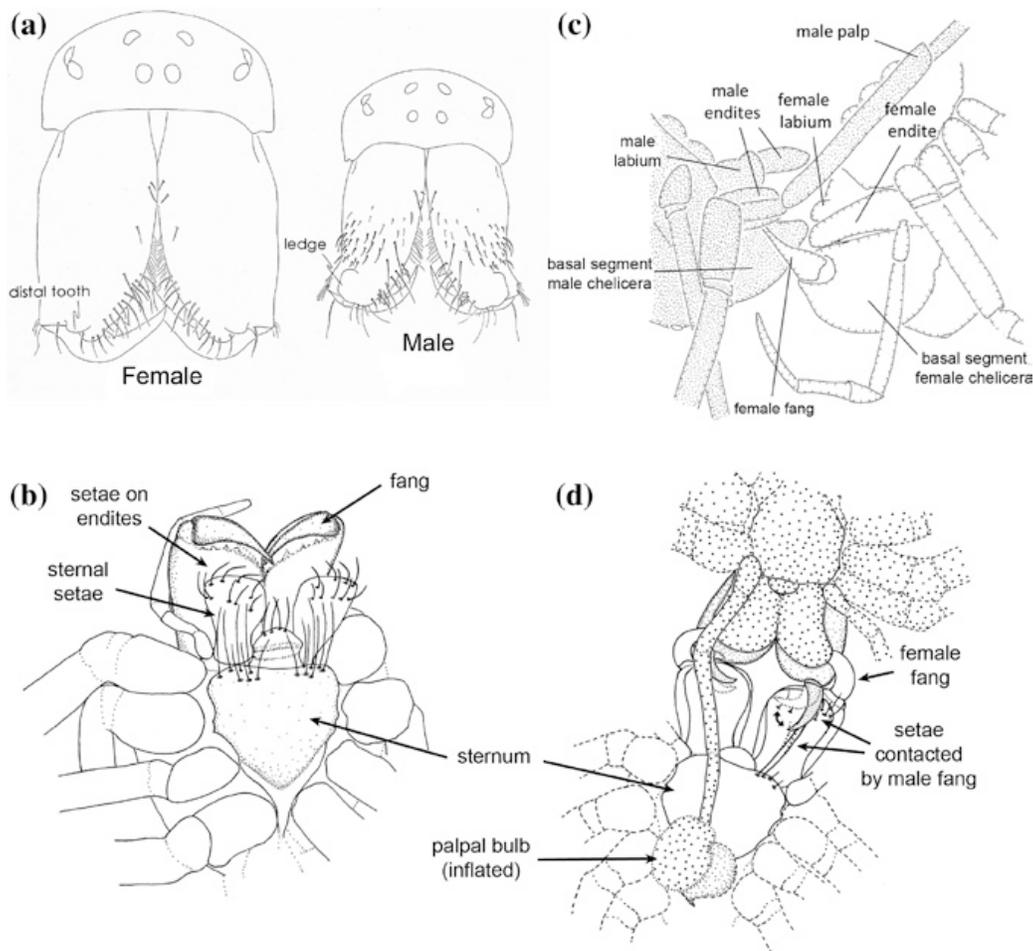


Fig. 4.2 Cheliceral dimorphism in anterior view (a), female endite and sternal setae in ventral view (b), and cheliceral clasp in lateral (c) and ventral (d) view in *L. mariana*. The anterior surface of the male chelicerae (a) has more setae and a small plate or ledge near the base of the fang. The anterior edge of the sternum of the female (b) has longer setae than those of the male, and these are deflected by movements of the male fangs during a clasp (c, d). The female's fang clamps the distal portion of the basal segment of the male's chelicera (c) (a from Eberhard and Huber 1998a; b–d from Aisenberg et al. 2015)

functions to induce these female acceptance responses. In addition, there is no way that a male can force a female to copulate in either species. For a mating to occur, the female must turn to face the male, spread her anterior legs, open her chelicerae to allow him to insert his basal cheliceral segment between her fang and basal segment, grasp the male's chelicerae with her fangs (Fig. 4.2), and tilt her abdomen ventrally to bring her epigynum within reach of the male's palpal bulb. The male has no way to force any of these female responses, even with a newly molted female. When a female fails to respond to a male's courtship, he eventually desists and leaves (interactions with newly molted females have not been observed often enough, however, to comment on male persistence in this context).

4.4 Copulation

Copulation is a complex process in *Leucauge*, and males perform highly repetitive leg and cheliceral movements in addition to the repeated movements with both their entire palps and with particular sclerites of the palpal bulb. Again, the most complete descriptions are available for *L. mariana* (Eberhard and Huber 1998a), and the descriptions that follow refer to this species (for comparisons, see Aisenberg and Barrantes 2011, Sect. 4.4.4 on *L. argyra*).

4.4.1 *Movements of the Female and Male's Body and Legs*

During copulation, males performed at least three types of the apparent courtship behavior that also occurred prior to copulation: leg tapping, abdomen bobbing, and rocking. Tapping often occurred during the first moments after the female grasped the male chelicerae and the male attempted to insert his palp, and also during the withdrawal of one palp and insertion of the other. Males also performed an additional behavior, leg pushing, which did not occur prior to cheliceral clasping. Each of the male's four anterior legs contacted the corresponding legs of the female, and the male's legs were repeatedly extended synchronously to push gently against the female's legs. Usually, the male contacted the female with the distal portions of his legs I and II (tarsi, metatarsi). In most cases, the male's legs III and IV were immobile. Bursts of leg pushing began when the basal hematodocha of the palp was inflated.

These behavioral patterns are important in the context of possible CFC in two respects. First (assuming that male behavior is adaptive), they demonstrate that there has likely been selection on males to elicit further female cooperation with their mating attempts, even after the pair has coupled. Secondly, they continue the theme seen in pre-copulatory interactions, in that the male's behavior was not physically coercive. Tapping, abdomen bobbing, rocking, and leg pushing in no way physically force the female to respond in any particular way.

4.4.2 *Cheliceral Clasping*

Most species in Tetragnathidae have large and conspicuous chelicerae which are larger in males, and the female and male clasp their chelicerae prior and during mating (Bristowe 1929; Eberhard and Huber 1998a; Álvarez-Padilla et al. 2009, 2011). Male–female dimorphism (with male chelicerae larger) has evolved at least five times in this family (Álvarez-Padilla and Hormiga 2011). The morphological designs and use reflect the typical male–female roles in *Tetragnatha* and *Pachygnatha*. The basal segment of the male chelicera has a distal, spurlike process which appears to serve the defensive function of arresting the female’s fang and preventing it from closing, while the male’s own fang clamps the basal segment of the female and appears to hold the pair of spiders together (Bristowe 1929). Cheliceral locking may thus anchor the male more securely to the female’s body during mating, and this could facilitate the coupling of their relatively simple genitalia (Levi 1981; Kraus 1984).

The chelicerae of *Leucauge* are used differently. It is the female who seizes the male’s chelicerae during copulation, rather than vice versa (Castro 1995; Eberhard and Huber 1998a). The female spreads her basal segments and opens her fangs, and the male then inserts the distal portion of each basal segment (with his fangs closed) between the female’s fang and her basal segment; she then closes her fang, clamping the distal portion of the male’s basal segment (Fig. 4.2). The chelicerae are not sexually dimorphic in length (Fig. 4.3), but, at least in *L. mariana*, *L. argyra*, and *L. venusta*, the male chelicerae have sexually dimorphic macrosetae, distal lateral plates, and corrugations (Figs. 4.2 and 4.3a). The designs of these modifications do not show any signs of being mechanically useful to defend against the female or to stabilize male–female cheliceral grasps. These sex-specific structures seem to be more appropriate for stimulating the female. There is further diversity in the genus; another, unidentified species of *L. sp.* (perhaps near *moerens*?) did not perform cheliceral clasps and lack sexual dimorphism in male and female chelicerae (Barrantes et al. in press).

Male chelicerae also show other modifications in tetragnathids. A sexually dimorphic, rugose cuticular texture of the male chelicerae has evolved at least five times (Álvarez-Padilla and Hormiga 2011). The basal segments of the chelicera of male *L. argyra* have a rugose area near the distal, lateral corner, but those of *L. mariana* are smooth (Fig. 4.3). Males of both of these species as well as other *Leucauge* and the related genera *Mesida* and *Opadometa* have more abundant macrosetae on the anterior surfaces of their chelicerae than do females (Álvarez-Padilla and Hormiga 2011). This sexually dimorphic cheliceral trait may be species-specific in at least some *Leucauge* species (H.W. Levi unpublished figures; Castro 1995).

In both *L. mariana* and *L. argyra*, the female’s chelicerae seize the male’s chelicerae before palpal insertion begins, and the cheliceral clasp is usually maintained throughout most if not all the copulation (Eberhard and Huber 1998a; Aisenberg 2009; Aisenberg and Eberhard 2009; Aisenberg and Barrantes 2011). Mating ends when the female releases the male’s chelicerae. Male chelicerae are narrower in the region (just short of the tip) that the female clasps with her chelicerae (Fig. 4.2a; Eberhard and Huber 1998a; Aisenberg et al. 2015).

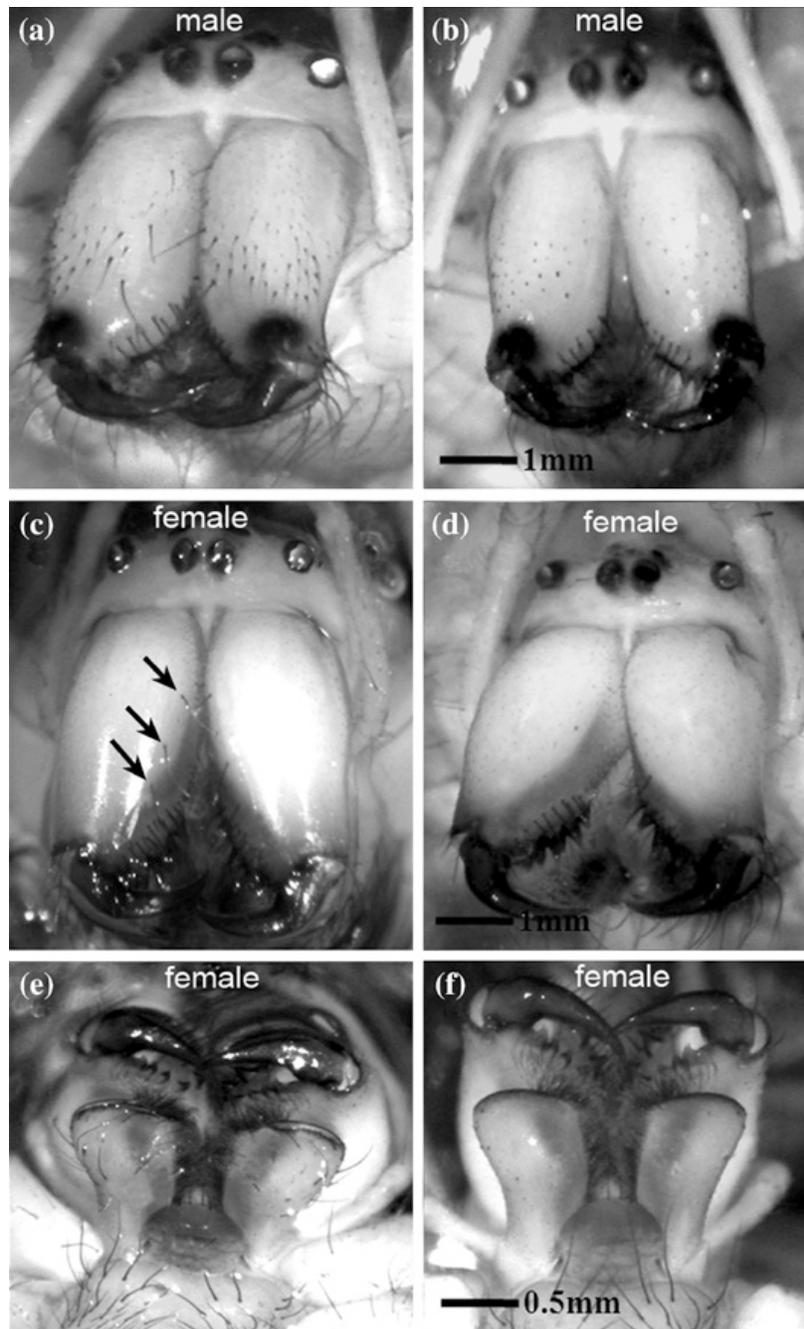


Fig. 4.3 Frontal views of the chelicerae of female (a, b) and male (c, d) *Leucauge mariana*; a, c are unmodified, while b, d have their setae shaved off. The endites and labium of female, before (e) and after (f) setae, were shaved off. The setae along the margins of both the chelicerae and the endites, which presumably function when the spider bites and ingests prey, were left intact (figure from Aisenberg et al. 2015)

The morphological details of the cheliceral clasps observed in *L. mariana* were as follows (Eberhard and Huber 1998a; Aisenberg et al. 2015). The female always opened her chelicerae wide as the male approached (usually with his own

chelicerae closed) and then grasped the distal portions of the basal segments of the male's chelicerae by closing her fangs. The inner surface of her fang grasped the posterior surface of the male's chelicerae rather than his endites (Fig. 4.2d). The distal portion of the male's basal segment projected beyond the female's chelicerae, and his fangs often opened and closed while he was being clasped (Fig. 4.2d). In some cases, his fangs, when extended, deflected long setae on the anterior margin of the female's sternum (Fig. 4.2b, d). The spiders often separated and then the female clasped the male again several times during a given pairing. Between clasps, the spiders moved apart, in some cases several body lengths. The male often courted again before the next cheliceral clasp. In some cases, the female's behavior just after a pair broke apart appeared to be aggressive, and she made rapid bursts of movement and gave relatively violent jerks on lines running toward the male. The male nevertheless often courted and successfully induced her to approach again (or to allow him to approach) and to assume the acceptance posture. Copulations with virgin females were longer and included more cheliceral clasps than copulations with non-virgins (Aisenberg and Eberhard 2009; Aisenberg and Barrantes 2011).

4.4.3 L. mariana: Movements of Male Genitalia When the Female Lacked a Plug

The movements of the male's genitalia in copulations with females that lacked a mating plug form an important baseline for comparisons with the movements of *L. argyra* (Sect. 4.4.4), those involving in plug removal (Sect. 4.4.5) and the effects of cutting off male sclerites (Sect. 4.5.2.2), so we will describe them in detail (based on Eberhard and Huber 1998a). To copulate, the male of *L. mariana* extended a palp one or more times so that the dorsal surface of his cymbium contacted the ventral surface of the female's abdomen just anterior to her epigynum. At least, some of the many setae on the cymbium, especially those on its basal half, were interlaced among the setae on the surface of the female's abdomen near her epigynum and may have helped couple the cymbium mechanically to the female. After the cymbium was seated, the basal hematodocha expanded. This moved the more distal portions of the palp away from the cymbium and rotated them nearly 180°. The conductor and embolus moved toward and usually contacted the female's epigynum, inserting (or apparently attempting to insert) the conductor and the embolus into the opening of an insemination duct (the "atrium"). There were two types of insertions of the male palps—"long" and "short."

4.4.3.1 "Long" Insertions

Long insertions (average duration >1 min) usually occurred early in copulations, while "short" insertions (as short as a second or so) tended to occur later (with exceptions). In a long insertion, the palp usually made only a single long insertion,

Table 4.1 Female reproductive processes that are capable of affecting male paternity success and that were correlated with particular male sexual traits in *Leucauge mariana* and *L. argyra*

Correlations between variation in male traits and female responses		
Male trait	Female response	Species
More bouts of copulatory courtship (leg pushing)	Form copulatory plugs more frequent	<i>L. mariana</i>
More duration of copulatory courtship (leg pushing)	Form copulatory plugs more frequent	<i>L. mariana</i>
More short insertions of palps	Form copulatory plugs more frequent	<i>L. mariana</i>
More twangs during pre-copulatory courtship	Form copulatory plugs more frequent	<i>L. argyra</i>
<i>Experimental manipulations</i>		
Remove male setae chelicerae	Remates more	<i>L. mariana</i>
Remove female setae to sense male chelicerae setae	Remates more, forms copulatory plug less frequent, and interrupts copulation more	<i>L. mariana</i>
Remove tip of conductor male pedipalp ^a	Less sperm transferred to female	<i>L. mariana</i>
<i>Additional female effect</i>		
Earlier, more abundant production of plug liquid	Trap and kill male	<i>L. argyra</i>

^aControl treatments were not exactly equivalent, leaving doubts in interpreting results

but there were repeated hematodochal expansions and collapses. The conductor and the embolus, which were driven against the epigynum by the movements produced by the first hematodochal inflation, remained in contact with the epigynum, while the basal and medial hematodochae repeatedly collapsed partially and then reinflated (Table 4.1). The base of the embolus moved toward the tip of the conductor at the beginning of the insertion and then remained there immobile. During each inflation, the conductor twisted slightly around the point where its tip contacted the atrium (the embolus was apparently inside the insemination duct—see below). The twisting movement caused the hook process on the conductor to sweep antero-laterally on the female's epigynum until it was arrested by encountering the hood at the anterior margin of the atrium. The tip of the embolus projected 155–165 μm beyond the tip of the conductor in three males. This distance was nearly the same as the distance travelled by the base of the embolus toward the tip of the conductor, confirming that the movement of the embolus base caused the embolus to be exerted. Thus, the tip of the embolus must have passed through the insemination duct and then entered deep into chamber I of the spermatheca, because the length of the insemination duct of the female was only about 60–80 μm (Eberhard and Huber 1998a). Since the base of the embolus did not move after the first hematodochal inflation, the embolus presumably remained inserted in chamber I throughout each long insertion. A long insertion ended when the conductor and embolus pulled away from the epigynum, and the cymbium and the entire palp were withdrawn from the female's abdomen.

Sometimes, a male appeared to have difficulty freeing the conductor and embolus from the epigynum following a long insertion, so that only after he had pushed the female with his legs (and sometimes, the female had released her cheliceral grip) did his palp come free with a snap. It is possible that the triangular tip of the embolus (Fig. 4.4; Méndez and Eberhard 2014) snagged at the junction of the insemination duct and spermathecal chamber I.

Sperm and additional material were transferred to the female during long insertions. Sperm introduced into the large, soft-walled chamber I of the spermatheca caused it to inflate. At least, some sperm in this chamber become decapsulated within an hour or so (Eberhard and Huber 1998a).

4.4.3.2 “Short” Insertions

Short insertions occurred in bursts of several short insertion movements of the conductor during a single period of cymbium–abdomen contact. In each insertion, the conductor tip was inserted into the atrium and the embolus base moved distally along the conductor. The conductor was withdrawn from the atrium after each insertion (it always came away smoothly, without a snap), and the embolus base withdrew to its resting position at the base of the conductor. Each time the palpal sclerites rotated again to bring the tips of the conductor and the embolus into contact with the epigynum, the base of the embolus gradually moved toward the tip of the conductor (as in a long insertion). Most inflations during short insertions resulted in a viscous white material with a consistency similar to that of toothpaste emerging from the tip of the palp during the period in which the base of the embolus moved distally. In most cases, the white plug material adhered only very poorly to the female. Sometimes, it came away still stuck to the male’s palp when the embolus and conductor were withdrawn. Often when the tip of the conductor and the embolus were reinserted, they dislodged and removed a mass of material that had been deposited previously. During one copulation, for instance, the male more or less filled one side of the atrium with white material three different times, but each time eventually dislodged the accumulation during subsequent insertions. Most copulations with virgin females ended with the female still lacking a plug, even though the male had deposited material. In two cases, the plug material assumed a more liquid consistency and flowed into the atrium and presumably at least into the mouths of the insemination canals, where it condensed into a single, smooth mass that remained in place at the end of copulation.

A burst of short insertions lasted on average less than half as long as a long insertion (mean durations 128 and 359 s, respectively; Aisenberg 2009) and included only about one-fourth as many hematodochal inflations. Following a burst of short insertions, the cymbium was withdrawn, the palp was retracted, and the other palp was extended to the female’s abdomen. The order of long and short insertions varied to some extent, and sometimes, a long insertion occurred after several short insertions had been performed on the same side of the epigynum.

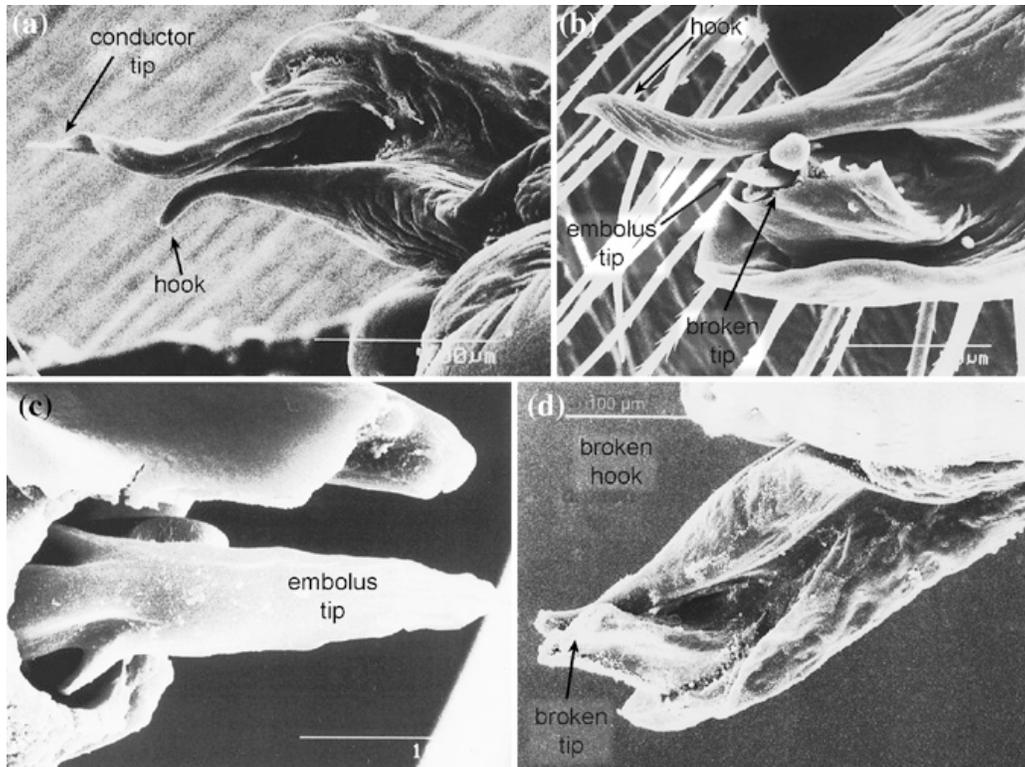


Fig. 4.4 Intact and modified palps of *L. mariana*. **a** Intact hook and conductor tip; **b** hook intact but tip of conductor removed and tip of embolus exposed; **c** triangular tip of embolus; and **d** both conductor tip and hook removed from Barrantes et al. 2013

4.4.3.3 “Flubs”

A third type of palpal contact consisted of what appeared to be failed attempts to insert the conductor (“flubs” in the terminology of Watson 1991). Inflation of the hematodochae caused the tips of the conductor and the embolus to scrape across the face of the epigynum without engaging it or only briefly engaging it, at an inappropriate site and then snap free. The male often repositioned his cymbium, lifting it briefly from the female’s abdomen and then setting it down at a slightly different site after a flub. Flubs were more common later in copulation, when short insertions tended to occur.

4.4.4 *L. argyra*: Movements of Male Genitalia When the Female Lacked a Plug

Copulations in *L. argyra* differed in several ways. They included only long palpal insertions, and there were multiple hematodochal inflations after each palpal insertion; flubs were rare (Aisenberg and Barrantes 2011). Matings usually ended

with a vigorous struggle, in which the female appeared to attempt to grasp the male's body or legs with her legs while maintaining her chelicerae locked. The male, for his part, frantically attempted to break away and escape. The female often attempted to wrap the male with silk during the struggle, as occurs during prey capture. Sexual cannibalism sometimes occurs (in 1 of 32 copulations of virgin females, and 4 of 13 in females with mating plugs) (Aisenberg and Barrantes 2011), both prior to and during copulation (it has never been seen in *L. mariana*).

As might be expected from the sharply different male and female genital morphology in this species, the copulatory behavior of the male genitalia of *L. argyra* differed in many aspects from that of *L. mariana* (Barrantes et al. 2013). Males of *L. argyra* have two major derived genital structures; one of them, the strong cymbial hook, locked his palp to the female's epigynum as follows. The male first inserted his cymbial hook into the laterally facing atrium on one side of the epigynum and then held it there while he inserted his conductor into the atrium on the opposite side (Figs. 4.5 and 4.6). Both the cymbial hook of the male and the large, widely flared, laterally oriented atria of the female are derived traits and may be unique to *L. argyra*. A second derived male genital structure, a curved tooth on the margin of the cymbium, probably served to lock the cymbial hook against the epigynum after it was inserted into the atrium. Both derived male structures of *L. argyra* may have evolved to stabilize the male's genitalia during intromission, perhaps in response to the frequently violent and dangerous resistance behavior of females of *L. argyra* during and at the end of copulation, or to perforate the especially strong mating plug (below). Clamping the female with the male's genitalia is not common in spiders (Eberhard and Huber 2010), in contrast to some other groups such as insects (e.g., Tuxen 1970; Scudder 1971). Possibly, the difference is due to spiders being unable to exert strong mechanical force with their genitalia because they lack in the palpal bulb muscles (Eberhard and Huber 2010).

4.4.5 Movements of Male Genitalia When the Female Has a Plug

In both species, some males which copulated with females that bore mating plugs were able to remove or penetrate the plug, but others failed to gain access to the insemination ducts despite sustained attempts and eventually left. Detailed observations of palp behavior are only available for *L. mariana*, in which the male succeeded in overcoming the plug in 68 % of 28 pairs (Méndez and Eberhard 2014) and we will describe them first. Copulations with unplugged non-virgins consisted mainly of short insertions and almost never included long insertions. Males employed three different mechanical ways to overcome plugs: snag the plug and pull it off; break it and then penetrate through it; and break its adhesion to the epigynum by injecting material under it. The genital bulb lacks muscles and

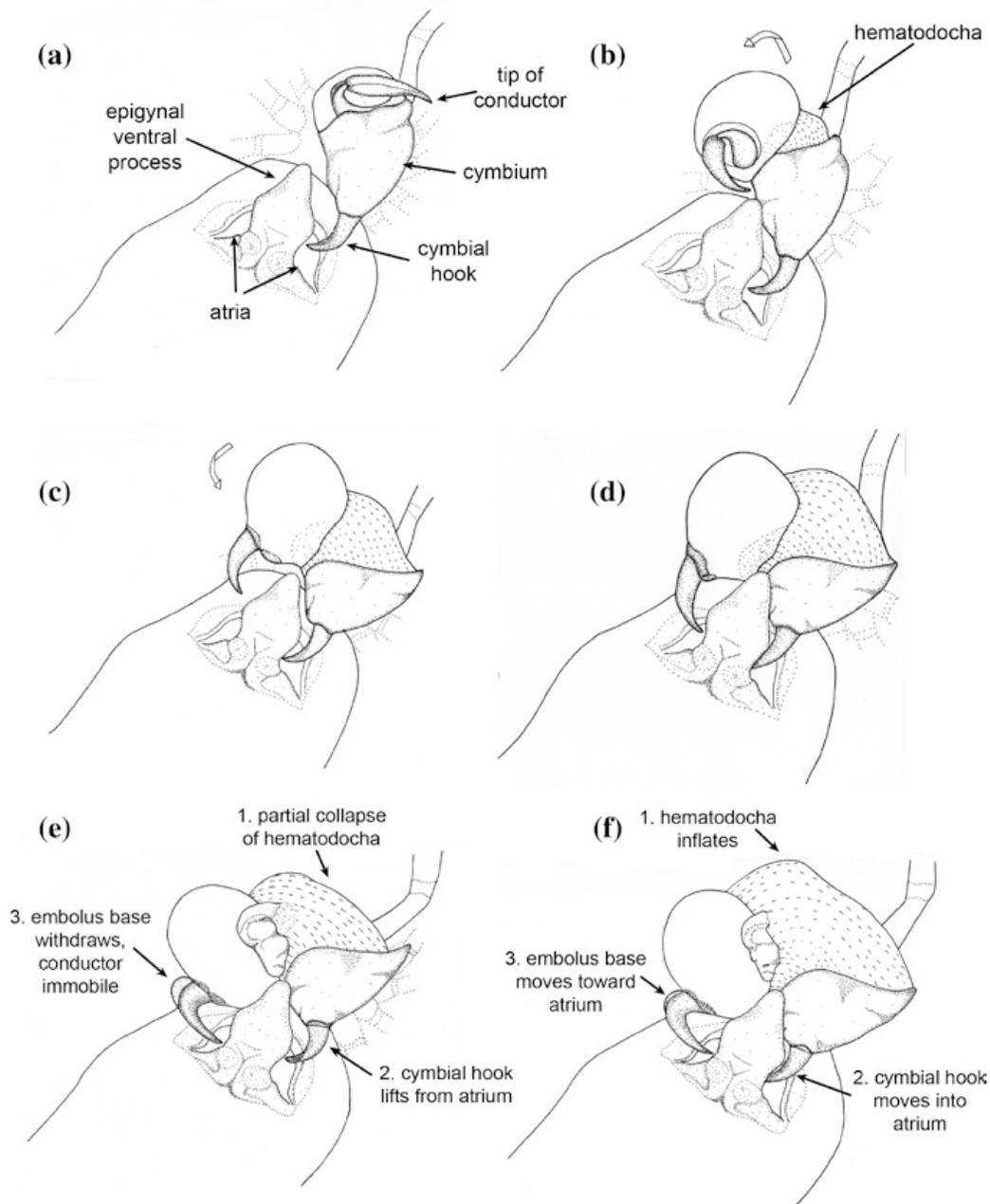
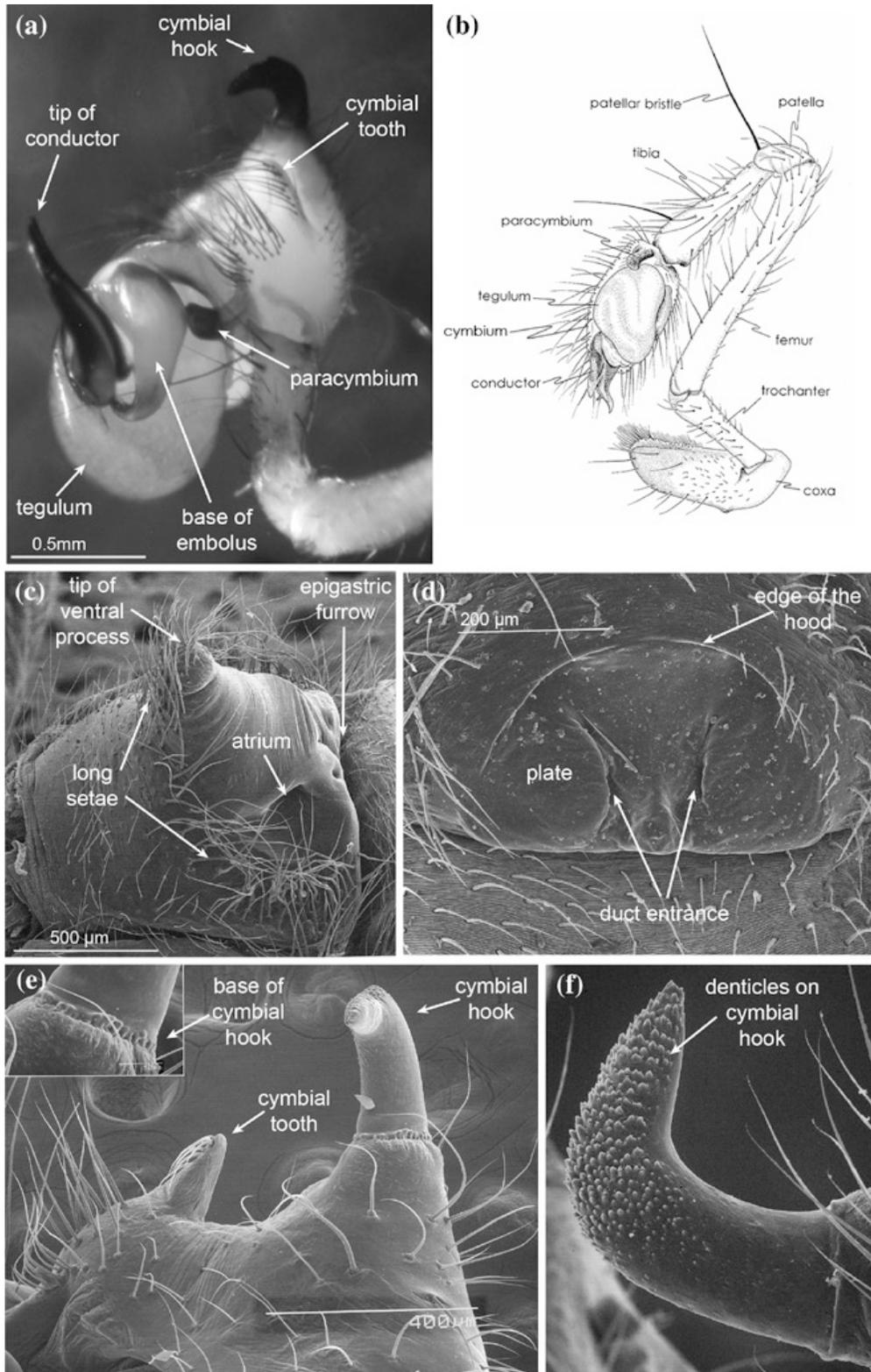


Fig. 4.5 Schematic drawings (in posterior–ventral views) that show how the male palp clasps the anterior portion of the female’s epigynum by inserting the cymbial hook in one atrium and the conductor in the other atrium in *L. argyra* (his palp is partially hidden behind the ventral projection of the epigynum). Insertion of the conductor (a–d) follows insertion of the hook, as the basal hematodocha expands and drives the conductor into the atrium. Next, the hematodocha collapses partially and the conductor remains immobile, but the embolus base withdraws (e). Finally, the hematodocha inflates and the embolus base moves toward the atrium (f), presumably driving the embolus tip into the insemination duct (figure from Barrantes et al. 2013)



◀ **Fig. 4.6** Portraits of the male and female genitalia of *L. mariana* and *L. argyra* that document several derived traits in *L. argyra*. **a** The palp of the male of *L. argyra* has a long process on the cymbium (cymbial hook), with a smaller toothlike process (cymbial tooth) near its base on the border of the cymbium (**e, f**); **b** both are lacking in the palps of *L. mariana* (from Eberhard and Huber 1998a, b). **c** The epigynum of a *L. argyra* female has long, robust ventral process; the entrances of the two insemination ducts (the atria) are large and open laterally; and there are abundant long setae around the atria as well as on the anterior surface of the ventral process. None of these traits occurs in the epigynum of *L. mariana*. **d** Although there is no phylogeny of *Leucauge* currently available, the absence of all of the *L. argyra* traits in other *Leucauge* and other tetragnathids indicates clearly that they are all derived in the lineage of this species (Barrantes et al. 2013)

innervation, so the male's ability to guide these genital movements precisely seems likely to be limited (Eberhard and Huber 1998b, 2010). Neither movements nor sequences during plug removal showed any special traits that occurred when the female was plugged or that seemed especially designed for removing plugs. Further, more detailed analyses of palp behavior are needed, however (Méndez and Eberhard 2014). After overcoming a plug and making one or more insertions, the male deposited plug material of his own, as was described above.

Males of *L. argyra* also sometimes failed and sometimes succeeded in coupling their palps to the epigynum when it was plugged. The details of the palp movements have not been studied in detail, however, and it was usually not certain whether the conductor or the embolus entered the atrium. Typically, a male made repeated failed attempts to engage the cymbial hook with the epigynum, first with one palp and then the other, before succeeding in clasping the epigynum with the hook and then the conductor. The plugs in *L. argyra* are so hard and adhere so tightly to the epigynum that it is difficult to imagine a male being able to penetrate a plug unless it were somehow first softened. Nevertheless, males did sometimes penetrate them without removing them; in one case, the male broke the plug on one side free and then inserted the conductor along the side of the plug on the other side (Aisenberg and Barrantes 2011; A. Aisenberg, unpub.). More detailed observations of plug removal and penetration are needed.

4.4.6 Female Participation During Copulation

Because the events of copulation are played out on and inside the female's body, her morphology and behavior inevitably have important effects on copulation success. "Passive" female morphological participation is involved at all stages of copulation. The shape of her epigynum, the length of her spermathecal duct, the positions and shapes of her spermathecae (Eberhard and Huber 1998a; Triana and Quesada, in prep.), the shape and size of her chelicerae, and the presence of sense organs on her chelicerae, endites, and epigynum, etc., all have potentially important roles in determining the success of a copulation. Most of these female traits

seem relatively free to evolve without having disadvantageous consequences under natural selection. Different forms could bias mating success with respect to different male traits (and thus exercise sexual selection on them), so this kind of female participation is not evolutionarily trivial.

Female *Leucauge* also participated actively in several aspects of copulation. Occasionally, a female *L. mariana* pushed away a male's palp that had been inserted in her epigynum with her leg IV. In both species, the female also sometimes terminated copulation itself by opening her chelicerae and pulling away. In addition, several lines of evidence showed that the females of both species also played active roles in the formation of mating plugs.

4.4.6.1 Copulatory Plugs

Copulatory plugs that adhere to female genitalia occur in various animal groups, including nematodes, insects, spiders, scorpions, reptiles, snakes, birds, and mammals (Drummond 1984; Andrade 1996; Gomiendo et al. 1998; Knoflach 1998; Simmons 2001; Wigby and Chapman 2004; Mattoni and Peretti 2004; Aisenberg and Eberhard 2009; Leonard and Córdoba-Aguilar 2010; Timmermeyer et al. 2010). Their composition varies and includes mucous, sperm, remains of spermatophores, parts of male genitalia that have broken off, and the male's entire body (Uhl et al. 2010). Traditionally, copulatory plugs have been considered male adaptations to gain advantage in sperm competition. By plugging the female after mating with her, a male imposes a physical barrier against other males, thus eliminating or reducing the chances that the female will receive sperm from additional males (Simmons 2001; Uhl et al. 2010).

Although mating plugs are widespread, their biological function is not always clear. The copulatory plugs of some spiders challenge the traditional interpretation because they are composed of a mixture of substances produced by the female as well as the male; those of still others are formed from substances produced exclusively by the female (Knoflach 1998; Aisenberg and Eberhard 2009; Uhl et al. 2010; Aisenberg and Barrantes 2011; Kuntner et al. 2012).

There are several additional possible functions for the plugs. Plugs could benefit females by preventing sperm desiccation or minimizing sperm loss (Boorman and Parker 1976; Huber 1995, 2005; Simmons 2001; Uhl et al. 2010). They could also act as mechanisms of CFC: By making a plug or allowing one to be made, a female could bias the chances of paternity for her offspring in favor of those future mates which are able to remove plugs. It is important to realize that a mating plug in *Leucauge* is probably of little significance from the female's point of view with respect to remating per se. This is because a female *Leucauge* need not rely on a plug to prevent additional copulations. Females can and do sometimes refuse males easily by failing to performing one or more of the several receptive responses that are necessary for copulation to occur (above).

The active female participation in plug formation in *Leucauge*, which has been documented in several ways, highlights the possible importance of females in

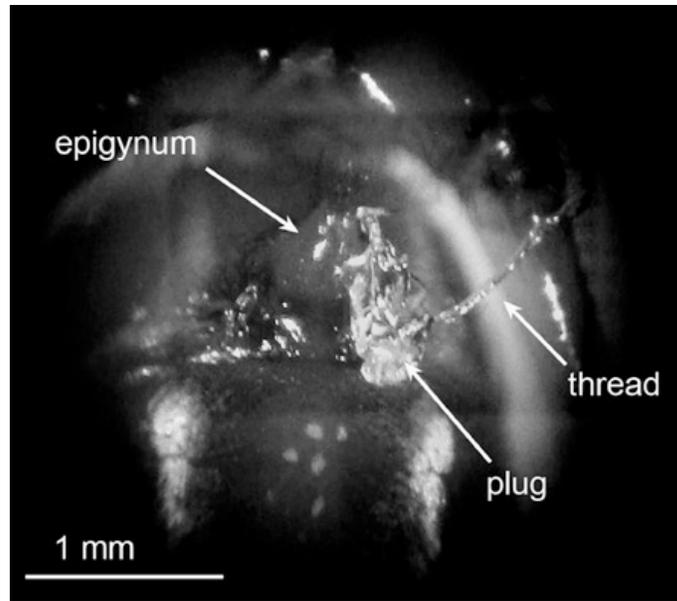
determining the conditions under which remating occurs. Most importantly, we have made occasional direct observations (when viewing angles were favorable) of liquid surging up from the insemination duct of an unplugged female and hardening to form a plug in both species (Eberhard and Huber 1998a; Aisenberg and Barrantes 2011). In *L. mariana*, plug material in some females had acquired a clearer, less-white appearance and was still very liquid in appearance an hour after copulation ended. The prominent, lumpy pile of white material that had emerged from the male's palp and accumulated on the epigynum sometimes later acquired a more level, smooth surface; in one case, we observed that this transformation occurred when liquid welled up from the insemination duct (Eberhard and Huber 1998a). Similar smooth-surfaced masses were common in many field-collected *L. mariana* females.

Direct observations showed that female *L. argyra* had an even more exclusive role in plug formation. The male never deposited any material on the surface of the female's epigynum during copulation, as in *L. mariana*. A clear liquid was seen welling up from inside the insemination ducts up to an hour or more following some copulations (Aisenberg and Barrantes 2011). While the possibility that this liquid contained male products cannot be ruled out definitively, this seems unlikely. In only 5 % of examined plugs were there any sperm present (and then in only very small quantities) (Barrantes et al. 2013). Even if male products are included in the liquid, the movement of the liquid to the surface of the epigynum from inside the insemination ducts was presumably performed by the female. The timing of copulatory plug production *L. argyra* varied substantially; plug material was first seen at the beginning of mating, during palpal insertions, immediately after copulation, and many hours later in different pairs (Aisenberg and Barrantes 2011). The hardened plugs of *L. argyra* were more rigid and adhered more tightly to the epigynum than those of *L. mariana* (Méndez 2002; Barrantes et al. 2013; Méndez and Eberhard 2014).

4.4.6.2 Copulatory Plugs as Lethal Traps in *L. argyra*

An unusual feature of plugs in *L. argyra* is that while the male has his palp inserted, apparent plug material sometimes emerged from the female's genitalia and then hardened into a strongly adhesive state and caused the male's palp to adhere tenaciously to her epigynum (Aisenberg and Barrantes 2011). The male struggled frenetically to pull his palp free, pushing with his legs against the female. Because the palp bulb has no nerves or sense organs (Eberhard and Huber 1998b, 2010), it is not clear whether or not the male was able to sense that the liquid was beginning to accumulate before he attempted to pull his palp free. In some cases, the male succeeded in freeing his palp easily; in other cases, it was more difficult, and the rapidly hardening substance was pulled into a peak or thread during the process of pulling free and hardened in this form (Fig. 4.7). On some occasions, the male was unable to pull free, and he was then attacked and killed by the female. The danger to the male was greatest when plug liquid was more abundant and emerged while the male was still copulating. In addition, some

Fig. 4.7 This thread extension of the mating plug on the epigynum of a female *L. argyra* tells a story of female treachery. The female produced an abundant substance that adhered to the male's palp and made it difficult for him to pull out (from Barrantes et al. 2013)



plugs seemed to harden more rapidly; others remained liquid for a longer time. It thus appears that the male's chances of survival are affected by three female-controlled variables: the quantity of plug material; the timing of its emission onto the epigynum; and the rate of hardening. The frequency with which females in the field were found that had peaks or threads of plug material (21 % of 113) indicates that struggles by males to free themselves from plugs are not uncommon in nature (Aisenberg and Barrantes 2011).

In sum, active female cooperation is crucial in both species in determining copulation success at three stages: whether copulation would occur, when it would end, and whether a mating plug would be formed. In *L. argyra*, the female's behavior also affects the male's chances of survival.

4.5 Factors Affecting the Outcomes of Copulation

4.5.1 Correlations with Courtship Behavior

Higher numbers and durations of one type of copulatory courtship (male leg pushing) and greater numbers of short genital insertions during copulation were correlated in *L. mariana* with a higher probability that the female would form a mating plug (adding her liquid to the white substance deposited by the male) (Aisenberg and Eberhard 2009). In *L. argyra*, twanging during pre-copulatory courtship was also correlated with a greater tendency for females to form copulatory plugs (Aisenberg and Barrantes 2011).

4.5.2 Evidence from Experimental Manipulations

Data from experimental manipulations have an advantage over those from correlations because they allow cause and effect to be distinguished. However, many direct experimental tests of the functions of sexually dimorphic traits are incomplete; most experiments focus mainly on modifying one of the sexes (usually males) and recording the consequences of these modifications on female choice (Eberhard 2011). In addition, many studies fail to demonstrate unambiguously that it is stimulation from male genital or non-genital traits rather than some other associated trait that is under sexual selection through female choice, and do not eliminate the possibility that the behavior of morphologically modified animals remains unchanged (Eberhard 2011).

We have confronted these challenges by using experimental tests in *Leucauge* in which both male structures and female sensitivity to them are modified in two contexts. Experimental modification of some of the rigid cuticular structures involved in sexual interactions in *Leucauge* was relatively easy. The manipulations performed to date, however, have involved only relatively gross alterations; they do not test the consequences of particular designs of structures, but rather test the effects of the presence or absence of the structure.

4.5.2.1 Experiments with the Male's Cheliceral "Beard"

Aisenberg et al. (2015) tested the possibility in *L. mariana* that tactile stimuli from the male's dimorphic cheliceral setae (Fig. 4.3a) and/or the movements of his fangs during cheliceral clasps constitute courtship. We modified both the male stimulatory structures and the suspected female receptors (setae on her chelicerae and endites) and then checked whether these manipulations affected female sexual responses that could alter a male's chances of paternity. We shaved male cheliceral setae in one experimental group and shaved the setae on the female's chelicerae and endites which are often contacted by male's chelicerae on another group (Fig. 4.3). We analyzed the effects of these manipulations on the female's mating behavior (e.g., her tendency to interrupt copulation), her receptivity to remating, and the formation of copulatory plugs compared with controls.

Removing the male cheliceral setae resulted in a female response that seems likely to decrease the male's chances of paternity: The female was more likely accept a remating (Aisenberg et al. 2015). However, the effects were even more extreme when female cheliceral and endite setae were removed: Chelicerae disengagement was more frequent, and the female was more likely to accept remating and less likely to form a copulatory plug. These experiments were incomplete in that they did not include masking the setae on the female sternum that may also be implicated in female sexual responses to male stimulation (Fig. 4.2), nor did they include removal of her cheliceral setae without also removing her endite setae.

4.5.2.2 Experiments with Genitalia: Cutting Palpal Sclerites in *L. mariana*

In *L. mariana*, the tip of the male conductor, or the tip of the conductor and that of the nearby conductor hook were cut (Fig. 4.4) (Méndez and Eberhard 2014). In each case, the other palp was left intact as a control, and the male's ability to remove plugs and to inseminate the female with his two palps was observed. The effects of these manipulations, combined with direct observations of the morphology and behavior of these structures, indicated that the relatively flexible conductor tip is important in sperm transfer (its removal reduced sperm transfer). Plugs were snagged and penetrated, in contrast, by the combination of the protruding conductor tip and hook. Of the two, the hook was probably more important in removing plugs; it is much stiffer than the conductor tip, and the form of its pointed, hooked tip is appropriate to snag the edge of the plug or irregularities on its surface. In addition, experimental removal of the hook and the conductor tip reduced the palp's ability to break the plug, compared with the intact palp of the same male, while removal of only the conductor tip did not result in differences in plug breakage between the experimental and the control palp.

Interpretation of the results is complicated, however, for several reasons: The plugs were not bilaterally symmetrical in these tests; there was less than complete certainty whether insemination followed plug removal because we were not able to distinguish with certainty whether or not the embolus was inserted into the insemination duct when the tip of the conductor was in the atrium; and there was at least occasional uncertainty regarding which palp was the first to loosen a plug.

4.6 Discussion

4.6.1 *The Non-passive Females of Leucauge*

Perhaps one of the most striking aspects of the sexual biology of *L. mariana* and *L. argyra* is the pervasive need for the female to actively cooperate if a male's attempts to copulate and sire her offspring are to be successful. She performs six active responses prior to coupling, two during copulation, and one following copulation. Some of these same cooperative responses also occur in *L. regnyi* (Alayón 1979) and *L. venusta* (Castro 1995). One of these, the ventral flexion of the female abdomen to bring it into range of the male's palps appears to be widespread in Tetragnathidae (Levi 1981; Álvarez-Padilla and Hormiga 2011), as it is indeed, in many other spiders (Huber 1998).

Physical coercion of a female *Leucauge* by the male to oblige her to mate is simply not possible, and male *L. mariana* and *L. argyra* do not attempt to coerce females. Instead, they perform several types of behavior that are apparently designed to induce females to cooperate. Cooperation in female *L. argyra* is less complete, and females often attack and sometimes kill males either prior to or part way through a copulation.

4.6.2 *Why Do Females Influence Copulation Outcomes?* *Functions of Male and Female Traits*

The active female influences just mentioned are in accord with the possibility that CFC occurs in *L. mariana* and *L. argyra*. Several of the requirements for demonstrating CFC (Eberhard 1991, 1996) are fulfilled: Females are polyandrous; female copulatory and post-copulatory behaviors can determine male paternity success; and females decide to favor certain sexual partners over others based on both male genital (number and duration of short insertions) and non-genital (number and duration of leg pushes) copulatory behaviors. As discussed in Chap. 1, however, there are several alternative types of selection that need to be considered (for more detailed descriptions of these hypotheses, see Chap. 1).

4.6.2.1 Species Isolation

Although we have not attempted to make crosses of *L. mariana* and *L. argyra*, their genitalia seem likely to be exclude each other. The form of the epigynum of *L. mariana*, with its small atria that open medially rather than laterally, seems highly likely to preclude successful intromission by a male *L. argyra* (there is nowhere for the cymbial hook to be inserted). Epigynal designs similar in general form to that of *L. mariana* occur in many *Leucauge* species (Levi, unpub.). We doubt, however, whether selection favoring species isolation was responsible for the evolution of these mechanical incompatibilities, or for the many other traits that we have discussed which only come into play after a female has received various pre-copulatory male courtship stimuli. Terminating cross-specific sexual interactions earlier rather than later in an interaction would be advantageous for both males and females and would be expected to evolve. As expected from this argument, Castro (1995) found that in none of the 47 crosses among four species of *Leucauge* that she made in captivity did the male even perform clear pre-copulatory courtship behavior, much less approach and clasp chelicerae with the female. We thus suppose that most if not all of the traits we described above evolved due to sexual selection, rather than natural selection to isolate species.

4.6.2.2 Sperm Competition

One type of post-copulatory sexual selection that probably occurs in *Leucauge* is sperm competition. Removal of mating plugs deposited during previous copulations and thus gaining access to the female's insemination ducts is a male mechanism to win out in sperm competition. One genital trait in *L. mariana*, the conductor hook, appears to function in plug removal and is thus probably an adaptation to sperm competition (though some details remain to be resolved). The cymbial hook in *L. argyra* may also function in plug removal, but further observations are needed to test this speculation.

With respect to the deposition of mating plugs that are capable of excluding future males, however, it is the females of *Leucauge* rather than the males that appear to be in control, so the traits discussed here that are related to the formation or lack of formation of a sperm plug are not attributable to sperm competition. In fact, as argued above, they are not even likely to be attributable to selection on females to prevent future insemination, because females can easily prevent any interaction with an unwanted male from reaching the stage of contact with his genitalia. A female need not rely on a physical covering of her genitalia to avoid insemination. The most likely sexual selection advantage of plugs to females is as filters for potential mates to allow CFC (below).

Except for copulatory plugs, none of the other male traits that are discussed here seem to have any physical relation to competition involving deposition or removal of sperm.

4.6.2.3 Sexually Antagonistic Coevolution

Having eliminated species isolation and sperm competition as potential explanations for the evolution of male chelicerae in *L. mariana*, the male genitalia of *L. argyra*, and the female traits that interact with these structures, only two hypotheses remain to be tested: SAC (Arnqvist and Rowe 2005) and CFC (Eberhard 1996).

SAC can be classified into two basic types (Arnqvist and Rowe 2005; Chap. 1). One possibility is that SAC results from forceful, physical male manipulations of females (coercive manipulations) (Alexander et al. 1997; Arnqvist and Rowe 2005). This type of sexual selection is highly unlikely in either species of *Leucauge*, because there is no way males can physically oblige females to cooperate. As noted above, females must cooperate in four different ways for copulation to begin; once copulation begins, they can terminate it at any time by opening their chelicerae or pushing the male's palp away from the epigynum with their tarsi; and they are free to produce or not produce sperm plugs following copulation. Furthermore, the male traits that correlate with female cooperation, such as gentle leg pushing, short pedipalp insertions, and contact with setae on the female's chelicerae and endites, are not appropriately designed to physically force the female to cooperate.

Thus only an alternative, signaling version of SAC could work with *Leucauge* (a third, physiological type of male manipulations is not relevant to the traits discussed here). One possible objection to the signaling version of SAC is that we did not find female morphological defensive structures or devices to block and protect her sense organs in the areas contacted by male genitalia and cheliceral setae, as SAC might predict. This failure is suggestive, but not convincing, as evidence against SAC, however, because female resistance might be in her nervous system (lack of synapses between particular neurons, lower responsiveness of certain neurons, etc.) rather than in her external morphology.

A more convincing objection to SAC involves female sensitivity and responses to male stimuli. SAC supposes that males manipulate females via sensory traps. A sensory trap can occur when the male mimics a stimulus to which females have evolved under natural selection to respond in a way that would favor the male. For

instance, the male *Calopteryx haemorrhoidalis* damselfly induces the female to discard sperm from previous males that are stored in her spermatheca, even though it may be in her best interests to conserve them. He uses a sensory trap (Córdoba-Aguilar 1999, 2002). He rubs his genitalia on receptors in the wall of the female's oviduct that evolved to sense the presence of an egg descending the oviduct; these receptors trigger the female to move sperm from her spermatheca to the oviduct and fertilize the egg. The male triggers this female sperm transport response and then removes the sperm from the oviduct with his genitalia. Sexual selection on the male (sperm competition) thus favors his use of a female response that evolved under natural selection, and continued natural selection on the female to sense and fertilize eggs in her oviduct prevents her from ceasing to respond to this stimulus in this way, even though her responses to males may reduce her own fitness.

Male *Leucauge* do not seem likely to be using such sensory traps. This is because it seems unlikely that the female sensitivities and responses to the male's stimuli are restrained by natural selection. Take, for instance, stimuli from the setae on the male's chelicerae, which increase the female's tendency to make a mating plug and to reject future mating attempts. Assume for the moment that there is conflict and that it is disadvantageous to the female to lose these mating opportunities. The female will lose nothing (via natural selection) from simply readjusting her threshold of response to the stimuli from the male's chelicerae (or eliminating her responses entirely), thus avoiding the cost of being manipulated. A similar argument could be made for the correlation between pre-copulatory male "twangs" during pre-copulatory courtship and the production of copulatory plugs. In this case, one might make the (strained) argument that sensitivity to the kinds of vibrations that a male produces with a twang might be under natural selection in another context, such as prey capture. But this neglects an additional necessity for the sensory trap argument: The female's sensitivity should be intrinsically linked, due to natural selection, to copulatory plug production. We see no logical reason to expect that such a linkage will occur under natural selection.

A limitation of these arguments is that they are not direct, and they involve theoretical predictions rather than empirical measurements (e.g., of the lack of costs to the females).

4.6.2.4 Cryptic Female Choice

The CFC hypothesis is favored as an explanation of several of the morphological and behavioral characteristics of male *L. mariana* and *L. argrya* that are employed during mating, both by the elimination of other alternatives and by the fact that several of the necessary prerequisites for CFC to occur are fulfilled (above). Our experimental manipulations of male signals and of female receptors demonstrated that stimulation from secondary sexual modifications of the male chelicerae (in *L. mariana*) and his courtship behavior (in both species) influence multiple female reproductive processes, including receptivity to remating (in *L. mariana*), female interruption of copulation (in *L. mariana*), copulatory plug formation (in *L. mariana*), and the consistency of plugs and the timing of their production (and thus both

their probable effectiveness in resisting intromission attempts by future males and the possibility that the male will be cannibalized) (in *L. argyra*). In addition, some correlations suggested additional possible male effects on post-copulatory female reproductive processes: Two male copulatory courtship behavioral patterns correlate with greater plug formation (in *L. mariana*), and one pre-copulatory courtship behavioral pattern in males correlates with plug formation (in *L. argyra*) (Table 4.1).

The effects of experimentally reducing the female's sensitivity to stimuli from the male's chelicerae mirrored those of modifying the male chelicerae, confirming that stimulation itself rather than some other associated factor was responsible for the changes in female responses. If anything, modifying the female produced greater effects than modifying the male. This pattern, to obtain more dramatic effects from sensory "blinding" of the female than from modifying the male, is similar to those in studies of stimulation by male genitalia in tsetse flies (Briceño and Eberhard 2009a, b) and by species-specific male front leg clamping organs in sepsid flies (Eberhard 2001, 2002). The greater response to experimental blinding is logical, taking into account that the modifications of the females (complete or nearly complete elimination of signals from her sense organs) were probably more radical than those in the males (a change in the details but not complete elimination of the male stimuli).

The male and female genitalia and behavior of *L. argyra* are derived in several respects with respect to typical traits in *Leucauge* (Fig. 4.6). Two derived male genitalic clasping and clamping devices in *L. argyra* may be associated with the extremely aggressive female behavior in this species. In contrast to predictions from SAC theory, the female genitalia of this species have a derived trait (highly accessible, large atria) that facilitates rather than impedes male genital clamping. In contrast to predictions regarding lock-and-key mechanical fit between male and female, one prominent, derived female genital structure (the ventral projection) does not mesh physically with any male structure during copulation. Barrantes et al. (2013) speculated that it may instead function in sensing male genital movements.

4.6.3 Why Do Female *Leucauge* Make Mating Plugs?

Producing a mating plug is clearly to a male's advantage if it prevents at least some future males from being able to inseminate the female, but the advantage to a female *Leucauge* of making plugs is less obvious. In the first place, it is not reasonable to suppose that, from the female's point of view, the mating plugs function to prevent future males from inseminating her in either species (above). The active female participation in plug formation in both species is thus paradoxical in terms of blocking all future males.

One possible CFC explanation for female production of plugs is that the plugs serve as filters for males. In both species, some males but not all overcame or removed mating plugs. By forming a mating plug, a female could thus both favor the current male and filter future males by allowing only those able to remove the plug to inseminate her. Plug formation by females may favor males that are

able to perform some types of both pre-copulatory and copulatory courtship (Table 4.1). These data are not completely convincing, however, because we were not able to confidently distinguish which plugs were sufficiently strong enough to exclude future males, as opposed to more fragile plugs, though in *L. argyra*, only relatively thick plugs that covered the atria completely were counted. In fact, the existence of some very fragile plugs in *L. mariana* (Méndez 2002; Méndez and Eberhard 2014) argues that they have additional, as yet undetermined functions (see Uhl 2010 for further discussion).

A second possibility relates to cannibalism of *L. argyra* males. In some spider species, females determine insemination rates and copulation durations by timing the attack on their sexual partners (Elgar et al. 2000; Schneider and Elgar 2001; Fromhage et al. 2003) (see also Andrade and MacLeod, this volume). Female *L. argyra* could exert mate choice through the timing and sizes of plugs that they form, which is in turn affected by the number of bursts of male twanging during pre-copulatory courtship via CFC (Aisenberg and Barrantes 2011). The data on this point are only preliminary, however, because only plug formation per se rather than the effectiveness in excluding future males was shown to correlate with pre-copulatory twanging. The general questions of why *L. argyra* females appear to attempt to use plugs to capture some males but not others and why they attack some males more intense than others during pre-copulatory courtship and at the end of copulation (while *L. mariana* females do not kill males) are still unanswered. As far as we know, the mating plug of *L. argyra* is the first plug known to function as a sticky mortal trap for males, and it opens multiple doors for future research.

Females also participate in producing mating plugs in some other spiders, such as the theridiid *Therion varians* (Knoflach 1998) and *Nephila pilipes* (Kuntner et al. 2012). Not enough is known in these cases to evaluate whether some of the same or other explanations also apply to their formation.

4.7 Directions for Future Research

The results described here, particularly the unusually detailed descriptions of the behavior of different portions of the male genitalia, shed new light on the general pattern in spiders of rapid evolutionary diversification of male genital morphology. The movements of the male genitalia are basically quite different in *L. mariana* and *L. argyra*. If, as in these species, differences in morphology in other *Leucauge* species are accompanied by differences in behavior, and if the movements of male palpal sclerites are controlled by differences in the form, elasticity, and twisting of the flexible membranes (hematodochae), then it seems likely that there is an entire character system in the forms and extensibility and folding abilities of hematodochae that could be used to discriminate closely related species. However, this possibility has not yet been exploited by taxonomists.

Although there are reasons to suppose that neither the first nor the last male to mate with a female achieves complete sperm precedence (above), the preference

of male *L. mariana* to associate with penultimate rather than mature females (Eberhard et al. 1993) predicts that the first male to mate with a female enjoys a paternity bias; quantitative paternity analyses when a female has mated with more than one male are needed. An additional important aspect of paternity is the numbers of sires of clutches laid by females in the field. The data available to date generally concern only matings with only two males and younger females; it is possible that expanding the range of ages and sexual experience of females would reveal further important details. More complete reproductive histories of females (visits by males, matings, and durability of plugs) in the field would also be useful to put present data into context.

The discovery of sexually dimorphic setae on the sternum of female *L. mariana* that may be stimulated by the male's chelicerae during copulation opens the exciting possibility of a female specialization whose function is to screen males on the basis of copulatory courtship stimulation and needs to be followed up by checking for effects of experimental modifications of these setae. Further experimental modifications of the tall epigynal process and the male cymbial hook in *L. argyra* are also needed to clear up the mystery still surrounding these derived structures. Even the use of an observation technique (e.g., mirrors) that would change in the observer angle of viewing copulation might help determine whether the male's palps ever deflect the setae on this female process. Finer modifications of male and/or female setae could permit finer conclusions regarding the functions of particular designs. It is still a mystery whether male *L. argyra* are able to penetrate the extremely hard mating plugs of this species, and if so, how they accomplish this feat.

Of course, there are many species of *Leucauge* yet to be observed. Some have mysterious structures (e.g., the pits on the epigynum of *L. venusta*, Barrantes et al. 2013), while others have interesting variant behavior (e.g., the lack of a cheliceral clasp in at least one *Leucauge* species, Barrantes et al. in press). A phylogeny of the genus would be of great help in interpreting further studies. It would also be useful to extend Castro's (1995) tests to see whether cross-specific pairs consistently fail to court and attempt to mate, in order to further test our conclusion that species isolation is an unlikely explanation for the rapid divergence in morphological and behavioral traits in *Leucauge*.

Acknowledgments We thank Rosannette Quesada, Catalina Sánchez, Emilia Triana, and Vivian Méndez for providing access to unpublished data; Jairo Moya-Ramirez for photographs; Rafael Lucas Rodriguez and A.V. Peretti for useful suggestions on the manuscript; and the Smithsonian Tropical Research Institute (AA, WE), Agencia Nacional de Investigación e Innovación (AA), Programa de Desarrollo de Ciencias Básicas (AA), and the Universidad de Costa Rica (GB, WE) for financial support.

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