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Seasonal variation in sexual behavior and web aggregation in a little-known long-jawed spider (*Tetragnatha straminea*) (Araneae: Tetragnathidae)

Victoria Simkovic and **Maydianne C.B. Andrade**: Integrative Behaviour & Neuroscience Group, Department of Biology, University of Toronto Scarborough, ON, Canada, M1C 1A4; E-mail: vsimkovi@uwo.ca

Abstract. General insights in sexual selection can be enhanced by the addition of data from under-studied species. Understanding the natural history of these species is acritical first step. Here, we report detailed field observations of *Tetragnatha straminea* Emerton, 1884, a long-jawed orb weaver common in riparian meadows. Relatively little is known about this spider, in which the elongate chelicerae of males lock with those of females during copulation. We assessed courtship, mate-guarding, intrasexual competition and predictors of female receptivity to mating. As inter-sexual fang-lock is critical to successful mating, we also examined male and female cheliceral allometry. Finally, we observed patterns of aggregative clustering during peak-mating periods, as this may indicate elevated levels of intra-sexual aggression. Overall, females were polyandrous, and it was not possible to predict female receptivity based on the number of males present or female aggressive behavior towards males. Courtship was not evident prior to mating attempts, although some males remained in close proximity to females for extended periods. Towards the middle-to-end of the mating season, multiple females would aggregate in clusters, where elevated intra-sexual aggression and frequent copulations occurred. Our observations suggested that fang-lock clasping may be important in intra-sexual agonistic interactions as well as mating, particularly during mid-season web aggregations. Consistent with this, morphological data suggest a trend towards larger chelicerae in males rather than females. *Tetragnatha straminea* may be ideal for testing hypotheses regarding effects of seasonal variation in sexual selection, and the evolution of structures that serve more than one sexually-selected function.

Keywords: Tetragnathidae, sexual selection, allometry, chelicerae, web-site tenure

Spiders may be ideal models for addressing important questions in sexual selection research (Huber 2005; Eberhard & Huber 2010), but the limited number of well-studied species hampers general insights and comparisons at a range of taxonomic levels (e.g., across broad taxonomic groups: Schneider & Andrade 2011; within genera: Andrade & MacLeod 2015). Further, overall patterns and inferences can be significantly altered by the addition of comparative data from under-studied groups. For example, a hypothesis about the links between male life history, sex ratio and the evolution of female-biased size dimorphism in spiders received early support (Vollrath & Parker 1992), but this was later challenged based on a phylogenetic analysis that included more taxa (Prenter et al. 1998). Similarly, broad differences in the genital morphology of 'haplogyne' and 'entelegyne' were used to predict differences in sperm use patterns (first vs. last-male sperm precedence; Austad 1984), but consideration of a wider range of species suggested complexities not captured by initial predictions (Elgar 1998). Thus, observations on the behavior and ecology of under-studied species may prove to be valuable for later analyses of broad hypotheses.

One relatively under-studied spider taxon that may be useful for testing general hypotheses about the evolution of sexual traits is the Tetragnathidae, or long-jawed orb weaver family. This widespread family includes 48 genera with 1,000+ species (World Spider Catalog 2018) and is named after an interesting and variable aspect of morphology (Dondale et al. 2003; Bradley 2013); in most species the chelicerae are as long as, or longer than the cephalothorax (Bristowe 1954). The adaptive significance and constraints posed on the evolution of these elongate chelicerae is an open question, as, in addition to their function in feeding, the chelicerae are used in mating and in most species may also be important in intra-sexual combat. In a novel behavior observed in some Tetragnathidae and a few Dictynidae species, males and females clasp chelicerae during mating, with the male's fangs appearing to hold the female's fangs apart and away from the male's cephalothorax (Bristowe 1954; Foelix 1982; Preston-Mafham 1993; Huber 1998; Álvarez-Padilla & Hormiga 2011). Since this fang-lock behavior is key to a successful mating attempt, the evolution of elongate chelicerae might be mediated by the mechanical requirements of successful mating (Eberhard & Huber 1998; Álvarez-Padilla & Hormiga 2011). For example, there may be an optimal ratio of male to female chelicera size that is necessary for ensuring that the fang remains propped open or locked to facilitate genital alignment, which would impose limits on sexual dimorphism in chelicera size. Alternatively, chelicerae may function in sexual selection, either indirectly by signalling male size to choosy females or directly if well-locked fangs reduce the likelihood of mating disruption by rival males, or if long-jawed males have higher success in inter-male combat (Andersson 1994). Finally, the presence of antagonistic coevolution between the sexes (sexual conflict) could influence the evolution of elongate chelicerae if the fang-lock reduces the risk of sexual cannibalism by females, or allows males to prolong copulation or prevent the retreat of non-receptive females (Arnqvist & Rowe 2005). The Tetragnathidae are also interesting because they include the subfamily Tetragnathinae, a mainly tropical subfamily with simple female reproductive organs, which lack an epigynum and have a single duct for insemination and fertilization ('haplogyne-like', Danielson-François et al. 2002). This modification is associated with last-male sperm precedence rather than first-male as in most other 'entelegyne' spiders, and has important consequences for predicting mating behavior patterns (West & Toft 1999; Danielson-François 2006).

Testing hypotheses about the origin and function of jaw elongation and genital simplification require comparative data regarding cheliceral allometry, mating systems and behavior, sperm competition, and general morphology in addition to well-supported phylogenies. Gillespie and colleagues have made extensive molecular phylogenetic analyses of Hawaiian Tetragnathinae species (Gillespie et al. 1994; Gillespie 1997, 2004), but there are only a handful of phylogenetic or behavioral studies of Tetragnathinae from other regions (Hormiga et al. 1995; Eberhard & Huber 1998; West & Toft 1999; Danielson-François et al. 2002; Aisenberg 2009; Barrantes et al. 2013; Aisenberg et al. 2014).

The purpose of this study was to obtain detailed field observations and morphological data on the mating behavior and natural history of a little-studied long-jawed orb weaver, *Tetragnatha straminea* Emerton, 1884 (Tetragnathinae). Similar to other common tetragnathid species (*T. laboriosa* Hentz, 1850, *T. elongata* Walckenaer, 1841 and *T. viridis* Walckenaer, 1841), *T. straminea* is restricted to riparian habitats, such as along streams, marshes and lakes (Williams et al. 1995; Aiken & Coyle 2000).

In this study, we first use field observations obtained during the mating period of *T. straminea* to describe the steps of mating and conspecific interactions in detail. We then ask whether behavioral aspects of inter-sexual interactions may predict female receptivity and the likelihood of successful mating. Second, we assess the degree of sexual size dimorphism and examine whether there are sex-differences in cheliceral allometry (Fromhage & Kokko 2014). Finally, we examine web-site tenure and report patterns of web distribution and aggregative clustering during peak-mating periods, as this has not been previously reported. We then observed clusters of female spiders, looking for evidence of mate-guarding, female competition, and sex ratios within the aggregate, as these could indicate elevated levels of sexual selection or sexual conflict in aggregations.

METHODS

Natural history of tetragnathids.—Previous literature suggests that *Tetragnatha straminea* has a one-year life cycle, with six post-emergence instars (Aiken & Coyle 2000). Individuals overwinter in the antepenultimate instar, emerging in early May, maturing and mating throughout May–July. Spiderlings and mature female tetragnathids build delicate, haphazard orb webs, often horizontally oriented in vegetation. Webs are rebuilt and ingested daily, with web building occurring in late evening through the early morning (Gillespie 1987a; Smallwood 1993). Tetragnathids have been found to aggregate in prey-rich areas, but similar to other species, web-site tenure at a single location varies, with the level of prey density being a primary determinant of web re-location (Gillespie 1987a,b; Smallwood 1993).

Observations of closely related species in the genus *Tetragnatha* indicate a relatively simple mating pattern; mate-guarding and courtship are frequently absent in species such as *T. elongata* (Danielson-François et al. 2002) and *T. extensa* (Linnaeus, 1758) (Preston-Mafham 1993; West & Toft 1999). The male appears to enter the female's web without hesitation, and if the female is receptive to mating, she orients towards the male with widened chelicerae (Preston-Mafham 1993). This behavior is then usually followed by chelicerae clasping and several alternating palpal insertions (Preston-Mafham 1993; West & Toft 1999; Danielson-François et al. 2002). In lab and field studies, both sexes of *T. elongata* copulate multiple times throughout the mating period; females lay multiple egg sacs throughout the season, and display no clear pattern of mate preference (Danielson-François et al. 2002).

Field site & survey technique.—*Tetragnatha straminea* is found in moderate abundance during the early summer months (May – June) in riparian habitats within the Greater Toronto Area (GTA) and Toronto, Ontario, Canada (V.S., pers. obs.). One of us (V.S.) observed the mating behavior of *T. straminea* along stream banks, or within 10 m inland of a Credit River island in Mississauga, Ontario, Canada (43.355306N, 79.432322W) in 2009, 2010 and 2012. Surveys were initiated in early May to locate populations of immature spiderlings within a designated 5 × 10 m site. The mating season was defined as the first day a copulating pair was observed until no spiders were seen for three consecutive days. Mating observations primarily took place during the early evening, 7:30 – 9:30 pm, as this was the time frame when females would first begin web building, and most inter-sexual interactions were observed, based on approximately 150 hours of observation. No spiders were found during preliminary surveys in the early morning or mid-afternoon (V.S., pers. obs.), nor was any mating activity seen during the late-evening (11pm – 1am) at this site or at another site in Toronto (Ashbridge Bay, 43.392889N, 79.184768W, M.C.B.A., pers. obs.). On May 29, 2012 and June 5, 2012, time-lapse photographs were taken of focal females at 5-second intervals throughout the night (using a Brinno TLC100 Time Lapse camera) to determine the amount of activity occurring outside of the timeframe observed at the Credit River field site.

Observations were suspended on cool, rainy evenings, when these spiders show marked reduction in web building (V.S. and M.C.B.A., pers. obs.), and observations were not possible on days following heavy rains as flooding made the site inaccessible. Spiders at the Credit River were surveyed over 36 days in 2009, 25 days in 2010 and 30 days in 2012. The field was surveyed by walking the same transect each day, to minimize trampling of vegetation and disturbance to the spiders. These transects were repeatedly travelled to locate adults and mated pairs throughout the evening, as new individuals would appear as the evening progressed. Whenever spiders were found, web locations were marked and numbered with surveyor's tape placed on nearby vegetation. Tetragnathids were very sensitive to disturbance; as the site was surveyed, females would frequently elongate and assume a cryptic pose or drop to the leaf blades below. For this reason, movement during transect sampling was minimized when behavioral interactions were observed, which necessarily reduced the number of observations that could be made each night.

Approximately 2–3 other tetragnathid species were occasionally found during surveys, although none mated during the observation timeframe. *Tetragnatha straminea* is morphologically distinct from other species found in the area, and confirmation of species identification was obtained by sending voucher specimens to systematist Dr. Gergin Blagoev at the Canadian Centre for DNA Barcoding (Biodiversity Institute of Ontario, University of Guelph).

Mating observations.—Whenever at least one male was seen at or below a female's web during surveys, the location was noted as a spot where mating might occur that evening, and was subject to more intense observations (recurrent visits, or sustained visits if inter-sexual interactions were observed). Broadly, we define inter-sexual interactions as male-female behaviors that occurred between the time when the male's first presence was first noted on the web and the time when the male left the web, including interactions during male entry or departure from the female's web, female chases of males, copulation (possibly) and behaviors during the male's departure from the web.

During behavioral observations, we recorded the following inter-sexual interactions: (1) mating latency (time between male's first presence below web and first successful mating), (2) the number of times the male departed or was chased from the web prior to mating, (3) copulation duration (defined as the interval between initial clasping of chelicerae until they unclasped), (4) number of palpal insertions, (5) post-copulatory departure and (6) evidence of mate-guarding (males found in close proximity to females for extended periods when not mating). In addition, the number of males at or below the female's web was recorded, as was any male-male competition (i.e., a rival male entering the web and contacting or disrupting a copulating pair, or chasing another male prior to entering the web). Finally, the number of inter-sexual interactions resulting in mating was recorded for each female.

In 2012, all observed mating interactions were filmed (Sony Handycam HDR-CX700). Ethogram illustrations were created (by V.S.) using this footage and digital photographs taken in the field (Sony Cyber-Shot digital camera).

Sexual dimorphism.—We compared the allometric relationship between sexes by regressing body size (chelicerae width) against basal segment, fang or total chelicera length (basal segment + fang). Nineteen adult males and fifteen adult females were collected from the field during the mid mating season, frozen, and stored in 75% ethanol. Spiders were dissected, digital images taken of the chelicerae (basal segment, fang) and prothorax at 1.25x and 0.65x respectively with a compound microscope (Zeiss Stemi 2000C), and body segments were measured using ImageJ (Rasband 2014). The width of the carapace was used as a proxy for body size. There was a strong positive correlation between the left and right body segments of each spider, suggesting our measurements were accurate ($r_{\text{basal segment}} = 0.96$, $r_{\text{fang}} = 0.99$, $r_{\text{chel (BS + fang)}} = 0.98$, $P < 0.001^*$, $n = 34$).

Web-site distribution & related interactions.—To test for the presence of aggregations of female webs during peak-mating periods, web proximity was noted by marking a focal web, and noting the number of additional webs within a 1 m radius of this web. In addition, to determine whether spiders moved between sites in successive nights, during May 23, 25, and 26, 2012 (the start of the mating season), 35 adult spiders (32 female, 3 male) were given unique color identification patterns on their abdomen with either yellow, pink or blue fluorescent paint (BioQuip non-toxic luminous paint), in order to determine typical duration of tenure at one location, and to get a better sense of how frequently each female mated. Mature adult spiders were distinguished from juvenile or penultimate instars based on behavioral and morphological changes observed during the field season. Both immature males and immature females build prey-capture webs at the start of the season (early May), while mature males will cease web-building activity and adopt a vagabond lifestyle. Males are also larger with visibly swollen and dark pedipalps, while females have larger and more swollen abdomens (abdomen appears more curved, particularly in the anterior portion) and build larger webs higher up in the vegetation. Past dusk, a portable black light was used to find tagged individuals within and adjacent to the study site.

Statistical analyses.—Given variable sample sizes and non-normal data distributions, we used separate Mann Whitney U tests to examine two possible predictors of female receptivity to mating, (1) the number of males present at the web, and (2) the number of times a male is chased by the female or departs from the female. We compared the allometric relationship between sexes by regressing the log of body size (chelicerae width) against the log of basal segment, fang or total chelicera length (basal segment + fang) using reduced major axis regression (RMAR, Warton et al. 2018). The allometric coefficient was determined for each sex and compared with those expected by isometry ($\beta = 1$). Sex differences in allometric slopes or intercepts were then compared by testing for an effect of the factor 'sex' in an ANCOVA model, with body size as the covariate.

RESULTS

Mating observations.—Mating first occurred on 22 May in 2012, but not until mid-June in 2009 (June 13) and 2010 (June 15), potentially influenced by cooler spring temperatures (available online at http://climate.weather.gc.ca/index_e.html). In every year, the mating season was brief and intense, ranging from 25 (2009, 2010) to 26 days (2012).

In the three years of study, detailed observations for nineteen females with one or multiple males at or below the web were recorded (Fig. 1). We defined the first male to approach the female as male A, and the second or third male to approach as male B and C respectively. Of the nineteen observations, fourteen females mated with one or more males between 7:30 and 9:00 pm, while inter-sexual interactions occurred without mating in five observations.

In focal interactions, males were typically first observed travelling up and down leaf blades and foliage below the female's web. Within 3–5 minutes of the male first being visible near the female's web, he would make an initial entry into the outer frame. Males would depart the web shortly after entering if the female continued to web-build or eat prey within the hub rather than orienting in the male's direction. Occasionally (approximately 40% of the time), the female would chase the male from her web. Males would quickly leave the web when chased, or drop to the grass blades below. In the five inter-sexual interactions observed with no mating, males would repeat the behavior of traveling up and down nearby foliage, occasionally entering the female's web and traversing to the opposite side, and exiting quickly if the female showed no response or chased the male from her web. In some cases, these interactions would later end in mating (Fig. 1), or the male would depart after a number of unsuccessful forays (although it is possible that the male returned to mate later in the evening, unobserved). In other

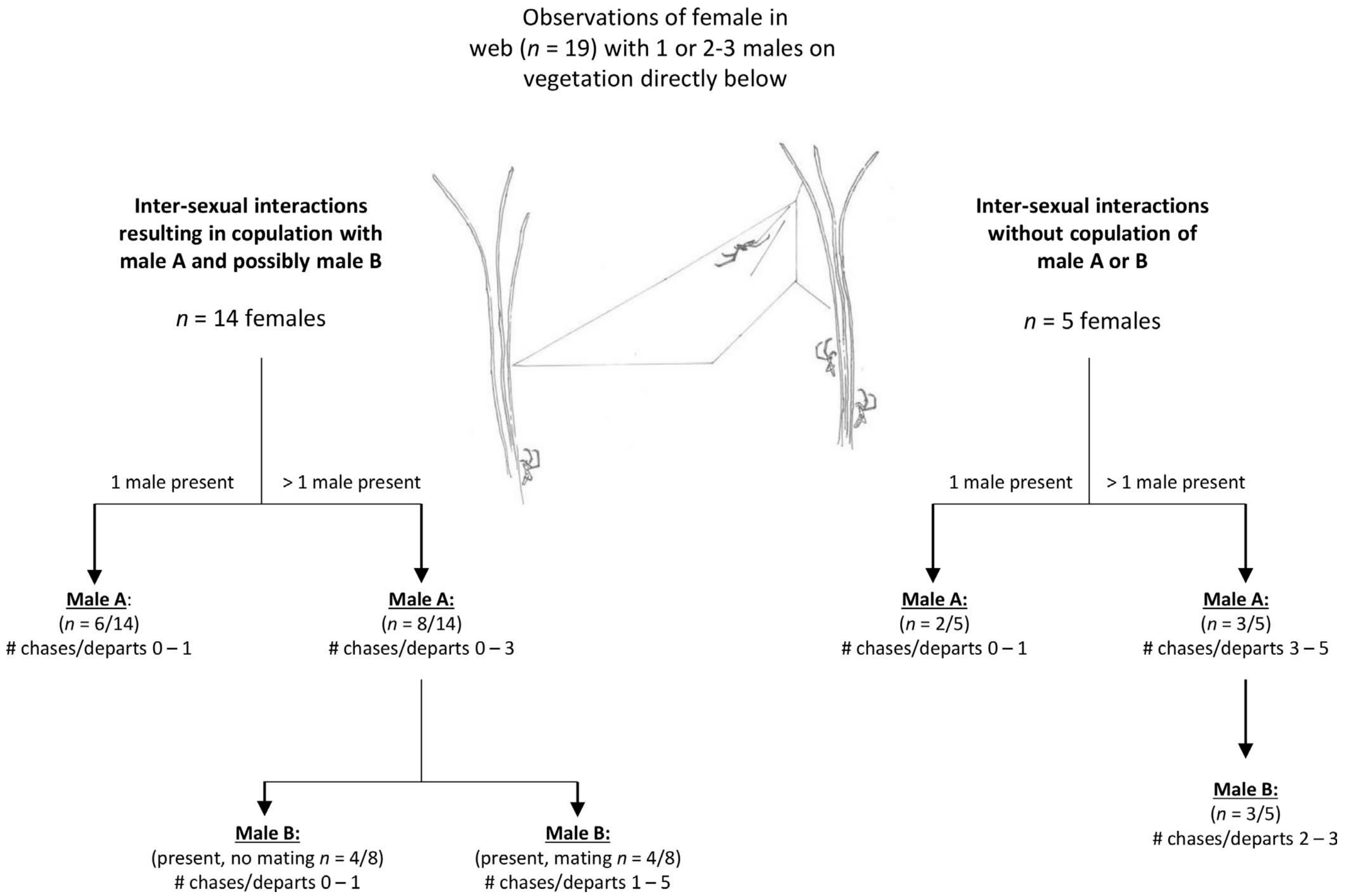

Figure 1.—Summary of inter-sexual interactions. $n = 19$ is the number of separate female webs observed with one or more multiple males below the web during the 2009, 2010, and 2012 field seasons. If copulation occurred, it usually happened within 3–5 minutes of the male first being visible at or below the female's web. Inter-sexual interactions without copulation were noted if no copulation occurred within 45–50 minutes of observation.

cases, male foliage traversing would continue for well over an hour, with no mating apparent. Pairs that did not mate were categorized as 'nonresponsive' (Fig. 1). Time-lapse video of a nonresponsive male-female pair (2012) confirmed no mating past the 7:30–9:00 pm observation period. In these observations of nonresponsive pairs, no evidence of courtship (distinct movements on the web likely to generate vibrations) (Lesar & Unzicker 1978) or mate-guarding (males remaining in close proximity to, but not approaching females, and rebuffing other males) was observed. It was not known how many other males were present but off-camera during time-lapse videos.

In contrast, forays that lead to successful matings were characterized by females ceasing other activity when the male entered the web, then orienting toward the male with widened chelicerae. The male would then return to the margin of the web from which he had entered and tap the outer radial thread before moving quickly towards the female with widened chelicerae. If the male and female clasped chelicerae, mating would begin (Fig. 2).

Prior to a successful mating, the number of times male A was chased or would depart from the web ranged from 0–3 for interactions resulting in mating ($n = 14$, females chased the male 43% of the time) and 0–5 for interactions not resulting in mating ($n = 5$, females chased the male 60% of the time, Fig. 1). The number of chases or departures was not significantly influenced by the number of males present (one or more males) at or below the female's web (Mann Whitney U test, $P = 0.25$, $n = 14$). The first male (male A) was more likely to mate if he was chased or departed the web less frequently, but these trends were not significant (all $P > 0.05$).

Once cheliceral clasping occurs, the female curves her abdomen towards the male, and the male grasps the abdomen with the third and fourth leg pairs while inserting a single left or right pedipalp into the female's gonopore (Figs. 3a–c). The male maintains the cheliceral clasp and leg position while alternating insertion of left and right pedipalps 1–2 times, minimally repositioning the female with his legs (Fig. 3d). The female then uncurls her abdomen, and, following some grappling (rapid movement of anterior legs), the chelicerae unclasp rapidly, after which the male is quick to drop on a drag-line or travel down the radial thread, often with the female in pursuit for several seconds. Females eventually end pursuit and return to the hub, often devouring any prey present in the web. On one occasion, a female was observed consuming a male in the hub of her web, but the behavioral interactions preceding this were not observed.

Copulation duration of male A ranged from 6.00 – 16.00 mins (median = 11.3 mins, $n = 14$). In many cases (57% of

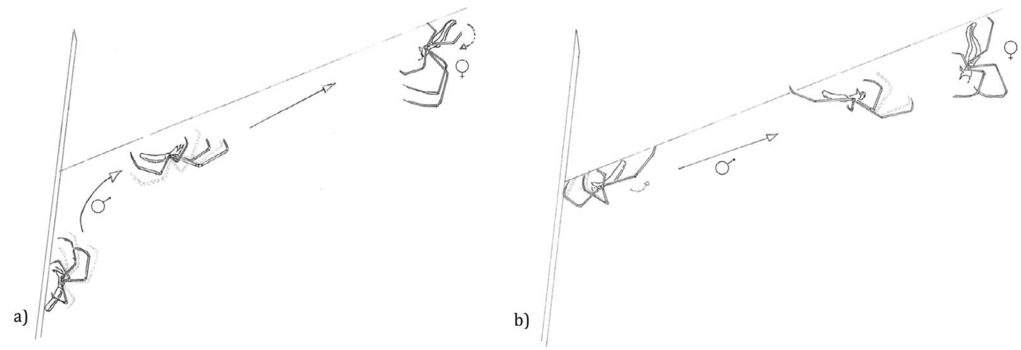

Figure 2.—Pre-copulation. a) Male enters web and approaches female, as female turns and faces male. b) Male turns, taps the outer radial thread female rests on, and rushes forward quickly, while widening chelicerae. Female positions body vertically and opens chelicerae to accept male.

interactions observed), at least one other male was present during focal observations (Fig. 1). In over half of the observations, rival males moved quickly towards the copulating pair and would make contact using their anterior legs or by using their chelicerae to bite the basal segment of the fang-lock, towards the center of the cheliceral clasp ($n = 6$ of 11 observed rival males below web). In all these cases, the copulating female responded to rivals by vigorously jerking her body and legs repeatedly, until the rival male dropped to the leaf litter below. Nevertheless, the number of males present (one or more) had no significant influence on copulation duration of male A ($P = 0.37$, $n = 14$), and rivals were never observed to break the cheliceral clasp or terminate copulation. At times, the rival male attempted to re-enter the web once the previous mating was complete. Second males (male B) were usually chased by females and would depart the web numerous times. If B males were persistent, females sometimes showed receptive behavior and a second mating would occur. In four of the 14 interactions in which females mated with male A, they also mated with male B within the same observation period. Thus, at least 29% of females are estimated to be polyandrous within a single night.

Copulation duration of second males appeared to be half as long as that of first males (range: 4.87 – 5.85 mins, median = 5.0 mins, $n = 4$), but the small sample size for these matings precluded a statistical test. Inter-mating interval varied between 5–45 minutes. Time-lapse images confirmed that focal females mated repeatedly outside the time frame typically observed and suggest that nightly polyandry rates are high. For example, following observations of a mated pair on June 5, 2012 at 8:00pm, the female mated a second time at 9:30pm (copulation duration = 5.85 mins) and at 11:30pm (copulation duration = 18 mins). It was unknown whether mating occurred with the same or different males. There was no evidence of aggression between males when they encountered one another below the female's web. Instead, males were quick to depart in the opposite direction if they came into contact with other males.

Sex dimorphism.—There were no differences in the slope of regression between males and females, as indicated by a lack of interaction between the factor sex and body size for basal segment (F = 0.17, $P = 0.68$), fang (F = 1.74, $P = 0.20$) or chelicerae (F = 0.37, $P = 0.55$) in the ANCOVA model. For both sexes, the common slope of the major axis regression did not differ from $\beta = 1.0$ (isometry) for basal segment (X^2 (2) = 0.54, $P = 0.76$), fang (X^2 (1) = 0.001, $P = 0.97$), or chelicerae (X^2 (2) = 0.58, $P = 0.75$) respectively (see Table 1 for individual slopes and confidence intervals, and Figs. 5a–c).

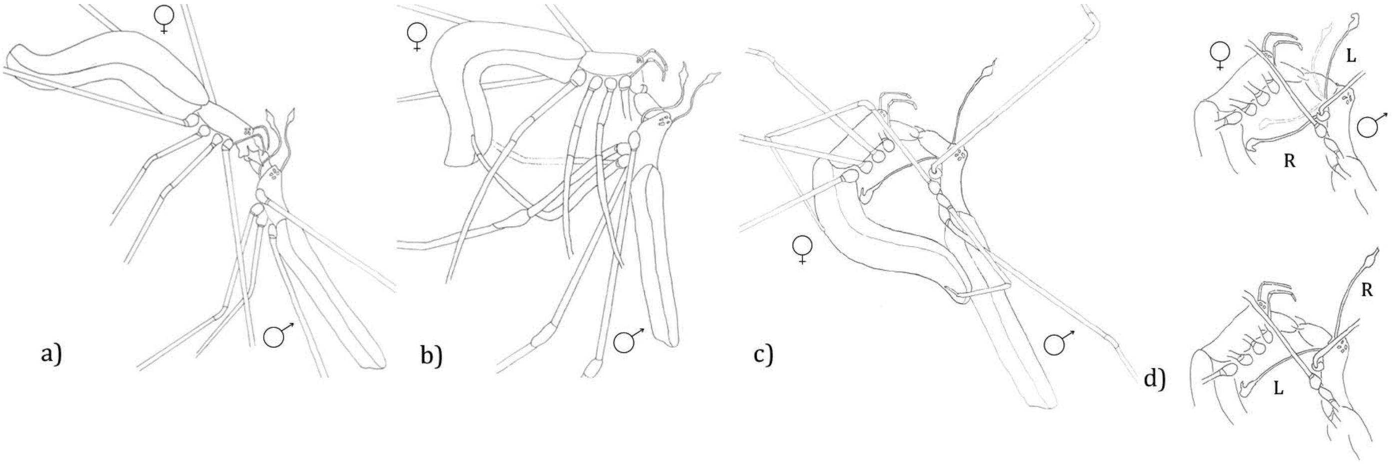

Figure 3.—Steps of Copulation. a) Chelicerae clasp b) Male grasps female's abdomen with third leg pair, as female curls her abdomen c) Male grasps female with third and fourth leg pair, inserts a single palp into the female's gonopore. d) Palps alternate 1–2x.

Table 1.—Summary of reduced major axis regression of *T. straminea*, for male and female log-transformed body size vs fang, basal segment and chelicerae length (basal segment + fang); (female: *n* = 15, male: *n* = 19). β = slope, and 95% confidence interval of slopes are shown.

	Male				Female			
	β	Confidence Interval (95%)	R^2	*P*	β	Confidence Interval (95%)	R^2	*P*
Log body size vs log basal segment	1.05	0.66 - 1.67	0.11	0.16	1.19	0.72 - 1.97	0.23	0.07
Log body size vs log fang	1.51	0.94 – 2.41	0.10	0.20	-1.53	-2.69 - -1.87	0.02	0.65
Log body size vs log chel (BS + fang)	1.19	0.75 - 1.90	0.11	0.15	1.00	0.58 - 1.75	0.05	0.43

Although slopes were non-significant, the intercepts significantly differed between sexes, indicating differences in body size. Male *T. straminea* were smaller (carapace width = 1.51 mm ± 0.11, *n* = 19) than females (carapace width = 1.63 mm ± 0.11, *n* = 15), but had longer chelicerae (male: 3.34 mm ± 0.29, female: 3.03 ± 0.22; F = 15.4, *P* = 0.0004*), basal segment (male: 1.78 mm ± 0.14, female: 1.71 ± 0.15; F = 5.32, *P* = 0.03*), and fang (male: 1.57 mm ± 0.17, female: 1.27 ± 0.10; F = 18.4, *P* = 0.0002*) lengths relative to body size (Fig. 4).

Web-site distribution & related interactions.—Females built their webs using the structural support of tall grasses, and on forbes such as *Solidago sp.*, *Hesperis matronalis, Alliaria petiolata* and *Arctium lappa* within the field site. Webs varied in size and were oriented at approximately 30° relative to horizontal. Females typically began web-building at about 7:30pm and web building was complete by 9:00pm.

Occasionally, notably towards the mid to end point of the mating season (∼ 10–12 days after the first females were observed), aggregations of multiple females and males would form and persist for 1 or more days (Table 2). Aggregations were formed when 2–5 females built webs within 1 m of each other, often side by side. The cluster would remain for 1–2 days before disappearing (sometimes weather-related disturbance may have influenced this).

In aggregations, males travelled along silk draglines below and between the adjacent webs of females. Females rarely interacted in these aggregations, although they would occasionally travel across a conspecific's web. A direct intra-sexual interaction was seen on only one occasion (on day 1 of observation of aggregations in cluster 1) when two females clasped chelicerae and vigorously jerked their body and legs after one female had entered another's web. Sex ratios varied among aggregations (Table 2). In aggregations, frequent polyandrous matings (female mating with 2–3 different males in a single night) were observed, but opportunities for multiple mating varied among females. One female within the cluster would often have several males near her, simultaneously attempting to enter the web. Other females, in contrast, would have no males attempting to mate or enter the web, even though multiple males were present in the aggregation. Increased male-male competition (chases, departures, attempts to break pair bonds) was observed in clusters. Male-male interactions included aggressive attempts to break pair bonds, and these were more frequent and more persistent than when female's webs were solitary.

Moreover, when males encountered each other beneath clustered webs, they engaged in intra-sexual cheliceral clasping and leg grappling, rather than retreating from contact as was seen when webs were solitary. Cheliceral clasping between males was never observed outside of these aggregations.

Among the 35 spiders (32 female, 3 male) that were marked with color tagging (yellow, pink, blue), seven females were found at the site on the day after they were tagged, and three females persisted at the site for four days. Residual paint was noted on a few additional spiders, indicating some of the paint may have worn off. No tagged spiders were observed within 1 m outside the research site boundaries. Unmarked spiders

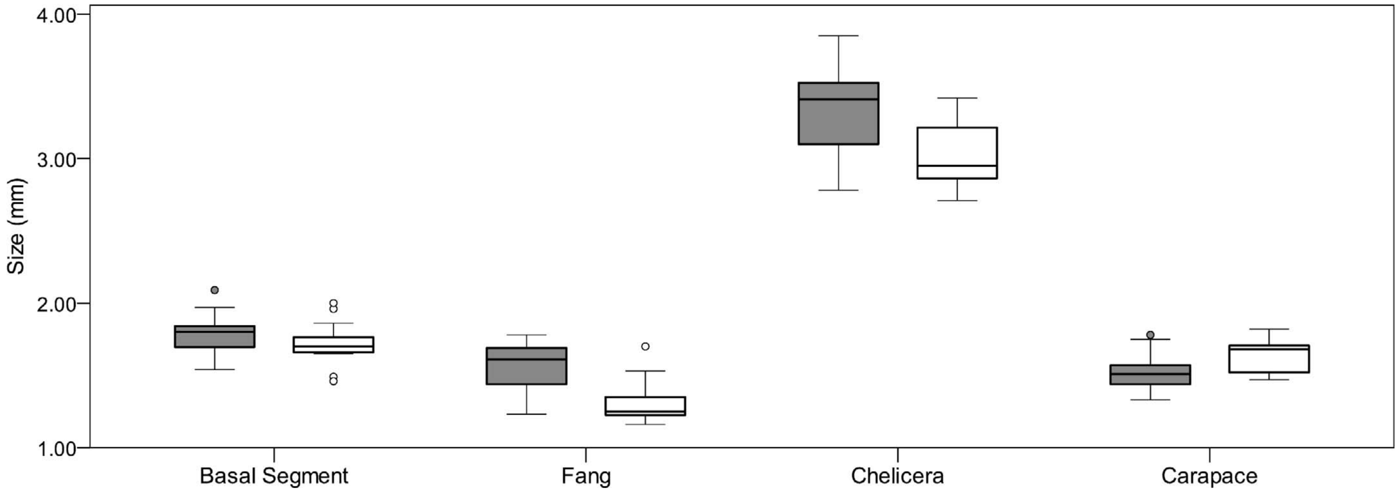

Figure 4.—Boxplot showing sexual dimorphism in *T. straminea* measured as mean lengths of basal segment, fang, chelicera (BS + fang) and carapace width (mm) for males (gray boxes, *n* = 19) and females (white boxes, *n* = 15). Plots show 1^{st} to 3^{rd} Quartile (upper and lower edge of box), median (central line), measure of variation (whiskers, 1.5 x interquartile range) and outliers (circles).

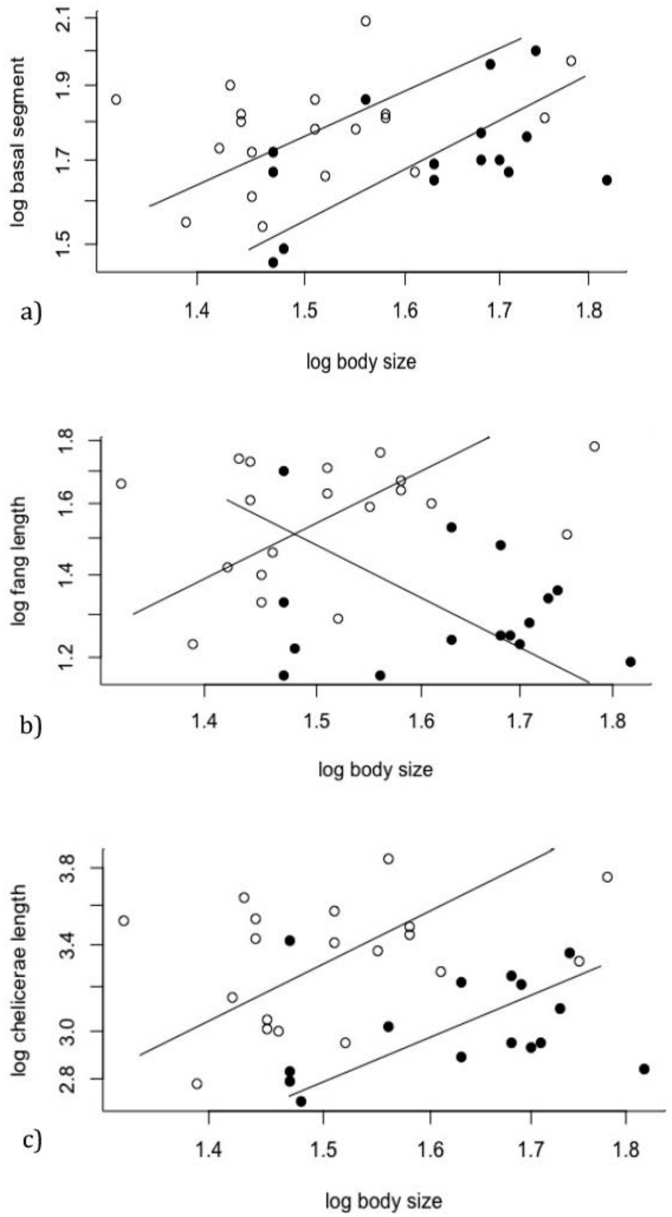

Figure 5.—Reduced major axis regression plots of log-transformed a) log body size vs basal segment length, b) log body size vs fang length, c) log chelicerae (fang + basal segment) length with allometric slope (correlation coefficient). Open point = female ($n = 15$), black point = male ($n = 19$).

were frequently found on subsequent days, which were likely new individuals, as no evidence of residual paint was found.

Table 2.—Clustering of *T. straminea* towards the mid-end of the mating season, including sex ratio and number of days a cluster of spiders would persist.

	# spiders (male:female)	# days persisting
Cluster 1	2:5	2
Cluster 2	5:3	2
Cluster 3	3:2	1
Cluster 4	2:4	3

DISCUSSION

This is the first paper to describe the mating behavior of the riparian long-jawed spider *Tetragnatha straminea*. Detailed field observations confirmed that females are polyandrous, with short and frequent copulations throughout the night beginning in the late evening. Female receptivity to mating could not be predicted based on the number of males present below the web or the number of times the male was chased, although the first mated male was more likely to be successful if chased less frequently. There was no evidence of male courtship and no evidence of mate-guarding when females were solitary, as males generally avoided other males early in the mating season. However, an increase in male-male competition (including cheliceral clasping) and frequent polyandrous mating occurred in the aggregative clusters that appear towards the mid to end of the mating season.

The brief and frequent copulations typical for *T. straminea* were consistent with predicted mating patterns of a haplogyne spider (West & Toft 1999; Danielson-François & Bukowski 2005; Danielson-François 2006). Although paternity patterns can be variable for any one male (Elgar 1998), males generally have an increased chance of siring offspring with the female if they mate just prior to oviposition (Danielson-François 2006). Last male paternity would be predicted to promote increased male-male competition and post-copulatory mate-guarding when oviposition is imminent (West & Toft 1999). In our study, although oviposition patterns are not yet known in this species, male behavior was consistent with the proximity to oviposition hypothesis. Early in the season, male competitive interactions were rare, and males generally avoided one another, but later in the season, escalated inter-male aggression was common, with males clasping chelicerae and interrupting copulating pairs. We predict that female aggregations during the mid-end of the mating season may reflect seasonal variation in female readiness to oviposit. If females derive benefits from mating with higher quality males, then competition between males may be advantageous to females at this time, and web clustering may enhance female fitness by inducing male-male competition (Watson 1990), or by promoting benefits of residing in groups during oviposition.

Interestingly, within a cluster of multiple females, the males would compete more vigorously for certain females, and these females displayed high rates of polyandry, while no mating attempts were made with females in nearby webs. It is possible that preferred females were those that were closest to oviposition, when the chance of a male siring her eggs was highest. Whether males engage in mate-guarding was unclear, but it may be indicated by the increased inter-male aggression (i.e., chelicerae clasping) within aggregations. Males that were observed traversing up and down foliage below the female's web for over an hour without attempting copulation may also have been engaging in mate-guarding. It was not possible to know whether mate-guarding was pre- or post-copulatory, as it is likely that some mating observations were missed. Overall, these observations suggest a number of intriguing questions in this species, including: why are females aggregating, what is the fitness effect of aggregation, and how do fluctuations in the intensity of sexual selection over the mating season affect mating tactics of males and females. This also suggests this species could provide interesting data regarding the effects of fluctuating sexual selection on population structure and phenotypic variation (Elias et al. 2011).

Although there was no evidence of courtship, males were frequently hesitant upon initial web entry, being quick to depart the web if the female showed no signs of receptivity, or if the female chased them from the web. This is in contrast to previous studies of *T. versicolor* and *T. extensa* (West & Toft 1999; Danielson-François et al. 2002), which describe males as entering the web without hesitation and mating immediately upon initial web entry. Assessing female receptivity to mating may be critical to reproductive success in *T. straminia* since there is a risk of mistaken identity (we observed more than one syntopic congener) and sexual cannibalism. Although we observed cannibalism in the field only rarely, the risk may be sufficient to drive male behavior, and the incidence may increase in habitats where prey is less abundant (e.g., hungry female spiders are more likely to be cannibals in several species (Andrade 1998; Herberstein et al. 2002; Roggenbuck et al. 2011)).

Using color identifications, we also confirmed that individuals in populations of *T. straminea* cycle through locations quickly. Although it is possible that tagged spiders were not found on subsequent days due to increased likelihood of predation, or that the paint may have worn off on some, frequent cycling is consistent with previous literature (Gillespie 1987b; Smallwood 1993). Therefore, the new, untagged spiders found on subsequent days were likely new individuals, with web relocation to distant sites occurring frequently. Thus, our observations likely represent new individuals each night engaging in polyandry, rather than the same spiders repeatedly mating on subsequent days.

Many tetragnathid spiders have remarkably elongate chelicerae, which serve the important and unique function of maintaining an inter-sexual fang-lock clasp during mating. This fang-lock behavior is crucial to successful mating and palpal insertion and as such, may be subject to elevated selection pressures. In this study, males were smaller in body size but had larger chelicerae compared to females, and rates of growth (allometric coefficient) did not differ between sexes, or appear to vary from isometric growth. Larger chelicerae in males may be due to elevated intra-sexual competition compared to females, may signal size to choosy females, or larger chelicerae may be necessary to overcome female reluctance to mate (sexual antagonism). We saw little evidence for cheliceral-size mediated female choosiness, as there was no chelicerae-based display evident prior to mating, and fang-locks always led to copulation. Moreover, in this highly polyandrous species, there is likely to be very little sexual conflict over mating frequency. Further, the readiness of females to mate multiple times, her willingness to bend the abdomen to facilitate palpal insertion, and her attempts to ward off rival males by vigorously shaking the web are evidence against antagonistic coevolution in this species (Arnqvist & Rowe 2005).

However, our data suggest that fang-locks may be important in intra-sexual agonistic interactions, particularly between males, and particularly in mid-season web aggregations. Selection for larger chelicerae in the context of aggression may be less important for females; females were observed interlocking jaws on only one occasion. Male chelicera size may be an evolutionary balance between the need to maintain efficient chelicerae clasping during mating (which may impose limits on overall size differences between the sexes), and the intensity of additional competition-mediated selection for increased fang length. This idea could be tested by examining selection gradients on relative cheliceral size in males and females as a function of social context within species (solitary or aggregated) and in comparative analyses that include species that vary in the intensity and form of sexual selection.

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