1997. The Journal of Arachnology 25:307–320

NATURAL HISTORY AND COPULATORY BEHAVIOR OF THE SPINY ORBWEAVING SPIDER *MICRATHENA GRACILIS* (ARANEAE, ARANEIDAE)

Todd C. Bukowski and **Terry E. Christenson**: Department of Psychology, Tulane University, New Orleans, Louisiana 70118 USA

ABSTRACT. We examine copulatory behavior and the reproductive natural history of the spiny orbweaver, *Micrathena gracilis*. Censuses were conducted on free-ranging, individually marked spiders. After molting to adulthood, males induct sperm into their palps and then search for mates. Females inhabit solitary, individually-constructed webs. Males preferentially remain with penultimate-instar females, those about to molt and mate for the first time. After a female molts and constructs a viscid spiral, males build mating threads on which they court. After copulating, the male must dismount and reapproach the female to inseminate her second reproductive tract. Two copulations are therefore required for a complete mating between male and female. Some males, however, obtained only one copulation and two males often copulated with a given female. Staged encounters in the field revealed the important observation that when a male did copulate twice with a female, the duration of the second copulation was more than twice as long as the first. Shortly after the second copulation, the male inducted sperm into the palps and moved away. Females remained sexually receptive throughout their lives and apparently mated with any male. Females oviposited about 30 days after molting and mating. Egg sacs were cryptic in appearance and yet clutch mortality was high. Copulatory behavior is discussed in relation to this reproductive natural history.

Spiders offer an intriguing model for the study of reproduction. To begin, the sexes often differ dramatically in both morphology and behavior (Foelix 1980; Vollrath & Parker 1992). The female orbweaver, for example, is generally a relatively large, sedentary predator while the adult male is smaller and, at least as an adult, a wanderer. The shape and presence and arrangement of sperm intake and fertilization ducts of the female spider's reproductive tracts is thought to have a strong influence on male sexual maturation rates and the pattern of cohabitation with females. Conduit spermathecae, those with separate insemination and fertilization ducts (entelegynes), are thought to promote a first male advantage in fertilization due to a serial ordering of sperm and a first-in/first-out usage pattern (Austad 1984). Consequently, males of such species usually mature before females and cohabit with penultimate-instar females approaching the final molt and sexual maturity (Christenson & Goist 1979; Robinson & Robinson 1980; Jackson 1986; Watson 1990; Dodson & Beck 1993). Cul-de-sac spermathecae, those with common insemination and fertilization ducts (haplogynes), are thought to promote a last male advantage because the sperm from the last mating are nearer to the exit of the duct and a last-in/first-out usage pattern (Austad 1984). This is supported by Kastner & Jacobs (1997; but see Eberhard et al. 1993). In this case there should be no selective pressure for early male maturation and preferential cohabitation with females approaching sexual maturity. Males mature at about the same time or after females and cohabitation with juvenile females is not noted (Huff & Coyle 1992; Eberhard et al. 1993)

While the morphology of the female reproductive tract may influence male advantage patterns for fertilization of a female's eggs, sexual maturation rates and male cohabitation patterns, the relationship between the female's reproductive tract morphology and copulatory behavior is uncertain. It is known that males of species with cul-de-sac spermathecae often simultaneously insert both palps during copulation (Foelix 1980). The patterns of palpal insertion and duration of copulation among species with conduit spermathecae show extreme variability within and between species (Robinson & Robinson 1980; Elgar 1995). Males can insert one palp several times before switching to the opposite palp or insert each palp once. Perhaps features of the natural history can influence, or at least help explain, male reproductive behaviors.

Our work focuses on the natural history and copulatory behavior in the spiny orbweaving spider, *Micrathena gracilis* (Walckenaer 1805). The genus *Micrathena* Sundevall 1833 contains over 100 species distributed throughout the New World tropics, with three found in North America (Levi 1978, 1985). The two species native to Louisiana, *M. gracilis* and *M. sagitatta* (Walckenaer 1841), have one generation a year at our study site. *Micrathena* are characterized by prominent spines on the female abdomen. The male is only a fraction of the size of the female with little similarity in form. Female *Micrathena* are also entelegynes, having separate insemination and fertilization ducts (Levi 1978, 1985). Limited observations of mating in captive *M. gracilis* were recorded by Montgomery (1903). Observations have also been conducted on *M. gracilis* macrohabitat preferences (Hodge 1987a), site tenacity (Hodge 1987b) and prey selection (Uetz & Biere 1980; Uetz & Hartsock 1987).

During the course of field observations of *M. gracilis*, we noted that a complete mating between a given male and female entails two copulations (insertions), one for each of the two male and female copulatory organs. After the first copulation, the male must dismount and reapproach the female to copulate again. Staged encounters revealed the important observation that the durations of the two copulations are asymmetrical. As we report here, the duration of the second copulation is more than twice as long as that of the first. Others have noted such differences in copulatory durations (Bristowe 1929; Huber 1993, 1995; Sasaki & Iwahashi 1995); but to our knowledge, no one has yet addressed how a copulatory pattern might be related to a species' natural history.

We conducted a census of marked, unrestrained animals and observed staged encounters to describe copulatory behavior of *M. gracilis* and to place it within a framework of reproductive natural history. We describe the timing of sexual maturation, architecture of the female web, behavior of males on the female web, courtship and copulation, the likelihood that copulation will occur, sperm induction, female oviposition and egg sac mortality. We then discuss how male copulatory pattern may relate to these natural history data.

METHODS

Study site.—Observations were conducted at the F. Edward Hebert Center of Tulane University, 20 km south of New Orleans, Louisiana. The studies were conducted on a 30 × 40 m plot in a hardwood, bottomland forest. *M. gracilis* occur there in relatively large numbers. The study area, located next to a lagoon, is frequently flooded during the spider's mating season.

Census procedures.—To describe reproductive natural history, census observations were made during June through September, 1990 and 1991. Census animals were individually marked with fast-drying acrylic paint and were observed daily. Females were marked on the tips of the spines and males on the dorsal surface of the abdomen. When animals molted they were re-marked. Paint-marking had no obvious effects on the spiders' behavior. All web sites were tagged. During the 1990 field season, a total of 143 females was observed between 0700–1600 h; no males were marked. During the 1991 field season, a total of 102 females and 105 males was observed between 0700–1600 h. Each day the census area was thoroughly scanned for animals and all animals were briefly observed at least once. All unmarked animals located in the census area were marked. Noted were the presence of a viscid spiral (prey catching surface) and web support strands, presence and identity of the female and males on a web, pattern of residency, molting, mating, oviposition, and disappearance or movement from the web site. For analyses of male residency on a female's web we used the term "male days". For example, if two males were present on a particular day on a given female's web, we scored this as two male days for that female on that day.

Every other day in late July and August of 1990, two plots about one km apart were searched for new egg sacs. Tagged intact sacs were examined daily. Five sacs were collected mid-season and their clutches were removed and weighed on a Mettler analytical balance. Eggs were then separated from each clutch with a paintbrush and 10 groups of 20 eggs

(n = 200 eggs per clutch, most of the eggs in the clutch) were weighed. The length and width of 10 eggs from each clutch were also measured.

Timing of first sperm induction.—Five penultimate-instar males approaching the final molt were placed in separate 250 ml collection vials that contained two small twigs. The vials were examined daily for molting or exoskeletons. The date molted was recorded. Three days later the males were brought into the lab and examined for sperm content. The methods for determining sperm content are reported elsewhere (Bukowski & Christenson 1997). Here we simply note whether sperm were present in the palps or not.

Procedures for staged matings.—To facilitate the observation of complete mating sequences, we conducted staged encounters between males and females in 1990 and 1991. To be certain females were virgin, they were monitored for an impending molt, which is preceded a day or two by failure to construct a viscid spiral. The abdomen of a penultimate-instar female approaching the final molt is spherical with relatively short spines while that of the newly-molted female is elongate with longer spines. Penultimate-instar females were placed in 250 ml plastic collection vials where they molted to adulthood. This ensured they did not copulate overnight when not observed. The females were released the next day at their original web site. Males were collected from the webs of penultimate-instar females and usually held a couple of days prior to testing. After collection they were examined under a dissecting microscope for bodily damage. Over the 1991 field season, 176 males were also examined for bodily damage to determine if they are typically injured in male-male interactions. The reproductive histories of the males were not known.

Staged encounters were initiated only under dry conditions. Females were released at 0800 h and allowed to build webs. A randomly chosen male was placed on an upper frame thread after the female had constructed the viscid spiral. Copulation is defined as the interval between palpal insertion and male dismount from the female, usually occurring immediately after removal of the palp. The intercopulatory interval refers to the amount of time between an animal's first and second copulation.

Frequency and duration of copulation: Males (n = 20) were each presented to a female (n = 20) and allowed to copulate twice and depart the web. Males were observed until after sperm induction or until 1½ h had elapsed. Both male and female were then collected and weighed (wet weight) that evening in the laboratory on a Mettler analytical balance. Both males and females were released at the field station the following day.

Phases of copulation: We examined in detail the phases (inflation state of the hematodocha) of the two copulations. The males (n = 13) used were taken from studies involving other factors that influence frequency and duration of copulation (Bukowski & Christenson unpubl. data). We recorded how long the hematadochae were inflated, the length of time deflating, intervals between deflation and palp removal, and palp removal and dismount.

Prolonged second copulation: We tested whether the second copulation would be prolonged when a male (n = 15) copulated with one palp with one virgin female and was then given another virgin female.

Statistics.—All summary statistics are reported as $\bar{x}$ ±SD.

RESULTS

Sexual maturation.—Mating occurred from mid-June to mid-August. Early in June 1991 all females (n = 24) and all (n = 19) but one male in the study area were juveniles. By late June, only 10.7% of the females (n = 51) had matured compared to more than 70% of males (n = 21, χ^2 = 24.97, $P < 0.00001$). Sex ratio appeared to change over time as well. In early June, the ratio was nearly at unity (n = 24♀, n = 19♂, 1.3:1; χ^2 = 0.58, P = 0.44) and by late June females (n = 51) outnumbered males (n = 21; 2.4:1; χ^2 = 5.4, P = 0.02). Wandering adult males are difficult to find so these numbers represent, for the most part, males on females' webs.

After molting to adulthood, males remained on a single strand of their last web for 3.0 ±1.2 days (n = 22). By this time, the dorsum remained white, but other body parts had turned from a gray to a rusty-red or black. In contrast, females were tan, black and white, or black and appeared not to change color at sexual maturation. Adult males weighed 3.3 ±0.3 mg (n = 20) and newly-molted females weighed 45.0 ±6.6 mg (n = 20). Adult males appeared not to feed, although they can drink water from leaves or silk surfaces.

Timing of first sperm induction.—It is not known when or where free-moving males first induct sperm to the palps. Presumably this is done before they leave their last web site. Males presented to females did not induct sperm prior to mating, but did so after mating (see post-copulatory sperm induction below). However, the best evidence for sperm induction prior to mate searching was found with males that had molted to adulthood in collection vials. Three days after the final molt all males (n = 5) had sperm in both palps.

The female's web.—After sunrise, females built radially symmetric orbs usually within 4 m of the ground. The web is essentially two-dimensional with a relatively small (about 20 cm in diameter) viscid spiral situated within triangular frame threads and maintained under high tension (Uetz & Hartsock 1987). The viscid spiral is sloped between 0–45° off of vertical. There are no support or barrier strands adjacent to the hub. Females remained at the hub with the head down, abdomen tilted back, and the dorsum parallel to and facing the ground. This is an unusual position made possible by the relatively long fourth femora (see photos and drawings in Levi 1985). At dusk, the female ingested virtually every strand except frame threads, on which she remained until morning.

Two to four days before the final molt, the female removed her viscid spiral and did not construct another until after the molt. Generally, all that remained was the top horizontal support strand and this was usually shortened within a day before the molt. Females would move within 2–3 cm of one end of the strand and there they molted. After shedding the exoskeleton, the top foundation strand was lengthened and used as a foundation thread for the next viscid spiral. The exoskeleton was removed from the molting thread and reconnected on the upper foundation strand near the edge of the viscid spiral. This pattern of molting behavior was similar for juvenile females and males.

Census females in the penultimate instar (n = 17) remained at their web sites for 11.4 ±5.8 days and moved 0.35 ±0.6 times between the penultimate and final molts. The interval between the penultimate and final molts was 15.4 ±2.1 days. Only 29% ever moved during this time. Adult census females (n = 81) remained at their web sites for 19.8 ±12.3 days and moved 2.2 ±1.3 times before death or disappearance.

Table 1.—Census females: number of census female observation days, number of female observation days with one or more males present, and number of observation days that males were found on the webs of ante-penultimate instar or younger females, penultimate-instar females and adult females. Some individual females are represented in more than one age category.

Female instar	Number of female days	Number of days with at least one male present	Number of male days
≤Ante-penultimate	621	30	38
Penultimate	726	217	297
Adult	174	7	8

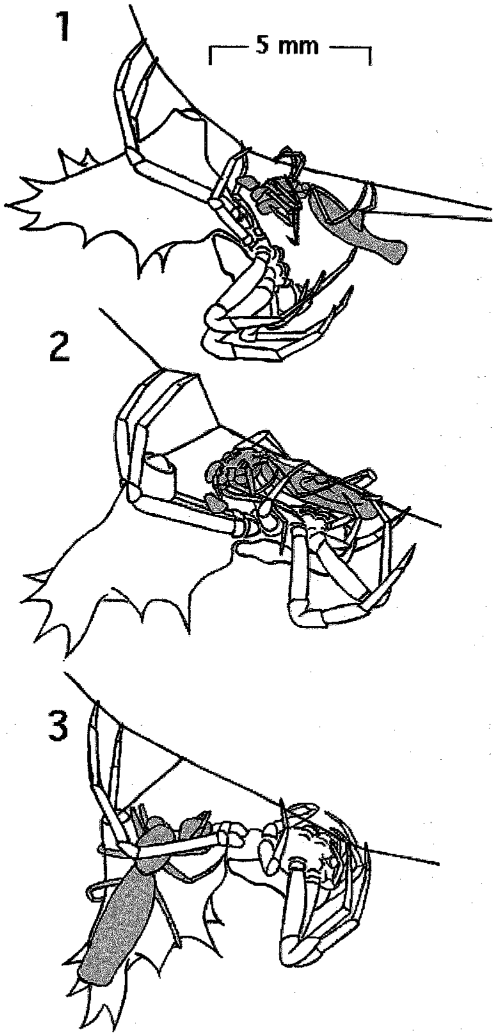
Male residency on females' webs.—Of 73 individually-marked adult males, 40 were marked the day of their final molt or were found with an exoskeleton. Nearly half of the males (n = 19) that were marked the day of their final molt were never found after leaving their tagged sites. Most marked males were found on the web of one (n = 28) or two (n = 15) females, but some were found on the webs of three (n = 8), four (n = 2) or seven (n = 1). Overall, males were observed to visit 1.3 ±1.25 females.

Males were more likely found with penultimate-instar than with younger juvenile or non-virgin adult females (Table 1; $\chi^2 = 170.8$, $P < 0.00001$). Over the census period, only 21 males were noted with ante-penultimate females and they stayed 2.2 ±2.1 days. Seven of those males were present when the female molted to the penultimate instar and all left the web that day. Males (n = 80) did not remain significantly longer ($\bar{x}$ = 3.3 ±2.6 days) with penultimate-instar than ante-penultimate instar females ($F_{1,99} = 2.98$, $P = 0.09$). Males were infrequently found with adult females; they were always observed *in copula*.

As each female approached her final molt the number of males on her web increased. We sampled three periods of equal length during the penultimate instar: (1) just after the molt to the penultimate instar, (2) mid-instar, and (3) just prior to the final molt. The duration of each period was not constant across females. The durations of the periods were slightly different for some females and were based (*post hoc*) on the number of days a given female did not construct a viscid spiral prior to the final molt, 3.6 ±1.3 days. Therefore, the average number of days for each of the three periods is also 3.6 ±1.3 days. Male presence significantly differed across these three time periods (male days, $F_{2,32}$ = 14.8, $P <$ 0.0001; number of males, $F_{2,32}$ = 21.46, $P <$ 0.0001). *Post-hoc* means comparisons showed that male presence increased with each time period. Few males were found with females that had just molted to the penultimate instar (0.2 ±0.7 male days, 0.1 ±0.3 males). A few more males were present at a point midway through the penultimate instar (1.5 ±2.3 male days, P = 0.056; 0.6 ±0.9 males, P = 0.032). Even more males were found with the female immediately prior to the final molt (3.6 ±2.9 male days, P = 0.0018; 1.5 ±1.1 males, P = 0.0002).

Of 33 known penultimate-instar census females, 79% (n = 26) had at least one male ($\bar{x}$ = 1.6, range 1–6) present the day before they molted. Males were usually stationary and found near the ends of foundation or peripheral strands. One to two days prior to the final molt, when no viscid spiral was constructed, males seemed to become more active and to be located nearer the female. A detailed description of intermale encounters was difficult to attain with the unaided eye, given male size and speed of engagement. Male encounters might be described as chases with brief physical contact. Males generally maintained all eight legs and examination revealed no signs of bodily damage. Of the 176 adult males collected from the webs of penultimate-instar females (1991) and examined under a dissecting microscope, only five males were missing a leg. Five others were missing a palp.

Courtship and copulation.—Just after the female's final molt, when she became sexually receptive, she mated while on a single strand or after she constructed a viscid spiral. In the latter case, the male constructed a mating thread between the end of the primary foundation strand and the outer end of a radial strand. The male courted vigorously by bouncing, bobbing and abdomen wagging, as defined for other *Micrathena* species by Robinson & Robinson (1980). The sequence of events leading to insertion and the copulatory position of *M. gracilis* is very similar to that of *M. schreibersi* (Perti 1833), also described by Robinson & Robinson (1980).



Figures 1–3.—Lateral view of copulatory mounting of *Micrathena gracilis*. Male spider is shaded and female spider is white. 1. Female acceptance posture and male approach; 2. Insertion; 3. Final copulatory position. See text for details.

When a female at the hub of a viscid spiral responded to a courting male, she moved across the viscid spiral, on the radial strand connected to the male's mating thread. She moved onto the mating thread, and then let go with the first (I), second (II), and sometimes third (III) pairs of legs (see Fig. 1). If the female were hanging by legs II–IV, the male bit and pulled at legs II until the female released them from the mating thread. This acceptance posture, necessary for coupling, placed the female at the proper angle for insertion, about 40–45° below the horizontal silk strand. The male then moved toward the female so they were head to head. Unsuccessful attempts at insertion were often followed by the male jumping from the female. The male then hung from his dragline, which was connected to the mating thread.

When the male inserted one palp, the female grasped the male's abdomen with legs I and II and chelicerae and appeared to pull him in further toward her epigynum (see Fig. 2). The female then rubbed legs I and II against his ventrum. Through an apparently joint effort, the male was flipped over 180° so that they were positioned ventrum to ventrum and facing essentially the same direction (see Fig. 3). The male was positioned with his cephalothorax midline at the epigynum and his abdomen at an angle of 30–45° to the major axis of the female's abdomen. A male that was positioned over the female's left side had the right palp inserted into the female's right genital pore. The male's body was bent at the pedicel, following the contour of the female's body. The male was connected by a thread to the female's abdomen which was later used when dismounting.

Once inserted and flipped over onto the female's abdomen, the male was firmly locked in place. Shortly after being properly positioned, the hematodocha expanded to full size. As with females of many *Micrathena* species, *M. gracilis* females have an epigynal protuberance (scape) oriented just below and medial to the spermathecae (Levi 1985). During copulation, the male's hematodocha nearly surrounded the end of this structure. Sclerotized parts of the palp closely gripped the scape at defined indentations. No contractions or changes in the hematodocha could be seen with the unaided eye and neither males nor females showed rhythmic movements while *in copula.*

After copulation was initiated, the female returned to the hub. The female's mobility was not compromised and she was able to capture and feed on prey items while mating. Within a few minutes the hematodocha deflated and within a few seconds the male removed the palp from the female's copulatory pore. Occasionally a male would begin pulling at the engaged palp at about the time most males would dismount. If the palp were not removed immediately, the male usually remained inserted for a relatively long period, apparently stuck. On a few rare occasions free-moving females in the field were noted with a dismembered palp in the epigynum.

Complete insemination of the female requires two distinct copulations. Due to the specific orientation required for insertion, males must dismount the female in order to re-mount and copulate a second time. Thus, once mounted, males intromit and copulate once with one palp and can inseminate only one spermatheca during that mount.

If more than one male were present with a receptive female, they would sometimes sever one another's mating threads, occasionally causing a male to fall from the web (Bukowski & Christenson unpubl. data). If one was engaged in copulation, another would often court, causing the female to move out onto his mating thread and exhibit the acceptance posture. This male would approach and attempt insertion which was precluded by the position of the copulating male. It then bit at the legs and palps of the engaged male but never dislodged it. A few males severed web foundation strands, causing the web to collapse.

Method of dismounting the female.—At the termination of copulation, the male (n = 19) either climbed (n = 10) off the female and moved up across the viscid spiral to the top frame thread or it jumped (n = 9) off and hung below the web, connected to her abdomen by the dismount thread. Movement of males opting for the former tactic elicited vigorous jerking and pursuit by the female. After reaching a foundation strand, the male would immediately construct a mating thread and court. During staged encounters, the intercopulatory interval for males that climbed was 203 ±241 sec (n = 9).

Males that jumped off occasionally attempted to climb back up the dismounting strand. The female usually jerked her abdomen vigorously, breaking the dismount strand and disconnecting the pair. Those that remained suspended released silk that usually connected to the bottom foundation strand or to nearby vegetation. If the released silk did not connect to the web, the male sent out additional strands until one attached. If several attempts failed, the male moved away from the web. Once the released silk connected, the male climbed onto the foundation strand, constructed a mating thread, and courted. During staged encounters, the intercopulatory interval for males that jumped (440 ±278 sec, $n = 6$) was not significantly shorter than for males that climbed (203 ±241 sec, $n = 9$, $F_{1,13} = 3.1$, P = 0.10). However, one male that climbed off the female courted the female nearly immediately, but was not able to insert until 834 sec later. When this outlier is removed, the intercopulatory interval for males that climbed (123.4 ±49 sec, $n = 5$) was significantly shorter than for males that jumped ($F_{1,12} = 10.23$, $P = 0.008$). Males did not groom legs or palps between copulations. The likelihood of obtaining a second copulation did not differ for males that climbed or jumped ($\chi^2 = 0.72$, $P = 0.40$).

Frequency of re-mating.—Females were sexually receptive throughout their lives and mated with virtually any male that encountered her web. Of the 57 adult census females (1990) that were observed briefly each day until oviposition, 34 (60%) were observed to mate on the day of the final molt, generally within 1 h after the molt. An additional 23 were observed to re-mate at a later date; 15 did so at least once, three twice, four thrice and one five times. Newly-molted females often alternated copulations with two or more males. Overall, census females were observed to mate with 1.7 ±1.1 males. The frequency of female mating is likely to be much higher, however, because copulations are brief and males do not remain after mating.

Copulations during staged encounters.—Staged encounters were held to determine more accurately the likelihood and duration of copulation under more controlled conditions.

Frequency and duration of copulation: Of the 20 newly-molted females presented a male, 19 were receptive. Fifteen (78.9%) copulated with the male on both sides or reproductive tracts, while four (21.1%) copulated on only one side. Of these four females, one did not respond to the male's courtship after the first copulation, one had a male possessing only one palp (the male pulled the other palp off after it was examined under the microscope and before it copulated) and he did not court a second time; and the other two males jumped after the first copulation and became disconnected. The female that did not copulate was presented a total of four males. She severed their mating threads or bit and threw the males to the ground.

For the second copulation, males employed the unused palp and inserted it into the virgin epigynal opening. All males ($n = 15$) copulated for a longer duration during the second coupling (1448 ±1265 sec) than the first (630 ±178 sec, $F_{1,14} = 5.93$, $P = 0.029$). The copulatory duration for males that mated on one side only (548 ±232 sec, $n = 4$) did not significantly differ from the duration of the first copulation of males that mated on both sides ($F_{1,17} = 0.56$, $P = 0.46$). All males terminated the second copulation by jumping off the female and hanging by the dismount strand. After mating, males departed the web, thus postmate defense was not noted.

Phases of copulation: We examined in detail the inflation phases of copulations of an additional group of males ($n = 13$). Once the palpal conductor was inserted, the hematodochae inflated, reaching full size (several times larger than the unused palp) by the end of the first minute. At this time the hematodochae appeared a translucent tan color. After several minutes (see Table 2 for time course of copulatory events), the hematodocha turned opaque white as it began deflating. The conductor remained inserted for a few minutes after deflation. It was then removed and the male dismounted. Overall, the second copulation was significantly longer than the first ($F_{1,12} = 30.17$, $P < 0.0001$; Table 2). Significantly different amounts of time were spent in each phase within a copulation ($F_{3,36} = 29.99$, $P < 0.0001$); most of the copulatory time was spent with the hematodochae inflated and less time was spent in each successive phase. The copulation (first or second) by phase (the four phases) interaction was significant ($F_{3,36} = 3.06$, $P = 0.041$); while the duration of the inflation phase was twice as long in the second copulation, the time taken for deflation and the time from deflation to palp removal showed a five and eight-fold increase, respectively, in the second copulation (Table 2).

Longer hematodochal inflation time did not necessarily result in a longer time to deflate.

Table 2.—Mean durations (seconds) of copulatory phases and mean copulatory durations for the first and second copulations with newly-molted virgin females (n = 13).

	First copulation		Second copulation	
	Mean	SD	*Mean*	SD
Duration of hematodochal inflation	507	127	1036	508
Duration of hematodochal deflation	95	86	408	454
End of deflation to palp removal	61	60	429	505
Palp removal to dismount	6	9	28	31
Total copulatory duration	670	201	1898	862

There were no significant relationships between the duration of hematodochal inflation and the amount of time taken to deflate for the first (r = +0.29, P = 0.33) or the second copulation (r = + 0.05, P = 0.88). There was no significant relationship between the durations of the first and the second copulations for males copulating twice (r = +0.09, P = 0.63, n = 28). However, one male's palp became stuck in the female during the second copulation and he could not remove the palp until 5684 sec. When data on this male are removed, there was a significant positive relationship between the duration of the first and second copulation (r = +0.44, P = 0.02; Fig. 4).

We examined the distributions of the durations of the first and second copulations for all animals that copulated twice with a single female (n = 28). We followed the method used by Suter (1990) to examine copulatory durations in a linyphiid. When the beginning of copulation is set at time 0, the times to completion of the first copulation fall along a straight line (r^2 = 0.98; Fig. 5). This suggests that the copulations were nearly uniformly distributed. However, the second copulation was better described by a negative logarithmic (r^2 = 0.95) than a linear function (r^2 = 0.85). While measures of latency (in this case duration of copulation) are often positively skewed, first copulations (skew = 0.27; normality = 0) were less positively skewed than second copulations (skew = 0.97).

Prolonged second copulation: Males (n = 15) that had mated on only one side of a newly-molted female were presented to a second newly-molted virgin female. All males employed the unused palp. They followed the same pattern of copulatory durations as a male mating on both sides of a single female. There

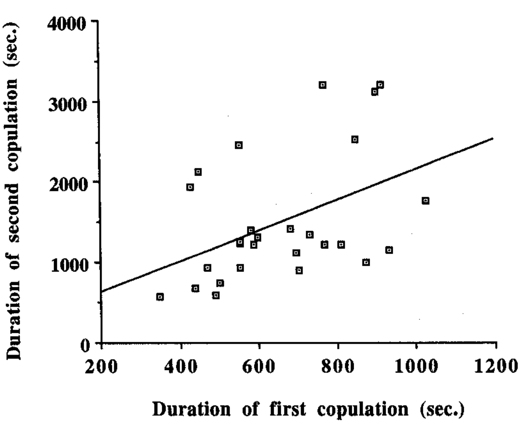


Figure 4.—Relationship between the durations of the first and second copulations. Males (n = 27) that copulated longer for the first copulation also copulated longer during the second copulation (r = +0.44; $Y = 248.8 + 1.91X$).

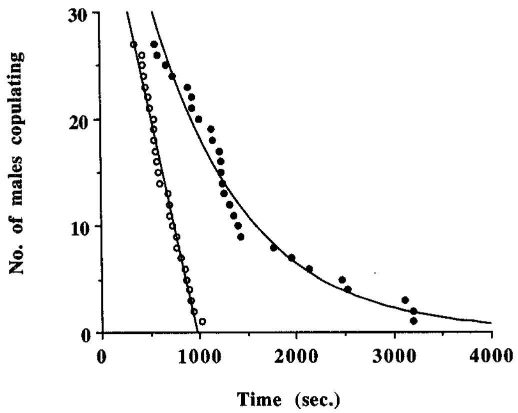


Figure 5.—When the start of copulation is set at time 0, the number of males remaining *in copula* over time for the first copulation (○) is best described by a linear function ($Y = 42.4 - 0.004X$) and for the second copulation (●), a negative exponential function ($Y = 53.146\ (10^{-0.0005})X$).

was no significant overall difference in the copulatory durations of males given one or two females ($F_{1,28}$ = 0.29, P = 0.60). Overall, second copulations were significantly longer than first copulations ($F_{1,28}$ = 26.28, $P <$ 0.0001). However, the durations of the first and second copulations did not differ as a function of the number of females with which the males mated ($F_{1,28}$ = 0.75, P = 0.39). For all males that mated with two females, first copulations (572 ±147 sec) were much shorter than second copulations (1721 ±814 sec).

Post-copulatory sperm induction.—After a male mated twice with a virgin female, it remained near, and rarely on, the female's web for a short time, grooming palps, legs and gonopore. The male moved 1–3 m from the female's web and resumed grooming until sperm web construction was initiated, 47.5 ±16.9 min (n = 15) after the final dismount. We do not know if males that mated only once with a female induct sperm prior to another mating.

The sperm web was constructed on a single horizontal strand 5–60 cm in length. After making several passes along this line, the male laid another strand, 3–4 cm in length, basically parallel to and near the middle of the first. The two strands were held apart by legs I and II and legs III and IV. The latter pairs were extended and held out in front of the body so that an elongated, horizontal hexagon was formed. Silk was then laid in a zigzag manner between the two lateral sides of the hexagon. The area proximal to the cephalothorax was completely covered with silk, and it was to the ventral side of this area (approximately 1.0 × 1.5 mm) that the male applied the gonopore. After many (about 100) applications, the male tipped its body up so it was perpendicular to the plane of the sperm web. He then reached over the dorsal side of the sperm web and applied the palps. It should be noted that one male (not included in this data set) had a broken fourth femur and could not keep the sperm web from collapsing before induction even though it constructed five webs.

Palps were individually dipped a mean of 4.6 ±1.9 times before they were switched. Dips were extremely shallow, a travel distance of less than 1 mm. Observation of this behavior was difficult and made worse by the slightest breeze. Males (n = 15) exhibited 7.4 ±1.9 and 7.9 ±1.8 induction bouts for the right and left palp, respectively. Sperm induction took 5.3 ±2.0 min (n = 15) to complete. The male then moved off the sperm web, which immediately collapsed, and departed. Observations were made only until the first sperm induction was completed, so males could have built additional sperm webs later.

Oviposition.—Females oviposited between July and October with most clutches laid in August and September. Of 101 marked adult females in 1990, 57 survived and remained in the study area to lay at least one clutch. Of the others, 13 were seen only the day they were marked and 31 disappeared before oviposition, after an average of 17.8 ±9.6 days. Females (n = 8) that were observed molting, mating and ovipositing laid their first clutch an average of 30.7 ±8.2 days after the molt.

Most oviposition occurred within 5 m of the web site and within 1–3 m of the ground (2.5 ±0.88 m, n = 97). Most egg sacs (66%, n = 63) were found in boxelder (*Acer negundo*), one of the more common trees in the area. The eggs were laid near the center of the underside of a leaf that was folded transversally and sealed tightly. The resulting sac was triangular in appearance with silk threads connecting the egg sac to the intersection of the leaf petiole and connecting branch. We have not observed *M. gracilis* constructing egg sacs but females left the web site sometime after dusk, and the procedure was usually completed by 0900 h the following morning. By this time most females had returned to their previous web site.

Females that had oviposited at least once (n = 57) produced 1.6 ±0.8 clutches before they died or disappeared. Examination of five clutches laid during the middle of the egg-laying season revealed 266.8 ±11.5 eggs. Clutches weighed 67.1 ±4.2 mg, and individual eggs 0.25 ±0.02 mg. The eggs were ovoid, 0.80 ±0.03 mm (n = 50) in length and 0.66 ±0.02 mm in width. By multiplying the mean number of egg clutches (1.6) by the mean number of eggs per clutch (266.8), the average female might lay 425 eggs throughout her lifetime.

M. gracilis egg sacs appeared to us to be cryptic, yet most suffered predation. Of 75 egg sacs found in the egg sac census areas (sacs laid by unmarked females), 62 (83%) were already destroyed at initial examination. Intact egg sacs (n = 13) lasted for a mean of only 3.9 days before we found that they had been opened and contents removed. Another 22 clutches laid by individually-marked census females (the female was found on the egg sac) did not fare much better. Overall, these lasted a mean of 10 days, however, many (41%; n= 10) were eaten within 48 h of being laid. Four egg sacs lasted between 18–57 days before either being eaten or falling to the ground. Only two egg sacs were intact as of mid-October. They hatched 37 and 41 days, respectively, after being laid. Within two weeks of hatching the spiderlings had molted.

When there was evidence of predation, the entire egg mass had usually been pulled from the sac. The white silk that normally surrounded the eggs was outside the sac, in an elongated cotton-like mass. Except for egg sacs that fell to the ground (n = 3), they all (n = 94) appeared to have been destroyed by a similar means of attack.

DISCUSSION

In many spider species, males mature in fewer molts than females. Consequently, males mature before females and are often smaller (Vollrath & Parker 1992). We found that these sex differences apply to *M. gracilis*. Sexual bimaturation is thought to be related to male sperm priority patterns (Parker 1984; but see Head 1995). When the first male to mate with a given female fertilizes most of her eggs, selection should favor males that mature early in the season so they are present when the female molts to adulthood and becomes sexually receptive (Austad 1984). Male advantage pattern for fertilizing a female's eggs has been determined for six entelegyne spiders, those with a heavily sclerotized female reproductive tract and separate sperm uptake and fertilization ducts. Most show basically a first male advantage (Jackson 1980; Vollrath 1980; Austad 1982; Martyniuk & Jaenike 1982; Christenson & Cohn 1988; Watson 1991; Masumoto 1993; but see Andrade 1996). Given that *Micrathena* is an entelegyne and shows early male maturation, we suspect it, too, will show a first male advantage pattern for fertilizing a female's eggs.

Male *M. gracilis* are found more frequently on the webs of penultimate-instar females, particularly those approaching the final molt. Jackson (1986), Watson (1990), Dodson & Beck (1993) and others (cited in Christenson 1984) have noted cohabitation with females just prior to their final molt and initial sexual receptivity. If *M. gracilis* does show a first male advantage pattern for fertilizing a female's eggs, the tendency of males to remain with penultimate-instar females is understandable as the female mates for the first time just after her final molt. We suspect that male *M. gracilis* can monitor an impending molt. As has been noted by Dodson & Beck (1993), the monitoring of the female must be frequent or continuous so that the male does not miss the female's molt and lose her to another male. We don't know how a male recognizes such a female, but there are at least four possibilities. First, females do not build a viscid spiral within a few days prior to the final molt, so a changing vibratory environment could cue the male. This would not, however, help the male determine when molting has occurred because the viscid spiral is not constructed three days prior to the final molt. Second, *M. gracilis* have stridulatory organs (Hinton & Wilson 1970; Uetz & Stratton 1982) that could be used to signal an approaching molt. Such communication may be relatively cryptic to the human observer and we may have missed it. Third, Hill & Christenson (1988) note that smaller juvenile *Nephila clavipes* (Linne 1767) females are more aggressive toward the male than are penultimate-instar females, so males may be responding to female aggressiveness. We did not note such a change in aggressiveness in *M. gracilis* females. Fourth, the female could produce a male-attracting pheromone. Female spiders are known to produce pheromones (Olive 1982; Watson 1986; Prenter et al. 1994) that have only recently been isolated and identified (Schulz & Toft 1993).

Male *M. gracilis* are unable exclude other males from approaching the female. At best, a male might interfere with another's courtship activities. It is worth noting that a male on a female's web is cohabiting and not actually defending or guarding the female. We think that there are two major reasons that guarding may not have evolved. First, guarding may not occur because it is impossible to guard the female and her web effectively. The viscid spiral of the female *M. gracilis* web is not held in place by a complex of barrier strands and is essentially two-dimensional. Thus, there is no central location near the female a male could occupy and prevent access by other males. This hypothesis is further supported by that observation that a male must attract a female to his location to mate via the mating thread. Competing males could entice the female to mate from any point along the perimeter of the viscid spiral. A male on one side of the viscid spiral is unable to prevent another male from accessing the female on the opposite side of the viscid spiral. In contrast, some orb-weavers, such as *Nephila clavipes*, construct an enduring three dimensional orb-web, one containing the viscid spiral held in place by many barrier or support strands. Many of the barrier strands connect near the hub opposite the female and a male can remain there, centrally located, and fend off other males as they approach from virtually any direction (Christenson & Goist 1979). Copulation occurs just after the final molt at the hub. Therefore, a male occupying the hub position almost always mates while males at the periphery of the web rarely ever mate with that female (Christenson & Goist 1979). Further evidence of a lack of functional guarding is that the male cannot fend off rival males while copulating and he must dismount the female after the first copulation. The female responds to courting males, even when *in copula*, so if courting is occurring during a dismount, the female can mate with another male.

A second major reason that guarding may not have evolved is that guarding one mate might be less profitable than searching for and mating with other females and thus it has not been selected for in males. The interval between mating just after the final molt and oviposition is about one month. This is a relatively long period of time for a male to remain and defend a sexually active female against other male visitors. If there is a strong first male advantage pattern for fertilizing a female's eggs, then little might be gained from guarding a mate given that subsequent males will fertilize very few of the female's eggs. In addition, the male's investment with a particular female might well be lost due to predatory pressure on egg sacs. Our data indicate that female *M. gracilis* lay one or two egg sacs that are likely not to produce spiderlings in the spring. Loss of investment through spiderling mortality is a relatively understudied phenomenon. We agree with Pitnick & Markow (1994) that in species where the female produces relatively few egg sacs that suffer relatively high mortality, a male would benefit from mating with as many females as possible rather than guard a single female. This would increase the likelihood that he would sire or contribute to a surviving clutch.

Like many spiders, a *M. gracilis* male must mount the female twice to inseminate both of the female's pores. Proximately, males must dismount the female between copulations because of the relatively complicated process of genitalic coupling. Male spiders must often assume very specific orientations to the female in order to insert the palp (Foelix 1980). The evolution of male genitalia is thought to be influenced by sexual selection on females (Eberhard 1985). Ultimately, females might influence the evolution of male specific insertion orientations and structures that prevent the male from inserting both palps in a single mounting. Coyle & O'Sheilds (1990) have suggested that female spiders might have evolved multiple spermathecae to prevent a male from monopolizing access to all of her sperm storage sites. We agree and suggest that female spiders might also have evolved specific insertion orientation requirements to prevent a male from monopolizing both tracts in a single mounting. Such an arrangement might allow a female to gain information about the quality of a male during the first copulation and between the first and second copulation. She could then allow or refuse the male access to the opposite tract based on that information.

A precondition for the kind of asymmetry in copulatory duration that we document here is a male mating once on each side of the female. Insertions separated by a dismount and preceded by overt courtship appears to be the rule in the spiny orbweavers (sub-family Gasteracanthinae; Robinson & Robinson 1980) and other Araneidae (Bristowe 1929; Robinson & Robinson 1980). What aspects of araneid natural history would drive the evolution of copulations consisting of one insertion on each side of the female? Our descriptive work indicates that general aspects of *M. gracilis* reproduction are fairly typical for the family, that is, females are solitary, sedentary predators while males move from female to female. Males do not remain and defend mates, they often copulate with several females, and females mate with more than one male. We suspect that their pattern of mating behavior, two copulations separated by a dismount with the second copulation being much longer than the first, is influenced by the ease with which a second male can copulate with the female within a relatively brief period of time.

Under certain circumstances selection should favor males that rapidly transfer sperm to one reproductive tract in a single insertion (copulation) rather than over a series of insertions. If a male is required to dismount in order to re-insert in the same epiginal opening, a second male could usurp the first male on that side. Our observations suggest that the likelihood of such usurpations is related to particular elements of reproductive natural history: males mature before females and many males may simultaneously cohabit with a single female, a web structure that does not allow a male to defend the female from competitors, and the use of a mating thread to entice the female to mate. There is no central area on the web near the female that the male can occupy, consequently a male must entice the female to his location in order to insert.

Male *M. gracilis* appear highly motivated to copulate with both tracts of the virgin female. If the suspected first male advantage pattern in fertilization is related to the conduit shape of the spermathecae, then the sperm priority pattern would be determined separately for each tract. The advantage would not go to the first male to mate with a female, *per se*, but to the first male to mate with a given tract. Therefore, a male should attempt to copulate on both sides. A male that copulates only once would leave the other virgin tract available for insemination by other males. Unless females can preferentially use sperm from one tract over the other at oviposition, the first male is likely to cede 50% or more of the fertilizations to a second male that mates with the remaining virgin tract.

The failure to obtain two copulations with a given female is likely to have important implications for the male's subsequent reproductive activities. About 25% of the males failed to copulate twice with a given female. These males would leave the females with one palp filled with sperm and one empty palp. A male in this circumstance might either refill the empty palp or mate with the next female using one full and one empty palp. It is unclear whether two copulations are required to trigger the onset of sperm induction and whether a male could preferentially fill the empty palp. Given the rhythmic and stereotyped organization of sperm induction behaviors, it is unlikely that a male could preferentially fill one palp. The filling of the empty palp may, in turn, influence whether a male copulates once or twice with the next female.

The distributions of the durations of first and second copulations suggest that different selective forces might be operating. The durations of the first copulations were short and nearly normally distributed which suggests that stabilizing selection is operating. The durations of the second copulation were longer and more positively skewed, suggesting directional selection on longer copulatory durations. Given that a male cannot exclude the advances of other males, selection should operate on speed and efficiency of sperm transfer during the first copulation so that he can switch sides before another male moves onto the web. That directional selection is operating on longer second copulations suggests that the second copulation may serve somewhat different functions than the first. All phases of the second copulation were prolonged. We have shown that the prolonged copulation can facilitate sperm storage on both sides of the female and may serve a mate guarding function as well (Bukowski & Christenson 1997, unpubl. data). Such differences in copulatory durations have been reported in other species of a number of spider families (Bristowe 1929; Huber 1993, 1995; Sasaki & Iwahashi 1995) and similar functions might be served.

ACKNOWLEDGMENTS

This paper was improved by comments by Petra Sierwald and two anonymous reviewers. An earlier draft of this paper was improved with comments by Paul J. Watson. Financial support was provided by the Robert E. Flowerree Fund of Tulane University. Thanks to Robin Richards for support in and out of the field.

LITERATURE CITED

Andrade, M.C.B. 1996. Sexual selection for male sacrifice in the Australian redback spider. Science, 271:70–72.

Austad, S.N. 1982. First male sperm priority in the bowl and doily spider, *Frontinella pyramitela*. Evolution, 36:777–785.

Austad, S.N. 1984. Evolution of sperm priority patterns in spiders, Pp. 223–249, *In* Sperm Competition and the Evolution of Animal Mating Systems (R.L. Smith, ed.). New York: Academic Press.

Bristowe, W.S. 1929. The mating habits of spiders, with special reference to the problems surrounding sex dimorphism. Proc. Zool. Soc. London, 21:309–358.

Bukowski, T.C. & T.E. Christenson. 1997. Determinates of sperm release and storage in the spiny orbweaving spider, *Micrathena gracilis*. Anim. Behav., 53:381–395.

Christenson, T.E. 1984. Alternative reproductive tactics in spiders. American Zool., 24:321–332.

Christenson, T.E. & J. Cohn. 1988. Male advantage for egg fertilization in the golden orbweaving spider, *Nephila clavipes*. J. Comp. Psych., 102: 312–318.

Christenson, T.E. & K.C. Goist. 1979. Costs and benefits of male-male competition in the orb-weaving spider *Nephila clavipes*. Behav. Ecol. Sociobiol., 5:87–92.

Coyle, F.A. & T.C. O'Shields. 1990. Courtship and mating behavior of *Thelechoris karschi* (Araneae, Dipluridae), an African funnelweb spider. J. Arachnol., 18:281–296.

Dodson, G.N. & M.W. Beck. 1993. Precopulatory guarding of penultimate females by male crab spiders, *Misumenoides formosipes*. Anim. Behav., 46:951–959.

Eberhard, W. 1985. Sexual selection and animal genitalia. Harvard Univ. Press, Cambridge, Massachusetts.

Eberhard, W., S. Guzman-Gomez & K. Catley. 1993. Correlation between spermathecal morphology and mating systems in spiders. Biol. J. Linn. Soc., 50:197–209.

Elgar, M.A. 1995. The duration of copulation in spiders: Comparative patterns. Rec. Western Australia Mus., 52:1–11.

Emlen, S.T. & L.W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. Science, 197:215–223.

Fincke, O.M. 1982. Lifetime mating success in a natural population of the damselfly, *Enallagma hageni* (Walsh) (Odonata: Coenagrionidae). Behav. Ecol. Sociobiol., 18:405–412.

Foelix, R.F. 1982. Biology of Spiders. Harvard Univ. Press, Cambridge, Massachusetts.

Head, G. 1995. Selection on fecundity and variation in the degree of sexual size dimorphism among spider species (class Araneae). Evolution, 49:776–781.

Hill, E.M. & T.E. Christenson. 1988. Male residency on juvenile female *Nephila clavipes* (Araneae, Araneidae) webs. J. Arachnol., 16:257–259.

Hinton, H.E. & R.S. Wilson. 1970. Stridulatory organs in spiny orb-weaving spiders. J. Zool., London, 162:481–484.

Hodge, M.A. 1987a. Macrohabitat selection by the orb weaving spider, *Micrathena gracilis*. Psyche, 94:347–361.

Hodge, M.A. 1987b. Factors influencing web site residence time of the orb weaving spider *Micrathena gracilis*. Psyche, 94:363–371.

Huber, B.A. 1993. Genital mechanics and sexual selection in the spider *Nesticus cellulanus* (Araneae: Nesticidae). Canadian J. Zool., 71:2437–2447.

Huber, B.A. 1995. Genital morphology and copulatory mechanics in *Anyphaena accentuata* (Anyphaenidae) and *Clubiona pallidula* (Clubionidae: Araneae). J. Zool., London, 235:689–702.

Huff, R.P. & F.A. Coyle. 1992. Systematics of *Hypochilus sheari* and *Hypochilus coylei*, two southern Appalachian lampshade spiders (Araneae, Hypochilidae). J. Arachnol., 20:40–46.

Jackson, R.R. 1980. The mating strategy of *Phidippus johnsoni* (Araneae, Salticidae): II. Sperm competition and the function of copulation. J. Arachnol., 8:217–240.

Jackson, R.R. 1986. Cohabitation of males and juvenile females: A prevalent mating tactic of spiders. J. Nat. Hist., 20:1193–1210.

Kastner, J.L. & E.M. Jakob. 1997. Last male sperm priority in a haplogyne spider (Araneae: Pholcidae): Correlations between female morphology and patterns of sperm usage. Ann. Entomol. Soc. America, 90:254–259.

Levi, H. 1978. The American orb-weaver genera *Colphepeira*, *Micrathena* and *Gasteracantha* north of Mexico. Bull. Mus. Comp. Zool., 148: 417–442.

Levi, H. 1985. The spiny orb-weaver genera *Micrathena* and *Caetacis* (Araneae: Araneidae). Bull. Mus. Comp. Zool., 150:429–618.

Martyniuk, J. & J. Jaenike. 1982. Multiple mating and sperm usage patterns in natural populations of *Prolinyphia marginata* (Araneae: Linyphiidae). Ann. Entomol. Soc. America, 75:516–518.

Masumoto, T. 1993. The effect of the copulatory plug in the funnel-web spider, *Agelena limbata* (Araneae: Agelenidae). J. Arachnol., 21:55–59.

Montgomery, T.H. 1903. Studies on the habits of spiders, particularly those of the mating period. Proc. Acad. Nat. Sci., Philadelphia, 55:59–149.

Olive, C.W. 1982. Sex pheromones in two orb-weaving spiders, (Araneae, Araneidae): An experimental field study. J. Arachnol., 10:241–245.

Parker, G.A. 1984. Sperm competition and the evolution of animal mating strategies. Pp. 1–60, *In* Sperm Competition and the Evolution of Animal Mating Systems (R.L. Smith, ed.). Academic Press, New York.

Pitnick, S. & T.A. Markow. 1994. Male gametic strategies: Sperm size, testes size, and the allocation of ejaculate among successive mates by the sperm-limited fly *Drosophila pachea* and its relatives. American Nat., 143:785–819.

Prenter, J., R.W. Elwood & W.I. Montgomery. 1994. Assessments and decisions in *Metellina segmentata* (Araneae: Metidae): Evidence of a pheromone involved in mate guarding. Behav. Ecol. Sociobiol., 35:39–44.

Robinson, M.H. & B. Robinson. 1980. Comparative studies of the courtship and mating behavior of tropical araneid spiders. Pacific Insects Monogr., 26:1–218.

Sasaki, T. & O. Iwahashi. 1995. Sexual cannibalism in an orb-weaving spider *Argiope aemula*. Anim. Behav., 49:1119–1121.

Schulz, S., & S. Toft. 1993. Identification of a sex pheromone from a spider. Science, 260:1635–1637.

Suter, R.B. 1990. Courtship and assessment of virginity by male bowl and doily spiders. Anim. Behav., 39:307–313.

Uetz, G.W. & J.M. Biere. 1980. Prey of *Micrathena gracilis* (Walkenaer) (Araneae: Araneidae) in comparison with artificial webs and other trapping devices. Bull. British Arachnol. Soc., 5: 101–107.

Uetz, G.W. & S.P. Hartsock. 1987. Prey selection in an orb-weaving spider: *Micrathena gracilis* (Araneae: Araneidae). Psyche, 94:103–116.

Uetz, G.W. & G. Stratton. 1982. Acoustic communication and reproductive isolation in spiders, Pp. 123–159, *In* Spider Communication, Mechanisms and Ecological Significance (P.N. Witt & J.S. Rovner, eds.). Princeton Univ. Press, Princeton, New Jersey.

Vollrath, F. 1980. Male body size and fitness in the web-building spider *Nephila clavipes*. Z. Tier., 53:61–78.

Vollrath, F. & G.A. Parker. 1992. Sexual dimorphism and distorted sex ratios in spiders. Nature, 360:156–159.

Watson, P.J. 1986. Transmission of a female sex pheromone thwarted by males in the spider *Linyphiia litigiosa* (Linyphiidae). Science, 233:219–221.

Watson, P.J. 1990. Female-enhanced male competition determines the first mate and principal sire in the spider *Linyphia litigiosa* (Linyphiidae). Behav. Ecol. Sociobiol., 26:77–90.

Watson, P.J. 1991. Multiple paternity and first mate sperm precedence in the sierra dome spider, *Linyphia litigiosa* Keyserling (Linyphiidae). Anim. Behav., 41:135–148.

Manuscript received 27 February 1996, accepted 5 March 1997.