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NATURAL HISTORY OF *GLENOGNATHA EMERTONI* (ARANEAE, TETRAGNATHIDAE): MATING BEHAVIOR AND SPERM RELEASE IN A HAPLOGYNE

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ABSTRACT. *Glenognatha emertoni* (Simon 1887) is a small haplogyne orb-weaver collected near streams and dry streambeds in southern Arizona whose habits are unknown. Field observations revealed that *G. emertoni* are commonly found in vegetation overhanging streams and, more rarely, under streamside rocks. Mating pairs were observed on or near adult female webs. Males lack mate-guarding behavior and leave the female immediately after copulation. To examine mating behavior in a controlled setting, juveniles and adults were collected from the field and maintained in the lab. Matings were arranged between wild-caught adults and also between laboratory-reared virgins in order to describe mating behavior and sperm release during copulation. Unlike most other orb-weaving spiders studied, the number of sperm released and overall duration of copulation are not influenced by female mating history in *G. emertoni*. Male *G. emertoni* release equivalent numbers of sperm to virgin and non-virgin females. Given this pattern of sperm release and the lack of mate-guarding behavior by males, sperm competition should be intense in this species. Based only on the numbers of sperm released by each male in the study, doubly-mated females would be expected to produce egg sacs of mixed paternity, if all else were equal.

Keywords: Araneae, sexual selection, sperm competition, spermatheca, spider

Glenognatha emertoni (Simon 1887) is a small, haplogyne tetragnathine orb-weaver (adult body length 4.5–5 mm) known from Arizona and New Mexico (Map 8, Levi 1980). The habits of *G. emertoni* are unknown. The genus *Glenognatha* includes 20 named species from the Americas (North, Central, and South), Africa, and the Caribbean, Galapagos, and Pacific Islands, but it is possible that many tropical species remain undescribed (Hormiga & Döbel 1990; Platnick 2006). This genus is a member of the sub-family Tetragnathinae, a haplogyne clade within the entelegyne family Tetragnathidae (Hormiga et al. 1995). Within the tetragnathines, the mating behavior of species in the haplogyne genera *Tetragnatha* and *Pachygnatha* have been well-described (Gerhardt 1921, 1927; LeSar & Unzicker 1978; Huber 1998; West & Toft 1999; Danielson-François 2002; Danielson-François & Bukowski 2005). However, the behavior of species within the haplogyne genus *Glenognatha* is essentially unstudied apart from Barrows (1919) observations of *Glenognatha foxi* (McCook 1894) and a single published note by Edwards & Senske (2001) on the mating behavior of *Glenognatha heleios* (Hormiga 1990), a recently discovered species (Hormiga & Döbel 1990).

Haplogyne taxa are especially intriguing to study because they provide a counter-point to studies on entelegynes, arguably the largest and most well-researched group of spiders. Haplogyny is the basal condition in the Araneomorphae and haplogynes are defined, in part, by their simple genitalia (Wiehle 1967). In particular, haplogyne females have *cul-de-sac* spermathecae with one duct for insemination and fertilization (Simon 1895). Entelegyne females have more complex conduit spermathecae, each with a specialized duct for insemination whose opening is located externally in the epigynum, and a separate duct for fertilization (Simon 1895). Although they are located within the entelegyne suborder (Hormiga et al. 1995; Griswold et al. 1998, 1999), a reversal to haplogyny has occurred within the tetragnathines (Wiehle 1967), which have *cul-de-sac* spermathecae with one duct for insemination and fertilization (Danielson-François 2002).

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Such morphological differences in female spermathecae might underlie the diversity of paternity patterns in spiders, which range from first- to last-male advantage—unlike the last-male advantage common to insects, whose spermathecae often resemble a simple *cul-de-sac* (Walker 1980; reviewed in Simmons & Siva-Jothy 1998; Elgar 1998; Eberhard 2004). To explain this diversity, Austad (1984) proposed that spermathecal morphology and ejaculate stratification generated first-male advantage in entelegynes and last-male advantage in haplogynes. Austad (1984) also argued that haplogyne males would be indifferent towards, but that entelegyne males would prefer, virgin females as mates and that haplogyne males would guard mates after copulation. To date, mate-guarding, mating behavior, and paternity have been studied in relatively few haplogyne taxa.

Post-copulatory mate-guarding should be common in haplogyne taxa if Austad's (1984) predictions hold. Of the haplogyne species studied thus far, co-habitation of adult pairs has been found in seven pholcid taxa (Eberhard & Briceño 1983; Eberhard et al. 1993; Blanchong et al. 1995; Kaster & Jakob 1997). Contrary to Austad's hypothesis, in at least one pholcid species males co-habit equally with penultimate-molt and mature females (Eberhard et al. 1993). Yet overall, a strong preference for co-habitation with penultimate-stage females, rather than mature females, appears to be lacking in the pholcid species studied (Eberhard et al. 1993). Of the *Tetragnatha* species examined, both co-habitation and mate-guarding appear to be absent; males leave the female immediately after copulation (West & Toft 1999; Danielson-François 2002; Danielson-François & Bukowski 2005; but see LeSar & Unzicker 1978). No information on the co-habitation or mate-guarding behavior of *Glenognatha* species is available at present.

Austad (1984) predicted that last-male paternity advantage would be common among haplogyne taxa, but empirical evidence to date offers equivocal support. Paternity patterns have been examined in three pholcids and one *Tetragnatha* species (reviewed in Elgar 1998; Eberhard 2004). A last-male biased paternity pattern has been shown for *Pholcus phalangioides* (Fuesslin 1775), *Holocnemus pluchei* (Scopoli 1763), and *Tetragnatha extensa* (Linnaeus 1758) (Yoward 1996, 1998; Kaster & Jakob 1997; West & Toft 1999; Schäfer & Uhl 2002). In contrast, *Physocyclus globosus* (Taczanowski 1873) has a paternity pattern that does not significantly differ from random sperm mixing (Eberhard et al. 1993). Haplogyne paternity patterns generally exhibited a high level of variation within a species: males sired from 0 to 100% of the progeny—similar to other spider paternity studies (reviewed in Elgar 1998). Even within an individual female, paternity patterns may vary: subsequent egg sacs from mated *T. extensa* females generally revealed an anti-chronological order of mating priority; however, a series of egg sacs from individual females defied any such general rules (West & Toft 1999). From these studies, it seems clear that paternity patterns are highly variable both within and among haplogyne species.

Although many different factors certainly underlie these complex behavioral and paternity patterns (e.g., cryptic female choice; Eberhard 1996, 2004), differences in sperm release among males might explain part of the variation. The amount of sperm transferred by males to virgin and non-virgin females may vary, as has been demonstrated in several entelegyne taxa in which males release little or no sperm to non-virgin females (Christenson & Cohn 1988; Andrade 1996; Bukowski & Christenson 1997; Bukowski et al. 2001). In contrast, the only data available for a haplogyne species thus far showed that males release equivalent numbers of sperm to virgin and non-virgin females (Danielson-François & Bukowski 2005). Data from only one species prevents generalizations about haplogyne sperm release behavior: more research on haplogyne species is needed.

This study focused on an Arizona population of *G. emertoni* from the Huachuca mountains, an appropriate location because the type specimen for the species was also collected in southeastern Arizona (Levi 1980). Here, I describe the natural history and mating behavior of *G. emertoni*, while offering the second demonstration of sperm release behavior in a haplogyne species.

METHODS

Natural History and Collection.—Previous records for *Glenognatha emertoni* stated that specimens were collected under rocks

near streams or in dry streambeds in the southern corner of Arizona and New Mexico (Levi 1980). Over the course of this study, approximately 175 immature, penultimate-molt and adult *G. emertoni* were collected from vegetation overhanging streams in mid-elevation riparian areas in the Huachuca Mountains of southeastern Arizona (31°28′N, 110°20′W). Voucher specimens were deposited in the arthropod collection of the Department of Entomology, University of Arizona, Tucson, Arizona 85721 USA. Of the spiders collected, 40 penultimates were brought into the laboratory, and reared to adult stage in individual 20 ml polystyrene vials from March–May 2000. Spiders were fed *ad lib.* daily on fruit flies (*Drosophila melanogaster*) and walnut flies (*Rhagoletis juglandus*). The animals were observed daily, and the date of molting was recorded. Adult virgins were used in staged mating experiments in the laboratory. Several wild adults were collected from March–May 2000 and additional wild adults were collected from March–April 2001. Collection sites were far downstream of field observation areas in order to avoid influencing the natural behavior of individuals at the observation sites.

Field observations of mating behavior were recorded during the late afternoon into the early evening (15:00–22:00 h) on 31 April and 2, 4, 6 May 2000 under ambient light conditions whenever possible. Occasionally, when natural light was insufficient for observations, a flashlight also was used. When used, the flashlight was not focused directly on the spiders, but directed instead at the substrate beneath the web such that the individual was silhouetted against a brighter background. Two females on webs with males nearby were observed each evening. Web surfaces were horizontal (or nearly so) and built directly above the stream, usually within five cm of the water surface. Field observations of mating behavior were comparable to the results obtained in the laboratory, suggesting that the staged matings were an appropriate surrogate.

Procedures for Staged Matings.—Laboratory-reared virgin males were at least three days post-molt before being used in staged matings to ensure they had inducted sperm into their palps. Prior to mating, virgin laboratory-reared females that were at least seven days post-molt were individually placed into glass aquaria (30.5 × 15.0 × 20.5 cm) containing about 4 cm of water and several twigs. Females were allowed five minutes to settle onto their twig before a randomly chosen male was placed nearby. All encounters were videotaped with a NEC color CCD camera model NX18A with attached macro lens (Micro Nikkor Nikon 105 mm) mounted on a wooden platform with a Bogen ball head for camera rotation. During recording, live signal was relayed to a monitor, resulting in ~20× magnification. Similar to other tetragnathine species studied, *G. emertoni* also use chelicerae as holdfasts to steady the pair as the male inserts his pedipalps during mating. After copulation, staged encounters were considered terminated when the pair disengaged chelicerae and retreated from one another for at least 15 min. Pairs have never been observed to resume copulation more than 15 min after cheliceral disengagement (Danielson-François pers. obs.). If mating did not occur within 15 min, the previous male was removed and a new male was introduced to the mating arena until mating occurred. Adult females were mated to two adult males within fifteen minutes of each other. Males were immediately frozen and stored at −80° C for sperm quantification. Procedures for mating wild-caught males to wild-caught females were similar to those described above for mating laboratory-reared virgins.

Videotapes were later transcribed and latency to cheliceral engagement (in sec), latency to courtship after cheliceral engagement (in sec), which palp was inserted (left or right), duration of palp insertion (in sec), number of insertions, and total time spent *in copula* (in sec) were recorded. Palp insertion was defined as the complete insertion of the tip of the embolus into the female's gonopore that was succeeded by at least one full hematodochal inflation and deflation.

Procedures for Sperm Quantification.—The methods for sperm quantification are based on a modification of the protocol of Bukowski & Christenson (1997). Male palps were removed at the femur under a dissecting microscope. Right and left palps were then labeled and separated. Each palp was placed into a labeled 1.5-mL polypropylene centrifuge tube (Brinkman) containing 300 μL of a sonication solution of 1 mL of 0.9% saline and 10 μL of 10% Triton-X100 detergent

drawn from a common stock. Treatment with detergent was necessary to prevent sperm aggregation and facilitate a homogeneous distribution within a sample. Each palp was crushed with forceps and centrifuged at 1000 g for 5 min. After centrifugation, each sample was ultrasonicated using a Branson sonicator with a 3.2 mm probe at a low level (approximately 2% of total power output) for about 20 s. Exactly 300 μl additional sonication solution was added to the preparations that were then vortexed for approximately 10 s. A subset of the homogenized sample was immediately withdrawn and placed on a hemacytometer. Sperm were counted under a light microscope at 400× magnification. All values reported are estimates of total numbers of palp sperm based on linear extrapolations of actual counts to the total sample volume.

Estimating Sperm Release.—To estimate the amount of sperm available prior to mating, a subset of males were sacrificed and their sperm was counted (see below). The resulting average sperm count was used as an estimate of the amount of sperm available prior to copulation. Sperm release is calculated by subtracting the amount of sperm remaining in the palps after copulation from the amount of sperm available prior to copulation. This is a conservative estimate of sperm release for males used in the mating studies. A more accurate estimation would be to restrict all males in the mating studies to one palp insertion, and compare used and unused palps for each male to directly determine sperm release. However, limiting males to only one palp insertion would create other artifacts when examining the overall length of mating, so this method was only used for this subset of males.

To measure sperm release, a subset of virgin males were allowed to copulate and transfer sperm from only one palp to a virgin female before being sacrificed for sperm counting (i.e., half-virgin males). After the male completed his first palp insertion (i.e., removed the first palp and began to position his other palp for insertion), the male was immediately removed and placed at −80° C for sperm counting later. These males are described as ''one-insertion'' males. This allowed a direct comparison between the amount of sperm in the unused and used palps, i.e. the amount of sperm available prior to copulation and the amount released during copulation, for each individual. The average sperm count of the unused virgin palps was used to estimate the amount of sperm available prior to copulation in the staged matings. The amount of sperm available prior to copulation for wild-caught adult males was unknown, as males may be sperm-depleted or have higher reserves from re-inducting sperm multiple times. To be conservative, calculations of sperm release from wild-caught adult males were based on the average sperm count of the unused palps of virgin laboratory-reared males. For comparison, several wild-caught males were allowed to mate using only one palp (in a similar fashion as above) to directly determine how much sperm was available before copulation and released during palp insertion for those particular males.

Statistical Analyses.—The Mann-Whitney U test was used to assess differences in the amount of palp sperm among and within virgin and mated males. The Kruskal-Wallis test was used for comparisons between males mated to virgin, non-virgin, and wild-caught females. Non-parametric Spearman rank correlation was used to test for associations between copulation duration and sperm release. All summary statistics of continuous variables are reported as $\bar{X} \pm$ SE.

RESULTS

Field Studies.—The following section describes the natural history of *G. emertoni*, including observations on mating behavior.

Habits: Overall, 112 adult, 40 penultimate-molt and 23 immature individuals were observed. During daylight hours, adult and penultimate individuals (respectively, $n = 69$, $n = 40$) were found near the stream under leaves, rocks or overhanging vegetation, whereas immatures ($n = 23$) were found in miniature webs suspended in between grass stems approximately three cm above the stream. When resting on vegetation (e.g., grass) diurnally, *G. emertoni* adopt a cryptic posture by extending leg pairs (1, 2) parallel to and wrapping leg pairs (3, 4) around a stem. Occasionally, adult females were found on the remnants of a web during the late afternoon on overcast days ($n = 7$), but were more often in their daytime retreats ($n = 19$). Retreats consisted of a few silk lines attached to the vegetation. Diurnally, adult and penultimate individuals were found in retreats (respective-

ly, $n = 43$, $n = 40$). After dusk, female adults were found on webs ($n = 27$) and male adults were either found moving between aggregations of females ($n = 5$) or resting beside a female's web ($n = 11$).

Adults were observed rebuilding their webs nightly. Typically, individuals emerged after dusk and began to spin webs three to five cm above the water surface. On multiple occasions, adult females built webs inside the webs of *Tetragnatha versicolor* (Walckenaer 1842), but no territorial aggressions between the two species were seen either during or after web construction.

Avoidance Behavior: When disturbed, *G. emertoni* dropped into the water beneath and traveled downstream, floating along with the current. To emerge from the water, they extended their legs, which retarded their downstream movement, and climbed up streamside vegetation. During collection, several *G. emertoni* traveled approximately one meter downstream on the current, but more escaped until an appropriate technique was developed: placing a mesh net underneath the individual as collection was attempted.

Mating Behavior: Three matings were observed in the field. Males approached females on webs ($n = 2$) or resting on vegetation ($n = 1$, this female left before sperm transfer and is excluded from analyses below, see Mate-Avoidance Behavior). Upon contact, adults immediately paired in a ventral-to-ventral mating position by interlocking their chelicerae. Then males began a vibratory courtship phase ($n = 2$, latency to courtship $\bar{X} = 11 \pm 6$ sec, courtship duration $\bar{X} = 123 \pm 37$ sec); this phase was considered courtship because it occurs before palp insertion and sperm transfer. During courtship the male rapidly flicked leg pairs (1, 2) over the female, until she exhibited an acceptance posture—curling her abdomen towards the male—a necessary step to bring her gonopore within reach of his palps.

In the field, a male inserted one palp, and then inserted the other palp, using each palp only once ($n = 2$, copulation duration $\bar{X} = 803 \pm 270$ sec). The insertion of pedipalps appears to be ipsilateral, but further confirmation of this would require freezing pairs *in copula* to determine the placement of the palp because the openings to the female spermathecae are located interior to the epigastric fold and, therefore, are not visible during mating. The two complete matings observed in the field were shorter in duration ($n = 2$, total duration $\bar{X} = 936 \pm 240$ sec) than those observed in the laboratory.

Males left females immediately after mating in the field, suggesting that there is no post-copulatory mate guarding in this species. Yet, in the late spring when most of the matings were observed, females were rarely collected from their daytime retreats without at least one male nearby. More often, a female was collected from her retreat with two or three males nearby ($n = 11$, $n = 4$, respectively). Occasionally, an adult pair was collected with up to five males in the surrounding vegetation (within 10 cm of the pair, $n = 4$). It is unknown if males compete for access to females within these groups during the day (see Discussion).

Mate-Avoidance Behavior: On one occasion in the field, an unusual mate-avoidance behavior was observed between a pair of adults with interlocked chelicerae on a stem of grass overhanging the stream. The female appeared to be resisting copulation: she held her abdomen away from the male and began moving backwards, pulling the male down the stem towards the water. Once she made contact with the stream, she released her hold on the grass entirely and floated on the water, still held by the male's chelicerae interlocked with her own. Seconds later, after touching the female with leg pairs (1, 2), the male disengaged his chelicerae and during this momentary release the female was quickly pulled away by the current. Approximately one meter downstream, the female extended her legs to grasp another grass stem and emerged from the water. The male remained on the original grass stem, skimming leg pairs (1, 2) across the water surface for about 30 s before returning to a resting position higher up on the grass stem. Another example of this mate-avoidance behavior also occurred in the laboratory.

Egg Sacs: Egg sacs were between pairs of moist leaves stitched tightly together with silk located in streamside litter, just above the water line. The base of the egg sac was spun onto a leaf surface, eggs were deposited, and a second tightly woven sheet was placed over the eggs before being attached loosely to the other leaf. The egg sac was distinctly flattened, its upper surface being slightly concave. Two

Table 1.—Summary of mating behavior for laboratory-reared virgin males mated to virgin and non-virgin females, and wild-caught males mated to wild-caught females in the laboratory, $\bar{X} \pm$ SE (n).

	Female type		
	Virgin	Non-Virgin	Wild-Caught
Latency to courtship (s)	40 ± 7.6 (8)	42 ± 7.9 (8)	<1 (3)
Courtship duration (s)	126 ± 34 (8)	151 ± 14 (8)	110 ± 5 (3)
Copulation duration (s)	1,000 ± 180 (8)	700 ± 110 (8)	1,734 ± 400 (3)
Total duration (s)	1,250 ± 180 (8)	1,050 ± 160 (8)	2,035 ± 626 (3)

sacs were collected, each of which had 20–30 eggs inside. The egg sacs were fairly large (3–4 mm across) relative to the adult body size of *G. emertoni* (4.5–5 mm). In the lab, females laid multiple sacs (maximum = 4) ranging from 16 to 26 eggs per sac. The hatchlings had a well-developed stout cephalothorax and, upon casual visual inspection, appeared to be larger than hatchlings of *Tetragnatha versicolor* (Walckenaer 1842) and *Leucauge venusta* (Walckenaer 1842).

Laboratory Studies.—The following section describes the experimentation on mating behavior and sperm release.

Mating Behavior: In the laboratory, just as in the field, males and females interlocked chelicerae immediately upon contact and males began courtship less than a minute later (Table 1). Vibratory courtship consisted of the male flicking his legs (pairs 1, 2) dorsally over the female until she adopted an acceptance posture. The male then used his legs to move the female's abdomen closer, sometimes shaking her until he successfully inserted his first palp.

Upon insertion, the palp hematodochae inflated and deflated repeatedly. In the staged matings, both laboratory-reared virgin and wild-caught males alternated palps, and in most cases, inserted each palp only once before disengaging chelicerae. There were no significant differences in the number of palps used by virgin males when mating with virgin and non-virgin females (Fisher's exact test, $P = 0.23$), but two males mating non-virgin females did insert a palp twice. In each case, the palp inserted a second time was the one with the shortest initial insertion duration. These matings were otherwise as described above with cheliceral engagement lasting the duration of the mating. Out of 16 matings, two laboratory-reared males (not the same males from the previous case) briefly disengaged their chelicerae after the first palp insertion and then immediately re-engaged chelicerae and re-courted the female before inserting the second palp. All wild-caught males inserted each palp only once. Sperm extrusion was not observed during or after any staged mating.

Mating propensity, latency to courtship, courtship duration, palp insertion duration, and overall mating duration were compared for all females. Laboratory-reared males were equally as likely to mate virgins as non-virgins ($n = 18$, $\chi^2 = 1.80$, $P = 0.18$); ten virgin females were mated immediately by the first male presented to them and eight mated with the second male presented to them. (The two singly-mated females and their mates were excluded from the analyses.) Latency to courtship (after cheliceral engagement), was not significantly different for males courting virgin and non-virgin females, but was lengthier than that of wild-caught males (Mann-Whitney, $U = 29$, $P = 0.75$; Kruskal-Wallis, $df = 2$, $H = 7.3$, $P = 0.03$, respectively; Table 1). In contrast, the courtship duration was significantly longer for laboratory-reared males courting non-virgin than virgin females, but neither was significantly different from courtship of wild-caught males (Mann-Whitney, $U = 13$, $P = 0.05$; Kruskal-Wallis, $df = 2$, $H = 5.3$, $P = 0.07$, respectively; Table 1). When all palp insertions were considered jointly, insertion duration was not different between first and second laboratory-reared males although there was a trend for insertions with non-virgins to be shorter in duration ($n = 16$, virgin $\bar{X} = 503 \pm 71$; $n = 16$, non-virgin $\bar{X} = 348 \pm 65$; Mann-Whitney, $U = 83$, $P = 0.14$). When only first insertions were considered, males mated to non-virgin females had shorter insertions than those mated to virgin females (Mann-Whitney, $U = 11$, $P = 0.05$); however, when second insertions were compared, there was no difference (Mann-Whit-

Table 2.—Total sperm released and sperm remaining for both palps of virgin males mated to virgin and non-virgin females in the laboratory, $\bar{X} \pm$ SE (n). *To calculate release, the sperm retained in the mated palp was subtracted from the estimate of sperm available prior to copulation.

	Female type	
	Virgin	Non-Virgin
Sperm released*	710,000 ± 87,000 (8)	740,000 ± 87,000 (8)
Sperm remaining	300,000 ± 110,000 (8)	200,000 ± 78,000 (8)

ney, $U = 29$, $P = 0.79$; Table 1). Wild-caught males had lengthier palp insertions than laboratory-reared males (Kruskal-Wallis, $df = 2$, $H = 6.1$, $P = 0.05$; Table 1). There was no significant difference in total copulation duration between males mated to virgin and non-virgin females and those mated to wild-caught females (Mann-Whitney, $U = 22$, $P = 0.29$; Kruskal-Wallis, $df = 2$, $H = 3.1$, $P = 0.22$, respectively; Table 1); however, there was a trend for copulation to be shortest with non-virgin females and longest with wild-caught females. Wild-caught males observed in the laboratory had a total copulation duration that was longer, but not significantly so, than those observed in the field (Mann-Whitney, $U = 1$, $P = 0.25$; Table 1).

Mate-Avoidance Behavior: In one case, the female mate-avoidance behavior already noted in the field was seen again in the laboratory. During one staged mating between wild-caught spiders, after pairing and engaging chelicerae, the female began resisting sperm transfer. She kept her abdomen away from the male's palps and walked backwards down the branch towards the aquarium water. Upon contacting the water, the female released her hold on the branch and floated on the water surface next to the branch. Yet, perhaps because there was no current pulling her away, the male was able to keep his chelicerae interlocked with the female and continue courting. Courtship lasted one hour and 31 s, possibly another measure of female resistance as typical courtship duration was approximately three minutes. The male managed to insert only his right palp for 11 min, but did not release any sperm (as determined afterwards by comparing the sperm remaining in the used palp to that in the unused palp), which may indicate that a more cryptic form of female resistance exists (this male was excluded from all mating behavior and sperm release analyses).

Sperm Release From Laboratory-Reared Males: All males released sperm ($n = 16$). Males mated to virgins and those mated to non-virgins (i.e., first and second males to mate) did not have any significant differences in the sperm remaining in their palps and, as a consequence, no difference in the sperm released to females (Mann-Whitney, sperm remaining $U = 20$, $P = 0.21$; sperm released $U = 25$, $P = 0.46$, respectively; Table 2). The sperm available prior to copulation was estimated from the average sperm count of palps from virgin laboratory-reared males. Although there were no differences in sperm release between males, there was variation in sperm release and palp use within a male. Most males released sperm from each palp and used both palps exactly once during mating ($n = 10/16$). Two males mating non-virgin females released sperm from both palps but inserted the first palp twice, after the insertion of the second palp ($n = 2/16$). Some males released sperm from only one palp ($n = 4/16$). These four males (three mated to virgin females, one mated to a non-virgin female) inserted the first palp only once during copulation. The sperm release by a male was not correlated with the overall duration of copulation (Spearman rank correlation, $rho = 0.250$, $Z = 0.97$, $P = 0.33$).

Sperm Release From ''One-insertion'' Males: To look at natural variation in sperm number as well as sperm release, a subset of virgin laboratory-reared males ($n = 3$) and wild-caught males ($n = 3$) were allowed to copulate with only one palp before being sacrificed for sperm counting. These males are described as ''one-insertion'' males. Unused palps from virgin laboratory-reared males contained significantly less sperm than unused palps from wild-caught males ($\bar{X} = 483{,}000$

Table 3.—Summary of palp insertion, sperm release, and sperm remaining for the first palp only for virgin males mated to virgin and non-virgin females, and wild-caught males mated to wild-caught females in the laboratory, $\bar{X} \pm$ SE (n). *To calculate release, the sperm retained in the mated palp was subtracted from the estimate of sperm available prior to copulation.

	Female type		
	Virgin	Non-Virgin	Wild-Caught
Palp insertion (s)	563 ± 49 (8)	294 ± 88 (8)	659 ± 86 (3)
Sperm released*	477,000 ± 12,000 (8)	456,000 ± 23,000 (8)	470,000 ± 9,000 (3)
Sperm remaining	19,000 ± 9,000 (8)	27,000 ± 23,000 (8)	14,000 ± 9,000 (3)

± 129,000, $\bar{X}$ = 1,026,000 ± 240,000; Mann-Whitney, $U = 9$, $P = 0.05$). This subset of males did release fully from the palp used in mating, as the amount of palp sperm remaining was similar for virgin laboratory-reared and wild-caught males ($\bar{X}$ = 14,500 ± 9,000, $\bar{X}$ = 14,000 ± 9,000; Mann-Whitney, $U = 4$, $P = 0.83$).

Sperm Release from First Palp Insertion: First palp insertion duration and sperm release were compared for males mated to virgin, non-virgin and wild-caught females (Table 3). The duration of the first palp insertion was significantly shorter for males mated to non-virgin females than for males mated to either virgin or wild-caught females (Kruskal-Wallis, $df = 2$, $H = 7.5$, $P = 0.03$; Table 3). Using the conservative method, the sperm retained and the sperm released from the first palp was not significantly different for males mated to virgin, non-virgin and wild-caught females (Kruskal-Wallis, sperm retained, $df = 2$, $H = 1.57$, $P = 0.46$; sperm released, $df = 2$, $H = 0.56$, $P = 0.76$; Table 3). However, the calculated sperm release for wild-caught males would be twice as much if a less conservative estimate was used: taking the average wild-caught, rather than virgin sperm count, to approximate the amount of sperm available before copulation (resulting in wild-caught male sperm release of $\bar{X}$ = 998,000 ± 9,000 per palp, Kruskal-Wallis, $df = 2$, $H = 7.3$, $P = 0.03$; compare to Table 3). Two males did not release any sperm and so were not included in these statistical analyses (one male was mated to a non-virgin female, the other male was mated to a wild-caught female).

Sperm Release Patterns: I calculated the relative sperm release by the first and second laboratory-reared male for each doubly-mated female in the study. This calculation is useful to provide a null hypothesis for future tests of paternity patterns in this species. S_2 is the proportion of sperm from the second male to mate ($S_2 = \text{Sperm}_{\text{2nd Male}}/\text{Sperm}_{\text{Total}}$). Using the convention of Christenson & Cohn (1988), sperm release patterns were categorized as first-male biased ($S_2 \leq 0.33$), mixed ($S_2 = 0.33$–0.66), and last-male biased ($S_2 \geq 0.66$). Sperm release patterns were variable: mixed ($n = 4$), last-male biased ($n = 3$) and first-male biased ($n = 1$). Each case of bias, either first- or last-male, resulted from a male releasing sperm from only one palp whereas the other male released from both palps, possibly gaining an advantage in sperm competition. Overall, S_2 values averaged 0.51 ± 0.05 ($n = 8$).

DISCUSSION

Glenognatha emertoni are well-hidden in the daytime, and emerge at dusk to spin orb webs over the stream. Male *G. emertoni* do not cohabitate with or guard females on their orb-webs. Instead, males leave females immediately after mating, similar to *Tetragnatha* species (LeSar & Unzicker 1978; West & Toft 1999; Danielson-François 2002; Danielson-François & Bukowski 2005), but unlike pholcid species that commonly have cohabitation (Eberhard & Briceño 1983; Eberhard et al. 1993; Blanchong et al. 1995; Kaster & Jakob 1997). During the day, *G. emertoni* females are found in retreats, but not alone—typically more than one male is nearby. Some females had as many as five surrounding males, but whether this was due to chance, mate-guarding of or a male preference for particular females remains to be tested (c.f. Danielson-François et al. 2002).

Males do not appear to discriminate between virgin, non-virgin, and wild-caught females, being equally likely to court and mate each type of female. Despite this lack of discrimination, there were some differences in

mating behavior. Laboratory-reared males courted non-virgins significantly longer than virgin females, and wild-caught adult males tended to get to the courtship phase more quickly than laboratory-reared virgin males. *Glenognatha emertoni* mating behavior was unique in having a vibratory phase of courtship that was absent in its congeners, but otherwise was similar to that of other tetragnathines in using chelicerae as holdfasts during mating (Barrows 1919; LeSar & Unzicker 1978; Huber 1998; West & Toft 1999; Danielson-François 2002; Danielson-François & Bukowski 2005). After cheliceral engagement, latency to courtship was brief (less than one min) and vibratory courtship (leg-tapping) lasted several min. Overall mating duration with laboratory-reared males was approximately 20 min, similar to the 15 min observed for *G. foxi* (Barrows 1919). Wild caught-males mated in the laboratory had a 40 min duration, whereas those observed in the field had a shorter duration, about 17 min.

Consistent with the lack of overt discrimination between females based on mating history, *G. emertoni* males released equivalent amounts of sperm to virgin, non-virgin, and wild-caught females. This pattern of sperm release was also noted in the related haplogyne *Tetragnatha versicolor* Walckenaer 1842 (Danielson-François & Bukowski 2005), but was distinctly different from the sperm release pattern of the related entelegyne orb-weaver *Nephila clavipes* (Linnaeus 1767), whose males preferentially release sperm to virgin females, but release none, or only a greatly reduced amount, during mating to non-virgin females (Christenson & Cohn 1988).

Despite a lack of significant differences in sperm release to virgin and non-virgin females, there was variation among *G. emertoni* males in sperm release. Calculated for each doubly-mated female in the study, the resulting proportion of sperm released by the second male to mate resulted in a range of first- to last-male bias (0.33–0.68), and yielded an average sperm release of 0.51 ± 0.05, similar to that observed for *T. versicolor* (Danielson-François & Bukowski 2005). First and second males typically released sperm from both palps, and all males inserted both palps at least once during mating, but sometimes a male would release sperm from only one of his palps and not the other, resulting in a bias in sperm release. Whether releasing sperm from only one palp during mating was due to cryptic actions by the male or female remains to be seen.

Huber & Eberhard (1997) suggest that haplogyne males might be able to influence paternity by repositioning rival males' sperm due to the short insemination duct found in most haplogyne taxa. In the haplogynes *Physocyclus globosus* and *Leucauge mariana* (Taczanowski 1881), males had extensive palpal movements that differed for virgin and non-virgin females (Huber & Eberhard 1997; Eberhard & Huber 1998). In the haplogyne *Pholcus phalangioides*, second males gain higher paternity even though they copulate for a shorter amount of time than first males (Yoward 1998). A detailed examination of *P. phalangioides* revealed that paternity was influenced by the number of pedipalp movements made by the second male, and Schäfer & Uhl (2002) suggest that these movements could remove rival's sperm. Consistent with the above studies, the flexible and short common insemination duct in *G. emertoni* would seem to make them particularly susceptible to this sort of manipulation (Danielson-François 2002).

The short and flexible nature of the haplogyne insemination duct may also increase the evolutionary lability of palp insertion patterns. Huber & Senglet (1997) argued that the absence of a ''lock-and-key'' fit between male and female genitalia in taxa within the entelegyne family Tetragnathidae made it possible to switch from the usual ipsi- to contra-lateral insertion, inserting the right palp into the left side of the epigynum. In contrast to the rest of the tetragnathine genera (i.e., *Tetragnatha* and *Pachygnatha*) and *Leucauge*, *G. emertoni* insertion patterns appear to be ipsilateral. Similarly, *G. heleios* also has an ipsilateral palp insertion pattern (Edwards & Senske 2001). The palp insertion patterns of *G. emertoni* were based on behavioral observations. Further confirmation of this result would require examination of pairs flash-frozen *in copula* (c.f. Huber & Senglet 1997) to physically determine which side (right or left) the palp enters.

Female *G. emertoni* appear to have a suite of resistance behaviors, one of which may have its origins in a startle behavior common to both sexes—dropping out of the web and

into the water below. The floating ability of spiders is influenced, in part, by the hydrophobicity of the cuticle and the density of hairs (Suter et al. 2004). Although the hydrophobicity of cuticle in *G. emertoni* is unknown, this small spider has a dense set of hairs covering the body that may aid its ability to float. Dropping into the current and floating downstream may prove to be a common mode of transport once more species are examined, but currently there is less information on floating as a means of travel even though locomotion across the water surface (e.g., walking, rowing, and galloping) is a well-documented phenomenon in spiders (Suter et al. 2003; Stratton et al. 2004).

Eberhard (2004) suggested that spiders are excellent organisms for the study of sexual selection, and in particular sperm competition. As a general method, examining the amount of sperm released per male during mating can be used to generate a null hypothesis for testing the mechanisms underlying paternity patterns in spiders. One such null hypothesis would be that the proportion of sperm from the second male to mate, S_2, would be equivalent to—under a simplistic ''lottery'' model (Parker 1998)—the expected paternity for the second male (P_2, *sensu* Boorman & Parker 1976). This is a null hypothesis that can and should be explicitly tested in future paternity studies. Given the pattern of sperm release found in *G. emertoni*, the null hypothesis based only on sperm release would be mixed paternity, all else being equal. The relationship of sperm release to paternity patterns has yet to be studied for *G. emertoni*, and if male or female manipulation of sperm release and storage occurs, it is unlikely that such a simplistic scenario will hold. Future work is needed to determine the actual paternity pattern by obtaining empirical P_2-values and comparing them to sperm release within the same study.

In summary, *Glenognatha emertoni* mating behavior was fairly simple, with a short latency to copulation and a phase of vibratory courtship before a single palp insertion (ipsilateral) from each palp (right and left) to transfer sperm. Males left immediately after copulation and did not cohabitate with females on their orb webs. Males released equivalent amounts of sperm to females, regardless of their mating history, similar to the only other haplogyne studied for sperm release behavior. Females actively resisted courting males by releasing from them and floating downstream, and may have a cryptic method of preventing sperm release during copulation. Future studies will examine the relationship among sperm release, mating behavior, and paternity patterns in *G. emertoni* and other haplogyne species.

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