Journal of Insect Behavior, Vol. 18, No. 1, January 2005 (© 2005)
DOI: 10.1007/s10905-005-9352-x

Female Mating History Influences Copulation Behavior but Not Sperm Release in the Orb-Weaving Spider *Tetragnatha versicolor* (Araneae, Tetragnathidae)

Anne M. Danielson-François[1,3] and Todd C. Bukowski[2]

Accepted September 12, 2002; revised September 30, 2004

We examined the influence of female mating history on copulation behavior and sperm release in the haplogyne spider Tetragnatha versicolor. *Despite significant behavioral differences during mating, males released equivalent amounts of sperm to virgin and non-virgin females. When mating with non-virgin females, males showed twice as many pedipalp insertions and half the copulation duration as compared to virgin females; however, males were as likely to mate with non-virgin as virgin females. Even with these overt behavioral differences, males released half of the sperm contained within their pedipalps during mating, regardless of female mating history. With respect to male mating order, first or second, we suggest the numbers of sperm released would lead to an expectation of unbiased paternity. In this species, sperm release is not directly proportional to total copulation duration.*

KEY WORDS: sperm competition; sexual selection; arthropod; arachnid.

[1]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona.
[2]Center for Insect Science, University of Arizona, Tucson, Arizona.
[3]To whom correspondence should be addressed at Rice University MS-170, P.O. Box 1892, Houston, Texas 77251-1892. e-mail: danielsn@rice.edu.

INTRODUCTION

For most species, we know little about the relationship between copulation duration and sperm release. Depending on the underlying mechanism of sperm competition, a male's fertilization success will generally increase as the number of his sperm stored within a female is increased, relative to those of other males (Parker, 1970; Parker *et al.*, 1990). When sperm release is proportional to copulation duration, males that copulate longer have higher fertilization success (Birkhead and Moller, 1998; Simmons, 2001). Yet, copulation may serve functions in addition to the release of sperm, such as courtship (Eberhard, 1996) and implementation of sperm competition avoidance mechanisms (Birkhead and Moller, 1998; Simmons, 2001). Studies that examine the relationship between natural variation in copulation behavior and numbers of sperm released *via* direct counts are relatively rare (but see Pitnick and Markow, 1994; Cook and Gage, 1995; Markow, 1996; Bukowski and Christenson, 1997; De Jong *et al.*, 1998; Wedell and Cook, 1999; LaMunyon, 2000; Snook and Markow, 2001) and yet quite useful in interpreting the mechanisms underlying paternity patterns (Cook *et al.*, 1997; Simmons and Siva-Jothy, 1998).

We examined copulation duration and male sperm release directly by quantifying the amount of sperm released to virgin and mated females in the orb-weaving spider *Tetragnatha versicolor* (Walckenaer Tetragnathidae). Spiders are unusual in that they exhibit indirect sperm transfer such that all sperm available for transfer during mating are readily quantifiable. Some species exhibit first-male advantage in paternity, a pattern rarely found in other arthropod taxa, while others exhibit last-male advantage (Lewis and Austad, 1990; Elgar, 1998). Austad (1984) noted that the paternity pattern (first- or last-male priority) is associated with a particular type of female reproductive morphology. Female spiders have sperm storage organs that can be divided into two morphological categories, entelegyne and haplogyne, although not all taxa conform to these types *sensu stricto* (Wiehle, 1967; Uhl and Vollrath, 1998). Entelegyne taxa typically show first-male advantage (reviewed in Elgar, 1998; Elgar *et al.*, 2000; Schneider *et al.*, 2000; but see Watson, 1990), whereas haplogyne taxa often show last-male advantage (Kaster and Jakob, 1997; Yoward, 1998; West and Toft, 1999; Schafer and Uhl, 2002; but see Eberhard *et al.*, 1993). Austad (1984) proposed that these differences were due to ejaculate stratification within the reproductive tract. Yet, evidence is mounting that, in entelegyne spiders, the relative numbers of sperm released by two males to mate with a female appear to be a primary factor determining the paternity patterns (Christenson, 1990; Suter, 1990; Masumoto, 1993; Andrade, 1996; Bukowski and Christenson, 1997; Elgar *et al.*, 2000; Schneider *et al.*, 2000; Bukowski *et al.*, 2001; but

see Watson, 1990). In some cases, paternity is positively correlated with copulation duration, suggesting that sperm release is directly proportional to copulation duration (Andrade, 1996; Elgar *et al.*, 2000; Schneider *et al.*, 2000). Yet in other cases, copulation duration is *not* related to the numbers of sperm released; however, female mating history does influence sperm release behavior (Austad, 1982; Christenson, 1990; Bukowski and Christenson, 1997; Bukowski *et al.*, 2001). Haplogyne spider taxa have been less studied and the relationships between copulation duration and paternity are less understood. Copulation duration is known for four haplogyne species, three with a last-male biased paternity and one with unbiased paternity, and in all cases the second male copulates for shorter duration than the first male (Kaster and Jakob, 1997; Eberhard *et al.*, 1993; Yoward, 1998; Schäfer and Uhl, 2002). The mechanism underlying the significant paternity advantage of the last male is not clear. Two species, one with paternity biased towards the second male and one with paternity unbiased with respect to male mating order, show sperm extrusion during or immediately after copulation (Uhl *et al.*, 1995; Huber and Eberhard, 1997). No one has examined sperm release in any haplogyne taxa. Consequently, it is difficult to generalize relationships between female mating history, copulation behavior, and sperm release in haplogyne spiders.

Here, we examine sperm release and copulation duration in the haplogyne orb-weaving spider *Tetragnatha versicolor* (Walckenaer Tetragnathidae). We presented virgin male *T. versicolor* to either virgin or mated females and documented copulation duration. In order to understand the relationship between copulation duration and sperm release, we assessed the numbers of sperm released by each male. We used the relative number of sperm released by the first and second male to predict what paternity pattern, P_2 (*sensu* Boorman and Parker, 1976), would be expected from sperm release alone.

METHODS

Tetragnatha versicolor were collected along streambeds in Garden and Ramsey Canyons in the Huachuca Mountains in Southeast Arizona. The collection sites are characterized as mid-elevation riparian areas with permanent streams running throughout. Adults have been observed mating in the field throughout the summer and fall seasons (Danielson-François, unpublished data). Juvenile males and females were collected between November 1998 and November 1999, and brought into the laboratory and held in 20 ml polystyrene vials. Spiders were fed *ad lib*. daily on houseflies (*Musca domestica*) and walnut flies (*Rhagoletis juglandis*). The animals were observed daily for molting and the date was noted.

Procedures for Staged Matings

Spiders are unusual in that they exhibit indirect sperm transfer, that is, sperm are transferred from the testes, which are located in the abdomen, to modified appendages, pedipalps, located on the prosoma (Montgomery, 1903). Consequently, all sperm available for transfer during mating are readily quantifiable. One can compare the quantity of sperm in the pedipalps of unmated males with those of mated males and determine the numbers of sperm released to the female. Males were at least 3 days post-molt before being used in staged encounters to ensure they had inducted sperm into their pedipalps (hereafter 'palps'). Virgin ($N = 22$) females were presented their first males between 5 and 31 days of molting to adulthood. Once mated, these females were given their second male 48 h after their first mating. All but three females were given a second male and these are included in the virgin category only. Of the three females that were included in the virgin category only, two laid eggs during their 48 h remating interval and were not used for a second mating, and the third was inadvertently mated to a male that had only one functional palp and was removed from the study (virgin females $N = 21$, non-virgin females $N = 19$). Because the overall variance in female age was greater than the 48 h that females were given between matings, we feel that our results can interpreted as an experience effect, rather than an age effect. Age was defined as the number of days since the final molt, including the first day.

Prior to mating, females were individually placed into glass aquaria (30.5 cm × 15.0 cm × 20.5 cm) containing about 2 L of water and several twigs. Females were allowed about 5 min to settle onto one of the twigs and then a male was randomly selected and placed onto the twig nearest to the female. All encounters were videotaped with a macro lens (Micro Nikkor Nikon, 105 mm) and displayed on a video monitor resulting in ~20× magnification. Staged encounters were considered terminated when, after a series of pedipalp insertions, copulation ended and the mating pair then disengaged their chelicerae. This indicates the end of sexual interaction as pairs have never been observed to re-couple and resume copulation after cheliceral disengagement (Danielson-François, personal observations). With the exception of one errant male which did not mate within 15 min, all encounters resulted in mating almost immediately on introduction. Immediately after cheliceral disengagement, males were removed from the aquarium, killed by hypothermia and tibia-patella leg I length was measured. Thus, males were unable to re-fill their palps with sperm after mating.

Videotapes were later transcribed and we recorded latency to mating (in seconds), which palp was inserted (left and right), duration of palp insertion (in seconds), number of insertions, and total time spent *in copula*.

Mating was defined as the total series of palp insertions from initial cheliceral engagement until cheliceral disengagement, after which the male departs the female. Palp insertion was defined as the complete insertion of the embolus into the female's gonopore that was followed by at least one full inflation and deflation of the hematodochae of the palp. The video recording method allowed us to see the embolus being inserted and each inflation thereafter. Insertions were distinguished from flubs, where the male would scrape his palp along the female's gonopore and release the embolus but not inflate the hematodochae. Videotapes were also carefully examined for the presence of sperm extrusion from the female's reproductive tract.

Procedures for Sperm Quantification

In order to characterize sperm release patterns in relation to female mating history, we first examined virgin males to determine the typical number of sperm a male has prior to copulation. We quantified the number of sperm in the palps of virgin males ($N = 18$) over the course of the study period. To determine the number of sperm released, we compared sperm counts obtained from virgin males with those in palps of mated males.

The methods for quantifying sperm in palps were based on the revised sperm counting methods of Bukowski and Christenson (1997). Males' palps were removed by cutting through the palpal femur under a dissecting microscope. Right and left palps were then labeled and kept separate. Each organ was placed in a 1.5 ml polypropylene centrifuge tube (Brinkman) with 400 μl of a solution of 1 ml of 0.9% saline and 10 μl of 10% Triton-X100 detergent drawn from a common stock. Sperm tended to aggregate, so treatment was required to facilitate a homogeneous distribution within a sample. Each organ was ground with forceps, and centrifuged at $1000 \times g$ for 10 min. Within 5 min, the samples were 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. The preparations were then vortexed for approximately 10 s and a sample was immediately drawn and placed on a hemacytometer. Sperm were then counted under a light microscope at 400×. All of the reported values are estimates of total numbers of sperm in the palps based on linear extrapolations of actual counts to the total sample volume.

Calculations for Predicted P_2

Given that the relative sperm release by each male to a given female was quantified, we estimated the P_2 value predicted for each female in this study. We estimated the relative numbers of sperm released to the female by the first and second male mated to a particular female and determined

the P_2 values for each female based on these sperm release patterns. The number of sperm each male released was estimated by subtracting the total number of sperm remaining in the palps after mating from the mean total number of sperm contained within the palps (left + right) of unmated males. From the estimates of numbers of sperm released, we then calculated the predicted P_2 values, that is, the predicted percentage of offspring sired by the second male to mate. Using the convention of Christenson and Cohn (1988), we categorized predicted paternity patterns as first-male biased ($P_2 \leq 0.33$), mixed paternity ($0.33 < P_2 < 0.66$), and second-male biased ($P_2 \geq 0.66$).

Statistics

Differences in continuous variables such as male sperm release were compared using repeated measures ANOVA, unless otherwise noted, or correlation. Because we used repeated measures, many analyses did not include data from the three virgin females that were not remated. Sample sizes were lower in some analyses because camera placement prevented observation of specific measures. Differences in discrete data were compared with a chi-square test or Fisher's Exact test. All summary statistics of continuous variables are reported throughout the 'Results' section as $\bar{x} \pm$ SE.

RESULTS

Patterns of Copulation Behavior

Overt courtship in *T. versicolor* appeared very subtle or non-existent, similar to the congener *Tetragnatha elongata* (Danielson-François *et al.*, 2002), but contrary to that seen in most orb-weaving spiders (LeSar and Unzicker, 1978; Robinson and Robinson, 1980; Robinson, 1982). A brief description follows. As a male contacted the web structure, the female oriented towards the point of contact from her location at the hub of the web. If the female did not chase away the male at this stage, the male continued advancing onto the female's web and they paired immediately. Pairing involved rapid locomotion towards each other with chelicerae held apart, and then vigorous grappling as they interlocked cheliceral fangs. Once the chelicerae were engaged, the pair then assumed a ventral-to-ventral mating position. The male then used his third pair of legs to contact the female's abdomen and often exerted force to move her abdomen nearer, sometimes even shaking her until he successfully inserted his palp. The insertion of pedipalps appears to be contralateral, but further confirmation would be

needed because the spermathecal ducts are interior to the epigastric furrow. During mating, the male inserted his left and right palps sequentially into the female's reproductive tracts. During palp insertion, the hematodochae inflated and deflated repeatedly. In most cases, each palp was inserted more than once. Sperm extrusion from the female was not observed during or after any staged mating.

Consistent with the observation that courtship appeared subtle or nonexistent in this species, most pairs of spiders readily mated when placed together. The likelihood that a particular pair of spiders would mate was not influenced by female mating history. All females, virgin ($N = 22$) and nonvirgin ($N = 19$), mated with the male placed in their aquaria. Most of the matings were initiated within 30 s on placement of the male into the aquaria. Two males required more time prior to mating, but this was not related to female mating history (one male spent 3.5 min with one non-virgin female before mating and a different male spent 5.0 min prior to mating a different virgin female). Although spiders readily mated, there were significant differences in male behavior towards virgin and non-virgin females in several measured variables. The duration of individual palp insertions, the number of palp insertions, and overall copulation duration were all influenced by female mating history and each will be considered separately later.

First, we considered variation in the total length of time spent *in copula*. The length of time spent *in copula* was significantly influenced by female mating history: males spent more time with virgin females. Overall, males spent nearly twice as long *in copula* with virgin than non-virgin females (repeated measures ANOVA $F_{1,18} = 19.07$, $P = 0.0004$; $N = 663.8 \pm 31.2$, $N = 423.7 \pm 44.2$, respectively). However, total copulation duration was not significantly related to other measured variables, such as male and female body size or age, within the treatment groups of virgin and non-virgin females (all $r < 0.18$, P-values ranged from 0.15 to 0.98, Table I). The overall time spent *in copula* is influenced by the length of each palp insertion, so next we examined the variation in insertion duration.

Second, we considered variation over the course of the mating in the length of each palp insertion. The duration of palp insertion was significantly influenced by female mating history: virgins had a fewer number of palp insertions of longer duration than non-virgins, which had more overall insertions of shorter duration ($F_{1,18} = 56.52$, $P < 0.0001$; $N = 243.3 \pm 21.2$, $N = 70.4 \pm 7.1$, respectively; Fig. 1). The overall distribution in duration of palp insertion with virgins was bimodal with one cluster less than 150 s (described as short) and the other greater than 150 s (described as long; Fig. 2). While individual palp insertions with virgin females tended to be bimodally distributed ($N_{\text{short}} = 31$, $N_{\text{long}} = 40$), insertions with non-virgin females were usually short in duration ($N_{\text{short}} = 98$, $N_{\text{long}} = 5$; Fisher's exact

Table I. Correlation coefficients (N) for relationships between size and age and total number of insertions, total duration of copulation and total sperm remaining in palps after mating with virgin or non-virgin females

Female history	Sex	Regressor	# Insertions	Duration of copulation	Total sperm remaining
Virgin	Male	Age	0.0000 (9)	0.035 (10)	0.172 (10)
		Size	0.109 (20)	0.038 (22)	0.011 (22)
	Female	Age	0.001 (13)	0.0001 (15)	0.006 (15)
		Size	0.0001 (17)	0.0001 (18)	0.08 (18)
Non-virgin	Male	Age	0.137 (12)	0.177 (12)	0.036 (13)
		Size	0.043 (17)	0.003 (18)	0.0001 (19)
	Female	Age	0.019 (12)	0.171 (13)	0.002 (14)
		Size	0.0003 (13)	0.065 (15)	0.0001 (16)

Note. None of the relationships were significant (all P-values > 0.12).

test, $P \leq 0.0001$; Fig. 2). Considering only *the first* insertion of the first palp used, those with virgin females were as likely to be short ($N = 11$) as long ($N = 11$) while those with non-virgin females were all categorized as short ($N = 16$; virgin *vs.* non-virgin, Fisher's exact test, $P = 0.0007$). (For three non-virgin females in the study, the initial insertion was not captured on videotape due to movement during mating obscuring the mating pair until the camera was re-adjusted). When males mated virgin females, 11 out of 22 showed an initial long duration palp insertion followed by a long duration insertion of the opposite palp before the mating ended, although occasionally additional long palp insertions occurred. The remaining 11 males showed short duration first insertions followed by a long duration insertion of the opposite palp and a long insertion of the initial palp before the mating ended. When males mated non-virgin females, all first palp insertions were short in duration and followed by many repeated short insertions with both palps. First insertions with virgins were, on average, three times longer than those with non-virgins ($F_{1,15} = 12.38$, $P = 0.0031$; $N = 16$; 173 ± 30 s and 57 ± 7 s, respectively). This trend continued with the second insertions, which were also longer in duration for virgins than non-virgins ($F_{1,15} = 21.16$, $P = 0.0001$; $N = 16$; 261 ± 36 s and 69 ± 14 s, respectively). While length of each palp insertion influences overall copulation duration, the number of times each palp is used by the male also affects the time spent *in copula*.

Third, we considered variation in the number and pattern of palp insertions during mating. The number of palp insertions was different between the treatment groups: all non-virgins had more insertions overall ($F_{1,15} = 8.02$, $P = 0.013$; Fig. 1b). The number of times a given palp was used during mating was highly dependent on the duration of the first insertion for that given palp. All males mated to non-virgin females had

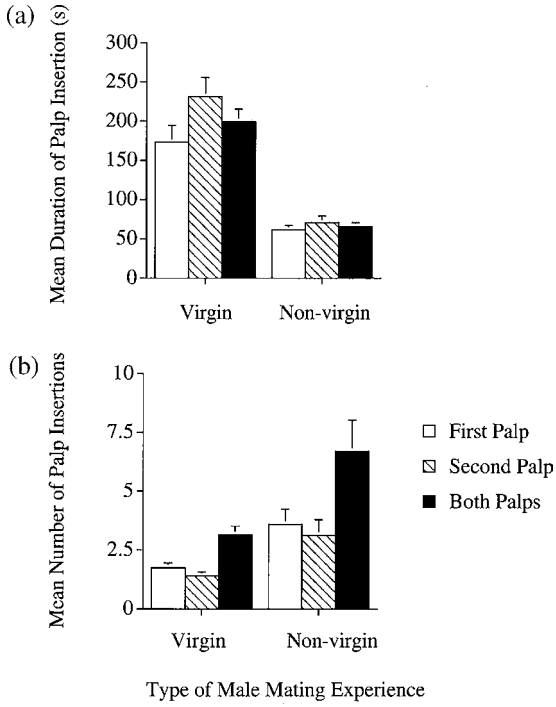

Fig. 1. Mean (+S.E.) duration (a) and number of palp insertions (b) for the first and second palps used in mating as well as for both palps combined for males copulating with virgin ($N = 22$) or non-virgin ($N = 19$) *T. versicolor* females. Males showed a lower number of palp insertions of longer duration with virgin females and a greater number of palp insertions of shorter duration with non-virgin females.

short initial insertions ($N = 16$). Whereas, of the males mated to virgin females ($N = 22$), only half had short first insertions ($N = 11$). When the first insertion was short (<150 s) the palp was always used again ($N_{\text{virgin}} = 11$, $N_{\text{non-virgin}} = 16$) and when the first insertion was long (>150 s), the palp was rarely used again ($N_{\text{virgin}} = 1$ out of 11; Fisher's exact test, $P \leq 0.0001$). Next, we considered the pattern of palp insertions. Palp insertion almost always proceeded in a strictly alternating pattern. Males often completed mating with a given female using the same palp that they used initially, thus first palps were used once more than second palps (virgins $F_{1,20} = 10.0$, $P \leq 0.005$; non-virgins $F_{1,15} = 11.67$, $P \leq 0.004$). This trend of inserting the first palp more often than the second was not

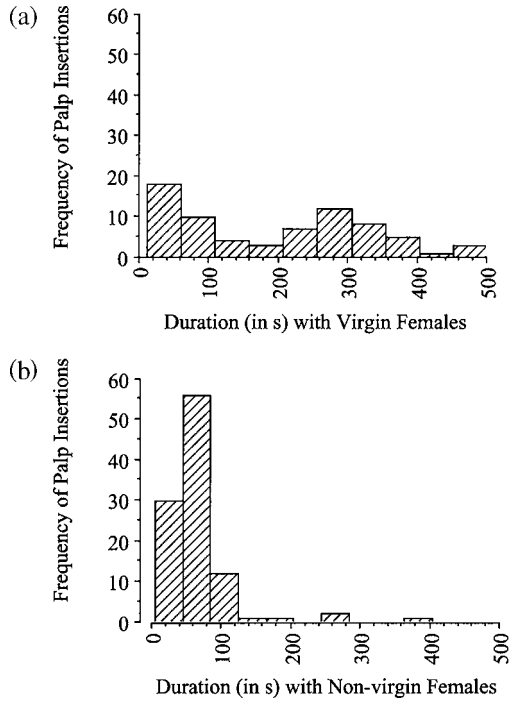

Fig. 2. Frequency distribution of the duration of all palp insertions with virgin (a, $N = 71$) and non-virgin (b, $N = 103$) female *T. versicolor*. Overall, insertion duration was bimodal. Most palp insertions with virgin females were relatively long (>150 s) while with non-virgin females most insertions were relatively short (<150 s) in duration.

significantly different between virgin and non-virgin females (Fisher's exact test, $P = 0.99$; Fig. 2b). Two males were clearly outliers in the number and pattern of insertions, and neither were included in the analyses: one male did not follow the typical alternating pattern and repeatedly inserted its right palp while another male inserted his palps 26 times, rather than two to eight as was typical. The overall number of insertions was not significantly related to other measured variables, such as male and female body size or age, within the treatment groups of virgin and non-virgin females (all $r < 0.14$, all P-values ranged from 0.12 to 0.97, Table I). Although right and left palps used by the male may have been inserted a different number of times, individual males appear to be unbiased and during the course of mating used each palp for equivalent lengths of time (when the

sum total duration of all palp insertions while *in copula* is considered). For all males, there was no difference in the sum total of insertion duration between the first and second palp used, regardless of treatment group (first and second palp total copulation duration compared for males mated to virgin $F_{1,20} = 0.80$, $P = 0.38$; non-virgin $F_{1,15} = 0.06$, $P = 0.82$).

Patterns of Sperm Release

In contrast to the differences in copulation duration and male behavior noted earlier, sperm release by males was consistent across female mating history. In order to evaluate sperm release, comparisons were made between sacrificed virgin males and males used in the mating study. During mating, males did release sperm. A comparison of the number of sperm contained within the palps of virgin (unmated) males and males that mated with females revealed a significant difference among the groups (factorial ANOVA $F_{2,55} = 14.91$, $P \leq 0.0001$; Fig. 3). Sperm numbers were consistent within males: there were no significant differences in sperm number between left and right palps for any male—mated or unmated (factorial ANOVA $F_{1,56} = 0.01$, $P = 0.92$). Males mating with females had slightly more sperm in the first than second palp used, while virgin males had slightly more sperm in the right than the left palp. Sperm numbers were consistent across treatments: males mated to virgin females and males mated to non-virgin females released equal amounts of sperm. The number of sperm remaining in the palp was not significantly influenced by male mating experience (virgin female, non-virgin female) or order of palp usage (first and second) ($F_{1,38} = 0.009$, $P = 0.93$; Fig. 3). Newman–Keuls *post-hoc* tests revealed that the palp sperm content of males mated to virgin or non-virgin females did not differ significantly ($P > 0.05$); even though both groups had significantly fewer sperm than the sacrificed virgin males (both P-values <0.01). An analysis of the total sperm remaining in the palps (left + right palps) also showed a significant overall difference among sacrificed virgin males and mated males (factorial ANOVA $F_{2,55} = 14.91$, $P \leq 0.0001$; Fig. 3). Newman–Keuls *post-hoc* tests also revealed no significant difference in total sperm remaining between males mated to virgin and non-virgin females ($P > 0.05$), but once again there were significant differences between unmated virgin males and both males with virgin females and males with non-virgin females (both P-values <0.01). A repeated measures ANOVA conducted on the total sperm (left + right palps) of the first and second males to mate with a female also revealed no significant difference in total sperm remaining ($F_{1,18} = 0.014$, $P = 0.91$). The first males to mate with a female did not release significantly more

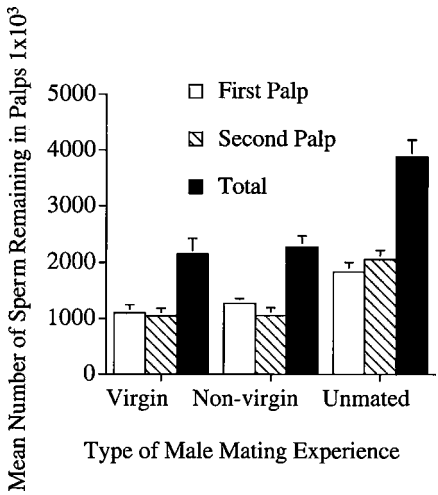

Fig. 3. The mean (+S.E.) number of sperm (1×10^3) remaining in first and second palps used in copulation and both palps combined (total) after male *T. versicolor* mated with virgin or non-virgin females as well as the sperm contents in the palps of virgin males. For virgin males, left palps were cast as first palp used while right palps were cast as second palp used. Males released about half the sperm contained in their palps regardless of whether mating with virgin or non-virgin females.

or less sperm than second males to mate with a female ($F_{1,18} = 0.006$, $P = 0.94$).

Despite differences in the total duration of copulation and the number of palp insertions, mated males released similar numbers of sperm to virgin and non-virgin females. Mated males released about half of the sperm contained within their palps to a female, regardless of her mating history (Fig. 3). The estimated total sperm contained within individual palps of virgin males ranged from 385×10^3 to 1673×10^3 sperm and for palps combined ranged from 923×10^3 to 2901×10^3 sperm. For virgin males, body size was positively, but not significantly, correlated with total sperm number (left + right palps) contained within the palps ($r = +0.44$, $F_{1,16} = 3.92$, $P = 0.0653$). Larger males tended to contain more sperm within their palps than smaller males. Total sperm number for virgin males was not significantly related to male age ($r = +0.17$, $F_{1,10} = 0.11$, $P = 0.76$). Furthermore, the number of sperm in the left palp was significantly correlated with the number of sperm in the right palp ($r = +0.54$, $F_{1,16} = 6.55$, $P = 0.0210$).

The total duration of copulation was not significantly related to the number of sperm remaining in the palps after copulation with virgin or non-virgin females (both $r < 0.23$, both P-values >0.31; see also Table I). Similarly, the number of palp insertions was not significantly related to the number of sperm remaining in the palps after copulating with virgin or non-virgin females (both $r = 0.10$, both P-values >0.66; see also Table I). The number of sperm remaining in the palps after copulation with virgin or non-virgin females was not significantly related to other measured variables, such as male or female body size or age (all $r < 0.18$ and P-values ranged from 0.23 to 0.98, Table I).

Results for Predicted P_2

The most common predicted P_2 was mixed paternity ($N = 14$), yet there were a few cases where first ($N = 2$) or second ($N = 3$) male biased paternity was predicted. The few cases where either first or last-male advantage were predicted are situations where males of different sizes where mated to the same female. As noted earlier, a trend exists that larger males have more sperm in their palps and, therefore, would release more sperm during mating than smaller males. In these cases, the larger male always had the advantage regardless of the mating order. Overall, predicted P_2 values averaged 0.50 ± 0.05 ($N = 19$).

DISCUSSION

Male *T. versicolor* released equal numbers of sperm to virgin and non-virgin females despite dramatic differences in copulation behavior. Although males were as likely to mate non-virgin as virgin females, males showed twice as many palp insertions and half the copulation duration with non-virgins as compared to virgins. Clearly, total copulation duration is not proportional to sperm release in *T. versicolor*. Males apparently gain information about female mating history as indicated by the differences in copulation behavior. These findings suggest that copulation behaviors may serve functions beyond sperm release in *T. versicolor*.

This is the first spider studied where males release equal numbers of sperm to females regardless of their mating history. Whether or not *T. versicolor* exhibit a biased (last-male) or unbiased paternity pattern, the special case of equivalent sperm release by two males has been modeled theoretically. A model by Parker (1998) predicts equivalent sperm release by both males when there is a loaded raffle (i.e., a bias towards one male),

males have perfect information about their role and males are randomly either in the favored or disfavored role. Another model by Parker (1998) predicts equivalent sperm release by males in a fair raffle when females mate with more than two males, regardless if males have perfect information or no information about the average ejaculate expenditure in a population (Parker, 1998).

Not only do the two male *T. versicolor* mated to a given female release approximately equal numbers of sperm, but they also release approximately half of the sperm contained within their palps. It is unclear why males do not release their full complement of sperm. Males clearly have the capacity to release more sperm during mating because approximately half of the palp sperm remained in the palps. Males of at least one related tetragnathid species release their entire complement of sperm during copulation (*Nephila clavipes*) but are limited to this one ejaculate (Christenson, 1989). *T. versicolor* are apparently not as limited in total sperm numbers, as they are able to re-induct sperm after copulation, resulting in at least one new full complement of palp sperm for future mating opportunities (Danielson-François, submitted). How males apportion their sperm among females depends on the average number of mates that both males and females will obtain over their lifetime, the number of sperm a male can produce and the level of sperm competition (Parker, 1998). Once again, Parker's (1998) models reveal that as the sperm competition risk increases above two ejaculates in direct competition, there should be a decrease in ejaculate expenditure and, therefore, would be allocated equally across females, irrespective of female mating history or local competition. It may be that releasing half of the sperm within their palps is the optimal male strategy, if there is a high level of sperm competition in the population.

Under some conditions, male *T. versicolor* might release the full complement of sperm in their palps. Males in several taxa release greater numbers of sperm in the presence of other males (Bellis *et al.*, 1990; Gage, 1991; Gage and Baker, 1991; Simmons *et al.*, 1993). Multiple males of *T. versicolor* have been observed competing for access to a single female (Danielson-François, personal observation). Perhaps male *T. versicolor* act similarly and release their entire complement of sperm when mating in the presence of another male. Our method of presenting males sequentially, rather than simultaneously, may have precluded such a finding. Full sperm release might be expected when two male spiders compete for a female and mate in rapid sequence and has been predicted by game theoretical models (Parker, 1998). In their natural setting, *T. versicolor* females tend to aggregate (Danielson-François, personal observation) so it is possible that males would encounter multiple females as well as potential rivals in the same location. Full sperm release might also be expected when females are gravid

and nearing oviposition, as male preference for gravid females has been shown in the congener *T. elongata* (Danielson-François *et al.*, 2002).

That males release equal amounts of sperm suggests that sperm competition alone could generate mixed paternity. The predicted P_2 values, estimated by relative sperm release to each female in this study, suggest that (all other things being equal) most double matings would result in mixed paternity but a few would result in first- or second-male biased paternity patterns. When males of equal size (and equal numbers of palp sperm) are mated to a female, mixed paternity is predicted. The few cases of predicted bias (first or last-male advantage) can be explained by males of different sizes being mated to the same female. Larger males tend to have more sperm in their palps and, therefore, larger ejaculates than smaller males. In the cases where first- or second-male advantage was found, larger males always had the advantage regardless of male mating order. Overall, predicted P_2 values averaged 0.50 ± 0.05 ($N = 19$), a value that compares favorably with paternity values obtained in this species (Danielson-François, submitted).

The differences in copulation behavior seen with virgin and mated females might reflect differences in requirements for storing sperm. Male *T. versicolor* appear to gain information about female mating history, they deliver a greater number of insertions of much shorter duration when mating with non-virgin females. The trend toward males with virgin females showing a short initial insertion followed by a long insertion with the opposite palp, suggests that half of all males do not receive or differentially act on information about the female's virgin status until they have gained copulation experience with both of her reproductive tracts. Female secretory processes related to reproduction may be dependent on copulatory stimulation (e.g., Eberhard, 1996) and virgin females might require different patterns of stimulation to initiate this process. Copulation behavior might influence the sperm storage process as it does sperm uptake and storage in another spider (Bukowski and Christenson, 1997). The greater frequency of insertions when mating with non-virgin females may also reflect re-positioning of the first male's sperm (Schäfer and Uhl, 2002). The greater number of insertions and the 'cul-de-sac' spermathecal morphology of *T. versicolor* would suggest *a priori* that a second-male advantage is most likely in this species (Austad, 1984), although our sperm release data suggest mixed paternity. Future studies should examine how copulation behavior relates to sperm use in this species.

In summary, male *T. versicolor* release equivalent amounts of sperm to females, regardless of female mating history; however, overall copulation duration is not directly proportional to sperm release in *T. versicolor*. Copulation duration is probably equivalent to sperm release up to the point in time where sperm release is complete, and after this point copulation is

serving functions beyond sperm release. Future studies should determine how predicted P_2 based on relative sperm release accurately reflect observed P_2 values from paternity studies. The ability to measure sperm release provides an excellent situation for testing the underlying mechanisms of sperm competition in this species.

ACKNOWLEDGMENTS

We thank Sheridan Stone and the Wildlife Management Office of Fort Huachuca, for support and access to Garden Canyon. We thank Dave Gori, Mark Pretti and The Nature Conservancy for access to spiders in Ramsey Canyon. We are grateful to Wayne Maddison for the use of his video equipment and Michael Wells for the use of his ultrasonicator. We also thank Terry E. Christenson, Therese Markow, Leticia Avilés, Kim Powers, Tamar Erez, Asher Cutter, and two anonymous reviewers for comments on an earlier draft of this manuscript. This material is based upon work supported by the National Science Foundation grant No. 0073266 to A.D.F.

REFERENCES

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. In Smith, R. L. (ed.), *Sperm Competition and the Evolution of Animal Mating Systems*, Academic Press, New York, pp. 223–249.

Bellis, M. A., Barker, R. R., and Gage, M. J. G. (1990). Variation in rat ejaculates is consistent with the kamikaze sperm hypothesis. *J. Mammol*. **71:** 479–480.

Birkhead, T. R., and Moller, A. P. (1998). *Sperm Competition and Sexual Selection*, Academic Press, New York.

Boorman, E., and Parker, G. A. (1976). Sperm (ejaculate) competition in *Drosophila melanogaster*, and the reproductive value of females to males in relation to female age and mating status. *Ecol. Entomol*. **1:** 145–155.

Bukowski, T. C., and Christenson, T. E. (1997). Determinants of sperm release and storage in a spiny orbweaving spider, *Micrathena gracilis*. *Anim. Behav*. **53:** 381–395.

Bukowski, T. C., Linn, C. D., and Christenson, T. E. (2001). Copulation and sperm release in *Gasteracantha cancriformis* (Araneae: Araneidae): Differential male behavior based on female mating history. *Anim. Behav*. **62:** 887–895.

Christenson, T. E. (1989). Sperm depletion in the golden orb-weaving spider, *Nephila clavipes* (Araneae, Araneidae). *J. Arachnol*. **17:** 115–118.

Christenson, T. E. (1990). Natural selection and reproduction: A study of the golden orb-weaving spider, *Nephila clavipes*. In Dewsbury, D. A. (ed.), *Contemporary Issues in Comparative Psychology*, Sinauer, Sunderland MA, pp. 149–174.

Christenson, T. E., and Conn, J. (1988). Male advantage for egg fertilization in the golden orb-weaving spider (*Nephila clavipes*). *J. Comp. Psych*. **102:** 312–318.

Cook, P. A., and Gage, M. J. G. (1995). Effects of risks of sperm competition on the numbers of eupyrene and apyrene sperm ejaculated by the moth *Plodia interpunctella* (Lepidoptera, Pyralidae). *Behav. Ecol. Sociobiol*. **36:** 261–268.
Cook, P. A., Harvey, I. F., and Parker, G. A. (1997). Predicting variation in sperm precedence. *Phil. Trans. R. Soc. Land. B* **352:** 771–780.
Danielson-François, A. (submitted). Natural variation in sperm release behavior among males generates inter- and intra-specific paternity patterns.
Danielson-François, A., Fetterer, C., and Smallwood, P. (2002). Body condition and mate choice in *Tetragnatha elongata* (Araneae: Tetragnathidae). *J. Arachnol*. **30:** 20–30.
De Jong, P. W., Brakefield, P. M., and Geerinck, B. P. (1998). The effect of female mating history on sperm precedence in the two-spot ladybird, *Adalia bipunctata* (Coleoptera, Coccinellidae). *Behav. Ecol*. **9:** 559–565.
Eberhard, W., Guzman-Gomez, S., and Catley, K. (1993). Correlation between spermathecal morphology in mating systems in spiders. *Biol. J. Linn. Soc*. **50:** 197–209.
Eberhard, W. (1996). *Female Control: Sexual Selection by Cryptic Female Choice*, Princeton University Press, Princeton.
Elgar, M. A. (1998). Sperm competition and sexual selection in spiders and other arachnids. In Birkhead, T. R., and Moller, A. P. (eds.), *Sperm Competition and Sexual Selection*, Academic Press, New York, pp. 307–337.
Elgar, M. A., Schneider, J. M., and Herberstein, M. E. (2000). Female control of paternity in the sexually cannibalistic spider *Argiope keyserlingi*. *Proc. R. Soc. Lond. B* **267:** 2439–2443.
Gage, M. J. G. (1991). Risk of sperm competition directly affects ejaculate size in the Mediterranean fruit fly. *Anim. Behav*. **42:** 1036–1037.
Gage, M. J. G., and Baker, R. R. (1991). Ejaculate size varies with socio-sexual situation in an insect. *Ecol. Entomol*. **16:** 331–337.
Huber, B. A., and Eberhard, W. G. (1997). Courtship, copulation, and genital mechanics in *Physocyclus globosus* (Araneae, Pholcidae). *Can. J. Zool*. **74:** 905–918.
Kaster, J. L., and Jakob, E. M. (1997). Last-male sperm priority in a haplogyne spider: Correlations between female morphology and patterns of sperm usage. *Ann. Am. Entomol. Soc*. **90:** 254–259.
LaMunyon, C. W. (2000). Sperm storage by females of the polyandrous noctuid moth *Heliothis virescens. Anim. Behav*. **59:** 395–402.
Lewis, S., and Austad, S. (1990). Sources of intraspecific variation in sperm precedence in red flour beetles. *Am. Nat*. **135:** 351–359.
LeSar, C., and Unzicker, J. (1978). Life history, habits and prey preferences of *Tetragnatha laboriosa* (Araneae: Tetragnathidae). *Environ. Entomol*. **7:** 879–884.
Markow, T. A. (1996). Evolution *ofDrosophila* mating systems. *Evol Biol* **29:** 73–106.
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.
Parker, G. A. (1970). Sperm competition and its evolutionary consequences in the insects. *Biol. Rev*. **45:** 525–567.
Parker, G. A. (1998). Sperm competition and the evolution of ejaculates: Towards a theory base. In Birkhead, T. R. and Moller, A. P. (eds.), *Sperm Competition and Sexual Selection*, Academic Press, New York, pp. 307–337.
Parker, G. A., Simmons, L. W., and Kirk, H. (1990). Analysing sperm competition data: Simple models for predicting mechanisms. *Behav. Ecol. Sociobiol*. **27:** 55–65.
Pitnick, S., and Markow, T. A. (1994). Male gametic strategies: Sperm size, testes size and the allocation of ejaculate among successive males by the sperm-limited fly *Drosophila pachea* and its relatives. *Am. Nat*. **143:** 785–819.
Robinson, M. (1982). Courtship and mating behaviour in spiders. *Ann. Rev. Entomol*. **27:** 1–10.

Robinson, M., and Robinson, B. (1980). Comparative studies of the courtship and mating behavior of tropical Araneid spiders. *Pacific Insects Monog*. **36:** 1–218.
Schäfer, M., and Uhl, G. (2002). Determinants of paternity success in the spider *Pholcus phalangioides* (Pholcidae: Araneae): The role of male and female mating behaviour. *Behav. Ecol. Sociobiol*. **51:** 368–377.
Schneider, J. M., Herberstein, M. E., De Crespigny, F. C., Ramamurthy, S., and Elgar, M. (2000). Sperm competition and small size advantage for males of the golden orb-web spider *Nephila edulis*. *J. Evol Biol*. **13:** 939–946.
Simmons, L. W., Craig, M., Llorens, T., Schinzig, M., and Hosken, D. (1993). Bushcricket spermatophores vary in accord with sperm competition and parental investment theory. *Proc. R. Soc. Land. B* **251:** 183–186.
Simmons, L. W., and Siva-Jothy, M. T. (1998). Sperm competition in insects: Mechanisms and the potential for selection. In Birkhead, T. R. and Moller, A. P. (eds.), *Sperm Competition and Sexual Selection*, Academic Press, New York, pp. 307–337.
Simmons, L. W. (2001). *Sperm competition and Its Evolutionary Consequences in the Insects*, Princeton University Press, Princeton.
Snook, R. R., and Markow, T. A. (2001). Mating system evolution in sperm- heteromorphic *Drosophila*. *J. Insect Physiol*. **47:** 957–964.
Suter, R. B. (1990). Courtship and assessment of virginity by male bowl and doily spiders. *Anim. Behav*. **39:** 307–313.
Uhl, G., Huber, B. A., and Rose, W. (1995). Male pedipalp morphology and copulatory mechanism in *Pholcus phalangioides* (Fuesslin 1775) (Araneae, Pholcidae). *Bull. Br. Arachnol. Soc*. **10:** 1–9.
Uhl, G., and Vollrath, F. (1998). Genital morphology of *Nephila edulis*: Implications for sperm competition in spiders. *Can. J. Zool.* **76:** 39–47.
Watson, P. J. (1990). Female-enhanced male competition determines the 1st mate and principal sire in the spider *Linyphia litigiosa* (Linyphiidae). *Behav. Ecol. Sociobiol*. **26:** 77–90.
Wedell, N., and Cook, P. A. (1999). Butterflies tailor their ejaculate in response to sperm competition risk and intensity. *Proc. R. Soc. Land. Series B* **266:** 1033–1039.
West, H. P., and Toft, S. (1999). Last male sperm priority and the mating system of the haplogyne spider *Tetragnatha extensa* (Araneae: Tetragnathidae). *J. Insect Behav*. **12:** 433–450.
Wiehle, H. (1967). Meta,-eine semientelegyne Gattung der Araneae (Arach.). *Senckenberg. Biol*. **48:** 183–196.
Yoward, P. J. (1998). Sperm competition in *Pholcus phalangioides* (Fuesslin, 1775) (Araneae, Pholcidae)—Shorter second copulations gain higher paternity reward than first copulations. *Proc. I7th Eur. Coll. Arachnol. Edinburgh 7997*, pp. 167–170.