Cohn, J. 1990. Is it the size that counts? Palp morphology, sperm storage, and egg hatching frequency in *Nephila clavipes* (Araneae, Araneidae). J. Arachnol., 18:59-71.

IS IT THE SIZE THAT COUNTS? PALP MORPHOLOGY, SPERM STORAGE, AND EGG HATCHING FREQUENCY IN *NEPHILA CLAVIPES* (ARANEAE, ARANEIDAE)

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ABSTRACT

This study investigated the relationship between male size and reproductive success in *Nephila clavipes*, a neotropical orb-weaving spider. Gross and palpal size variation were examined in relation to copulatory behavior. sperm transfer/uptake, and utilization by the female. The effect of conductor breakage was also evaluated by assessing the timing of its occurrence and its influence on sperm transfer.

There was less variation in palp size of male *N. clavipes* than in other aspects of male morphology. Gross male body size correlated most highly with how much sperm was produced, transferred to, and stored by the female. Size of the male was not related, however, to the percentage of sperm actually transferred. The number of sperm retained by the female was influenced by the time of mating, but not by copulatory behavior. Approximately twice as many sperm were found in the palps of virgin males as were found in combined totals from mated pairs. This suggests that a substantial percentage of sperm transferred by the male is not stored by the female. None of the variables analyzed in this study greatly influenced the percentage of eggs eventually hatching. Conductor breakage seriously interfered with sperm transfer but occurred less often than expected and did not appear to result from copulatory activity.

INTRODUCTION

Individual differences in invertebrate male morphology may influence copulatory behavior (Jackson 1980; Thornhill and Alcock 1983; Christenson 1984). Male morphological variation may differentially affect internal processes in the female as well. Eberhard (1985) postulated that females in a wide variety of taxa may copulate with many males but discriminate based upon characteristics of the males' genitalia, fertilizing her eggs with sperm from the most desirable male. This might be accomplished through control of intromission, and differential uptake of sperm, among other mechanisms (Eberhard 1985). Once copulation has begun, females could monitor such variables as intensity or quality of stimuli received, thereby affecting the timing and consequences of copulation including uptake and storage (Jackson 1980; Thornhill and Alcock 1983; Eberhard 1985, 1986).

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The genitalia of male golden orb-weaving spiders (*Nephila clavipes* L.) are not noted for great complexity (Schult and Sellenschlo 1983). One outstanding characteristic, however, is the size of the conductor. Males of similar weight and/or body length, differing in conductor size, will almost certainly differ in the stimulation they provide the female, possibly affecting how much sperm is stored and later utilized by the female. Selective pressures determining conductor size could be open-ended, i.e., continuous pressure for ever larger (or smaller) size, or restrictive, i.e., males with an optimal genitalic size having an advantage over males with larger or smaller conductors. In this study, variation in *N. clavipes* palpal morphology was first assessed and compared to variation in more gross aspects of male size and sperm production. The relationships of natural and experimentally induced palp variation with transfer/storage, copulatory behavior, and egg hatching percentage were then evaluated. Because reproductive behavior of *N. clavipes* differs depending upon the age of the female (Christenson et al. 1985), palpal variation could have different effects on the uptake of sperm by young and mature adult females. Males were therefore mated with females either immediately following the final molt or two weeks post-molt.

METHODS

Study site.—The study was conducted at the F. Edward Hebert Center of Tulane University, approximately 20 km south of New Orleans in Belle Chasse, La. The facility is situated on 500 acres of hardwood, bottomland forest of elm, maple, oak, hackberry, and box elder. The site is transected by dirt roads, drainage ditches, and a series of lagoons.

Subject selection.—One hundred sixty-seven male and 157 female *N. clavipes* were collected at either the Hebert Center or the Barataria unit of Jean Lafitte National Historical Park in Barataria, La., in July and August 1987. Males were selected based upon coloration, web structure, and the presence of sperm webs, thus ensuring all were approximately the same age, that is, within one or two days after their final molt (Myers and Christenson 1988). Seventeen males, to be included in the virgin male analysis, were selected for very small size (less than 6 mm cephalothorax-abdomen length) or very large body size (greater than 9 mm). Those to be included in the two mated male studies were not selected for size. Females selected were between 18-20 mm in cephalothorax-abdomen length. This ensured that they were in their penultimate instar (Moore 1977). The spiders were housed in 123 × 62 × 62 cm boxes constructed of wood furring strips sided with Fiberglas® screening. Female subjects were presented one or two mealworm larvae each day.

Female *N. clavipes* were divided into four groups. The first variable was the female's age at mating: Day of final molt (Day 0) or two weeks post-molt (Day 14). The second variable, was the measure of reproductive success: Number of sperm found in female's sperm storage sacs (Sperm) or percent of clutch hatched (Egg). This resulted in a 2 × 2 (age vs measure) factorial design.

Initial palp evaluation.—In daily groups of approximately 20, 100 male subjects were brought into the lab before assignment to females. Males were subjected to hypothermia by placing them in a refrigerator for a few minutes and then checked for the occurrence of conductor breakage. Those found to have broken

conductor tips were excluded ($N = 4$). Males were not kept out of the field for more than 24 hours.

Mating procedure.—Males in the Day 0 groups were housed together until a female's web showed signs of degeneration, indicating a molt was to occur within a few days. At this time a male was randomly selected from the storage box (similar to female boxes) and placed via a stick near the hub position above the female. Among Day 14 dyads, virgin females were supplied with males 14 days after their final molt. After placing the male, a mealworm was added to the web to facilitate female receptivity (Christenson et al. 1985). Males in both conditions were rarely housed apart from females for more than two days.

Behavioral records.—Serial recording was conducted for a minimum of one hour on the day of the female's final molt in Day 0 females and following prey capture or the onset of copulation in Day 14 females (whichever occurred first). Specific behaviors recorded included amount of time spent *in copula* (min per h), the number of copulatory bouts (BOUT - the number of observed palpal insertions of at least 5 sec duration), rates of hematodochal bulb contractions (BC - mean rate per min), number of palp pounding bouts (PP - male rapidly drums his palps on epigynum of the female, 1 sec separating bouts), and number of female fends (FF). The latter was defined as any female behavior which either immediately terminated a copulatory bout or immediately caused a male to move off of or away from her venter. Fends generally included a brisk brushing of the male with the female's third pair of legs.

Subsequent analyses of male size.—Males were sacrificed by hypothermia. Wet weights were taken and measurements of cephalothorax-abdomen length (CthA) and tibia-patella length (TiPt) were made. Conductors were rechecked to determine frequency of breakage in non-virgin males. Palps were then removed. If not broken, the right palp was measured on a Quantimet 970 Image Analyzer®, otherwise the left palp was used. Four separate measures of palpal length were made (Fig. 1): 1. overall palp length along its retrolateral axis (PLRA); 2. length of conductor along its prolateral axis (CLPA); 3. length of conductor along prolateral axis below the conductor buttress (CLBB); 4. width of conductor at widest point (CndW). Gross and palpal measurements were taken twice on 10 males. Correlations between first and second measurements were greater than or equal to 0.98.

Some slight differences in morphology were found between males assigned to Day 0 and Day 14 females. As males were randomly assigned to these groups, and since both groups were run in equal numbers throughout the summer, these differences were likely due to chance. There were trends toward significantly larger tibia-patella length ($F1,135 = 3.20$; $p = .076$) and greater weight in Day 0 males ($F1,135 = 3.88$; $p = .051$). There was a tendency for Day 0 males to have larger conductors in three of four measures: PLRA ($F1,135 = 2.98$; $p = 0.086$), CLPA ($F1,135 = 3.52$; $p = 0.063$), CLBB ($F1,135 = 0.00$; $p = 0.973$), CndW ($F1,135 = 4.03$; $p = 0.047$).

Conductor manipulation.—To determine the effects of conductor breakage on copulatory behavior and sperm transfer/storage, conductor tips of 10 males were severed with a scalpel blade. The cuts were made approximately 0.2 mm from the distal end of the conductor, about the length which is occasionally broken off in nature. Males were maintained outdoors in separate boxes for two days after this procedure to await placement on a female's unrepaired web. Ten additional males

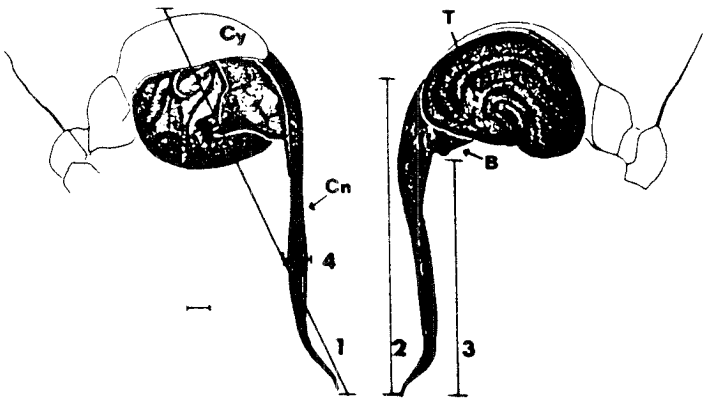

Figure 1.—Measurements of palp morphology. Retrolateral view on the left, prolateral on the right. 1 = PLRA - Palp length retrolateral axis, 2 = CLPA - Conductor length prolateral axis, 3 = Conductor length below buttress, 4 = CndW - conductor width at widest point (retrolateral axis), B = conductor buttress, Cn = conductor, Cy = cymbium; T = tegulum. Adapted from Levi (1980). Used by permission of the Museum of Comparative Zoology, Harvard University. Scale = 0.1 mm.

serving as controls were similarly handled but not cut. Females mated with these control males were part of the Day 0 Sperm group.

Histological procedure.—Mated pairs in the Sperm groups were brought into the lab five days following their initial copulation, ensuring that female sperm storage sacs had hardened. The storage sacs were removed under a dissecting microscope then placed in a 4 ml centrifuge tube with 200 ml of Ringer's solution. Following analyses on the image analyzer, male palps were treated in the same manner. The palps (or sacs) were ground thoroughly with forceps and then vortexed for approximately one min. The tubes were then centrifuged for 25 min at 1000 *g*. The tubes were removed, and the grinding, vortexing, and centrifuging were repeated two more times. The tubes were vortexed one more time, and then 5 ml samples were immediately removed, placed on acid-cleaned gel-coated slides, dried ovenight, and stained with hematoxylin. In the study of sperm availability in virgin males, the procedure was identical.

Sperm counts.—Sperm counts were performed on a Quantimet 970 Image Analyzer®. To facilitate counting, 5 ml samples were used (2.5 percent of the total). The image analyzer was programmed to count all objects with an area of between 3 μ^2 and 25 μ^2. Within-field editing allowed for the exclusion of extraneous material.

Egg sac analyses.—Following mating, females in the Egg groups were maintained until oviposition. Egg sacs were brought into the lab approximately five weeks after oviposition, sufficient time for spiderlings to have hatched and molted to the second instar. Number of spiderlings, unhatched eggs, and egg sac parasites were counted.

RESULTS

Palpal and gross morphological variation among mated males.—Overall palp length (PLRA) ranged between 1.75 and 2.33 mm, a difference of about 25 percent. The distribution was normal with a skew of well under 1.00 (normality)

Table 1.—Mean ($\bar{x}$) and standard deviations (SD) for Day 0 and Day 14 subjects. The number of sperm found in the male has been omitted from Day 0 data, as only a few sperm were found in only two males. Sperm number refers to sample size (2.5% of the total) in Day 0 and Day 14 Sperm subjects (n = 35, 36 respectively). Percentage of clutch hatched refers to Day 0 and Day 14 Egg subjects only (n = 31, 38 respectively). PLRA = Palp length along retrolateral axis; CLPA = Conductor length along prolateral axis; CLBB = conductor length below buttress; CndW = Conductor width at widest point.

	Day 0 (n = 66)		Day 14 (n = 74)	
Measure	$\bar{x}$	SD	$\bar{x}$	SD
Cephalothorax abdomen (mm)	7.67	1.24	7.47	1.12
Tibia Patella (mm)	6.89	1.22	6.53	1.12
Weight (g)	0.033	0.016	0.028	0.010
PLRA (mm)	2.08	0.12	2.04	0.11
CLPA (mm)	1.60	0.07	1.58	0.08
CLBB (mm)	1.22	0.06	1.22	0.06
CndW (mm)	0.10	0.01	0.10	0.01
Sperm remaining in male palps	—	—	4401	4592
Sperm stored in females	8037	3682	1834	1404
Egg hatching percentage	0.90	0.24	0.88	0.27
In copula (min/h)	26.7	13.0	10.6	9.4
Hematodochal bulb contraction rate (n per min)	36.2	16.0	0.4	9.1
Female fends (per h)	21.4	16.2	1.7	7.0
Copulatory bouts (per h)	10.4	7.4	1.5	1.4
Palp pounds (bouts per h)	25.9	21.2	3.1	4.9

in Day 0 and Day 14 males. In comparison, tibia-patella length varied by over 100 percent, ranging between 4.0 and 9.4 mm. Indices of skewness and kurtosis exhibited trivial differences from normality between all morphological measures. Means and standard deviations for morphological and behavioral data are presented in Table 1.

Palps were less variable than more general measures of body size. This was determined by calculating coefficients of variation (standard deviation/(mean $\times$ 100)) and testing for significance using log transformations of each of the morphological variables in Day 0 and Day 14 males. Log transformation allowed the variance of each variable to be compared directly (Lewontin 1966). An F-ratio was formed between the coefficient for each palpal measure and each gross morphological measure. Coefficients for palpal measurements were significantly smaller than those for weight, tibia-patella length, or cephalothorax-abdomen length ($p < 0.007$). Among gross morphological variables, the coefficient for weight was significantly larger than that for tibia-patella length or cephalothorax-abdomen length ($p < 0.001$). Coefficients and variance of log transformed data are presented for Day 0 and Day 14 subjects in Table 2.

There was a positive correlation between palp size and gross body size. The highest correlation found was between PLRA and tibia-patella length in Day 14 subjects ($r = 0.82$; $p < 0.00001$).

Male size and available sperm in virgins.—In virgin males, the amount of sperm found in palps was highly related to gross and palpal morphology. The highest correlation was with weight ($r = 0.82$; $p < 0.0001$) and the lowest was with tibia-patella length ($r = 0.72$; $p = 0.002$). The various measurements of palp structure correlated equally with the amount of available sperm. Variables PLRA, CLBB, and CndW correlated with sperm at $r = 0.75$ or 0.76 ($p < 0.002$). Variable

Table 2.—Coefficients of variation (C. V.) (Mean/(Standard deviation X 100)) and variance of gross morphological and palpal measures using log transformation. CthA = cephalotharax-abdomen length; TiPt =Tibia-Patella length; PLRA = Palp length along retrolateral axis; CLPA = Conductor length along prolateral axis; CLBB = conductor length below buttress; CndW = Conductor width at widest point.

	Day 0		Day 14	
Measure	C. V.	$s^2(\text{Log}(x))$	C. V.	$s^2(\text{Log}(x))$
Gross				
CthA	15.84	5.18×10^{-3}	15.03	4.36×10^{-3}
Weight	50.05	6.56×10^{-3}	45.05	5.62×10^{-3}
TiPt	19.05	8.12×10^{-2}	17.30	4.04×10^{-2}
Palp				
PLRA	6.06	6.76×10^{-4}	5.33	5.29×10^{-4}
CLPA	4.62	4.00×10^{-4}	4.91	4.41×10^{-4}
CLBB	5.01	5.29×10^{-4}	4.90	4.41×10^{-4}
CndW	7.11	5.29×10^{-4}	7.71	4.41×10^{-4}

CLPA correlated with sperm at $r = 0.61$ ($p = 0.009$). Selection bias for very large and very small males resulted in somewhat exaggerated Pearson's *r*s.

Male size and sperm storage by females.—Male weight was the best predictor, among male morphological characteristics, of the amount of sperm stored by the female. Stepwise multiple regression performed on collapsed Day 0 and Day 14 data yielded a multiple R of 0.31 for the variable WGT. This score accounted for a significant amount of the variance (F 2,68 = 7.42; $p = 0.001$). The variable CthA accounted for a significant proportion of the remaining variance. When included in the equation, CthA increased the multiple R to 0.41 ($F2,68 = 6.84$; $p = 0.002$). The relationships between male weight and the amount of sperm stored by the female in Day 0 and Day 14 dyads are presented in Fig. 2.

Male size and proportion of sperm transferred.—When the amount of sperm found in the female was expressed as a percentage of the total available sperm in the female (SP-F) and male (SP-M) combined (SP-F/(SP-M + SP-F)), no significant relationships were found between the proportion of sperm found in the female and any aspect of male morphology. To test whether males with average-sized palps had an advantage over males of either extreme, proportions of sperm transferred from Day 14 males were converted to *z*-scores and Pearson *r*s calculated for the four palpal variables vs the *z*-scores' absolute values. Once again, no significant relationship was found.

Male size and copulatory behavior.—To examine whether small males exhibit differences in copulatory behavior to compensate for a deficit in the ability to facilitate sperm storage, the 10 largest (M CthA = 9.50; SD = 0.81) and 10 smallest (M CthA = 5.90; SD = 0.43) males were selected from the Day 0 groups and the 11 largest (M CthA = 9.20; SD = 0.38) and 11 smallest (M CthA = 6.00; SD = 0.57) from the Day 14 groups. Each group was divided in half again based upon palp size (large or small palps using PLRA as an index), resulting in a 2×2 body size vs palp size design. Two-way analyses of variance were conducted to determine whether these divisions resulted in significant size differences.

Day 0 subjects.—As expected, big males had significantly larger palps than small males ($F1,16 = 196.904$; $p < 0.0001$). When the data were collapsed across body size, a significant difference was still found between the largest and smallest

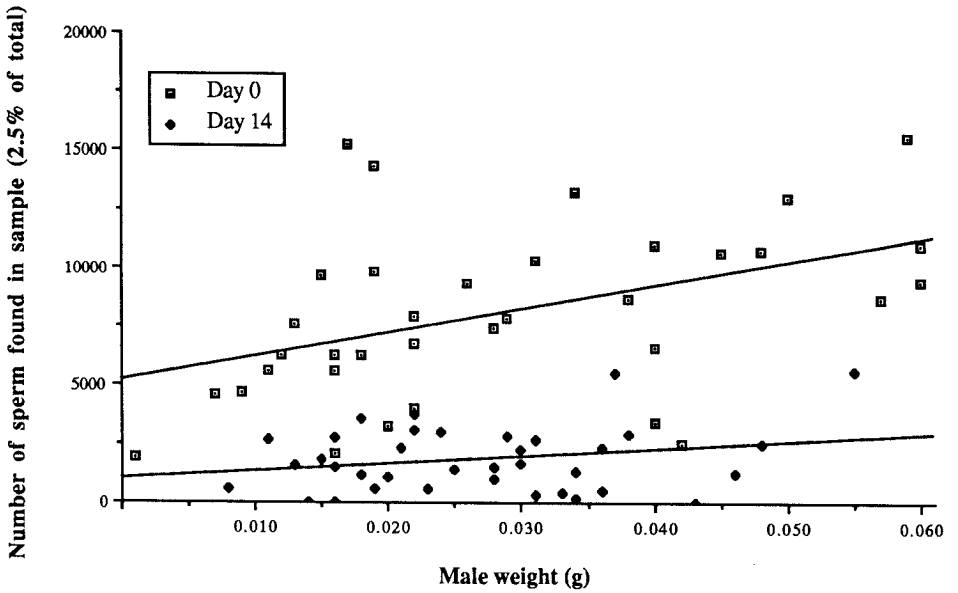

Figure 2.—Scatterplot for male weight (g) and sperm (samples) found in female storage sacs in Day 0 and Day 14 dyads with regression lines. Pearson r for Day 0 animals = 0.46 (p = 0.0002). Regression equation is Y = 5125 + 1.0163e+5x. For Day 14 animals the correlation is 0.25 (p = 0.05) and the regression equation is Y = 991.1 + 3.1331e+4x.

palps (PLRA, large bodied males, M = 2.12; SD = 0.05; PLRA, small bodied males, M = 1.81; SD = 0.04; $F1,16 = 44.109$; $p < 0.0001$). The palp size × body size interaction was not significant ($p < 0.267$). No behavioral differences related to palp size or body weight were uncovered using MANOVA.

Day 14 subjects.—Large and small males displayed means and differences in palp size nearly identical to those found in Day 0 males. Higher rates of some copulatory behaviors were observed in larger males during the one hour serial record: COP ($F1,18 = 5.98$; $p < 0.025$), BOUT ($F1,18 = 4.77$; $p < 0.043$), PP ($F1,18 = 7.82$; $p < 0.012$). The overall multivariate F of behavioral differences based on male weight was significant ($F6,13 = 3.83$; $p = 0.02$). Higher rates of palp pounding in large-palped males ($F1,18 = 18.58$; $p < 0.0004$) and more copulatory bouts ($F1,18 = 4.77$; $p < 0.043$) contributed to a trend towards significance in the multivariate F of differences based on palp size ($F6,13 = 2.74$; $p = 0.06$). The overall multivariate F for the palp size by body weight interaction was not significant ($p = 0.34$).

Male size and egg hatching.—Hatching percentage was not dependent upon the size of the male. The highest correlation was with cephalothorax-abdomen length in Day 0 subjects ($r = 0.25$; $p = 0.05$). This relationship was not apparent in the Day 14 Egg group.

Female age at mating, sperm storage, and copulatory behavior.—When mating with a newly-molted female, males nearly always transferred their entire supply of sperm (M > 99 percent). When copulation was delayed for two weeks, mated males retained about 24 percent of the sperm found in virgin males. A one-way analysis of variance between Sperm groups indicated that significantly more sperm were found in Day 0 females ($F1,69 = 35.70$; $p < 0.0001$). A mean of 8037 sperm was found in Day 0 samples (SD = 3682), versus 1834 in Day 14 samples

(SD = 1404). These means reflect sample sizes of 2.5 percent of the total sperm. When the number of sperm transferred to Day 14 females was expressed as a percentage of the total available sperm (SP-F/(SP-M + SP-F)), no relationship was found to exist between any of the behavioral variables and the proportion of sperm transferred.

A MANOVA was performed to determine if any aspects of copulatory activity were related to female age at mating. Due to missing data, three dyads were dropped (for this analysis only) leaving a total of 137. The overall multivariate F was significant ($F14,122 = 24.75$; $p < 0.0001$), indicating that the overall pattern of variable scores differed between Day 0 and Day 14 subjects. Subsequent analyses revealed significantly higher rates of copulatory activity in Day 0 subjects: more time spent copulating per one hour serial record ($F1,135 = 68.87$; $p < 0.0001$), a higher number of copulatory bouts ($F1,135 = 105.79$; $p < 0.0001$), higher rate of hematodochal bulb contractions ($F1,135 = 143.50$; $p < 0.0001$), and more palp pounding ($F1,135 = 77.40$; $p < 0.0001$). There were more fends by the female as well ($F1,135 = 87.73$; $p < 0.0001$).

Females fended males more often per unit time spent copulating on Day 0 ($F1,115 = 10.498$; $p < 0.002$); the mean fend/cop ratio was 1.04 on Day 0 versus 0.33 on Day 14. Cases where no copulations were observed during the one hour observation period were dropped from this analysis ($N = 23$) leaving a final N of 117. To determine if females were influencing the number of times a male attempted to mate, 10 Day 0 dyads and 10 Day 14 dyads were randomly selected from those dyads in which at least one mating attempt and fend were observed. The above analysis was then repeated using the ratio of fends to copulatory attempts. A copulatory attempt was defined as occurring when the male descended to the ventrum of the female followed by either successful copulation or insertion of less than 5 sec. No significant difference was found between Day 0 and Day 14 dyads ($p = 0.346$). Day 0 males were fended a mean of 1.1 times per copulatory attempt. Day 14 males were fended a mean of 0.8 times per attempt.

Do females influence copulation duration?—Gross female activity had little effect on male reproductive behavior. Female fends of males were not correlated with the amount of copulation and only a slight negative correlation was found with the amount of sperm later obtained in the female (Day 0 $r = -0.23$; $p = 0.06$; Day 14 $r = -0.26$; $p = 0.05$). Fends were positively correlated with BC rates in Day 0 males ($r = 0.38$; $p = 0.001$), but this relationship was not found in Day 14 dyads.

Copulatory behavior and sperm storage.—Among Day 0 subjects, total copulation time was the best behavioral predictor of the amount of sperm found in the female. This variable had a correlation with SP-F of 0.47, and was the only variable accounting for a significant proportion of the total variance ($F1,32 = 8.89$; $p < 0.001$). No behavioral variables were related to the amount of sperm found among Day 14 females. The predictive value of behavioral variables were determined by stepwise multiple regression analysis. Because of behavioral differences between Day 0 and Day 14 mating, the analysis was run under each condition.

Amount of sperm transferred during feeding bouts.—Day 14 Sperm dyads were analyzed to determine how much sperm were transferred during each mating bout. These copulations took place almost exclusively after mealworms were added and when females were observed feeding. The numbers of bouts are only

an approximation as clearly not every one occurring within these dyads was recorded. In three cases, sperm were found in females even though no copulation was observed. Because final molts were observed, it is clear that insemination could only have been carried out by the introduced males. These dyads were included and scored as having the minimum possible one copulatory bout. A mean of 2.8 copulatory bouts were observed among Day 14 Sperm subjects over the 4 days of observations (SD = 1.6). Each bout resulted in the transfer of a mean of 37 750 sperm (SD = 46 886). These were the true numbers, obtained by multiplying the sample size by 40. As the mean amount of sperm found in virgin males (total, not sample size) was 520 898 (SD = 257 779), each bout transferred about seven percent of the male's total sperm. There was, however, a large amount of variation among males.

"Lost" sperm.—Because the combination of SP-F and SP-M always appeared to be less than the amount of sperm found in similarly-sized virgin males, a comparison was made between the two totals. Seventeen mated males were matched for weight with the virgin males. Virgin males contained significantly more sperm than were found in mated pairs ($F1,34 = 17.64$; $p = 0.0002$). There was a mean of 13 022 sperm in the virgin male samples (SD = 6444) and 6261 in the mated pair samples (SD = 2647). There was no significant difference in weight between the mated and virgin males (M = 0.029 g and 0.021 g, respectively), hence a reasonable matching ($p = 0.30$).

Copulatory behavior, time of mating, and egg hatching.—Egg hatching percentage was not greatly influenced by male behavior. The highest correlation found was with hematodochal bulb contraction rate ($r = 0.25$; $p = 0.05$). This correlation was identical for both the Day 0 and the Day 14 groups. Females of both groups had a mean 89 percent of their clutch hatch. Time of mating did not affect egg hatching percentage ($p = 0.727$).

Differences in egg parasitism between Day 0 and Day 14 clutches.—Many egg sacs contained parasites. The majority were larvae of the insect family Mantispidae. One sac contained a small unidentified spider. Twelve of 31 Day 0 egg sacs (39%) were found to contain at least one parasite. Nine of 38 Day 14 egg sacs (24%) were also parasitized. Chi-square analysis indicated no significant association of time of mating with rates of egg parasitism ($p = 0.177$).

Frequency of conductor breakage.—Only the first 100 virgin males collected for this study were checked for broken conductors prior to their introduction to females. Four had a broken conductor tip and were excluded. When the 140 mated males included in this study (excluding those that were artificially broken) were examined, eight had a single broken conductor. No males had two broken conductors. Chi-square analysis indicated that conductor breakage was equally likely in virgin and non-virgin males ($p = 0.54$). Chi-square analysis further indicated that, following mating, sperm remaining in males with broken conductors equalled that of intact males matched for weight and time of mating ($p = 0.59$). As conductors were not found to be broken more frequently following mating, it is clear that copulation is not a major cause of conductor breakage.

Cut palp study.—Severing the tips of conductors had adverse effects on male reproductive behavior. Hematodochal bulb contractions, an index of copulation intensity, were observed in only two of the experimental males tested. A small amount of sperm (about 250/sample) were found in one female paired with a cut male. A MANOVA was performed to evaluate differences in copulatory behavior

between these two groups. The overall multivariate F was significant ($F9,9 = 9.49$; $p = 0.001$). Intact males were observed copulating significantly more often than cut palp animals ($F1,17 = 29.08$; $p < 0.0001$). Hematodochal bulb contractions were significantly faster in intact males as well ($F1,17 = 7.31$; $p = 0.015$). The damaged palp was clearly preventing successful copulation. This was also reflected in the number of copulatory bouts ($F1,17 = 19.73$; $p = 0.0004$). Motivation to mate, however, seemed unaffected. The number of copulatory attempts made by the males were compared to evaluate whether damaged males were less active. There was no significant difference between intact and cut palp males ($p = 0.65$) nor were differences in palp pounding observed ($p = 0.19$). Females did not distinguish between intact and damaged males. Cut palp males were not fended away any more often than intact males ($p = 0.12$). Behavioral data for cut palp and intact males are presented in Table 3.

DISCUSSION

Palpal Variation and its relation to gross male morphology.—Variation in palp size does not exhibit a range comparable to that found in more gross measurements such as weight or cephalothorax-abdomen length. Small males with exceptionally large palps or large males with small palps were not observed in the sample studied. The reduced variance in palpal size is consistent with results obtained in other genera such as *Pardosa* (Barnes 1959), *Castianeira* (Reiskind 1969), and *Hypochilus* (Coyle 1985). This consistency is an important reason for the use of male genitalia as taxonomic markers (McCrone 1963; Coyle 1985), and suggests that any selective forces at work favor a narrow range of palp sizes rather than a trend towards ever larger (or smaller) palps. While there are likely to be genetic constraints on the overall size of males, there appear to be stronger constraints on palp size. Ecological variables such as prey availability and temperature exert a much stronger influence on gross morphology than on palp morphology (Vollrath 1980). Growth rates among unrestrained populations during critical periods of development are highly variable, changing with shifts in these factors (Coyle 1985).

Determinants of sperm storage by males and females, and its utilization.—The amount of sperm stored in male palps prior to mating is closely related to overall male size. The correlation between size and sperm availability could be due to two factors. Larger males probably have more gonadal tissue with which to manufacture sperm and larger palps in which to store sperm until the opportunity to mate arises.

The amount of sperm stored by female *N. clavipes* is related to the gross size of the male and to the size of his palps. When the amount of sperm found in Day 14 females was expressed as a percentage of the total, however, no advantage was found for exceptionally large, small, or average-sized males. As a large proportion of the available sperm was "misplaced" somewhere between copulation and laboratory analysis, this statement is made with some caution.

Twice as many sperm were present in virgin males as were later recovered from mated dyads. Some of the difference in numbers can be attributed to experimental procedures as the SP-M + SP-F group went through the sperm counting procedure twice and the virgin male group once. Sperm taken from

Table 3.—Descriptive statistics for intact and cut palp males in the conductor manipulation study. N = 19. $\bar{x}$ = Mean; SD = Standard deviation.

Measure	Intact palp		Cut palp	
	$\bar{x}$	*SD*	$\bar{x}$	*SD*
Copulatory attempts (per h)	21.67	13.13	19.22	20.36
Palp pounds (bouts/h)	32.67	33.46	15.90	18.73
In copula (min per h)	22.00	11.47	1.67	3.54
Copulatory bouts (per h)	8.33	5.20	0.80	1.32
Hematodochal bulb contraction rate (n per min)	34.00	16.08	11.00	22.51
Female fends (per h)	23.00	20.37	10.78	14.65

females also had a tendency to clump together occasionally, sometimes making an accurate count more difficult. However, the very large difference indicates some loss of sperm and warrants further investigation.

Male body size, palp size, and behavior were not related to the percentage of eggs hatching. This is logical as females were not mated with second males and may be expected to use any sperm available to them at the time of oviposition. It remains to be seen whether the aforementioned variables influence paternity when females mate with more than one male.

Timing of the initial copulation.—The timing of mating greatly influences copulatory behavior and the amount of sperm ultimately stored by the female. These results are consistent with past studies of *N. clavipes* (Brown 1985; Christenson et al. 1985). There was no reduction in female reproductive success when her initial mating was delayed for two weeks. Surprisingly, females fended off males significantly more often just after molting. This is in part due to the increased amount of copulatory behavior occurring at this time. When the proportion of fends to observed copulation time is compared for the two groups, however, it is clear that females were more reactive following the final molt.

Females in the Day 14 Egg group fertilized their entire clutch despite receiving only 24 percent of the males' sperm. This is interesting as it calls into question why a male transfers his entire supply of sperm when mating with a newly-molted adult. Some recent modeling by T. E. Christenson and W. P. Dunlap (Pers. comm.) proposes that total sperm transfer is the best strategy for a male mating with a newly-molted adult. Their model suggests that total transfer may be a consequence of the extended copulation necessary to insure a first male precedence effect (Christenson and Cohn 1988). One advantage may be to dilute the effectiveness of subsequent mating by the female. Sperm "dumping" may also result from the rather low probability of successful copulation (about 20 percent of males) and the even lower probability of a mated male either making it to the hub of another receptive female or defending his mated partner until oviposition (unpublished data).

Conductor breakage.—Conductor breakage did not occur in mated males at a higher rate than in virgins. The overall rate of breakage was low, less than seven percent. While the occasional broken conductor tip may inhibit further sperm uptake, the low rate of breakage suggests that this is not a typical occurrence. When conductor tips were experimentally severed, behavioral deficits were observed. Males with severed conductors did not mate successfully as only one male transferred a small number of sperm. Motivation to mate seemed unaffected

as there were no significant differences in the number of copulatory attempts or palp pounds.

Male copulatory behavior, morphology, and uptake.—The copulatory behaviors evaluated in this study did not vary systematically with either male size or uptake of sperm by the female. Hematodochal bulb contraction rates were higher in Day 0 males, and females in this group acquired more sperm. These differences seem to be related to the softer, unscleroticized epigynal tissues in newly-molted females and not to individual male variation (Christenson and Cohn 1988). Among Day 0 subjects, copulation time correlated most strongly with the number of sperm found in the female. This is surprising as all males in this group were virtually depleted of sperm. These results, and the finding that a good deal of sperm may be "lost", suggest that increased amounts of copulatory activity could facilitate storage of sperm and not just release. However, no relationship was found between observed copulation time and the amount of sperm later found in Day 14 females. These results suggest that larger amounts of sperm simply take longer to transfer. In *N. clavipes*, however, all sperm is transferred within the first three hours while copulation continues for up to 48 h (Christenson and Cohn 1988). It seems unlikely, therefore, that the higher proportion of time larger males spent *in copula* was due to the volume of sperm they carried. The meaningfulness of the relationship between copulation time and sperm storage by the female remains unclear. Twenty-four h serial records need to be conducted on older adult females mating for the first time, with sampling of the amount of sperm found in each member of the dyad occurring at different times after the first observed copulation. Time sampling methodology as employed in the present study may not be able to yield data of sufficient accuracy.

Sexual selection in *N. clavipes*.—The following conclusions can be drawn regarding sexual selection in *N. clavipes*. Intense intrasexual selection, through agonistic encounters among males, takes place before, during, and after copulation (Goist 1983; Cohn et al. 1988). No evidence for intersexual selection was found in the present study. This investigation was, however, conducted within very narrow parameters, during and immediately after mating with a single male. Female *N. clavipes* can, of course, influence their reproductive processes in ways not addressed in the present study. For example, Christenson and Cohn (1988) demonstrated that the first male advantage typical in *N. clavipes* can be significantly reduced if males are limited in the amount of copulation time following sperm transfer. Post-transfer copulation may reduce future sexual receptivity in the female (Christenson and Cohn 1988). Fifteen percent of females leave their orb within 24 h of their final molt with little likelihood of successful pursuit by the male (Cohn et al. 1988). These early departures may provide a means for intersexual selection to operate.

In summary, a close relationship was found between the size of the male, the amount of sperm available for transfer, and the amount of sperm later found in the females' storage sacs. Females who mate with the largest males store the most sperm, but even the smallest males transfer enough to fertilize a clutch. While female *N. clavipes* may exercise several reproductive options, no preference for males of a particular size was found within the parameters of this study.

ACKNOWLEDGEMENTS

I would like to thank T. E. Christenson for his guidance and support during the course of this study. I would also like to thank H. W. Levi for his permission to include figures from one of his publications, W. G. Eberhard and S. N. Austad for their comments and suggestions, and the many field and laboratory assistants without whom this work could not have been done.

LITERATURE CITED

Barnes, R. D. 1959. The lapidicina group of the wolf spider genus *Pardosa* (Araneae, Lycosidae). Am. Mus. Nov., 1960:1-20.

Brown, S. G. 1985. Mating behavior of the golden orb-weaving spider, *Nephila clavipes*: II. Sperm capacitation, sperm competition, and fecundity. J. Comp. Psychol., 99:167-175.

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

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

Christenson. T. E., S. G. Brown, P. A. Wenzl, E. M. Hill and K. C. Goist. 1985. Mating behavior of the golden orb-weaving spider. *Nephila clavipes*: I. Female receptivity and male courtship. J. Comp. Psychol., 99:160-166.

Christenson, T. E. and J. Cohn. 1988. Male advantage for egg fertilization in the golden orbweaving spider. J. Comp. Psychol., 102:312-318.

Cohn, J. and T. E. Christenson. 1987. Resource utilization in the male golden orb-weaver *Nephila clavipes*. J. Arachnol., 15: 185-192.

Cohn, J., F. V. Balding and T. E. Christenson. 1988. In defense of *Nephila*: Post-mate defense in the golden orb-weaving spider. J. Comp. Psychol., 102:319-325.

Coyle, F. A. 1985. Two-year life cycle and low palpal character variance in a great smoky mountain population of the lamp-shade spider (Araneae, Hypochilidae, Hypochilus). J. Arachnol., 13:211-218.

Eberhard, W. G. 1985. Sexual Selection and Animal Genitalia. Harvard University Press, Cambridge.

Eberhard, W. G. 1986. Why are genitalia good species characters? Int. Congr. Arachnol., 9:53-59.

Goist, K. C. 1983. Male-male competition in the orb weaving spider. *Nephila clavipes*. Unpublished doctoral dissertation, Tulane University, New Orleans, Louisiana.

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.

Levi, H. W. 1980. The orb-weaver genus *Mecynogea*, the subfamily metinae and the genera *Pachygnatha, Glenognatha*, and *Azilia* of the subfamily tetragnathinae north of Mexico (Araneae: Araneidae). Bull Mus. Comp. Zool., 149:1-75.

Lewontin, R. C. 1966. On the measurements of relative variability. Syst. Zool., 15:141-142.

McCrone. J. D. 1963. Taxonomic status and evolutionary history of the *Geolycosa pikei* complex in the southeastern United States (Araneae, Lycosidae). Am. Midl. Nat., 70:47-73.

Moore. C. W. 1977. The life cycle, habitat, and variation in selected web parameters in the spider *Nephila clavipes* Koch (Araneidae). Am. Midl. Nat., 98:95-108.

Myers, L. and T. E. Christenson. 1988. Transition from predatory juvenile male to mate-searching adult in the orb-weaving spider *Nephila clavipes* (Araneae, Araneidae). J. Arachnol., 16:254-257.

Reiskind, J. 1969. The spider subfamily castianeirinae of North and Central America (Araneae, Clubionidae). Bull. Mus. Comp. Zool., 138:163-325.

Robinson. M. H. and B. Robinson. 1980. Comparative studies of the courtship and mating behavior of tropical araneid spiders. Pac. Ins. Monogr., No. 36. Bishop Museum, Honolulu.

Robinson, M. H. 1982. Courtship and mating behavior in spiders. Ann. Rev. Entomol., 27:1-20.

Schult, J. and U. Sellenschlo. 1983. Morpholgie und funktion der genitalstrukturen bei *Nephila* (Arach., Aran., Araneidae). Mitt. Ham. Zool. Mus. Inst., 80:221-230.

Thornhill, R. and J. Alcock. 1983. The Evolution of Insect Mating Systems. Harvard University Press, Cambridge.

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

Manuscript received August 1988, revised June 1989.