SPERM DEPLETION IN THE ORB-WEAVING SPIDER
NEPHILA CLAVIPES (ARANEAE, ARANEIDAE)

In the spider literature, it is generally assumed that individual males can and do inseminate more than one female. This would appear to be adaptive from the male point of view, particularly since spider males invest nothing in care of egg sac or young, and, in all three species examined so far, it seems that the first male to mate with a given female fertilizes most of her eggs (Austad 1984; Christenson and Cohn 1988). If a male defended a juvenile female for a period of time and then mated with her just after her final molt, he might well be expected to search for and mate with another female. However, the data showing that male spiders can inseminate that second female are sparce. Breene and Sweet (1985) have

demonstrated that male black widow spiders can inseminate at least three females. In this study, the limitation on insemination was determined by placing male *Nephila clavipes* (L.) with up to three sexually receptive virgin females and later examining both sexes for the presence of sperm. It would seem that any assessment of male tactics, in any species, would benefit from taking into account such information.

A number of ante-penultimate females (18-21 mm cephalothorax-abdomen length) and newly-molted adult males (7-9 mm cephalothorax-abdomen length) were gathered in July of 1986. To insure males were virgin, I gathered only those on orbs with sperm webs and with the newly-molted animal's coloration (see Myers and Christenson 1988). All subjects were housed in 123 x 62 x 62 cm boxes screened with Fiberglas®. These were situated in the hardwood forest at the F. Edward Hebert Center of Tulane University, located about 20 km outside of New Orleans, La. Females were fed two mealworm larvae per day.

Males were held in the field boxes, in male groups, for a few days to a week prior to presentation to a female. About two days before the female's molt, determined by pattern of web deterioration, each virgin male ($n = 11$) was placed in the upper barrier strands of a female's orb. After the female's molt, mating behavior was time sampled at about ten-minute invervals for two hours. This is sufficient observation to determine the nature of mating as copulations are frequent after the female's final molt, occurring an average of 28 minutes out of the hour for more than a day (Christenson et al. 1985). Males were removed from the first female's web at different times, ranging from 5 to 35 days after the female's molt. Time of removal varied in order to increase the longevity of some males, and to allow some males ($n = 3$) to mate with three females, and as a consequence of availability of females who were ready to molt. After male removal, first females ($n = 11$) were sacrificed, and the spermathacae removed, squashed, and examined with a phase contrast microscope for sperm. Female maturity was verified for each female by the sclerotized condition of the spermathecae.

Immediately after removal from the first female's cage, each male ($n = 11$) was placed with a second female in the manner already described. Second females molted anywhere from 10 to 34 days after the first female. On the day of second female molt, mating behavior was observed during a one-hour serial record, to give a more accurate account of mating frequency and time. Eight males were removed and the palps checked for sperm. Second females ($n = 11$) were removed and spermathecae checked in the manner already described.

The remaining three males were placed with a third female; those females molted 47, 51, and 53 days after the first females. Mating was observed for one hour after the female's molt. The three males and females were removed a few days after her molt and were checked for sperm. So, the eleven males had been sacrificied and checked for sperm between 2 and 9 weeks after molting to adulthood. This is about as long as adult male *N. clavipes* live under these captive conditions (Cohn and Christenson 1987) and probably much longer than they live under unrestrained conditions. To be included in the following analysis, the male and all females he mated with had to have been examined for sperm. Due to deaths and a few possible male escapes, data for an additional 8 males and 15 females were excluded.

Nine of the eleven males were observed to mate with the first female. Mating was of the usual vigor noted in *N. clavipes* just after the female's final molt (Christenson et al. 1985); mating was noted in 60% of the time samples and bulb contraction frequencies averaged 41 per min. Insertions of the conductor were numerous, and prolonged hematodochal bulb contractions were evident. All of the eleven first females' spermathacae contained many sperm.

Seven of the eleven males demonstrated some level of sexual behavior with the second female. However, the vigor of mating was relatively low. Prolonged conductor insertion with rhythmic and countable hematodochal bulb contractions occurred in only two cases. In the first, the male mated eight times for a total of 16 minutes; the bulb contraction rate was 30 per min. In the second, the male mated three times for almost 20 minutes; bulb contraction rate was 8 per min. The matings of five males included an average of seven brief conductor insertions with intromittent bulb contractions, the frequency of which were impossible to count. None of the 11 second females' spermathecae contained sperm.

None of the males with a third female showed any mating behavior. Third females contained no sperm. Eventually, all 11 males tested were sacrificed; none of their palps contained sperm. No sperm webs were noted in any female orb.

One can not take for granted that an active male spider can inseminate more than one female. Robinson and Robinson (1980) noted that eunuchs, males with terminal portions of the palps broken off, still actively fight with one another while on a female's orb. Although embolus damage is commonly thought to reduce or eliminate the ability to transfer sperm (Foelix 1982), it should be pointed out that Breene and Sweet (1985) found that even with damage to the tip of the embolus, male black widows were capable of inseminating the female. When a male *N. clavipes* mates, that does not necessarily mean that the male has sperm available to the female. Two males in this study demonstrated mating with a second female that could easily have been confused with mating typical of the day after the female's final molt (Christenson et al. 1985). The intermittent insertions and bulb contractions noted in five other males could have been confused with mating typically noted with older adult females (ibid.).

No sperm were found in the palps of any of the 11 males. This was somewhat surprising; we had suggested at one time that because males near the hub (and thus the female) had a feeding advantage over males remaining on the web periphery, the hub male would have a relatively greater chance of moving to and then mating with a second female (Christenson and Goist 1979). Anyway, it is unclear why no sperm were available. Perhaps spermatogenesis is terminated at the final molt, or there is some constraint on sperm transfer via sperm web construction or via prolonged copulations (up to 48 hours; Christenson et al. 1985) leading to embolus damage which in this species might inhibit sperm transfer. These possibilities are being examined histologically.

One might expect males of *N. clavipes* to be somewhat choosey concerning females that could be assessed at the time of their final molt. I do not think that males have an ample opportunity to do so. First, our boxed females do not attract males, indicating that molting females do not appear to produce a distance-acting pheromone, as can happen in other species (Olive 1982). Second, males have a limited opportunity to visit adult females inhabiting aggregated webs. Female *N. clavipes* aggregate later in the season, after having matured and

mated for the first time (Brown et al. 1985). Further study of male choice is, however, warranted.

The discussion of alternative mating tactics in spiders must include limitations on insemination. Consider what is perhaps the most fundamental decision a male orb-weaving spider has to make, to remain on a female web or to search for another female. Austad (1984) has argued that phyletic constraints, such as the structure of the female sperm uptake and storage anatomy, may be closely related to whether the male should guard the female. Results of the present study indicate that constraints relating to insemination limits should also be considered. Male *N. clavipes* deplete sperm after mating with a newly-molted female but do not leave to search. Most remain with the female after mating (Cohn et al. 1988). There is no compelling reason for males to leave their mate and search for another because they could not father additional offspring by doing so. The comparative study of spider species whose males routinely show limited guarding and frequent movement between females would clarify the importance of insemination limitation in the prediction of male behavioral tactics.

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