X-RAY STERILIZATION OF MALE GOLDEN-WEB SPIDERS *NEPHILA CLAVIPES* (ARANEAE)

In some spider species, females mate with more than one male (Austad 1984). Male sterilization can be used to determine whether a particular male fertilizes a disproportionate number of a given female's eggs (Parker 1970). A popular method requires some females to mate first with an intact male and second with a sterile; other females are presented males in the reverse order. Differential rates of egg hatching between these two groups reveal patterns of male priority in fertilization. There are, however, only a few published papers on irradiation and male sterility in spiders (Jackson 1980, Vollrath 1980, Austad 1982). Austad (1982) presented a relatively detailed methodology which we used as a guide. Because Austad's bowl and doily spiders (*Frontinella pyramitela* (Walck.)) and our *Nephila clavipes* (L.) differ in size and aspects of natural history, a presentation of our methods and findings might be useful to others contemplating sterilization procedures.

This study was done during the summer of 1984 at the F. Edward Hebert Center of Tulane University, about 15 km south of New Orleans. Subjects were selected in mid-July, early in the mating season, because early season eggs are more likely to hatch than later eggs (Christenson, Wenzl and Legum 1979). Sixty adult males were collected from small orbs (probably of their own construction) that contained sperm webs. This insured that males were in the same phase of spermatogenesis at the time of irradiation. Light brown abdomen coloration and the presence of the sperm web indicated that collections were made within three days after the final molt.

After collection, males were refrigerated until inactive and then placed singly in a 1 cm cardboard cube. Twenty received 2000 rad of X-ray radiation, 20 received 5000 rad, and 20 served as controls, being treated in the same manner as experimentals except for irradiation. Radiation was generated by a General Electric Maximar® machine at 250 kv through a filter of 1/4 mm copper and 1 mm aluminum situated 39 cm above the center of the holding box. Output was 129 rad/min. These specifications are based upon those that proved effective for Austad (1982).

Males were placed individually with a female collected in her penultimate instar. Subjects were housed in Fiberglas screen boxes (123 × 62 × 62 cm). We placed males with females prior to the female's final molt since unrestrained *N. clavipes* females mate just after the final molt (Christenson, Brown, Wenzl, Hill, and Goist 1985). However, in this species, irradiated males could have been mated to virgin adults since adult females will mate while feeding and such matings result in egg hatching (Wenzl 1980). Males were removed 25 days after irradiation.

When assessing fertilization priority between two males, it is necessary to demonstrate that both males actually mated with the female. Or, if one wished to study eggs fertilized by irradiated sperm, it would be necessary to observe mating to insure that fertilization had occurred since unfertilized eggs and eggs fertilized by inviable sperm are similar in appearance. To document mating, we observed subjects during a morning census and several 1-min time samples taken throughout the day. Mating and egg data presented in this paper are based upon

the 38 pairs that were observed to mate and in which the female lived long enough to produce an egg clutch.

Females laid eggs in the field boxes, with the first appearing about four weeks after the final molt. Eggs were left in the boxes until hatching since laboratory conditions tend to reduce hatching success (unpublished observations). Fourteen of the controls, 13 of the 2 krad group, and 11 of the 5 krad group produced a clutch of eggs. Eggs of thirteen of the control sacs hatched compared to none of the 2-krad or 5-krad clutches ($\chi^2 = 33.874$, $df = 2$, $p < 0.0001$). Eggs of the three groups were of a normal yellow coloration and could not be distinguished by the unaided eye. Eggs fertilized by control males ($n = 3$ clutches) and irradiated males ($n = 3$ clutches) were examined microscopically. Group differentiation was possible about one week prior to hatching, with developmental details obvious on the surface of the eggs fertilized by sperm of control males.

Irradiation did not seem to affect the positioning of males on the female web nor the vigor of overt male sexual behavior. A male who appears on a unrestrained female's web will position himself near the hub; if two or more are present, usually the largest male will defend this position (Christenson and Goist 1979). All males but one (a 2-krad animal) had assumed position near the hub by the morning after introduction. The remaining male did so by the following day. Groups did not statistically differ in terms of males who mated the day the female molted; all but one control, one 5-krad, and three 2-krad males were observed mating ($\chi^2 = 1.856$, $df = 2$, $p < 0.395$). All males were observed to mate for the first time within two days of the female's final molt. All initial mating occurred within 15 days after irradiation.

As Austad (1982) notes, to assess the effectiveness of sterilization procedures used to examine sperm competition and determine paternity, it is necessary to note if the irradiated sperm are motile. To assess motility, we examined microscopically sperm for six females (two controls, two 2-krad, and two 5-krad) 26 days after males were irradiated and at least two weeks after mating had occurred. Collection was made at this time since *Nephila* sperm do not become flagellate (and motile) in the spermathecae until about ten days after mating (Brown 1985). Spermathecae were removed from the abdomen, placed on a clean slide in an uncontrolled drop of saline, cut with a razor, the halves squashed, and sperm immediately examined under a Leitz® phase-contrast microscope. All six sets of spermathecae contained many flagellate, motile sperm. Inviability of eggs of females in the irradiated groups was not due to sperm being aflagellate since oviposition occurred an average of 27 days after copulation, when most sperm are still motile (Brown 1985).

Irradiation influences the lifespan of sperm. Five pairs of spermathecae were removed and examined sixty days after irradiation. One control female and two 2-krad females contained many flagellate sperm, while two 5-krad females contained mostly dead sperm. This presented no problem in *N. clavipes* as fertilization occurs at oviposition, about 27 days after final molt.

Irradiation did not influence male longevity. Sixteen control males, 17 2-krad males, and 16 5-krad males ($\chi^2 = 0.223$, $df = 2$, $p < 0.895$) were alive twenty five days after irradiation. To more fully assess effects on longevity, fifteen males were placed with a second female, after the death of the original female with which they were housed. Males residing with an adult female live longer than those which are not (unpublished observations). Eight controls lived an average of 52.6

(s.d. = 13.6) days and seven 5-krad animals 46.9 (s.d. = 9.4) days (F 1,13 = 0.887, $p > 0.10$).

We do not know exactly how long the initial radiation treatment affects sperm production. As our males were housed with the female for 25 days, they had ample opportunity to mate more than once, first after the final molt, and during subsequent days while the adult female fed. If an irradiated male eventually produced viable sperm, then it is possible that at least some of the eggs of a second female sac would hatch. Thirty three of our females produced a second clutch. Of the 12 second control clutches, 12 second 2-krad clutches, and 9 second 5-krad clutches, 11 (91.7%), 0%, and 0% hatched, respectively ($\chi^2 = 28.875$, $df = 2$, $p < 0.0001$). Our initial radiation treatment was effective for at least 25 days which is comparable to "at least 10 days" noted by Jackson (1980) for the jumping spider *Phidippus johnsoni*. To assess longevity of irradiation effects, further data are needed on the normal course of spermatogenesis in this species and egg productivity of females mated with one irradiated male.

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