Herpetologica, 40(4), 1984, 410–415

THE POTENTIAL FOR LONG-TERM SPERM COMPETITION IN A PLETHODONTID SALAMANDER

LYNNE D. HOUCK AND KURT SCHWENK

ABSTRACT: Fourteen *Desmognathus ochrophaeus* females each laid a complete clutch of fertilized eggs in the laboratory. The spermatheca of each female was examined histologically to determine whether stored sperm were retained after ovulation. All 14 females retained apparently viable sperm which were histologically indistinguishable from spermathecal sperm observed in pre-ovulatory females. Multiple mating and sperm storage are necessary criteria for sperm competition within a single oviposition period, but the storage of viable sperm even after oviposition creates the potential for long-term sperm competition from one oviposition period to the next.

Key words: Sperm competition; Plethodontidae; *Desmognathus ochrophaeus*

COMPETITION for mates can be an important agent of sexual selection, and typically it is males that compete for access to mates. Differences among males in the number of offspring sired, however, are not necessarily a simple function of the number of females each inseminates. Determining paternity can be difficult if a female has multiple mates and if she can store sperm for extended periods until it is needed for fertilization. In such species, paternity is a component of male fitness which can be affected not only by competition for mates but also by sperm competition.

Sperm competition is defined by Parker (1970:527) as "the competition within a single female between the sperm from two or more males for the fertilization of the ova." The particular outcome of sperm competition (first male, mixed, or last male paternity) can have significant effects on behavior, physiology and morphology. In one example, Waage (1979) showed for damselflies (*Calopteryx maculata*) that the male's intromittent organ functions first to

remove most of the sperm already stored by the female and then to deliver its own sperm to her. The act of sperm removal greatly reduces the chance that previous mates will produce progeny while increasing the likelihood that the female's last mate will fertilize all or most of her ova.

Sperm competition is well documented for some animal groups, particularly for insects (Gwynne, in press; Parker, 1970; Walker, 1980) and spiders (Austad, 1982, in press). Compared with arthropods, sperm competition is not well known for vertebrates, although several studies (see Smith, in press) now are examining reproduction in terms of the criteria for sperm competition. Parker (1970, in press) considered these criteria to include insemination by multiple males and storage of viable sperm through at least one oviposition period. Among vertebrates, most salamander species meet these criteria. The female possesses a specialized sperm storage organ, the spermatheca, in which viable sperm can be maintained for months (and in some cases years) before ova are fertilized (Boisseau and Joly, 1975).

Promising candidates for studies of sperm competition are plethodontid salamanders, such as *Desmognathus ochrophaeus*, which have a completely terrestrial courtship (Organ, 1961). Unlike aquatic-breeding species of ambystomatids and salamandrids, the courtship season for *D. ochrophaeus* is lengthy. The reproductive season typically includes two multi-month courtship periods, autumn and spring, which are separated by a winter period of inactivity due to unfavorable weather (Houck, 1977; Tilley, 1973). The female stores sperm from fall and spring inseminations until late spring or summer when ova are fertilized just prior to oviposition. Male parental care is absent, but the female guards the eggs for ca. 2 mo while hatchlings develop. A female can live for several years and lay many clutches during her lifetime; oviposition occurs annually, or perhaps biennially depending on food availability and energy reserves (Organ, 1961; Tilley, 1973).

The actual effects of sperm competition are poorly known for salamanders (Halliday and Verrell, in press) although the capabilities of species such as *D. ochrophaeus* for long-term sperm storage and multiple mating suggest that sperm competition can strongly affect individual male fitness via differences in paternity. We hypothesize that, in addition to the possibility that sperm competition is a factor of major importance for male reproductive success within each reproductive season, such competition potentially can be carried over to successive reproductive periods. The purpose of this study was to determine whether females of *D. ochrophaeus* continue to retain stored sperm even after the fertilization and oviposition of a complete clutch.

Materials and Methods

We collected *Desmognathus ochrophaeus* from two different localities in the Appalachian Mountains: Unaka Mountain, Unicoi Co., Tennessee (August 1980); and Mt. Rogers, Smyth Co., Virginia (April 1981). Immediately after collection, we brought salamanders to the laboratory and housed them individually at 15 C on a natural (Chicago) photoperiod and fed them with *Drosophila* spp. ad libitum.

The number of inseminations that may have taken place in the field is unknown for the 14 females examined here. In the laboratory, two females were not inseminated, and the remaining 12 were inseminated from 1–12 times (mode = 2) between the date of collection and May 1981. Each of the 14 females subsequently laid a complete clutch of fertilized eggs (larval development was observed for all eggs) in the laboratory during July 1981. No female had access to a potential mate for at least 4 wk before oviposition and at no time after oviposition.

Within 4 wk after oviposition, we anesthetized females in 33% chloretone solution, preserved them in 10% neutral-buffered formalin for 24 h, soaked them in water for 24 h, and then placed them in 70% ethanol. We dissected the spermathe-

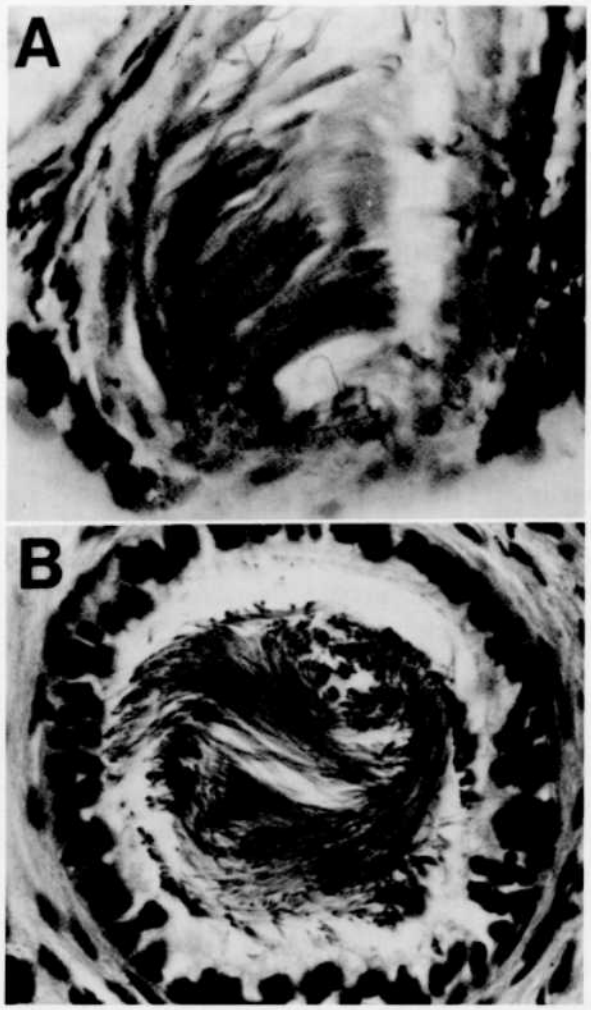

FIG. 1.—Sperm storage by females is revealed in cross-sections of spermathecal tubules from two *D. ochrophaeus*: (A) from a female preserved before ovulation, and (B) from a female preserved after oviposition of a complete clutch of fertilized eggs. Diameter of each tubule is ca. 150 μm. Sperm heads are oriented towards spermathecal cells and sperm tails appear as thread-like clumps extending into the spermathecal lumen. Some differences in appearance are due to different stains: (A) with alcian blue, and (B) with hematoxylin and eosin. Thickness of sections (10–14 μm) precludes sharp focus in photographs.

ca and surrounding tissue from each female. Ten of the specimens included part of the vertebral column so we decalcified the tissue in 0.1% HCl for 4 h. We embedded specimens in paraffin, sectioned at 6, 7 or 10 μm, and stained with hematoxylin and eosin using standard histological techniques (Humason, 1979). We photographed histological sections of spermathecae from pre- and post-ovulatory females using Olympic (Fig. 1A) and Nikon (Fig. 1B) photomicroscopes. The histological section for the preovulatory female is courtesy of David Sever (slide #5547-3 AB).

RESULTS

Spermatozoa were in the spermatheca of each of the 14 females that had oviposited previously (see Fig. 1B). In all but two, sperm were abundant (similar to fig. 1 in Marynick, 1971). Sperm were organized in clumps with their heads oriented towards the spermathecal epithelium and their tails extending into the lumina of spermathecal tubules, sometimes entirely filling them. There was no evidence of sperm degeneration; all sperm were intact and appeared histologically similar to stored sperm from preovulatory females (Fig. 1A). We observed no relationship between relative abundance of sperm and number of laboratory inseminations.

A secretory epithelium ranging from cuboidal to columnar in cell type lined spermathecal tubules. The epithelium was most highly developed (columnar) in the distal portions of the tubules where sperm were most abundant. There was no regular association between sperm and the secretory cells, although a few sperm had their heads apparently buried within or between cells. There was a direct link between spermatozoa and secretory epithelial cells of the spermatheca for another plethodontid (*Eurycea quadridigitatus*; Pool and Hoage, 1973; Trauth, 1983), and evidence available for salamandrids also indicated that spermathecal epithelium was definitely involved in the maintenance of stored sperm (Boisseau, 1971; Boisseau and Joly, 1975; Dent, 1970).

DISCUSSION

Evidence presented here documents that females of at least one salamander species, *Desmognathus ochrophaeus*, retain stored sperm not only within one courtship season but even after oviposi-

tion. Significance of long-term retention of sperm depends on whether this sperm is capable of fertilizing ova when the female next ovulates. We assume that sperm retained by a post-ovulatory female are viable because, at the light microscopy level, they are abundant, the sperm appear in good condition, and are histologically identical with sperm stored prior to oviposition. Histological analysis alone cannot determine viability of spermatozoa, but this analysis does indicate a potential for sperm competition which has not previously been considered. Thus sperm from a male that inseminates a female during a given mating season might fertilize ova not only at the end of that season (when oviposition occurs) but could also fertilize ova at the end of a subsequent mating season. Male–male competition for mates potentially can affect the number of progeny that a male sires in clutches laid not only during one season but also during the next. Retention and storage of viable sperm after oviposition is a mechanism that could lower the fertilization success of a female's "second-season" mates. To the extent that this mechanism increases the variance in male reproductive success (number of progeny sired), the general level of male–male competition can be intensified and the opportunity for sexual selection is increased (Wade and Arnold, 1980).

Retention of viable sperm after oviposition may be common among plethodontid salamanders, but few studies provide direct evidence concerning sperm storage and oviposition in this group. In his pioneering work on the spermatheca and fertilization in a variety of American salamanders, Kingsbury (1895) noted that *Desmognathus fuscus* differed from *Plethodon* spp. in that *D. fuscus* ". . . taken in the summer, fall, winter and spring were examined, and in all the spermathecas were well filled" (ibid., p. 287). Kingsbury's work was based on a relatively small number of specimens, but his observations are supported by more recent studies. Marynick (1971), for example, examined spermathecae from 38 adult females of *Desmognathus fuscus*. He found sperm to be present throughout the year, even in months directly after the general period of oviposition for his study population. Marynick did not mention whether any female in his sample had recently oviposited (as indicated by relatively enlarged oviducts and the lack of enlarged ovarian eggs). Also, females that he collected potentially had access in the field to courting males; we cannot completely rule out the possibility that a spent female was inseminated shortly after having oviposited, although this seems unlikely. In a study of two related plethodontids, *Plethodon cinereus* and *P. dorsalis*, Sever (1978) found that the presence or absence of stored sperm varied seasonally among adult females (operationally defined as females that possessed at least some enlarged yolky ova). Oviposition for these populations occurs in late May or early June, and every female collected in April (11 *P. cinereus* and nine *P. dorsalis*) had sperm in her spermatheca. In contrast, all females collected between June and August (27 *P. cinereus* and 12 *P. dorsalis*) lacked stored sperm. These data suggest that sperm are not retained after oviposition in these *Plethodon* species, since it is unlikely that Sever's sample of 39 females collected between June and August included only those that had never previously mated or laid eggs. Sayler (1966) also showed, for a different population of *P. cinereus*, that females collected after the oviposition period lacked stored sperm, although sperm were present in spermathecae in months preceding oviposition. Trauth (1983) found the same phenomenon of no stored sperm after oviposition in another plethodontid, *Eurycea quadridigitata*, and Sever (personal communication) the same for *Eurycea bislineata*. Thus, among the species for which there are data, only the two *Desmognathus* species show evidence for sperm retention after oviposition.

The significance of retained sperm in *Desmognathus* spp. could be determined experimentally. Females inseminated in

the laboratory subsequently could be sequestered from males through two oviposition periods. Production of fertilized eggs during the second period would show that retained sperm were viable. Methods for facilitating spontaneous oviposition in the laboratory are described in Houck et al. (in press).

The potential for sperm competition is high in salamanders, especially among plethodontid species with a prolonged courtship season preceding oviposition. Females may be inseminated by many different males during the season. In an electrophoretic study of *D. ochrophaeus* populations, Tilley and Hausman (1976) compared females and their clutches and found that at least 7% of clutches contained offspring sired by two or more different males. The 7% value is a minimum estimate, however, since electrophoretically identical sires could not be distinguished. A different study of *D. ochrophaeus* (Houck et al., in press) suggests that last-male paternity is not the case, and that the number of offspring sired by a given male may depend on the number of times the female has previously been inseminated by other males. We could better assess the importance of sperm competition (both within and between seasons) and its effect on male reproductive success with field studies that estimate the natural incidence of multiple inseminations and the nature of male–male competition for mates.

Acknowledgments.—We thank Marvalee Wake for the use of her laboratory facilities for our histological work. David Sever kindly provided the histological section photographed for Fig. 1A. Constructive comments on the manuscript were made by S. Arnold, D. Fraser, T. Halliday, D. Sever, J. Spotila, P. Verrell and M. Wake. This work was supported by NSF BNS 80-14151 to S. Arnold. KS was supported in part by NSF DEB 80-05905 to M. Wake.

Literature Cited

Austad, S. N. 1982. First male sperm priority in the Bowl and Doily spider, *Frontinella pyramitela* (Walckenaer). Evolution 36:777–785.

———. The evolution of sperm priority patterns in spiders. *In* R. L. Smith (Ed.), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, New York. In press.

Boisseau, C. 1971. Etude en culture organitypique du role de la spermatheque et du canal de Wolff dans la survie des spermatozoides de *Salamandra salamandra* (L.). Ann. Sci. Nat. Zool. 13:163–180.

Boisseau, C., and J. Joly. 1975. Transport and survival of spermatozoa in female Amphibia. Pp. 94–104. *In* E. S. E. Hafez and C. G. Thibault (Eds.), The Biology of Spermatozoa. S. Karger AG, Basel, Switzerland.

Dent, J. N. 1970. The ultrastructure of the spermatheca in the red spotted newt. J. Morphol. 132: 397–423.

Gwynne, D. T. Male mating effort, confidence of paternity, and insect sperm competition. *In* R. L. Smith (Ed.), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, New York. In press.

Halliday, T., and P. Verrell. Sperm competition in amphibians. *In* R. L. Smith (Ed.), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, New York. In press.

Houck, L. 1977. Life history patterns and reproductive biology of neotropical salamanders. Pp. 43–71. *In* D. H. Taylor and S. I. Guttman (Eds.), Reproductive Biology of Amphibians. Plenum Publ. Corp., New York.

Houck, L., S. G. Tilley, and S. J. Arnold. Sperm competition in a plethodontid salamander: preliminary results. J. Herpetol.: In press.

Humason, G. L. 1979. Animal Tissue Techniques. 4th Ed. W. H. Freeman and Co., San Francisco.

Kingsbury, B. F. 1895. The spermatheca and methods of fertilization in some American newts and salamanders. Trans. Am. Micros. Soc. 17:261–305.

Marynick, S. P. 1971. Long term storage of sperm in *Desmognathus fuscus* from Louisiana. Copeia 1971:345–347.

Organ, J. A. 1961. Studies of the local distribution, life history, and population dynamics of the salamander genus *Desmognathus* in Virginia. Ecol. Monogr. 31:189–220.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. 45:525–568.

———. Sperm competition and the evolution of animal mating systems. *In* R. L. Smith (Ed.), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, New York. In press.

Pool, T. B., and T. R. Hoage. 1973. The ultrastructure of secretion in the spermatheca of the salamander, *Manculus quadridigitatus* (Holbrook). Tissue Cell 5:303–313.

Sayler, A. 1966. The reproductive ecology of the red-backed salamander, *Plethodon cinereus*, in Maryland. Copeia 1966:183–193.

Sever, D. 1978. Female cloacal anatomy of *Plethodon cinereus* and *P. dorsalis* (Amphibia, Urodela, Plethodontidae). Herpetologica 12:397–406.