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REMOVAL OF COPULATORY PLUGS BY FEMALE TREE SQUIRRELS

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Copulatory plugs form in the vagina of females from the semen of male fox squirrels (*Sciurus niger*) and eastern gray squirrels (*S. carolinensis*). Plugs are opaque white with waxy to rubbery consistency resembling those of other rodents. Although copulatory plugs frequently are hypothesized to prevent the successful copulation of subsequent males, female tree squirrels often remove the plug within 30 s of copulating and either discard or consume it. The removal by the female of a copulatory plug suggests that conflict may exist between the reproductive strategies of the sexes.

Key words: *Sciurus*, reproduction, copulatory plugs

Copulatory plugs are reported from a variety of vertebrate taxa, but are especially well known from the rodents (Baumgardner et al., 1982; Hartung and Dewsbury, 1978; Voss, 1979). Rodent plugs form in the vagina solely from the coagulation of male semen including secretions from the prostate, seminal vesicles, and Cowper's glands (Mann and Lutwak-Mann, 1981). At least five functions of copulatory plugs are hypothesized (Voss, 1979): sperm storage, plugs release spermatozoa as they disintegrate in the female; sperm leakage, plugs prevent leakage of sperm from the cervix or vagina; pseudopregnancy, plugs provide stimulation of the cervix to induce pseudopregnancy; sperm transport, plugs facilitate the transport of sperm across the cervix; chastity enforcement, plugs prevent subsequent inseminations of the female by additional males. Voss (1979) concluded that the available literature best supports the chastity enforcement hypothesis.

Several sciurids, including white-tailed prairie dogs (*Cynomys leucurus*; Asdell, 1964), tassel-eared squirrels (*Sciurus aberti*; Farentinos, 1980), eastern gray squirrels (*Sciurus carolinensis*; Deanesley and Parkes, 1933), California ground squirrels (*Spermophilus beecheyi*; Tomich, 1962), Colum-

bian ground squirrels (*S. columbianus*; Murie and McLean, 1980), Arctic ground squirrels (*S. parryi*; Murie and McLean, 1980), Richardson's ground squirrels (*S. richardsonii*; Michener, 1984), and thirteen-lined ground squirrels (*S. tridecemlineatus*; Foster, 1934) produce copulatory plugs. Sciurid copulatory plugs are moist, opaque, creamy white, and rubbery (Michener, 1984; Murie and McLean, 1980). Structurally, plugs consist of a central acellular core and thin layers of external epithelial cells and sperm (Michener, 1984); the core likely forms from secretions of male accessory glands (Mossman et al., 1955). Therefore, copulatory plugs of the Sciuridae are similar to those of other rodents.

Copulatory plugs in ground squirrels are retained for as long as 19 h (Michener, 1984; Murie and McLean, 1980). Some, and possibly the majority, of recently mated females did not possess plugs when captured several hours after copulation (Michener, 1984; Murie and McLean, 1980). However, because ground squirrels usually mate underground, the fate of copulatory plugs prior to capture is unknown. Most rodent plugs form in <15 min (Hartung and Dewsbury, 1978) and likely form in <1 min (Dewsbury, 1988). Herein, I describe the copu-

latory plugs of eastern gray squirrels (*S. carolinensis*) and fox squirrels (*S. niger*), report field observations of the immediate fate of copulatory plugs during the first few minutes after copulation, and interpret these results in light of knowledge of plug retention and positioning gleaned from other studies.

MATERIALS AND METHODS

The study area was a 4.2-ha black walnut (*Juglans nigra*) dominated parkland located on the University of Kansas campus, Lawrence, Douglas Co., Kansas. The ground cover was mowed grass; the absence of a shrub stratum and the habituation of squirrels to humans facilitated observations. Since May 1986, squirrels were trapped and marked for identification at a distance; methods were described elsewhere (Koprowski, 1991a). Observations focused on the winter breeding season, due to difficulties in observing copulations during the May–June breeding period because of leaf cover. Each morning prior to dawn from mid-December to mid-February 1988–1989 and 1989–1990, I traversed the study area in search of males congregating in the vicinity of the den of an estrous female. When a mating bout was detected, the activities of the female were followed continuously until bout termination. The behavior of the female after each copulation was observed and the fate of the copulatory plug described. Discarded plugs were collected immediately and frozen at -5°C until measurement. Volume was determined by water displacement. Plugs were fixed in a paraffin block, sliced at a thickness of $4\ \mu\text{m}$, stained with hematoxylin and eosin, and examined at a magnification of $100\text{--}400\times$. Sample sizes differ due to damage incurred when the plug was thrown to the ground by the female and because I collected plugs even if I was unable to observe all postcopulatory behavior.

RESULTS

The post-copulatory behavior of females from 22 copulations involving eight female fox squirrels and 26 copulations involving eight female gray squirrels occurred in unobscured locations permitting continuous and detailed observation. Following a copulation, females immediately groomed their genitalia (Horwich, 1972; McCloskey

and Shaw, 1977). While grooming, females frequently used their incisors to remove the copulatory plug from the vagina within 30 s of copulation (*S. niger*: 72.7%, 16 of 22 copulations; *S. carolinensis*: 50.0%, 13 of 26 copulations). All eight female fox squirrels and seven of eight female gray squirrels removed at least one plug during their mating bout. The female either consumed the plug (*S. niger*, 75.0%, 12 of 16 plugs; *S. carolinensis*, 61.5%, 8 of 13 plugs) or discarded it. Plugs likely form during most or all copulations yet I never observed a female dislodge a plug >30 s after copulation during the remainder of the day of estrus. The fate of plugs that are not removed immediately by the female is unknown, but a female that I captured after a copulation had retained a copulatory plug for >2.5 h. For mating bouts in which I observed all copulations, females were equally likely to remove plugs after the first copulation (8 of 15 plugs removed; 5 of 8 plugs of *S. niger*, 3 of 7 plugs of *S. carolinensis*) and the final copulation (8 of 15 plugs removed; 4 of 8 plugs of *S. niger*, 4 of 7 plugs of *S. carolinensis*).

Plugs were milky white and slightly opaque with a moist waxy to rubbery consistency. Small plugs ($<10\%$ of the volume of fully formed plugs) resulting from short, prematurely terminated, copulation attempts (<10 s) apparently without ejaculation were transparent and brittle. Plugs apparently begin to form from seminal fluids released before ejaculation. In this study, I only considered plugs that resulted from copulations that were not terminated prematurely. Copulatory plugs of fox squirrels averaged 1.66 ± 0.25 cm long ($n = 7$), 1.04 ± 0.20 cm wide ($n = 6$), and 0.85 ± 0.19 cm high ($n = 4$). Copulatory plugs of gray squirrels averaged 1.57 ± 0.21 cm long ($n = 5$), 0.93 ± 0.14 cm wide ($n = 5$), and 0.85 ± 0.11 cm high ($n = 5$). Plugs of four fox squirrels and three gray squirrels were completely undamaged and available for volumetric analysis. Plugs of fox squirrels averaged 0.70 ± 0.14 ml and the single plug that could be weighed before desiccation was

1.11 g; plugs of gray squirrels averaged 0.53 ± 0.13 ml and the only plug that could be weighed before desiccation was 0.75 g.

Histologically, copulatory plugs of both species were composed of an acellular core with an outer layer of columnar and squamous epithelial cells likely from the vaginal wall. Spermatozoa were found on and shallowly imbedded in the outside of the plug.

DISCUSSION

The copulatory plugs of eastern gray squirrels and fox squirrels were similar in appearance and structure to the plugs reported for other sciurids (Michener, 1984; Murie and McLean, 1980). Because most ground squirrels mate in burrows, the formation time and immediate fate of their copulatory plugs are unknown. Evidence from wild-caught Richardson's ground squirrels suggests that plugs likely form in <1 h (Michener, 1984). Plugs of tree squirrels form within a few seconds of ejaculation.

The majority of female ground squirrels lacked copulatory plugs when captured within hours of mating, but it is not known whether plugs failed to form, were lost, or were removed (Michener, 1984; Murie and McLean, 1980). Because small plugs form even without ejaculation in tree squirrels, plugs likely form from all copulations with some removed by females soon after copulation. The removal of copulatory plugs by females was not reported in other rodents; however, Michener (1984) noted that two plugs apparently were consumed by female Richardson's ground squirrels. Observations of mammals in the wild or under semi-captive conditions are necessary to examine how widespread this potentially important behavior might be.

Why do females remove copulatory plugs? At least three hypotheses that are not mutually exclusive can be postulated. First, females may simply remove a protruding copulatory plug while grooming much as an ectoparasite is removed and eaten. There-

fore, plug removal is of no adaptive significance and is solely an artifact of another behavioral pattern. Second, if the position of the plug (deeply imbedded or protruding from the vagina) in some way indicates the quality or effectiveness of a copulation then the removal of the plug could be significant if the plug differentially influences the reproductive success of males or females. Plugs of ground squirrels are found in a variety of depths and to adhere with different tenacities in the female reproductive tract (Michener, 1984; Murie and McLean, 1980). Twice I observed a male begin ejaculating outside of the female after the penis momentarily dislodged during pelvic thrusting, but the male quickly replaced his penis in the vagina. Protruding plugs formed and were removed by the female after copulation. If the plug serves as a physical barrier to future copulations by males and a protruding plug indicates an ejaculation with insufficient sperm transfer near the proximate end of the vagina, females may remove the plug to permit other males to copulate. Finally, the removal of plugs by females may represent a conflict in the reproductive strategies of males and females. The interpretations of the function of copulatory plugs that have the most empirical support focus on maximizing the reproductive success of the male (Dewsbury, 1988; Voss, 1979). In several sciurids, multiple-mating by the female results in multiple paternity (Foltz and Schwagmeyer, 1989; Sherman, 1989) and likely reduces the reproductive success of any one male. Multiple-mating by female tree squirrels (Koprowski, 1991*b*), as hypothesized for ground squirrels (Schwagmeyer, 1984), may assure fertilization and genetic heterogeneity of offspring. The removal of the plug by the female may permit her to accrue the benefits of multiple-mating, but to the detriment of males due to the dilution of paternity.

Order of mating affects reproductive success in many rodents (Dewsbury, 1984; Foltz and Schwagmeyer, 1989; Sherman, 1989). Copulatory plugs effectively reduce the suc-

cess of subsequent males in some species (Martan and Shepherd, 1976), but not in others (Dewsbury, 1988). If a last-male advantage exists, then the cost to the male of plug removal by the female may be great if the plug influences male reproductive success. Given the frequent removal of plugs, guarding the female may represent a behavioral alternative to assure paternity (Sherman, 1989). Guarding is an evolutionarily stable strategy in *Spermophilus brunneus*, a species with an apparent last-male advantage (Sherman, 1989). *S. tridecemlineatus* and *S. beecheyi*, species with a first-male advantage, do not guard (Schwagmeyer and Parker, 1990; Sherman, 1989). The high frequency (>95% of copulations) of guarding by both gray squirrels and fox squirrels (Koprowski, 1991b) implies that a last-male advantage may exist in both species. Unfortunately, the function and significance of copulatory plugs and their removal by females will remain speculative until the influence of the presence of the copulatory plug, multiple-mating, and mating order on male and female reproductive success is ascertained.

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