Journal of Mammalogy, 83(4):1035–1038, 2002

COPULATORY PLUGS IN MASKED PALM CIVETS: PREVENTION OF SEMEN LEAKAGE, SPERM STORAGE, OR CHASTITY ENHANCEMENT?

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Several hypotheses have been advanced to explain the functions of copulatory plugs: to store spermatozoa, reduce postcopulatory leakage of semen, or prevent further matings. These hypotheses were examined in captive masked palm civets (*Paguma larvata*). The results showed that spermatozoa were absent in histological sections of copulatory plugs, and copulatory plugs were dislodged from the vagina during a female's 2nd copulation (time to dislodgment 10.8 min ± 1.2 *SE*). Time to dislodgment was significantly reduced if copulations with 2 males occurred in rapid succession (6.3 ± 2.7 min). However, some plugs were retained up to 18–22 h after 2nd copulations. Females sometimes mated with 2 partners during 1 night, and plugs of the males could be distinguished morphologically. These results suggest that the copulatory plug functions primarily to prevent leakage of spermatozoa from the reproductive tract of the female.

Key words: copulatory plug, masked palm civet, *Paguma larvata*, sperm competition

Copulatory plugs have been reported in diverse animal groups including insects, reptiles, bats, rodents, and primates (Baumgardner et al. 1982; Dixson 1998; Hartung and Dewsbury 1978; Stockley 1997; Voss 1979). Plugs are formed primarily from the male's accessory sexual secretions (Mann and Lutwak-Mann 1981; Voss 1979). In the well-studied Norway rat (*Rattus norvegicus*), copulatory plugs are formed immediately after ejaculation, and mass of the plug is correlated positively with mass of the seminal vesicles. Formation and structure of copulatory plugs depend upon secretions of the seminal vesicles, and the plug does not attach to walls of the vagina if seminal vesicles are partly removed (Carballada and Esponda 1992, 1993). Voss (1979) listed 5 possible functions of copulatory plugs in rodents: to facilitate gradual release of spermatozoa within the female reproductive tract; to prevent semen leakage; to induce pseudopregnancy; to facilitate sperm transport; to prevent subsequent insemination by other males (''chastity-enforcement hypothesis''). He also stated that only the last had empirical support. Michener (1984) found no direct evidence for the chastity-enforcement hypothesis in Richardson's ground squirrels (*Spermophilus richardsonii*) and proposed that plugs might prevent sperm leakage. Similarly, Dewsbury (1988) found no support for the chastity-enforcement hypothesis in deer mice (*Peromyscus maniculatus*). Thus, functions of copulatory plugs remain controversial.

Copulatory plugs have been reported from only 1 species of Carnivora, the masked palm civet (*Paguma larvata*). During the mating season, estrous females may copulate with more than 1 male, and the

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copulatory plug from a previous copulation can become dislodged (Jia et al. 2000, 2001, 2002). This report examines possible functions of copulatory plugs in captive masked palm civets, with respect to 3 hypotheses: sperm storage, semen leakage, and chastity-enforcement hypothesis.

Materials and Methods

The civets we used came from a captive population in Hunan Agricultural University, China. All animals were cared for in accordance with guidelines of the Chinese Wildlife Management Authority. Detailed information concerning captive management is in Jia et al. (2001, 2002).

Two groups (treatments) of civets were established in mid January 1997 with different sex ratios: 1 male and 1 female (this will be referred to as 1:1 SR) and 2 males and 1 female (2:1 SR). All animals were sexually mature. The experimental sex ratios are similar to those in natural populations (Zhang 1997). Each group was kept in a cage measuring 140 by 93 by 190 cm. Each had an activity room and a dark room (85 by 64 by 59 cm). Walls of the cages were made of split bamboo. We collected copulatory plugs that fell through gaps between the bamboo slats. Behavioral observations were made from 1700 to 0900 h under artificial illumination (15-W red light). After a copulation was observed, flashlights wrapped with red cloth were used to identify the dislodgment of a copulatory plug. Red lights had no apparent effects on mating behavior. The interval between the beginning of copulation and dislodgment of plugs was recorded to the nearest second (Jiang 1999). Plugs were wrapped in aluminum foil and stored at −10°C.

We used 18 males and 15 females in the study, including a few individuals in both years and some individuals in both treatments (sex ratios of 1:1 and 2:1). In 1998 we studied 1 pair plus two 2:1 treatment groups (5 males and 3 females in the latter). In 1999 we studied 10 pairs (9 males, 10 females) plus three 2:1 treatment groups (10 males, 10 females). In one 2:1 treatment during 1999, only 1 male copulated, so this was considered to be a 1:1 treatment. Overall 209 plugs from 11 males and 13 females in 1:1 treatments and 7 mixed plugs from 2:1 treatments were collected.

Four plugs produced by 4 different mating pairs in the 1:1 treatment in 1999 were examined for presence of sperm. Slices of frozen plugs were examined under a light microscope (100×). We also cut longitudinal sections of plugs (10 μm thick), stained them with hematoxylin and eosin, and examined them under a light microscope (100×). We also compared plugs of old (≥2-year-old) and young (1-year-old) males. Data are presented as mean ± *SE*.

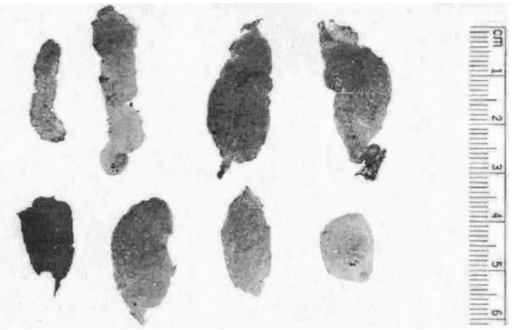

FIG. 1.—Copulatory plugs of masked palm civet (*Paguma larvata*), showing varied shapes. All plugs are from old (≥2-year-old) females and males, except the 2nd one from right, 2nd row, which is from a pair of 1-year-old animals.

Results

Morphology of copulatory plugs.—All plugs were hollow. Most were reddish-orange, some were saffron-yellow or grayish-white. Most were lozenge-shaped, and many had protuberances (Fig. 1). Plugs of subadult males (12 months old) were round or oblong (Fig. 1). Wet mass of plugs from the 1:1 SR group was 886 mg ± 25.9 *SE* (n = 209; range 164–2,226); dry mass was 228 ± 7 mg (n = 208; range 42–560). We observed no spermatozoa in histological sections (Fig. 2).

In the 2:1 SR groups, 2 males in each group copulated repeatedly in turn or 1 male copulated after the other had finished. In such cases, females often disgorged a mixed plug (Fig. 3; 2 instances in 1998, 5 in 1999). Wet mass of mixed plugs averaged 2,089 ± 292 mg (n = 7; range 1,544–3,766), significantly greater than that of plugs from 1:1 SR groups (Levene's test: P = 0.02; t = 4.31, $d.f.$ = 6, 32, P = 0.004); dry mass was 514 ± 65 mg (n = 7; range

FIG. 2.—Structure of copulatory plug of masked palm civet (*Paguma larvata*), showing small cavities and absence of sperm.

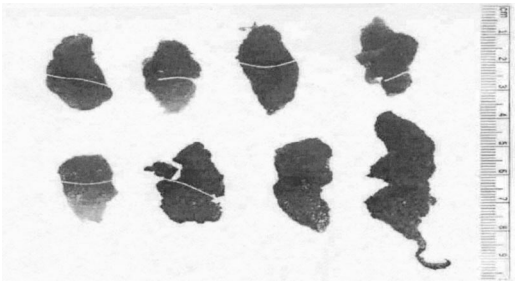

FIG. 3.—Mixed copulatory plugs of masked palm civet (*Paguma larvata*). The white lines mark junctures between plugs in most specimens; junctures are evident in the others.

378–874), significantly different from that of the 1:1 SR treatment (Levene's test: $P = 0.02$; $t = 4.54$, $d.f. = 6, 45$, $P = 0.003$).

Latency to plug dislodgment.—In 1:1 SR, plugs were dislodged soon after the beginning of copulation. The time from 1st mount to dislodgment averaged 10.7 min ± 1.2 *SE* ($n = 37$; range 1.1–35). If a female mated with both males in the 2:1 SR treatment, males typically mounted and ejaculated in turn. After ejaculation by the 1st male, the female always accepted the 2nd male's attempt to mount. The interval between 2 ejaculations was as brief as 20 s. Generally, the female dislodged the plug from the 1st male within 6.5 ± 0.7 min ($n = 10$; range 1.2–10 min), significantly briefer than the 1:1 SR treatment (Levene's test: $P = 0.007$; $t = -2.85$, $d.f. = 45, 68$, $P = 0.006$).

After their last copulation, females sometimes retained the plug from the last male for many hours (18 and 22 h were the longest periods observed). Females that were disturbed by being handled for transfer to other cages dislodged plugs sooner (at 10–15 h).

DISCUSSION

No spermatozoa were identified in copulatory plugs we examined, and all plugs were dislodged completely. Based on these observations, we reject the sperm-storage hypothesis. Our observations also do not support the chastity-enforcement hypothesis. Copulatory plugs from 1st-copulating males did not prevent females from remating, and females always ejected a copulatory plug soon after a 2nd ejaculation. The occurrence of mixed copulatory plugs and the brief interval between ejaculations by 2 males indicate that the plug of the 1st male did not prevent insemination by the 2nd male. This suggests the possibility of mixing of ejaculates and of sperm competition.

Our study provides indirect support for the semen-leakage–prevention hypothesis (Dewsbury 1988; Michener 1984; Voss 1979). Copulatory plugs of masked palm civets are hollow and similar to plugs of Richardson's ground squirrels. They probably result from rapid coagulation of semen (Michener 1984). Plugs became dislodged after a variable period (1 min to 22 h). Thus plugs are formed rapidly and may endure for extended periods. We conclude that the plug is likely to prevent leakage of semen and spermatozoa from the female's reproductive tract after copulation.

ACKNOWLEDGMENTS

We thank the College of Animal Science and Technology, Hunan Agricultural University, for allowing us to use the palm civet colony and for providing housing to observers. We also appreciate the assistance of X. Qu, W. Li, and M. Kang. We thank Y.-T. Lin for assistance with

preparing the manuscript and the Chinese Academy of Sciences Innovation Program (C2999082, KSCX2-1-03, KZ-951-A1-105), the Ministry of Sciences and Technology (G2000046805), and the Chinese Natural Scientific Foundation (3770104) for financial support.

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Submitted 23 October 2001. Accepted 13 May 2002.

Associate Editor was Edward H. Miller.