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Short communication

Mating behavior during the estrus cycle in female Mongolian gerbils (*Meriones unguiculatus*)

By R. Weinandy, Sylvia Hofmann, and R. Gattermann

Institute of Zoology, Martin-Luther-University Halle-Wittenberg, Halle, Germany

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Mongolian gerbils (*Meriones unguiculatus*) are common socially living rodents in the Steppe and semi-desert regions of Mongolia and Mandchuria (Gromov 1990). In their natural habitat, families, grouped around a founder pair, are strictly territorial (Ågren et al. 1989). Male behavior has been shown to be influenced by females (Probst and Lorenz 1987). Since the current literature on the female estrus cycle is limited and ambiguous (Marston and Chang 1965; Nishino and Totsukawa 1996), a redescription appears to be necessary. The aim of the present study was to obtain detailed data on the four stages of the estrus cycle in the Mongolian gerbil.

Adult Mongolian gerbils (*Meriones unguiculatus*) of both sexes from different litters aged 12–28 weeks were selected for this study. They were derived from our own laboratory stock (Zoh:CRW) and were kept in climatised windowless rooms under a photoperiod of LD = 14:10 (lights on at 0500 h CET; 200–300 lx during the light phase, approximately 5 lx during the dark phase). The room temperature was 23 ± 2° C and the relative humidity varied between 65 and 70%. The animals were housed in plastic cages (55 × 33 × 20 cm) with a wire mesh top. Tap-water and food pellets (Altromin® 7024, Altromin GmbH, Lage) were provided ad libitum. The animal bedding (Allspan®, NL) was renewed every two weeks.

Initially, the four different stages of the estrus cycle were defined in adult females (n = 18) by taking vaginal smears daily between two to four hours after lights on, over a period of two months. The stained smears were microscopically analysed (Leica®, Type DMRBE, ×200).

In figure 1 the respective pattern of the four stages of the estrus cycle is depicted. Some females remained in diestrus for up to 14 days, i. e., the cycle became irregular or was arrested for that period of time. However, it was always followed by the preestrus and the estrus cycle proceeded regularly.

Mating tests were performed during the four different stages of the estrus cycle of the gerbils. To prevent gravidity, adult but sexually unexperienced males were sterilized by vasectomy. Two weeks after surgery they were taken to perform mating tests. Vaginal smears were taken from all 24 females to evaluate their stage of estrus cycle two hours before the start of the mat-

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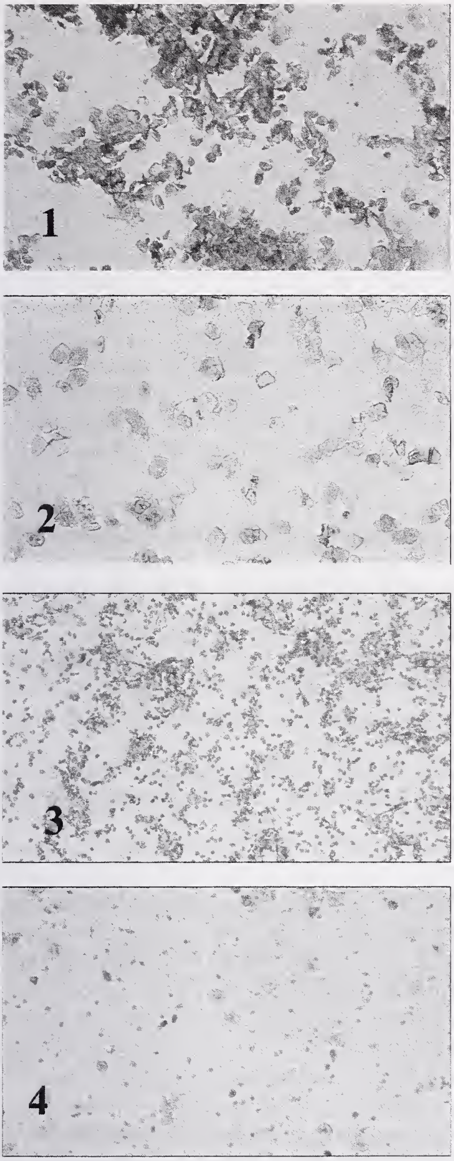

Fig. 1. Vaginal smears during the estrus cycle. Photographed under microscope (×200).
1. Preestrus: high number of squamous epithelial cells, absence of leukocytes and almost no cornified epithelial cells; 2. Estrus: low number of squamous epithelial cells, high number of dispersed cornified epithelial cells and no leukocytes; 3. Metestrus: mainly leukocytes, isolated squamous epithelial cells and/or cornified epithelial cells; 4. Diestrus: low number of leukocytes, no or only a few squamous epithelial cells and/or cornified epithelial cells.

ing tests (20–30 minutes after lights off). The lowest number of females, to which a stage could be unambiguously assigned, counted 11. In the following, always 11 out of 24 females were randomly chosen before every mating test. For each stage the animals were tested in a clean cage with new animal bedding. Ten minutes before the female was introduced, a vasectomized male was put into the cage. Each test lasted for ten minutes and the frequency of the following activities of the females was registered: copulation [c]: female is mounted by the male combined with friction movements; copulation trials [ct]: female presses tail to bottom and prevents the male, which tries to mount the female; lordosis [l]: female remains in front of the male with bent hind paws and lifted tail; copulation avoidance behavior [cab]: female poses head towards the male, vocalizes and/or avoids the male, genitals and tail are directed away. Kruskal-Wallis analysis of variance and subsequent two-tailed Mann-Whitney U-test (Winstat V 3.1) were used to assess differences in the mating tests. Since multiple tests were run on the same basic dataset, the resulting p-values were corrected by the standard Bonferroni procedure. Differences were accepted as significant at $p < 0.05$ (* in Fig. 2).

Figure 2 shows the results of the mating tests. The copulation behavior occurred exclusively in estrus (Kruskal-Wallis H-test: H-value = 20.23, $n = 11$, $p < 0.05$; Mann-Whitney U-Test estrus vs. preestrus, metestrus and diestrus: in all cases $U = 27.5$, $p = 0.0346$). The number of copulation trials was highest during the preestrus and lowest at diestrus. This difference was significant (Kruskal-Wallis H-test: H-value = 10.86, $n = 11$, $p < 0.05$; Mann-Whitney U-Test preestrus vs. estrus: $U = 31$, n. s.; preestrus vs. metestrus: $U = 26.5$, n. s.; preestrus vs. diestrus: $U = 16$, $p = 0.0188$; estrus vs. metestrus: $U = 48$, n. s.; estrus vs. diestrus: $U = 35.5$, n. s.; metestrus vs. diestrus: $U = 50.5$, n. s.). The lordotic behavior was mainly shown in the estrus (Kruskal-Wallis H-test: H-value = 18.37, $n = 11$, $p < 0.05$; Mann-Whitney U-Test preestrus vs. estrus:

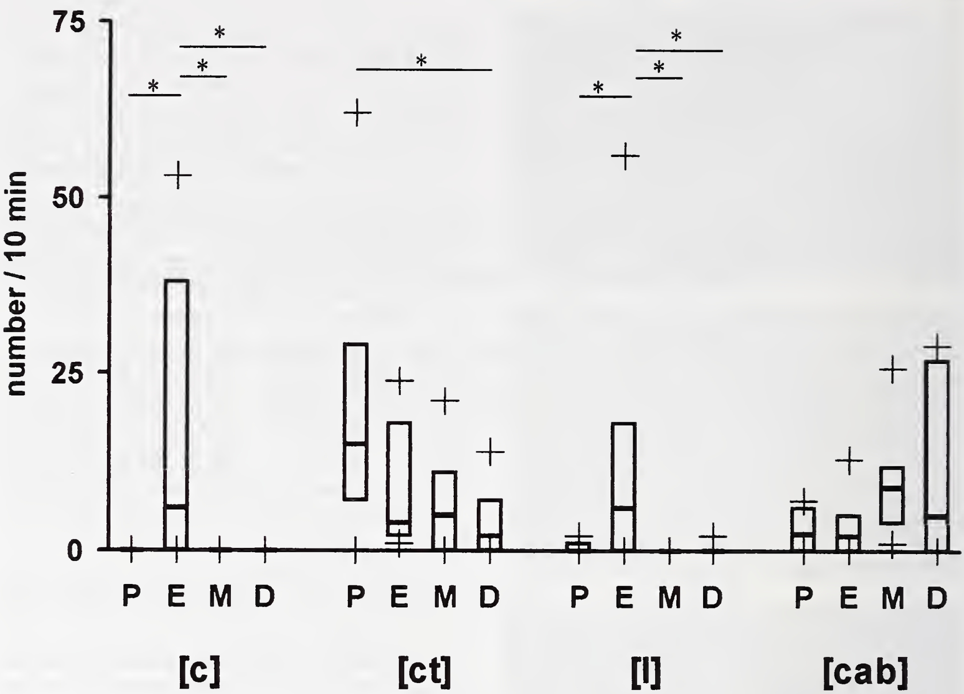

Fig. 2. Frequency of behavioral parameters during the mating tests. Females (n = 11) were tested during preestrus (P), estrus (E), metestrus (M) and diestrus (D).
[c] = copulation; [ct] = copulation trials; [l] = lordosis; [cab] = copulation avoidance behavior. Median values and the interquartils are shown, differences are significant at $p < 0.05$ and given as asterisks in the graph.

U = 21.5, p = 0.0369; preestrus vs. metestrus: U = 44, n. s.; preestrus vs. diestrus: U = 57, n. s.; estrus vs. metestrus: U = 16.5, p = 0.0048; estrus vs. diestrus: U = 20.5, p = 0.0244; metestrus vs. diestrus: U = 12.5, n. s.). There were no significant differences concerning copulation avoidance behavior towards the males during the estrus cycle (Kruskal-Wallis H-test: H-value = 7.21, n = 11, n. s.). The morning after the females were tested in estrus, 7 of the 24 tested females developed a vaginal plug.

In various rodents the uterus and the vagina as targets of ovarian hormones show cycle-dependent proliferation and apoptosis of luminal and glandular epithelium (SATO et al. 1997). The periodical increase and decrease of squamous epithelial cells, leukocytes and cornified epithelial cells in vaginal smears is a consequence of these changes and has already been described for rats (OTHA 1995) or golden hamsters (SANDOW et al. 1979; GATTERMANN et al. 1985) and reliably indicates the estrus. In gerbils, the preestrus used to be characterized by an increased number of squamous epithelial cells and the absence of leukocytes (NISHINO and TOTSUKAWA 1996). The aggressiveness of the females was low and they displayed only minor copulation avoidance behavior towards the males. This belongs to precopulatory behavior which may have a proceptive function (HOLMAN et al. 1985). The estrus stage is a period of characteristic behavior including sexual receptivity (lordotic posture) in confrontation with males and the related vaginal smear pattern has already been described (BARFIELD and BEEMANN 1968; ADAMS and NORRIS 1973; VICK and BANKS 1969). A further indicator for the receptivity in ***Meriones unguiculatus*** is a vaginal plug (Marston and Chang 1965; Norris and Adams 1981). Due to the receptive stage, the interactions initiated by the females were not aggressive during the mating tests. The typical cellular pattern of metestrus was in some cases preceded by clustered cornified cells and isolated leukocytes. This has already been described in a previous study and classified as "estrus II" (Nishino and Totsukawa 1996). Our data do not confirm this suggestion, because our vaginal smear alike was always connected to metestrus behavior. A possible explanation for these contradictory results may be found in the diverging procedure, i. e., in the cited investigation the animals were injected with pregnant mare serum gonadotropin and human chorionic gonadotropin. The elevated level of gonadotropin might have extended the estrus without affecting the vaginal epithelium. In the present study the females displayed no sexual behavior in that stage. The diestrus is generally defined as a "state of rest" between met- and preestrus, when the female was not fertilized. As described in an earlier study (Ågren and Meyerson 1977) the behavior of the females is agonistic and biased towards avoidance. Our analysis of the estrus cycle revealed characteristic changes in mating behavior of female gerbils.

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Authors' address:
René Weinandy, Sylvia Hofmann, and Rolf Gattermann, Institut für Zoologie, Martin-Luther-Universität Halle-Wittenberg, Domplatz 4, D-06108 Halle, Germany
(e-mail: weinandy@zoologie.uni-halle.de)