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THE ESTROUS CYCLE AND INDUCTION OF ESTROUS BEHAVIOR IN FOUR SPECIES OF HAIRY-FOOTED GERBILS (GENUS *GERBILLURUS*)

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Little information is available on the reproductive biology of species of the genus *Gerbillurus*, endemic to southern Africa (Smithers, 1983). A captive colony including the species *G. paeba paeba*, *G. paeba exilis*, *G. tytonis*, *G. setzeri*, and *G. vallinus* has been maintained for approximately 4 years. Breeding occurs sporadically and unpredictably.

Recently, a project was initiated to investigate behavioral mechanisms whereby potential mates are recognized. For purposes of this study, it was decided to attempt to determine the length and class of estrous cycle, and to induce estrous behavior artificially by means of hormone injections (Dewsbury and Hartung, 1982).

Thirty-four wild-caught females representing all species were caged singly in laboratory rodent cages (40 by 24 by 12 cm) or in terraria (60 by 30 by 30 cm). A 4-cm layer of sand was placed in the cages and grass was supplied as nesting material. Temperature was maintained at 25°C and relative humidity at 35%. The light cycle was reversed and set at 16L:8D, with a 100-W white light bulb providing illumination. Approximately 50 animals were housed in a single 16-m^2 animal room. Food and water were provided ad libitum and consisted of mixed seeds, "Pronutro" (commercial cereal mixture), fresh plant material, and insects.

The taking of vaginal smears from 16 females having perforate vaginae commenced approximately 6 weeks after capture and was performed daily for 4 weeks. Smears were taken by gently rotating a small cotton swab moistened with normal saline against the vaginal wall (Snell, 1941). Smears were stained with methylene blue and examined immediately. The presence or absence of nucleated epithelial cells, cornified cells, and leucocytes was recorded and their relative abundances in the smear noted. The cytological characteristics of the four stages of the estrous cycle have been described elsewhere (Champlin et al., 1973; Dewsbury et al., 1977). Two females that had shown changes in vaginal smears were sacrificed and their ovaries sectioned, stained with haematoxylin-eosin, and examined for presence of corpora lutea.

Cyclical changes in vaginal smears were exhibited by three *G. p. exilis*, four *G. p. paeba*, two *G. tytonis*, and two *G. vallinus*. Cycle lengths were assessed as the interval between smears that showed a virtual absence of leucocytes. Mean ($\pm SD$) cycle length in days was 7.2 ± 1.5 for *G. p. exilis* (nine cycles), 6.8 ± 1.7 for *G. p. paeba* (eight cycles), 6.2 ± 1.2 for *G. tytonis* (five cycles) and 11.3 ± 1.2 for *G. vallinus* (three cycles). Smears entirely devoid of nucleated cells were rare in all species.

Mongolian gerbils (*Meriones unguiculatus*) were reported to be spontaneous ovulators, but a regular, predictable cycle was difficult to detect (Marston and Chang, 1965). Vaginal smears were useful only as indicators of approaching estrus in this species, most matings coinciding with a smear containing epithelial and cornified cells (Barfield and Beeman, 1968). The only reliable method for identifying estrus was daily pairing with reliable indicator males (Vick and Banks, 1969). Burley (1980), however, identified the smear associated with estrus as being devoid of leucocytes, but showing a transition from nucleated to cornified cells.

The results of vaginal smears suggest that *Gerbillurus* species are spontaneous ovulators, supported by the presence of corpora lutea in the ovaries of two unmated females. Stutterheim and Skinner (1973) reported the presence of primary and secondary follicles, but no corpora lutea, in 21 wild-caught *G. p. paeba*. Among muroid rodents, microtines are induced ovulators with irregular cycles, whereas virtually all other muroid species are spontaneous ovulators with short (<8 day) cycles and induced corpus-luteum formation (Dewsbury et al., 1977).

The predictive value of the vaginal smear for determining the stage of estrus of *Gerbillurus* species is limited. Insufficient data preclude the determination of the length and class of the estrous cycle, although indications are that *Gerbillurus* species are spontaneous ovulators, with conflicting evidence as to induced or spontaneous luteal formation.

Vaginal casts formed from the accumulation of vaginal secretions and sloughed epithelial cells have been described for *Dipodomys deserti* (Butterworth, 1961). Similar casts were found in the vaginae of five unmated *Gerbillurus* females.

Estradiol-17β (BDH Chemicals Ltd.) and progesterone (Sigma Chemical Co.) were dissolved in sweet oil and injected subcutaneously in the neck of 31 females (perforate and imperforate). Ovariectomies were performed on two *G. p. paeba*, both of which died within 2 months. It was decided not to ovariectomize remaining females owing to difficulties involved in obtaining replacement animals and lack of local expertise in performing ovariectomies. Females were selected randomly from all five taxa for each of three hormonal regimes, with an interval of ≥3 weeks separating consecutive treatments of individual animals. Hormonal regimes were applied in the following order:

1.—0.006 mg estradiol-17β followed after 36 h by 0.4 mg progesterone; tests for sexual behavior were conducted 10 h later (33 animals).

2.—0.06 mg estradiol-17β followed after 68 h by 0.6 mg progesterone; tests for sexual behavior after 4 h (10 animals).

3.—0.006 mg estradiol-17β, repeated after 24 h, followed after 36 h by 0.4 mg progesterone; tests for sexual behavior were conducted after 10 h (12 animals).

At the time of the first injection, a female and male were placed in a terrarium with a wire-mesh screen separating the pair. Behavior tests were conducted in the first 3 h of the artificial dark phase. The screen was removed and the first 20 min of interaction was observed under red light. The number of times that one animal mounted the other was used as an index of sexual activity. Complete copulation was confirmed by the presence of a copulatory plug in the vagina after mounting with intromission was observed.

Vaginal smears were taken immediately after testing. Forty pairs of animals remained together and females were reexamined for evidence of copulation (swollen vagina, copulatory plug, or sperm in vaginal smear) 24 h after the initial observation.

Seven imperforate females at the start of hormone treatment became perforate 36–60 h later. Leucocytes were absent in vaginal smears of 28 (85%) of 33, six (60%) of 10, and six (50%) of 12 *Gerbillurus* receiving the three treatments, respectively. Reproductive behavior (>1 mount) was observed in only 16 (49%) of 33, three (30%) of 10, and five (42%) of 12 females receiving the three treatments, respectively. Only two of 55 females copulated (both treatment 1), but two additional females exhibited evidence of having copulated after 24 h (one each treatments 1 and 3). In total, <50% of the pairings resulted in sexual behavior even though 73% exhibited leucocyte-free smears.

Kuehn and Zucker (1968) found that six ovariectomized Mongolian gerbils subjected to the same regime as treatment 1 of our study became sexually receptive. This hormonal regime did not reliably induce estrus in intact *Gerbillurus* species.

Treatment 2 induced estrus reliably in intact female voles of three species and also served as an effective contraceptive (Dewsbury and Hartung, 1982). This technique failed to induce copulatory behavior in any of 10 pairs of *Gerbillurus* species.

Another effective method of inducing estrus is by repeated injections of estrogen (McDermott et al., 1980). Ovariectomized Mongolian gerbils given 0.0066 mg estradiol benzoate daily displayed lordosis after 7 days. Progesterone injections (0.4 mg/day) resulted in increased lordosis quotients after 4–6 h. As with the previous regimes, this method was not effective with *Gerbillurus*.

The low rate of breeding in captivity indicates that some environmental cue to initiate reproduction is lacking, and this may be reflected in irregular estrous cycles and lack of behavioral response to hormone injections. *G. paeba* previously was reported to be difficult to breed in the laboratory (Hallett and Keogh, 1971; Stutterheim and Skinner, 1973), although maintaining the photoperiod at 16L:8D and temperature at 25°C resulted in greater uterus mass and diameters of seminiferous tubules (Stutterheim and Skinner, 1973). All males used in the present study had large scrotal testes, which did not regress during the study period.

This study raises doubts about the general usefulness of various laboratory techniques used to study copulatory behavior in rodents. Different researchers have used vastly differing quantities of hormones to induce estrous behavior with few standardized regimes being adopted. The assessment of estrus based on vaginal smears appears to be unreliable for some rodent species (Hoffmeyer, 1982), including *Gerbillurus* species. Finally, the presence of a vaginal plug as an indication of copulation was ineffective for *Gerbillurus* species, and possibly for other rodent species as well. Our suggestion is that rodent species should be selected for laboratory studies of copulatory behavior only if they breed readily in captivity.

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SHORT NOTE

Ancient tracks near Tsondab vlei

by
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At present the authorities do not have the means to continue with a full scale investigation of the finds reported on here. Until time and money is available to do so this publication is to elicit useful comments. It is known that the Tsondab River reached the ocean during earlier times, cutting its bed into the tertiary dune surface (Martin, pers. com.). When dune sand was once more deposited in the west it blocked the river from entering the sea and the river sediments could no longer reach the ocean. They consisted of highly calcareous silts which were derived from the calcareous crusts and calcretes in the catchment area of the Tsondab. These sediments were deposited in the arms and ponds of the Tsondab formed by the engulfing dunes. In such a way pans and vleis developed which probably resembled those in evidence at Sossus Vlei.

Water stored in this way was used by the animals in the area. In the course of the year the level of the water in these pans dropped due to evaporation, seepage and usage by the animals. Consequently the surface of the silt layers along the higher lying edges became increasingly exposed towards the inner, lower lying areas. The animals followed the receding water across sediments which would still be wet and soft, leaving behind their tracks. In the course of drying out the sediments would shrink and harden. While the tracks were being preserved numerous mud cracks occurred.

These silt layers were often covered by aeolian sand as well as by more recent river sediments. A part of such an older silt layer was recently uncovered.

Within the framework of an archaeological project reconstructing past environments in the Namib stone tools had been found west of Tsondab Vlei (Seely & Sandelowsky: 1974). A surface collection of fresh water snail shells was dated to approximately 10 000 years.

In August 1975 the site where the snail shells had been collected was once more inspected. Along an eroded edge of a clay layer the imprint of a bird's foot became visible on a covered silt surface. This suggested that foot prints of other animals might be found beneath the present day surface. Consequently another visit to the site was arranged in November.

In the process of uncovering one of the lower silt-layers a series of eleven large impressions in the surface were found. Five pairs of prints could be observed while one end pair appears to have been disturbed by a later silt layer. They have an oval shape and are between 60 cm and 80 cm long with a breadth of 20 cm to 25 cm. At the deepest point the impressions are 10 cm deep (fig. 1). These eleven tracks extend over a total area of 4,60 m x 1,20 m. The form of the silt indicates that the impressions were made while the surface was still soft and wet. Tracks of birds and other animals which are less striking on account of their smaller size and shallower impression can be observed on this surface as well. In other parts of the Namib similar tracks of animals today foreign to the area have been observed (Wendt, 1976).

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It is not yet known what caused the pattern of large prints, but their repetitive regularity implies the movement of a living creature.

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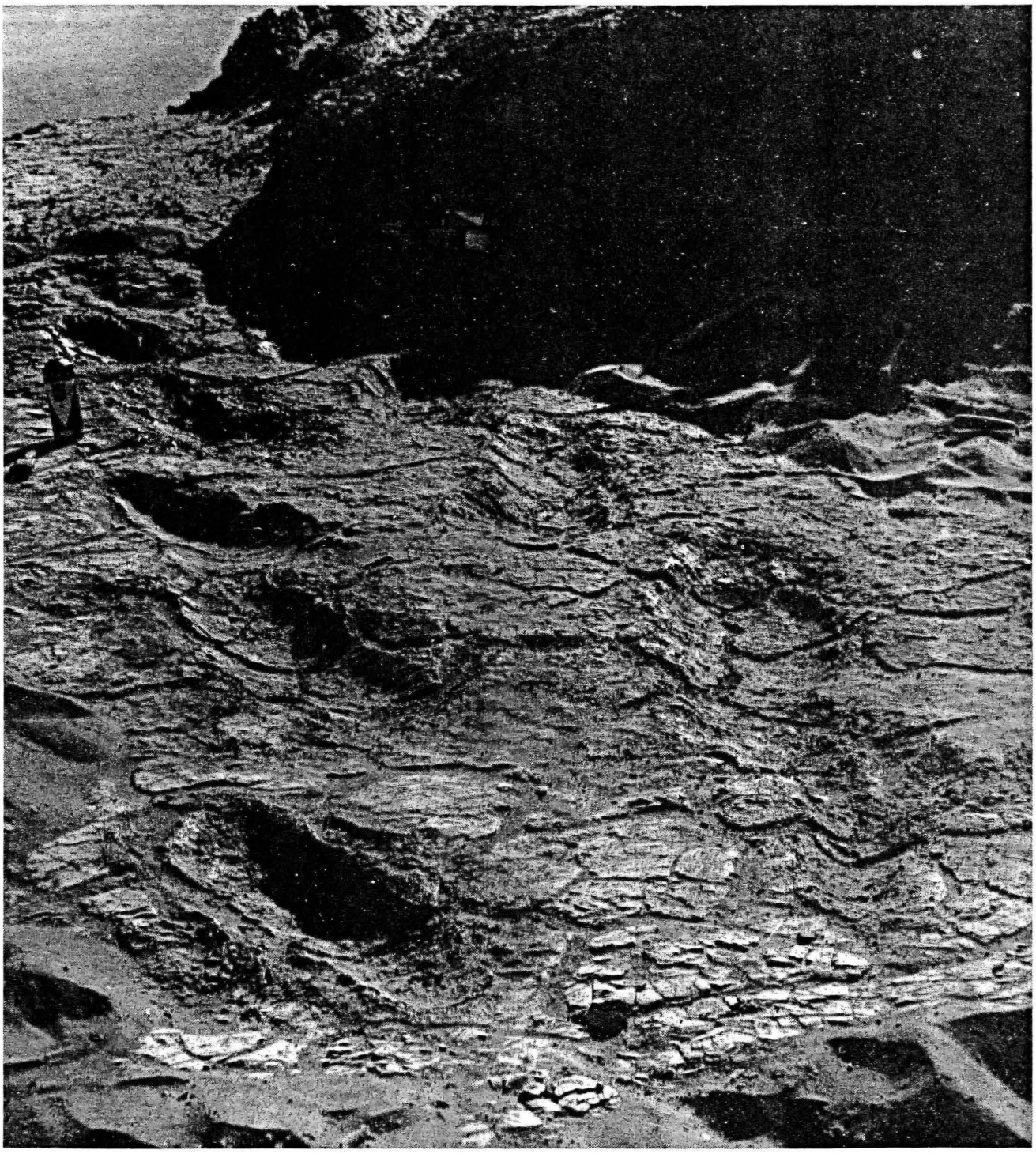

Plate 1: Five pairs of impressions in the resisual silts.