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# THE ESTROUS CYCLE OF TWO SPECIES OF KANGAROO RATS (*DIPodomys microps* AND *D. merriami*)

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**ABSTRACT.**—The estrous cycle of the kangaroo rats *Dipodomys merriami* and *D. microps* entails spontaneous ovulation and a spontaneous luteal phase. Corpora lutea are present throughout the cycle. The modal cycle length is 12 days in *D. microps* and 13 days in *D. merriami*, a significant difference. The duration of the cycle is unaffected by mating with a sterile male. Vulvar swelling and vaginal opening are conspicuous external indicants of estrus. The examination of vaginal cytology provides little information beyond that available from these overt signs. In both laboratory and field, the spontaneous activity of females in estrus is elevated severalfold compared to the anestrous state.

Kangaroo rats (*Dipodomys*) are a genus of some 20 species endemic to arid and semiarid habitats of North America. Although kangaroo rats have been much studied by ecologists, their reproductive biology is not well known. Individual females may produce three successive litters in one season (e.g., *D. spectabilis*, Holdenried, 1957; *D. merriami*, Behrends, 1984) and probably sometimes more. In *D. microps* and *D. merriami*, gestation periods are 31 and 33 days, respectively, and most litters are of two or three pups (Daly et al., 1984). Several investigators have noted changes in vulvar swelling, vaginal opening, and vaginal cytology of unmated females (Butterworth, 1961; Chew, 1958; Day et al., 1956; Eisenberg and Isaac, 1963; Fitch, 1948; Holdenried, 1957; Pfeiffer, 1960), but these have not been systematically described. We recently established successful breeding colonies of *D. merriami* and *D. microps* (Daly et al., 1984), and in so doing studied the estrous cycle with respect to changes in the external genitalia, vaginal cytology, and behavior.

## MATERIALS AND METHODS

**Animals.**—Progenitors (27 females and 10 males) of the laboratory *D. microps* colony were captured in shadscale (*Atriplex confertifolia*) scrub in the vicinity of Big Pine, Inyo Co., California in 1978–1979. Progenitors of the *D. merriami* colony were captured in the same shadscale habitat (17 females and one male) or in creosote bush (*Larrea tridentata*) habitat near Palm Springs, Riverside Co., California (30 females and 7 males). Estrous cycle characteristics did not differ between *D. merriami* from the two locales, and the two populations will not be distinguished in this report.

The kangaroo rats were maintained individually in clear Plexiglas cages (33 by 17 by 28 cm) filled to a depth of about 3 cm with washed sand and furnished with a 0.5-l cardboard tube or can. The two species were housed in separate but adjoining rooms maintained at 22°C with a 13-h daylength (lights on at 2200 h and off at 1100 h). They were fed sunflower seeds, wheat kernels, and rolled oats ad lib, and fresh lettuce or spinach daily. Water was available ad lib.

**Characterization of the estrous cycle.**—Twenty-seven *D. microps* and 14 *D. merriami* females were examined daily for 204 consecutive days from March to September 1979, in order to monitor changes in the appearance of the external genitalia. During this time, 20 of the 27 *D. microps* were successfully bred; daily examinations were then suspended for 57 days during gestation and lactation. No matings of *D. merriami* took place during the same period, despite various efforts to breed them; however, estrous cycle characteristics recorded at this time were not different from those observed once successful breeding was established in 1980 (see Daly et al., 1984).

Calipers were used to measure the diameter (mm) of the vulva. In addition to this measure a standardized catalog of descriptors was used to record the appearance of the females' external genitalia. The degree of swelling or tumescence of the vulvar skin was assigned to one of the five ordinal categories ranging from "flat" to "swollen." Swelling is correlated with vulvar diameter, but is not redundant therewith since cyclic variation in diameter may occasionally be unaccompanied by any evident swelling. The vaginal orifice was categorized as open or closed. Vaginal discharges were noted and characterized as (1) sloughed, dried, crusty

and flakey material, usually seen only at the vaginal orifice or occasionally in such quantity as to cover much of the vulva; (2) a whitish, striated "vaginal cast" up to 2 cm in length, exuded from the orifice intact; (3) a copulatory plug (which could be confused with a vaginal cast, but upon close examination lacked the ridges of the striated cast); and (4) fresh or dried blood. Independent observation and recording of appropriate descriptors for the same animals by three people yielded interobserver reliabilities exceeding 0.85 for these classifications.

Handmade cotton swabs (toothpicks with short fiber cotton batting) dipped in normal saline were used to sample vaginal cytology. The smears were taken at the time of the daily genital examination, within 2 h before lights-out. When the vagina was not evidently open, a smear was taken from the area of the orifice without forcing insertion of the swab, so that the cells obtained probably varied in age. The vaginal smears were fixed in a 50:50 solution of ether and alcohol, and subsequently stained with Harris hematoxylin, OG-6 and EA-36 (Fisher Scientific Ltd.) using a modification of the Papanicolaou method (Drury and Wallington, 1980). The numbers of blue- and pink-stained nucleated and nonnucleated epithelial cells, leukocytes, and red blood cells were counted under 400 $\times$  magnification for each of 10 separate fields.

In more than 1,000 mating tests over a 5-year period, including over 150 fertile matings, females of both species invariably proved receptive on a single day of their cycle, if at all. The day of behavioral receptivity is invariably one in which vulvar tumescence and diameter are maximal or nearly so for that individual animal (see Behrends, 1981; Daly et al., 1984). Thus, to determine cycle length, we define the *day of estrus* according to a hierarchy of criteria: (1) day of copulation; (2) if no copulation, then day of maximal vulvar swelling; (3) if two days of equivalent maximal swelling then day vaginal orifice open and/or day of greatest vulvar diameter; (4) if no maximal swelling then maximal vulvar diameter, if at least 2.5 mm greater than minimum diameter of last cycle.

*Hormone replacement after ovariectomy.*—Twelve *D. merriami* and 11 *D. microps*, ovariectomized 6 months previously, were given subcutaneous injections of 20  $\mu$ g estradiol benzoate (Steraloids, Wilton, New Hampshire) twice daily for 5 days. Eight of these females (four of each species) were additionally given a single injection of 0.5 mg progesterone (Steraloids) on the day when vulvar diameter reached 5 mm (the third, fourth, or fifth day of estradiol treatment). Daily genital examinations were conducted from 2 weeks before hormone treatment until 2 weeks after.

*Ovarian histology.*—Eighteen *D. microps* and six *D. merriami* were bilaterally ovariectomized (under 3 mg intraperitoneal Ketamine hydrochloride, "Vetalar," Parke-Davis) on pre-selected days of the estrous cycle (one or two *D. microps* on each day from 0–12 days after vaginal estrus; one *D. merriami* on each of days 0, 1, 2, and 4 and two on day 10 after vaginal estrus). Females were selected on the basis of having exhibited several reliable consecutive cycles just before the date of ovariectomy. The ovaries were fixed in Bouin's solution of 10% formalin, mounted in paraffin, serially sectioned at 6 micra, and stained with hematoxylin and eosin. The presence of corpora lutea, Graafian follicles, and subantral follicles was ascertained under 100 $\times$  magnification for each of the ovaries (Duke, 1940).

*Matings with sterile males.*—Two *D. microps* males were castrated and testosterone-treated (twice-weekly injections of 5.0 mg testosterone cypionate, Upjohn) so that they behaved sexually like fertile stud males. These sterile males were mated with receptive females in order to assess effects of copulatory stimulation upon estrous cycle length.

*Spontaneous activity over the cycle.*—Activity of *D. merriami* females as a function of stage of the estrous cycle was assessed in an automated apparatus. The home cage of each female was individually housed within a dark, quiet styrofoam box, from which the resident had access via an opaque tunnel, 1 m in length, to an open arena 1 by 1 m, with a sand-covered floor. Photocells connected to a microcomputer permitted the monitoring of each animal's trips through the tunnel. Daily examination of the external genitalia, as described above, was conducted between 1 and 2 h before lights-out, and the daily ration (mixed seeds and a leaf of spinach) was placed in the arena. Activity was then recorded automatically over the last hour of light and the first 5 h of darkness. Eight cycles were recorded and analysed from four females habituated to the apparatus for at least 10 days before the data were collected.

## RESULTS

*Cyclicity of external genital characteristics.*—Vulvar diameter exhibited a clear cyclicity in females of both species, with maximal diameters tending to be associated with tumescence of the surrounding tissue and opening of the vaginal orifice (Figs. 1, 2). Distinctive vaginal discharges were observed in a minority of cycles, but tended to occur at predictable times in the cycle: vaginal bleeding was observed primarily 1 to 3 days before estrus; vaginal casts were recorded immediately after estrus; crusty, flaking material was prevalent after estrus.

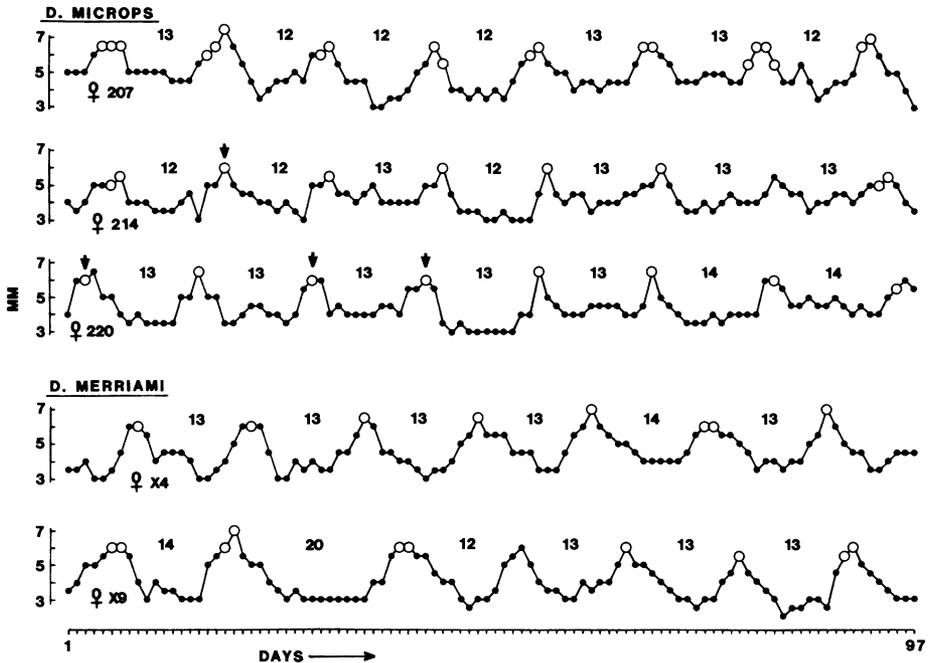


FIG. 1.—Cyclic changes in the external genitalia of three female *D. microps* and two *D. merriami* examined daily for 97 days. The scale represents the diameter (mm) of the vulvar skin. Open circles indicate days of vulvar swelling. Superscripted numbers indicate the duration (days) of successive cycles. Arrows indicate days on which female *D. microps* copulated with a castrated, androgen-treated male.

*Length of the estrous cycle.*—The median length of 242 *D. microps* cycles by 27 females was 12.5 days (mode = 12). For 106 *D. merriami* cycles by 13 females, the median was 13.4 days (mode = 13). This species difference was significant ( $P < 0.003$  by Mann-Whitney test, with the individual animal as the unit of comparison).

*Vaginal cytology.*—In both *Dipodomys* species, vaginal smears at estrus tend to be characterized by the absence of leukocytes and by a high incidence of “cornified” epithelial cells (Fig. 2). Variability in the incidence of cornification, however, makes this feature almost useless as a diagnostic: Individual smears ranged from 0% to 100% cornified for each day of the cycle, so that the pattern in Fig. 2 is apparent only after averaging large numbers of smears. Leukocytes are prevalent for several days after estrus, suggesting the presence of progestational hormone influence at this stage.

Mating often does not occur even when a sexually experienced male is paired with a female in vaginal estrus (swollen vulva). Vaginal cytology did not distinguish those swollen females who did and those who did not mate. Less than 1% of all cells in the smears of swollen females were leukocytes, for example, whether mating occurred or not. In *D. microps*, 76% of epithelial cells were cornified in those who mated, 77% in those who did not; in *D. merriami*, discrimination was no better. Thus, the taking of vaginal smears would not improve the prediction of behavioral receptivity beyond the level of prediction obtained from external examination.

*Effects of hormone replacement after ovariectomy.*—All ovariectomized females exhibited a continuously “flat” anestrus-like vulvar condition until hormone replacement. Twice-daily 20  $\mu\text{g}$  injections of estradiol benzoate induced vulvar swelling and vaginal opening within 3 to 5 days of the start of treatment in both species. The estradiol-induced swelling persisted as long as 2 weeks after treatment was terminated, but a single injection of 0.5 mg progesterone was sufficient to induce a rapid vulvar detumescence within 1 to 2 days.

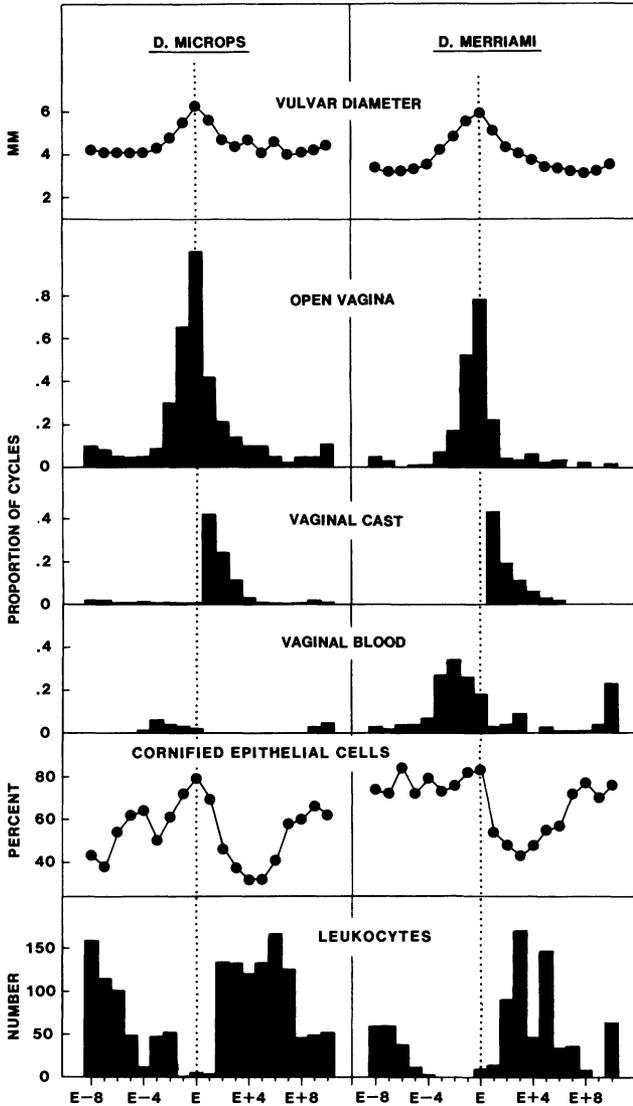


FIG. 2.—Estrous cycle of kangaroo rats: Changes in external genitalia from 8 days before until 10 days after the day of estrus (E). Vulvar diameters and the incidence of vaginal opening, vaginal casts, and vaginal blood are based on 286 cycles by 27 *D. microps* and 118 cycles by 13 *D. merriami*. Measures of vaginal cytology are based on 54 cycles by 11 *D. microps* and 60 cycles by 10 *D. merriami*.

*Ovarian histology.*—Corpora lutea were present in the ovaries of all 18 *D. microps* females and hence on every day of the cycle, increasing in size until 8 days after estrus, and then regressing (Fig. 3). Graafian follicles were present in 7 of 18 females: on the day of vaginal estrus and 8–12 days later. Subantral follicles were present in ovaries taken 1–11 days after vaginal estrus.

Corpora lutea were present in five of the six female *D. merriami*. Graafian follicles were present on the day of vaginal estrus and 10-days post-estrus but not 1, 2, or 4 days post-estrus. Subantral follicles were present 2, 4, and 10 days post-estrus.

None of the females of either species had access to a male at the estrus before ovariectomy.

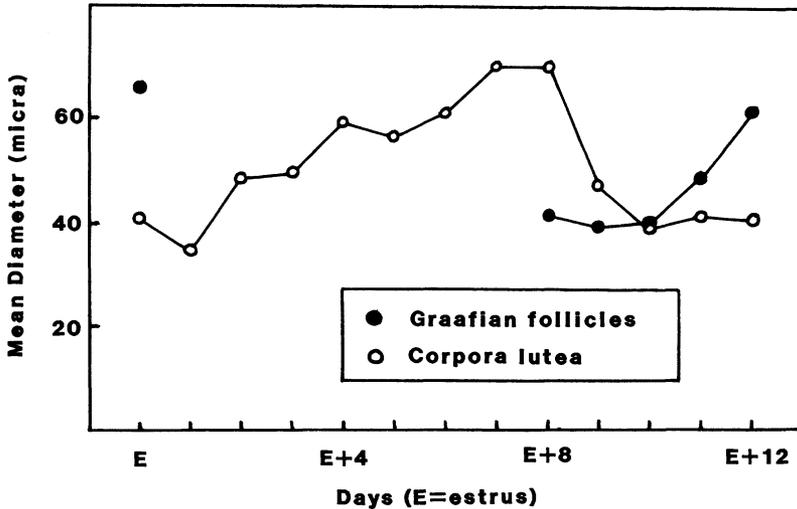


FIG. 3.—Mean maximal diameter of corpora lutea and Graafian follicles in *D. microps* by day of the cycle.

The data therefore indicate that the presence of corpora lutea throughout the estrous cycle does not depend upon stimulation during copulation; in *Dipodomys*, ovulation and formation of corpora lutea evidently occur spontaneously.

*Effects of copulation with sterile males in D. microps.*—Between June and September 1979, eight females copulated when paired with a castrated testosterone-treated male. Seven of the females had delivered litters earlier in 1979. Daily changes in the appearance of the females' external genitalia were then compared with changes when the same females had no access to a male at estrus or had failed to mate when paired with a fertile male. There were no significant differences in the mean  $\pm$  SD interval to next estrus for the three conditions: sterile male mating (interval =  $12.6 \pm 0.9$  days;  $n = 14$ ), fertile male but no mating ( $13.5 \pm 1.1$  days;  $n = 15$ ), and no male ( $13.1 \pm 1.5$  days;  $n = 20$ ). In the days following these three estrous conditions, there were no significant differences in the schedule of observed changes in appearance of the external genitalia. Figure 1 illustrates the persistent cycling of females that experienced sterile matings.

*Spontaneous activity of D. merriami over the cycle.*—Females in the photocell apparatus exhibited cyclicity of locomotory activity, with a peak occurring on the day before vaginal estrus (Fig. 4).

#### DISCUSSION

The estrous cycle of the kangaroo rat entails spontaneous ovulation and corpus luteum function. This is most clearly shown by the results of ovarian histology, corpora lutea being present in the ovaries of unmated females. That estrous cycles are unaffected by the experience of mating with sterile males further indicates the irrelevance of stimulation from the male to the establishment and maintenance of luteal function. Continued progesterone secretion during pregnancy (whether of luteal or other origin) must therefore result from some active influence of the conceptus.

The effects of hormone replacement in ovariectomized females suggest that the vulvar changes observed during normal estrous cycling are induced by ovarian hormones: vulvar swelling by the mounting estrogen levels during the follicular phase, and vulvar detumescence by progesterone of luteal origin (see also Pfeiffer, 1960).

For the practical prediction of receptivity for purposes of captive breeding, taking vaginal

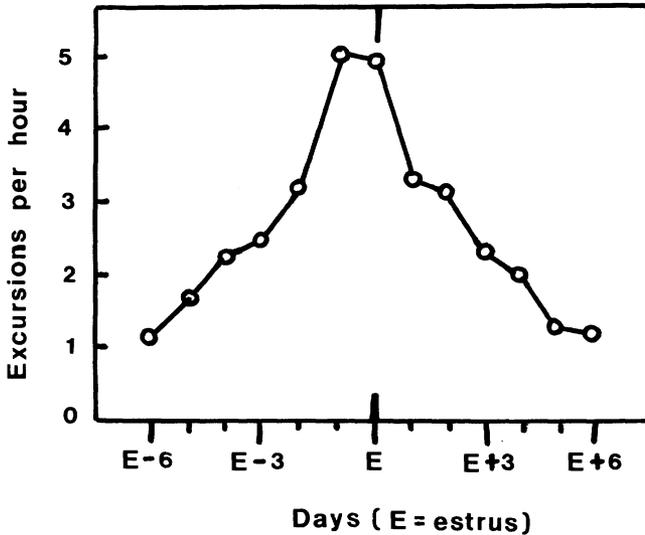


FIG. 4.—Spontaneous activity by *D. merriami* in relation to the estrous cycle. An “excursion” is one round trip between the home cage and an open arena accessible by a tunnel. Values are means for eight cycles by four females.

smears does not appear worthwhile. Better standardization of smear sampling methods might reduce the observed variability, but swelling and vaginal opening are easily observed and are, at present, the only useful indicators of probable behavioral receptivity.

The estrous cycle of *Dipodomys* corresponds to Conaway's (1971) type IA in that ovulation is spontaneous and the duration of pseudopregnancy is unaffected by sterile copulations. However, the kangaroo rat cycle of 12–13 days with a luteal phase of one week is shorter than his characterization of type IA as 2–5 weeks including a 2-week luteal phase.

Conaway suggested that mammalian cycle types are adaptively related to the intensity of predation and expected lifespan, with the relatively long type I cycle characteristic of relatively long-lived species of low reproductive capacity. With a typical litter size of two or three (Daly et al., 1984), the maximal annual production of offspring by a female kangaroo rat is unlikely to exceed ten, far below the maximum for rodents with Conaway's cycle types II (induced ovulation, e.g., microtines) and III (spontaneous ovulation and induced luteal function, known only in certain large-litter murids and cricetids). Kangaroo rats can also survive and reproduce far beyond the age at which most muroids with type II or III cycles senesce. Under Conaway's scheme, then, cycle type IA would have been predicted for *Dipodomys*.

The high activity levels of females at estrus (Fig. 4) are reminiscent of findings in rats (e.g., Birke and Archer, 1975; Finger, 1969) and some other rodents. Female *D. merriami* exhibit a similar cyclicity of activity in the field. Behrends et al. (in press) found that radio-implanted females moved about three times as far between successive hourly radio fixes when in estrus as did the same individuals when in anestrus. An especially intriguing field observation is that females in estrus sometimes travel exceptional distances and remain away from their usual day burrows for at least a day, then return (Behrends et al., in press). These travels raise the possibility that estrous females, more than merely being restless and especially likely to attract males, may actively choose among potential mates.

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