

BRIEF REPORT

Vaginal Estrus in Unmated Belding's Ground Squirrels

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Belding's ground squirrels are seasonally breeding rodents that have a single annual mating season (ca. 3 weeks long) which begins shortly after their vernal emergence from a 7-month period of hibernation. In this study, changes in vaginal estrus were assessed among unmated captive females. Following a 7-month period in a coldroom, vaginal lavages were taken daily to monitor changes in estrous condition. Females were in vaginal estrus within 24-48 hr of removal from the coldroom. Rather than exhibiting repeated cycles, adults (≥ 2 years old) remained in prolonged estrus (typically 3-4 weeks, but 8-10 weeks in some cases), whereas yearlings exhibited similar but shorter and possibly periodic changes in vaginal condition. The difference between the two age classes persisted in a second year of testing, indicating that the preadult status of yearlings (in the first year of testing) did not primarily account for the difference. In another experiment, removal from the coldroom was delayed for 24 days relative to adults removed at a time coincident with emergence from hibernation of free-living females. The "delayed" adults showed persistent vaginal estrus for a shorter total duration, such that both groups reached anestrus at approximately the same time. This implies that the latency to anestrus is not simply a fixed period from the time of removal from the coldroom. © 1986 Academic Press, Inc.

Belding's ground squirrels (*Spermophilus beldingi*) that live near Tioga Pass, California (elevation ca. 3000 m) are seasonally breeding rodents (family Sciuridae) that typically mate during a 2-week period beginning in mid-May. Females copulate 4-6 days after emerging from a 7-month hibernation period and produce one litter per year (Morton and Sherman, 1978; Hanken and Sherman, 1981). Yearlings emerge from hibernation later than adults (Morton and Sherman, 1978) and are less likely to produce litters (Morton and Gallup, 1975; Sherman and Morton, 1984). Little is known about estrous cycles in free-living *S. beldingi*, in part because mating occurs a few days after females emerge and they are not receptive again until the following spring (see also Michener, 1980, on *S. richardsonii*).

We sought to describe vaginal conditions in captive Belding's ground squirrels by collecting daily vaginal lavages from females held apart from

males. Our purposes were (1) to determine whether unmated females would show recurring patterns of vaginal estrus, (2) to compare the vaginal conditions of virgin yearling vs nonvirgin adult (≥ 2 years old) females, and (3) to compare the vaginal conditions of adults taken from the coldroom coincident with when free-living females emerge from hibernation vs adults removed 24 days later.

METHODS

Young were born (25 June–7 July 1982) and reared in captivity by females trapped (when pregnant) near Kirkwood, Placer County, California (39° N, latitude; elevation ca. 2500 m). Following weaning (ca. 25 days of age), the young remained with their dams and littermates in the laboratory ($20 \pm 2^\circ\text{C}$, natural light and photoperiod; 42°N, latitude) and were given food (Purina mouse breeder chow daily, lettuce twice weekly) and water daily. On 28 September 1982, dams and their juvenile daughters weighed 303.4 ± 8.3 and 299 ± 6.2 g, respectively (mean \pm SEM). They were placed individually in a coldroom (ca. 5°C , constant darkness, no food or water) where they remained for 31 weeks until 3 May 1983, when they were taken from the coldroom. Upon removal, eight adult females and eight yearling females (juveniles the previous summer) were housed individually in plastic cages ($38 \times 33 \times 18$ -cm) under the same laboratory conditions as they experienced the previous summer.

Vaginal lavages were taken (see Michener, 1980) from females within 3 hr of removal from the coldroom (Day 1, Week 1), followed by daily collections for 3 weeks, and six collections per week thereafter until a female entered anestrus (see below). Lavages were classified immediately under a microscope ($100\times$ magnification), according to standard criteria (Turner and Bagnara, 1976). Briefly, estrus (E) = a clear predominance of cornified cells; diestrus (D) = a majority of leukocytes; metestrus (M) = approximately equal proportions of cornified cells and leukocytes; and proestrus (P) = a majority of nucleated cells. Lavages were also described as borderline between two states (e.g., P/E). Intra- and interrater reliability ($N = 4$) in the classification of 30 randomly chosen slides was $>95\%$. Hereafter, "estrus" refers to vaginal estrus as determined from vaginal lavages, unless stated otherwise.

For the statistical analyses, an estrous period was defined as a series of consecutive days that an animal was rated as E, E/P, or E/M. The criterion for anestrus (and termination of sampling) was 21 consecutive days without being rated as E, E/M, or E/P. The latency to anestrus was the number of days from coldroom removal to the first day of the anestrus criterion period. Adult vs yearling comparisons were analyzed by the Student's *t* test.

After their first summer as subjects, all animals were returned to the coldroom on 23 September 1983. On 1 May 1984, four adults and four "yearlings" (now referred to as 2-Year-Olds) were removed from the

coldroom and returned in individual cages to the laboratory as described previously. Since free-living females typically emerge in early May (Morton and Sherman, 1978), females removed on 1 May were labeled as Normal Adults and Normal 2-Year-Olds, respectively. On 25 May, 4 more adults were removed from the coldroom, and labeled Delayed Adults (Delayed 2-Year-Olds were not available due to mortality in the coldroom). All subsequent procedures for these animals were the same as for 1983. Comparisons among the three groups were analyzed by one-way analyses of variance, with subsequent paired comparisons assessed by the HSD test. To be consistent with the statistical procedures used in 1983, comparisons were also assessed using the *t* test where appropriate.

To permit a qualitative inspection of daily records, estrous ratings for all 1983 and 1984 females were graphed as a function of the percentage of animals on each day that were rated E, E/P, or E/M over the first 7 weeks after removal from the coldroom.

RESULTS

In 1983, fifteen of the sixteen females (94%) were rated P/E or E within 24 hr (i.e., by Day 2) of being taken from the coldroom (Table 1). Adults entered and remained in estrus for about 3–4 weeks, whereas yearlings entered but came out of estrus during the second week, only to return to estrus in the third week (Fig. 1A). For 7 out of 8 adults, the initial period of estrus was their longest uninterrupted period, whereas for 7 out of 8 yearlings, their longest estrous period was their second period (Table 1). The duration of both the first period of estrus and the longest period of uninterrupted estrus was significantly longer for adults than for yearlings.

The pattern of results from 1984 was similar to that of 1983 (Fig. 1B). Thus, the "yearlings" (now referred to as Normal 2-Year-Olds) came out of estrus and reentered estrus during the first 3 weeks after removal from the cold, while the Normal Adults remained in estrus throughout this period. As they did in 1983, the Normal Adults and Normal 2-Year-Olds differed significantly on the durations of their first estrous period and their longest estrous period (Table 1). In 1984, unlike the previous year, both Normal Adults and Normal 2-Year-Olds reached anestrus at approximately the same time (Table 1 and Fig. 1).

Adults and 2-Year Olds that were removed from the coldroom in 1984 at the "normal" time were also compared with adults whose removal was delayed by 24 days (Delayed Adults). The longest estrous period of Delayed Adults was about half that of Normal Adults and about twice that of Normal 2-Year-Olds (Table 1). In addition, the difference in time to reach anestrus for Delayed (ca. 5 weeks) and Normal (ca. 8 weeks) animals was about equal to the additional time that Delayed animals spent in the coldroom. The difference between anestrus latency for Normal

TABLE 1
Mean Durations and Latencies for Vaginal Estrus and Anestrus

	Duration of		Latency to	
	Longest E	First E	First E	Anestrus
Belding's Ground Squirrels: 1983				
Adults	34.3 ± 6.7 <i>a</i>	31.4 ± 7.8 <i>b</i>	2.0 ± 0.0	45.0 ± 6.6
Yearlings	15.0 ± 4.2	5.6 ± 1.8	2.0 ± 0.2	32.4 ± 4.4
Belding's Ground Squirrels: 1984				
Normal Adults	41.3 ± 7.0	38.3 ± 9.8	1.3 ± 0.3	52.5 ± 4.5
Normal 2-Year-Olds	11.5 ± 2.2	9.0 ± 1.4	1.3 ± 0.3	59.5 ± 6.7
Delayed Adults	19.3 ± 0.8 <i>c,d</i> <i>a,b</i>	18.8 ± 0.5 <i>c</i> <i>a</i>	1.0 ± 0.0	36.8 ± 1.8 <i>e</i> <i>c</i>

Note. Data expressed as mean days ± SEM. E = estrous period as defined by consecutive days of E, E/M, or E/P. Anestrus latency = first of 21 consecutive days of no scores of E, E/M, or E/P. *N* = 8 per group (1983) and 4 per group (1984).

^a *P* < 0.05, adults vs yearlings.

^b *P* < 0.01, adults vs yearlings.

^c Normal Adults vs Normal 2-Year-Olds: *P* < 0.05.

^d Normal Adults vs Delayed Adults: *P* < 0.05.

^e Normal 2-Year-Olds vs Delayed Adults: *P* < 0.05.

Adults and Delayed Adults was significant, based on the *t* test (*t* = 2.73, *P* < 0.05), though it did not attain significance using the HSD test. Once taken from the coldroom, the Delayed Adults' pattern of estrus was similar to that of Normal Adults (Fig. 1B).

DISCUSSION

Unmated Belding's ground squirrels were in vaginal estrus shortly (24–48 hr) after being taken from the coldroom. The adult nonvirgin females were not polycyclic, but rather exhibited relatively constant vaginal estrus for about 3–5 weeks, with three adults exhibiting constant estrus for more than 8 weeks before entering anestrus. In contrast, nulliparous yearlings entered estrus for a briefer initial time and then reentered estrus for at least one additional time approximately 3 weeks after removal from the coldroom. Since the "yearlings" exhibited a similar pattern in 1983 and 1984, it seems unlikely that age alone was the critical variable distinguishing the adults from the "yearlings." Of perhaps greater sig-

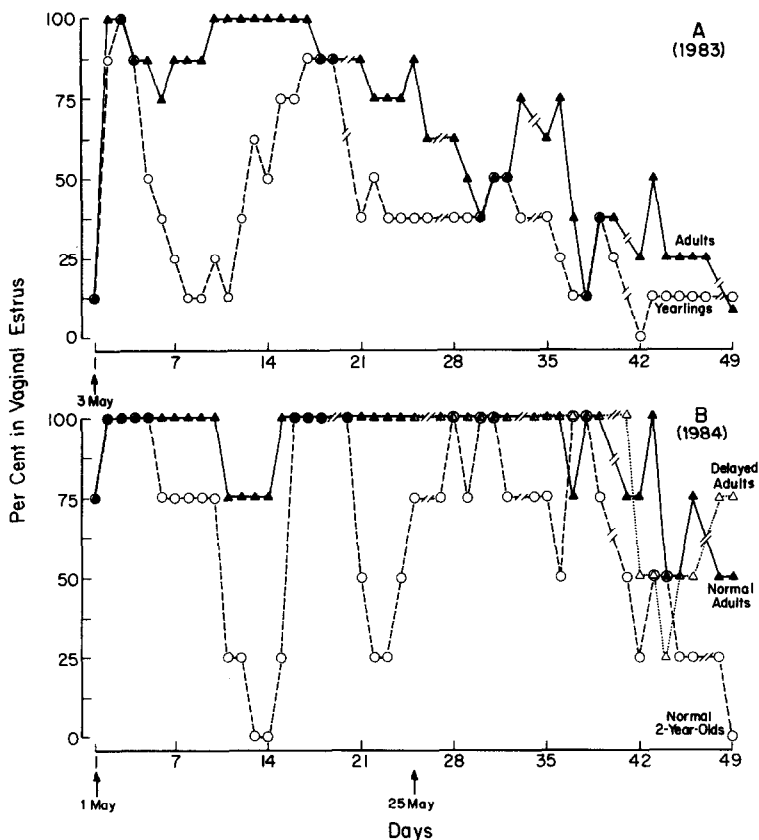


FIG. 1. (A) Percentage of animals ($N = 8$ per group) in vaginal estrus (Adults vs Yearlings) each day for the first 7 weeks after removal from the coldroom on Day 1 (3 May). (B) Percentage of animals ($N = 4$ per group) in vaginal estrus each day after removal from the coldroom. Normal Adults and Normal 2-Year-Olds were removed from the coldroom on Day 1 (1 May), whereas Delayed Adults were removed on Day 24 (25 May).

nificance, yearlings differed from adults in that the younger females were nulliparous and had never experienced a field environment (e.g., photoperiod, natural hibernation conditions).

In nature, mating by female *S. beldingi* marks the end of receptivity until the following spring (Hanken and Sherman, 1981). Our results demonstrate that unmated females show a prolonged period of vaginal estrus. We do not know, however, whether females are sexually receptive during this entire period or whether pregnancy would ensue if mating occurred.

Delayed Adults remained in estrus for a shorter total duration than did Normal Adults, such that both groups entered anestrus at approximately the same time. Thus, the duration of estrus and the latency to anestrus are not determined primarily by the date of removal from the coldroom

or conditions on that day. Instead, it may be that some current environmental cue (e.g., the length of the natural photoperiod) or an endogenous circannual cycle (the periodicity of which was established prior to hibernation) determines the onset of anestrus.

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