Sexual Maturation in Female Townsend's Voles

DOJRZEWANIE PŁCIOWE SAMIC *MICROTUS TOWNSENDII*

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MacFarlane J. D. & Taylor J. M., 1981: Sexual maturation in female Townsend's voles. Acta theriol., 27, 7: 113—117 [With 3 Tables].

Earliest evidence of vaginal perforation in both field and laboratory *Microtus townsendii townsendii* (Bachman, 1839) in the spring-summer season occurs at a body weight of 15—19 g. Virtually all field females are perforate in the 25—45 g weight category, but laboratory females tend to be somewhat heavier before vaginal introitus takes place and first estrus may not occur until several weeks thereafter. Sterile cycles were not perceived in females that were approaching puberty; rather, the interval between vaginal perforation and first estrus was characterized by a diestrous smear.

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1. INTRODUCTION

The age of attainment of sexual maturity in wild populations of mammals is a parameter of great interest to both ecologists and reproductive physiologists. Early maturation is viewed as a major driving force behind population increments (Krebs & Myers, 1974). The physiology of *quasi* sexual maturation, reflected in sterile cycles or in estrus and mating followed by pregnancy failure, is poorly understood in young mammals and poses confusing interpretative problems in field investigations. Voles are target species for analysis of these problems since they reach pubescence at the earliest age known for a mammal (Greenwald, 1957), are seasonal polyestrous breeders, and produce several young per litter.

Our study examines variability in attainment of sexual maturation under both field and captive spring-summer conditions in the female Townsend's Vole, *Microtus townsendii townsendii* (Bachman, 1839), including temporal associations of vaginal introitus, first estrus, ovulation, and evidence of fertility. On the basis of mean body weights of field samples, this same species has already been shown to vary widely in the onset of puberty between summer and winter (LeDuc & Krebs, 1975).

2. MATERIALS AND METHODS

Field samples and the laboratory stock of *M. t. townsendii* were obtained from Ladner, British Columbia, in delta farmland near the Fraser River. All field specimens were taken as live captures during the main breeding season from late February to September. The laboratory stock was housed in a controlled environment room under a light: dark regime of 16 : 8 hours, respectively, and at a temperature of 24.4—25.6°C. The mice were given commercial mouse pellets, greens, and water *ad libitum*. Twenty laboratory-born mice were weaned and

isolated individually in metal basins 30 cm in diameter and 15 cm deep that contained bedding of sawdust 4 cm deep. Once each week the mice were weighed and then transferred to sterilized basins containing fresh sawdust. They were kept in adjacent cages and in the same rack that housed adult females, but they were isolated at all times from males of any age.

Vaginal smears were taken with a pipette by expressing a drop of water into the vaginal orifice and aspirating the flush. By avoiding insertion of the pipette directly into the vagina, precaution was taken against traumatizing the vaginal wall and possible induction of ovulation. The smears were stained with Giemsa solution.

Average weight for the 20 laboratory-born females, weaned at 15—17 days of age, was 15.1 g (11.4—20.0 g). They were checked daily for vaginal perforation. On the day of introitus a vaginal smear was taken to ascertain the stage of the estrous cycle (MacFarlane & Taylor, unpubl.) and, in addition, to the weekly weighing, the mice were weighed on this day. Daily vaginal smears were taken on 11 of these females from the day of perforacion until the first estrus.

3. RESULTS

3.1. Laboratory Colony

Sixteen of the 20 laboratory females underwent vaginal perforation at 2 1/2—7 1/2 weeks of age (Table 1). The vaginal smear was diestrus

Table 1

Body weights and ages of vaginal perforation and first estrus in laboratory-born *Microtus townsendii*.

Body Weight (g) at Perforation	Age Day of First Perforation	Age Day of First Estrus	Mean Body Wt. (g) at First Estrus; Age, Day
19.9	18	45	39
20.0	19	—	—
21.0	20	49	41
22.5	25	—	—
31.5	32	—	—
36.2	34	35	36
32.5	36	38	37
42.1	38	62	44
35.7	38	72	46
37.0	40	—	—
39.0	43	79	48
45.0	43	49	41
40.0	45	—	—
39.3	47	80	48
37.7	50	65	44
26.2	54	—	—
39.2	59	76	47
41.6	66	—	—
39.0	70	—	—
53.8	82	—	—

from the day of perforation to the day of first estrus in each of the 11 females for which daily vaginal smears were taken. After becoming perforate for 2—5 days, the three youngest and smallest females underwent temporary vaginal reclosure for 5—17 days. Wide variation

occurred in age of vaginal perforation (range=64 days) and of first estrus (range=45 days) and in the interval between the two events (1—36 days), even under relatively constant laboratory conditions. Body weight was a better correlate than age since over half of the occurrences of both vaginal perforation and first estrus were between weights of 35—45 g (Table 1).

3.2. Field Captures

Females under 15 g body weight were imperforate, but between 25—45 g virtually all had attained vaginal introitus (Table 2). The smallest females with uterine implants weighed 30—31 g (embryos removed), a laboratory age equivalent of 27 days. Since embryos are not readily visible until well into the second week of pregnancy, they were probably the product of a successful mating at first or second estrus.

Table 2

Occurrence of vaginal perforation in 53 field *Microtus townsendii*.

Weight Range (g)	Number of females	Number Perforate	Number Imperforate
<15.0	2	0	2
15.0—24.9	14	9	5
25.0—34.9	17	16	1
35.0—44.9	20	16	4

The vaginal smear stage was compared with the ovarian histology in seven females weighing 16—21 g (Table 3). The 16-gram female had a proestrous smear but, unlike the others, showed no sign of preovulatory follicle development. The remaining females had enlarged follicles and were in estrus. The heaviest female had mated, ovulated, and had young hemorrhagic *corpora lutea* which were still hollow centrally. Five two-celled ova were present in her Fallopian tubes. No *corpora albicantia* were observed in the ovaries of any of these proestrous or estrous mice. The ovaries wer all normal; polyovular follicles, that characterize sterile cycling in *Microtus californicus* (Peale, 1848) at puberty (Greenwald, 1956), were not present.

The body weight range at which estrus was first apparent in these field females agrees closely with the lower portion of the weight range at which sexual maturity is reached, according to the criterion of lactation (Redfield *et al.*, 1978). Both studies are of unmanipulated spring-summer populations of *M. t. townsendii*.

3.3. Comparison between Laboratory and Field Females

The field population underwent vaginal perforation at a lighter body weight than did the laboratory bred stock although, in lower weight range of introitus and in range of highest occurrences, the differences are not pronounced. In both situations the minimum was within 16—

20 g, but between 20—45 g the field voles were proportionately in advance of the laboratory stock.

The comparison is more pronounced in the body weight range at the time of first estrus. Whereas in the field evidence of estrus could be detected in most mice 18.5 g and heavier, laboratory females were twice that weight before estrus first became apparent (Tables 1 & 3). Differences between these two groups are undoubtedly a consequence of profoundly different environmental exposures.

Table 3

Ovarian histology of vaginally perforate field *Microtus townsendii*.

Capture Weight (g)	Number of Females	Vaginal Smear Stage	Max Ovarian Diameter (mm)	Largest Ovarian Follicle (μ)	Number of *corpora lutea*
16.0	1	Proestrus	0.63	142	0
18.5—20.0	5	Estrus	0.97—1.28	315—360	0
21.0	1	Estrus (sperm)	1.81	675	5

4. DISCUSSION

In a study of laboratory *Microtus pennsylvanicus* (Ord, 1815), both vaginal perforation and first estrus were substantially delayed when, from weaning to sexual maturity, the mice were housed in all-female groups (Pasley & McKinney, 1973). The delay in sexual maturation among our female laboratory-raised *M. t. townsendii*, that were housed singly and were isolated from males, may also have been the consequence of isolation from the opposite sex.

Delays also occur in field circumstances. Onset of sexual maturation in a field environment may differ in the same season of successive years at a given site or simultaneously in two populations. *Microtus californicus* reached sexual maturity at a average body weight of 35 g one breeding season and 30 g the next (Greenwald, 1957). The median weight of *M. pennsylvanicus* and of *Microtus ochrogaster* (Wagner, 1842) at sexual maturity was higher in a population peak than in other phases, but equal in both expanding and declining phases (Keller & Krebs, 1970). In a manipulated population of *M. t. townsendii*, females were shown to delay maturation when sex ratios were altered (Redfield *et al.*, 1978).

Variation in mean weights at puberty within a single population can also differ markedly at different seasons. In another study of female *M. t. townsendii*, puberty was attained at 35 g mean body weight in spring, while the following winter the mean weight was 71 g (LeDuc & Krebs, 1975). Changes in photoperiod are known to affect sexual maturation in *Microtus* (Lecyk, 1963; Breed & Clarke, 1970; Breed, 1972) as does seasonal availability of edible green foliage (Pinter & Negus, 1965; Berger & Negus, 1974; Berger *et al.*, 1977).

The minimum age at which vaginal perforation occurs in *M. t. townsendii* is consistent with that of several other species of microtines.

For example, laboratory *Microtus agrestis* (Linnaeus 1761) undergo perforation at age 19 days (Ranson, 1934), and in wild *M. californicus* not only vaginal perforation but fertilized tubal ova have been found in a female of the age equivalent of 15 days in the laboratory (Greenwald, 1957). A laboratory-bred female *M. ochrogaster* mated at 13.4 g, or 27 days of age, was subsequently released into the field, and produced a litter from this mating (Fitch, 1957). In a constant laboratory environment, however, this same species rarely becomes perforate before 40 days of age or mates to carry a successful pregnancy before 60 days of age (Richmond & Conaway, 1969).

Our records of sustained diestrus before first estrus, of a female with two-celled tubal ova at an age equivalent of 20 days in the laboratory, of field females in advanced pregnancy at an age equivalent of 25 days, and evidence of lactation at weights as light as 16 g (Redfield *et al.*, 1978), collectively provide strong evidence that sterile breeding cycles are insignificant in young *M. t. townsendii* and that successful pregnancies can result from matings at first or second estrus.

Acknowledgements: A grant from the National Research Council of Canada A-3462 (Taylor) and graduate fellowships from this Council (MacFarlane) provided financial support for the study. Drs. H. and D. Chitty gave helpful advice on animal husbandry, and Mrs. Sarah. C. Smith aided in library searches and in preparation of the manuscript.

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Accepted, October 2, 1980.