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Reproduction in a Laboratory Colony of the Female Pine Voles, Pitymys subterraneus

Bożena JEMIOŁO

Jemioło B., 1982: Reproduction in a laboratory colony of the female pine voles, *Pitymys subterraneus*. Acta theriol., 28, 12: 197—207 [With 5 Tables & 2 Figs.]

Reproduction of females of pine voles *Pitymys subterraneus* (de Sélys-Longchamps, 1836) has been tested under constant laboratory conditions. Mated 37 days old females have given litter. The duration of pregnancy in primiparous females has been 22.9 days (from 21 to 25 days). Litter size has ranged from 1 to 5 with a made of 2.47 young born per litter. Both primiparous and multiparous females have given birth to the litter similar in size. The number of litters amounted to 18, however, a full reproductive capacity lasted up to birth 9. In most multiparous females *post partum* mating occurred. The interbirth intervals have lasted from 18 to 25 days. In the females of *Pitymys subterraneus* neither lactation anestrus nor implantation delay subsequent to lactation occurred. In constant conditions of breeding, seasonal effects on females reproduction have been observed. In winter, litter sizes were smaller and the mortality rate higher as compared with spring and reproduction periods. Body weight increase in weanlings depended on the litter size and season.

[Dept. Genet. and Evol., Inst. Zool., Jagiellonian Univ., M. Karasia 6, 30-060 Kraków, Poland.]

1. INTRODUCTION

Recently, numerous studies on reproduction of vole species bred in laboratory conditions have been carried out. The data obtained for the reproduction of *Microtus* and *Clethrionomys* seem to be typical for the whole *Microtidae* family. Their common feature are: lack of a regular sexual cycle and ovulation provoked by copulatory. A number of information has been also reported on the pregnancy duration, litter size and lactation in the females of *Microtus* and *Clethrionomys* (Richmond & Conaway, 1969; Richmond & Stehn, 1976; Schadler & Butterstein, 1979; Landgford & Clulow, 1979; Gustafsson *et al.* 1980; German, 1981). As to the reproduction of *Pitymys subterraneus*, a representative of the *Microtidae* family, common in Europe, the information is very scanty.

The present study is an attempt at determining pregnancy duration, number and size of litters, survival rate of young and effects of seasons on reproduction activity of the females of *Pitymys subterraneus*.

2. MATERIAL AND METHODS

Animals used in the experiments were obtained from the laboratory colony maintained by the Department of Genetics and Evolution, Jagiellonian University in Cracow. Young voles were weaned at 21 days of age and were kept in groups of four or five animals of the same sex until pairing. The females and males were maintained on a photoperiod of 14 L : 10 D at a temperature of 20—22°C, in metal cage (40×20×25 cm). Food and water was provided in excess once daily and consisted of seeds, red beet and apples.

Experiment 1. The females 37, 57 and 90 days old were mated with fertile males. Every morning, the opening of the vagina was checked and vaginal smears were taken by vaginal irrigation with physiological saline. The following data were recorded for each tested female: date when the spermatozoa or copulatory plug were found in the vagina, day of delivery and litter size.

Experiment 2. Adult voles, 90 days old, were kept in monogamous pairs. The following data were recorded: day of mating, days in which successive litters were found, litter size, and number and weight of young weaned up to day of age 21. As *Pitymys subterraneus* in the field and in the laboratory reproducts all the year round, the effects of season on female reproduction cycle and on young weaning and development were studied. Three periods were assumed and called as follows: I — Spring period — lasting three months, from mid January to mid April. In the field, this period is characteristic of a violent increase of day length and beginning of reproduction period in rodents. II — Reproduction period — lasting 6 months, from mid April to mid October, entirely corresponding with the reproduction period of rodents in natural populations. III — Winter period — lasting 3 months, from mid October to mid January. Rapid shortening of the day and the end of reproduction period in wild rodents.

3. RESULTS

Duration of Pregnancy and Litter Size in the Primiparous Female Voles

All females from the three groups have their vagina closed in the day of mating. Females, 90 days old, were covered in the mating day or, at the very latest, after two days. On the other hand, in 37 and 57 days old females the spermatozoa or copulatory plug were found in the vagina as late as 3 to 8 days after mating. The vaginal smears taken from females with open vagina contained vaginal epithelial nucleated and cornified cells and leucocytes. No regular changes in the presence of these three types of cells were found. Vaginal smears from females in coitus were different. In 51.7% females cornified cells prevailed, in 25% leucocytes prevailed, and in 23.3% both cornified and leucocytes occurred. Nucleated cells were present in the smears in a small quantity. The permanent leucocyte predomination was observed only from 2—3 day of pregnancy. This state lasted until mucus with blood appeared in the vagina (day of pregnancy 11—15). The duration of pregnancy

and litter size were similar in all females of the three groups, though 6 out of 15 mated females 37 to 57 days of age gave birth to the litter (Table 1).

Interbirth Intervals in the Multiparous Female Voles

The pregnancy (counted from copulation to delivery) takes 22.9 ± 0.3 days in 28 sexually matured primiparous females. Multiparous females delivered their successive litters in various intervals; they were classified into 3 groups (Fig. 1):

Group I — includes the females delivering their successive litter 18 to 25 days after previous delivery. Such short intervals between the deliveries were equal to 66.1% of all examined intervals. 36.3% fell to the reproduction period and only 16.7% to winter period, and 13.1% to spring period.

Table 1

The mean gestation period and number of offspring in the primiparous female voles.

Age of females, days	Perforation the vagina	Days from mating until copulation	Number of females Mated/Breeding	Duration of pregnancy, S.E.	Number of offspring, S.E.
37	no	3—5	7/2	22.5	3.0
57	no	3—8	8/4	22.7±0.2	2.2±0.5
90	no	0—2	28/28	22.9±0.3	2.4±0.2

Group II — contained females delivering after 26 to 36 days. This group included only 13.5% of all examined interbirth intervals. The group was represented abundantly in the reproductive period (8.4%) and scarely in the spring (2.7%) and the winter (2.4%) periods.

Group III — contained females delivering in 37 to 88 days after their previous delivery. This group contained 20.4% of all examined interbirth intervals. In spring and reproduction periods, such long intervals occurred only in 5.9% and 5.2%, respectively. The most of them were found in winter period (9.3%). No relationship between the duration of intervals and sequence of litters was found (Table 2). Group I in all deliveries from 1 to 18 was always over 50%.

Number and Litter Size

The females of *Pitymys subterraneus* delivered 1 to 5 young in one litter; most often 3 young per litter (41.6%). Primiparous and multiparous females gave the same number of progeny (Tables 1, 4). The number of young born and weaned in 317 litters was 2.65 ± 0.1 and 2.36 ± 0.1 (S.E.) respectively. However, this value was not constant in

all periods (Table 3). In spring and reproduction periods, more young were born and weaned as compared with winter period ($F=3.36$, $p<0.05$; $F=7.59$, $p<0.01$). In those periods, the rate of mortality of

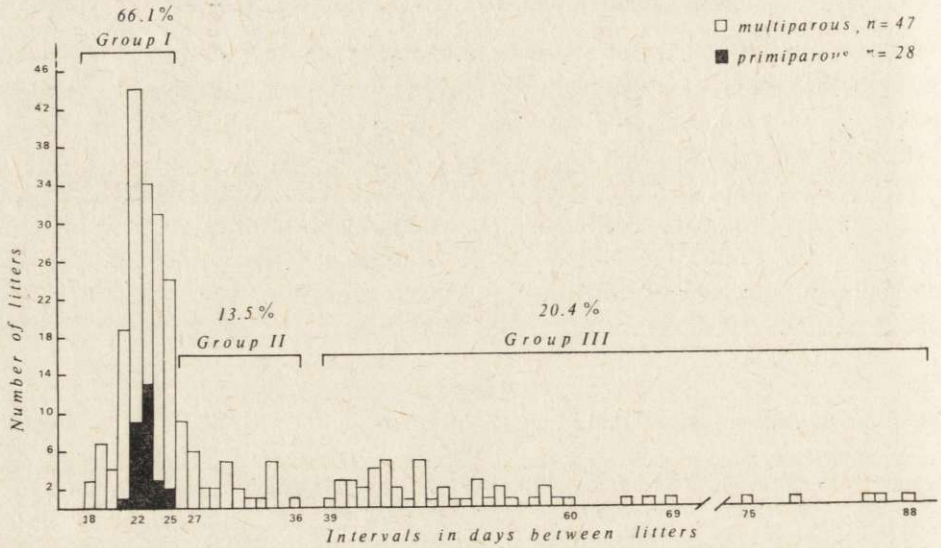

Fig. 1. Intervals in days between litters born to permanently paired breeding stock (distribution of 279 interbirth intervals).

Table 2

Distribution of three groups interbirth intervals in successive litters for the multiparous females voles.

Order of litter	Number of litters	Intervals in days between litters (%):		
		Group I (18—25)	Group II (26—36)	Group III (37—88)
1	28	100.0[1]	—	—
2	44	59.1	6.8	34.1
3	38	71.0	7.9	21.1
4	29	82.9	3.4	13.7
5	28	75.0	10.7	14.3
6	25	76.0	12.0	12.0
7	23	73.9	17.4	8.7
8	21	76.2	19.0	4.8
9	17	58.8	5.8	35.3
10	15	60.0	20.0	20.0
11	9	55.6	33.3	11.1
12	5	60.0	20.0	20.0
13	5	80.0	—	20.0
14	4	—	25.0	75.0
15	2	100.0	—	—
16	2	50.0	—	50.0
17	2	100.0	—	—
18	1	—	100.0	—

[1] For primiparous females

Table 3

Mean number of born and weaned young (21 day) by the primiparous and multiparous female voles in three periods of the year.

Period	Number of females: Primi-parous	Multi-parous	Number of litters	Number of young born, S.E.	Number of young weaned, S.E.	Mortality rate, %
Spring	7	31	87	2.74±0.10[1]	2.51±0.12[2]	8.4
Reproduction	14	30	149	2.78±0.07[1]	2.52±0.08[2]	9.7
Winter	7	30	81	2.44±0.11[3]	2.04±0.13	16.4

[1] As compared with the winter period, $F=3.36$, $p<0.05$; [2] As compared with the winter period, $F=7.59$, $p<0.01$; [3] As compared with weaned young, $t=2.24$, $p<0.02$.

young below 21 days of age was low, *i.e.* 8.4% and 9.7%, respectively, whereas the mortality increased to 16.4% in winter period. In winter period a considerable difference between young born nad those weaned is well seen to ($t=2.24$, $p<0.02$). Table 4 shows litter size delivered by multiparous females. Out of 47 females kept continuosly with a male, as many as 7 females gave 15 to 18 litters. The average number of young delivered in successive litters increase slightly from 2.6 ± 0.1 in the first litter to 3.3 ± 0.2 in the litter 9 ($F=1.24$, $p<0.05$), whereas in the litter 10 and successive ones, a significant drop in the number of young is observed ($F=193$, $p<0.05$) as compared with litters 8 and 9 (Table 4).

Table 4

The number of offspring per litter and survival during the suckling period in 317 successive litters of *Pitymys subterraneus*.

Order of litter	No. of litters	Mean no. in litter, S.E.	Percent surviving
1	47	2.60±0.10	91.3
2	44	2.52±0.15	80.2
3	38	2.71±0.14	92.2
4	29	2.68±0.25	80.8
5	28	2.67±0.23	89.3
6	25	2.84±0.14	85.9
7	23	2.95±0.16	95.6
8	21	3.15±0.22	85.7
9	17	3.30±0.22	96.2
10	15	2.57±0.22[1]	89.5
11	9	2.22±0.33	85.0
12	5	1.80±0.37	100.0
13	5	2.00±0.44	90.0
14	4	2.00±0.70	87.5
15	2	1.00	50.0
16	2	1.50	100.0
17	2	2.50	80.0
18	1	1.00	100.0

[1] $F=1.93$, $p<0.05$; litters 8, 9 *vs* litters 10—18.

Survival of Young

The survival rate of young nursed by pregnant females up to the age of 21 days was independent of litter sequence and ranged from 80 to 96% (Table 4). However it is strongly influenced by the litter size. The females nurse their whole litter composed of 1, 2, and 3 young more often than those composed of 4 or 5 young (Table 5). The females weaned only 59% of 4-young and 50% of 5-young litters.

Table 5

Reproduction on multiparous females *Pitymys subterraneus* in the laboratory.

Litter size	Litters	Offspring born	Offspring weaned	Percent offspring weaned	Percent litters weaned intact
1	40	40	36	90.0	90.0
2	97	194	164	84.5	80.4
3	132	396	358	90.4	81.2
4	42	168	137	81.5	59.0
5	6	30	18	60.0	50.0

Growth of Young

The body weight of one day old young born in the three assumed periods was similar. Although there were no statistically significant differences in body weight of young born in different size litters, it was noticed that the weight of young from 3 and 4-specimen litters was lower than that of young born in 2-specimen litters (Fig. 2).

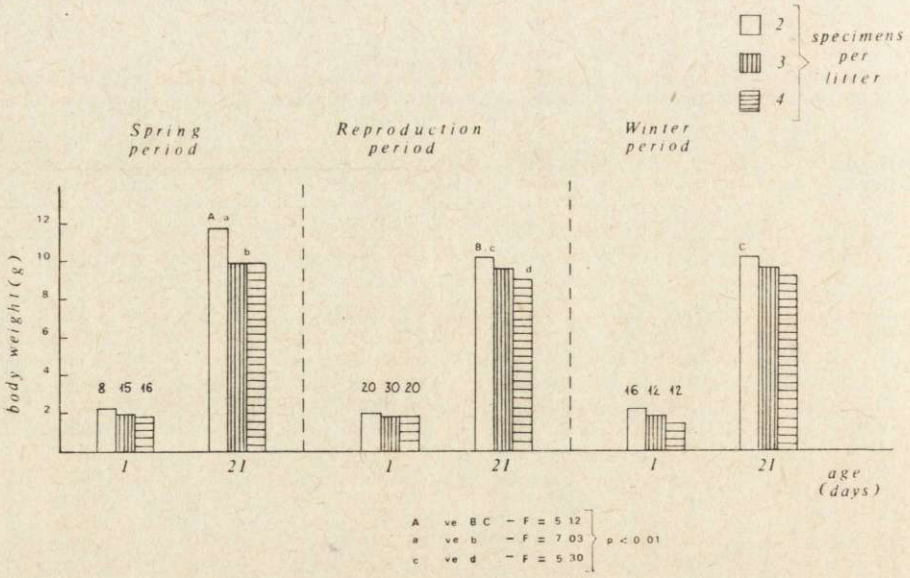

Fig. 2. Body weight of 1 and 21 days old individuals during weaning as related to litter size and season.

The growth of animals up to 21 day of age depended both on litter size and period in which they were born and weaned. In spring, young from 2-specimen litter were significantly heavier than those from 3 and 4-specimen litters ($F=7.03$, $p<0.01$) and from those born in reproduction and winter periods nursed in 2-specimen litters ($F=5.12$, $p<0.01$). In the reproduction period, young from 2-specimen litters were also significantly heavier than the young from 4-specimen litters ($F=5.3$, $p<0.01$). On the other hand, in the winter period no statistically significant differences in body weight of 21 days old young from different size litters were found. Nevertheless, it was noticed that the body weight of specimens nursed in larger litters tended to decrease.

The Student *t* test and single-factor analysis of variance were made on the data (Zar, 1974).

4. DISCUSSION

In the laboratory conditions the first fertile mating of females of microtine species was obtained at a various female age. For instance, the females of *Lagurus lagurus* were 21 days old (Gębczyńska, 1969), the females of *Microtus oeconomus* were 37 days old (Linn, 1957) or 40 to 50 days old (Gębczyńska & Buchalczyk, 1969), those of *M. pinetorum* were 77 days old (Schadler & Butterstein, 1979), those of *Clethrionomys glareolus* 67 days old (Buchalczyk, 1969) or 27—32 days old (Gustafsson *et al.*, 1980), those of *C. gapperi* 30—60 days old (Langford & Clulow, 1979). In the present experiments, the females *Pitymys subterraneus* 37 and 57 days old were covered by a male at least after several days after mating (3 to 8) and only 40% of them gave birth to young. Mature 90 days old animals copulated usually at their mating with no regard to the state of the vagina. Similar phenomenon was reported by Clarke & Clulow (1973) and Milligan (1974) in the females of *Microtus agrestis*. In the females of *Pitymys subterraneus* the spermatozoa or copulatory plug in the vagina evidenced their previous copulation. Some authors believe that as the vaginal smears are concerned an immediate leucocyte invasion occurring after cornified cell phase can also evidence the copulation. It seems to agree with case of *M. agrestis* (Clulow & Clarke, 1968; Clarke & Clulow, 1973), whereas it can not be applied to the species such as *C. glareolus* (Clarke & Clulow, 1973), *C. gapperi* (Langford & Clulow, 1979) and the examined *Pitymys subterraneus*.

Microtines are characteristic of short pregnancies. In the females of *Pitymys subterraneus* the pregnancy lasts 22.9 ± 0.3 days (from 21 to

25). This period is slightly longer as compared with that the females of *Clethrionomys* (18—20 days) and it is similar to *Microtus* (20—24 days). Both the primaparous and multiparous females *Pitymys subterraneus* gave birth to similar number of young per litter. In litters from 1—9 this number slightly increased. A significant decrease occurred only in litter 10 and continued to decrease in subsequent litters, up to litter 18. Similar phenomenon in the females of *M. agrestis* was reported by Egan *et al.* (1980). In this species, a significant drop in mean number of young took place between litter 8 and 14. Hence, it can be assumed that litter 9 is that last one where *Pitymys subterraneus* females show their reproductive capacity and that up to this period, the litter size does not depend on the sequence of litters.

Post partum mating is quite common in microtine species (Richmond & Stehn, 1976). In the females of *Pitymys subterraneus*, short 18 to 25 days intervals made as many as 66.1% of all examined interbirth intervals. This is the case, undoubtedly of an immediate *post partum* mating occurring not later that after 3 days. Similar number (61.5%) of short interbirth intervals (19—23 days) was reported by Richmond & Conaway (1969) in *M. ochrogaster*. The presence of short intervals indicates that in females of the discussed species no phoetus implantation delay subsequent to lactation occurred as it was the case of *e. g.* laboratory mouse, and *C. glareolus* (Andersson & Gustafsson, 1979). Graafian follicles occuring in ovaries of lactant females of *M. agrestis* evidence the fact that there is no effects of lactation on gonadotrophic hormone secretion in some microtine species (Breed, 1969). Also in the females *Pitymys subterraneus*, 26—36 days long intervals indicate the possibility of fertile matings during an advanced lactation. The duration of these intervals may suggest their delay susequent to implantation. However, it is little probable to assume that this phenomenon occurrs only in 13.5% females. Longer intervals are probably induced by the lack of *post partum* mating. Many behavioral factors occurring between both sexes can contribute here. Breed & Clarke (1970) and Richmond & Conaway (1969) reported already the possibility of mating in any day of lactation in *M. agrestis* and *M. ochrogaster*.

The genus *Pitymys* is characterized by small litters. The females of *Pitymys subterraneus* in laboratory conditions delivered 2.47 (Jemioło, unpubl.) or 2.7 young per litter (Buchalczyk, 1961) and in the field 2.7 (Wasilewski, 1960) and *Pitymys pinetorum* 2.2 per litter (Paul, 1966). These values are significantly lower from the mean values for other genera, *e. g.* for *Microtus* they are 2.9 to 6.0 (Goto *et al.*, 1977), and for *Clethrionomys* 4.0 to 4.6 (Drożdż, 1963; Clarke & Clulow, 1973; Langford & Clulow, 1979).

Despite the constant and uniform breeding conditions, influence of season on young weaning and female reproduction was observed. In the discussed winter period marked by shorter days and reproduction setback, a drop in reproduction activity of females was observed. It is shown by a reduced number of litters (longer interbirth intervals), number of young per litter and their high mortality rate. Seasonal changes in reproduction of rodents seem to be a very constant feature. Even in laboratory rats bred for many generations in the very same conditions, *i. e.* temperature, pressure, humidity and the same food, the rate of their sexual maturation is influenced by seasons. Animals born in spring reach maximum testosterone level in blood between 50 and 70 day of life, whereas animals born in autumn reach the same hormone level as late as between 80 and 90 day of life (Mock & Flankel, 1980). Similar seasonal pattern of sexual maturation was found in the laboratory mouse. Animals born from November to March attained their sexual maturity slower that those born from April to August (Vandenbergh *et al.*, 1975).

The presented results on reproductive features of the females of *Pitymys subterraneus* evidence a great affinity of this species to other microtine species. Their common feature are: short pregnancies, fertile copulation during lactation. A distinctive feature for this species is a small litter size. Limited number of ova released by their ovaries seems to be a well developed feature in the evolution of this species. Even an increased stimulation of ovary with high doses of gonadotropic hormone will not induce superovulation (Jemioło, 1983).

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Bożena JEMIOŁO

ROZRÓD SAMIC *PITYMYS SUBTERRANEUS* W WARUNKACH LABORATORYJNYCH

Streszczenie

Rozród samic *Pitymys subterraneus* badano w stałych warunkach laboratoryjnych. Pierwsze płodne kojarzenie samic uzyskano w wieku 37 dni. Ciąża u pierworódek trwała 22,9 dni (w zakresie od 21 do 25 dni). Wielkość miotu wahała się od 1 do 5 młodych (średnio 2,47). Zarówno pierworódki jak i wieloródki rodziły podobnej wielkości mioty (Tabela 1, Tabela 4). Liczba miotów dochodziła do 18, lecz pełna zdolność rozrodcza samic utrzymywała się do 9 wykotu (Tabela 4). U większości wieloródek zachodziło krycie poporodowe. Odstępy pomiędzy wykotami najczęściej (66,1%) trwały od 18 do 25 dni (Ryc. 1). U samic *Pitymys subterraneus* nie występuje ani laktacyjny anestrus ani opóźnienie implantacji z powodu laktacji. Przy stałych warunkach hodowli zaznaczył się wpływ pór roku na rozród samic. W sezonie zimowym rodziło się mniej młodych oraz ich śmiertelność (16,4%) była wyższa niż w sezonie wiosennym i rozrodczym (Tabela 3). Przyrost ciężaru ciała osobników odchowywanych przez matki zależał od wielkości miotu oraz pory roku (Ryc. 2).