Annual Reproductive Cycle of the Formosan Wood Mouse, *Apodemus semotus*

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Bu-Miin Huang, Liang-Kong Lin and Paul S. Alexander (1997) Annual reproductive cycle of the Formosan wood mouse, *Apodemus semotus*. Zoological Studies 36(1): 17-25. The annual reproductive pattern of *A. semotus* was studied. *A. semotus* specimens were trapped at monthly intervals from October 1984 through March 1986 at about 2 300 m elevation in the Hohuanshan area of central Taiwan (24° latitude). Reproductive parameters of 267 mice were measured. Seasonal changes in weights of male gonads showed peaks in April-May and September-October, whereas gonadal weights were sharply reduced and there was histological evidence of regression in June-July and December-February. Ovarian monthly mean weights also showed 2 peaks in April and September. Pregnant or lactating females were found in April-May and August-December. Breeding was more frequent in autumn than in spring. The mean litter size of 23 pregnant mice was 3.83 ± 0.88 (range 2-6). The difference between litter size and ovulation rate (4.24 ± 0.22) indicates that prenatal mortality was 12.8%, including 7.5% pre-implantation mortality and 5.3% post-implantation mortality. No more than 2 sets of placental scars were found in any individual. Analyses of age structure and the time of appearance of juveniles give further evidence of a bimodal annual reproductive pattern for *A. semotus*.

Key words: Rodent, Reproduction, Bimodal, Gonad, *Apodemus*.

Rodents exhibit a variety of annual reproductive patterns, some with year-round reproductive activity and others with cyclical periodicity related to environmental factors (Rapp 1985). There are 13 species of *Apodemus* in the Palearctic region (Honacki et al. 1982), and reproductive patterns for several species have been reported (Adamczewska 1961, Bergstedt 1965, Ashby 1967, Larsson et al 1973, Murakami 1974, Kimura 1977, Green 1979, Nishikata 1979, Gibson and Delany 1984).

Only 2 species of *Apodemus* are found in Taiwan. *A. agrarius*, the Formosan striped field mouse, is very common in the lowlands and has not been reported above 1 000 m. *A. semotus*, the endemic Formosan wood mouse, distributed at 1 200-3 500 m (Chen and Yu 1984), is common above 2 100 m in central and southern parts of Taiwan (Aoki and Tanaka 1941). References to *A. semotus* taxonomy (Thomas 1908), karyotype (Tateishi 1935), food resources (Aoki et al. 1941), distribution (Aoki and Tanaka 1941), and ectoparasites (Phillips 1966) are brief; postnatal growth and development (Lin et al. 1992), demography (Lin and Shiraishi 1992a), and reproductive biology (Lin and Shiraishi 1992b) of *A. semotus* in the Alishan area (23° 31'N; 2 200 m elevation) have been documented recently.

In Taiwan, *A. agrarius* was found to breed at low elevations for 10 months from April to January (Chou and Lin 1980), and *A. semotus* in the Alishan area was found to breed in spring and autumn (Lin and Shiraishi 1992b). In Japan, a 6-month breeding season during late spring to autumn has been reported for *A. argenteus* in Hokkaido (Kimura 1977); an 8-month breeding season during late

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autumn to spring has been reported for *A. speciosus* in Kyushu (Murakami 1974); and bimodal patterns in spring and autumn have been reported for *A. speciosus* (Murakami 1974) and *A. argenteus* (Nishikata 1979) in the Kyoto area. In Europe, many reports indicate that *Apodemus* species have breeding seasons continuing for 4 to 9 months during spring to autumn depending on the latitude (Bergstedt 1965, Green 1979, Montgomery 1980). A bimodal pattern was reported for *A. flavicollis* in Poland (Adamczewska 1961). If good food resources are associated with a warm autumn, winter breeding of *A. sylvaticus* and *A. flavicollis* may occur in Europe (Smyth 1966, Larsson et al. 1973, Montgomery 1980).

In this report, we describe the annual reproductive pattern and the seasonal appearance of different reproductive stages in male and female *A. semotus* in the Hohuanshan area, central Taiwan.

**MATERIALS AND METHODS**

**Study area**

This study was carried out mainly at Tsuifeng, Nantou County (24°10′N; 120°12′E) at an elevation of 2300 m in the Hohuanshan area, 18 km north-east of Wushe. Additionally, 8 collections were made at Tayuling (2550 m elevation, 10 km from Tsuifeng) and Sungkang (2100 m elevation, 6 km from Tsuifeng).

About 2/3 of the 2.5-ha study site at Tsuifeng was covered with grassland and patches of predominantly deciduous trees, and about 1/3 had vegetable crops. The trapping site at Sungkang was very similar to that of Tsuifeng. The habitat at Tayuling had fewer vegetable crops and more evergreen trees.

Mean temperatures at Tayuling (Fig. 1) were obtained from the Central Weather Bureau.

**Trapping procedure**

*A. semotus* specimens were trapped mostly with snap traps at monthly intervals from October 1984 through March 1986. Sherman live traps (20 to 30) were also used in 6 monthly collections for maximizing mouse sample size. More than 100 traps baited with sweet potato and peanut butter were set in late afternoon in suitable places with dense vegetation at intervals of 8 to 10 m along 5 main trap lines for 1 to 3 nights each month.

**Morphological observations**

For males, weights of testes, epididymides and seminal vesicles were recorded to the nearest 1.0 mg. For females, the variables observed were a) ovary weight (to the nearest 1.0 mg); b) the condition of mammary glands; c) number of healthy embryos, plus number of resorbing embryos in each uterine horn; and d) number of placental scars in the right and left uterine horns (Corthum 1967).

**Histological methods**

Paired gonads and accessory reproductive organs were removed and fixed in Bouin's fluid and embedded in paraffin wax. Sections of 6-8 μm thickness were cut and subsequently stained with Harris hematoxylin and eosin. These sections were microscopically examined to assess spermatogenetic activity of males and follicular development of females.

**Analysis of reproductive status of mice**

The reproductive status for each of 267 *A. semotus* was determined from the histological analyses of gonads and accessory organs.

Male mice were categorized into 4 reproductive stages: a) juvenile, b) pubertal, c) mature functional, and d) mature non-functional, on the basis of their spermatogenetic activity (Pudney 1976, 20).

![Fig. 1. Monthly changes of the means of minimum, maximum and the average of minimum and maximum temperatures (°C) at Tayuling from October 1984 to March 1986.](image-url)

The female breeding condition was categorized into 3 stages: a) juvenile, b) pubertal, and c) mature, based on the classification described in Rose and Gaines (1978).

Statistical analysis

One-way ANOVA and the Fisher-PLSD multiple comparison procedure were used to examine monthly differences among the gonadal indices and weights. Chi-square was applied to examine the sex ratios in different reproductive stages.

RESULTS

Sample size and sex ratio

Table 1 presents the numbers of male and female *Apodemus semotus* in each monthly collection from October 1984 through March 1986. The monthly sample size was 10 or more except for the months of October 1984, February and April 1985, and January 1986. Gonads and accessory glands of all mice were examined except for the 10 mice collected in December 1984. Among 267 mice studied histologically, 24 mice were juvenile, 53 mice were pubertal, and 190 mice were mature. Their sex ratios (male: female) were 1:1 for juvenile ($p > 0.05$), 1.00:1.04 for pubertal ($p > 0.05$), and 1.38:1.00 for mature mice ($X^2 = 4.74, p < 0.05$), respectively. Only in mature mice was there a significant difference in number of each sex captured, with males exceeding females.

Reproductive pattern of the male mouse

Based on histological analysis of 148 male mice, 12 were juvenile, 26 were pubertal, and 110 were mature (65 functional and 45 non-functional).

The annual pattern of changes in reproductive organs are described below.

a) Gonadal-somatic index (GSI): Figure 2 presents monthly means for GSI {$\frac{\text{testes weight} \times 1000}{\text{body weight}}$} in mature males. Mean values reached peaks in April (44.48 ± 1.95) and in October (43.27 ± 5.17). The lowest mean GSI value occurred in January 1985 (3.37 ± 0.92), but in January 1986 the GSI value was 28.02 ± 10.74. The second lowest mean GSI value was found in June (13.78 ± 5.44), and the value for July (19.23 ± 3.39) was not significantly different from that for June. Monthly mean values of GSI for October 1984 to January 1985 were significantly lower than for the same months of 1985-1986.

b) Epididymis index: Figure 3 shows the monthly means for epididymis index {$\frac{\text{epididymis weight}}{\text{body weight}}$}.
weight \times 1000)/body weight). Peaks also occurred in spring (15.31 ± 2.98) and autumn (15.48 ± 0.57), and depressions in summer (4.51 ± 1.87) and winter (2.78 ± 0.48 in 1984-1985 and 4.44 ± 2.26 in 1985-1986). The monthly fluctuations of this index are similar to those of GSI.

c) Seminal vesicle weight: Figure 4 shows the monthly means for seminal vesicle weight. Peaks also occurred in the spring (284.00 ± 74.22 mg) and autumn (405.38 ± 27.92 mg), and depressions in summer (23.46 ± 7.83 mg) and winter (45.30 ± 7.61 mg in 1984-1985 and 20.00 ± 6.00 mg in 1985-1986). These monthly fluctuations are also comparable with those of the above parameters.

High values of GSI, epididymis index, and seminal vesicle weight indicate that high breeding activities occurred in spring and autumn, whereas low values of those indices indicate low breeding activities in summer and winter.

d) Presence of sperm in epididymis: Figure 5 shows the changes in abundance of sperm in the cauda epididymis. Reproductive mice with the epididymis full of sperm can be found in the breeding season. In other seasons mice have variable amounts of sperm in the cauda epididymis. Mature sexually non-functional mice without epididymal sperm can be found, particularly during summer and winter. No sperm was found in the cauda epididymis of 4 and 2 mice, respectively, in January and December of 1985. Also, 8 of 9 mature mice had no epididymal sperm in July of 1995, while only one of those 9 had very few sperm in the cauda epididymis.

Reproductive pattern of the female mouse

Among 119 female mice, 12 were juvenile, 27 were pubertal, and 80 were mature (18 nulliparous, 43 primiparous, and 19 multiparous). Among 62 primiparous and multiparous female mice, 23 were pregnant and 10 of 39 non-pregnant mice were lactating. Graafian follicles and corpora lutea were found in non-pregnant mice every month.
The annual pattern of changes in percentage of pregnant and lactating mice and in ovarian weight were analyzed.

a) Pregnant and lactating mice: Figure 6 presents the monthly percentages of pregnant and lactating mice. In April-June 2 pregnant and 3 lactating females (33% of a sample of 15 adults) were found and in August-December 21 pregnant and 7 lactating females (49% of 57 adults) were found. Pregnant or lactating mice were not found in January-March of 1985 and 1986 nor in July of 1985, and pregnant mice were also absent in June.

b) Ovarian weight: Figure 7 presents the monthly mean ovarian weights, showing 2 peaks: one in April (35.5 ± 7.5 mg) and one in September (36.0 ± 5.8 mg). Ovarian weights were lowest in July and in January.

**Estimation of litter size and prenatal mortality**

a) Ovulation rate: A. semotus in this study had an ovulation rate of 4.24 ± 0.22 ova/female based on counting the number of corpora lutea of 62 mature females.

b) Litter size: The litter size was 3.83 ± 0.88 embryos/female based on the number of embryos found in 23 pregnant mice. There was no significant difference in the number of embryos found in the right horn of the uterus (2.00 ± 0.23) as compared with the left horn (1.83 ± 0.22) (p > 0.05).

c) Placental scars: The mean number of older and more recent placental scars of primiparous and multiparous females was 3.72 ± 0.16, nearly the same as the estimated litter size (p > 0.05). The numbers of placental scars from the 1st brood of parous females (3.68 ± 0.19) and from the 2nd brood of multiparous females (3.81 ± 0.30) were not significantly different. No more than 2 sets of placental scars were found in any individual.

d) Pre-implantation and post-implantation mortality: The difference between ovulation rate and litter size indicates failure of fertilization or loss of embryos before birth. Pre-implantation loss is calculated by subtracting the number of implanted embryos of a pregnant mouse from the number of corpora lutea and dividing by the number of corpora lutea. Post-implantation loss is calculated by dividing the number of resorbing embryos by the number of implanted embryos. Pre-implantation loss in A. semotus was 7.5%, and post-implantation loss was 5.3%.

**Seasonal changes in reproductive status**

Reproductive status: Figure 8 shows seasonal changes of different reproductive status in the combination of males (juvenile, pubertal, mature functional, and mature non-functional) and females (juvenile, pubertal, and mature). Males and females with the same status were combined together. Mature female mice and mature sexually functional males were pooled together. Juvenile and pubertal mice were found in May-July and November-February following breeding seasons. Non-functional mature males were found from October
DISCUSSION

Monthly distribution of male GSI values and of pregnant and lactating females provide evidence that A. semotus has a bimodal annual reproductive pattern. Peaks of high breeding activity occurred during spring (April-May) and autumn (August-November), whereas depressions of breeding activity occurred in summer (June-July) and winter (December-February). The annual pattern of monthly changes in epididymis index, seminal vesicle weight, and ovarian weight generally show a corresponding bimodal tendency to that described above. That juvenile and pubertal mice appeared in May-July and November-February following breeding seasons further confirms the bimodal pattern.

Bronson (1985) pointed out that a bimodal pattern of spring and autumn breeding is common for Peromyscus species near 40° latitude in North America, whereas the same species may show a unimodal pattern further north or south. In Japan A. speciosus (Murakami 1974) and A. argenteus (Nishikata 1979) also show bimodal reproductive patterns with breeding in spring and autumn in Kyoto near 40° latitude. A. semotus specimens in the present study were collected at 24° latitude at about 2300 m elevation which has a similar climate to that of Kyoto.

Our finding of A. semotus with a bimodal annual reproductive cycle in the Hohuanshan area is similar to that reported from the Alishan area (Lin and Shiraishi 1992b). However, the summer depression (June-July) in breeding of A. semotus in the Alishan area is not as obvious as it is in the present study.

Spermatogenetic activity in many mammals is seasonal and may be in complete abeyance or regression during the non-breeding season (Grocock and Clarke 1975). Resumption of such activity is usually preceded by an increase in testis size and growth of the accessory sex glands (Grocock and Clarke 1974). It has been reported that high temperatures reduced the formation of sperm (Watts 1969). Kenagy and Bartholomew (1981) suggested that high temperatures could also operate indirectly via effects on energy metabolism or water balance. Parker (1966) reported that thyroid hormone influences the initiation and maintenance of normal spermatogenesis and reproductive activity. High temperatures may stimulate thyroid hormone secretion which then inhibits androgen secretion, spermatogenesis, and the estrous cycle. Kimura (1977) concluded that food scarcity and periods of rapid rise or fall of temperature were correlated with decreased breeding activity in A. argenteus. Also, Murakami (1974) pointed out that the variation of breeding seasons in A. speciosus at different geographical locations seems to be related more intimately to temperature. In the present study of A. semotus, the June-July depression in breeding might be due to the high maximal mean temperature (over 20°C) and the distinct daily rise and fall between mean maximal and minimal temperatures (Fig. 1). However, this speculation of a temperature effect on summer depression of breeding in A. semotus still needs to be confirmed. Although Lin and Shiraishi (1992b) concluded that the cause of summer depression in breeding of A. semotus in the Alishan area is the yearling adults entering into the population in the summer, the effect of temperature still cannot be ruled out. In fact, high population density (Rose and Gaines 1978), aggressive behavior (Watts 1969, Flowerdew 1974), proliferation of ecto- and endo-parasites (Langley and Fairley 1982), and phytoestrogens (Labov 1977) have also been illu-
strated to depress breeding in the summer season. A. semotus shows potential to breed throughout most of the year, as evidenced by presence of sperm in the testis of at least 1 male mouse in each month, except January and December of 1985. Males showed an increasing GSI from January to March of 1985 and 1986 when neither pregnant or lactating females were found. This phenomenon refers to that initiation of spring breeding which is characterized by a slower female response to environmental cues, one of which may be the male pheromone (Bronson 1971). Graafian follicles and corpora lutea were found in non-pregnant female mice every month, suggesting that A. semotus is a spontaneous ovulator (Breed and Clarke 1970, Conaway 1971) and also that females show potential for breeding throughout the year.

The percentage of pregnant and lactating females among all adult females was greater in autumn (28/57 = 49%) than in spring (5/15 = 33%). It is possible that lower breeding activity in spring is due to the effects of winter stress from low temperatures and scarce food resources, causing increased mortality and a reduction in population as well as depression of reproductive activity. Fairbairn (1977) suggested that the number of females declined in spring because of mortality due to food storage aggravated by the attempts to breed early in spring. In autumn the mice born in spring join the breeding population as temperatures and food supplies are more favorable for reproduction. Without considering the small sample size in April of 1985, the percentage of juvenile and pubertal mice of A. semotus being higher in late autumn as compared to spring confirms other evidence of higher breeding activity in autumn. Adamczewska (1961) also found that the autumn breeding of A. flavicollis created a greater number of young in spring and summer.

A. semotus has a mean litter size of 3.83, which is not significantly different from that reported from the Alishan area at 3.49. Loss of A. semotus pre-implantation embryos is estimated to be 7.5%, while post-implantation loss is 5.3%. It is possible that pre-implantation loss results from mating with a male of reduced fertility or from a qualitative inadequacy of the uterine mucosa, and post-implantation loss may result from genetic or developmental abnormalities or endocrine factors (Christian and Davis 1964, Batterm and Berry 1967, Rose and Gaines 1978, McClinton 1983). No more than 2 sets of placental scars were observed in any female in this study. This is also consistent with results from the Alishan area. Breeding females need much more food to compensate for increased energy expenditure connected with pregnancy and lactation (Harland and Millar 1980, Wolton 1983). Hence, too many litters might possibly endanger their lives.

In summary, we have demonstrated that A. semotus has a bimodal annual breeding pattern with high breeding activity occurring during spring and autumn and depression of breeding in summer and winter. This result is consistent with the study in the Alishan area (Lin and Shiraishi 1992b). However, the causes for the summer and winter suppression in breeding are still undetermined. Laboratory experiments to evaluate the effects of high temperatures, xeric conditions, or yearling adults entering the population might answer these questions.

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Wolton RJ. 1983. The activity of free ranging wood mice
本研究在探討臺灣森鼠各月份生殖之表現，按月於臺灣中部合歡山地區(北緯24度)海拔約2300公尺處，
以捕鼠器採集臺灣森鼠樣本。採樣期間自民國73年10月起至75年3月止。有效之臺灣森鼠樣本共267隻，
雄性森鼠之生殖腺重量在4月-5月份及9月-10月份間達到最高峰。相反的，在6月-7月份及12月-2月份間
生殖腺重量明顯下降，雌性森鼠的卵巢重量在4月及9月份亦達到最高峰，雌性森鼠的懷孕及泌乳現象在4
月-5月份及8月-12月份間均被發現。23隻懷孕雌鼠之平均胎兒數為3.83 ± 0.8 隻(2-6 隻)，平均排卵
率(4.24 ± 0.22 個)及平均胎兒數之差異顯現臺灣森鼠胎兒出生前之死亡率為12.8%。其中包含7.5％著床前死
亡率及5.3％著床後死亡率。雌性森鼠子宮內不曾發現二組以上之胎瘟。在年齡結構及幼鼠出現時間的分析
上，更顯示出臺灣森鼠具有雙峰型之年生殖周期型態。在環境因子及天候的探討上，年溫度變化與臺灣森鼠雙
峰型之年生殖周期具有較密切的關係。

關鍵詞：臺灣森鼠，生殖，小老鼠，生殖腺，雙峰型。

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