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Annual Reproductive Patterns of Female House Shrew, *Suncus murinus*, in Taiwan

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ABSTRACT—Reproductive patterns of female house shrew (*Suncus murinus*) inhabiting central Taiwan were studied from September 1990 to February 1992 with animals collected monthly. The annual average body weight was 37.4 g for adults (N=198), and 23.2 g for juveniles (N=18), respectively. The reproductive females (pregnant and lactating) were found all the year round with higher percentages (more than 80%) in spring-summer. The average litter size was 2.9 (range: 1–6). The levels of plasma estradiol-17 β in adult females were higher in March-June (140 pg/ml), and decreased gradually to 98 pg/ml in January-February. Plasma levels of estradiol-17 β in pregnant and lactating females were significantly greater than those of non-pregnant adults and of juveniles. Ovarian weights, plasma levels of estradiol-17 β, and pregnancy rate were correlated with day length. The pre-implantation mortality and post-implantation mortality of the embryos were 6.9% and 1.6%, respectively. The present study has demonstrated that the female *S. murinus* inhabiting central Taiwan breeds all year round with higher reproductive activity in spring and summer.

INTRODUCTION

The house shrew or musk shrew (*Suncus murinus*) is a primitive eutherian mammal of the order Insectivora, which inhabits tropical and subtropical regions throughout Asia (Churchfield 1990; Ruedi *et al*., 1996). This species breeds opportunistically, and pregnant females and fertile males can be found all the year in most of the regions studied (Louch *et al*., 1966; Hasler *et al*., 1977; Brooks *et al*., 1980; Yang and Zhuge, 1989).

In Taiwan, *S. murinus* is commonly found in gardens, villages, and backyards of urban houses, but seldom found in the wild environs. The altitude of their habitation is seldom over 500 meters (Chang, 1992). Previous studies on *S. murinus* inhabiting Taiwan were concerned about serological survey, cytogenetical study and a preliminary investigation with respect to the shrews' reproductive ecology (McNeill *et al*., 1968; Yosida, 1982; Lin and Chang, 1996). However, no work has been attempted to investigate the seasonal reproductive patterns of *S. murinus*, and their relations to environmental factors.

We have investigated the annual reproductive patterns of *S. murinus* of males and females inhabiting central Taiwan. In previous paper, we reported the male reproductive patterns (Chang *et al*., 1999). The present paper reports on monthly patterns of reproduction in female *S. murinus* with respect to: 1) changes in weights of body and ovary, 2) changes in reproductive status (pregnancy, lactation, and non-pregnancy), 3) levels of plasma estradiol-17 β and their relations to reproductive status, 4) litter size and embryonic mortality, and 5) correlations among changes of various reproductive parameters and environmental factors.

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MATERIALS AND METHODS

Trapping of animals, handling techniques and histological method

This study was carried out in the dormitory area of Tunghai University, located on the central Taiwan (24°10.68’N, 120°35.90’E; 184 m in altitude). From September 1990 to February 1992, house shrews were captured in the middle of each month with metal live-traps baited with sausages by setting different traplines with 100 traps (about 4 hectares) for 4 consecutive days. The traps on the study sites were examined every morning (0800–0830 h). The captured animals were immediately transported to the laboratory for examination. Animals were sacrificed within 2 hr after they were collected from the study sites. They were anesthetized with ether, and body weights and lengths (body and tail) were measured before they were bled by puncture of the vena cava with a heparinized syringe. The blood was centrifuged at $10^3 \times g$ for 10 min at 5°C, and plasma was collected and then stored ar –20°C until the assay of estradiol-17 β. Ovaries were checked for the Graafian follicles and the number of corpora lutea by the routine histological methods (Ann, 1978). The teats in females were also examined for any sign of lactation. The conditions of the uterus and the number, size and weight of embryos were recorded.

Classifications of reproductive status

The trapped females were assigned a reproductive status as follows: 1) Pregnant females had visible embryos in their uteri; 2) Lactating females were those in which milk could be expressed from one or more teats by hand pressing. During lactation the nipples became enlarged, bare, dark, and roughened; 3) Non-pregnant females were those having no visible embryos, and their nipples were minute and light-colored, but their ovaries were developed with Graafian follicles; 4) Juveniles whose ovaries were not much developed.

Radioimmunoassay of estradiol-17 β

Blood plasma samples were extracted with diethyl ether and allowed to freeze in a dry ice-ethanol medium. The ether was decanted into another tube and dried in a water bath at 38°C under continuous flow of nitrogen gas in a ventilator. The dried residue was dissolved in 0.01 M phosphate buffer saline (pH 7.40) containing 0.1% gelatin (PBSG) and incubated at room temperature (25°C) for one hour. Aliquots of the PBSG-dissolved ether-extracted plasma sample were incubated with ^{3}H-estradiol-17 β [2,4,6,7,16,17-^{3}H (N), New England Nuclear, Boston, MA, USA] and antiserum of estradiol-17 β; the antibody bound estradiol-17 β was separated with dextran-coated charcol and then counted in a liquid scintillation counter. The antiserum was produced in rabbits by immunization with estradiol-6-CMO:BSA (Steraloids Co.), and was highly specific for estradiol-17 β (Yu *et al.*, 1988). The coefficients of variation (CV) of intra- and inter-assays were 3.0% and 12.1% respectively (N=5).

Climatic data and statistical analysis

Data on rainfall, temperature, photoperiod and relative humidity were obtained from the Taichung Weather Station, and are summarized in Fig. 1.

Analysis of variance (ANOVA) was used to examine differences among the numerous variances. The correlations between reproductive parameters and climatic factors were examined by the methods of Pearson's correlation coefficients. Duncan's multiple range test was used to analyze the differences among reproductive status. Probability levels of 0.05, 0.01, and 0.001 were taken to indicate significance in comparison of means and correlations. Mean values were given with standard errors.

RESULTS

Seasonal changes in body weight and body length

A total of 216 female shrews were examined with 198 adults and 18 juveniles (Table 1). The mean body weights of adults was 37.4±6.1g (SEM), and changed monthly with the lowest in May (31.7±7.0 g) and the highest in April (40.8±6.4 g), but there was no significant monthly difference ($F_{17,180}$ =1.52, P>0.05). The lowest and highest body weights in collected 198 adult females were 22.3 g found in February 1991 and 59.2 g found in November 1991, respectively. As for juveniles, the mean body weight was 23.2±2.3 g (SEM), with the highest being 27.9 g found in February 1991, and the lowest 20.1 g in May 1991.

The mean monthly body length was 205.4±10.2 mm with a range from 165 mm to 225 mm (Table 1). The average body

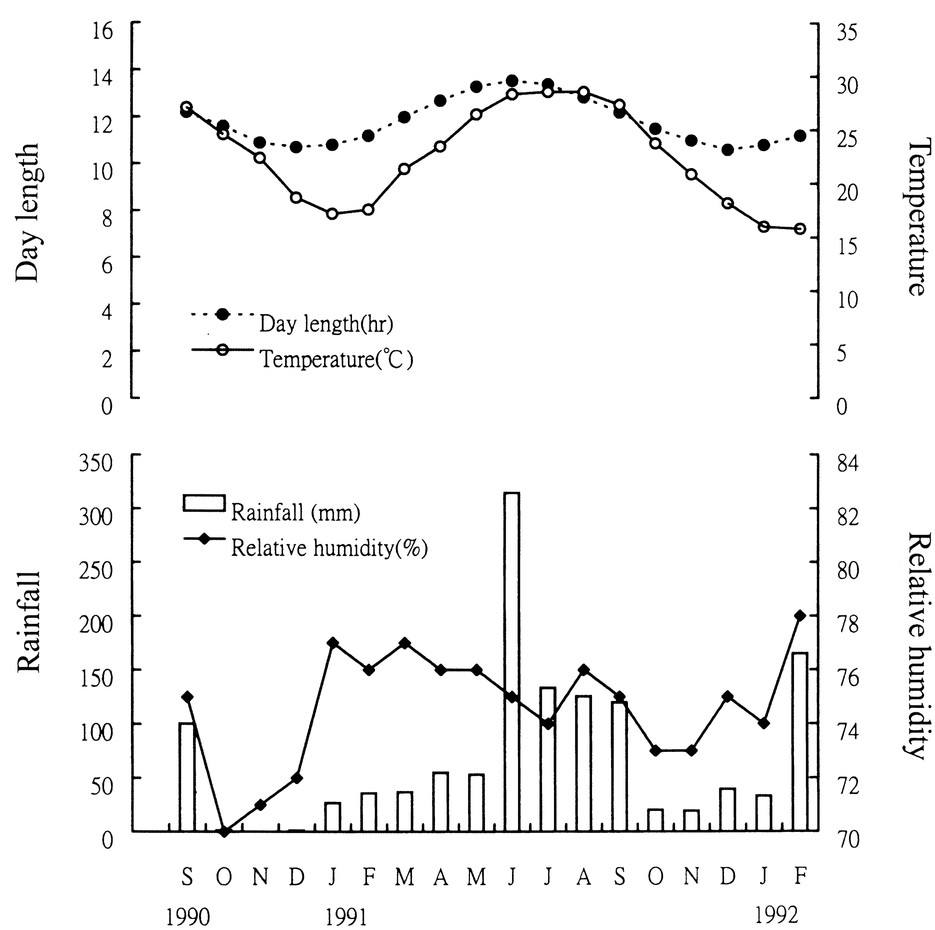

Fig. 1. Monthly changes in climatic factors (temperature, day length, rainfall, and relative humidity) in Taichung, Taiwan. (1990–1992)

Table 1. Mean monthly body weight and body length in adult and juvenile female Suncus murinus inhabiting central Taiwan

Year and Month	Adult			Juvenile		
	No. of Animals	Body weight (g) Mean±SE (range)	Body length (mm) Mean±SE (range)	No. of Animals	Body weight (g) Mean±SE (range)	Body length (mm) Mean±SE (range)
1990						
September	22	35.0±3.5 (28.1–41.0)	203.2±13.0 (165–215)	1	24.0	167
October	14	37.3±6.2 (26.9–50.0)	199.9± 9.4 (175–208)	2	22.9 (21.0, 24.7)	186.5 (182, 191)
November	13	36.3±4.5 (28.8–44.2)	203.9± 7.8 (185–215)	2	25.8 (25.5, 26.0)	191.0 (190, 192)
December	6	33.9±9.9 (22.9–45.5)	197.7±13.0 (179–212)	2	22.2 (21.9, 22.4)	177.5 (173, 182)
1991						
January	6	31.9±5.2 (26.0–40.7)	201.8± 6.0 (196–204)	1	24.9	185
February	8	36.3±7.5 (22.3–45.3)	201.9±11.2 (181–220)	1	21.3	170
March	11	39.7±6.6 (27.7–47.2)	206.0± 8.8 (191–213)	1	22.3	180
April	12	40.8±6.4 (31.2–52.3)	204.1± 9.3 (187–219)	0		
May	5	31.7±7.0 (27.7–44.2)	194.8±10.3 (184–214)	1	20.1	174
June	5	40.8±6.7 (33.1–46.7)	210.2± 8.6 (196–222)	0		
July	15	37.7±5.6 (28.1–48.0)	204.1± 6.6 (187–218)	0		
August	12	38.7±5.1 (26.5–46.7)	205.2±13.5 (180–219)	0		
September	17	38.6±3.7 (27.0–43.3)	208.9± 6.6 (194–220)	0		
October	11	39.4±7.5 (30.3–53.9)	207.5±10.1 (184–219)	2	23.0 (22.8, 23.1)	181.5 (180, 183)
November	14	36.5±8.3 (25.3–59.2)	203.8± 9.7 (184–225)	1	22.9	179
December	10	38.1±5.6 (31.2–47.2)	206.1±13.5 (172–223)	1	26.3	196
1991						
January	7	33.4±5.1 (25.9–40.2)	205.5± 8.9 (192–220)	1	21.2	183
February	10	39.9±6.3 (28.4–48.3)	204.6±10.0 (180–215)	2	23.6 (19.3, 27.9)	163.5 (163, 164)
Total	198	37.4±6.1 (22.3–59.2)	205.4±10.2 (165–225)	18	22.2±2.3 (19.3–27.9)	179.7±9.3 (163–196)

length of the juveniles was 179.7±9.3 with a range from 163 mm to 196 mm.

Seasonal changes in reproductive status and characters

Both pregnant and lactating females were found throughout the year and the prevalence ranged from 20% to 100%. There were higher proportions (over 70%) of both pregnant and lactating females in the population from March to September (Fig. 2). Thus, the intensity of reproduction were higher in spring-summer and lower in autumn-winter.

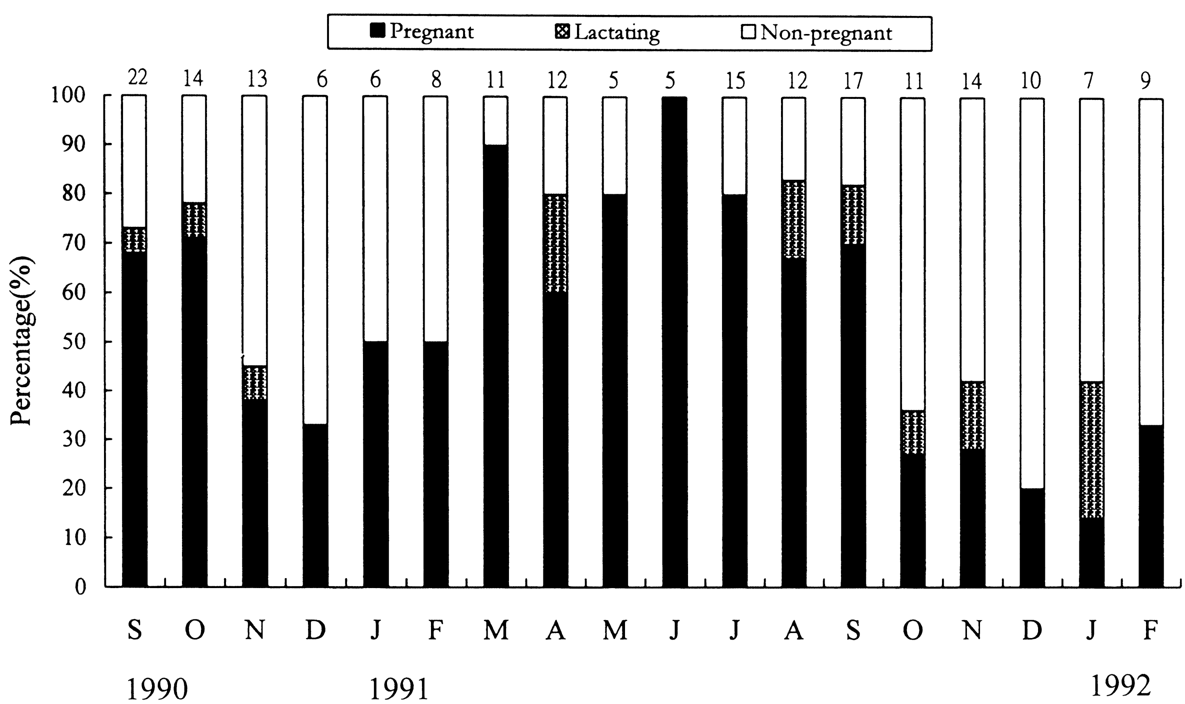

Fig. 2. Seasonal changes of the percentages of reproductive status of adult females of *Suncus murinus*. Sample sizes are shown at the top of respective columns.

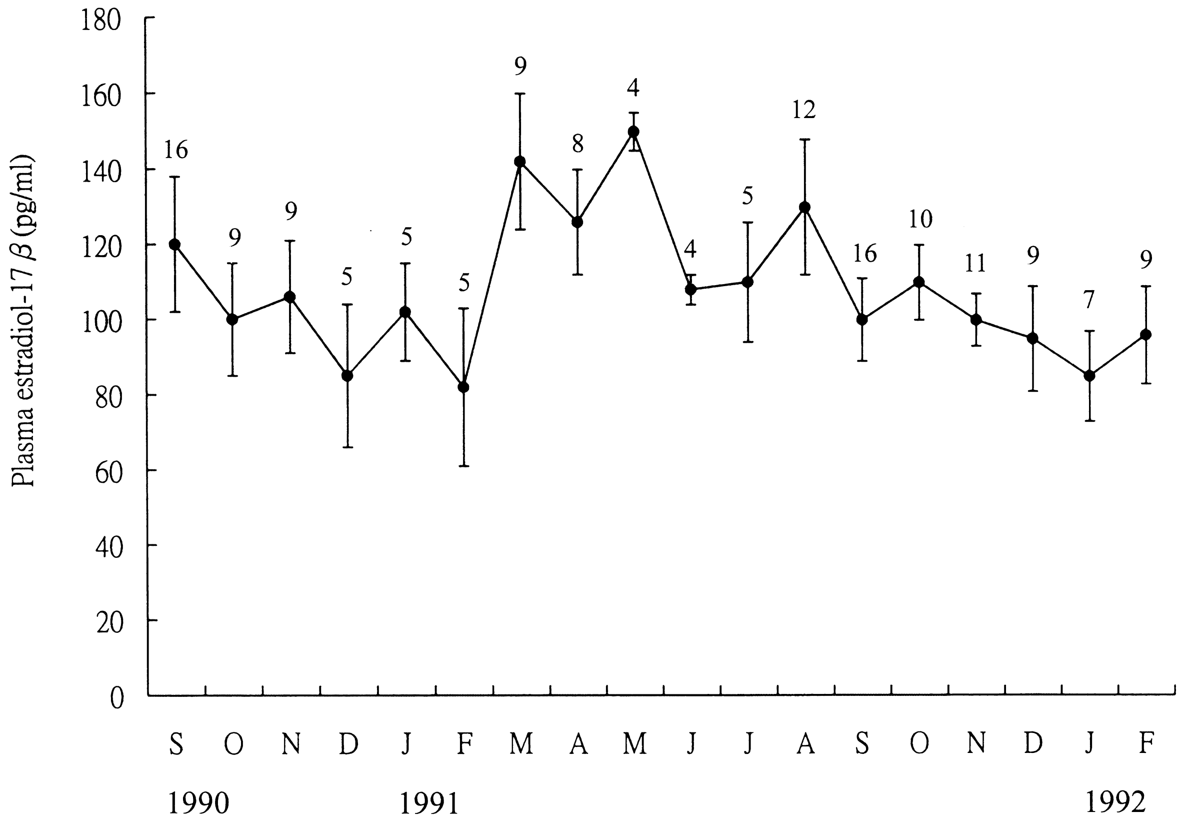

Fig. 3. Monthly changes in plasma estradiol-17 β levels of adult female *Suncus murinus*. The data are expressed as mean±SEM. Arabic numerals above the bars are the numbers of animals.

Table 2. Reproductive parameters among different reproductive status of female *Suncus murinus* (Mean±SEM). Different letters indicate a significant difference (p<0.05)

Reproductive status	Number of animals	Body weight (g)	Body length (mm)	Ovary weight (mg)	Plasma estradiol-17 β (pg/ml)
Juvenile	18	23.2^a ±2.3	179.7^a± 9.3	4.4^a±1.1	79.7^a±18.0 (N=12)
Non-pregnant	67	33.8^b ±5.1	201.1^b±12.2	5.7^b±1.6	99.4^b±12.8 (N=51)
Pregnant	108	40.7^c ±4.9	207.7^b±12.1	7.6^c±1.9	136.8^c±16.2 (N=88)
Lactating	23	$38.9^{b,c}$±6.1	206.9^b±10.4	6.8^c±1.8	122.7^c±18.7 (N=14)

The average plasma estradiol-17 β concentration in adult females was 109.8±18.9 pg/ml, and there was no significantly monthly change ($F_{8,145}$=0.86, P>0.05). There appeared to be an elevation in plasma levels of estradiol-17 β in March-May (140 pg/ml) in contrast to other periods (Fig. 3).

Comparisons and differences in various reproductive parameters among different reproductive stages are indicated in Table 2. There were significant differences in body weights, body lengths, ovary weights and plasma estradiol-17 β levels between adult and juveniles. There were also differences between non-pregnant and pregnant adults in body weight, ovary weight and plasma estradiol-17 β levels. There were no differences between pregnant and lactating females in body weight, body length, ovary weight, and plasma estradiol-17 β levels.

Embryo numbers were obtained by counting of the number of visible uterine swelling. The number of embryos per female ranged from 1 to 6, with an average of 2.94±1.00 (N=107)(Table 3). Out of a total of 315 embryos, only 5 embryos were in the processes of re-absorption, i.e., the post-implantation mortality was 1.6%.

The pre-implantation mortality was assessed by comparing the differences between the numbers of corpus lutea through histological sections of ovaries (Fig. 4) and the implanted embryos. In 78 pregnant females examined, there were 288 corpora lutea in ovaries, but only 268 embryos present in uterus; the pre-implantation mortality was thus 6.9%.

Climatic factors and reproductive patterns

The correlations between reproductive parameters of adult female *S. murinus* and the climatic factors (mean temperature, rainfall, relative humidity, and day length) were compared. As shown in Table 4, ovary weight, pregnancy rate, and levels of plasma estradiol-17 β were all correlated with

Table 3. Monthly distribution of litter sizes in *Suncus murinus* inhabiting central Taiwan

Year and Month	No. of embryos per pregnancy						Total No. of litters	Total No. of embryos
	1	2	3	4	5	6		
1990								
September	1	2	10	2			15	43
October	1	2	5	2			10	28
November		2	3				5	13
December	1	1	1				3	6
1991								
January			2	1			3	10
February			2		1	1	4	17
March		1	3	4			8	27
April	1	2	2	2			7	19
May	1		1	2			4	12
June		1	2	1	1		5	19
July	1	5	4	2			12	31
August		2	3	3			8	25
September	1	3	4	3			11	31
October	1	1	1				3	6
November	1		2				3	7
December				1	1		2	9
1992								
January			1				1	3
February		1	1	1			3	9
Total	9	23	45	26	3	1	107	315

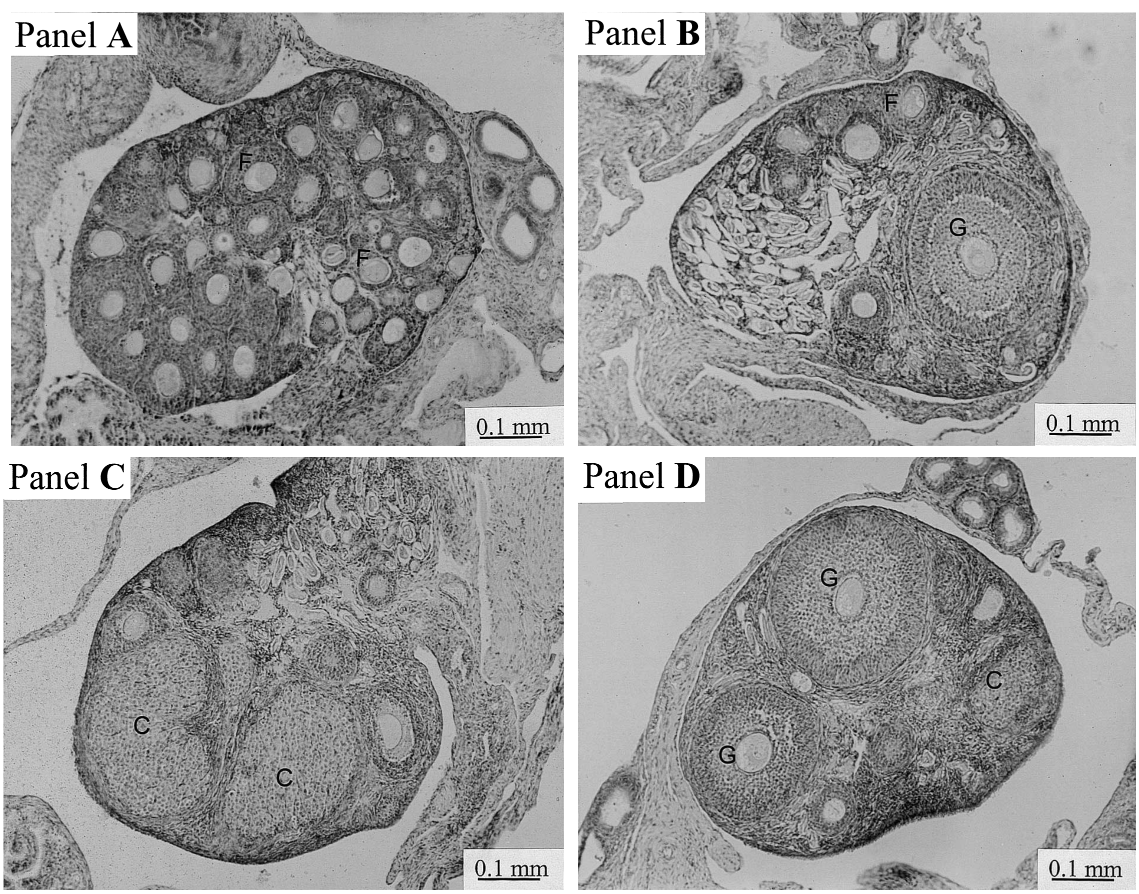

Fig. 4. Histological photographs showing different ovarian follicles and/or corpora lutea at various reproductive status of *Suncus murinus*. Panel A: A juvenile with immature follicles (F) in ovary. Panel B: A mature but non-pregnant female *Suncus murinus* with a Graafian follicle (G) in ovary. Panel C: A pregnant female Suncus murinus with corpus luteum (C) in ovary. Panel D: A lactating female with Graafian follicles and regressed corpus luteum in ovary. Scale = 0.1 mm.

Table 4. Pearson's correlation coefficients between reproductive characters of adult *Suncus* females and the climatic factors (* p<0.05; ** p<0.01)

Climatic factor	Ovary weight	Pregnancy rate	Levels of plasma estradiol-17 β
Temperature	0.552*	0.726**	0.571*
Length of day	0.586**	0.833**	0.665**
Rainfall	0.374	0.528*	0.119
Relative humidity	0.233	0.222	0.327

temperature or day length. In addition, the pregnancy rate was also correlated with rainfall. Although their correlations were statistically significant at $p<0.01$ or $p<0.05$, their correlation coefficients were not high except those between pregnancy rate and length of day or temperature.

DISCUSSION

The annual breeding patterns of *S. murinus* have been investigated in various tropical and subtropical regions. Except in certain desert areas (Rajasthan, India and Punjab, Pakistan), the pregnant female *Suncus* were found to be present all, or nearly all year round (Table 5). In the present study, we observed that the pregnant female *Suncus* were present every month, with higher prevalency in March-September and lower prevalency in November-February, exhibiting large difference between seasons. The seasonal variation of reproductive activity of the female *Suncus* were essentially synchronized with those of the male Suncus that exhibiting maximal spermatogenic activity in March-September (Chang *et al.*, 1999). As indicated in Table 6, the female *Suncus* in Rangoon had more stable prevalency of pregnancy on seasonal basis; while those in Calcutta, Guam, Malaya, Chekiang and Taiwan showed more variable prevalency of pregnancy. It is interesting to note that female *Suncus* in Chekiang, China exhibited a bimodal reproductive activity occurring in March-April and July-August (Yang and Zhuge, 1989). The observations of previous investigators from different laboratories revealed that female *Suncus* is either a

Table 5. Reproductive characters of Suncus murinus at 9 localities

Locality	Latitude	Body weight	Breeding season	Litter size	Source
Rajasthan	25° 7' N	(M) 33.20±1.31 (F) 23.53±1.39	March to September	4.71±0.23 (Range 2–7)	Rana & Prakash (1979)
Punjab	31°47' N	(M) 52.6 ±1.26 (F) 37.4 ±0.9	April to October	4.17±0.39 (Range 2–6)	Khokhar (1990)
Calcutta	22° 5' N	(M) 105.6 (F) 67.7	All the year	3.8 ±0.3	Louch *et al.* (1966)
Rangoon	17° 0' N	(M) 60.9 ±13.7 (F) 41.6 ±11.3	All the year	2.99±1.21 (Range 1–7)	Brooks *et al.* (1980)
Malaya	5° 0' N	45	All the year	2.7 ±0.1 (1977)	Hasler *et al.*
Guam	23° 0' N	(M) 30 (F) 21	All the year	2.1 ±0.1	Hasler *et al.* (1977)
Okinawa	25°10' N	(M) 50.5 (F) 33.8	—	3. 21 (Range 1–5)	Oda & Shigehara (1978)
Chekiang, China	30° 0' N	(M) 54.9± 1.13 (F) 37.9± 0.16	Nearly all the year	3.5 ±0.2 (Range 1–7)	Yang & Zhuge (1989)
Taichung, Taiwan	20° 10' N	(M) 53.1±14.5 (F) 37.4± 6.1	All the year	2.94±1.00 (Range 1–6)	Chang *et al.* (present study)

Table 6. Comparison of prevalence by seasons in *Suncus murinus* of different localities

Locality	Percentage of Pregnancy				Sample size	Mean pregnancy percent	Reference
	Jan.–Mar.	Apr.–June	Jul.–Sep.	Oct.–Dec.			
Taiwan	49	80	73	37	198	60	Chang *et al.* (This study)
Rangoon	60	63	57	54	679	59	Brooks *et al.* (1980)
Calcutta	46	52	47	33	92	48	Louch *et al.* (1966)
Guam	38	54	50	38	144	44	Hasler *et al.* (1977)
Malaya	25	19	22	42	290	24	Hasler *et al.* (1977)

Data from Yang and Zhuge (1989) on percentage of pregnancy of *Suncus murinus* on basis of every 2-month intervals were as follows: Jan.–Feb., 10%; Mar.–Apr., 88%; May–June, 17%; Jul.–Aug., 55%; Sep.–Oct., 46%; Nov.–Dec., 6%.

continuous or non-continuous breeder depending on the localities of habitation (Table 5). For those *Suncus* being continuous breeders, however, they all exhibited, more or less, seasonal variation in reproductive activity. Thus, the variability of prevalency of pregnancy and other reproductive status of *S. murinus* among various localities studied are correlated to and reflected by, different climatic factors and other ecological conditions.

The litter size in *S. murinus* shows a vast geographical variation (Table 5). The two largest litter sizes are from India and Pakistan with 4.7 and 4.2, receptively, from Rajasthan (Rana and Prakash, 1979) and Punjab (Khokhar, 1990). Rana and Prakash (1979) suggested that it is a compensated way for the shorter breeding season of shrews in the desert environment to maintain a higher turnover rate of the population in the yearly cycle. At other localities with continuous breeding all the year round, the litter size appears to be a function of body weight in female shrews. The Calcutta shrews were heaviest in body weight (67.7 g) and possessed the relatively large litter size (3.8)(Louch *et al.*, 1966); while the Guam shrews were the lightest (21 g) and had smallest litter size of 2.1 (Hasler *et al.*, 1977). The litter size of shrews in central Taiwan as observed in this study was 2.9, which is similar to those in Rangoon (Brook *et al.*, 1980), Okinawa (Oda and Shigehara, 1978) and Chekiang (Yang and Zhuge, 1989). It can be inferred that the shrews inhabit in central Taiwan exhibit higher reproductive efficiency with a lower pre-natal mortality (both pre- and post-implantation mortality) due likely to a more stable ecological environment.

Studies indicated that musk shrews are induced ovulators, but exhibit no cyclic changes in reproductive structure or in sexual behavior (Dryden, 1969; Fortune *et al.*, 1992). Sexual maturity in female *Suncus* is generally reached by 30 days of age and mating behavior is induced by contact with a male, and its gestation period lasts about 30 days (Dryden, 1969; Fortune *et al.*, 1992). Dryden and Anderson (1977) reported that uterine and vaginal weights and histologies were not altered by ovariectomy or estrogen treatment in female shrews. Hasler and Nalbandov (1978) also revealed that the levels of plasma estradiol-17 β were not detectable in adult female shrews. Thus, the role of ovarian estradiol in the control of reproductive status of this species was once considered not conformable to the accepted mammalian pattern. However, Rissman and Bronson (1987) demonstrated that ovariectomy eliminated sexual behavior, and treatment with estradiol restored it in almost all shrews. Such observations indicated that estradiol plays a role in regulation of sexual behavior in house shrews. Fortune *et al.* (1992) further demonstrated that matings stimulated ovulation in musk shrews also triggered specific changes in ovarian steroidogenesis; increased estradiol-17 β production by the ovary and elevated level of estradiol-17 β in the blood during periovulatory period suggest a role of estradiol during the final stage of follicle development and/or ovulation. They also demonstrated that ovarian secretion of estradiol was increased during the early luteal phase, thus suggesting a role of estradiol in luteal function in shrews.

The present study also investigated the levels of plasma estradiol-17 β in female shrews during an annual reproductive cycle. We have demonstrated that the ovarian weights of both pregnant and lactating female *Suncus* were larger than those of non-pregnant adults and juveniles (Table 2), and that the levels of plasma estradiol-17 β were correlated to different reproductive status of the *Suncus* with higher levels of plasma estradiol-17 β present in both pregnant and lactating females in comparison to the non-pregnant and juvenile female (Table 2). In addition, the present study revealed that the levels of plasma estradiol-17 β tended to be elevated during March-September when the percentage of pregnancy and lactation of *Suncus* being greatest. Our findings thus support the proposal that ovarian estradiol-17 β plays a role at various stages of reproduction of female *Suncus*. Furthermore, we found that the elevated levels of plasma estradiol-17 β of female *Suncus* in spring-summer are very much synchronized with the elevated levels of plasma androgen of the male in the same period (Chang *et al.*, 1999).

Correlations between environmental cues and reproduction of female *Suncus* inhabiting in different localities have been investigated by various laboratories (Table 5). In general, the peak breeding activity of female Suncus occurs in spring-summer when the day lengths are longer. It was shown that the female *Suncus* housed in short day lengths had significantly lighter uterine cervices, and were less likely to demonstrate sex behavior than animals kept in under long day lengths, although ovarian and uterine weights did not differ (Rissman *et al.*, 1987; Wayne and Rissman, 1990). In the present study, we have observed that ovarian weight, pregnancy rate, and levels of plasma estradiol-17 β of the female *Suncus* were all correlated with day length and temperature, although their correlation coefficients were not consistently high (Table 4). The weights of testis, epididymis, seminal vesicle-prostate, and plasma levels of androgen of the male *Suncus*, investigated simultaneously, were all correlated to the day lengths and temperature (Chang *et al.*, 1999). Consequently, the long photoperiod and higher temperature are likely stimulatory to reproductive activity in house shrews. The effects of rainfall and relative humidity on reproductive activity of house shrews are variable and complex. Factors such as amount and length of rainfall, different latitude and elevation, desert or swamp area, and the length of rainy season may have different influences on reproductive activity. Rainfall was correlated with the reproductive peak of *S. murinus* (Rana and Prakash, 1979). We have observed that rainfall, but not relative humidity, was correlated with pregnancy rate of female *Suncus*. Our present study thus demonstrated that day length and temperature are likely the most important factors in relation to reproductive activity of *S. murinus* inhabiting central Taiwan.

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