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SEASONAL REPRODUCTIVE CYCLES IN MALE PLATEAU PIKA (OCHOTONA CURZONIAE)*

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Abstract

Annual changes of the reproductive activity in adult male plateau pika (*Ochotona curzoniae*), a small endemic mammal in Qinghai-Tibet Plateau, were investigated from January to December, 1991. All of the animals were killed and decapitated during the night (23∶00～24∶00) and the plasma, pineal glands, testes epididymis, seminal vesicles, deferent ducts were collected and used for biochemical, and histological studies. Significant changes associated with seasonal cycles were found. (1) In February～early April, the restoration phase, the weights of testes, epididymides and deferent ducts were increased; the process of spermatogenesis was strengthened and testosterone level in plasma was increased, but the pineal weight and its melatonin content were decreased. (2) During the middle of April～late May, the sexually active phase, a significant elevation of gonadal activity was observed. In this period, gonadal weights were increased, spermatogenesis was completed, pineal weights were decreased and melatonin contents were fluctuated at a low level. These results suggested the increasing in sexual activity as well as in the ability of testosterone secretion. (3) A striking reduction of testicular activity appears in June～August. In this inhibition phase, gonadal weight, process of spermatogenesis, plasma testosterone level were decreased while the pineal weight and pineal melatonin content were increased. (4) During September～January, the sexually quiescent phase, declining in weights of testes and epididymides, arrest of spermatogenesis, decreasing of plasma testosterone concentration, fluctuating in pineal weights and increasing in pineal melatonin level were observed. Our findings indicated that the male pikas under natural conditions exhibited an annual reproductive cycle. A possible relationship between pineal activity and reproductive function was also suggested.

Key words Plateau pika (*Ochotona curzoniae*); Reproduction; Pineal gland; Melatonin

Many seasonal breeding mammals are able to respond to annual climatic changes by

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adaptive alterations in physiological as well as in behavioral status. The switching on and off of reproductive function is the most striking example of such a photoperiodically induced process. The role of pineal gland in the regulation of seasonal reproductive cycle was demonstrated in several mammalian species. One of the most studied species is the Syrian hamster (*Mesocricetus auratus*), in which gonadal regression is induced by exposure to short photoperiod (Reiter et al., 1983).

Plateau pika (*Ochotona curzoniae*), as a small endemic mammal, lives in Qinghai-Tibet Plateau and shows typical characteristics of seasonal breeding. The circadian rhythm of the pineal melatonin content in the animals showed that pika's pineal gland was able to respond to photoperiods (Li et al., 1994a). The experimental studies of the reproductive endocrine of pika in different photoperiods also indicated that plateau pika might be classified as a long-day breeder (Li et al., 1994b, 1994c). The present study was designed to examine the annual reproductive cycle of the adult male plateau pika in natural habitat and analyze the possible relationship between pineal activity and reproductive function in naturally acclimatized animals.

MATERIAL AND METHODS

1. Animals

Nine groups (the total of 286 animals) of adult male pika were trapped in the field of Guinan county, Qinghai province during January～December. They were sent to laboratory of Northwest Plateau Institute of Biology, the Chinese Academy of Sciences (in Xining city) and housed in wire cages individually in a room with natural light through windows. Food (carrot and cabbage) were supplied ad libitum.

2. Collection of Samples

Five days after arrival in the laboratory, pikas in each groups were killed by decapitation during 23:00～24:00 under a dim red light (20～30 lux). The pineal glands were quickly removed, weighed, frozen in liquid nitrogen and stored at - 20℃ for melatonin assay. Trunk blood was collected with heparinized syringe, centrifuged and stored at - 20℃ for testosterone assay. Paired testes, epididymides, seminal vesicles and deferent ducts were dissected and weighed. Then they were fixed in Bouin's fluid and processed for histological studies. Cross sections (8 μm thickness) were examined under light microscope (Olympus BH-2, AD System). Photomicrographs were taken at random at 100 x, 200 x and 400 x and enlarged up to 2 x during printing.

3. Hormone Assay

Melatonin: Each pineal gland was homogenized by sonication (Sonics and Materials INC.) for 5 sec. in 110 μl chilled 0.1 mol perchloric acid containing 0.1% ascorbic acid. Pineal melatonin was measured by high-performance liquid chromatography (Variant 5000) with fluorimeter detection (Shimadzu RF530) described by Wakabayaski et al. (1986). The recovery rate of the pineal melatonin was 96.2±3.2 %.

Testosterone: Plasma testosterone was determined by radioimmunoassay kit (Diagnostic Product, Shanghai Institute of Endocrinology). The recovery rate was 96.9±2.5 %.

4 **Statistical Analysis**

Data are shown as mean SE. Differences between group were analyzed by student's *t* test and one-way-ANOVA.

RESULTS

1. **Body weights**

Seasonal profiles of body weights (BW) in male adult pikas are shown in table 1. Its weight gained during February～May, and reaching peak (147.5±1.8 g, $n = 25$) during June～July. Rapid loss of body mass occurred in August, with the lowest weight (128.9±3.4 g, $n = 22$) at early September. Slow recovery of body weight was observed during October～December.

Table 1 Seasonal changes of body weights, contents of plasma testosterone and pineal melatonin (mean±SE) in Plateau pika

Time of collection	Body weight (g)	Testosterone content (pg/ml plasma)	Melatonin content (pg/gland)
a. Feb. 9	132.0±1.6 (39)*	192.5±23.0 (15)**	154.6±15.9 (12)*
b. Apr. 3	137.6±2.0 (24)	1084.0±96.6 (14)**	109.6±2.0 (12)*
c. Apr. 27	140.3±3.5 (22)	2531.3±220.4 (16)	72.4±5.4 (11)
d. May 26	147.5±1.8 (25)*	2917.0±280.5 (15)**	85.5±11.3 (12)**
e. June 29	140.2±2.5 (21)	469.5±58.7 (17)	136.6±13.1 (14)**
f. Aug. 5	145.2±1.9 (22)**	327.9±53.5 (18)*	200.9±22.2 (14)
g. Sep. 3	128.9±3.4 (22)	199.1±20.5 (17)	186.6±22.8 (14)**
h. Oct. 11	133.6±2.1 (30)	129.8±18.3 (17)	422.3±33.0 (8)
i. Dec. 21	138.0±2.1 (19)*	89.4±10.9 (15)	336.5±45.0 (12)
ANOVA	$P < 0.01$	$P < 0.01$	$P < 0.01$

The sample size are given in parentheses

* $P < 0.05$; ** $P < 0.01$, comparison with the following collecting group

2. **Reproductive organ weights**

The annual changes in the weights of paired testes, epididymides, seminal vesicles and deferent ducts in pikas are shown in table 2. All their weight were low in February and the more elevated values were observed during April～May. The maximal values in late May were followed by rapid and significant ($P < 0.01$) decrease in June～July, and remained low through winter.

3. **Histology of reproductive organ**

Significant structural changes associated with the seasonal cycles of reproductive activity were found in natural population. During April～May, the contorted seminiferous tubules and the ducts of epididymides and deferent ducts were large in diameter and many mature spermatozoa were present in ducts of epididymides and deferent ducts. An inactive gonadal state was observed during July~ February. It was characterized with a gradual reduction of seminiferous tubules and loss of tubular lumen. Spermatozoa were absent in lumen. So the histological studies were well concordant with changes in gonadal weights in seasonal aspects.

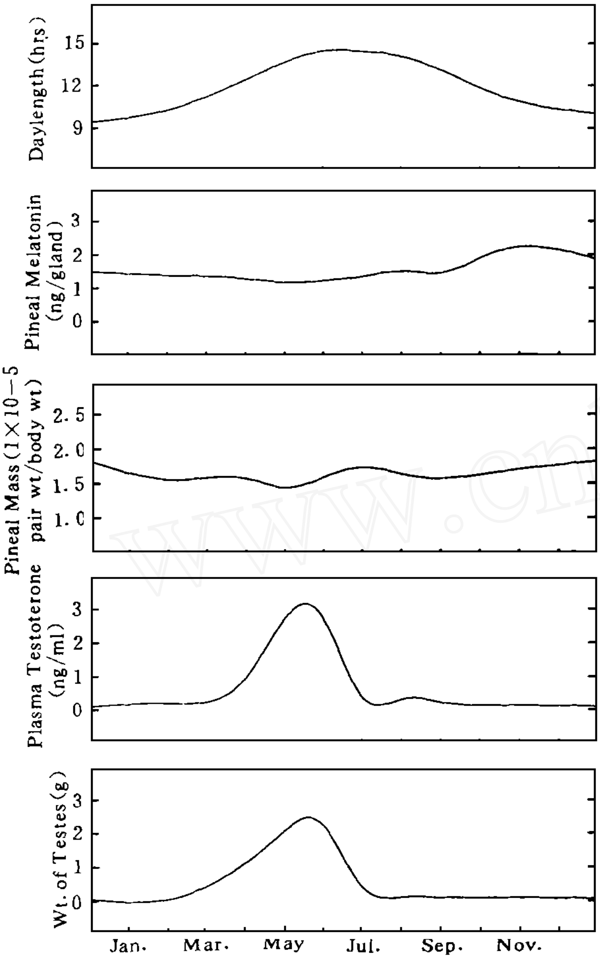

Fig. 1 Seasonal changes in testes-weight, plasms testosterone, pineal mass and pineal melatonin in plateau pika

4 **Plasma testosterone**

Plasma testosterone contents of natural male adult pikas showed a marked annual changes, with it concentration rising significantly ($P <$ 0.001) during April and May (table 1), peaking in late May, then fell sharply to basal levels during late June, remaining low through the winter.

5 **Pineal mass and melatonin**

The pineal mass was the highest in early February (1.32±0.08 mg/100 g, BW, n= 32) and the lowest in late May (0.94 ± 0.06 mg/100 g BW, n= 25). Pineal melatonin levels showed a marked seasonal pattern (Table 1): with contents significantly lower ($P <$ 0.05) between April~ May than that in early February, and rapid recovery of its content occurred in early July and reached the highest at middle October (422.3±33.0 pg/gland, n= 8).

DISCUSSION

1. **Seasonal cycle of reproduction**

Studies on the ecology (Wang et al., 1991), behavior (Wang et al., 1981) and reproductive function (present study) showed that there was a marked seasonal cycle of reproduction in adult male plateau pikas in nature. For the purposes of discussion, the pika's reproductive cycle in this paper was divided into four phases with reference to golden hamster (Reiter et al., 1983): (1) The restoration phase, from early February to early April, the daylength in Qinghai-Tibet Plateau becomes longer and the climate gets warmer (- 8.0~ 1.7℃, the average month temperature).

Pika's testes and their gametogenic function recrudesce, but there is still no evident external reproductive activity in the field. (2) The sexually active phase, from middle April to late May, the climate gets further warmer (the average month temperature is above 5℃) with a longer daylength. Pika's testes reach the maximal weight, this coincides the maximal reproductive capability in this period. (3) The inhibition phase, from June to late August, is characterized by rapidly atrophy of testes and termination of reproduction, while the climate is still warm (the average month temperature is 11.0℃ in June). (4) The sexually quiescent phase, from early September to January of next year, the testes reduce to minimal weights and gametogenesis has ceased, while the climate is getting colder and colder from 8.5 to - 11.7℃ (the average month temperature from September to January). Pikas remain in non-reproductive state throughout the long winter.

Table 2 Seasonal changes of the reproductive organ weight (mean±SE) in male plateau pikas

Time of Collection	Testes wt mean±SE	Epididymides wt mean±SE	Deferent ducts wt mean±SE	Seminal vesicle wt mean±SE
a. Feb. 9	68.4±3.9 (36)**	14.4±1.4 (36)**	1.8±0.1 (37)**	7.0±0.4 (38)**
b. Apr. 3	870.0±37.6 (24)**	32.0±2.1 (24)**	7.5±0.6 (24)**	20.1±1.7 (24)**
c. Apr. 27	1409.1±56.9 (23)**	156.0±10.7 (19)**	12.4±0.6 (18)**	60.0±4.7 (19)**
d. May 26	1624.6±53.5 (28)**	208.8±12.6 (28)**	19.8±1.2 (28)	119.9±14.4 (27)**
e. June 29	351.8±16.9 (21)**	149.7±1.9 (21)**	16.8±0.7 (19)**	36.5±2.3 (19)**
f. Aug. 5	78.9±3.3 (22)	17.8±0.8 (22)**	6.4±0.4 (22)	13.2±0.4 (22)*
g. Sep. 3	72.9±2.9 (20)	13.9±0.6 (18)**	5.7±0.5 (19)*	11.4±0.6 (19)**
h. Oct. 11	67.9±2.4 (30)**	10.0±0.6 (30)**	4.5±0.2 (30)**	8.5±0.4 (29)*
i. Dec. 21	43.7±2.5 (19)*	4.8±0.5 (18)**	1.9±0.2 (19)	6.9±0.7 (19)
ANOVA	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$

The sample size are given in parentheses; The unit of the weights is: 1×10^{-5} pair wt/body wt.

* $P < 0.05$; ** $P < 0.01$, comparison with the following collecting group

2 Photoperiod, pineal melatonin, and reproduction

As showed in Fig. 1, during the short day period, from late June to late December, there was a continued atrophy of gonads and low testosterone level in plasma of male adult pika, and a continued increasing in pineal melatonin contents. It seems like the golden hamster, the short days, acting by way of the pineal gland, maintained the gonads in the inactive state (the sexually quiescent phase). Experiments with different photoperiods in this phase also showed that pikas would develop their reproductive function and appeared mature spermatozoa in the testes under long photoperiod; while pikas under short photoperiod continued present the inhibitory state of the reproductive function (Li et al., 1994b). With increasing of daylength (early January～early April), the lower pineal melatonin level decreased its inhibitory influences to the neuroendocrine-gonadal axis. At this time, pikas would restore their reproductive function under both long photoperiod and short photoperiod. (Li et al., 1994c). As soon as the re-establishment of the reproductive functions, pikas are immediately capable of mating and breeding litters in the early spring of the Qinghai-Tibet Plateau (sexually active phase). The lower pineal melatonin level, high level of plasma testosterone concentration and the heaviest gonadal mass coincided with the maximal reproductive capability of the animals. It was still unclear to the reason of the strongly inhibitory effects on pika's reproductive function in June. As showed at Fig. 1, in June, before the daylength reaches its maximum, nature pika's population already showed the gonad atrophy and lowering of plasma testosterone contents. This is an interesting feature and might be specific to plateau pika, living in habitats of high elevation with shorter summer. The results of our experimental research indicated that plateau pika was a long-day breeder and long-day photoperiod induced increasing male reproductive function and decreasing pineal melatonin secretion (Li et al., 1994c), but its natural population showed decreasing reproductive function even in June, before daylength reached maximum. Therefore, it must have an another controlling factor beside the photoperiod. Secondly, Fig. 1 also showed that the increasing in pineal melatonin level began in May, that is, before the gonad atrophy and testosterone decline. So, pikas probably anticipated the changes in days to come, and the pineal secretory products caused the collapse of the neuroendocrine-gonadal axis (the inhibition phase from July to late August) to adapting shorter summer in high elevation. Therefore it seems that the controlling of reproductive function is a complicated phenomena and it is necessary to study it on different wild mammals with different reproductive strategy.

3 Pineal mass and pika's reproductive function

Studies suggested that both mammal's pineal mass and its metabolizing activities exhibited significantly seasonal variation, and this was in close relationship with photoperiods (Tamarkin et al., 1985). The pineal masses in pikas also present significantly seasonal changes, although the correlation between pineal mass and pineal melatonin level does not show statistically significant during the year. During the restoration phase both pineal masses and melatonin level showed a significantly decreasing tendency with the daylength increasing. Following those changes, gonads continuously recrudesced and reached its heaviest; while the pineal mass decreased continuously to its lightest with lower melatonin content from early April to early May. Then the reproductive activities gradually decreased and came into the inhibition phase. At the same time, pika's pineal mass got heavier and heavier with a higher melatonin level. It was more difficult to explain the sexually quiescent phase, while pika's gonads showed the strongest inhibiting with lighter pineal mass and higher melatonin content. This is during the time of shorter photoperiod.

4 Other factors affecting pika's seasonal reproduction

It was testified that photoperiod was of more importance to regulate pika's seasonal reproduction (Li et al., 1994b, 1994c). In the mean while, there are also some other possible factors participating in the regulation. During the inhibitory period (from late

May to late June) of pika's reproduction, temperature, humidity and rainfall in the field seems not effective to inhibiting the reproduction. According to the meteorological information of Qinghai Meteorological Observatory: during this period, the environmental temperature increases from 9.8℃ to 12.3℃, the rainfall amount of 24.1 mm decreases to 21.9 mm, both temperature and rainfall were not changed greatly during May～June. No paper had been reported on the effects of social factors on pika's seasonal reproduction. From our field investigation, it seemed that the behavior and the population density would not strongly inhibit the seasonal reproduction during May～June. But which factors would play the important role in inhibiting the reproduction during this period? It probably related to pika's foods, nutrients and energetic strategies. Researches showed that mammals needed outweigh more energy for their reproduction: growth of the reproductive organ, gametogenesis, nesting site, male dominance, territorial defense, pregnancy, lactation, give birth (Bronson, 1994). Female plateau pika would increase her food intake more than 30% when she became pregnant or gave birth (Wang et al., 1980). During the "green up" phenological period (April～May) in Qinghai-Tibet Plateau, the energy intake of a pika was about 150 Cal/day (Wang et al., 1980). Pikas would outweigh more caloric not only for their body growth (from 140 g to 148 g, $n = 25$, present study), but also for the birth. Another investigation showed that the stored fat decreased significantly during May～June (Wang et al., 1989). All those energetic changes probably would inhibited the reproduction at this time.

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中 文 摘 要

高原鼠兔的季节性繁殖*

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作者对成年雄性高原鼠兔 (*Ochotona curzoniae*) 野外繁殖进行了全周年的调查。同时, 对松果腺在其季节性繁殖周期调控中所起的作用进行了探讨。2月至4月上旬为其季节性繁殖的恢复期, 睾丸、附睾、输精管和精囊腺的重量增加, 血浆睾硐水平升高; 但松果腺重量和褪黑素 (Melatonin, MLT) 含量降低。4月中旬至5月下旬为性活跃期, 性腺器官显著增重, 睾硐水平升至最高; 而松果腺重量和MLT 含量呈现最低水平。6月至8月, 鼠兔的性腺功能明显减弱; 而松果腺重量和MLT 含量开始升高, 此时为抑制期。9月至1月为性休止期, 性腺器官的重量及血浆睾硐水平维持在最低水平; 而松果腺重量和MLT 含量在较高的水平波动。结果表明, 高原鼠兔的繁殖呈现明显的季节性。其松果腺活动的年周期反映了自然光周期的变化, 并与鼠兔的季节性繁殖密切相关。

关键词　高原鼠兔; 繁殖; 松果腺; 褪黑激素

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