Reproductive Cyclicity Based on Fecal Steroid Hormones and Behaviors in Sumatran Tigers, *Panthera tigris sumatrae*

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糞中の性ステロイドホルモンの動態と行動からみたスマトラトラの繁殖活動の周期性

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ABSTRACT. Fecal concentrations of progesterone (P_4), estradiol-17 β (E_2) and testosterone (T) were measured and daily behaviors were recorded in a pair of Sumatran tigers. The fecal P_4 profile of the female indicated cyclic changes, and the cycle length was 58.3 ± 2.7 days. The copulation interval was 58.0 ± 4.0 days. When fecal P_4 contents were low and began to increase, the female's flehmen and exhibiting the tail area to the male were observed. Fecal E_2 contents in the female and T contents in the male tended to be high around the time when copulation was seen, but not always; however, the male responded to the female estrous signs by vocalization, flehmen, chasing, mounting and copulation. It is possible to assess non-invasively reproductive activities by monitoring fecal P_4 concentrations in the female and the sexual behaviors of male and female Sumatran tigers.

Key Words: fecal steroid hormone, sexual behavior, Sumatran tiger.

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Indonesia is well known as a habitat for three tiger subspecies; however, about 70 years ago, Javan (*Panthera tigris sondaica*) and Bali tigers (*P. t. balica*) disappeared due to poaching and over hunting, and the Sumatran tiger (*P. t. sumatrae*) became the only living Indonesian tiger subspecies [1]. The population is predicted to be around 400 to 500 individuals and, based on the 2006 IUCN Red List of Threatened Species, it is categorized as a critically endangered species [2]. To improve the Sumatran tiger population *in situ* and *ex situ*, the breeding performance and reproductive physiology of Sumatran tigers should be understood. The Indonesian government created Tiger Conservation Units in Aceh, Riau, Jambi and Lampung provinces of Sumatra to secure the future of Sumatran tigers [3]; moreover, international zoos and breeding institutions are promoting breeding and conservation programs for this exotic felid.

There are limited scientific references on the reproductive

physiology and endocrinology of tigers [4-5], especially Sumatran tigers. This lack of information on reproductive physiology offers many research opportunities. The Sumatran tiger is the smallest tiger, reaching sexual maturity at about 3 years of age for females and 4 years of age for males. The estrous interval is about 3-9 weeks and females are receptive to males for about 3-6 days [6-7]. The gestation period is about 103 days [3].

Serum hormones are the most accurate reflection of gonadal activity. Most circulating steroid hormones are metabolized in the liver, and discarded into urine and feces as waste material to be removed from the body [8]. Recently, fecal hormones measurements have become the preferred method of assessing the reproduction status of felids such as Siberian tigers (*P. t. altaica*) [4-5], Asiatic lions (*P. leo persica*) [9], African lions (*P. leo*), snow leopards (*Uncia uncia*), cheetahs (*Acinonyx jubatus*) and clouded leopards (*Neofelis nebulosa*) [10]. Fecal sampling

for fecal hormone analysis is available as a non-invasive method with long-term advantages, and fecal samples from felids are widely used.

The purpose of the present study was to monitor the reproductive status of Sumatran tigers by assessing changes in fecal progesterone (P_4) and estradiol-17 β (E_2) in a female tiger, and testosterone (T) in a male tiger, and by understanding the relationship between fecal hormone changes and sexual behavior during natural estrus in their *ex situ* habitat.

The animals monitored were a pair of Sumatran tigers, a female named Yuri (International Studbook No. 1074, 6 years of age at the beginning of this study) and a male named Suntik (No. 842, 10 years of age) housed at Ueno Zoological Gardens, Tokyo, Japan. The tigers live together and have free access to the natural photoperiod in an outdoor paddock during the daytime, and are isolated in individual indoor chambers at night. They were sexually mature based on their individual ages during this study. They were fed a diet consisting of horse meat, chicken and rabbit meat. Drinking water was available *ad libitum*.

Fecal samples were collected within at least 18 hrs after excretion approximately once a week from July 2004 to November 2005 for both tigers; however, on several occasions, the collection of feces was difficult as they were excreted in a pond or drinking water container. Feces were stored at -20°C immediately after collection. The fecal extraction procedure was described in previous reports [4, 11]. Briefly, fecal samples were lyophilized for 48 hr and pulverized prior to extraction in order to separate digested fecal material from undigested bones and hair. Fecal powder (0.1g) was extracted with 5 ml of 80% methanol in water, mixed and shaken for 30 min. After centrifugation at 2500 rpm for 10 min, each aliquot of solution was diluted 10 times with assay buffer.

The fecal contents of P_4 , E_2 , and T were determined by enzyme immunoassay [4, 11]. P_4 antiserum (LC-28; Teikoku Hormone Mfg. Medical, Kanagawa, Japan) mainly cross-reacts with P_4 (100%), 5 α -pregnanedione (62.2%), pregnenolone (6.3%), 11-deoxycorticosterone (3.9%), 17 α -hydroxyprogesterone (2.3%), and 11 α -hydroxyprogesterone (1.2%). E_2 antiserum (QF-121; Teikoku Hormone Mfg. Medical) mainly cross-reacts with E_2 (100%), estrone-3-sulfate (8.0%), 16-epiestriol (5.3%), estrone (3.2%), and estriol (1.8%). T antiserum (ED-131; Teikoku Hormone Mfg. Medical) mainly cross-reacts with T (100%), 5 α -dihydrotestosterone (41.3%), and androstenedione (2.1%). The intra- and inter-assay coefficients of variation were 2.0 and 5.0% for P_4 , 2.6 and 11.2% for E_2 and 2.2 and 5.6% for T, respectively.

Daily observation of external sexual behavior of the Sumatran tigers was conducted by the animal keeper at no specific observation time during 9:00 to 16:30 from July 2004 to November 2005. Female estrous behavior included vocalization, flehmen, spray urination (marking) and female tail exhibit to the male. For the male tiger, sexual behavior included

vocalization, flehmen, penile erection (excluding copulation), chasing, mounting and copulation. The female estrous behavior in this study has been used for Asiatic lions [9], snow leopards [12] and leopards (*P. pardus*) [13].

All data are shown as the mean $\pm$ SEM. The ovarian cycle was calculated based on fecal P_4 cycles. Cycle length was calculated as the number of days from the first rise in fecal P_4 of one cycle to the P_4 rise in the following cycle. The copulation interval was calculated as the number of days from the first day of the copulation period to first day of the next copulation period. Each sexual behavior was scored as one (1) for the presence and zero (0) for the absence of external events which occurred daily. Total daily sexual behavior scores were expressed as weekly frequency.

There are some reports on the ovarian activities of tigers [4-5, 14]; however, this study is the first description of fecal gonadal hormone monitoring combined with observation data of external sexual behavior during natural estrus in Sumatran tigers.

In female Sumatran tiger No. 1074, fecal P_4 contents changed cyclically and ranged from 1.56 to 37.59 $\mu\text{g/g}$ ($12.17 \pm 9.89 \mu\text{g/g}$, $n = 52$ samples) (Fig. 1). It was evidenced for the first time in the female tiger that fecal P_4 profiles showed cyclical changes. Our results included 4 distinct cycles (Fig. 1). The cycle length based on changes in fecal P_4 was 58.3 ± 2.7 days ($n=4$ cycles). This fecal P_4 cycle length corresponded with the copulation period of 58.0 ± 4.0 days ($n = 8$ intervals). Other P_4 cycles are probably included in Fig. 1, but the cycle length could not be distinguished due to the low frequency of sampling. Fecal E_2 contents changed from 0.12 to 27.87 $\mu\text{g/g}$ ($2.36 \pm 4.50 \mu\text{g/g}$, $n = 52$ samples). Although only 3 increases were recorded in August and November 2004, and October 2005, no distinct cycles were shown in the fecal E_2 contents of this female. Fecal E_2 in August 2004 and October 2005 temporarily increased when fecal P_4 levels were basal. Some E_2 peaks might have been missed due to the low frequency of sampling.

When fecal P_4 contents were at the basal level and began to increase, flehmen and exhibiting its tail to the male were visually observed in the female (Fig. 1). During this period, the female was chased and mounted by the male and accepted copulation. Therefore, female flehmen and exhibition of its tail to the male are probably estrous signs in this tiger. Vocalization is an external event appearing to be common to many large felids during estrus and became a valid indicator of estrous status, as in Asiatic lions [9], snow leopards [12], leopards [13] and Siberian tigers [15]. It has been reported that vocalization was the most potent cue of estrous status in female felids and vocalization was the first noticeable and dominant sexual activity observed during natural and induced estrus in Asiatic lions [9]. It was also the dominant estrous activity in female snow leopards [12]. It has also been reported that, in the wild, chemical communication by spray urination/markings is a very important aspect of communication between tigers

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Fig. 1 Relationship between sexual behaviors and changes in fecal contents of progesterone and estradiol-17 β in female and testosterone in male Sumatran tigers. Filled marks indicate female data. Unfilled marks indicate male data.

[16]. In our study, several vocalizations and spray urinations were observed in the female; however, they could not be used as a clear indicator of estrus. In limited circumstances, such as in captivity, males can probably easily receive female estrous signs and females might not exhibit strong estrous behavior. In this study, we assumed that estrous behavior in the female stimulated the libido of the male. This was assumed from the male's vocalization and flehmen, which appeared following the female's estrus. Our data showed that the male responded to the female's signs by chasing and mounting, and finally by copulation.

In the male Sumatran tiger used in this study, fecal T contents varied from 0.21 to 6.69 $\mu\text{g/g}$ ($2.19 \pm 1.20 \mu\text{g/g}$, $n = 68$ samples). Fecal T contents tended to be high around the time when copulation was seen, that is, when fecal P_4 contents in the female were at the basal level, but not always. Fecal T of the male and P_4 of the female markedly changed throughout the year, and the female accepted copulation throughout the year. The Sumatran tigers in this study had no seasonal reproductive pattern. Although tigers in the wild can breed all year, they typically breed during the winter or spring (about the end of November to April) [6]. Bengal tigers in the wild seem to breed all year; however, the peak of breeding activities is from November to February [17]. Our study confirmed physiologically that Sumatran tigers can breed throughout the year.

Tigers are known as induced ovulators and rare individuals are spontaneous ovulators [5, 18]. A rise of fecal P_4 only after copulation has been reported, even though there were clear changes in fecal E_2 in female Siberian tigers [4]. Our results indicated that the female Sumatran tiger had a cyclical P_4 increase followed by cyclical estrus, and the result was a spontaneous ovulation-like pattern; however, this pattern did not show valid evidence to indicate that spontaneous ovulation occurred in this tiger, because copulation occurred at all basal P_4 levels and estrus. This pair of Sumatran tigers has been non-successful in breeding despite cyclic copulation and a P_4 pattern. The relationships between successful breeding and hormone patterns should be clarified and the reproductive/physiological mean of spontaneous ovulation in tigers should also be understood to improve the captive breeding of tigers in the future.

We conclude that it is possible to assess non-invasively ovarian events such as luteal activity and ovulation of Sumatran tigers by endocrine monitoring based on fecal P_4 and to understand reproductive physiology by combining with the observation of sexual behavior.

要 約

スマトラトラの雌のプロジェステロン (P_4) とエストラジオール- 17β (E_2) および雄のテストステロン (T) の各糞中含量を測定し、このペアの行動を毎日記録した。雌の糞中 P_4 含量には 58.3 ± 2.7 日間の周期性がみられ、交尾行動は 58.0 ± 4.0 日間隔で確認された。糞中 P_4 含量が増加し始める時期に雌

のフレーメンと雄への尻向け行動が多くみられた。雌の糞中 E_2 および雄の糞中 T 含量は、交尾行動がみられた時期に高い傾向が認められたが、毎回ではなかった。しかし、雄の発声、フレーメン、追尾、マウントおよび交尾行動は、雌の発情に合わせて発現した。スマトラトラの繁殖活動は、雌の糞中 P_4 含量と雌雄の性行動から非侵襲的に把握できることが示された。

キーワード: スマトラトラ, 性行動, 糞中ステロイドホルモン

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