Fecal reproductive steroid profiles for monitoring reproductive patterns in female Formosan black bears (*Ursus thibetanus formosanus*)

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Fecal sexual estradiol (E_2) and progesterone (P_4) analyses were conducted on three adult female Formosan black bears in order to establish non-invasive enzyme-linked immunosorbent assays (ELISA) for describing the endocrine profile in a simulated natural breeding environment. Moreover, we investigated whether the Formosan black bear living in subtropical habitats in Taiwan is a seasonal breeder with delayed embryo implantation, ovulation pattern, and maternal perinatal fasting. Our results indicate that mating behavior occurred both before and after the fecal E_2 peak of March–June. Fecal P_4 concentrations initially increased after mating, then decreased to lower levels, and finally increased again, which is indicative of a delayed embryo-implantation. Fecal P_4 levels decreased postpartum. Peaks of fecal P_4 levels were also observed in unmated female bears housed with male bears without coitus: their annual fecal P_4 levels were higher from winter to spring, similar to the pregnant female bear. Interestingly, only pregnant female bears displayed a physiological state of fasting before and after parturition. These results suggest that Formosan black bears' breeding is seasonal and takes place from spring to early summer. Delayed implantation, maternal perinatal fasting and induced ovulation from male stimulation independent of coital stimulation alone are typical for the species.

Introduction

The family Ursidae consists of two subfamilies: Ailurinae and Ursinae. The Formosan black bear (*Ursus thibetanus formosanus*) is an endemic subspecies of the Asiatic black bear and belongs to the genus *Ursus* (Wozencraft 2005, Hwang & Garshelis 2007). Their principal color is black, with a white "crescent moon" on the chest (Fig. 1). They are the largest carnivore endemic to Taiwan and live from 200 m a.s.l. in broadleaf forests to 3500 m a.s.l. in coniferous forests of the Central Mountain Range. However, less evidence is available to support their persis-

Fig. 1. Young Formosan black bear.

tence at elevations below 1000 m a.s.l., with the exception of foraging during winter (Hwang *et al*. 2000). Since 1989, this species has been protected in Taiwan under the wildlife conservation law, prohibiting hunting, possession and slaying (Chang *et al*. 2006). Close to a decade later, the bear had been included in the *Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), appendix 1*, as endangered, and in 2008 the World Conservation Union (IUCN) included it in the *Red List of Threatened Animals*, indicating a declining population and local extirpation caused by habitat degradation and illegal hunting. The act also makes it illegal to trade the Formosan black bear (Wang 1990, Chang *et al*. 2009).

Generally, Ursidae living within temperate habitats such as the American black bear, the Hokkaido brown bear and the Japanese black bear are seasonal breeders; mating takes place in spring or summer, and birth in winter (Erickson *et al*. 1964, Tsubota *et al*. 1985, Sato *et al*. 2000); tropical zone bears such as the sloth bear and spectacled bear mate year-round (Spady *et al*. 2007). Alternatively, polar bears mate between March and May, i.e. during the period of increasing day length (Parovshchiko 1964, Palmer *et al*. 1988). However, to date there has been little information regarding the reproductive characteristics of subtropical bears and a lack of detailed information on annual endocrine events, especially for female Formosan black bears.

Previous work on the profiles of sex steroid hormones has provided some information on the reproductive biology of female Formosan bears and has helped in estimating the number of female bears and their suitable habitats (Tsubota *et al*. 1998, Ishikawa *et al*. 2002). The hormones that we measured, estradiol (E_2) and progesterone (P_4), are reliable indices commonly used for detecting ovarian and luteal activity as well as identifying pregnancy status in Ursidae (Sato *et al*. 2000, Onuma *et al*. 2002). In this study, we attempted to describe the reproductive pattern of female Formosan black bears via a noninvasive method assay that monitored fecal E_2 and P_4 changes, which did not require immobilization of the animals. This technique was chosen because of its proven effectiveness on the reproductive cycles of bears and because of its unobtrusive nature in contrast to the dangers of anesthesia when blood is used for hormone assays (Onuma *et al*. 2001, Ishikawa *et al*. 2003).

Material and methods

Animals and study area

Three adult female Formosan black bears were used in this study. Female 1 was confiscated from a hunter, female 2 was obtained from a private amusement park, and female 3 was obtained from a private donation. They originated from the wild and were housed individually in covered outdoor enclosures (9 m × 5 m × 2.5 m), except during mating periods. Although during the study period female 1 and female 2 were in contact with two different male bears, they did not mate. Female 3 mated during 22–23 March 2001, 5–9 May 2002, and 15–18 April 2003. She produced a cub after each mating period (born in early November 2001, 2002 and October 2003). The study was carried out at the Low Altitude Experimental Station of the Endemic Species Research Institute (Taichung County, 120°56′52.47′′E, 24°16′24.39′′N, 1000 m a.s.l.). Climate at the station was typical for natural breeding conditions. An appropriate diet, according to Yang *et al*. (2001), was provided

throughout the study. The reproductive condition and physical status of all bears were observed and recorded by technicians.

Fecal sample collection

Fecal samples of females 1, 2, and 3 were collected every 2–3 days during January 2001–December 2003, January 2002–December 2003, and April 2001–December 2003, respectively. However, fewer fecal samples of female 3 were obtained during the period of parturition in November 2001, November 2002, and October 2003 due to very limited food intake before and after parturition. Furthermore, to avoid interference during the first parturition, samples were not collected from female 3 during November–December 2001. A total of 1090 fecal samples were collected and frozen at –20 °C until analyses were performed.

Fecal reproductive steroid extraction and analyses

Fecal reproductive E_2 and P_4 were extracted and assayed according to a modified version of the protocol described by Yang *et al.* (2003). In brief, 3 ml of assay buffer (0.01 M EDTA, 0.12 M NaCl, 0.04 M MOPS, 0.1% gelatin, 0.05% Tween 20, 0.005% chlorhexidine digluconate, pH 7.4) was added to 1 g of a wet fecal sample, agitated for 10 min at room temperature and then heated at 100 °C for 10 min and agitated again for 20 min. Supernatants were collected after centrifugation (30 min, 3500 rpm), and used as the fecal E_2 and P_4 assay samples. Fecal E_2 and P_4 were quantified using enzyme-linked immunosorbent assay (ELISA) analysis and assayed in triplicate. The antiserum for the E_2 and P_4 measurements was prepared in rabbit anti-estradiol-17β-6-CMO-BSA (Anti-E_2-6-BSA) at a final dilution of 1:10 000 as in Chen and Mao (1993) or obtained from mice anti-progesterone-11-BSA (Anti-P_4-11-BSA) at a final dilution of 1:80 000 (Chemicon International, CA, USA), respectively. Crossreactivity of both E_2 and P_4 with other endogenous steroids of this assay was minimal (≤ 2%). The detection

levels were 26 pg ml^{-1} and 60 pg ml^{-1} for the E_2 and P_4 assays, respectively. An intra-assay coefficient of variation was obtained via measuring the standard curve: for 4 individual replicates of the E_2 and P_4 assays, values of 4.9% and 6.4% were found, respectively. Two control samples (3 ng ml^{-1} and 200 p g ml^{-1}) gave coefficients of variation of 8.8% and 5.1% for the E_2 inter-assay ($n = 76$ for both comparisons), respectively; and 9.3% and 9.7% for the P_4 inter-assay ($n = 78$ for both comparisons), respectively. Mean recovery rates of E_2 (3 ng ml^{-1}) and P_4 (3 ng ml^{-1}) added to feces were 95.4% ($n = 12$) and 93.2% ($n = 13$), respectively. Using this procedure it was possible to extract and measure unconjugated E_2 and P_4 from feces.

Correlation between serum and fecal reproductive steroid concentrations

Blood samples that individually corresponded to the fecal samples were collected from the Formosan black bears' jugular veins during a routine physical examination carried out under anesthesia (intramuscular injection of Zoletil, Virbac Co. Ltd., Taipei, Taiwan; dosage 5010 mg kg^{-1}). Two to three samples were collected from each bear, for a total of seven blood samples.

Seven sample sets, each consisting of blood serum and extracted fecal reproductive steroids collected from the same individual on the same day, were used to examine the correlation between the serum and fecal concentrations of E_2 and P_4. Blood samples were cooled at room temperatures for 2 to 3 min and then centrifuged at 3000 rpm for 10 min; the separated serum was immediately collected. The serum samples were pipetted off and stored at –20 °C until analyses. Serum concentrations of E_2 and P_4 were measured by automatic immunoassay instruments (AxsymTM system; Abbott Laboratories, Taipei, Taiwan) using the ELISA analysis.

Statistical analysis

Seasonal changes in E_2 and P_4 were compared using a *t*-test for each bear and year. To evaluate relationships between serum E_2 and fecal E_2,
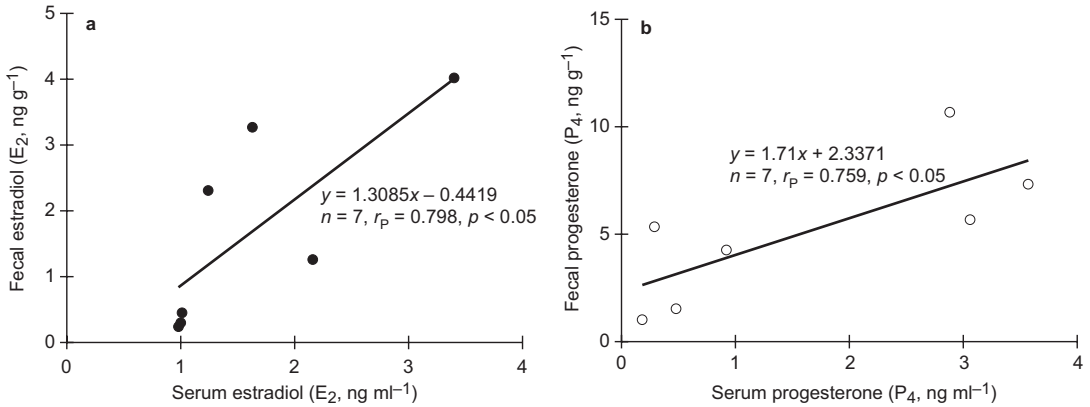
Fig. 2. Correlation of steroid hormone concentrations between serum and fecal samples in Formosan black bears. (**a**) estradiol, (**b**) progesterone.

and serum P_4 and fecal P_4, Pearson correlation (r_p) coefficients were calculated and a regression analysis performed.

The E_2 and P_4 concentrations are presented as means ± SE.

Results

Correlation between serum and fecal reproductive steroid concentrations

The fecal E_2 and P_4 concentrations correlated positively with concentrations in the blood serum ($r_p = 0.798$, $p < 0.05$, $n = 7$ for E_2 and $r_p = 0.759$, $p < 0.05$, $n = 7$ for P_4) (Fig. 2).

Annual changes of E_2 concentrations in feces

During the study period, fecal E_2 concentrations varied in all three bears (Fig. 3). In unmated female 1, fecal E_2 concentrations increased in the late winter, reaching peaks in June 2002 (15.04 ± 2.82 ng g^{-1}; $p < 0.001$) and May 2003 (30.43 ± 9.53 ng g^{-1}; $p < 0.001$). Concentrations markedly decreased after July and returned to a base value during November–December in 2002 (from 2.48 ± 0.45 to 2.05 ± 0.55 ng g^{-1}) and 2003 (from 0.59 ± 0.41 to 2.46 ± 1.16 ng g^{-1}).

No significant differences in monthly fecal E_2 concentrations in female 2 in 2002 were found, with the exception of low levels in November (1.41 ± 0.38 ng g^{-1}). E_2 concentrations were highest in April 2003 (8.87 ± 1.09 ng g^{-1}; $p < 0.01$), and decreased markedly after June until December 2003 (1.99 ± 0.58 ng g^{-1}). No mating behavior was observed during the periods of sample collection.

The mated bear, female 3, displayed a similar monthly trend in fecal E_2 concentrations: increasing in spring, reaching a peak in May 2001 (3.91 ± 0.04 ng g^{-1}; $p < 0.01$), May 2002 (17.01 ± 2.02 ng g^{-1}; $p < 0.001$), and March 2003 (13.48 ± 6.04 ng g^{-1}; $p < 0.001$), and then decreasing markedly, with the exception of a secondary peak in October 2002. Mating behavior was observed prior to or following the fecal E_2 peaks during the spring seasons of 2001, 2002 and 2003.

Determination of breeding and non-breeding seasons

Our findings showed that seasonal fecal E_2 concentrations were lower from mid-summer to early winter (July–December), and tended to increase after winter. Based on the fecal E_2 peaks that occurred during spring–early summer (March–June) and mating behavior that was observed both before and after fecal E_2 peaks, the breeding season lasts from March to June, and the non-breeding season from July to February. Fecal E_2 concentrations in the two seasons were significantly different ($p < 0.001$) (Table 1).
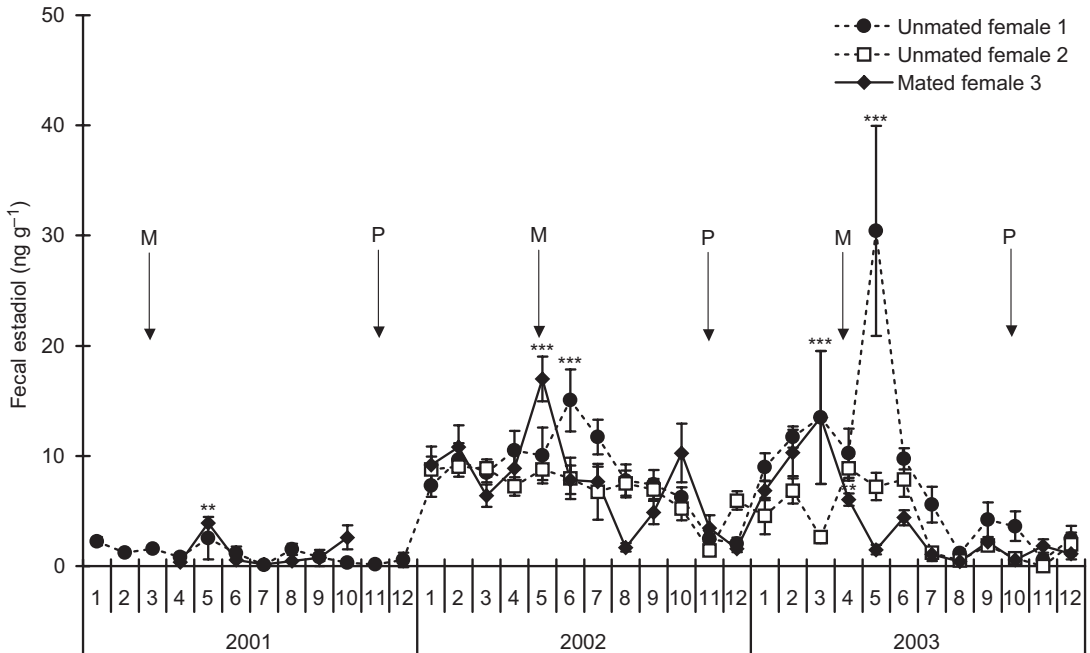
Fig. 3. Annual changes in fecal estradiol concentrations in three female Formosan black bears. Female 1, January 2001–December 2003; female 2, January 2002–December 2003: no significant differences were found during 2001 for unmated female 1 and in 2002 for female 2. Highest fecal estradiol concentrations were observed in June 2002 and May 2003 for female 1, and April 2003 for female 2. Mated female 3, April 2001–December 2003: highest fecal estradiol concentrations were observed in May 2001, 2002, and March 2003. Significant differences in fecal estradiol concentrations between months are indicated with asterisks: ** $p < 0.01$ and *** $p < 0.001$. The arrows indicate the times of apparent mating (M) and parturition (P) for female 3. Data are presented as means ± SE.

Moreover, the mean fecal E_2 concentrations during January–June (7.42 ± 0.78 ng g^{-1}) differed significantly ($p < 0.001$) from those during July–December (2.94 ± 0.83 ng g^{-1}) (Table 2).

Annual changes in fecal P_4 concentrations

In non-pregnant female 1, fecal P_4 concentrations increased from late winter until spring and declined thereafter (Fig. 4). Concentrations were the highest in March 2001 (87.71 ± 17.05 ng g^{-1}; $p < 0.001$), December 2002 (29.12 ± 7.24 ng g^{-1}; $p < 0.01$) and May 2003 (51.25 ± 11.23 ng g^{-1}; $p < 0.001$).

The same changes in fecal P_4 concentrations — i.e., increase in winter 2002, and then decline in March 2003 — were observed in non-pregnant female 2. Moreover, higher levels were measured in April–May 2002 (15.94 ± 1.32–16.50 ± 2.13

ng g^{-1}) and May–June 2003 (14.83 ± 3.94–15.11 ± 3.67 ng g^{-1}) than in June–October 2002 (3.43 ± 0.44–5.46 ± 0.75 ng g^{-1}; $p < 0.001$) or July–October 2003 (4.51 ± 0.89–8.66 ± 1.82 ng g^{-1}; $p < 0.01$). Concentrations were highest in December 2002 (31.89 ± 3.81 ng g^{-1}; $p < 0.001$) and February 2003 (16.45 ± 2.16 ng g^{-1}; $p < 0.05$).

Monthly fecal P_4 concentrations of pregnant female 3 increased after mating. A delayed implantation phase was characterized by a lower P_4 concentration detected at the beginning of the bear's gestation period (Ishikawa *et al*. 2002, Spady *et al*. 2007) that lasted for two–three months (July–September 2001, July–August 2002 and 2003). Concentrations then increased, which was apparently associated with implantation two months prepartum, and were the highest in October 2001 (56.89 ± 7.91; $p < 0.001$), December 2002 (39.06 ± 3.37; $p < 0.01$) and September 2003 (30.19 ± 14.01; $p < 0.05$), a month prior to or after parturitions. Moreover,

concentrations started to decrease from their peak and dropped to base levels (15.38 ± 2.39) within three months postpartum.

Monitoring of gestation length

The exact dates of mating and parturition recorded for female 3 revealed that gestation in the Formosan bear lasts for 214.7 ± 22.5 days, and cubs are born in the late autumn (Table 3).

Discussion

Measurements of steroid hormones in feces do not require stressful restraint procedures necessary for conventional blood sampling, and thus do not limit sampling frequency (Dehnhard *et al.* 2006). However, since different assay methodologies for fecal E_2 and P_4 exist, a method must be tested to ensure that comprehensive information on endocrine events will be collected from fecal samples. Here, our method of combining assay buffer extraction and the ELISA analysis was applied to measure fecal E_2 and P_4 concentra-

Table 1. Comparison of fecal estradiol (E_2) concentrations (mean ± SE) between breeding and non-breeding seasons for adult female Formosan black bears. Sample sizes (*n*) are shown in parentheses. Significance of differences are indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Bears	Year	Mate	E_2 (ng g^{-1})	
			Non-breeding season (July–February)	Breeding seasons (March–June)
Female 1	2001	No	0.86 ± 0.26 (*n* = 102)	1.52 ± 0.37 (*n* = 59)
	2002	No	6.83 ± 1.15 (*n* = 98)	11.02 ± 1.41** (*n* = 50)
	2003	No	4.79 ± 1.36 (*n* = 104)	15.98 ± 4.88*** (*n* = 43)
Female 2	2002	No	6.45 ± 0.85 (*n* = 87)	8.22 ± 0.39** (*n* = 47)
	2003	No	2.21 ± 0.82 (*n* = 101)	6.65 ± 1.38*** (*n* = 41)
Non-mated means	2001–2003		4.23 ± 1.17	8.68 ± 2.39**
Female 3[a]	2001	Yes	0.98 ± 0.54 (*n* = 49)	1.61 ± 1.14 (*n* = 22)
	2002	Yes	6.18 ± 1.34 (*n* = 89)	10.03 ± 2.38** (*n* = 52)
	2003	Yes	3.02 ± 1.25 (*n* = 95)	6.35 ± 2.55* (*n* = 51)
Mated means	2001–2003		3.40 ± 1.51	5.99 ± 2.43*
Total means	2001–2003		3.91 ± 0.46	7.67 ± 1.09***

[a] Fewer samples were obtained from pregnant female 3 due to sampling restrictions in 2001 (*see* text).

Table 2. Comparison of fecal estradiol (E_2) concentrations (mean ± SE) between the first (January–June) and secondary halves (July–December) of the years for adult female Formosan black bears.. Sample sizes (*n*) are shown in parentheses. Significance of differences are indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Bears	Year	Mate	E_2 (ng g^{-1})	
			July–December	January–June
Female 1	2001	No	0.58 ± 0.21 (*n* =79)	1.59 ± 0.27* (*n* = 82)
	2002	No	6.27 ± 1.47 (*n* = 75)	10.17 ± 1.08*** (*n* = 73)
	2003	No	2.94 ± 0.77 (*n* = 78)	14.11 ± 3.33** (*n* = 69)
Female 2	2002	No	5,63 ± 0.91 (*n* = 71)	8.45 ± 0.28*** (*n* = 63)
	2003	No	1.05 ± 0.32 (*n* = 72)	6.33 ± 0.95*** (*n* = 70)
Female 3[a]	2001	Yes	0.98 ± 0.54 (*n* = 49)	1.61 ± 1.14 (*n* = 22)
	2002	Yes	4.91 ± 1.41 (*n* = 69)	10.01 ± 1.52*** (*n* = 72)
	2003	Yes	1.08 ± 0.23 (*n* = 75)	7.08 ± 1.73*** (*n* = 71)
Total means	2001–2003		2.94 ± 0.83	7.42 ± 0.78***

[a] Fewer samples were obtained from pregnant female 3 due to sampling restrictions in 2001 (*see* text).
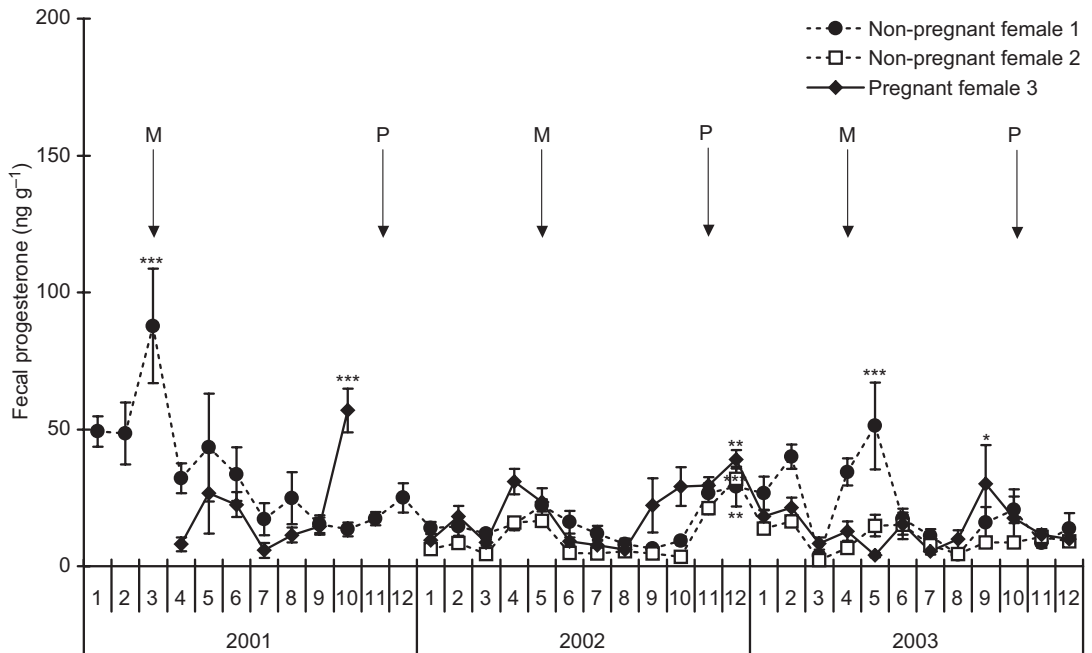
Fig. 4. Annual changes in fecal progesterone concentrations in three female Formosan black bears. Non-pregnant female 1, January 2001–December 2003; Non-pregnant female 2, January 2002–December 2003: highest fecal progesterone concentrations were observed in March 2001, December 2002 and May 2003 for female 1, and December 2002 and February 2003 for female 2. Pregnant female 3, April 2001–December 2003: highest progesterone concentrations were observed in October 2001, December 2002, and September 2003 near or following parturitions. Significant differences in fecal progesterone concentrations between months are indicated with asterisks: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. The arrows indicate the times of apparent mating (M) and parturition (P) for female 3. Data are presented as means ± SE.

tions. In addition, it was important to avoid variation in steroid levels in feces due to interference from fecal contents resulting from high-fiber diets or steroid conversion from conjugated to unconjugated forms by intestinal microorganisms. To maintain congruency during the study periods, all three bears were fed the same diet and samples were frozen at a fixed time after defecation. Thus, our fecal endocrine profile provided a more thorough and, therefore, credible set of data for research of sexual steroid hormones as compared with a few serum samples collected annually.

Results of our endocrine studies showed that fecal E_2 concentrations reached their highest levels during March–June, in parallel with mating behavior, and then decreased until December. These results corroborate our previous studies (Chang *et al.* 2004) in which we found FSH (follicle stimulating hormone) concentrations being highest in winter, suggesting that FSH stimulates follicular action and the ovaries recrudesce after winter. This may indicate that — similarly to bears of the temperate zone (Erickson *et al.* 1964, Tsubota *et al.* 1985,

Table 3. The length of gestation of a pregnant Formosan black bear.

Year	Mating date	Date of birth	Gestation length (days)
2001	22–23 March	4 November	227
2002	5–9 May	6 November	175
2003	15–18 April	3 October	171
Mean ± SE			214.7 ± 22.5

Sato *et al*. 2001) — the Formosan black bear is a seasonal breeder that starts the estrus cycle in the spring. Reports indicated that the breeding season of polar bears is in spring when food resources are abundant (Palmer *et al*. 1988, Spady *et al*. 2007). Sun bears in Malaysia do not hibernate and their breeding season occurs during the rainy season of August–September, corresponding with fruit abundance (Onuma *et al*. 2001). Thus, it is reasonable to speculate that the breeding season of the Formosan black bear, which do not hibernate, occur from spring to early summer, when food becomes abundant after winter. Moreover, results suggest that by increasing foraging activity and territory size, females increase their opportunities for multiple matings (Howell-Skalla *et al*. 2002, Fernandez-Gil *et al*. 2006).

In the northern hemisphere, matings of most Ursidae take place in spring or summer, when food resources are plentiful (Renfree & Calaby 1981, Yamamoto *et al*. 1998). Our results indicated that fecal E_2 concentrations were higher during the first half of the year as compared with those in the latter half of the year. On the other hand, records from 2002, 2005, and 2007 from the warmer habitat of Shou Shan Zoo in Kaohuiung showed that mating behavior occurred between June and September. These findings agree with those of Garshelis and Hellgren (1994), who reported that the breeding season of bears at lower latitudes started earlier and lasted longer. Moreover, variation in the reproductive season of the Asian black bear may also be due to higher temperatures of the subtropical zone (Yang *et al*. 2003, Chang *et al*. 2009). A similar dependence of the reproductive season on temperature was found for polar bears, American black bears (Palmer *et al*. 1988, Rogers 1992) and European brown bears (Fernández-Gil *et al*. 2006).

Although mating behavior was observed for female 3 in 2001, fecal E_2 levels during the breeding season were not higher than those of unmated females 1 and 2 in 2002 or 2003. In addition, mating behavior of female 3 occurred both before and after fecal E_2 level peaks in 2001 and 2003 indicating that the highest fecal E_2 concentrations did not correspond to the time of mating. Similar results were reported for the giant panda (Bonney *et al*. 1982) and Japanese black bears (Sato *et al*. 2000). The breeding season of American black bears lasts from May to July (Erickson *et al*. 1964), and E_2 concentrations increase only in June (Tsubota *et al*. 1998). This implies that minor E_2 elevations may be sufficient to stimulate the development of the gonad and that an appropriate increase in E_2 levels promotes sexual activity. Therefore, the mating behavior in Formosan black bears is not solely dependent upon E_2 levels.

Ursidae are mostly classified, according to ovulation patterns, into either spontaneous ovulators, like sun bears (Schwarzenberger *et al*. 2004) or induced ovulators, like American black bears (Boone *et al*. 1998), grizzly bears (Herrero & Hamer, 1997) and polar bears (Rosing-Asvid *et al*. 2002). The two ovulation patterns were indirectly identified by P_4 profiling (Boone *et al*. 2004, Okano *et al*. 2006). Here, we also observed peaks in fecal P_4 in non-mated female bears. Moreover, in both non-mating and mating bears, the mean fecal P_4 concentrations started to increase in winter after the mating season and continued to do so until the next spring. The mean fecal P_4 levels in non-pregnant and pregnant bears did not differ (18.12 ± 1.96 and 17.55 ± 2.11 ng g^{-1}, respectively). P_4 is primarily secreted from the *corpus luteum* and is accompanied by luteinization and ovulation in bears, irrespective of pregnancy (Tsubota *et al*. 2001, Boone *et al*. 2004, Yamane *et al*. 2009). Similar to a study on Hokkaido brown bears (Tsubota *et al*. 1987), all female bears in our study were considered to have ovulated despite the absence of coitus in the 2 unmated female bears. On the other hand, Chang *et al*. (1994) found lower levels of P_4 in female bears that did not have contact with a male. This suggests that female Formosan black bears are perhaps similar to Japanese black bears (Okano *et al*. 2006), i.e. they are induced ovulators that depend both on contact with a male and the opportunity to mate. Visual, olfactory and auditory stimuli might also contribute to induced ovulation in Ursidae (Boone *et al*. 2004, Okano *et al*. 2006). Further studies using laparoscopy or ultrasonography might help confirm whether ovulation in Formosan black bears is a combination of spontaneous and induced ovulation factors.

During the gestation period, fecal P_4 levels are low from ovulation to the peri-implantation stages and the *corpus luteum* remains relatively dormant. In Ursids, this is referred to as delayed implantation (embryonic diapause) (Tsubota *et al.* 1987, Schwarzenberger *et al.* 2004, Spady *et al.* 2007). Results from our study also revealed a reproductive strategy of Formosan black bears. After mating, fecal P_4 concentrations were low during delayed implantation and were sustained at low levels for two–three months, until reactivation of the *corpora lutea*, after which they sharply increased. Furthermore, relatively high P_4 concentrations may coincide with the blastocyst implantation phase (Foresman & Daniel 1983, Tsubota *et al.* 1987). Evidence showed that the initiation of the circannual gonadal rhythm and subsequent synchronization of the annual reproductive cycle in Ursids appears to be triggered by a photoperiod switch, especially in seasonal Ursini (Erickson *et al.* 1964, Spady *et al.* 2007, Chang *et al.* 2009). As such, our findings showed that reactivation of the *corpora lutea* occurred in the autumn, corresponding to the switch from long to short days, which was also reported for other Ursid species such as the Japanese black bear (Sato *et al.* 2000, Okana *et al.* 2006), Hokkaido brown bear (Tsubota *et al.* 1991), Brown bear and Spectacled bear (Dehnhard *et al.* 2006). This suggests that the carnivore mechanism for photoperiod regulation of *corpus luteum* reactivation (Legan & Winans 1981, Spady *et al.* 2007) that involves a neurotransmitter-hormonal cascade along the pineal–hypothalamic–pituitary–gonadal axis triggered by retinal perception of environmental photoperiod cues also exists in Ursids.

Reproductive records from this study indicate that Formosan black bear gestation lasts for 171–227 days. These results are consistent with those for other bear species where gestation length was found to be 110–161 days in giant pandas, 150–272 days in spectacled bears, 151–301 days in polar bears, and 190–260 days in brown bears (Masui *et al.* 1989, Schwarzenberger *et al.* 2004, Spady *et al.* 2007). A number of explanations exist for the variation in the gestation length in Japanese black bears (Sato *et al.* 2001) and giant pandas (Yu *et al.* 2003), but the most probable are those associated with stress,

ambient temperature, maternal age, and maternal physiological condition. Differences in gestation times in the Ursidae are also typically attributed to variation in the length of delayed implantation (Foresman & Gagnon 1982, Spady *et al.* 2007). It is interesting to note that in our study, gestation period of female 3 was shorter in 2002 and 2003 than in 2001.

Pregnancy of the Formosan black bear is similar to that of American black bears (Spady *et al.* 2007) and Hokkaido brown bears (Tsubota *et al.* 1991), where the embryo grows for approximately 60 days, followed by embryo implantation during the mid-to-latter period of gestation. Because of the relatively short embryonic growth period, newborn bears are extremely small relative to the maternal size (Tsubota & Kanagawa 1993). However, during pregnancies, the Formosan black bear in our study showed reduced activity levels and lower food intake from one month prior to parturition. Moreover, maternal fasting, non-excretion and reduced activity levels began one week before and continued until one week after each parturition. These characteristics parallel that observed in pregnant polar bears, the only time of polar bear to perform denning behavior (Palmer *et al.* 1988). On the other hand, evidence suggest that denning of American black bears, and fasting by pregnant polar bears can help retain near-normal body temperature and allow body fat to be converted into thermal energy (Nelson *et al.* 1984, Palmer *et al.* 1988, Hissa 1997). Furthermore, although Formosan black bears do not seem to hibernate (Hwang *et al.* 2000), we predict that this behavior , observed only in the pregnant Formosan black bear, also serves to maximize maternal energy to promote embryonic growth, which is similar to parturitions of Hokkaido brown bears (Tsubota *et al.* 1985) and Japanese black bears (Sato *et al.* 2001) during hibernation.

Age and interactions between bears are believed to be critical factors that induce mating behavior in Japanese black bears (Yamamoto *et al.* 1998, Sato *et al.* 2001). Although the exact ages of the three female bears were unknown, we believe that the fact that mated female 3 and male 3 were fed together since they were cubs, created an interaction that was conducive for mating and implies a mating incompatibility

phenomenon in Formosan black bears. However, further studies from larger mating-couple samples in the wild or captivity are necessary to determine whether this is true.

In conclusion, in this study a combination of extraction assays and the ELISA analysis was applied to measure fecal E_2 and P_4 levels for monitoring ovary/*corpora lutea* activities and the detection of pregnancy. Our results suggest that the Formosan black bear is a seasonal breeder and mates usually in spring. Moreover, the Formosan black bear shows a tendency towards a longer and earlier breeding seasons that are typical for subtropical zones. On the other hand, delayed implantation is likely and induced ovulation may also occur from exposure to a male even in the absence of coital stimulation. These findings allow for the evaluation of the reproductive potency for the management and conservation of wild Formosan black bears. An extended pregnancy detection technique using independent ultrasonography methods and further field studies consisting of larger samples are needed to assess the reproductive parameters of Formosan black bears.

Acknowledgements

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