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Estimating the success rate of ovulation and early litter loss rate in the Japanese black bear (*Ursus thibetanus japonicus*) by examining the ovaries and uteri

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Abstract

In order to develop a method for estimating the success/failure rates of reproductive processes, especially those of ovulation and neonate nurturing, in the Japanese black bear (*Ursus thibetanus japonicus*), we examined offspring status, corpora lutea (CLs), placental scars (PSs) and corpora albicantia (CAs) in 159 females (0-23 years old) killed as nuisances on Honshu Island of Japan during 2001-2009. PSs were found to remain in the uterus at least until November of the year of parturition. CA detectability began to decline after September of the year of parturition. Monthly and age-specific proportions of CL-present females revealed that the post-mating season starts in August, and that the age of first ovulation is 4 years. These results indicate that the success rate of ovulation (SRO: the probability that solitary/non-lactating mature females actually succeed in ovulation) can be estimated by calculating the proportion of CL-present females among ≥ 4 -year-old females without PSs captured from August to November; the early litter loss rate (ELLR: the probability that parenting females lose all of their cubs [0-year-old offspring] before mating season) can be estimated by calculating the proportion of CL-present females among those with PSs and CAs captured in August or later. The estimated values of SRO and ELLR were 0.93 (62/67) and 0.27 (6/22), respectively.

Key words: corpus albicans, corpus luteum, Japanese black bear, ovulation, placental scar

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Introduction

The reproductive cycle of female Japanese black bears (*Ursus thibetanus japonicus*) spans multiple years. Japanese black bears are considered to be induced ovulators¹⁾, and their reproductive cycle starts with ovulation at copulation in the mating season in summer¹⁸⁾. Following a period of delayed implantation, implantation occurs in early winter^{10-12,17)}. Parturition takes place in the den around late January⁶⁾. After parturition, Japanese black bears usually nurture their offspring for about 18 months⁹⁾. Females with cubs (0-year-old offspring) do not ovulate in the postpartum mating season because of lactational anestrus^{2,5,14)}. Although the time of weaning is not exactly known, it is thought that they probably wean their offspring before the mating season in the second year postpartum. After weaning, they enter a new reproductive cycle.

Major processes of the reproductive cycle include ovulation, implantation, fetus development, parturition, and neonate nurturing. It is of interest to know which of these processes has the greatest effect on the success of the whole cycle. One way to gain insight into this is to compare the success rates (the probability of success) of each of the processes. A process with a lower success rate is considered to contribute more to reproductive failure.

Ecological studies, such as biotelemetry, of female bears can reveal the success rates of some processes in the reproductive cycle¹³⁾. However, as ecological studies rely on observations of female's offspring status to determine the reproductive success, they are unable to deal with any processes whose success or failure is not evident through field observations, e.g. ovulation. In addition, as ecological studies require vast research efforts and funds, it is desirable to develop an alternative approach for the estimation of success rates.

In the present study, we took an anatomical approach, and examined the corpora lutea (CLs),

corpora albicantia (CAs) and placental scars (PSs) in female bears killed as nuisances. We focused on two reproductive processes: ovulation and neonate nurturing. Ovulation is the first process of the female's reproductive cycle, and its success rate determines the maximum potential success rate of a whole reproductive cycle. Survival or mortality of neonates also has an important effect on population dynamics. We defined the success rate of ovulation (SRO) as the probability that solitary/non-lactating mature females actually succeed in ovulation, and the early litter loss rate (ELLR) as the probability that parenting females lose all of their cubs (0-year-old offspring) before the mating season.

To estimate SRO and ELLR, it is necessary to know the offspring status, ovulation history and parturition history, as well as sexual maturity and mating season, of females in the year of their capture. Ovulation history can be determined by the presence of CLs^{4,8,15,16)}. Parturition history may be determined by the presence of PSs^{4,8,16)} or CAs^{4,16)}. However, for PSs or CAs in killed females to be evidence of parturition in the year of capture, they should be detected in the year of parturition, but not in the second year postpartum. Offspring status, which is rarely available for nuisance-killed females, can be determined from parturition history. Given that parturition history can be determined by PSs or CAs, SRO may be estimated from the proportion of CL-present females among mature females which had no PS or CA and were captured after the mating season; ELLR may be estimated from the proportion of CL-present females among females which had PSs or CAs and were captured after the mating season, assuming that virtually all solitary/non-lactating mature females succeed in ovulation.

In this study, we observed CLs, PSs and CAs in female Japanese black bears killed as nuisances in Honshu Island of Japan, and examined the postpartum durations of PS and CA presence, sexual maturity (age of first ovulation) and the mating season. Based on the

results, we established a method for the estimation of SRO and ELLR.

Materials and Methods

The ovaries and uteri of Japanese black bears were collected from 159 females killed as nuisances in Honshu Island of Japan during May–November, 2001–2009. The samples were stored frozen at -20°C , or fixed in 70% ethanol or 10% buffered formalin before examination. We gathered information about the offspring status of the females based on sightings of accompanying juveniles reported by cooperating officials or hunters. When accompanying juveniles were sighted, the female was classified as “female with offspring”, and the number of offspring and their estimated age were reported. In cases where no juveniles were sighted, the female was dealt with as an individual without available offspring data. The age of the females was determined by counting the cementum annuli of teeth³⁾, which were collected along with the ovaries and uteri.

The ovaries and uteri were grossly examined for CLs and PSs. The ovaries were sliced parallel to the hilum at several sections with the maximum spacing of 4 mm, and grossly examined for CLs. The uterine horns were cut longitudinally to reveal the endometrium, and grossly examined for PSs. When CLs and PSs in a sample were difficult to identify by gross

examination alone, we histologically examined the sample with hematoxylin-eosin stain.

Thirty-six pairs of ovaries from the females with PSs were histologically examined for CAs. The same slices of ovaries that were cut out for the gross examination were embedded in paraffin blocks, sectioned with a microtome into approximately $2\ \mu\text{m}$ thick sections, and stained with Weigert’s stain. We defined CAs as regressed corpora lutea in the stages of I and II classified by Katayama *et al.*⁷⁾. We focused only on the presence or absence of CAs and did not attempt to count them, because we were unable to determine whether the CA images on different histological sections originated from the same CA or different ones.

Results and Discussion

Eighteen females were sighted with offspring, and the remaining 141 females were without available offspring data (Table 1). All of the 18 females with offspring had PSs, and, of those, all 16 females whose ovaries were available were confirmed to have no CL. Among females without available offspring data, 44 females had PSs. The age of females with PSs ranged from 4 to 20 years. Table 2 shows the individual data of the 18 females with offspring. The offspring were cubs (0 year old) in all 9 cases where estimated age was reported. The number of PSs was equal to or larger than the number of

Table 1. Number of female Japanese black bears classified by the status of offspring, placental scar (PS) and corpus luteum (CL).

Offspring status	PS	CL		No OV ^{a)}	Total
		+	–		
With offspring	+	0	16	2	18
	–	0	0	0	0
Without available offspring data	+	21	23	0	44
	–	67	30	0	97
Total		88	69	2	159

+ : detected, – : not detected. ^{a)}Ovaries were unavailable. Females were killed as nuisances on Honshu Island of Japan during 2001–2009.

offspring in all cases, except one (Identification: 08Yasaka-2) in which the exact number could not be determined due to incomplete sampling of the uterus. The capture dates spanned from May 9 to as late as November 25. From this, we were able to judge that PSs remain at least until November of the year of parturition. Thus, we set the criterion for not giving birth in the year of capture as the absence of PSs in females captured no later than November. Katayama *et al.*⁷⁾ found PSs in all of the 3 female Japanese black bears that were sighted with offspring and killed during October-December. Their observations support our criterion.

Table 3 shows the CA status of 36 females with PSs whose ovaries were histologically examined. Females in Table 3 correspond to the fraction of the females with PSs in Table 1, *i.e.*

11 females (6-16 years old) out of 18 females with offspring in Table 1, and 25 females (4-20 years old) out of 44 PS-present females without available offspring data. The monthly distribution of CA status is shown in Fig. 1. Among 11 females with offspring, CAs were undetectable in 3 females, and the monthly trend of CA detection shows that the detectability of CAs decreased after September. We judged, from this trend, that CAs are no longer detectable when the capture period (May and later) starts in the second year postpartum, and employed the presence of CAs in females with PSs as the criterion for giving birth in the year of capture. A similar trend in CA detection was found among 25 females without available offspring data. This supports the above criterion, although some of the 7 females without CAs may have given birth

Table 2. Individual data of 18 female Japanese black bears sighted with offspring at their capture sites.

Identification	Capture date	Age (yr)	Sighted offspring		No. of PSs	No. of CLs
			Number	Age (yr)		
HR337	5/ 9	16	1	0	1	0
FB20060829-1	8/29	13	2	ND	2	0
Ena0603	9/ 2	8	2	ND	2	0
03Mito-3	9/12	11	1	ND	1	0
HR218	9/20	6	2	0	2	0
06Hikimi-8	10/ 3	10	1	0	2	0
HR241	10/ 7	12	2	0	2	0
HR127	10/19	16	2	0	2	0
Ibigawa0604	10/20	9	2	ND	2	0
HR134	10/24	14	2	ND	3	0
06Kanagi-4	10/24	7	1	0	$\geq 1^a$	0
HR295	10/27	8	1	0	1	0
Nakatsugawa0601	10/29	15	1	ND	2	0
Nakatsugawa0602	11/ 3	7	2	ND	2	0
HR314	11/ 6	14	1	0	2	0
08Mito-9	11/20	5	2	ND	2	ND
HR328	11/21	9	1	0	2	0
08Yasaka-2	11/25	9	2	ND	$\geq 1^a$	ND

ND: No data available.

^aThe exact number could not be determined due to incomplete sampling of the uterus. Females were killed as nuisances on Honshu Island of Japan during 2001-2009.

Table 3. Number of 36 placental scar-present female Japanese black bears classified by the status of offspring, corpus luteum (CL) and corpus albicans (CA).

Offspring status	CL	CA		Total
		+	-	
With offspring	+	0	0	0
	-	8 (7)	3 (3)	11 (10)
Without available offspring data	+	7 (6)	1 (1)	8 (7)
	-	11 (9)	6 (6)	17 (15)
Total		26 (22)	10 (10)	36 (32)

+: detected, -: not detected.

Values in parentheses denote females captured in August or later (post-mating season).

Females were killed as nuisances on Honshu Island of Japan during 2001-2009.

	Capture month						
	May	Jun	Jul	Aug	Sep	Oct	Nov
Females with offspring	●			●	●●●	●●○	●○○
Females without available offspring data		●	●●	●●●	●●●●○	●●●●● ●●●○ ○○○○○	●○

Fig. 1. Distribution of 36 placental scar-present female Japanese black bears by offspring status, capture month and corpus albicans (CA) status. Filled circles denote females with CAs ($n = 26$), and open circles denote those without CAs ($n = 10$). Females were killed as nuisances on Honshu Island of Japan during 2001-2009.

in the year prior to their capture. Moreover, Katayama *et al.*⁷⁾ reported that they detected CAs (regressed corpora lutea in stage II) in 1 of the 3 female Japanese black bears that were sighted with offspring and killed during October-December, and this is consistent with our results.

Fig. 2 shows the distribution of 97 PS-absent females by age, capture month and CL status. These females met the criterion for not giving birth in the year of capture, and were, therefore, assumed to be solitary/non-lactating. The proportion of CL-present females was 2/12 at 2 years of age and 1/9 at 3 years of age, but 90% (64/71) of females ≥ 4 years old had CLs. Only 2 out of 6 females captured in June and July had CLs, but 71% (65/91) of females captured in August and later had CLs. To show this trend more clearly, the monthly proportion of CL-present females among females ≥ 4 years old is shown in Fig. 3A. The proportion reached its

highest level ($17/18 = 0.94$) in August. Since most females had ovulated before August, we regarded August as the first month of the post-mating season. The age-specific proportion of CL-present females among those captured in August or later, *i.e.* in the post-mating season, is shown in Fig. 3B. The proportion abruptly increased between 3 and 4 years of age, and reached its highest level ($5/5 = 1.0$) at 4 years of age. Thus, we determined that the age of first ovulation is 4 years, and designated females ≥ 4 years old as sexually mature. These findings and criteria regarding mating season and the age of first ovulation were consistent with previous studies. The age of first ovulation in the Japanese black bear has been reported to be 4 years, and a possibility of ovulation at younger ages has also been suggested⁷⁾. The mating season has been deemed to be from mid-June to early August based on observations of mating

		Capture month					
		Jun	Jul	Aug	Sep	Oct	Nov
Age	0-1				○○	○○	○
	2		○○	○	○○	●○○○	●○○
	3			●○	○	○	○○○○○
	4		●	●	●●●		●
	5		●	●●	●●●	●●	●
	6			●	●	●	●●
	7	○		●●	●●●	●	
	8-9			●●●	●●●●	●●●	
	10				●	●●○	●
	11			●	●	●●	●●●
	12			●●	●	●○	○
	13-14		○	●●●	●●○	●●	
	15-23			●●○	●	●●●	

Fig. 2. Distribution of 97 placental scar-absent female Japanese black bears by age, capture month and corpus luteum (CL) status. Filled circles denote females with CLs ($n = 67$), and open circles denote those without CLs ($n = 30$). The ages with a sample size < 5 were combined with the adjacent age(s) to form an age group with a sample size ≥ 5 . Females were killed as nuisances on Honshu Island of Japan during 2001–2009.

behavior in captive bears¹⁸⁾.

Based on these results, we calculated SRO as the proportion of CL-present females among ≥ 4 -year-old females without PSs captured from August to November. There were 67 females which met the criteria, and of those, 62 females had CLs. Thus, SRO was $62/67 = 0.93$. ELLR was calculated as the proportion of CL-present females among those with PSs and CAs captured in August or later. We found 22 females which met the criteria, and of those, 6 females had CLs. Thus, ELLR was $6/22 = 0.27$.

We believe that our method for estimating SRO and ELLR provides a new way to monitor the reproductive status of the Japanese black bear. In the following, we discuss the points which should be noted when applying our method.

1) Validity of the absence of PSs as a criterion for not giving birth: Because we determined the criterion from a limited number of females with offspring ($n = 18$, Table 2), there remains a possibility that some females without PSs had a parturition history in the year of capture,

especially among those captured around November. In such cases, SRO will be underestimated. In addition, this criterion was implicitly based on the assumption that weaning occurs before the mating season in the second year postpartum. Although this is considered a general pattern of weaning, some females may nurture their offspring longer. This raises the possibility that some parenting females are included in females without PSs. In that case, SRO will be underestimated.

2) Validity of the presence of CAs as a criterion for giving birth: We did not employ the presence of PSs alone as a criterion for giving birth in the year of capture, because we did not obtain any information about the time of PS's disappearance. Because CAs are assumed to be no longer detectable when the capture period (May and later) starts in the second year postpartum, we used the presence of CAs in females with PSs as a criterion for giving birth in the year of capture. However, as we determined this criterion also from limited data ($n = 11$), we cannot completely deny a possibility that CAs remain in the capture

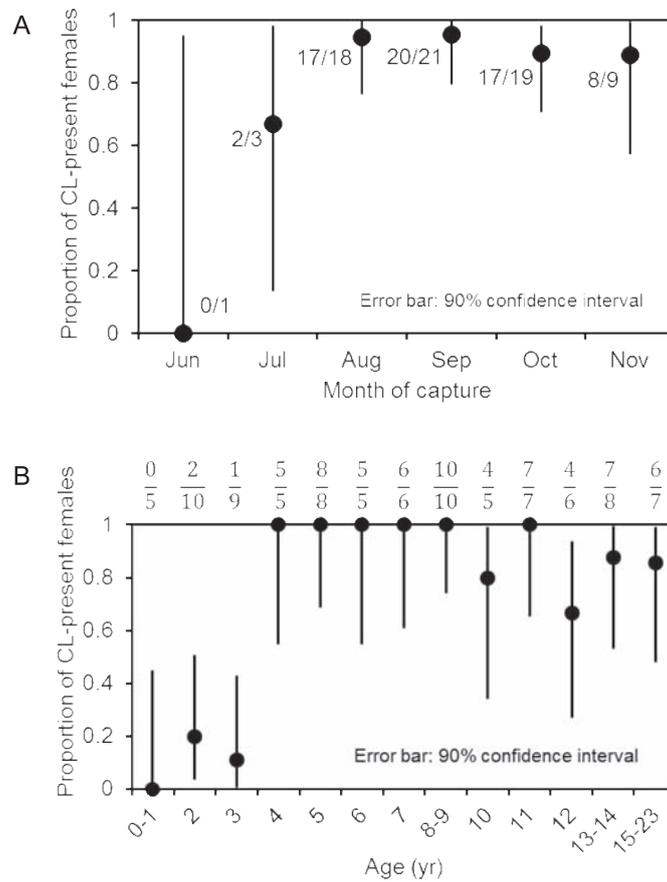


Fig. 3. Monthly proportion (A) and age-specific proportion (B) of females possessing corpora lutea (CLs) among females without placental scars. A: the age of females is limited to ≥ 4 years of age; B: the capture month of females is limited from August to November. Error bars show 90% confidence intervals. Fractions denote (number of individuals with CLs) / (sample size) in each month or age. The ages with a sample size < 5 were combined with the adjacent age(s) to form an age group with a sample size ≥ 5 . The females were killed as nuisances in Honshu Island of Japan during 2001–2009.

period in the second year postpartum. If some CAs remain until August or later (the post-mating season) in the second year postpartum, ELLR will be overestimated.

3) *Assumption in ELLR calculation:* In the calculation of ELLR, we assumed that virtually all solitary/non-lactating mature females succeed in ovulation, *i.e.* $SRO \approx 1$. We considered that this assumption was valid for a rough estimation of ELLR, because our estimate of SRO was 0.93. It should be noted, however, that the estimation of SRO is a prerequisite for that of ELLR.

The magnitude of the possible errors described above, if any, may be revealed in future studies with larger sample sizes, and those studies may show the need for refinement of our

present criteria or assumptions. Nevertheless, the principles and approaches presented in this study surely serve as a basis for estimation in future studies. To reveal the process which contributes most to the failure of a reproductive cycle, success rates of other processes, *e.g.* implantation, fetus development and parturition, should also be examined in future studies. Our present estimates of SRO and ELLR suggest, at least, that ovulation is a stable process with a high success rate, and that nurturing neonates is a less stable process in the Japanese black bear.

In conclusion, we developed a method for the estimation of SRO and ELLR in the Japanese black bear based on the examination of CLs, PSs and CAs in killed females. We showed that SRO

can be estimated as the proportion of CL-present females among ≥ 4 -year-old females without PSs captured from August to November. ELLR can be estimated as the proportion of CL-present females among those with PSs and CAs captured in August or later. Our method provides a new way to monitor the reproductive status of the Japanese black bear, and may help better understand it as a complementary approach to conventional ecological methods.

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