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Methods of estrus detection and correlates of the reproductive cycle in the sun bear (*Helarctos malayanus*)

Cheryl Frederick[a],*, Randall Kyes[b], Kathleen Hunt[c], Darin Collins[d], Barbara Durrant[e], Samuel K. Wasser[f]

[a] *Department of Psychology, University of Washington, Seattle, WA, USA*
[b] *Department of Psychology, University of Washington, Seattle, WA, USA*
[c] *Department of Biology, University of Washington, Seattle, WA*
[d] *Woodland Park Zoo, Seattle, WA, USA*
[e] *San Diego Zoo's Institute for Conservation Research, Escondido, CA*
[f] *Department of Biology, University of Washington, Seattle, WA*

Abstract

The objective was to explore multiple methods for detecting and characterizing the reproductive cycle of the sun bear (*Helarctos malayanus*). Thirteen *H. m. euryspilus* females, loaned from the Malaysian government to US zoos, were used. Fecal metabolite concentrations of estrogen and progesterone were compared to vaginal cytology, changes in genital appearance, and behavior (videotapes and zookeeper observations). Cytology and video behavior were characterized during five hormonally defined states: high, low, and baseline progesterone, estrus, and high estrogen. Among states, there were significant differences in cytology and behavior. Sexual, affiliative, and stereotypic behaviors were highest during estrus, whereas affiliative and social behaviors were lowest during high progesterone. In this captive breeding population, 30.8% of females cycled two or three times a year, 30.8% cycled once a year, and 38.5% did not cycle during this study. Inter-estrus intervals were (mean ± SEM) 115.7 ± 6.3 d (range, 101–131). Spearman rank correlations were significant between both ordinal sexual and affiliative behaviors and vulva swelling and color. Sexual behavior was significantly positively correlated with superficial and keratinized cells, but negatively correlated with parabasal and basophilic cells in cycling females (opposite pattern for appetitive behavior). In conclusion, data for cytology, vulva changes and behavior were consistent with, and complementary to, hormonal data; collectively, they delineated estrus and identified specific reproductive types.
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1. Introduction

Sun bears (*Helarctos malayanus*) are distributed throughout the low and tropical forests of Southeast Asia [1]. However, their range is shrinking, with further decline and fragmentation anticipated [2]. The subspecies *H. m. euryspilus* is found only on the island of Borneo and is notably smaller than their mainland counterparts [3,4]. The sun bear is listed by Convention on International Trade in Endangered Species (CITES) as Appendix I and falls under the IUCN category "Vulnerable" [5]. Captive breeding efforts in North American zoos have met with limited success, leading to a steady decline in the captive population [6].

Present address: CWCE Program, Unity College, Unity, ME, USA
* Corresponding author. Tel.: 207 948 3131.
E-mail address: cfrederick@unity.edu (C. Frederick).

Very few studies of female sun bear reproductive biology have been conducted. Schwarzenberger et al [7,8] studied hormone profiles of captive female sun bears in European zoos; however, their behavioral data were limited to the timing of mating [7,8]. They also presented hormone metabolite data on two free-ranging females [8]. Onuma et al [9,10] examined bears that were captive *in situ*. They collected fecal samples and immobilized a female to obtain vaginal smears on two occasions (1 y apart) [10]. Thus, these studies have focused almost exclusively on fecal hormone metabolite concentrations, and to our knowledge there are no reports that systematically assess multiple behavioral and biological measures of the reproductive cycle of this species.

Urinary and fecal hormone metabolite assays are well-established means to assess reproductive status in a diversity of mammalian species, and have the added advantage of being non-invasive [11–15]. Therefore, they often serve as a standard against which other techniques for detecting estrogenic cues may be measured or compared. Moreover, these hormone assays provided information regarding both the follicular and luteal phases of the reproductive cycle. A rise in estrogens (follicular phase) culminating in a peak value, followed by a rise in progestins (luteal phase), indicates that ovulation occurred, followed by an active CL producing progestins [16].

Hormonal concentrations are also reflected in the appearance of cells of the vaginal epithelium. Cytology has been used to elucidate phases of the estrous cycle and general timing of ovulation in domestic [17,18] and exotic [19–21] species. A series of smears is required for accurate estimation of cycle state [22,23]. Operant conditioning to gain the animal's cooperation for repeated reproductive data collection has become increasingly common [24] and has been used successfully to collect vaginal smear data on bears [20]. Further, when the animals are in a position for vaginal swabbing, visual assessment of the vulva can be done and retrospectively related to endocrine status [25–27].

The progressive maturation of cells through the layers of the vaginal epithelia results in the ratio of parabasals:intermediates:superficial cells known as the Maturation Index (MI). Shifts in the MI correspond to cycle stage changes from follicular phase to pregnancy [22,28]. Papanicolaou (PAP) staining can aid in further delineating the reproductive cycle by monitoring shifts among basophilic (blue), acidophilic (pink), and keratinized (yellow) epithelial cells in some species [19,20,29]. Similar to the MI, this continuum of color changes reflects circulating hormone metabolite concentrations, for example, with keratinized cells corresponding to very high estrogen and estrus [22].

Finally, behavioral assessment can play a crucial role in identifying and understanding reproductive issues or problems faced by species in captivity [30]. Several studies reported giant panda *(Ailuropoda melanoleuca)* reproductive behavior in detail [31–34]. Incorporating caretaker observations adds other potential cycle-associated behavioral cues, e.g. changes in appetite, activity levels, and voiding patterns.

The goals of the present study were to examine and inter-relate multiple methods for characterizing the reproductive cycle of the sun bear, describe the states of the cycle, and facilitate detection of estrus in this species. The present study drew from methodology developed for the giant panda [20,26,32]. Specifically, we assessed: 1) hormone metabolites from feces; 2) vaginal cytology and vulva appearance; and 3) observer and keeper-collected behavioral data. We also sought to categorize the reproductive potential of the entire US zoo founder population of *H. m. euryspilus* by assessing whether or not each female had estrous cycles. Our longterm objective was to promote improved reproductive management of this species, both in participating zoos and in *in situ* facilities.

2. Methods

2.1. Subjects

Thirteen female wild-caught, former-pet, Bornean sun bears on loan to eight US zoos from the Malaysian Government were used (Table 1). These animals represented the entire US captive female founder population of *H. m. euryspilus* sun bears when the study was conducted (2001–2006). Age estimates ranged from ~2–19 y (at the start of the study). One of the San Diego Zoo females (No. 665) became pregnant during the study; data collected during pregnancy were not included in this study. Based on the intensive monitoring of all subjects in this study, we are reasonably confident that no additional, undocumented breedings and unsuccessful pregnancies occurred. All bears were exhibited and socially housed in semi-naturalistic enclosures. Ten of the 13 females were housed with a male for at least part of the study period (Table 1). Social and other potential influences on the sun bear's reproductive cycle will be addressed in another publication.