Journal of Mammalogy, 89(1):50–57, 2008

REPRODUCTIVE ENDOCRINOLOGY OF A SMALL TROPICAL BAT (FEMALE *SACCOPTERYX BILINEATA*; EMBALLONURIDAE) MONITORED BY FECAL HORMONE METABOLITES

CHRISTIAN C. VOIGT* AND FRANZ SCHWARZENBERGER

Research Group Evolutionary Ecology, Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Strasse 17, D-10315 Berlin, Germany (CCV)
Department of Natural Sciences, Institute for Biochemistry, Veterinärmedizinische Universität Wien, Veterinärsplatz 1, A-1210 Vienna, Austria (FS)

We tested the suitability of fecal sexual steroid hormone metabolites for use in endocrinological studies of free-ranging, small bats. We monitored estrogen and 20-oxo-pregnane metabolites in fecal samples collected daily from 19 free-ranging female *Saccopteryx bilineata* during the assumed mating season. Median fecal estrogen metabolite levels equaled 0.09 ng/g for the pre- and postovulatory periods and 4.2 ng/g for the follicular phase of the estrous cycle in female *S. bilineata*. Median fecal 20-oxo-pregnane metabolite levels were 50.9 ng/g in pre-estrous, 88.8 ng/g in estrous, and 190.2 ng/g in postestrous females. Reliability of fecal steroid metabolite analysis was supported by results of video observations. Two successful copulations for a single male–female pair were recorded; mating coincided with peak fecal estrogen levels (7.0 ng/g) in the copulating female. In contrast, 2 observed mating attempts in a 2nd male–female pair occurred approximately 19 days after the female's physiological estrus and fecal estrogen metabolites in the rejecting female were only 0.2 ng/g. Fecal 20-oxo-pregnane levels were almost 3 times lower in fecal samples collected from the copulating female than those from the rejecting female (157 ng/g versus 410 ng/g). All females that were still present in the colony 6 months later gave birth to a single offspring between late May and early June. Gestation length averaged (mean ± *SE*) 169 ± 2 days. During the 20 days preceding parturition, fecal 20-oxo-pregnane metabolites were approximately 100 times higher than during the mating period. Fecal 20-oxo-pregnane metabolites of lactating females were comparable to those during the mating season. Noninvasive fecal steroid analysis can be a valuable tool for monitoring reproductive events in colonies of free-ranging small-sized bats.

Key words: Chiroptera, estrus, fecal estrogen metabolites, fecal pregnane metabolites, mating, noninvasive analysis, ovulation, seasonal reproduction, tropics

Although our knowledge of many ultimate aspects of mating systems has greatly benefited from the development of molecular genetic tools (e.g., review in Clutton-Brock 1989; Isvaran and Clutton-Brock 2007), we still lack information on many equally important proximate aspects of mating systems, such as the reproductive endocrinology of individuals. Recent behavioral and genetic studies suggest a great diversity of social and mating systems within the order Chiroptera, especially among tropical bat species (e.g., Dechmann et al. 2005; Heckel et al. 1999; Heckel and von Helversen 2003; Storz et al. 2001; reviewed in McCracken and Wilkinson 2002), but the reproductive endocrinology of these bats is still unknown. Tropical animals face an almost constant environment with respect to photoperiodicity and temperature, but most tropical bats reproduce seasonally (Baker and Baker 1936; Baker et al. 1936; O'Brien 1993; Porter and Wilkinson 2001). This general trend obscures the many variations on the theme. Tropical bats are known to exhibit postpartum estrus, cyclic ovarian activity, sperm storage, or delayed embryonic development (e.g., Fleming 1971). All of these strategies are largely controlled by hormones, but hormonal data—especially in the context of mating systems and sex-specific mating tactics—are scarce for almost all of the several hundred bat species inhabiting the tropics. In part, this may be attributable to the difficulties of conventional blood sampling in small mammals.

* *Correspondent: voigt@izw-berlin.de*

Recently, noninvasive analytical techniques have been developed that allow the study of metabolites of sexual hormones in fecal, salivary, or urinary samples (reviewed in Goymann et al. 2005; Schwarzenberger et al. 1996, 1997; Touma and Palme 2005). Collection of fecal samples for hormone analysis was particularly suitable in our study because the body mass of *Saccopteryx bilineata* averages only 8 g, and our study animals were free-ranging and thus collection of plasma samples on a regular basis would not have been possible. In this paper, we used the technique of fecal hormone metabolite analysis to shed light on the female reproductive endocrinology of an insect-feeding neotropical bat, the greater sac-winged bat, *S. bilineata* (Emballonuridae). The social system of this species is exceptionally well known and thus it can be used as a model species to assess the role of hormones in the reproductive biology of females in general, or the endocrinological basis of individual reproductive tactics in particular, or both.

Saccopteryx bilineata has a polygynous mating system (Bradbury and Emmons 1974; Bradbury and Vehrencamp 1976, 1977a). The species lives in colonies that consist of 1 or several harems. Each harem includes on average 2 or 3 females that are defended by males against competitors throughout the year. In Central America, female *S. bilineata* give birth to a single offspring per year in May or June (Bradbury and Emmons 1974; Tannenbaum 1975; Voigt et al. 2006). Gestation lasts for several months because fetuses are already present in late January or early February (Tannenbaum 1975). From September through November most juveniles disperse from the natal colony. Previous studies suggest that the mating season is restricted to a few weeks in December or January (Tannenbaum 1975; Voigt et al. 2006). We investigated the reproductive biology of female *S. bilineata* in a Costa Rican colony by fecal steroid analysis. We monitored fecal estrogen metabolites secreted from the growing follicle, and fecal 20-oxo-pregnane metabolites (progesterone metabolites) secreted during the luteal phase of the ovarian cycle. We predicted that estrogen peaks occur around the 2nd half of December or early January followed by an increase of 20-oxo-pregnanes. In addition, we asked whether ovulation is related to unusual climatic conditions such as cold temperatures or heavy rainfall at the end of the rainy season.

Materials and Methods

Study site and sample collection.—Sample collection and field observations took place between 3 December 2001 and 23 January 2002 (52 days) because previous studies indicated that this time period would cover the mating season of *S. bilineata* in Central American populations of *S. bilineata* (Bradbury and Emmons 1974; Bradbury and Vehrencamp 1976; Tannenbaum 1975). Our study colony was located in an abandoned building in the protected forest of La Selva Biological Station, Province Heredia, Costa Rica (10°25′N, 84°00′W). At dusk, we captured emerging colony members with mist nets (6 × 2 m) set at a minimum distance of 5 m from the daytime roost. For individual identification we placed colored or numbered plastic bands (AC Hughes Ltd., Hampton Hill, United Kingdom) on the forearms of captured bats. Females were marked on the left and males on the right forearm.

Because colony members have individual roosting sites that are defended against other colony members, we were able to collect fecal pellets below the individual roosting sites on a regular, almost daily basis. For this purpose, we installed a 1-m-long, 3-cm-wide wire mesh trough below each roosting site in 5 focal harem territories. We visited the colony 4 times each day and noted the roosting sites of all colony members from a distance of at least 3 m with minimal disturbance to the animals. During the first 3 visits at 0900, 1100, and 1500 h, we noted the roosting positions of all colony members in the roost. For each day we calculated the probability for a given female to be present in her harem. The probability equaled 100% when she was present in her territory during all 3 censuses, 67% when she was present in 2 of 3 censuses, 33% when she was present in 1 of 3 censuses, and 0% when she was present in the colony but not in her territory. For each female we calculated the mean daily probability of presence in her harem for the 53 days of the study period.

During the 4th visit to the colony, shortly after the colony members had left the daytime roost, we transferred the individual fecal pellets with forceps into plastic tubes and then stored the samples in a freezer at −20°C. Based on the census data from the 3 previous visits to the roost, we attributed each pile of fecal pellets to an individual bat. In total, we collected fecal material from 19 adult females. The frozen fecal samples were shipped on dry ice to the laboratory where samples were again stored at −20°C before analysis.

Video observations.—During the mating season we set up a video camera (Conrad Electronics, Nuremberg, Germany) and a portable videocassette recorder (Panasonic Inc., Secaucas, New Jersey) in front of 1 or several harem territories before the bats returned to the daytime roost. We repeatedly recorded the activity of harem individuals in all 5 focal territories in a rotational routine, that is, each territory was recorded every 6th day. In total, we collected video material from each territory from 9 sampling days, which equaled 108 h for each focal group. For better illumination we used a headlamp that was covered with an infrared filter. Video material was analyzed with respect to mating activity.

We defined harem males or harem holders as adult males defending a territory with females, peripheral or nonharem males as adult males having either no female in their territory or not defending a territory, cryptic males as males of the juvenile cohort still roosting next to their mother, and harem females as females roosting in a specific territory defended by a harem male (Bradbury and Emmons 1974; Bradbury and Vehrencamp 1976).

Monitoring of parturition days and gestation length.—We visited the colony again in May, June, and July 2002 (4 May, 18–20 May, 29 May, 4 June, 19 June, 26 June, 29 June–12 July, 15 July, 17 July, 23 July, and 30 July) to assess the timing of parturition. During these days we again collected fecal material from focal females for analyses of hormone metabolites. We estimated the mean duration of pregnancy with a Weibull model in a survival analysis (SYSTAT Software Inc. 2004) based

either on the exact gestation length if both the day of estrus and the day of parturition were known, on right-censored data if the timing of estrus was known but the bat disappeared from the harem later, or on interval-censored data if the time interval between the last day females were seen pregnant and the 1st day females were seen with young were known.

Analyses of hormone metabolites.—We extracted 20-oxo-pregnane and estrogen metabolites from fecal samples using methanol and diethylether as described for several other mammalian species (Schwarzenberger et al. 2004, and references therein). Because only 0.05 g of feces was available on average, daily samples were placed into test tubes and then the weight was increased to 0.5 g by adding water. The extraction procedure continued with the 0.5-g feces and water mixture by adding 1.0 ml of water and 4.0 ml of methanol. After vortexing for 30 min, samples were centrifuged. One milliliter of the supernatant methanol was transferred into a new vial, mixed with 0.5 ml of a 5% $NaHCO_3$ in water solution, and reextracted with 3.0 ml diethyl ether. The ether phase was transferred into a new vial, and evaporated. The residue was redissolved and diluted with assay buffer (1:10 to 1:1.00 according to concentrations) and aliquots were analyzed by enzyme immunoassays as described in Schwarzenberger et al. (2004). Extraction efficiency using this method was tested previously and was shown to yield constant recoveries of added radioactively labeled steroids greater than 80% (Schwarzenberger et al. 2004, and references herein).

Fecal extracts were analyzed by enzyme immunoassays for immunoreactive progesterone and estrogen metabolites. The group-specific antibodies used in the enzyme immunoassays were raised in rabbits and assays included 20-oxo-pregnanes (antibody: 5a-pregnane-3β-ol-20-one 3-HS:BSA) and total estrogens (antibody: estradiol-17β-OH 17-HS:BSA). Significant cross-reactivities in the progesterone metabolite assay were those with 5-reduced steroid metabolites (pregnanes); the estrogen assay detected estrone and estradiol. Assays were validated by demonstrating parallelism between standard curves and serial dilutions of fecal extracts. The intra- and interassay coefficients of variation for these assays were similar to those described previously (Schwarzenberger et al. 2004) and ranged between 10% and 15%, respectively.

We timed the estrus of female *S. bilineata*, based on the increase and decrease of estrogen and pregnane metabolites. Preliminary analysis based on fecal samples from the previous year showed that estrogen metabolites increase sharply for 1 or 2 days and then decrease during the following 1 or 2 days in estrous females. This estrogen peak is followed by a shallower, but steady increase in concentration of pregnane metabolites over the following 3 weeks. We defined a follicular phase and hence physiological estrus in *S. bilineata* by elevated levels of estrogen metabolites over 2–3 days that exceed baseline estrogen levels by 3 standard deviations. Baseline values for fecal estrogen metabolites refer to the time period exclusive of the 3 days of ovulation (= preovulation and postovulation period). The transient increase in fecal pregnane metabolites parallel to the estrogen peak is thought to result from progesterone being the precursor of estrogen synthesis.

Table 1.—Presence of female *Saccopteryx bilineata* in the colony during the study period (53 days) and loyalty to a territory in relation to the number of assigned fecal samples and the percentage of days covered with hormonal data. Estrogen peaks indicating days of estrus were detected in fecal samples of females F_1–F_{15}, whereas no estrogen peaks were detected in fecal samples of females F_{16}–F_{19}.

Female no.	Harem no.	Days present in the harem	Mean daily presence probability in harem (%)	Number of assigned fecal samples	Percentage of days covered with hormonal data (%)
F_1	7	53	100	43	81
F_2	7	53	100	20	38
F_3	9	50	71	28	56
F_4	7	52	72	35	67
F_5	1	53	100	37	70
F_6	9	53	100	39	74
F_7	10	53	100	36	68
F_8	1	53	100	42	79
F_9	7	12	89	9	75
F_{10}	1	53	100	33	62
F_{11}	10	53	99	27	51
F_{12}	9	52	76	27	52
F_{13}	10	53	61	23	43
F_{14}	1	18	89	9	50
F_{15}	10	53	93	40	75
F_{16}	11	53	98	43	81
F_{17}	10	52	95	36	69
F_{18}	10	37	95	20	54
F_{19}	1	28	98	21	75
Median		53	98	33	68
$\bar{X}$		47	91	30	64
SD		13	12	11	13

We compared timing of estrus in females with measurements of daily minimum and maximum temperature (°C), and daily rainfall (mm/day) collected by the Organization for Tropical Studies (2002) at a weather station approximately 400 m away from the study colony during the same time period.

All values are given as means ± 1 *SD* unless stated otherwise and all statistical tests were performed 2-tailed with SPSS (SPSS Inc. 1998) if not otherwise stated. This study followed the guidelines of the American Society of Mammalogists (Gannon et al. 2007) and was approved by the corresponding animal care and use committees.

Results

Between December 2001 and January 2002 the study colony consisted of 25 adult females, 10 harem males, and 9 nonharem males. Colony females roosted in 10 territories each defended by a single harem male. In 4 territories, we noted a single cryptic male roosting next to his mother and in 1 territory, we noted 2 juvenile males hanging each next to their mother. Females were present in their harem during an average of 47 ± 13 days (median 53 days) of the study period (Table 1). The mean probability to detect a female in her harem was 91% ± 12% (median 98%). We assigned an average of 30 ± 11 fecal samples (median 33) to the 19 individuals, covering an average of 64% ± 13% (median 68%) of the days in which each female was present in the colony (Table 1).

TABLE 2.—Estrus and parturition data of 19 female *Saccopteryx bilineata*. Median fecal 20-oxo-pregnane (P; ng/g) and estrogen (E; ng/g) metabolite concentrations for pre-estrous, estrous (3 days of peak E levels), and postestrous periods. Three females (F_9, F_{14}, and F_{19}) left the colony (NA = not available; *SE* = standard error of mean).

Female no.	Harem no.	Estrus date (2001)	Baseline	Pre-estrus	Estrus		Postestrus		Date of parturition	
			E	P	E	P	E	P	Earliest (2002)	Latest (2002)
F_1	7	5 Dec.	0.21	NA	0.5	90.3	0.21	196.1	21 May	3 Jun.
F_2	7	5 Dec.	0.00	NA	4.7	68.2	0.00	205.5	21 May	3 Jun.
F_3	9	6 Dec.	0.00	NA	3.2	39.5	0.00	258.5	20 May[a]	
F_4	7	7 Dec.	0.48	213.4	9.6	187.3	0.96	146.6	21 May	3 Jun.
F_5	1	8 Dec.	0.00	90.7	4.9	52.8	0.00	149.7	19 May[a]	
F_6	9	8 Dec.	0.06	29.9	5.7	48.4	0.05	217.7	21 May	3 Jun.
F_7	10	10 Dec.	0.01	25.7	12.6	42.4	0.00	267.8	20 May[a]	
F_8	1	11 Dec.	0.35	46.2	4.2	88.8	0.33	190.2	21 May	3 Jun.
F_9	7	11 Dec.	0.09	52.1	3.5	57.9	NA	NA	NA	NA
F_{10}	1	13 Dec.	0.28	15.5	2.0	23.2	0.28	164.2	21 May	3 Jun.
F_{11}	10	13 Dec.	0.08	74.3	1.8	173.5	0.07	60.1	4 Jun.	18 Jun.
F_{12}	9	14 Dec.	0.42	21.4	3.2	111.1	0.38	126.0	21 May	3 Jun.
F_{13}	10	18 Dec.	0.00	50.2	6.8	132.0	0.00	189.0	21 May	3 Jun.
F_{14}	1	24 Dec.	0.58	145.4	24.6	210.8	NA	NA	NA	NA
F_{15}	10	25 Dec.	0.27	51.7	2.2	157.3	0.37	219.2	21 May	3 Jun.
F_{16}	11	No	0.07	266.2					21 May	3 Jun.
F_{17}	10	No	0.39	223.3					4 May	18 May
F_{18}	10	No	0.03	328.8					21 May	3 Jun.
F_{19}	1	No	0.00	206.2					NA	NA
Median[b]			0.09	50.9	4.2	88.8	0.07	190.2		
$\bar{X}$[b]			0.19	68.0	6.0	98.9	0.20	183.9		
SE[b]			0.05	16.8	1.6	15.5	0.08	15.5		

[a] Exact date of parturition.
[b] Calculated only for females with estrogen peaks (F_1–F_{15}).

Endocrinological monitoring of female estrus.—We detected a peak of estrogen in 15 of 19 females during the study period (Table 2). We considered whether a possible absence of females from the colony or harem may be responsible for our inability to detect estrus in 4 of the 19 females. However, females for which no estrogen peaks were detected were present in the colony during the study period for a similarly long time as females for which an estrogen peak was detected (Mann–Whitney *U*-test: $n_1 = 15$, $n_2 = 4$, $U = 18$, $P = 0.18$). In addition, both groups of females were as likely present in their harem regardless of detection of a peak in estrogen (Mann–Whitney *U*-test: $n_1 = 15$, $n_2 = 4$, $U = 28$, $P = 0.84$). Last, the percentage of fecal pellets that could be assigned to the females was not significantly different between these 2 groups (Mann–Whitney *U*-test: $n_1 = 15$, $n_2 = 4$, $U = 19.5$, $P = 0.29$).

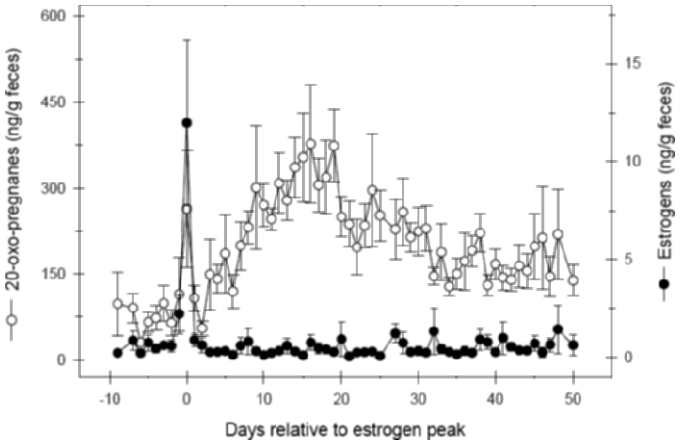

FIG. 1.—Concentrations of fecal 20-oxo-pregnane (open circles) and estrogen metabolites (filled circles) of female *Saccopteryx bilineata* ($n = 15$) in relation to peak estrogen levels indicating day of estrus. For each animal we set the day of peak fecal estrogen metabolite values as day 0 and then calculated mean fecal hormone metabolite values (± 1 *SE*).

During estrus, values of fecal estrogen metabolites usually rose within 1 or 2 days by a factor of 30 from baseline values to a maximum (2-tailed Wilcoxon test: $n = 15$, $Z = -3.4$, $P < 0.001$) and then dropped again to baseline levels during the following 1 or 2 days (Table 2; Fig. 1). During the 3 weeks after the peak in estrogen metabolites, fecal pregnane metabolites steadily increased from moderate levels during estrus (2-tailed Wilcoxon test: $n = 13$, $Z = -2.4$, $P = 0.013$; data from 2 females were not available because they left the colony after ovulation). Afterward, fecal pregnane metabolite values remained elevated above basal values. We could not detect a 2nd estrogen peak for any of the 13 females that were still present in the colony at the end of the study period. Median baseline values of fecal estrogen metabolite concentrations were not significantly different between females for which estrogen peaks were or were not detected (Mann–Whitney *U*-test: $n_1 = 15$, $n_2 = 4$, $U = 25$, $P = 0.61$; Table 2). However, median baseline levels of fecal pregnane metabolites of females for which an estrogen peak was not detected were significantly higher than corresponding values of females for which estrogen peaks were detected (Mann–Whitney *U*-test: $n_1 = 15$, $n_2 = 4$,

$U = 1$, $P = 0.005$). The majority of females came into estrus during the 1st half of December. Ovulation coincided with changes in precipitation and temperature in early December (Fig. 2)

Video observations.—We recorded 2 copulations (25 December 2001) and 2 copulation attempts (24 December 2001) in the 2-month study period. The harem male of territory 1 (M_1) copulated twice with female F_{15} from harem 10 and the harem male of territory 9 (M_9) tried to copulate twice with a female (F_6) of his own territory.

On the day of copulation, the fecal estrogen metabolite level of F_{15} was 7.0 ng/g and the fecal pregnane metabolite level was 157.3 ng/g. F_{15} entered the territory of M_1 at 0559 h, and repeatedly left and reentered. During her visit, F_{15} copulated twice with M_1 (0614 h and 0616 h). Before the 1st copulation, F_{15} returned twice to her former territory, each time followed by M_1 and each time both came back to territory 1 after a few seconds. During the 15-min interval before the 1st copulation, the territory male hovered 7 times in front of the female and fanned scents from his wing sacs toward the female. The 2nd copulation was preceded by 5 hovering flights with scent displays by M_1. F_{15} returned to her former territory 3 times before she copulated with the male the 2nd time. The 1st copulation lasted 4.4 s and the 2nd lasted 3.0 s. In both cases, M_1 landed on the wall above F_{15} and crawled downward to mount her. Each time, F_{15} interrupted the copulation by getting airborne. Before both copulations, F_{15} changed her roosting site frequently: 7 times before the 1st copulation and 9 times before the 2nd copulation. After the 2nd copulation, M_1 hovered 3 more times in front of F_{15} and 14 times in front of other harem females. F_{15} aggressively beat her forearms twice toward M_1 roosting next to her. F_{15} finally left the territory of M_1 at 0653 h, approximately 50 min after her initial entry into the territory. F_{15} was never observed in the territory of M_1 before the day of copulation, but was observed there on 36% of the following 30 days.

Male M_9 attempted to copulate with female F_6 in his territory on 24 December 2001 at 1227 h. F_6 had a fecal estrogen metabolite level of 0.21 ng/g and a fecal pregnane metabolite level of 409.7 ng/g, indicating that she was not in estrus on that day. M_9 landed above F_6 and tried to crawl downward onto her back. Each time, F_6 evaded the copulation attempts of M_9 by stepping sideways and beating her folded forearms toward the male. M_9 performed 5 hovering displays before and after the copulation attempts and stopped his mating attempts after approximately 2 min.

Monitoring of parturition day and gestation length.—For 3 females, we noted both the day of estrus and the day of parturition (either 19 May or 20 May 2002) and for 2 individuals we noted an estrogen peak but not the parturition of young, because the 2 bats disappeared from the colony, whereas for 10 females we noted the date of estrus and the time interval when females were seen the last time pregnant and the 1st time with an offspring (Table 2). From these data we calculated an average gestation length of 168.9 ± 1.8 days (mean ± *SE*; $n = 15$) for *S. bilineata*, assuming that peak secretion of fecal estrogen metabolites reflected true physiological estrus. The median concentrations of fecal pregnane metabolites was 9,605 ng/g for the 20-day period before parturition (Fig. 3). After parturition, values of fecal pregnane metabolites dropped by a factor of almost 100 to a median value of 100 ng/g (2-tailed Wilcoxon test: $n = 7$, $Z = -2.4$, $P = 0.018$).

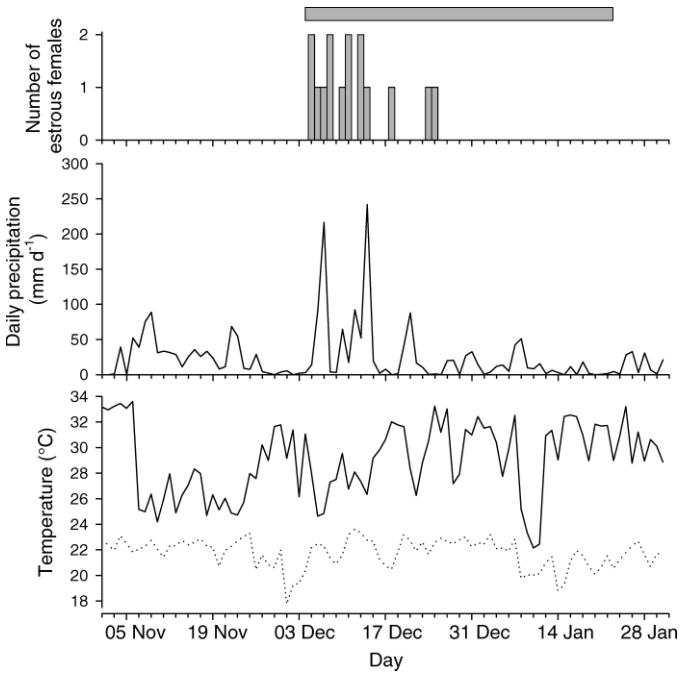

Fig. 2.—The timing of estrus in *Saccopteryx bilineata* in relation to climatic conditions such as daily precipitation (mm/day), maximum temperature (solid line; °C), and minimum temperature (dotted line; °C).

Discussion

Hormonal data of female S. bilineata during the mating season.—We monitored the timing of ovulation in female greater sac-winged bats by analyzing metabolites of sexual steroid hormones in minute fecal samples collected daily from individual roosting sites in a free-ranging colony. To our knowledge, our analyses represent the 1st endocrinological assessment of ovulation in a free-ranging bat species. Female *S. bilineata* showed a pattern of rise and decline of estrogen concentrations followed by an increase of fecal pregnane metabolite concentrations typical for ovulation and postovulation periods of mammals (reviewed in Nelson 2000): concentrations of estrogen metabolites peak during follicular development and indicate ovulation, whereas concentrations of progesterone metabolites indicate the presence of a corpus luteum. Peak levels of estrogen metabolites were on average 30 times higher than baseline values and correlated with observed mating in 1 female. Fecal 20-oxo-pregnane levels increased from pre- to postovulation periods in all but 2 females (F_4 and F_{11}), which may be attributed to the small sample size available for the preovulation time period, at least in F_4 ($n = 4$). In all other females, fecal 20-oxo-pregnane metabolite levels increased from pre- to postovulation periods.

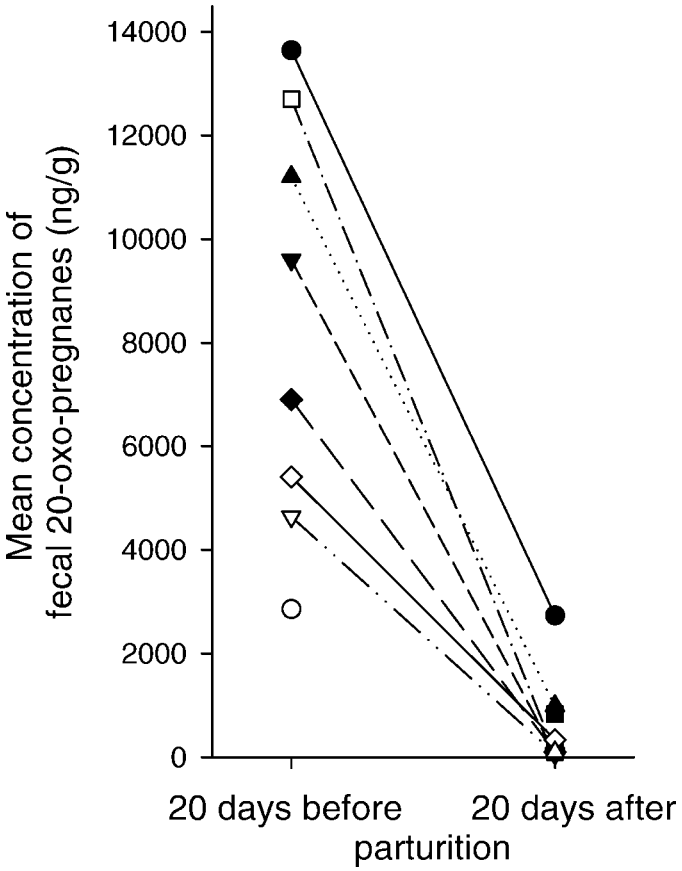

FIG. 3.—Concentrations of fecal 20-oxo-pregnane metabolites of female *Saccopteryx bilineata* averaged over 20 days before and 20 days after parturition (each symbol and line indicates an individual; note that fecal samples could only be assigned unambiguously to 8 individuals because bats were more active in the colony during the parturition period and because the presence of young complicated individual assignment of fecal pellets).

Examination of the hormonal data of female *S. bilineata* suggests that most of the focal females came into estrus during the 1st half of December. This coincides with the observed mating activity of *S. bilineata* during previous years in the same colony (Voigt et al. 2006) and also in colonies of other Central American populations (Tannenbaum 1975). One female (F_{15}) in estrus was observed to copulate in the daytime roost, whereas a 2nd female (F_6) refused to copulate with a male 16 days after her presumed ovulation. These observations suggest that female *S. bilineata* mate at the days of peak estrogen levels. Thus, physiological and behavioral estrus are likely to coincide in *S. bilineata*.

Three females for which we could not detect an estrous peak in December or January each gave birth to young between 21 May and 3 June (F_{16} and F_{18}) and 4 and 18 May 2002 (F_{17}). If these 3 females were pregnant for a similarly long time period as the other females (169 days), the corresponding ovulation period ought to be in the 1st half of December 2001 for F_{16} and F_{18}, and the 2nd half of November 2001 for F_{17}. November was not included in our study and therefore it is likely that we missed the estrus of F_{17}. For the other 2 individuals, we are able to evaluate retrospectively whether we missed the ovulation period of these females. F_{16} was present in her harem during all days of the 1st half of December except for 3 December when she roosted outside in a territory close to a nonharem male. During the 2 weeks she was present in her harem, her median fecal estrogen and median fecal pregnane metabolite concentration were 0.07 ng/g and 315 ng/g, respectively. The peak concentration of fecal estrogen metabolite for F_{16} was 0.31 ng/g on 8 December, suggesting F_{16} could have ovulated on 3 December and mated with the peripheral male outside her harem. However, because concentrations of fecal pregnane metabolite for F_{16} were significantly higher than those of other pre-estrous females, we find it likely that F_{16} ovulated and mated in November and thus gestation length in this female was longer than the average 169 days calculated. F_{18} was present in her harem in only 5 days between 3 and 16 December 2001 and no fecal pellets could be assigned to F_{18} in early December; thus, ovulation in this female remained unnoticed.

Behavior of females during estrus.—Although we recorded 540 h of video observations, mating activity (copulations lasted less than 5 s) was seen in only 1 single female–male pair (M_1 and F_{15}). Before the day of mating, F_{15} roosted exclusively in a territory of another male. She visited M_1 only for the brief period of mating during the day of estrus, but was observed in the territory of M_1 during approximately one-third of the remaining days in our study. Males trying to mate with the estrous or anestrous females courted frequently in front of the females by performing hovering displays. These hovering displays have already been described as part of the courtship display of male greater sac-winged bats (Voigt and von Helversen 1999).

The observation of extraharem mating by female *S. bilineata* agrees well with the paternity data collected from individuals of this colony during the 6 years preceding our study. On average 70% of the offspring in a harem were sired by males other than the current harem holder (Heckel et al. 1999; Voigt et al. 2005). The observed mating activity suggests that females have control over mating. It seems as if females of *S. bilineata* visit males for copulations when they are in estrus, and refuse to mate when they are not in estrus. This is most likely caused by the larger size of females (Voigt et al. 2006), which makes females physically superior to males. Thus, males of *S. bilineata* cannot defend their harem efficiently, and they cannot coerce copulations.

Gestation and parturition in S. bilineata.—Each focus female *S. bilineata* that remained in the colony until June gave birth to a single offspring in late May or early June. Fecal 20-oxo-pregnane metabolites dropped to values (median of 100 ng/g) comparable to those observed before ovulation. The gestation length averaged 169 days or 5.6 months in *S. bilineata*, which is long for an 8-g normothermic mammal that does not store sperm or delay embryonic development (Genoud and Bonaccorso 1986; Tannenbaum 1975). Sympatric normothermic bats of similar body mass usually have shorter gestation lengths (Hayssen et al. 1993); for example, 10-g *Glossophaga soricina* (Phyllostomidae) have gestation lengths

of approximately 3.5 months, 7-g *Peropteryx macrotis* (Emballonuridae) approximately 4.5 months, and 12-g *Peropteryx kappleri* (Emballonuridae) approximately 5 months. However, in contrast to most vespertilionids and phyllostomids, newborns of *S. bilineata* are well developed when they are born, being fully furred and having almost 40% of adult body mass (C.C. Voigt, in litt.). The extensive gestation period underlines that reproduction is a large investment for female *S. bilineata*, and it also implies that female greater sac-winged bats should synchronize parturition with peak insect abundance (Bradbury and Vehrencamp 1977b).

Zeitgeber of reproduction in females.—We observed that the majority of females of *S. bilineata* came into estrus during a few weeks in early December and that ovulation coincided with the adverse climatic conditions of this time period. Other field studies support that reproductive events are synchronized and seasonal in tropical bats (Baker and Baker 1936; Baker et al. 1936; Heideman 1995; Heideman et al. 1992; O'Brien 1993; Porter and Wilkinson 2001). For example, Baker et al. (1936) argued that *Miniopterus schreibersii* from populations close to the equator has a highly synchronous and short mating period. For neotropical nectar-feeding bats, it was argued that minor changes in intensity of precipitation and temperature in combination with photoperiodic changes trigger reproduction (Heideman and Bronson 1994). Possibly, ovulation is triggered in female *S. bilineata* by adverse climatic conditions such as cold weather, heavy precipitation, or both. However, further studies are required to test this hypothesis. Other nonchiropteran mammals that co-occur in the forest around La Selva Biological Station have either aseasonal reproduction (e.g., collared peccaries [*Tayassu tajacu*], primates, or some rodents such as *Cuniculus paca*), or seasonal reproduction (e.g., some other rodents—Timm 1994). For example, Desmarest's spiny pocket mouse (*Heteromys desmarestianus*) exhibits a sharp decline in reproductive performance in May and June in the forest around La Selva Biological Station (Fleming 1983). Different selective forces acting on flying and terrestrial mammals of varying body sizes are most likely responsible for the diverse reproductive patterns found among mammals of the same rain-forest habitat.

General remarks on fecal hormone metabolite studies in free-ranging bats.—Our study demonstrates that analysis of fecal hormone metabolites is suitable for the study of bat reproductive physiology, especially in the context of mating tactics. In general, we consider it a prerequisite for long-term endocrinological studies that fecal samples collected in the daytime roost can be assigned to individuals. This is the most likely problem encountered when working with gregarious mammals. We monitored the position of our study individuals in the daytime roost 3 times each day and thus are confident that fecal samples were correctly assigned to specific individuals. The small size of our study species prevented conventional sampling of blood with a needle because of the stress posed on the animal, the small blood volume of the bats, or both. In addition, a long-term study based on daily blood sample collection would be impossible in free-ranging animals. Studying free-ranging, gregarious mammals of small size also requires compromising the frequency of sample collection. In order to not disturb social settings of our study group, we observed the animals from a distance of at least 3 m. It certainly would have been advantageous to collect samples immediately after defecation to prevent possible degradation of fecal hormone metabolites by bacteria, and to collect samples at the same time each day to account for diurnal rhythm. Although this was not possible in our study, we are certain that results are comparable among individuals of our study group, because conditions were similar for all samples collected. In addition, results for estrogen and progesterone metabolite excretion seem to be less affected by storage in ambient temperature (i.e., Galama et al. 2004; Pettitt et al. 2007; Schwarzenberger et al. 1996) than results for fecal glucocorticoid metabolites (Palme et al. 2005; Touma and Palme 2005). Further, in a parallel study of male *S. bilineata*, we tested for but could not find a significant effect of prolonged exposure of fecal samples to ambient conditions on values of fecal testosterone metabolite (Voigt et al. 2007). In conclusion, analysis of fecal steroid hormone metabolites is a promising tool for evaluating the reproductive activity of bats, especially in free-ranging animals, when collection of other sample material (i.e., blood) is impossible on a continuous basis.

Acknowledgments

We are most thankful to B. Caspers and S. Brass, who helped during the fieldwork. We are grateful to D. Kelm for censusing the colonies, to K. Rex for her endurance during the video analysis, and to D. Fichte for logistic support. Special gratitude is extended to D. Thaller and E. Leitner for their excellent technical assistance with fecal steroid assays, and to E. Möstl for preparation of antibodies and biotinylated labels. We are most grateful to W. Goymann for discussing hormonal data of *S. bilineata* with us and to D. Dechmann and K. Rex for commenting on an earlier version of the manuscript. We thank the Costa Rican authorities, especially J. Guevara, and the Organization for Tropical Studies for permission to work at La Selva Biological Station. The study complied with the current laws of Costa Rica.

Literature Cited

Baker, J. R., and Z. Baker. 1936. The seasons in a tropical rainforest (New Hebrides). 2. Fruit bats. Journal of the Linnean Society, Zoology 40:123–142.

Baker, J. R., D. Phil, and T. F. Bird. 1936. The seasons in a tropical rainforest (New Hebrides). Part 4. Insectivorous bats (Vespertilionidae and Rhinolophidae). Journal of the Linnean Society, Zoology 40:143–161.

Bradbury, J. W., and L. Emmons. 1974. Social organization of some Trinidad bats I Emballonuridae. Zeitschrift für Tierpsychologie 36:137–183.

Bradbury, J. W., and S. L. Vehrencamp. 1976. Social organization and foraging in emballonurid bats I. Field studies. Behavioral Ecology and Sociobiology 1:337–381.

Bradbury, J. W., and S. L. Vehrencamp. 1977a. Social organization and foraging in emballonurid bats III. Mating systems. Behavioral Ecology and Sociobiology 2:1–17.

Bradbury, J. W., and S. L. Vehrencamp. 1977b. Social organization and foraging in emballonurid bats IV. Parental investment patterns. Behavioral Ecology and Sociobiology 2:19–29.

CLUTTON-BROCK, T. H. 1989. Mammalian mating systems. Proceedings of the Royal Society of London, B. Biological Sciences 236:339–372.

DECHMANN, D. K. N., E. K. V. KALKO, B. KÖNIG, AND G. KERTH. 2005. Mating system of a neotropical roost-making bat: the white-throated, round-eared bat, *Lophostoma silvicolum* (Chiroptera: Phyllostomidae). Behavioral Ecology and Sociobiology 58:316–325.

FLEMING, T. H. 1971. *Artibeus jamaicensis*: delayed embryonic development in a neotropical bat. Science 171:402–404.

FLEMING, T. H. 1983. *Heteromys desmarestianus*. Pp. 474–475 in Costa Rican natural history (D. H. Janzen, ed.). University of Chicago Press, Chicago, Illinois.

GALAMA, W. T., L. H. GRAHAM, AND A. SAVAGE. 2004. Comparison of fecal storage methods for steroid analysis in black rhinoceroses (*Diceros bicornis*). Zoo Biology 23:291–300.

GANNON, W. L., R. S. SIKES, AND THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 88:809–823.

GENOUD, M., AND F. J. BONACCORSO. 1986. Temperature regulation, rate of metabolism, and roost temperature in the greater white-lined bat *Saccopteryx bilineata* Emballonuridae. Physiological Zoology 591:49–54.

GOYMANN, W., S. JENNI-EIERMANN, AND U. BAUCHINGER (EDS.). 2005. Bird hormones and bird migrations: analyzing hormone in droppings and egg yolks and assessing adaptations in long-distance migration. Annals of the New York Academy of Sciences 1046:1–295.

HAYSSEN, V., A. VAN TIENHOVEN, AND A. VAN TIENHOVEN. 1993. Asdell's patterns of mammalian reproduction: a compendium of species-specific data. Cornell University Press, Ithaca, New York.

HECKEL, G., C. C. VOIGT, F. MAYER, AND O. VON HELVERSEN. 1999. Extra-harem paternity in the white-lined bat *Saccopteryx bilineata*. Behaviour 136:1173–1185.

HECKEL, G., AND O. VON HELVERSEN. 2003. Genetic mating system, relatedness and the significance of harem associations in the bat *Saccopteryx bilineata*. Molecular Ecology 12:219–227.

HEIDEMAN, P. D. 1995. Synchrony and seasonality of reproduction in tropical bats. Symposia of the Zoological Society of London 67:155–165.

HEIDEMAN, P. D., AND F. H. BRONSON. 1994. An endogenous circannual rhythm of reproduction in a tropical bat, *Anoura geoffroyi*, is not entrained by photoperiod. Biology of Reproduction 50:607–614.

HEIDEMAN, P. D., P. DEORAJ, AND F. H. BRONSON. 1992. Seasonal reproduction of a tropical bat, *Anoura geoffroyi*, in relation to photoperiod. Journal of Reproduction and Fertility 96:765–773.

ISVARAN, K., AND T. H. CLUTTON-BROCK. 2007. Ecological correlates of extra-group paternity in mammals. Proceedings of the Royal Society, B. Biological Sciences 274:219–224.

MCCRACKEN, G. F., AND G. S. WILKINSON. 2002. Bat mating systems. Pp. 321–362 in Reproductive biology of bats (E. G. Crichton and P. H. Krutzsch, eds.). Academic Press, San Diego, California.

NELSON, R. J. 2000. An introduction to behavioral endocrinology. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

O'BRIEN, G. M. 1993. Seasonal reproduction in flying foxes, reviewed in the context of other tropical mammals. Reproduction, Fertility and Development 5:499–521.

ORGANIZATION FOR TROPICAL STUDIES. 2002. Metereological data at La Selva Biological Station's East Bank (Bridge, ''Puente'') Weather Station. http://www.ots.ac.cr/en/laselva/metereological.shtml. Accessed 14 May 2006.

PALME, R., S. RETTENBACHER, C. TOUMA, S. M. EL-BAHR, AND E. MÖSTL. 2005. Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Annals of the New York Academy of Sciences 1040:162–171.

PETTITT, B. A., C. J. WHEATON, AND J. M. WATERMAN. 2007. Effects of storage treatment on fecal steroid hormone concentrations of a rodent, the cape ground squirrel (*Xerus inauris*). General and Comparative Endocrinology 150:1–11.

PORTER, T. A., AND G. S. WILKINSON. 2001. Birth synchrony in greater spear-nosed bats (*Phyllostomus hastatus*). Journal of Zoology (London) 253:383–390.

SCHWARZENBERGER, F., G. FREDRIKSSON, K. SCHALLER, AND L. KOLTER. 2004. Fecal steroid analysis for monitoring reproduction in the sun bear (*Helarctos malayanus*). Theriogenology 62:1677–1692.

SCHWARZENBERGER, F., E. MÖSTL, R. PALME, AND E. BAMBERG. 1996. Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. Animal Reproduction Science 42:515–526.

SCHWARZENBERGER, F., R. PALME, E. BAMBERG, AND E. MÖSTL. 1997. A review of faecal progesterone metabolite analysis for non-invasive monitoring of reproductive function in mammals. Zeitschrift für Säugetierkunde 62:214–221.

SPSS INC. 1998. SPSS Trends™ 6.1. SPSS Inc., Chicago, Illinois.

STORZ, J. F., H. R. BHAT, AND T. H. KUNZ. 2001. Genetic consequences of polygyny and social structure in an Indian fruit bat, *Cynopterus sphinx*. II. Variance in male mating success and effective population size. Evolution 55:1224–1232.

SYSTAT SOFTWARE INC. 2004. SYSTAT version 11. SYSTAT Software Inc., San Jose, California.

TANNENBAUM, R. 1975. Reproductive strategies in the white-lined bat. Ph.D. dissertation, Cornell University, Ithaca, New York.

TIMM, R. M. 1994. The mammal fauna. Pp. 229–237 in La Selva: ecology and natural history of a neotropical rain forest (L. A. McDade, K. S. Bawa, H. A. Hespenheide, and G. S. Hartshorn, eds.). University of Chicago Press, Chicago, Illinois.

TOUMA, C., AND R. PALME. 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Annals of the New York Academy of Sciences 1046:54–74.

VOIGT, C. C., G. HECKEL, AND F. MAYER. 2005. Sexual selection favours small and symmetric males in the polygynous greater sac-winged bat *Saccopteryx bilineata* (Emballonuridae, Chiroptera). Behavioral Ecology and Sociobiology 57:457–464.

VOIGT, C. C., G. HECKEL, AND O. VON HELVERSEN. 2006. Conflicts and strategies in the harem-polygynous mating system of the sac-winged bat *Saccopteryx bilineata*. Pp. 269–278 in Functional and evolutionary ecology of bats (G. McCracken, A. Zubaid, and T. H. Kunz, eds.). Oxford University Press, Oxford, United Kingdom.

VOIGT, C. C., W. J. STREICH, AND M. DEHNHARD. 2007. Assessment of fecal testosterone metabolite analysis in free-ranging *Saccopteryx bilineata* (Chiroptera; Emballonuridae). Acta Chiropterologica 9:463–475.

VOIGT, C. C., AND O. VON HELVERSEN. 1999. Storage and display of odor by male *Saccopteryx bilineata* (Chiroptera; Emballonuridae). Behavioral Ecology and Sociobiology 47:29–40.

Submitted 13 December 2006. Accepted 9 May 2007.

Associate Editor was Rodrigo A. Medellín.