BIOLOGY OF REPRODUCTION 50, 607–614 (1994)

An Endogenous Circannual Rhythm of Reproduction in a Tropical Bat, *Anoura geoffroyi*, Is Not Entrained by Photoperiod[1]

PAUL D. HEIDEMAN[2] and F.H. BRONSON

Institute of Reproductive Biology and Department of Zoology, University of Texas at Austin Austin, Texas 78712

ABSTRACT

Most species of mammals live in the tropics, and many breed seasonally, but little is known about the regulation of their seasonal cycles. Males of a tropical bat, *Anoura geoffroyi* (Order Chiroptera, Family Phyllostomidae), from 10° latitude in Trinidad, were studied to test the role of photoperiod in regulating seasonal reproduction in the deep tropics. Groups of males were subjected to five treatments: 1) constant photoperiod; 2) a 12-mo cycle of civil twilight photoperiods mimicking those occurring at 10° latitude; 3) civil twilight photoperiods of 10° latitude, but accelerated to a 9-mo cycle; 4) civil twilight photoperiods characteristic of 30° latitude, but accelerated to a 9-mo cycle; and 5) constant photoperiod, but with the timing of dark onset varied to match the timing of darkness at 10° latitude, and accelerated to a 9-mo cycle. In all treatments, the first cycle of testis growth and regression matched that expected in the wild population, as reported previously for some of these groups. Subsequently, the testis cycle of bats in constant conditions free-ran for 20 mo with a peak-to-peak period of 7.3 ± 0.3 mo. Period lengths in the four nonconstant groups, 7.2–7.7 mo, were not significantly different from that under constant conditions. Bats failed to entrain to any photoperiod cycle, including those mimicking changes at 10° or 30° latitude. They also failed to entrain to the cycle in which day length was held constant while time of sunset was varied, as occurs at the equator. This study provides the first evidence for deep tropical mammals of a truly seasonal and endogenous reproductive rhythm that is not regulated by photoperiod. By extension, these results imply that effective seasonal timing can be achieved in the deep tropics by use of a nonphotoperiodic predictive cue.

INTRODUCTION

Most species of mammals live in the tropics [1], and many breed seasonally (e.g., [2–6]), but little is known about the regulation and maintenance of their seasonal cycles. As in the temperate zone, the ultimate cause of most seasonal reproduction in the tropics is probably seasonal change in food availability. Mammals typically match peaks in food availability with lactation and/or late pregnancy because maternal energy and nutritional demands peak in lactation or late pregnancy. Food availability is determined mostly by the annual temperature cycle in the temperate zone, but by seasonal rainfall in the tropics. Mammals in seasonal environments have evolved two general strategies of reproductive timing. In some mammals, reproduction is opportunistic, depending upon immediate climatic and nutritional conditions [6]. Because good conditions are transient, this strategy is effective only for species that can produce offspring rapidly and therefore can complete lactation before the end of optimum conditions. Seasonally breeding species with a long gestation cannot use an opportunistic strategy, since the long delay between copulation and birth would cause them consistently to miss the optimum periods for the most difficult parts of their reproductive cycle. The proximate cause of reproduction in these species is usually an environmental cue that is a predictor of good conditions. The predictive cue used by most seasonally breeding mammals in the temperate zone is photoperiod [7].

Our knowledge of the ways in which photoperiod regulates seasonal cycles in temperate zone mammals is probably incomplete (for review see [8]), but we know that photoperiod can be used in at least two ways. First, photoperiod can be used to turn on or turn off a breeding season, but apparently not both. This strategy is seen in the Syrian hamster [9, 10] and probably many other shorter-lived mammals. Second, photoperiod can entrain an endogenously generated circannual rhythm of reproduction, as it does in sheep [11, 12] and probably many other longer-lived mammals (reviewed by [13]).

The amplitude of the annual change in day length declines as latitude decreases, to a constant day length on the equator. The question of how deeply into the tropics photoperiod can enforce seasonal breeding has not been answered (see [14]), but it obviously could not operate at or close to the equator. It is known only that some tropical mammals can respond reproductively to changes in photoperiod [15–20], and some cannot [6, 21–24]. The extent to which the reproductively photoresponsive species actually use photoperiod to regulate their reproduction remains an open question.

The object of this study was to examine the seasonal reproductive cycle of a deep tropical mammal, a nectar-feeding bat (*Anoura geoffroyi*), and test it for responsiveness to photoperiod. In a previous study, Heideman et al. [25] described the natural cycle of this species in the wild and asked whether the timing of the recrudescence phase of

Accepted October 19, 1993.
Received July 26, 1993.
[1]This research was supported by National Institutes of Health grant HD 24177 and National Institutes of Health National Research Service Award F32 HD 07294.
[2]Correspondence. FAX: (512) 471–9651.

the annual testis cycle was sensitive to photoperiod; it was not. The present paper reports a continuation of that study over a much longer period of time. In this longer study we established that an endogenously generated circannual rhythm underlies this species' annual cycle, and we still found no evidence that this rhythm is sensitive to regulation by photoperiod, suggesting that a cue of a different nature must be used as a seasonal synchronizer in the deep tropics.

MATERIALS AND METHODS

Geoffroy's hairy-legged, long-tongued bats, *Anoura geoffroyi* (Family Phyllostomidae), were collected from Tamana Cave (10°28′N, 61°12′E, 386.16 km elevation) in central Trinidad. This species feeds on nectar, pollen, soft fruit, and soft-bodied insects [26–30]. *Anoura geoffroyi* have relatively large eyes and presumably rely heavily on vision. They are small (12–19 g), agile fliers and are capable of hovering flight. The species ranges from central Mexico south across the equator to Peru, Bolivia, and east-central Brazil [31]. At Tamana Cave, *A. geoffroyi* roost in large groups in chambers 15–30 meters from the cave entrances. In these roosts, light intensity is below one lux (unpublished observations), but light from the entrances is apparent, and the bats can experience higher light intensities by circling near cave entrances at dusk and dawn [32].

Rainfall on central Trinidad is seasonal, with a dry season typically from January through April or May, and a wet season from June through December [25]. Mean monthly temperature varies only slightly, ranging from 24.6–26.6°C (Piarco Meteorological Service, Trinidad). In the wild population of *A. geoffroyi* on Trinidad [25], testicular recrudescence occurs at the end of the dry season in April and May, and females become pregnant during July and August, in mid wet season. Testicular regression occurs in August and September, with births at the end of the wet season in November and December, followed by approximately 2 mo of lactation.

Husbandry

Housing conditions in the laboratory have been described by Heideman et al. [25] and are briefly summarized here. Bats were individually marked and placed in groups of 11–13 in light-sealed, ventilated flight cages. Each cage had a feeding area 87 cm long, 62 cm wide, and 55 cm high, lighted during part of each day, and connected at one end by an opening to a roosting area 30 cm long, 62 cm wide, and 55 cm high, which received light only through the opening to the flight area. The bats were fed a diet slightly modified [25] from a formula devised by Rasweiler ([33]; diet XI) for keeping bats of this family. The diet was a mix of canned peach nectar, water, sucrose, nonfat and whole milk powder, high-protein baby cereal, wheat germ, and supplements of essential oils, vitamins, and minerals. The temperature was maintained at 30 ± 1°C, and relative humidity was maintained at 70 ± 10%. Light was provided by two 20-watt externally ballasted fluorescent bulbs, one at the ceiling of each end of the feeding area. The onset and end of the light period in each cage was controlled by a Hunter timer (model 41001; Hunter Fan Co., Memphis, TN) programmable to the minute, and accurate to within one second per day.

All of the experimental photoperiod treatments to be listed shortly were derived from the Astronomical Almanac [34]. Civil twilight photoperiods were chosen for this experiment because there is evidence that mammals may interpret the period of civil twilight just after sunset and before sunrise, during which light intensity is still high, as part of the light period [35].

At 2-wk intervals throughout the study, each bat was lightly anesthetized with methoxyflurane (Pitman-Moore Inc., Mundelein, IL) and weighed, its scrotum was moistened, and the greatest length and width of the right testis was measured with calipers. Testicular volume was then estimated by use of the formula for a prolate spheroid (= [width]^2 × length × 0.523). External testis measurements were significantly and highly correlated with measurements and weights taken from excised testes ([25]; Heideman, unpublished data). Data on testis volume were not adjusted for body weight because these two variables were not correlated significantly ($R^2 = 0.10$, $p > 0.05$ at the start of the study and $R^2 = 0.09$, $p > 0.05$ at the end of the study). Because body weight varied relatively little during the study, data on body weights are not presented here.

A large group of bats was captured in October 1990 and delegated to one of five treatments in which photoperiod was varied. A previous publication [25] presented data collected in the wild and data from three of these five groups between the time of capture and late July 1991, when conditions were changed for all five experimental groups. At that time, light intensity was decreased approximately 10-fold, to 100–200 lux in the flight area and 1–100 lux in the roosting area, for all treatment groups. The new light intensity within the roost area was still higher than that at the roost sites occupied by *Anoura* at Tamana Cave (unpublished observations), but light intensity in the flight area approximated that at the cave entrances near sunrise and sunset. The other changes made at this time are included below in the descriptions of the treatments.

The change in treatments in late July 1991 was timed to begin at or near peak testis size in all treatments in order to provide a clear starting point for the data reported here. Thus, the analyses in this paper use data from late July 1991 through March 1993. However, in the figures we include the full data set, starting in November 1990, in order to provide complete information on the prior history of the bats and their photoperiod treatments.

Experimental Treatments

One group ("Constant," Table 1) received a constant photoperiod of L12 h:19 min:D11 h:41 min (annual mini-

TABLE 1. Summary of photoperiod treatments from late July 1991 through March 1993 (for treatment details, see text of Methods).

Group	Photoperiod Treatment
Constant	Constant photoperiod of 12 h, 19 min
12-month 10°	12-month cycle mimicking natural cycle of civil twilight photoperiods of 10° latitude
9-month 10°	Accelerated, 9-month cycle of civil twilight photoperiods of 10° latitude
9-month 30°	Accelerated, 9-month cycle of civil twilight photoperiods of 30° latitude
9-month Sunset	Time of lights off varied to match the end of civil twilight at 10° north, with the annual cycle accelerated to 9 months; time of lights on a constant 12 h, 54 min after onset of dark

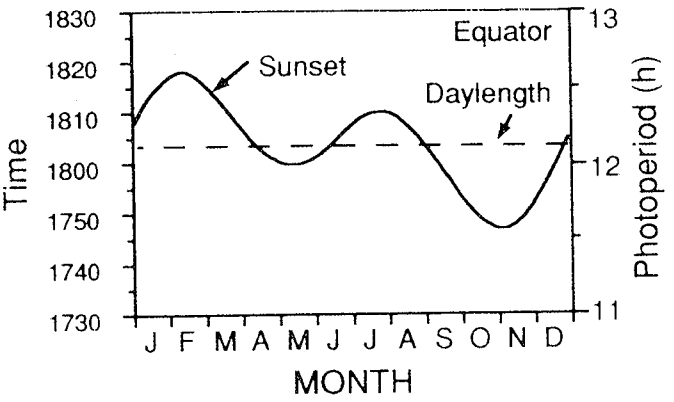

FIG. 1. Photoperiod (dashed line) and annual variation in the time of sunset at the equator (solid line).

mum of civil twilight photoperiod at latitude 10°) during the 21-mo period from July 1991 to April 1993. The photoperiod during the previous 8 mo had been a constant L12 h:54 min:D11 h:06 min. Reproductive data from this group were analyzed for evidence of an endogenous rhythm according to criteria described by Gwinner [13].

A second group ("12-Month 10°," Table 1) received two 12-mo cycles of natural change in photoperiod mimicking the civil-twilight cycle at 10° of latitude (amplitude of 1 h and 10 min). The photoperiod during the previous 8 mo followed the same schedule, but light intensity was higher, as described above. We predicted that these bats would follow a 12-mo reproductive cycle in phase with that of wild bats if photoperiod entrained their reproductive cycle.

A third group ("9-Month 10°," Table 1) received over two full cycles of change in photoperiod that mimicked the amplitude of natural change in the civil-twilight cycle at 10° of latitude while accelerating this cycle to 9 mo. During the previous 8 mo, this group had received the same cycle compressed to 6 mo [25]. We predicted that these bats would follow a 9-mo reproductive cycle out of phase with that of wild bats if photoperiod entrained their reproductive cycle.

A fourth group ("9-Month 30°," Table 1) received over two full cycles of change in photoperiod that mimicked the amplitude of natural change in the civil-twilight cycle at 30° of latitude (amplitude of 3 h and 52 min), while accelerating this cycle to 9 mo. During the previous 8 mo, this group received a 12-mo cycle of natural photoperiod change, following sunrise and sunset times for 10° latitude. As with the previous group, we predicted that these bats would follow a 9-mo reproductive cycle (out of phase with that of wild bats) if photoperiod entrained their reproductive cycle. Furthermore, reproductive timing in this group should differ from the 9-mo 10° group if these bats were sensitive to photoperiod, but were unable to respond to the lower amplitude of change in photoperiod of 10° latitude.

The treatment to which the fifth group was exposed was designed to test the importance of a phenomenon that occurs at the equator, where photoperiod is constant throughout the year, but the time of sunset and sunrise vary seasonally (Fig. 1). Specifically, in this group ("9-Month Sunset," Table 1), photoperiod was held constant at 12 h 54 min, but the time of lights-off was varied to mimic the natural cycle of the timing of sunset at 10° latitude, except that the cycle was accelerated to 9 mo in duration. Light onset always occurred 12 h 54 min after the lights were turned off. During the previous 8 mo, this group had received a cycle with a period of 6 mo in which the time of lights-off varied as above, but the time of lights-on was held constant. We predicted that the bats would entrain to the new cycle if they were able to use the changing time of sunset or sunrise to regulate their reproduction.

Data Analysis

Repeated-measures analysis of variance was used to test for differences among the groups of bats held under controlled photoperiods. One-way analyses of variance were used to test for differences among groups at each sampling point. Statistical significance was defined as probability values less than 0.05. Means are presented with their standard errors.

The period of each cycle of testicular growth and regression was defined as the time between midpoints of successive peaks. Peaks were defined as clusters of four or more points (e.g., 8 or more wk) during which testis volume was significantly higher than during the flanking 8-wk (or longer) periods, according to a Mann-Whitney U test with significance defined as $p < 0.05$. The midpoint of a peak was defined as the highest value of a running three-point mean within the peak. Terminal plateaus were considered to be peaks if they were at least 12 wk (6 testis measurements) long. For each bat, we calculated the mean length of its periods. These mean period lengths were used in an analysis of variance including all five treatments. Scheffe confidence intervals for the mean of each group were used to compare the observed period length of testis cycles with the period length of the experimental treatment or, for the constant treatment, with 12 mo. Varying the way in which peaks were defined or the probability value for statistical

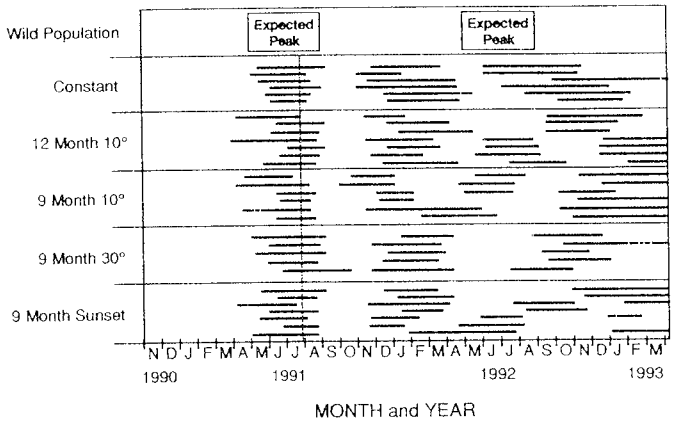

FIG. 2. Periods of large testes (black bars) for individuals in all five treatments. Each bar comprises the period during which testis volume was above the midpoint between the flanking peak and nadir of testis volume. The boxes at the top of the figure show the expected timing of large testes in the wild [25]. The dashed line represents the point at which photoperiod treatments were changed at the beginning of this study. See text for additional treatment details.

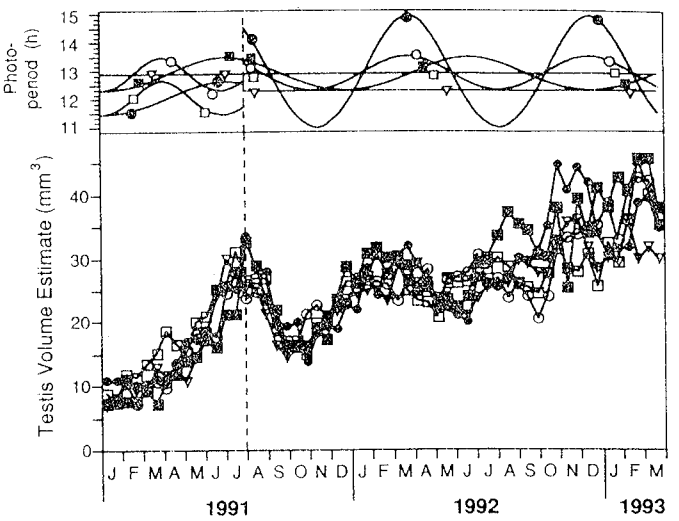

FIG. 3. Mean testis volume in each treatment during the course of the study. The dashed line represents the point at which photoperiod treatments were changed at the beginning of this study. The box at the top of the figure shows the photoperiod treatments and histories for each group. Standard errors have been omitted from the figure to improve readability; they ranged from 1–6 mm^3 in 1991, when individual cycles were well synchronized, to 1–10 mm^3 at the end of 1992 and in 1993, when individual cycles were asynchronous. (Constant: open triangle, 12-Month 10°: solid square, 9-Month 10°: open circle, 9-Month 30°: solid circle, 9-Month Sunset: open square; N = 6, 7, 6, 5, and 7, respectively; see text for treatment details).

significance of a peak altered Tau values slightly, but did not affect the conclusions of the study. Period lengths were converted to months for presentation, each month defined as 30.5 days.

Over the 2.5 yr of the study, several bats died under anesthesia, three died of unexplained causes, and others were removed as part of another study, leaving final group sizes of 8–10 individuals per treatment. In each group, two or three bats either produced ambiguous peaks or had no peaks by our definition, in most cases because they had small testes through all or most of the study, resulting in less accurate testis measurements and poor resolution of changes in testis size. These individuals were excluded from the analysis. All the bats used were at or near a peak in July 1991, when this study began, and period lengths for each bat were measured from that initial peak.

RESULTS

Endogenous Rhythm

Bats in the group housed in constant conditions demonstrated cycles of testis size that averaged 7.3 ± 0.3 mo from peak to peak. This period was significantly shorter than 12 mo ($p < 0.01$). The cycles of individuals within this group lost synchrony with each other over the course of the study; some individuals reached peaks in testicular size while others were at minima (Fig. 2). In addition, there was a gradual increase in testis size at both peaks and lows within cycles (Figs. 3 and 4).

Effects of Photoperiod Treatments on Reproductive Cycles

A repeated measures ANOVA showed no significant differences in testis volume among treatments ($F = 0.10$; $p > 0.90$), but there were significant changes in testis volume over time ($F = 44.11$; $p < 0.0001$), reflecting the gradual increase in testis size in all treatments. The interaction term between treatment and the repeated measure was significant ($F = 1.35$; $p < 0.001$), indicating that testis volume changed in different ways among the different treatments. This was probably due to slight differences among groups as they gradually lost synchrony in the timing of peaks and to the small differences in mean testis size during the last 6 mo of the study (Fig. 3).

Mean period length ranged from 7.2–7.7 mo across treatments (Fig 5), with no significant differences in period length among treatments ($F = 0.19$; $p > 0.90$). The period length of the 12-mo 10° treatment was significantly shorter than 12 mo ($p < 0.01$), and that of the 9-mo 30° treatment was significantly shorter than 9 mo ($p < 0.01$). In two groups, the 9-Month Sunset treatment group and the 9-Month 10° treatment group, the respective 7.7-mo and 7.6-mo average period lengths were only marginally significantly different from the 9-mo periods of their photoperiod treatments ($0.10 > p > 0.05$ and $p = 0.10$, respectively). However, these period lengths were also nearly identical to the bats' free-running rhythm under constant conditions (Fig. 5).

DISCUSSION

Our results show that testis size in *Anoura geoffroyi* is controlled in part by an endogenously generated circan-

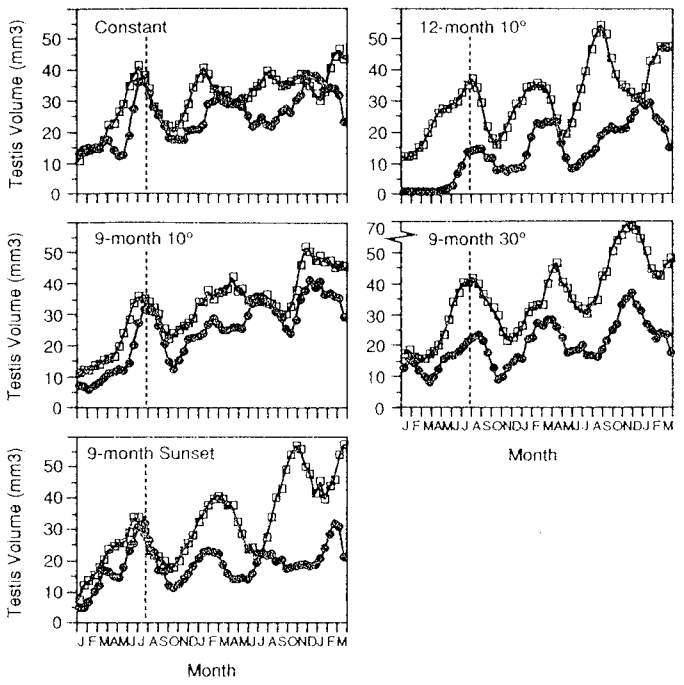

FIG. 4. Testis volume of two cycling individuals from each treatment from January 1991 through March 1993. The points on the figures are running means of three adjacent measurements. The dashed lines represent the point at which photoperiod treatments were changed at the beginning of this study.

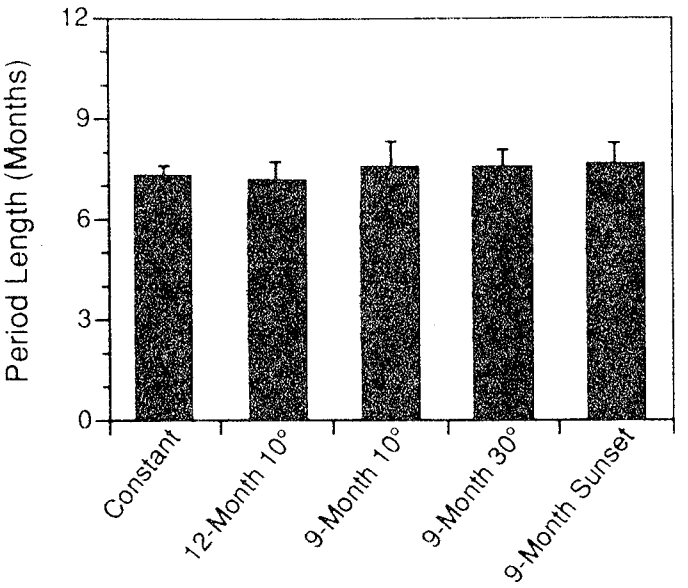

FIG. 5. Mean and standard error of period length in constant conditions (Constant; N = 6), varying photoperiod using civil twilight photoperiods for 10° latitude on a 12-mo cycle (12-Month 10°; N = 7), varying photoperiod using civil twilight photoperiods for 10° latitude on a 9-mo cycle (9-Month 10°; N = 6), varying photoperiod using civil twilight photoperiods for 30° latitude on a 9-mo cycle (9-Month 30°; N = 5), and constant photoperiod with varying onset of darkness (9-Month Sunset; N = 7).

nual rhythm, as defined by Gwinner [13]. The group of bats housed under constant conditions exhibited almost three full cycles of testicular regression and recrudescence in just under two years. Their mean period length, 7.3 mo, was significantly shorter than 12 mo and cannot be accounted for by any rhythmic change in the laboratory environment. Within treatments, individuals cycled independently of each other (Figs. 2–4), which indicates that the bats were not entraining their cycle to some unknown and uncontrolled cue in the laboratory. The free-running period under the conditions of our study was shorter than those of most other mammals examined (reviewed in [13]), but similar in length to those of several species of ground squirrels [36–38].

All groups underwent a gradual increase in mean testis size over time (Figs. 2 and 4). This may represent a dampening of rhythm characteristics with time, which is a common observation in long-term studies on endogenous rhythms (reviewed in [13]). However, our data suggest that testis volume tended to be higher at both peaks and nadirs in the last months of the study (see Fig. 4) and that the rhythms were not dampened. In either case, it may be that natural selection has favored a tendency to maintain reproductive readiness in the sustained absence of seasonal environmental signals, while rhythms may or may not be superimposed upon a higher baseline, as in this study.

In the temperate zone, several species of bats are known to have circannual endogenous rhythms of weight or testis size that are entrained by photoperiod [39, 40]. In bats from the subtropics, the effects of photoperiod vary among species. Males of the primarily temperate and subtropical grey-headed fruit bat *Pteropus poliocephalus*, collected at about 28°S latitude in Australia, are reproductively photoresponsive [15], but this is not true of males of a species from lower temperate to mid-tropical latitudes, the little red flying fox (*Pteropus scapulatus*), collected from the same region [24]. The presence of large pineal glands in many tropical bats [41, 42] suggests that elements of the photoperiod transduction pathway are functional at least through the pineal gland, as has been shown for a rodent from the deep tropics that is reproductively unresponsive to photoperiod [22, 23]. In their laboratory colony of a tropical Molossid bat, Häussler et al. [43] noted periodic births, implying that the bats might have an endogenous reproductive rhythm, but they had no data on potential entraining cues. There are, to our knowledge, no previous studies on the regulation of endogenous rhythms in seasonally breeding mammals from the deep tropics.

Overall, our results indicate that photoperiod does not regulate the annual cycle of testicular growth and regression in *A. geoffroyi* in the wild. We showed previously [25] that the recrudescence phase of the testis cycle could not be accelerated by a 6-mo photoperiod cycle. The experiments presented here have tested the potential role of photoperiod much more thoroughly and over a much longer period of time, and they allow several more conclusions. First, in the present study the photoperiod cycles were ac-

celerated only to 9 mo, which is longer than the free-running period length in constant conditions. This eliminates the possibility that the experimental cycles were too short for entrainment. Second, the failure of bats to entrain to two full cycles of photoperiod change eliminates the possibility that photoperiod might entrain an endogenous circannual rhythm of reproduction at only a single point in their annual reproductive cycle (e.g., [11, 12]). Third, the bats in the 9-mo 30° group failed to entrain even to a much greater amplitude of photoperiod change, 3 h and 52 min, than they experience in the wild. This suggests that *A. geoffroyi* may entirely lack the ability to respond reproductively to photoperiod, as has been reported for the tropical cane mouse [22, 23]. Finally, full entrainment of mammals to a photoperiod cycle can take several cycles if the treatment begins out of phase with the original rhythm of the animal [44]. This possibility cannot apply to our 12-mo cycle of change, which began in phase with the natural cycle, continuing the cycle of natural change described in Heideman et al. [25]. Similarly, the three accelerated photoperiod treatments were phased to match the cycle of the bats when they entered the experiment in July. These bats all experienced declining photoperiods as their testes decreased in size, just as they would have in the wild, but failed to entrain to the rhythm of the experimental photoperiod cycle.

It is impossible to prove that photoperiod has absolutely no affect on reproduction in male *A. geoffroyi*. It is possible that there are minor, subtle effects of photoperiod on reproduction in this bat, and that the experimental conditions (ad lib food, etc.) might block effects of photoperiod. However, because the phenomenon of reproductive photoresponsiveness is typically robust and demonstrable under a wide range of conditions in the laboratory, even in species from the higher tropics (see the many papers cited in [7, 8, 13]), we argue that it is extremely unlikely that photoperiod is an important regulatory cue for reproduction in male *A. geoffroyi* in the wild.

In total, our results suggest that the endogenous reproductive rhythm of this population may be entrained normally by an environmental cue other than photoperiod acting at one particular time of the year, in a manner similar to the requirement in domestic sheep of shorter days at a single time of year [11, 12, 45]. Our bats were collected in October 1990, just after completing testicular regression [25]. Their first cycle of testicular growth and regression matched the timing expected in the wild, including the period of testicular quiescence between capture and May 1991 (Figs. 2 and 3, and see [25]). However, subsequent cycles lacked a long quiescent period, free-running instead with an average period of about 7.5 mo. This suggests that environmental conditions during or just after the peak in testicular development, which our bats experienced for the last time in 1990, prior to capture, may set the subsequent time of testicular recrudescence, approximately 8 mo later. If so, then failure to receive the appropriate synchronizing cue during subsequent cycles in the laboratory permitted testis cycles to free-run at each individual's endogenous rhythm.

If photoperiod does not synchronize the reproductive cycle of *A. geoffroyi*, then what cue does serve this purpose? One possible cue tested here and found to be ineffective is the annual cycle of the time of sunrise and sunset. As noted earlier, the timing of sunrise and sunset varies according to a biannual pattern on the equator, although day length is constant (Fig. 1). The annual range in timing is 31 min on the equator and 51 min at 10° latitude. The mean period length in the treatment testing this cue in *A. geoffroyi* (9-mo Sunset) was 7.7 mo, almost identical to period lengths in the other treatments, suggesting that this is not the factor of interest. As suggested by Alibhai [46], plant secondary compounds may entrain seasonal rhythms in the tropics. This hypothesis is attractive because some plants possess photoperiodically entrained seasonal clocks that are accurate within 2–5° latitude of the equator [47, 48]. The plant compound 6-methoxybenzoxazolinone (6-MBOA) has been shown to stimulate reproduction in some temperate zone mammals [49, 50], but 6-MBOA has produced disappointingly slight effects on reproduction in tropical mammals (e.g., [46, 51]). Plant estrogens have been suggested to affect seasonal reproduction in primates [52], but the only evidence for their use as a seasonal cue is correlational. Both rainfall and temperature might be used as predictive cues, but both have low signal-to-noise ratios in most tropical environments, and hence make better proximate cues for opportunists than for species that use predictors. Seasonal changes in light intensity [53, 54] and in magnetic fields [55] are potential cues for which we have no experimental data on tropical species. A precise biological compass or orientation system could be used to track the changing position of the setting or rising sun (or the plane of maximally polarized light orthogonal to it), or their position relative to magnetic north. These aspects of sun position vary seasonally by 46 degrees of arc even on the equator. Although these have the potential to be cues with high signal-to-noise ratios, we know of no evidence that any animal actually uses any of them as predictive cues.

Ultimately, the signal-to-noise ratio of potential seasonal cues determines their precision, and hence their value as predictors. Similarly, the sensitivity of regulatory physiological pathways to seasonal cues determines the accuracy of seasonal responses that animals can evolve. Either increased noise in a potential predictive cue or constraints on the sensitivity of physiological pathways may result in evolutionary pressures favoring responses to alternative predictive cues and physiological pathways. It remains an open question whether seasonal change in photoperiod can be used to enforce seasonality in mammals in the deep tropics, or whether selection has favored the use of alternative predictive cues. The current study on *A. geoffroyi* provides the first test of potential entraining cues in a deep tropical mammal whose seasonality is regulated by an en-

dogenous circannual rhythm, and it shows that the entraining cue apparently is something other than photoperiod. The significance of this observation lies in the implication that effective seasonal timing can be achieved in the tropics through the use of a nonphotoperiodic predictive cue.

ACKNOWLEDGMENTS

This study would not have been possible without generous advice and support from John J. Rasweiler IV, both in the field and in the laboratory. We thank S. Beckwith, K. Hoskins, and T. Pierson for skilled assistance in handling and maintaining bats. Faculty and staff at the University of the West Indies in Trinidad, particularly Dr. J.S. Kenney, R. Mahabir, D. Peterkin, and G. White, provided invaluable logistical support in the field.

REFERENCES

1. Brown JH, Gibson AC. Biogeography. CV Mosby Co. 1983: 643.
2. Fleming TH, Hooper ET, Wilson DE. Three Central American bat communities: structure, reproductive cycles, and movement patterns. Ecology 1972; 53:555–569.
3. Bradbury JW, Vehrencamp SL. Social organization and foraging in emballonurid bats. IV. Parental investment patterns. Behav Ecol Sociobiol 1977; 2:19–29.
4. Bonaccorso FJ. Foraging and reproductive ecology in a Panamanian bat community. Bull Fla State Mus Biol Ser 1979; 24:359–408.
5. Heideman PD. The timing of reproduction in the fruit bat *Haplonycteris fischeri* (Pteropodidae): geographic variation and delayed development. J Zool (Lond) 1988; 215:577–595.
6. Heideman PD, Bronson FH. A pseudo-seasonal reproductive strategy in a tropical rodent, *Peromyscus nudipes*. J Reprod Fertil 1992; 95:57–67.
7. Bronson FH. Mammalian Reproductive Biology. Chicago: Univ. Chicago Press; 1989: 325.
8. Bronson, FH, Heideman PD. Seasonal regulation of reproduction in mammals. In: Knobil E, Neill JD (eds.), The Physiology of Reproduction, 2nd Edition. New York: Raven Press; 1994.
9. Elliott JA, Goldman BD. Seasonal reproduction: photoperiodism and biological clocks. In: Adler NT (ed.), Neuroendocrinology of Reproduction. New York: Plenum Press; 1981: 377–423.
10. Stetson MH, Watson-Whitmyer M. Hormonal regulation of the annual reproductive cycle of golden hamsters, *Mesocricetus auratus*. Gen Comp Endocrinol 1981; 45:329–344.
11. Malpaux B, Robinson JE, Wayne NL, Karsch FJ. Regulation of the onset of the breeding season of the ewe: importance of long days and of an endogenous reproductive rhythm. J Endocrinol 1989; 122:269–278.
12. Woodfill CJI, Robinson JE, Malpaux B, Karsch FJ. Synchronization of the circannual reproductive rhythm of the ewe by discrete photoperiodic signals. Biol Reprod 1991; 45:110–121.
13. Gwinner E. Circannual Rhythms. Berlin: Springer-Verlag; 1986: 154.
14. Heideman PD, Bronson FH. Sensitivity of Syrian hamsters (*Mesocricetus auratus*) to amplitudes and rates of photoperiodic change typical of the tropics. J Biol Rhythms 1994; (in press).
15. McGuckin MA, Blackshaw AW. Effects of photoperiod on the reproductive physiology of male flying foxes, *Pteropus poliocephalus*. Reprod Fertil Dev 1992; 4:43–53.
16. Petter-Rousseaux A. Observations sur l'influence de la photoperiode sur l'activite sexuelle chez *Microcebus murinus* en captivite. Ann Biol Anim Biochem Biophys 1970; 10:203–208.
17. Sicard B, Maurel D, Gautun J-C, Boissin J. Activation ou inhibition testiculaire par la photoperiode chez plusieurs especes de rongeurs saheliens: premiere mise en evidence d'une variation circadienne de la photogonadosensibilite. C R Acad Sci (Paris) 1988; 307(III):11–17.
18. Sicard B, Maurel D, Fuminier F, Boissin J. Circadian rhythm of photosensitivity and the adaptation of reproductive function to the environment in two populations of *Arvicanthis niloticus* from Mali and Burkina Faso. J Reprod Fertil 1992; 95:159–165.
19. Van Horn RN. Primate breeding season: Photoperiodic regulation in captive *Lemur catta*. Folia Primatol 1975; 24:203–220.
20. Wayne NL, Rissman EF. Tropical photoperiods affect reproductive development in the musk shrew, *Suncus murinus*. Physiol Behav 1991; 50:549–553.
21. Loudon ASI, Curlewis JD. Cycles of antler and testicular growth in an aseasonal tropical deer. J Reprod Fertil 1988; 83:729–738.
22. Heideman PD, Bronson FH. Photoperiod, melatonin secretion, and sexual maturation in a tropical rodent. Biol Reprod 1990; 43:745–750.
23. Bronson FH, Heideman PD. Lack of reproductive photoresponsiveness and correlative failure to respond to melatonin in a tropical rodent, the cane mouse. Biol Reprod 1992; 46:246–250.
24. O'Brien GM, Curlewis JD, Martin L. Effect of photoperiod on the annual cycle of testis growth in a tropical mammal, the little red flying fox, *Pteropus scapulatus*. J Reprod Fertil 1993; 98:121–127.
25. Heideman PD, Deoraj P, Bronson FH. Seasonal reproduction of a tropical bat, *Anoura geoffroyi*, in relation to photoperiod. J Reprod Fertil 1992; 96:765–773.
26. Alvarez T, Gonzalez Q. Analisis polinico del contenido gastrico de murcielagos glossophaginae de Mexico. An Esc Nac Cienc Biol Mex 1970; 18:137–165.
27. Goodwin GG. Mammals of Costa Rica. Bull Am Mus Nat Hist 1946; 87:275–458.
28. Goodwin GG, Greenhall AM. A review of the bats of Trinidad and Tobago. Bull Am Mus Nat Hist 1961; 122:191–301.
29. Howell DJ, Burch D. Food habits of some Costa Rican bats. Rev Biol Trop 1974; 21:281–294.
30. Sazima M, Sazima I. Bat pollination of the passion flower, *Passiflora mucronata*, in south eastern Brazil. Biotropica 1978; 10:100–109.
31. Eisenberg JF. Mammals of the Neotropics: Vol. 1, The Northern Neotropics. Chicago: Chicago University Press, 1989: 449.
32. Darlington JP. Studies on the ecology of the Tamana Caves with special reference to cave-dwelling cockroaches. Trinidad: University of West Indies; 1970: Ph.D. Thesis.
33. Rasweiler JJ IV. Care and management of the long-tongued bat, *Glossophaga soricina* (Chiroptera: Phyllostomatidae), in the laboratory, with observations on estivation induced by food deprivation. J Mammal 1973; 54:391–404.
34. Anonymous. The Astronomical Almanac for the Year 1990. Washington: United States Naval Observatory; 1990: 29.
35. Malpaux B, Wayne NL, Karsch FJ. Termination of the breeding season in the suffolk ewe: involvement of an endogenous rhythm of reproduction. Biol Reprod 1988; 39:254–263.
36. Pengelley ET, Kelly KH. A "circannian" rhythm in hibernating species of the genus *Citellus* with observations on their physiological evolution. Comp Biochem Physiol 1966; 19:602–617.
37. Kenagy GJ. Effects of day length, temperature, and endogenous control on annual rhythms of reproduction and hibernation in chipmunks (*Eutamias* spp.). J Comp Physiol 1981; 141:369–378.
38. Zucker I, Licht P. Circannual and seasonal variations in plasma luteinizing hormone levels of ovariectomized ground squirrels (*Spermophilus lateralis*). Biol Reprod 1983; 28:178–185.
39. Funakoshi K, Uchida TA. Annual cycles of body weight in the Namie's frosted bat, *Vespertilio superans superans*. J Zool (Lond) 1982; 196:417–430.
40. Beasley LJ, Zucker I. Photoperiod influences the annual reproductive cycle of the male pallid bat (*Antrozous pallidus*). J Reprod Fertil 1984; 70:567–573.
41. Bhatnagar KP, Frahm HD, Stephan H. The pineal organ of bats: a comparative morphological and volumetric investigation. J Anat 1986; 147:143–161.
42. Bhatnagar KP, Frahm H, Stephan H. The megachiropteran pineal organ: a comparative morphological and volumetric investigation with special emphasis on the remarkably large pineal of *Dobsonia praedatrix*. J. Anat. 1990; 168:143–166.
43. Häussler U, Möller E, Schmidt E. Zur haltung und jugendentwicklung von *Molossus molossus* (Chiroptera). Z Saugetierekunde 1981; 46:337–351.
44. Davis DE, Finnie EP. Entrainment of circannual rhythm in weight of woodchucks. J Mammal 1975; 56:199–203.
45. O'Callaghan D, Karsch FJ, Boland MP, Hanrahan JP, Roche JF. Variation in the timing of the reproductive season among breeds of sheep in relation to differences in photoperiodic synchronization of an endogenous rhythm. J Reprod Fertil 1992; 96:443–452.
46. Alibhai SK. Reproductive response of *Gerbillus hardwoodi* to 6-MBOA in the Kora National Reserve, Kenya. J Trop Ecol 1986; 2:377–379.
47. Dore J. Response of rice to small differences in length of day. Nature 1959; 183:413–414.
48. Njoku E. Seasonal periodicity in the growth and development of some forest trees in Nigeria II. Observations on seedlings. J Ecol 1964; 52:19–26.
49. Negus NC, Berger PJ. Experimental triggering of reproduction in a natural population of *Microtus montanus*. Science 1977; 196:1230–1231.
50. Sanders EH, Gardner PD, Berger PJ, Negus NC. 6-Methoxybenzoxazolinone: a plant derivative that stimulates reproduction in *Microtus montanus*. Science 1981; 214:67–69.
51. Neal BR, Alibhai SK. Reproductive response of *Tatera leucogaster* (Rodentia) to

supplemental food and 6-methoxybenzoxazolinone in Zimbabwe J Zool (Lond) 1991; 223:469–473.
52. Whitten PL. Flowers, fertility and females. Am J Phys Anthropol 1983; 60:269–270.
53. Pohl H. Grenzen der synchronisation circadianer rhythmen durch licht bei vogeln. Die Vogelwarte 1988; 34:291–301.
54. Davis DE. Seasonal change in irradiance: a zeitgeber for circannual rhythms in ground squirrels. Comp Biochem Physiol 1991; 98A:241–243.
55. Cremer-Bartels G, Krause K, Mitoskas G, Brodersen D. Magnetic field of the earth as additional zeitgeber for endogenous rhythms? Naturwissenschaften 1984; 71:567–574.