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Long-Term Entrainment of Circannual Reproductive and Metabolic Cycles by Northern and Southern Hemisphere Photoperiods in Woodchucks (*Marmota monax*)[1]

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ABSTRACT

Woodchucks were exposed to simulated Northern Hemisphere (boreal) or Southern Hemisphere (austral) natural photoperiods in groups of 17 males and 17–18 females at 20–23°C for 69 mo and examined monthly. Photoentrainment of endogenous cycles was evaluated based on dates of peak body weight, peak testis volume, increased serum testosterone in males, and increased serum progesterone in females. Boreal photoperiods entrained and synchronized annual cycles in 15 of 17 males and in all 17 females; 2 males never entrained and free-ran at 9- to 11-mo intervals. Austral photoperiods phase-advanced cycles by approximately 6 mo after 2.5 yr in 34 of 35 animals. Four entrained males became refractory after 4 yr, free-running at 6- to 10-mo intervals. Photoentrained boreal animals became phase-advanced by 1 mo during the first 2 yr, and then had 12-mo cycles for 4 yr. In Year 5, on average, boreal cycles included initial testosterone elevations in mid-January (vs. mid-July in australs), parturition in early March (vs. early September in australs), and peak body weight in mid-July (vs. late January in australs). The results confirm that endogenous circannual cycles of woodchucks can be entrained and synchronized for 6 yr by daily changes in photoperiod similar to those of midnorthern latitudes, and can be re-entrained and phase-advanced 6 mo by photoperiods of midsouthern latitudes.

INTRODUCTION

In the woodchuck (*Marmot monax*), the breeding season lasts for 4–6 wk in late winter following early-winter recrudescence of gonads. Pregnancy lasts 32 days and pups are weaned after 6 wk, in late spring. Gonads become fully regressed in the summer or early autumn [1–4]. Body weight increases by 25–100% in the summer to reach peaks of 3–6 kg, and then declines by 25–50% during the autumn and winter [4–11]. The annual reproductive and metabolic cycles persist nearly intact in the laboratory under constant temperature conditions and window light, and in the absence of hibernation-inducing conditions [4]. In constant photoperiods, cycles persist intact for 1–3 yr, but animals become asynchronous and free-run after 2–3 yr [12].

Seasonal breeding in woodchucks and related sciurid rodents, as in other orders of mammals, appears to involve an endogenous circannual cycle in which both reproductive status and various aspects of metabolism change in a predictable manner [7, 9, 13–17]. These endogenous cycles free-run at intervals of 8–11 mo when not photoperiod-entrained and, in the wild, they are entrained to 12 mo by natural changes in photoperiod [11, 12, 18–27]. The existence of endogenous circannual cycles independent of environmental cues has been demonstrated in various species by free-running cycles of variable duration observed after ablation of the pineal or photosensory pathways, or during long-term maintenance in unchanging photoperiods [23, 25, 28]. They are likewise reflected in cycles of 9- to 12-mo duration in woodchucks and other sciurid rodents maintained in constant 12L:12D photoperiods [7, 12, 19, 27]. In woodchucks, entrainment by photoperiod was suggested by the approximately 6-mo phase advancements in the body-weight cycle observed in two animals 2 yr after transportation from the Northern to the Southern Hemisphere [29]. It was also suggested by the 6-mo advancement of the gonadal cycle in 30 animals after 2.3-yr exposure to simulated natural photoperiods of Southern versus Northern Hemispheres, as well as by the synchrony among animals maintained in the Northern Hemisphere photoperiods [11]. In the latter study, temperature was maintained in a narrow range, further suggesting that any role played by temperature under natural conditions is probably not a requirement for entrainment of the cycle. However, studies confirming entrainment for longer than 2–3 yr have not been reported.

Most studies on photoperiod regulation of circannual reproductive cycles in ruminants, equids, and laboratory rodents have involved use of block changes in photoperiod to perturb or entrain the normal cycle. Such studies have been used to demonstrate the stimulatory or inhibitory effects of continuous long-day (> 14 h of light per day) or short-day (< 10 h of light per day) photoperiods, as well as refractoriness to such effects when the photoperiod remains unchanged for several months. Frequent or daily changes in photoperiod have typically not been used experimentally, although they have been used in some studies in deer [30], sheep [21], dormice [18], ground squirrels [24], flying squirrels [31], and woodchucks [11].

In marmotine sciurid rodents, including woodchucks, the endogenous circannual cycles not only persist nearly intact in constant 12L:12D photoperiods for 1–3 yr before reverting to overtly free-running intervals of 9–10 mo [12, 15, 20]; they also show no response to block changes in photoperiod. For this reason, earlier studies had concluded that the endogenous cycle in these species is not entrained by photoperiod [13, 15, 19, 32, 33]. Our observations on woodchucks using daily changes in photoperiod simulating those of the Northern (boreal) and Southern (austral) hemispheres suggested that natural changes in photoperiod probably do entrain their circannual cycle. This conclusion was based on the fact that during the first 28 mo of study, austral animals experienced precocious seasonal changes each of the first 2 yr, resulting in a cycle that was phase-advanced by nearly 6 mo after 2.3 yr. However, such results do not preclude the possibility that the altered photoperiod disrupted rather than re-entrained the annual cycle, allowing the animals to free-run, potentially synchronously. Since

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woodchucks can free-run at 9- to 10-mo intervals [12] (unpublished results), it is possible that the animals, if disrupted, would free-run and appear to be phase-shifted by approximately 6 mo after 2–3 yr. The fact that one animal in the study had free-running cycles of 10- to 11-mo intervals when exposed to austral photoperiods [11] was also a matter of concern, as was the advancement of the breeding season from March to February in the boreal, control animals (unpublished results).

Unqualified confirmation of photoentrainment, re-entrainment, and phase advancement of the circannual cycle by simulated natural photoperiods in this species, which is not affected by block changes in photoperiod, would be important. It would suggest a potentially important role of daily changes in photoperiod versus day length per se in the photoentrainment of cycles in this and possibly other species under natural conditions. The austral versus boreal photoperiod study in woodchucks was therefore extended to 5.8 yr to confirm phase advancement and photoentrainment by daily changes in photoperiod, and to evaluate the incidence of any spontaneously occurring free-running cycles. Continued study also allowed for a more complete evaluation of possible persistent differences in adult body weight due to age, treatment, or sex. Further, it would be useful to know whether reproductive and metabolic cycles maintained their normal relationships long-term during both photoentrained and free-running cycles. It was also considered important to determine whether such lighting conditions would maintain reproductive synchrony and yield fertility levels to permit maintenance of woodchucks in closed colonies.

MATERIALS AND METHODS

Animals, Treatments, and Observations

The study was initiated in late June, 1989, using groups of 14.5-mo-old yearling males (n = 14) and females (n = 16), and 2.5-mo-old juvenile males (n = 20) and females (n = 19), all recently captured locally (42.5°N, 72.5°W). Groups of 7–10 animals of each age and sex were then housed in artificial Northern Hemisphere (boreal) or Southern Hemisphere (austral) photoperiods, as previously described [11], through March 1995. Results for the initial 2.3 yr of the 5.8-yr study have been previously reported [11]. Also reported were the details of animal housing, daily changes in photoperiod, and monthly reproductive examinations; measurements of food intake and body weight; assay of testosterone and progesterone; and details of breeding and fertility assessment [11]. Rooms were maintained at 20–24°C throughout the study. Programmable timers (Chrontrol Model D; Lindberg Industries, Inc., San Diego, CA) were used to adjust the daily light period ± 0 to 4 min per day (in whole-minute increments) and to yield yearly solstices of 15.8–16.3 h light (summer) or 8.5–9.8 h light (winter) on December 15–25 or June 15–25 (Fig. 1). Illumination in each room was provided by six 60-watt florescent bulbs (GE-F96T12-SP35-WW-3500K) located 1.5–2.5 m from the front of the cages or pen at the level of the woodchucks. Light intensity at cage or pen level was 600–700 lux as measured with a photometer with a 2π collector. Testis volume was estimated based on minimum and maximum diameters measured during scrotal or abdominal palpations [34], except that nonpalpable testes were assigned a minimum volume of 0.3 cm^3. Blood samples were obtained under ketamine-xylazine anesthesia [12]. Serum progesterone and testosterone were determined by solid-phase

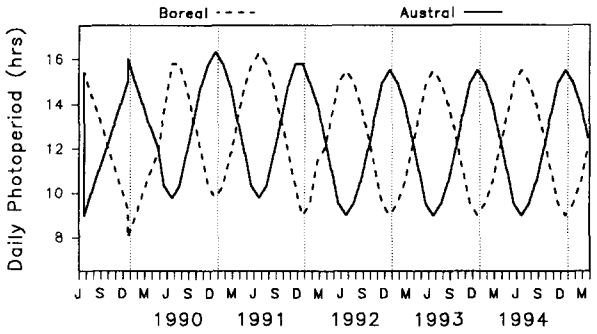

FIG. 1. Hours of light per day used to provide simulated Northern Hemisphere (boreal) and Southern Hemisphere (austral) photoperiods to groups of male and female woodchucks (*Marmota monax*).

RIA (Coat-A-Count; Diagnostic Products, Los Angeles, CA) without modification except for the use of testosterone standards prepared in serum from ovariectomized woodchucks [12]. Coefficients of variation within and between assays for testosterone and progesterone ranged from 4% to 12%. Animals were housed in same-sex pairs in 0.6-m^2 cages except for housing in mixed-sex groups in pens during the breeding season. Food intake was measured on a per cage basis [11]. The latter involved monthly weighing of feed daily for 3–4 consecutive days for each pair of animals and recording half the average daily change in food weight as the daily food intake per animal between Months 8 and 48 of study. Body weight was determined monthly at the time of blood sample collection. Food intake relative to body weight was estimated per cage using the combined food intake and combined body weight of the animals in the cage.

Breeding groups were established for all females in each photoperiod group during the breeding seasons of Years 2–6 of study (Table 1). Breeding management and husbandry did not differ between groups except for one breeding season (see *Results*). For 9 of the 10 breeding seasons, breeding groups were established 2 wk after 50% of the males had palpable scrotal testes. It involved housing groups of 2–3 females with one male in 1.5–2.0-m^2 pens and changing the male at 2- to 4-wk intervals until the end of the breeding season and/or until females were ascertained to be pregnant and removed from the pen. Breeding was delayed in the remaining breeding season (see *Results*). The breeding season was considered to have ended when testis regression was obvious or a female had shown no evidence of vulval swelling for ≥ 2 wk [2].

There were no seasonal changes or differences between groups in room temperature or humidity and no differences between groups in the schedule of room maintenance, exposure to building noise, or room air exchange rate. Males and females were housed in the same rooms when caged and in the same rooms when placed in breeding pens.

Statistics

Means are reported ± SEM. Data for 3 animals that had free-running cycles from the start of study were not included in the means and are reported separately. When it was observed that 6 other animals became distinctly asynchronous with others in the group later in the study, those data and those of the previous 3 mo were not included in group means and were also evaluated separately. Student's *t*-tests were used to determine differences between groups in peak body weights, testis volumes, or hormone concentrations or

TABLE 1. Timing of parturitions, testosterone elevations, and peak body weights in woodchucks maintained in austral vs. boreal photoperiods.

Variable	Year of study					
	1	2	3	4	5	6
Parturitions						
Austral	NA	Nov. 15 ± 4	Oct. 10 ± 4	Oct. 6 ± 1	Sep. 6 ± 4	Aug. 25 ± 1
Boreal	NA	Apr. 4 ± 3	Mar. 22 ± 1	Mar. 8 ± 2	Mar. 5 ± 2	Mar. 3 ± 4
Interval A-B[a]	—	4.7	5.4	5.0	6.0	6.2
Interval B-A[b]	—	6.2	6.5	6.0	5.7	—
Testosterone ≥ 0.3 ng/ml						
Austral	Dec. 10 ± 9	Sep. 18 ± 7	Aug. 13 ± 7	Jul. 12 ± 7	Jun. 28 ± 8	Jun. 24 ± 9
Boreal	Feb. 22 ± 4	Feb. 24 ± 5	Jan. 22 ± 5	Jan. 15 ± 6	Jan. 22 ± 5	Jan. 19 ± 0
Interval A-B[a]	2.4	5.2	5.3	6.1	6.9	6.8
Interval B-A[b]	6.9	5.7	5.7	5.4	5.1	—
Peak body weight						
Austral	Oct. 5 ± 10	May 1 ± 6	Mar. 8 ± 4	Feb. 12 ± 11	Jan. 29 ± 13	Jan. 2 ± 5
Boreal	Oct. 7 ± 9	Aug. 13 ± 4	Jul. 29 ± 3	Jul. 19 ± 4	Jul. 14 ± 5	Jul. 20 ± 5
Interval A-B[a]	0.1	3.4	4.7	5.1	5.5	6.5
Interval B-A[b]	6.8	6.8	6.5	6.3	5.7	—

[a] Interval (mo) from mean time in austral animals to mean time in boreal animals.
[b] Interval (mo) from mean time in boreal animals to mean time in austral animals.

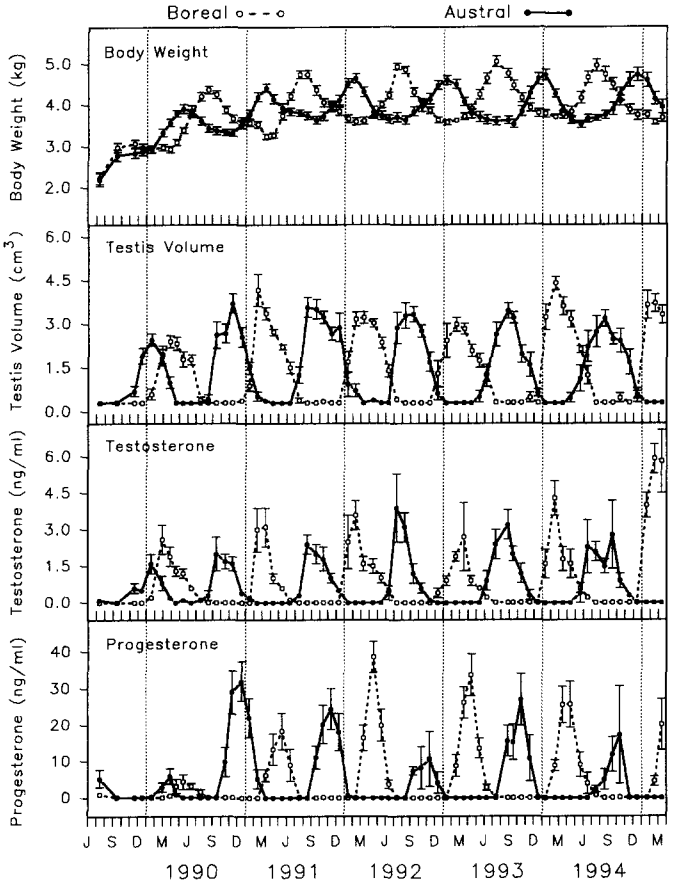

FIG. 2. Mean (± SEM) body weight, testis volume, serum testosterone of males, and serum progesterone of pregnant females for woodchucks maintained in experimental photoperiods simulating those of Northern (boreal) and Southern (austral) Hemisphere latitudes. The ranges in numbers of animals for each calendar year change due to losses of animals over time and removal of animals that were confirmed to free-run at 9- to 11-mo intervals. The number of animals per mean in 1990, 1992, and 1994 ranges from 30 to 34, 25 to 27, and 16 to 11, respectively, for body weight, and from 12 to 16, 8 to 12, and 6 to 8 for testis and testosterone.

in mean times of peak body weight, of increase in testis size ≥ 1 cm^3, of the initial rise in testosterone ≥ 0.3 ng/ml, of progesterone increases, and of parturition.

RESULTS

As evidenced by reproductive and body weight changes, the majority of males and females in both age groups not only were re-entrained and phase-shifted by the austral photoperiods but remained entrained throughout the 69 mo of study or until they died. However, failure or eventual loss of photoentrainment as evident in free-running cycles occurred in 3 of the 17 austral males and 2 of the 18 austral females. Similarly, the majority of the animals in the control boreal photoperiods remained entrained to the photoperiod and did not initiate free-running cycles. Free-running cycles were documented in 4 of the 17 boreal males but none of the 17 boreal females. The results for free-running animals were excluded from mean results from the start of study in 3 animals, and from 3 mo before obvious evidence of loss of entrainment in the others. Also reducing the number of animals per mean each year were 33 animals that died. These included, in Years 2, 3, 4, 5, and 6 of study, respectively, 5, 2, 2, 6, and 3 males and 3, 2, 1, 5, and 4 females. Most deaths involved cardiac infarction, aortic aneurisms, diaphragmatic hernia, or parasitic invasion of the central nervous system by *Baylisascaris procyonus*. The circannual cycle synchrony among animals within groups, excluding obvious free-running cycles, resulted in very distinct seasonal patterns of mean body weight, testis volume, serum testosterone, and serum progesterone in females that became pregnant (Fig. 2).

Body Weight

No consistent differences due to photoperiod treatment were observed in mature body weight as reflected in peaks and nadirs during the last 3 yr of study, but age at start of treatment appeared to affect sex differences in weight. Among animals captured as yearlings, mature peak body

weight was higher ($p < 0.05$) in males than in females in both the boreal (5.5 ± 0.2 vs. 4.8 ± 0.1 kg) and austral groups (5.4 ± 0.2 vs. 4.8 ± 0.1 kg) and was not affected by photoperiod. In contrast, in animals captured as juveniles, mature peak body weights in males were not larger than in females in either the boreal (4.9 ± 0.1 vs. 5.2 ± 0.1 kg) or austral group (4.6 ± 0.1 vs. 4.6 ± 0.1 kg).

Furthermore, in animals first treated as juveniles, austral photoperiods resulted in apparent reduced annual peaks in mean body weight. This was due to body weights in boreal females being larger ($p < 0.05$) than those of austral females in this age group (5.2 ± 0.1 vs. 4.6 ± 0.1). The latter differences among younger females appeared to account for all the differences in mature body weight caused by photoperiod.

Yearly phase shifts in peak body weight in austral compared to boreal animals are summarized along with those of reproductive parameters in Table 1. The intervals in the mean dates of peak body weight, including those for both groups during the last 4 yr of study (from April 1991 to March 1995), ranged from 4.7 to 6.8 mo and averaged 5.8 ± 0.2 mo (n = 8). The mean date of peak body weight during the last 4 yr was January 13 ± 4 days in austral animals and July 20 ± 2 days in borealis (n = 72), with the difference representing an advancement ($p < 0.05$) of 5.8 mo.

Testis Volume and Testosterone

There were no consistent differences between photoperiod groups in peak testis volumes or peak testosterone concentrations except for the advanced timing in the austral woodchucks. The annual initial increases and peak values for both parameters were advanced by about 6 mo in austral males in comparison to boreal males after 2 yr of treatment (Fig. 2). During the last 4 yr of study, mean dates on which testis volume increased to ≥ 1 cm^3 were July 16 ± 6 days in austral males versus January 15 ± 3 days in boreal males, and involved a phase shift of 6.0 mo. Intervals between successive mean dates for these initial increases in testis volumes for both groups combined ranged from 5.6 to 7.6 mo and averaged 6.0 ± 0.2 mo. During the last 4 yr of study, the mean date for the first increase in testosterone ≥ 0.3 ng/ml was July 17 ± 5 days (n = 42) in austral males and January 19 ± 2.7 days (n = 39) in boreal males, a mean advancement of 6.1 mo. The intervals between successive mean dates of testosterone increases ≥ 0.3 ng/ml for both groups ranged from 5.3 to 6.9 mo and averaged 5.9 ± 0.2 mo (Table 1).

During the last 4 yr, the intercycle interval in the boreal control animals remained 12 mo, with peak body weight in the last half of July and initial increases in testosterone in the last half of January (Table 1) being 1 mo earlier than in animals (other than 1-yr-olds) during the first 2 yr of study [11].

Progesterone and Pregnancies

There were no consistent differences between austral and boreal females in serum progesterone, except for the austral phase shift ($p < 0.05$) and lower mean values associated with fewer pregnancies in austral females in Year 3 of study (data not shown). For animals that became pregnant, progesterone profiles included elevated values during pregnancy and during lactation, and in some cases after lactation (Fig. 2). Progesterone was also seasonally elevated in females that did not become pregnant. Levels ≥ 1 ng/ml were

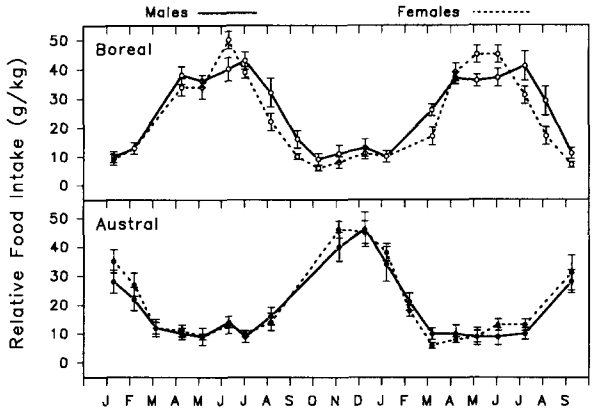

FIG. 3. Mean (± SEM) body weight and food intake relative to body weight in male and female woodchucks exposed to Northern Hemisphere (boreal) and Southern Hemisphere (austral) photoperiods.

observed for 1–4 mo in nonpregnant animals and for 1–5 mo in pregnant and postpartum animals. Progesterone peak values (4.9 ± 0.8 ng/ml, n = 80) in nonpregnant females were less ($p < 0.05$) than those seen during pregnancy or lactation (20 ± 2 ng/ml, n = 110). During the last 4 yr, the mean parturition date was September 21 ± 4 days in austral females and March 12 ± 2 days in boreal females, yielding a mean phase shift ($p < 0.05$) of 5.8 mo. During that time (April 1991-March 1995), the interval between successive mean dates of parturition for the two groups combined ranged from 5.0 to 6.4 mo and averaged 5.9 ± 0.2 mo (n = 8). There was a 1-mo phase advancement of parturition date in boreal females over the first 3 yr and maintenance of 12-mo intervals in the last 3 yr (Table 1).

Fertility

For the breeding season of Years 2 through 6 of study, fertility was confirmed based on pregnancies and birth of young. The pregnancy rates as a percentage of females paired with males were, in the last 4 yr, respectively, 87% (n = 15), 74% (n = 15), 92% (n = 12), and 46% (n = 11) in boreal animals and 77% (n = 17), 13% (n = 15), 60% (n = 15), and 50% (n = 12) in austral animals. Variation in fertility within the austral group appeared to be, in part, related to the timing of the pairing of males and females. During the third year of study, housing of austral females with males was delayed until 1.5 mo after 50% of males had scrotal testes. That 1-mo delay in housing as compared to that in other breeding seasons appeared to decrease the pregnancy rate (2 of 15, 13%) compared to the rate of the next breeding season in boreal (11 of 15, 74%) and austral (9 of 15, 60%) females. The October 6 mean parturition date was presumably later than it would have been if the animals had been placed with males earlier (Table 1).

Food Intake

Food intake results for the first 28 mo of study have been reported and discussed previously [11]. For Months 31–51 of study, reported here, there were no obvious differences in relative food intake between males and females of each group (Fig. 3). Relative food intake in each photoperiod group showed circannual changes relative to body weight that reflected periods of negative energy balance in later winter, during annual initial increases in food intake, and positive energy balance in summer, during annual initial decreases in food intake (Fig. 4). This resulted in circannual

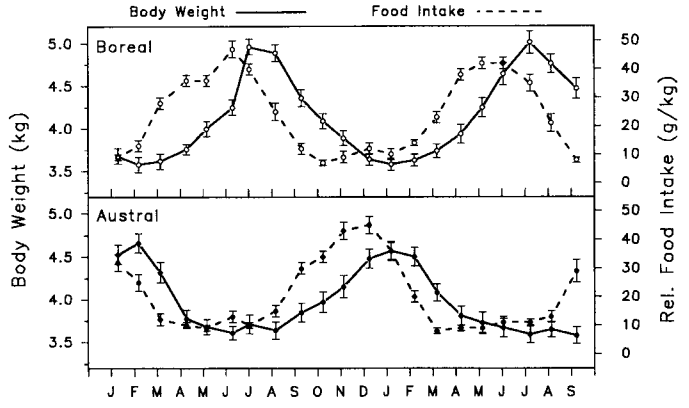

FIG. 4. Mean (± SEM) body weight and food intake relative to body weight in woodchucks maintained in austral versus boreal photoperiods.

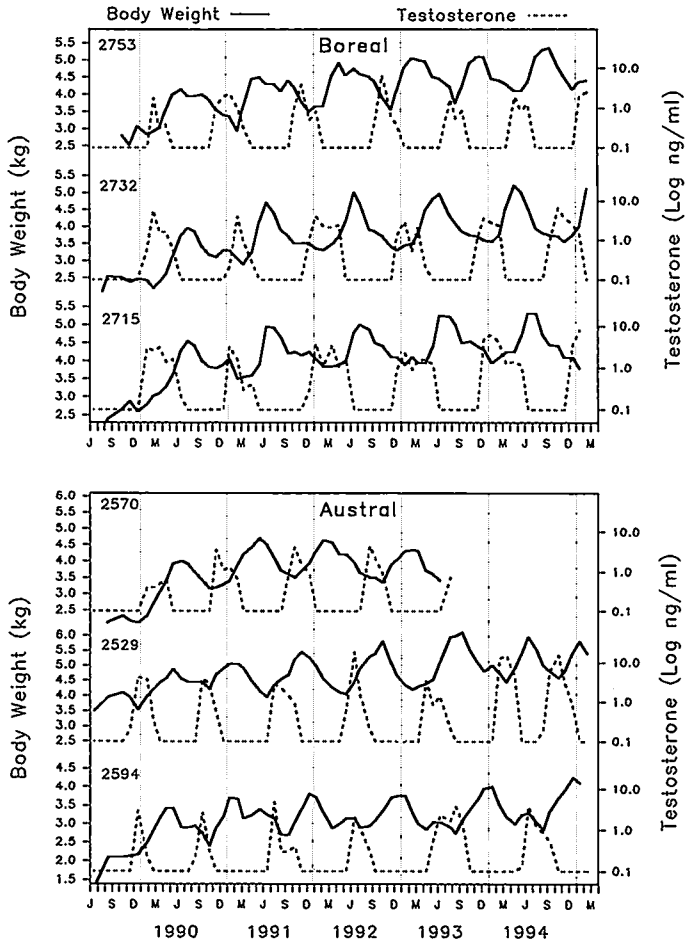

FIG. 5. Top panel. Serum testosterone concentrations and body weights of male woodchucks maintained in boreal photoperiods, including one male with free-running cycles throughout study (#2753), one with free-running cycles beginning after 4 yr of photoentrainment (#2732), and one that remained photoentrained throughout study (#2715). Bottom panel. Serum testosterone concentrations and body weights of male woodchucks maintained in austral photoperiods, including one with free-running cycles from birth (#2570), one observed to free-run after 2 yr of photoentrainment (#2529), and one that entrained and remained entrained to the austral photoperiod (#2594).

changes in the ratio of body weight to relative food intake that presumably reflect changes in energy expenditure and extent of conversion of caloric energy into adipose tissue versus heat and work (data not shown).

Free-Running Cycles and Photoentrainment Failure

Examination of testosterone and body weight profiles of individual males confirmed the occurrence of free-running cycles in four males maintained in boreal photoperiods and three males transferred to austral photoperiods (Fig. 5). Two boreal photoperiod males had free-running cycles throughout the study that were evident based on both serum testosterone and body weight when compared to cycles of photoentrained animals (#2753 in Fig. 5). They were both treated starting at 2.5 mo of age; one had five cycles within 4 yr, at repeated intervals of 10–11 mo, and the other had seven cycles in 5 yr, at repeated intervals of 9–11 mo, based on times that testosterone increased ≥ 1 ng/ml (#2753). Two other boreal males remained entrained to the boreal photoperiod for 4 yr, before having a 2–3-mo premature (October-November vs. January) rise in testosterone and recurrent intercycle intervals of 9–11 mo (#2753 in Fig. 5).

The three males that had free-running cycles in austral photoperiods included one that was studied first at 2.5 mo of age and from the start of study had intercycle intervals of 10–11 mo each (#2570). These cycles involved body weight and testosterone profiles (Fig. 5) nearly indistinguishable from those of a free-running male of the same age housed in boreal photoperiods until both were dropped from study after 4–4.5 yr. The other two males, first studied at 14.5 mo of age, entrained to the austral photoperiod and remained synchronous with others in the group until the fourth year of study. They each then had two abbreviated intercycle intervals of 8–9 mo and 6–10 mo, respectively, based on both serum testosterone and body weight (#2529 in Fig. 5).

Among females, there was no clear evidence for the occurrence of free-running cycles, based on breeding dates, progesterone, or body weight profiles, in either photoperiod group. Two possible exceptions were littermate austral photoperiod females, initially studied at 2.5 mo of age, that had two successive 11-mo intercycle intervals in peak body weight at the end of study.

DISCUSSION

The results of this study provide conclusive evidence that natural changes in daily photoperiod can not only entrain the endogenous cycle of a marmotine rodent to 1-yr intervals long-term, but can also phase-advance and re-entrain the cycles to the photoperiods of an austral latitude. The findings confirm and extend earlier observations suggesting such an entrainment by photoperiod. An approximately 6-mo phase shift was reported in woodchuck body weights 2.5 yr after transport to Australia [29]. Periods of simulated natural photoperiod (alternated with blocks of constant photoperiod) were also able to prevent free-running in ground squirrels for at least 3 yr [24]. The results of the first 28 mo of the present experiment also provided strong evidence for photoentrainment in the absence of changes in temperature [11]. However, the present observations of the absence of any entrainment in three animals, along with free-running cycles seen in all animals in neutral photoperiod [12], demonstrate that conclusions about entrainment to photoperiods that might be expected to advance cycles can be premature before 4 or more years, par-

ticularly in individual animals, or in studies using a small number of animals. The present results also demonstrate that constant dark and/or unchanging photoperiod during the period when the animals normally hibernate is not required to photoentrain the circannual cycle to 12 mo. Likewise, they confirm that photoperiod alone, in a constant-temperature environment, can entrain the cycle.

If the change in photoperiod had caused the disruption and/or termination of normal photoentrainment rather than re-entrainment, the subsequent occurrence of free-running cycles at intervals of 9–10 mo would result in animals that appeared to be 6-mo phase-shifted or -advanced after 2 or 3 yr. It is unlikely that such animals would phase-advance synchronously like the majority of photoresponsive animals in the present study. However, only by extending the study to 4 or more years could the response be confirmed to be one of photoentrainment and not one of entrainment-disruption followed by free-running. The photoentrainment results obtained with simulated natural photoperiods in this study, but not with block photoperiods [7, 13, 16], also raise questions about the appropriateness of using block changes in photoperiod as the standard approach to study the biology of photoentrainment in other species. That changes in photoperiod and not just absolute duration of photophase is important in providing cues has been demonstrated in other species including hamsters and voles [35–39].

The reasons some animals failed to entrain to either the austral or boreal photoperiod, or began to free-run after 1–4 yr of entrainment, are not clear. Of the initial 39 males, 3 showed no photoresponsiveness and were observed to free-run throughout the study. All 3 were started on the study at 2.5 mo of age. Their paternity varied, and their housing locations were not unique and did not differ in intensity of incident lighting. Such animals may be less responsive or nonresponsive to photoperiod cues because of a sensory, neural, or endocrine abnormality. Such nonresponsiveness may occur naturally in a percentage of individuals of the wild population. It would likely remove them from the breeding population and compromise their survival. Alternatively, it is possible that these animals were normal and capable of responding to photoperiod cues provided by sunlight but were not sensitive to the indoor lighting used to provide simulated natural photoperiods in the present study. The ambient light for this study was 600–700 lux, and thus only 1–20% of the intensity of natural daylight [40]. Photointensity appropriate or effective in other species varies, being as low as 1 lux or less for rats, which are a nocturnal species, and as high as approximately 1200 lux for humans [41]. The possibility that the light intensity used in the present study was near the threshold for woodchucks cannot be discounted. Lighting of 150 lux has been successfully used in photoperiod studies in European hamsters [42], and intensities of 350 lux have been successfully used with sheep [25, 43]. The lighting in our study was of sufficient intensity to entrain the majority of animals. However, three species of ground squirrels were observed to have a light intensity requirement of more than approximately 4000 lux, higher than that of most other species, for the acute suppression of nocturnal melatonin secretion [41].

The seasonal changes in the ratio of body weight and relative food intake reflected the underlying changes in efficiency of food conversion. However, data obtained from individual animals, and more frequently, would be needed to characterize the extent and time course of underlying endocrine and metabolic changes.

The observed changes in testis size, serum testosterone, and serum progesterone in pregnant and nonpregnant females were similar in both photoperiod groups and were similar to those previously observed in laboratory woodchucks [34, 44–47] and in animals in the wild [3, 34, 44, 48, 49]. The entrainment and re-entrainment of reproductive as well as metabolic parameters of the annual cycle, and the 1-mo long-term advancement of both parameters in boreal animals, suggest that the mechanisms of photoperiod entrainment for both aspects of the cycle are similar. They presumably involved central melatonin effects on pituitary secretion of gonadotropic hormones and metabolic hormones. There are no detailed reports of changes in gonadotropins in woodchucks. A preliminary report suggested that basal LH in male woodchucks was elevated at the time of initial elevations in testosterone, but not earlier [50]. A seasonal pattern of LH has been reported for gold-mantled squirrels, with elevations associated with the onset of the breeding season and declines at the end of the breeding season [51].

Our demonstration in laboratory woodchucks of photoentrainment by daily changes in photoperiod similar to those that occur in nature suggests that the circannual cycle in wild woodchucks is primarily entrained by photoperiod. However, there are differences between laboratory and wild woodchucks that can be considered in addition to restricted locomotion, forced interaction, and commercial diet imposed on laboratory animals. In the laboratory, the breeding season becomes progressively advanced by about 1 mo before stabilizing at 12-mo intervals, as seen in the boreal animals in the present study. These animals initially bred in March and gave birth in April, as in the wild [3, 5], but eventually bred in February and gave birth in March, with concomitant 1-mo advancements in the initial rise in testosterone, peak testosterone, and peak testis size. There was also a 1-mo advancement in the timing of peak body weight in adults, from late August, as in the wild [7], to late July. One reason for such advancements may be the absence of a normal hibernation as occurs for 4–6 mo in the wild from September to October until February to March [8, 32]. During that period, animals in the wild remain underground without food, and have frequent and prolonged bouts of hibernation torpor with an associated reduction in metabolic rate. In the laboratory, food remains available, bouts of hibernation-like torpor are less extensive (unpublished results), food intake declines but does not cease, body temperature cannot decline below room temperature, and overall metabolic rate is likely to be higher than in woodchucks hibernating in the wild. The 5–6 mo of constant darkness experienced in the wild during the hibernation period is not needed to entrain woodchucks to 12-mo cycles, as shown in the data for boreal animals in Years 3–6 of study. However, the associated hibernation may delay the rates and timing of reproduction and thyroid axis reactivation, simply due to the Q_{10} effect of low body temperature. Low temperature has been reported to phase-delay circannual rhythms in two species of ground squirrels [52, 53].

The absence of a normal hibernation depth and associated metabolic suppression might be expected to allow gonadal recrudescence to occur more rapidly in laboratory woodchucks, and for factors regulating seasonal increases in food intake to occur earlier, thus advancing the timing but not annual intervals of some parameters.

In conclusion, the results demonstrate that natural changes in photoperiod entrain the endogenous circannual cycle of woodchucks to 1 yr, independent of any effects of

temperature, and that such photoperiods can re-entrain the cycle to an austral latitude. However, the phase advancement of events by about 1 mo in laboratory-maintained animals compared to those in the wild suggests that other factors, such as the metabolic consequences of deep hibernation, may play a role in the specific timing of reproductive and metabolic changes within the 12-mo year. Whether failures to photoentrain and failures to remain photoentrained (documented in 9 of the 58 animals that survived to Year 4 of study or beyond) were due to the use of light intensities possibly marginal for this species, were the result of abnormalities in some animals, or were indicative of a wide spectrum of responses in normal animals was not determined.

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