

## Effect of Winter High Temperatures on Reproduction and Circannual Rhythms in Hibernating Ground Squirrels

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**Abstract** We tested whether prevention of hibernation in ground squirrels by midwinter exposure to high ambient temperatures influenced timing of the spring phase of reproductive maturation and the phase and period of subsequent circannual rhythms of reproduction and body mass. Exposing hibernating adult male *Spermophilus lateralis* to 30°C for 6 weeks beginning December 4 advanced the timing of testicular recrudescence by 4–5 weeks, compared to controls left at 4°C. Males exposed to 30°C for 6 weeks beginning at the average time of spontaneous end of hibernation (January 15) reached reproductive maturation at a time intermediate to those of controls and of the December 4 experimental group. However, neither the date of the subsequent fall's body mass peak, the date of the next year's reproductive maturation, nor the periods of circannual rhythms of body mass and reproduction differed among groups. Premature interruption of hibernation appears to allow early expression of reproduction, but does not affect the underlying timing mechanism.

Endogenous circannual rhythms, annual cycles in physiology and behavior that persist in the absence of seasonal cues, occur in a variety of long-lived animals living in arctic, temperate, and tropical environments (for reviews, see Pengelley, 1974; Mrosovsky, 1978; Gwinner, 1986; Zucker, 1988). Circannual rhythms were first described in the hibernation behavior of ground squirrels (Pengelley and Fisher, 1957, 1963; Pengelley and Asmundson, 1970); ground squirrels also show circannual cycles of feeding and drinking (Heller and Poulson, 1970; Kenagy, 1980), body mass (Pengelley et al., 1976), molt (Joy and Mrosovsky, 1982), and reproductive function (Heller and Poulson, 1970; Kenagy, 1980; Licht et al., 1982; Barnes, 1986). These cycles will generally maintain fixed internal phase relationships and free-run under constant conditions with a period shorter than 1 year (Gwinner, 1986).

Experimental attempts to entrain circannual rhythms in hibernators or to alter the phase relationships among rhythm components have met with limited success, at least when compared to similar experiments on circadian rhythms. Exposing ground squirrels to differences in the daily photocycle length (T cycles; Carmichael and Zucker, 1986); length of day, including constant dark (Pengelley et al., 1976); and temperature (Pengelley and Fisher, 1963) have not led to the identification of a simple, singular zeitgeber that will entrain circannual rhythms. However, treating ground squirrels with seasonal changes of photoperiod will synchronize circannual rhythms within groups (Lee and Zucker, in preparation), a long winter's exposure to low temperature phase-delays circannual cycles for 4.5 months (Mrosovsky, 1980a), and

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a combination of seasonal photoperiod and temperature cues apparently successfully re-entrained annual rhythms in woodchucks moved to a southern latitude and housed outdoors (Davis and Finnie, 1975). These experiments suggest that ground squirrels may be sensitive to such factors if they are presented in a seasonal fashion, rather than continuously. There remains an interest, therefore, in controlled laboratory experiments that can potentially allow one to distinguish specific exogenous factors that will phase-shift circannual cycles. Changing ambient temperature perhaps has had the most dramatic phase-shifting effect on circannual rhythms (Pengelley and Fisher, 1963; Mrosovsky, 1980a; Joy and Mrosovsky, 1983, 1985). Mrosovsky (1986) has proposed a model of thermal effects on circannual cycles, which predicts that increasing ambient temperatures in the spring are necessary for cycle persistence and that if low temperatures are maintained, cycles will be phase-delayed, with the result of increased period lengths or acyclicity.

The responses of hibernating animals to altered ambient temperatures must be interpreted cautiously, however, for the treatment will affect the body temperature pattern of animals during their heterothermic season, with unknown effects on the circannual clock, as well as potentially acting as a zeitgeber to entrain the rhythm. Low ambient temperatures that permit deep hibernation may also mask the expression of components of circannual rhythms that require a functional response. Gonadal maturation is a key feature of the spring phase of circannual rhythms in ground squirrels (Heller and Poulson, 1970; Kenagy, 1980; Barnes, 1986), yet *in vitro* and other experiments indicate that low hibernating body temperatures directly inhibit the secretion of hormones and gonadal growth and development (Barnes et al., 1986, 1987, 1988). Testes and pituitaries during hibernation will respond actively, however, when they are tested at high temperatures that are representative of euthermia (Goldman et al., 1986; Barnes et al., 1987). This suggests that the reproductive system could respond during hibernation, but that it is normally constrained from doing so by low body temperatures.

These considerations of circannual rhythms and temperature constraints lead to two alternative hypotheses: Reproduction occurs after hibernation in ground squirrels because it is inhibited from occurring earlier by the low body temperatures of torpor; or reproduction may follow hibernation as a result of a rigid, internal phase relationship between these two components of the circannual rhythm. To distinguish experimentally between these alternatives, we transferred groups of hibernating male golden-mantled ground squirrels (*Spermophilus lateralis*) in midwinter from their cold hibernacula temporarily into high ambient temperatures (30°C), and then monitored their reproductive development. The high ambient temperature arouses hibernating animals and prevents them from returning to a low body temperature, thereby prematurely terminating hibernation in the experimental groups compared to controls left in the cold. Such high ambient temperatures do not interfere with the expression of other components of circannual rhythms, such as eating, drinking, and body mass (Pengelley and Fisher, 1963; Pengelley et al., 1978). This experiment is an *in vivo* test of the previously mentioned *in vitro* results of a potential for reproductive responsiveness during hibernation if body temperatures are high. In addition, to determine whether high ambient temperatures would phase-advance the circannual rhythm, we monitored the timing of a second year's cycles of body mass and reproduction. We considered the following possible outcomes:

1. Prematurely interrupting hibernation will have no effect on the timing of reproductive maturation in the first spring, which will take place at the same time in each group. Furthermore, the timing of the second year's cycles of weight gain and reproduction will be the same

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among groups. This outcome would argue for a circannual clock that strictly schedules the period and phase relationships among cycles of body mass, hibernation, and reproductive maturation, and is not sensitive to phase shifting by high temperatures in winter.

2. Prematurely interrupting hibernation will advance the time of reproductive maturation in the first spring but not in the second, which would suggest that exposure to high temperatures lifts the constraint of low body temperatures on maturation, allowing it to occur earlier, but does not influence the circannual clock.

3. Reproductive maturation may be phase-shifted in the experimental group in both the first and second spring, which would imply that the transition to high ambient temperature is a potential zeitgeber of circannual rhythms.

## METHODS

### *ANIMALS AND GENERAL CARE*

Adult male golden-mantled ground squirrels were live-trapped in May 1987 in Sierra and Nevada Counties near Truckee, California. After 2 weeks in quarantine in a light-dark cycle (LD 14:10) at 20°C, animals were transported by air to Fairbanks, Alaska, where they were housed in LD 16:8 at 17°C. Over the next 3 months, daylength was shortened weekly to mimic natural daylength at the latitude of capture. Animals were kept in individual metal cages (21 × 25 × 20 cm) with cotton batting and burlap cloth for bedding. Food (Purina Rodent Chow and Mouse Chow) and water were provided *ad libitum*, and carrot slices were given twice weekly. Animals with maloccluded incisors had their teeth trimmed regularly and were given chow in water as mush.

### *EXPERIMENTAL TREATMENTS*

On September 24, 1987, all animals were transferred to an environmental chamber held at 4° ± 0.5°C and LD 12:12. Daylength continued to be shortened weekly until LD 8:16 was reached on October 31; this photocycle was maintained throughout the rest of the experiment. In early December 1987, after they had clearly entered the weight loss phase, animals were matched for body mass and divided into four experimental groups. Group 1 ( $n = 8$ ) remained at 4°C through winter and spring 1987–1988; group 2 ( $n = 9$ ) was exposed to 30°C for 6 weeks beginning December 4, 1987, and then returned to 4°C on January 15, 1988; group 3 ( $n = 11$ ) was exposed to 30°C for 6 weeks beginning January 15, 1988, and returned to 4°C on March 2, 1988; group 4 ( $n = 19$ ) remained at 4°C throughout the experiment. Groups 1, 2, and 3 were moved to 18°C on May 15, 1988. Group size reflects the number of animals at the beginning of the experiment; reductions reflect deaths or animals that did not hibernate (two animals in group 4) and therefore could not be used to calculate onset or end of hibernation or percentage of weight gained after hibernation.

### *HIBERNATION AND SCROTAL PIGMENTATION*

Animals were checked daily for hibernation by placement of sawdust on the dorsum of torpid animals; sawdust is displaced during an arousal from hibernation, and its presence or

absence each day indicates whether animals have remained continuously torpid or have aroused since the last check (Pengelley and Fisher, 1961). The hibernation season was defined to begin with the first day of a multiday bout of torpor and to end with the termination of torpor. Incidence of shallow torpor in animals held at 18°C was not monitored. Body mass and reproductive condition (testicular enlargement and descent and extent of scrotal pigmentation) were recorded every 2 weeks. Testes were palpated and described in size as small, medium, or large. Scrotal pigmentation was classified into one of four stages as follows: (1) no pigment visible; (2) slight pigment visible anterior to anus; (3) scrotum pigmented to halfway between anus and penis; (4) scrotum fully pigmented from anus to penis (Barnes et al., 1988). Scrotal pigmentation was used to assess the progress of testicular recrudescence. Measurements of testis size from 86 laparotomies (see below) performed during testis growth were compared to the scrotal pigmentation index at the time of laparotomy. The correlation of testis size and pigment index was significant ( $r = 0.72$ ,  $p < 0.001$ ); thus, the progress of scrotal pigmentation parallels increasing testis mass (see also Barnes et al., 1988). After peaking in mass, testes involute rapidly, leaving sperm in the enlarged epididymides. The scrotum remains pigmented for several weeks after testes have regressed.

#### LAPAROTOMY AND BIOPSY

To determine more precisely reproductive state in males that had ended hibernation, animals were periodically anesthetized with methoxyflurane (Metofane, Pitman-Moore, Inc., Washington Crossing, NJ) and laparotomized, and the length and width of one testis were measured. Paired testis mass was estimated using the regression equation for a ground squirrel given in Kenagy (1979). A biopsy of testis tissue was obtained by puncturing the testis with a 20-g needle and cutting off approximately 1 mm<sup>3</sup> of the tissue that was exuded. Alternate testes were sampled on successive biopsies; biopsy does not appear to interfere with normal gonadal growth and regression (Barnes et al., 1986). Biopsied tissue was preserved in Bouin's solution, dehydrated in 70% ethanol, and embedded using JB-4 Embedding Kits (Polysciences, Inc., Warrington, PA). Plastic blocks were sectioned at 3 µm using glass knives, and stained with hematoxylin and eosin. Sections were scored for spermatogenic state as in Barnes et al. (1986).

#### DATA ANALYSIS

Periods of circannual cycles in body mass were calculated as the number of days between yearly body mass peaks. Peaks were identified using three methods: (1) The date of the highest body mass was measured; (2) three-point running averages were computed, and the date of the highest average was chosen; (3) a curve was hand-fitted to the averaged data, and the date of the peak was determined from the highest point of that curve. These three methods were used on all body mass records. There were no significant differences in period lengths calculated using the three methods within groups, nor did outcomes comparing groups differ depending on the method used. The data reported were derived using method 3, since it yielded the median period in three of four groups and the smallest standard deviation (*SD*) when the three methods were compared for all groups.

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Nine noncycling animals in group 4 (see "Results") have been excluded from analyses that sought to identify differences among treatment groups in circannual period and in the timing of subsequent body mass peaks and reproduction.

A microcomputer statistics package, Microstat, was used for correlations, analyses of variance, Duncan's multiple-range test, and one-tailed *t* tests. Values are reported as means  $\pm$  *SD*.

## RESULTS

Animals that were forced to terminate hibernation prematurely by exposure to 30°C in early December 1987 (group 2) achieved full scrotal pigmentation on January 30, 1988, 4–5 weeks earlier ( $p < 0.02$ ) than did control animals (groups 1 and 4) that remained at 4°C and ended hibernation spontaneously (Table 1 and Fig. 1). Animals (group 3) exposed to 30°C for 6 weeks beginning January 15, 1988, the average date of spontaneous hibernation end in groups 1 and 4, became fully pigmented at a time intermediate between groups 1 and 2. Despite these differences in the timing of scrotal pigmentation in the first spring that followed experimental manipulations, there were no significant differences among groups in the timing or length of subsequent rhythms of body mass and scrotal pigment (Table 1).

Nine of 19 males that remained at 4°C from September 1987 on (group 4) did not undergo complete gonadal regression after recrudescence in spring 1988 (testes continuously medium or large and spermatogenic, and scrotal pigment  $\geq$  stage 3). These animals also stopped cycling in body mass (Fig. 2).

Early acquisition of scrotal pigment in group 2 reflected early reproductive maturation. Histological examination of biopsies from five group 2 males obtained on February 1, 1988 revealed all five to have tailed spermatozoa within their testes; examination of tissue taken from seven group 3 males within the same week revealed only spermatocytes and round spermatids. The early maturation in group 2 males was not accompanied by earlier gonadal regression (dates of regression in 1988 were May 31  $\pm$  29 days, May 29  $\pm$  20 days, June 5  $\pm$  23 days, and June 11  $\pm$  22 days for groups 1, 2, 3, and 4, respectively); thus, duration of reproductive function was longer in group 2 animals compared to other animals ( $p < 0.03$ ). After being moved to high temperatures and ending hibernation, however, group 2 males took longer to achieve full pigmentation than did group 3 males (57  $\pm$  27 days vs. 26  $\pm$  10 days;  $p < 0.006$ ).

Ground squirrels moved from 4° to 30°C in December transiently increased in body mass by over 20% while they were in the warm (Fig. 1, group 2). Males exposed to warm conditions beginning January 15 and males spontaneously ending hibernation also subsequently gained weight, but the increase was less (6–10%; Table 2). When squirrels first showed full scrotal pigmentation, their weights were not significantly different among groups (group 1, 241  $\pm$  36 g; group 2, 230  $\pm$  29 g; group 3, 235  $\pm$  34 g; group 4, 217  $\pm$  15 g).

The period of the circannual cycle in body mass was significantly longer than was the circannual period of acquisition of scrotal pigment (421  $\pm$  27 days vs. 371  $\pm$  45 days;  $p < 0.001$ ;  $n = 34$ ) in all groups. Six group 4 animals hibernated in both years. The circannual period of hibernation onset in this group was intermediate between the periods of body mass and reproduction cycles (389  $\pm$  35 days, Table 1).

TABLE 1. Dates ( $\pm$  SD) of Body Mass Peaks, Hibernation and Scrotal Pigmentation (Reproduction), and Period Lengths (in Days) of Body Mass, Reproduction, and Hibernation Cycles of Golden-Mantled Ground Squirrels

	Group 1 ( $n = 8$ )	Group 2 ( $n = 7-9$ )	Group 3 ( $n = 8-9$ )	Group 4 ( $n = 8-10$ )
Body mass peak I, 1987	August 22 $\pm$ 10	August 23 $\pm$ 12	September 7 $\pm$ 16	August 29 $\pm$ 9
Hibernation began, 1987	October 16 $\pm$ 31	October 13 $\pm$ 19	October 13 $\pm$ 24	October 11 $\pm$ 13
Hibernation ended, 1988	January 15 $\pm$ 8 (Spontaneous end)	December 4 (Forced end)	January 15 (Forced end)	January 26 $\pm$ 24 (Spontaneous end)
Pigmented scrotum (reproduction I) began, 1988	February 28 <sup>a,b</sup> $\pm$ 8 (Groups 1, 2, 3, moved to 18°C on May 15, 1988)	January 30 <sup>c</sup> $\pm$ 28	February 10 <sup>b,c</sup> $\pm$ 10	March 9 <sup>a</sup> $\pm$ 37
Body mass peak II, 1988	October 24 $\pm$ 26	October 24 $\pm$ 30	November 3 $\pm$ 23	October 12 $\pm$ 31
Pigmented scrotum (reproduction II) began, 1989	February 17 $\pm$ 28	February 18 $\pm$ 37	February 28 $\pm$ 33	February 28 $\pm$ 32
Circannual period of body mass	428 $\pm$ 24	426 $\pm$ 35	423 $\pm$ 22	412 $\pm$ 27
Circannual period of reproduction	358* $\pm$ 31	390* $\pm$ 45	385* $\pm$ 36	375* $\pm$ 29
Circannual period of hibernation ( $n = 6$ )				389 $\pm$ 35

Note. Animals were kept in LD 8:16, and at 4°C except where otherwise specified. Group 1 and group 4 animals spontaneously ended hibernation in mid-January; group 2 animals ended hibernation on December 4, 1987, after transfer to 30°C; group 3 animals ended hibernation on January 15, 1988, after transfer to 30°C. Groups 1, 2, and 3 were transferred to 18°C in mid-May 1988. Dates of body mass peaks reported were derived using method 3 (see "Data Analysis").

<sup>a,b,c</sup>Dates with different superscripts differed significantly ( $p < 0.05$ ) among groups.

\* The annual period of reproduction was significantly shorter than the annual period of body mass in all four groups.

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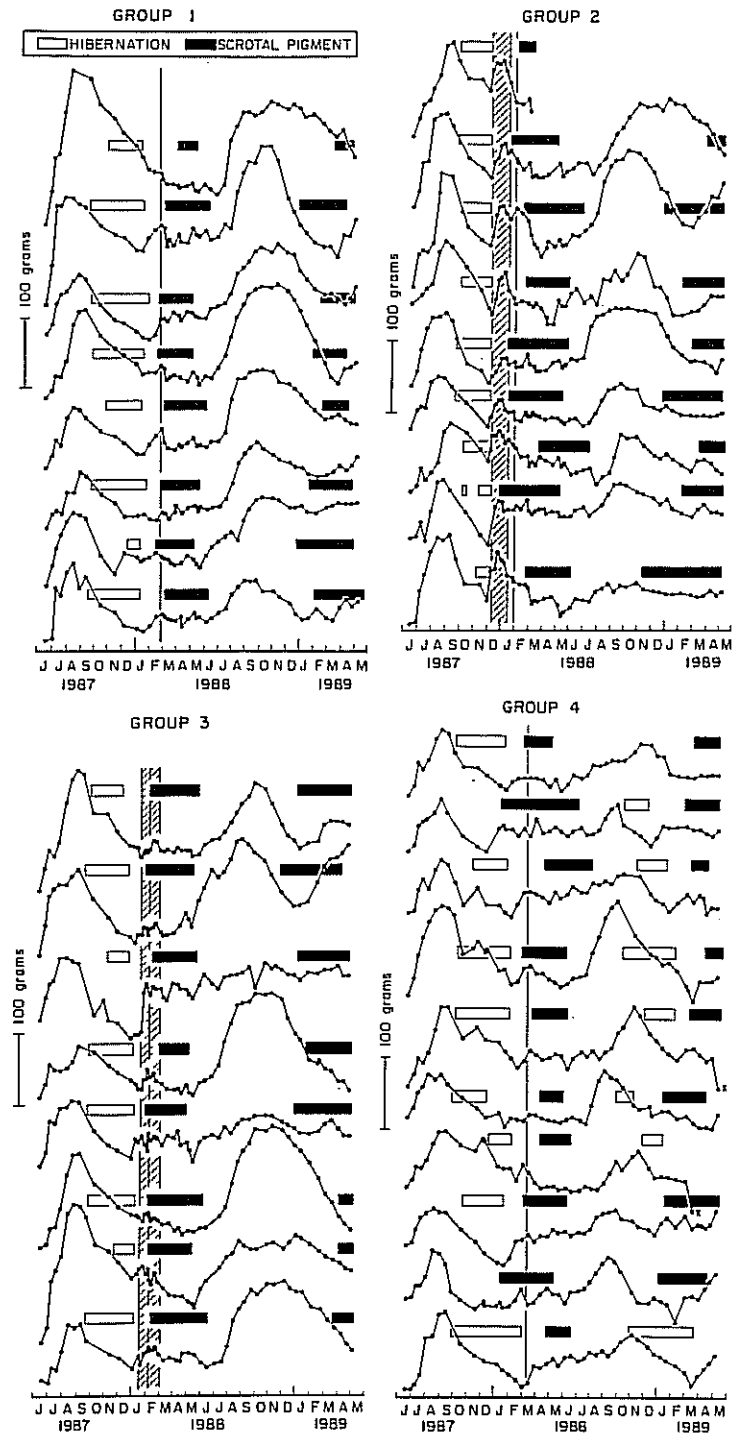


FIGURE 1. Individual plots of body mass (lines), hibernation season (open bars), and reproduction (period when testes had descended into a stage 4 fully pigmented scrotum; black bars) for groups 1-4. Vertical lines represent the average date of complete scrotal pigmentation for each group. Hatched areas in groups 2 and 3 indicate 6 weeks of exposure to 30°C.

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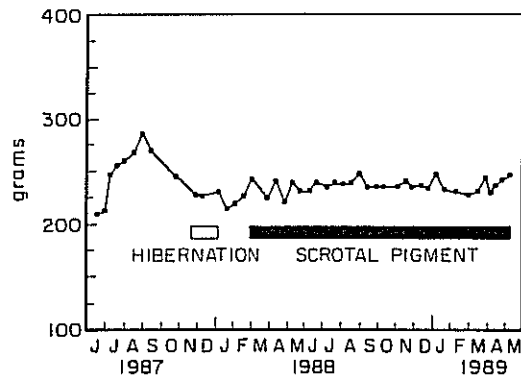


FIGURE 2. Individual plot of a representative group 4 animal, which ceased circannual cycles of reproduction and body mass when held at 4°C. Conventions as in Figure 1.

DISCUSSION

Premature termination of hibernation in male golden-mantled ground squirrels by experimental exposure to a high ambient temperature resulted in early expression of reproductive maturation. However, subsequent phasing and periods of circannual rhythms of reproduction and body mass were similar in all groups with and without warm treatment. The effects of early interruption of hibernation bear on several important aspects of the control over reproductive function and the nature of endogenous circannual rhythms in hibernating ground squirrels.

*INHIBITION OF REPRODUCTIVE MATURATION BY LOW BODY TEMPERATURES*

Since low tissue temperatures inhibit pituitary and testicular responsiveness to reproductive hormones in hibernating hamsters and ground squirrels (Goldman et al., 1986; Barnes et al., 1987), reproductive maturation may be held in check during hibernation by low body

TABLE 2. Mean Weight Gain and Percentage of Increase in Body Mass at End of Hibernation Represented by That Weight Gain of Ground Squirrels during the 6 Weeks after Ending Hibernation

Group	Weight gain (g) (mean ± SD)	% increase
1 (n = 8)	11.3 ± 19.5	5.8 ± 8.5
2 (n = 8)	46.0 ± 10.7*	23.0 ± 4.5*
3 (n = 7)	12.6 ± 8.6	7.8 ± 4.6
4 (n = 8)	20.6 ± 9.8	10.5 ± 5.7

Note. Groups 1 and 4 ended hibernation spontaneously while at 4°C; group 2 ended hibernation after transfer to 30°C beginning December 4; and group 3 ended hibernation after transfer to 30°C on January 15.

\* Significantly different ( $p < 0.001$ ) from groups 1, 3, and 4.

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temperatures, and may occur only after a sustained return to high body temperature lifts the low temperature constraint. Our new data suggest that there is a potential for male reproductive maturation to occur throughout a window of time that begins during hibernation and ends after hibernation is terminated. When this window opens is not clear; however, males transferred to 30°C in early December took 1 month longer to become mature after ending hibernation than did males transferred to 30°C in mid-January. This difference was mainly due to a delay in when most of the December group began to respond, rather than a difference in testis growth rate, suggesting that the ability to respond to high temperatures with reproductive maturation began between December and January, during the last month of hibernation. Two previous observations in golden-mantled ground squirrels may be relevant to this suggestion. Reproductive maturation occurs approximately 1 month earlier in animals that fail to hibernate than in those that do hibernate (Barnes et al., 1986), and an increase in plasma levels of gonadotropins and testosterone occurs during arousals in the last month of hibernation (Barnes et al., 1988). These results suggest a spontaneous increase in the activity of the pituitary-gonadal axis (which may reflect activation of the hypothalamic centers that regulate reproduction) that occurs in the weeks before hibernation ends.

### *HIGH AMBIENT TEMPERATURE AS A DISCRETE ZEITGEBER*

Ambient temperatures, particularly during the winter and spring phases of annual cycles in ground squirrels, have been previously shown to phase-shift circannual rhythms. Transferring golden-mantled ground squirrels from high to low temperatures in spring, but not in fall or winter, phase-advanced the timing of their subsequent hibernation (Pengelley and Fisher, 1963). Prolonging winter low temperatures into the spring delays the timing of the next fall's body mass peak in golden-mantled and 13-lined ground squirrels (*S. tridecemlineatus*), perhaps even indefinitely in animals that become acyclic (Mrosovsky, 1980a; Joy and Mrosovsky, 1983, 1985). The present experiment tested the ability of high temperatures presented during hibernation to phase-advance the circannual clock. Although the spring phase of reproduction was temporarily advanced in warm-treated groups, by the second year there were no significant differences in circannual phase or period among groups, indicating that no apparent phase shift had occurred. The similarity in second-year rhythms among groups also suggests that the transfer of groups 1, 2, and 3 into 18°C in mid-May 1988 did not entrain these animals, compared to those group 4 males left at 4°C that continued to show cycles in body mass and reproduction. Thus, these results are inconsistent with a role for ambient temperatures increasing near the end of hibernation in entraining circannual cycles (Mrosovsky, 1986). This conclusion is contingent on the ability of biweekly data collection to discern a 1-month phase advance in the second year. In addition, a treatment temperature of 30°C, which was chosen to ensure that animals could not become hypothermic, may not be relevant as a zeitgeber to golden-mantled ground squirrels in winter.

The phenomenon of the nine group 4 males that became arrested in the spring phase of reproduction and at low and even body mass ("cold-hold") has once previously been reported in this species (Mrosovsky, 1978), and three times described in *S. tridecemlineatus* (Wells and Zalesky, 1940; Joy and Mrosovsky, 1985; Ambid and Berges, 1986). The fact that approximately half of the males left at 4°C apparently stopped cycling in both reproduction and body mass, whereas no animals from other groups failed to cycle, lends support to Mrosovsky's (1986) model for the persistence of circannual rhythms, which requires temperatures

that are above a declining threshold of ambient temperature in order to break the spring phase and resume the cycles of reproduction, weight change, and hibernation. Males in groups 1, 2, and 3 presumably experienced temperatures above this threshold when they were transferred to 18°C in May 1988, whereas 4°C must have been a sufficiently high temperature for the 10 males in group 4 that did cycle, but not for the other 9, reflecting individual variability in threshold temperature levels. A temporary increase in ambient temperature (as in this experiment) may be involved in the persistence, but not the entrainment, of circannual rhythms in ground squirrels.

#### *BODY MASS SET POINT IN HIGH AMBIENT TEMPERATURES*

Body mass set point theory posits that hibernators defend a seasonally appropriate body mass against perturbation (Heller and Poulson, 1970; Mrosovsky and Fisher, 1970; Dark et al., 1986). In the present experiment, animals that experienced warm conditions in December but not in January showed a large but temporary weight gain, followed by a return to normal seasonal weight loss. However, when ground squirrels first showed full scrotal pigmentation, there was no difference in body mass among groups, suggesting that there is a seasonally appropriate body mass reached by all animals at the initiation of reproduction. When hibernation was ended prematurely, group 2 males apparently reset their set point in order to gain weight to achieve that appropriate body mass in late winter rather than early spring.

#### *CIRCANNUAL PERIODS ( $\tau$ )*

The circannual periods of body mass, reproductive function, and hibernation measured in males in the present experiment were notably longer than circannual  $\tau$ 's reported previously in this species. The average  $\tau$  of the circannual cycle of body mass, compiled from 10 separate studies of golden-mantled ground squirrels held in constant conditions (Heller and Poulson, 1970; Pengelley et al., 1976; Kenagy, 1980; Mrosovsky, 1980a,b; Zucker and Boshes, 1982; Joy and Mrosovsky, 1983; Zucker et al., 1983; Zucker, 1985; Carmichael and Zucker, 1986), is  $320 \pm 32$  days (first year  $317 \pm 32$  days, subsequent years  $338 \pm 29$  days). Animals in the present study also spontaneously ended hibernation earlier than might have been expected. The reasons for the longer  $\tau$ 's ( $421 \pm 27$  days) and early end to hibernation in the present study are not known, but could relate to the age or sex of our animals: adult males, trapped in spring. Most animals used in other studies summarized here have been female young of the year ( $n = 86$  females, 33 males all laboratory-born or trapped in the fall). Circannual period length and ambient temperature are predicted to be inversely related (Mrosovsky, 1986), which should have resulted in longer  $\tau$ 's in animals kept at 4°C (group 4) than in animals in other groups that experienced 30°C and 18°C; instead, there were no significant differences among groups.

#### *ADAPTIVE SIGNIFICANCE OF A FLEXIBLE RESPONSE*

Since female ground squirrels advance the timing of their emergence from hibernation and their sexual receptivity in years of early, warm springs (Shaw, 1925; Bronson, 1979; Michener, 1984), male ground squirrels should also respond to early springs with early emergence and sexual maturity, in order to maximize their potential for successful matings. That hibernating

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males respond to elevated temperatures by early reproductive development is consistent with a need for some flexibility in circannual timing in response to field conditions. An early spring in one year, though, is not predictive of springs in subsequent years, and thus it would not be advantageous to have permanently reset the clock.

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