A study of hibernation in the western jumping mouse, Zapus princeps
by Samuel Neil Luoma

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in Zoology
Montana State University

Abstract:
A field and laboratory study of hibernation in Zapus prinaeps was conducted. Males gained weight-more gradually. and reached a higher weight peak than female Z. prinaeps. Hibernation was preceded in all animals by a period of lethargy and weight loss during which body temperatures were very labile. Torpor was easily induced during the, summer by removing either food or water from Zapus at any temperatures below 220, Arousal from induced torpor during the summer. did not occur at temperatures below TOC,. probably due to a lack of a sufficient amount of brown fat. Animals kept at constant temperature (3±1C) in 24 hours of light per day hibernated two to three weeks later than animals caged outside. The characteristics of hibernation in Z, prinaeps appear to place it between the larger obligatory hiber-nators and the- smaller mammals that hibernate. .

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A STUDY OF HIBERNATION IN THE WESTERN JUMPING MOUSE, *ZAPUS PRINCEPS*

by 7B

SAMUEL NEIL LUOMA

A thesis submitted to the Graduate Faculty in partial
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in

Zoology

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ABSTRACT

A field and laboratory study of hibernation in *Zapus princeps* was conducted. Males gained weight more gradually and reached a higher weight peak than female *Z. princeps*. Hibernation was preceded in all animals by a period of lethargy and weight loss during which body temperatures were very labile. Torpor was easily induced during the summer by removing either food or water from *Zapus* at any temperatures below 22C. Arousal from induced torpor during the summer did not occur at temperatures below 10C, probably due to a lack of a sufficient amount of brown fat. Animals kept at constant temperature (3±1C) in 24 hours of light per day hibernated two to three weeks later than animals caged outside. The characteristics of hibernation in *Z. princeps* appear to place it between the larger obligatory hibernators and the smaller mammals that hibernate.

INTRODUCTION

Although the body of literature on hibernation is substantial, little work has been done on hibernation in the western jumping mouse, *Zapus princeps*. Quimby (1951) and Whitaker (1963) have done thorough ecological studies of *Zapus hudsonius*. Both considered the phenomenon of hibernation. Morrison and Ryser (1962) studied metabolism and body temperature during hibernation in *Z. hudsonius*. Brown (1967) related seasonal activity and gonadal activity in *Z. princeps*.

The purpose of this investigation was to study preparation for hibernation and hibernation in *Z. princeps*. Preparation for hibernation in this species appeared to involve a period of weight gain followed by a shorter period of weight loss. A phase of short periods of torpor preceded extended hibernation. Some of the significant physiological variables affecting these steps were studied. The ecological and evolutionary relationships of the hibernating physiology in western jumping mice were also considered.

METHODS AND MATERIALS

Thirty *Zapus princeps* were trapped with Sherman live traps at altitudes of 5000, 6000, 6500 or 7500 feet in the Bridger Mountains (lat. 45 45', long. 111 00') northeast of Bozeman, Montana. After July 1, mice were weighed when captured. Those weighing less than 18 grams were considered immature. Animals were marked by toe clipping.

Three animals were housed in each of three wire mesh cages 2 x 4 x 1½ feet which contained one to three inches of loose dirt and sod on the floors. These mice were captured in early June and early July. One cage was placed in the Bridger Mountains at an altitude of 7500 feet in the same meadow in which its inhabitants were trapped. The two other cages were placed at the base of the Bridger Mountains at an altitude of 5000 feet. One of these contained two mice trapped at 5000 feet and one, an immature mouse, trapped at 6500 feet. The three mice in the other cage were trapped at 7500 feet. All cages were placed in a shady area near water in the same type of area *Z. princeps* seems to prefer. The animals were given water and food (rolled oats and Ralston-Purina mouse pellets) "ad libitum" and long stems of grass in seed. From July 14 to September 10 all nine animals were weighed every other day, to the nearest 0.1 gm. on an Ohaus triple beam balance. After August 14, mice in the low-area cages were usually observed twice daily. In order to determine time of arousal from torpor, four or five observations per day were made. Microenvironmental temperatures were determined by inserting a general purpose thermistor into the nest of a *Zapus* and recording temperature with a Rustrak dual-channeled temperature recorder. Body temperatures in the field were taken using a small animal probe inserted 10-15 mm rectally and a Yellow Springs telethermometer. Access to records from a max-min thermometer located one-quarter mile from the low-area cages allowed ambient air temperature observations. Sex was verified by dissection in all cases.

Laboratory experiments involving constant light were conducted in an International refrigerator illuminated by a 15 watt light bulb at temperatures of 3±1C. Studies on the effects of cold or removal of food or water were conducted in a walk-in cooler (temperature 3±1C). These mice were placed in cages under a table over which heavy blankets were draped to help subdue disturbing noises and to provide a means of controlling photoperiod. A loud, constantly operating fan suppressed disturbances by providing a background noise. All mice studied in the laboratory were caged individually.

For continuous temperature readings, such as during arousals from or entrance into torpor, a small animal probe was taped to a mouse's tail after rectal insertion, and temperatures were recorded on a Rustrak dual-channeled temperature recorder. To facilitate a a study of anterior-posterior temperature differences, a small hole was cut in the skin on the back of a hibernating animal, through which a banjo type, flat surface thermistor was inserted to the interscapular area. Rectal and interscapular temperatures were then simultaneously recorded. Electrocardiograms were recorded by pin electrodes on an E&M Instrument Company Physiograph.

To prevent unnecessary disturbance during studies of arousal from torpor, the animals were either placed in a semi-darkened styrofoam cooler or under an overturned cooler. A small crack was left for observations. The darkened conditions seemed to relax the mice enough to prevent loss or leads of temperature probes due to either excess movement or chewing.

Mice were killed by cervical dislocation. Brown fat and white fat were removed by dissection and weighed to the nearest 0.1 mg. on a Mettler precision balance.

In laboratory studies of torpid animals, unobserved arousals were determined by placing a pile of sawdust on the animal's back as suggested by Pengelley (1961).

In cases where statistical tests were used an F distribution (Lentner, 1968) was employed to test the differences of the means.

RESULTS

Trapping

Trapping of *Zapus princeps* to determine time of spring arousal was begun on May 16 at 6000 feet. The first *Z. princeps* was caught on May 18. On May 22 trapping was begun at 7000 and 7500 feet. The first mouse was caught in this area on May 26. There were patches of snow only in the higher region.

Trapping continued sporadically throughout the summer; the last jumping mouse being trapped on August 13. Trapping was most successful in close proximity to water, usually in lush, shady areas.

Preparations for Hibernation in *Zapus princeps*

Weight Gaining Phase

From July 14, when this experiment was begun, until early August, the nine jumping mice studied in the outdoor cages gained weight rapidly. In the adult *Zapus* These weight gains varied from 43.9% to 79.7% of weights at the beginning of the experiment. It is possible that some of the animals were in the weight gaining phase of the yearly cycle when the experiment was begun.

Maximum weights varied from 42.9 to 34.9 gms. (Table I). Males reached a heavier maximum weight than females, although one female weighing 42.0 gms. and not listed in Table I was trapped in August. The weight gains of male *Z. princeps* were more often stretched out

TABLE I. The percent of initial weight gained and the percent of this weight gain put on in the last two weeks before maximum weight was reached.

	Date Wt.$_i$ **	Wt.$_i$ (Gm) **	Wt.$_{max}$ (Gm)	% Gain	% Gain in last 2 wks.	Date Reach Max.	Sex
Low-Area Animals							
L-4	7/16	20.2	34.9	72.8	57.5	8/13	♀
L-5	7/16	27.1	39.0	43.9	100	7/31	♂
Mean		23.7	97.0	58.4	78.8		
H-7	7/14	24.3	37.5	54.3	89.4	8/20	♀
H-15#	7/27	16.7	36.0	115.6	71.5	8/21	♀
H-3	7/14	25.0	40.9	63.3	53.5	8/15	♂
H-4	7/14	27.0	42.9	58.9	47.2	8/15	♂
H-8	7/14	22.6	41.0	79.7	48.4	8/19	♂
H-13	7/14	26.0	41.5	59.6	61.9	8/6	♂
H-9	7/14	24.7	37.2	50.6	100	8/4	
Mean		23.7	39.6	68.9	67.4		

** Wt.$_i$ indicates initial weight

# Indicates immature animal

over a longer period of time than those of the females, with both sexes showing some acceleration of weight gain in the two weeks immediately prior to maximum weight (Fig. 1, Table I). With one exception, females reached their maximum weight later than males in the small sample of animals studied here (Table I). The first male to reach its peak weight did so on July 31, and the last female to reach its weight peak did so on August 21.

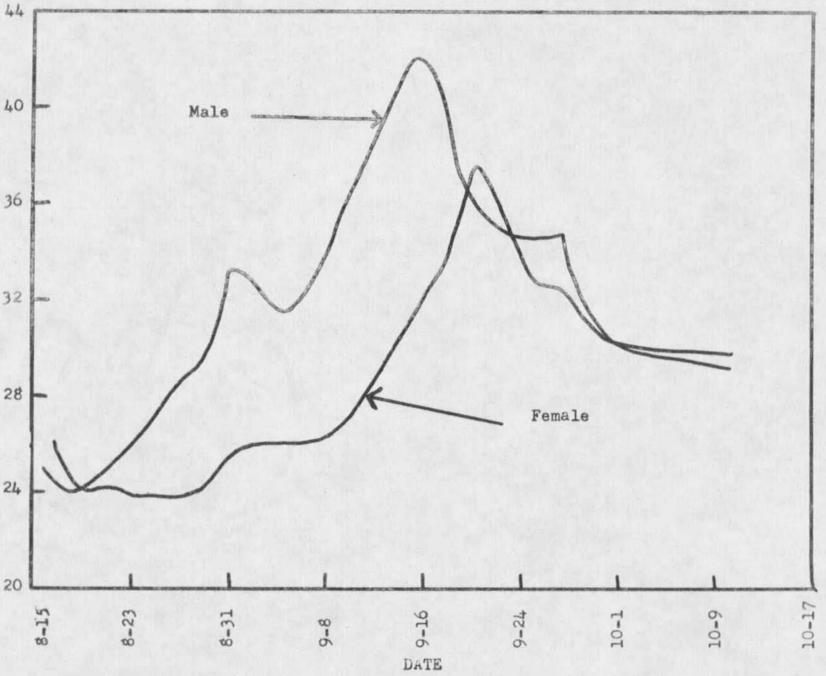

Fig. 1. Weight curves from a selected male and a selected female *Zapus princeps*. Weights were taken every other day. Female mouse is H-7 in Table I. Male mouse is H-3 in Table I.

The immature mouse studied reached a peak of 36.0 gms. on August 21, a gain of 115.6% of its initial (July 27) weight in 25 days. This suggests that some immature mice are capable of gaining weight faster than adults, reaching their peak at the same time and at approximately the same weight as adults.

Rectal body temperatures ranged from 36 to 39C during this period of weight gain. *Zapus* are typically nocturnal (Quimby, 1951; Burt, 1964), however, the animals in this study were extremely light sleepers during the day. Disturbance usually resulted in instant flight from the nest, and continued movement around the cage made them difficult to catch. A great deal of fecal material was found in the water, and much food was consumed during weight gain.

Period of Weight Loss and Lethargy

Once maximum weight was attained, the behavior and physiology of *Z. princeps* appeared to change considerably. A sharp drop in weight accompanied by lethargic behavior were the most obvious characteristics of this phase. An average of 15% (range 11-22%; n=8) of the maximum weight attained was lost over a period of two to nine days before initial torpor occurred (Table II). The animals that lost weight for the least number of days before becoming torpid usually lost more weight per day than the animals that lost weight for a greater number of days before reaching torpidity (Table II).

The animals were lethargic while they were losing weight. Body temperatures during the day ranged from 32 to 35C with occasional evening temperatures of 36-37C. The lower body temperatures were very labile, with increases from 33 to 36C observed to occur in as little as four minutes upon disturbance. When the animals did leave their nest there was an obvious loss of quickness, and there appeared to be an inability for extended locomotion. At this time *Zapus* could easily be picked up by the tail or the nape of the neck. From observations of both fecal material in the water and food disturbance, eating and activity appeared to be subdued.

TABLE II. A summary concerning weight loss immediately prior to initial torpidity.

	Sex	% Wt. Loss *	No. Days **	Gm/Day Loss	Wt. at Entrance ***	Date of Entrance ***
Animals						
L-5	♂	22.3	9	.97	30.3	8/14
H-8	♂	11.2	8	.58	36.4	8/25
H-15	♀	15.2	7	.79	30.5	8/27
H-3	♂	15.1	5	1.20	34.8	8/20
H-7	♀	10.9	4	1.03	33.4	8/25
H-13	♂	13.9	4	1.43	35.4	8/17
H-4	♂	13.8	3	1.96	37.0	8/17
L-4	♀	13.5	2	2.30	30.0	8/17
Mean		14.5	5	1.28	33.5	

* Percent of maximum weight lost before initial torpor.

** Number of days in weight losing-lethargic phase before initial torpor.

*** Entrance refers to initial entrance into torpidity.

Initial Short Periods of Torpidity

The two low-area mice averaged five grams less in body weight than the six high-area mice on initial entry into torpor. Mice from high and low areas did not appear to differ in time of entry into torpor. Little difference could be found in either weight at entry or time of entry between high-area mice caged at 5000 feet (H-13, H-8, H-15) and high-area mice caged at 7500 feet (H-3, H-4, H-7).

After L-5 initially became torpid on August 14, the low-area cages were observed at least twice daily. It appeared that periods of torpor during this phase were short and sporadic with more time spent active than torpid. During the day the mice were lethargic when active.

Animals which were not torpid in the morning were never observed torpid later in the day. From these observations and observations made at dawn it appeared entrance into torpidity occurred only during the dark hours. During this phase a single period of torpidity never lasted beyond 4:00 p.m. of the day it began.

Rectal body temperatures of torpid jumping mice ranged from 1.6 to 22C, and agreed closely with microenvironmental temperatures. When body temperature approached 22C, arousal followed.

These initial short periods of torpor could possibly have been a prolonged test drop phase as described for *Citellus lateralis* (Strumwasser, 1959), with the exception that the body temperature of *Z. princeps* appeared to drop all the way to microenvironmental temperature, whereas the body temperature of *C. lateralis* stops at a temperature higher than that of the microenvironment during initial periods of torpor (Strumwasser, 1959).

The mice caged at 7500 feet were disturbed less and provided with more nesting materials than in any other cage. After a normal preparatory period all three mice in the high-area cage were found deeply torpid on August 29. The temperature low the night before had been near 0C. Their nest had been moved from a shallow area under a piece of sod to a deeper, more well-constructed area. Here each mouse was in a tight-fitting, round nest of soft grass. Two mice were huddled against each other while the third mouse was approximately two inches away. A large hollow area which contained long stems of grass with seeds and grains of rolled oats, was found about a foot away from the nesting area. The nesting area and the storage area were connected by a tunnel. No other entrance could be found to the nesting area. Nest temperatures from this area were compared with air temperatures over a three day period in September (Fig. 2). They showed that while air

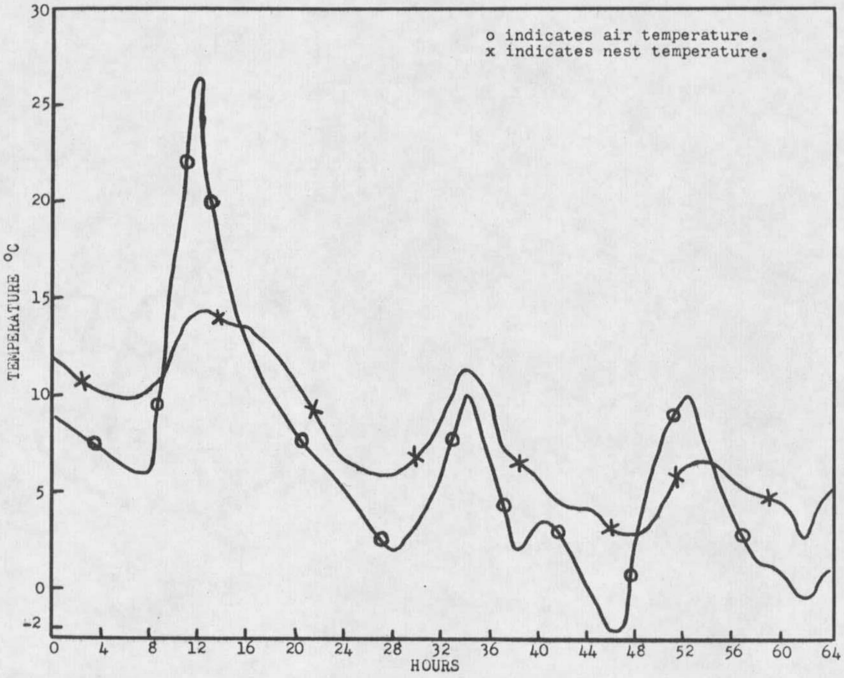

Fig. 2. A comparison of temperatures from a jumping mouse nest and ambient air temperatures.

temperature varied from -2.8 to 26.6C, nest temperature varied from 2.8 to 14.4C. These nest temperatures were well within the torpid range of *Zapus princeps*. Lack of fecal material in the water and disturbance of food indicated that activity was minimal from August 29 until these mice were killed on October 5.

The six animals caged at 5000 feet failed to progress from short periods of torpor into hibernation before October 5. The disturbances associated with daily observations and a lack of sufficient nesting materials seemed to play a role in this occurrence. The floors of these two cages were made with more loose dirt and less sod than the high-area cage, allowing only shallow nests and little microenvironmental temperature control. Daily temperatures often exceeded 22C during this time in this area. Near freezing night temperatures usually yielded morning torpor and in several cases coincided with nest movement in these cages.

Variables Possibly Causing Torpor

The main variables in nature which might control hibernation include food, water, cold and photoperiod. Each of these was considered under controlled conditions.

Food and Water

Two *Zapus* were found torpid in traps: one on the morning of May 28, the other on July 7. Controlled studies were begun on July 24 to determine what variables might play a role in causing torpor out of season in this species. These studies were terminated on August 14, the day after the first animal caged in the field became torpid.

Between July 25 and August 11, eight adult and three immature jumping mice were caged individually under controlled temperature conditions (3±1C) with nesting materials, and three hours of light per day. One adult and one immature mouse were kept under similar conditions but in constant light. Six of the mice in a three hour photoperiod were given water "ad libitum" but kept without food. Two of these mice were given both food and water "ad libitum". One of the mice in constant light was kept without water, but with food, the other without food but with water.

Six of the seven animals caged without food entered torpidity within three days after losing from 8.0 to 30.7% of their initial body weight. The seventh animal became torpid after five days and a loss of 47.1% of its initial body weight. The mouse kept in constant light without food was included in the former group and reacted similarly to the animals in a three hour photoperiod (Table III).

The four jumping mice studied without water all became torpid within three days after losing 17.2-23.0% of their initial body weight. The mouse in constant light reacted similarly to those with a three hour photoperiod (Table III).

The two mice with both food and water showed no signs of torpor.

TABLE III. Weight changes of mice from experiments inducing torpor by lack of food or lack of water.

	Wt._i (Gm) **	Wt. When Torpid (Gm)	% Wt._i Lost **	Gm/Day Lost	No. Days Before Entry	Sex
Animals w/o Food						
E-9	36.8	19.5	47.1	3.5	5	♂
E-3	36.2	25.7	30.7	3.5	3	♀
E-19	27.3	24.9	9.4	2.4	1	♀
E-5	25.2	22.8	10.1	2.4	1	♂
E-4	24.9	18.0	27.8	3.5	2	♀
E-1*	13.2	12.2	8.0	1.0	1	♂
Constant Light						
E-6	21.2	17.8	16.1	1.7	2	♀
Mean	26.4	20.1	21.3	2.6	2	
Animals w/o Water						
E-15	29.6	24.5	17.2	1.7	3	♀
E-8	25.2	19.3	23.0	1.3	3	♂
E-20*	14.2	11.2	21.3	2.0	1	♀
E-11*	18.3	11.9	35.2	2.2	3	♀
Constant Light						
E-7*	15.1	12.2	19.4	1.5	2	♀
Mean	20.5	15.8	25.2	1.8	2	

* Indicates immature mice.

** Wt._i indicates initial weight.

Between July 28 and August 14 five *Zapus* were caged individually outdoors (temperature ranged from 7-32C). Four animals were studied without food and one without water.

One of the animals without food became torpid in one day, one, a lactating female, died, and two were fed after two days due to weakness. The mouse without water entered torpidity in three days after losing 35.2% of its initial body weight.

Mature and immature mice did not differ significantly ($p > .25$) (Lentner, 1968) in the number of days required for entry into torpidity. A small sample of immature mice lost significantly less ($p < .025$) (Lentner, 1968) of their body weight than mature animals before entering torpor.

Arousal could be induced by disturbance in animals torpid at field temperatures. Arousal in the cold (3±1C) could not be initiated in animals in which torpor had been induced by a lack of food or water. In these animals noise or tactile disturbance yielded only reflex kicks typical of torpor in this species.

The torpid state produced by removal of food or water showed characteristics of both physiologically uncontrolled hypothermia and true hibernation. Failure to arouse in the cold and the death of four of the eight animals aroused at room temperature might indicate the former condition. Since one animal lived for 12 days in this torpid state without arousal and since only three of the eleven animals induced into torpor died in that state, some of the characteristics of true hibernation were also present. All animals studied under these conditions also showed position, piloerection, body temperature, ECG, and respiration typical of a normally torpid animal.

Photoperiod

On July 14 two mice were placed in a controlled temperature environment (3±1C), with food and water "ad libitum", and with 24 hours of light per day. Both mice gained weight slowly at first, mouse I-10 gaining 2.6 grams in 22 days and mouse II-2 gaining 2.6 grams in 33 days. On August 11, mouse I-10 began to gain weight rapidly, and on August 20, mouse II-2 also began a rapid weight gain. On August 29, mouse I-10 reached a peak weight of 34.7 grams and was first observed torpid on September 2. On September 10, mouse II-2 reached a weight peak of 35.1 and was first observed torpid on September 14. Both animals showed a weight loss period immediately prior to torpor, however, mouse II-2 did not show initial short periods of torpor (Fig. 3). After an initial five day period of torpor mouse I-10 spent more time active than torpid for eight days, after which the long periods of torpidity began (Fig. 3). As is typical of most hibernators (Twente and Twente, 1967), the periods of torpor in both animals were gradually increasing in length when the experiment was terminated on October 28 (Fig. 3).

Prior to torpidity both mice moved food into their nesting areas. There was evidence that mouse I-10 ate during arousals.

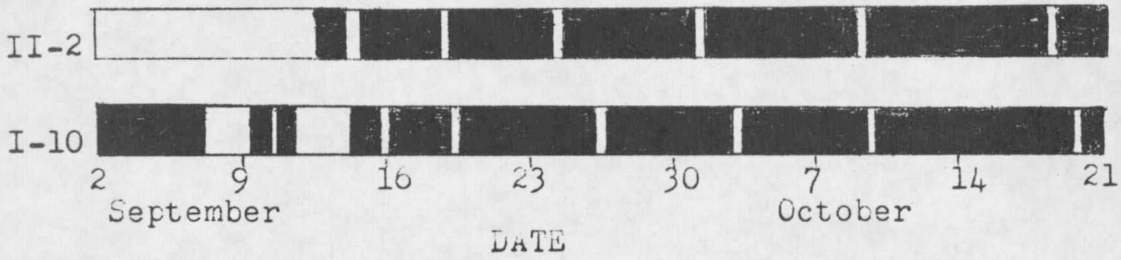

Fig. 3. Amount of time spent in torpor by two *Zapus princeps*.
* Shaded areas indicate time spent in torpor.

Cold

All experiments indicated that cold alone, out of season, will not induce torpor in *Z. princeps*. The possible accelerating effect of cold on weight gain was tested by comparing the weight gains of a female immature and a mature female animal in the cold (3±1C) with the gains in a mature female mouse and the immature female mouse caged in the field. The weight gains studied occurred in late July and early August. Although the animals in the cold appeared to gain weight faster, an F test of the means (Lentner, 1968) in this small sample indicated that the increase in rate of weight gain was not statistically significant ($p < .075$) (Table IV).

TABLE IV. Weight gain of females in the cold (3±1C) compared with weight gain of females caged in the field.

	Wt. Gain (Gm) From	Wt. Gain (Gm) To	Days	Gain/Day (Gm)	Gain/Day % Wt.$_i$ *
Animals in the Cold					
E-6	15.6	21.2	6	.94	5.96
E-12	27.6	41.6	8	1.75	6.30
Animals in the Field					
H-15	16.7	22.8	8	.76	4.56
H-7	24.2	37.5	27	.49	2.03

* Differences between percentages of initial weight gained per day in the two groups were not significant ($p < .075$) (Lentner, 1968).

Hibernation Characteristics

Typically, hibernation consists of three phenomena: entrance into torpor, torpor itself and arousal from torpor. Entrance into torpor apparently requires a lack of disturbance and thus is the most difficult to study.

Two attempts were made to obtain a general idea about body temperature during entrance into torpor in *Z. princeps*. In both cases it was necessary to hold the animal immobile in a plastic animal holder at 3±1C without food or water. Results (Fig. 4) indicate that entrance in this species resembles a series of test drops as described for *Citellus* (Strumwasser, 1959).

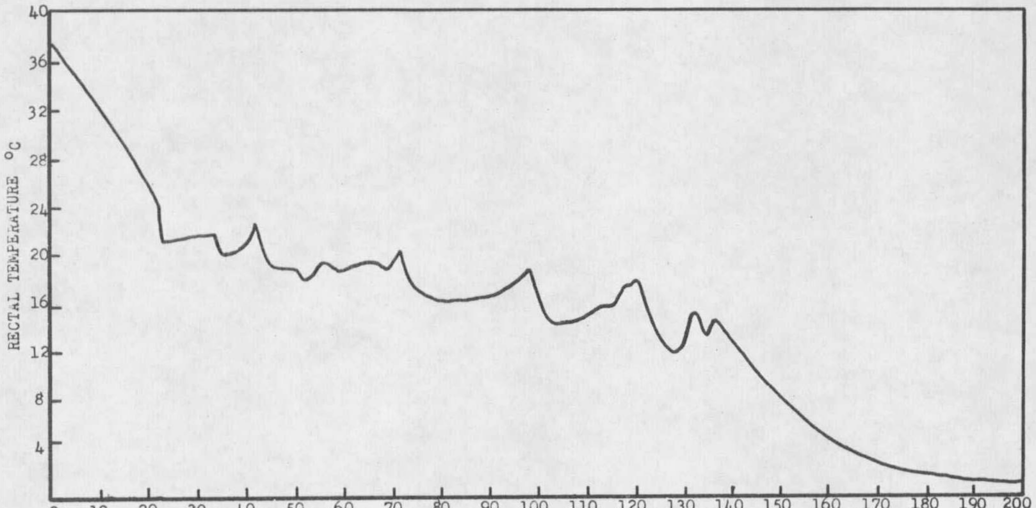

Fig. 4. Body temperature during entrance into torpor in a *Zapus princeps*.

The hibernating posture of Z. *princeps* resembles the ball-like posture of most hibernators. Postural adjustments, characteristic of many torpid ground squirrels, were not observed in hibernating jumping mice.

The body temperature of a torpid western jumping mouse was approximately equal to microenvironmental temperature until the latter dropped below 1.6C. Between ambient temperatures of 1.6 and -2C (the lowest temperature used in these experiments) body temperature remained at 1.6C. Respiration occurred in bursts with periods of apnea lasting as long as five minutes.

Dissection of hibernating jumping mice showed the urinary bladders to consistently contain urine if the animal had been torpid for more than 24 hours. Stomachs, intestines and rectums usually contained materials, even in a few of the cases where mice became torpid due to a lack of food. Stomachs of animals that had been torpid for over seven days were somewhat shrunken and did not always contain matter. In these cases the small intestines and rectums invariably did hold material, however.

The higher the body temperature of a torpid jumping mouse the more irritable and more easily aroused it became. The first reaction of a jumping mouse to tactile stimulation at any temperature was a slow kicking of the hind legs and increasingly frequent bursts of rapid breathing. Any handling usually resulted in arousal at body temperatures above 10C. In normal torpor (not induced by lack of food or water) at body temperatures below 10C excessive handling, noise, replacing the animal in its nest in an abnormal position or taking rectal temperatures invoked arousal.

Zapus princeps appears capable of two types of arousal from torpor. The most common type is the differential arousal, utilizing anterior-posterior temperature differentiation. The second type is typical of torpor induced by a lack of food or water. Here arousal is more passive, occurring only in temperatures above 10C, and if anterior-posterior temperature differentiation occurs, it is minimal.

In the early stages of a differential arousal from torpor, vasoconstriction occurs posterior to the diaphragm, allowing the anterior of the animal to heat first. When this vasoconstriction subsides, the posterior part of the animal heats in an explosively rapid fashion (Lyman, 1965). Figure 5 illustrates the difference between anterior temperature of a jumping mouse, taken subcutaneously from the interscapular region, and posterior (rectal) temperature in a differential arousal in the cold. Rectal temperature at first rose slowly and appeared to be a function of ambient temperature (Fig. 6). At this time both absolute temperature and rate of temperature increase were greater in the interscapular region than in the rectal region. When interscapular temperature reached 22-24C it began to level off, and rectal temperature then began its rapid ascent. The rectal

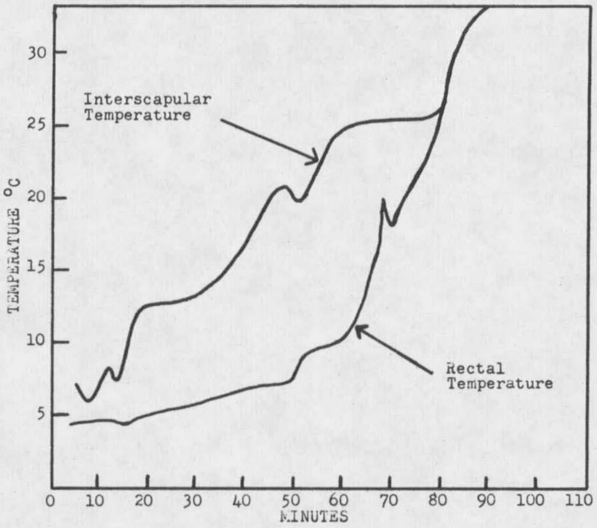

Fig. 5. A comparison of anterior and posterior temperatures during arousal from torpor in a *Zapus princeps*.

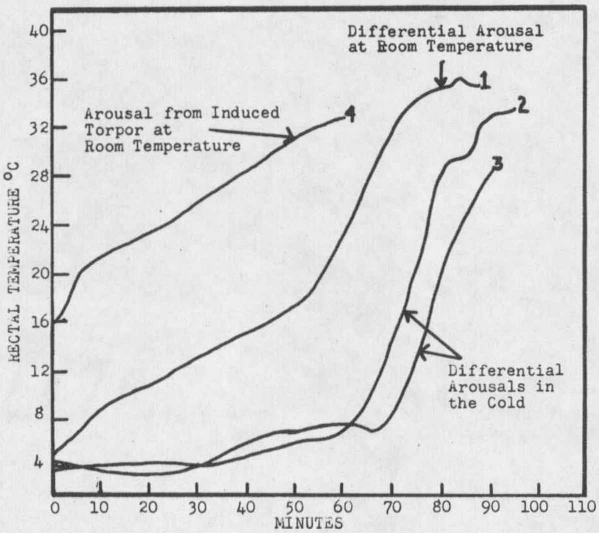

Fig. 6. Rectal temperatures during a differential arousal at room temperature (1), two differential arousals in the cold (2,3) and an arousal from induced torpor at room temperature (4). Curves 1, 2, and 3 are three separate arousals from the same animal and demonstrate the dependence of rectal temperature upon ambient temperature in early stages of a differential arousal.

temperature at which the rapid ascent began varied with ambient temperature (Fig. 6) and seemed dependent upon the heart rate reaching near 600 beats per minute (Fig. 7). Rectal temperature and interscapular temperature coincided at 24-25C.

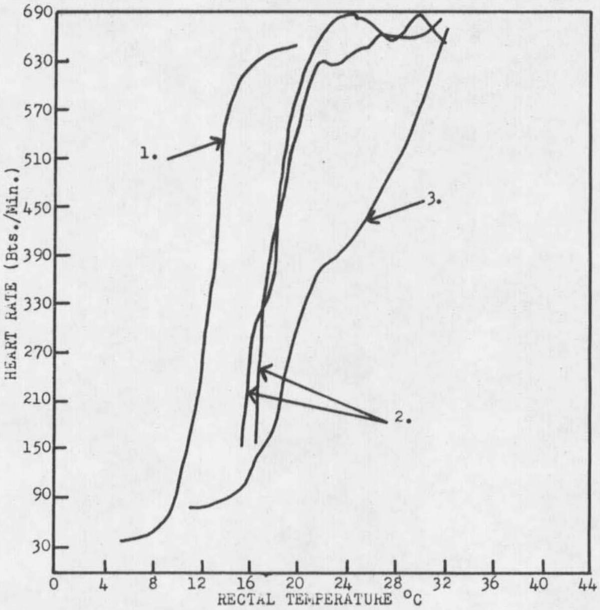

Fig. 7. Heart rate-body temperature increase during arousal from torpor in four *Zapus princeps*. 1. A differential arousal from body temperature below 10C. 2. Two differential arousals from body temperatures above 10C. 3. An arousal from induced torpor.

In a differential arousal from below 10C the heart rate-rectal temperature curve was S-shaped, with a linear acceleration of heart rate occurring between the rectal temperatures of 10 and 16C. The overall rate of heart rate increase, through the S curve, was 7.6 beats per minute (Fig. 7). In arousal from torpid body temperatures above 10C the curve lost its lower inflection point and rapid acceleration of heart rate began immediately when arousal was invoked (Fig. 7). The overall rates of increase were 42.5 beats per minute and 34.4 beats per minute in the two such cases studied.

The hind feet of a jumping mouse were usually very pink during torpor but became blanched upon initiation of a differential arousal, regaining their pinkness after the rapid rectal temperature acceleration began. Extremely heavy shivering anteriorly accompanied by hyperpnea usually occurred just prior to and during the early part of the rapid rectal temperature ascent, stopping when rectal temperatures reached 23-26C. The first muscular movements other than shivering occurred at rectal temperatures of 15-20C. From 26-30C the animal usually began shuffling around in an uncoordinated manner, sniffing and often eating if food was available. The eyes normally opened between 30 and 32C.

During the second type of arousal from torpor rectal temperature rose at a rate closely resembling the rate of the early ambient temperature dependent stage of a differential arousal at room temperature (Fig. 6). Heart rate also increased more slowly and did not reach 600 beats per minute until rectal temperature was in the 30-32C range (Fig. 7). The rate of heart rate increase was 3.9 beats per minute in the fastest arousal studied from induced torpor.

In both types of arousal from torpor, body weight loss due to arousal alone was usually around .15 grams (1.43 Calories if this loss was completely lipid), or 0.6% of body weight. The electrocardiograms in both kinds of arousal showed anamolies typical of most arousing hibernators (Nardone, 1955; Chatfield and Lyman, 1950) with the exception that dysrhythmias occurred only between 11 and 19C in *Z. princeps*. ECG's also showed response to both noise and tactile stimulation at temperatures as low as 3.3C.

Brown Fat

Brown fat, which plays an important role in heat generation during arousal (Joel, 1965), may also play a part in differentiating between the two types of arousals in *Z. princeps*.

All brown fat in *Z. princeps* appears to lie anterior to the diaphragm. According to Smalley and Dryer (1967), brown fat pads in hibernators typically lie in the interscapular, subscapular axillary, dorsal cervical, inferior cervical, suprasternal, thoracic, perianal inguinal and retrolumbar regions. All but the last three pads existed in hibernating *Zapus princeps*. The subscapular axillary pad in *Zapus* was actually three pads extending from under the scapulae laterally onto the rib cage. Over the top of the ventral-anterior corner of this pad was a pad unnamed by Smalley and Dryer, which Joel (1965) calls the axillary pad. With the exception of the thoracic pad, which is deeply embedded between the ribs around the spine, some mean weights of the brown fat pads in *Z. princeps* are given in Table V. The thoracic pad would probably have a weight between that of the dorsal cervical and inferior cervical pads.

TABLE V. Means and standard deviations of brown fat pad weights in *Zapus princeps*.

	Is. (Gm)	Ss. Ax. (Gm)	D.C. (Gm)	I.C. (Gm)	Ax. (Gm)	Sst. (Gm)
Animals						
Means	.1954	.3711	.1591	.0749	.2765	.2143
S_X	.0308	.0791	.0329	.0173	.0866	.1095
N	5	5	5	5	5	5

Is. indicates interscapular pad.
Ss. Ax. indicates subscapular axillary pad.
D.C. indicates dorsal cervical pad.
I.C. indicates inferior cervical pad.
Ax. indicates axillary pad.
Sst. indicates suprasternal pad.

The most important brown fat pads producing heat during arousal are probably the thoracic pad, which lies inside the thoracic cavity on either side of the spinal chord back to the diaphragm and has some tissue among the vessels at the cranial end of the heart; the suprasternal pad, which extends from under the salivary glands in the neck region to the sternum and in which all the major vessels leaving the thoracic cavity anteriorly are imbedded; the subscapular axillary which lies laterally over the lungs and surrounds the major nerve trunk to the forelegs; and finally the inferior cervical which lies directly over the stem of the brain. The remaining pads of brown fat appear to be located so as to supplement these pads in heating the vital organs of the body.

Most animals dissected in July had only a small pad of brown fat in the interscapular region and some brown fat under the salivary glands in the neck. Brown fat was completely lacking in several animals in which torpor was induced by lack of food or water. The animals in these categories either did not arouse from torpor in the cold or did not show any signs of anterior-posterior temparature differentiation in arousals from torpor at room temperature. Animals in which torpor had been induced usually had less brown fat than animals which had been living under normal conditions. All animals which were able to awaken from torpor in the cold showed some brown fat in every region listed as typical for *Zapus princeps*.

Table VI lists some brown fat and white fat weights from twelve animals killed between September 28 and October 2. All these animals

TABLE VI. The means and standard deviations of white fat and total brown fat weights in seven hibernating *Zapus princeps* compared with the white and brown fat weights of two animals active in early October. All animals were killed between September 28 and October 2.

	Body Wt. (Gm)	BF Wt. (Gm) *	BF Wt. (% Body Wt.)	WF Wt. (Gm) **	WF Wt. (% Body Wt.)
Torpid Animals					
Means	27.6	1.2056	4.33	4.2832	15.37
S_X	3.9	.2554	.52	.8989	2.42
Active Animals					
H-14	24.7	.7378	2.99	.7679	3.11
H-8	29.8	.5435	1.82	1.0587	3.55

* BF indicates brown fat.
** WF indicates white fat.

were torpid when killed with the exception on H-14 and H-8. Both of these animals had been torpid earlier for a period of time. However, they had been fully active and gaining weight for the seven to nine days before they were killed. Their low brown and white fat weights might have been responsible for this behavior.

DISCUSSION

Behavior of *Zapus princeps*

It appears that 10½-11 months of the yearly cycle of *Zapus princeps* is associated with hibernation. This period can be divided into several parts.

From early July until mid-August jumping mice appeared active and gained weight in preparation for a long period of torpor. During this time food and water consumption were high, body temperature was relatively stable, and activity was great. The end of sexual activity probably dictated the beginning of this period (Pengelley, 1967). Sexual activity appears to terminate in most female jumping mice with seven weeks of gestation and lactation (Brown, 1967). The present study indicated that rapid weight gain in female *Zapus* usually began in late July. This would lend support to Pengelley's (1967) theory that preparation for torpor begins after sexual activity ends and would agree closely with Brown's (1967) seven week period involved with bearing young after an early June fertilization. According to Brown (1967) early June is the time of peak testicular weight in males and probably the period of fertilization in *Z. princeps*. Thus one might expect the weight gain of the male *Zapus*, after a short period of sexual activity, to occur over a longer period of time and reach a higher peak, earlier than that of the female. This was shown to often occur in *Z. princeps* (Fig. 1, Table I).

The ability of *Z. princeps* to become torpid during the weight gaining preparatory period when food or water was removed could very well be a protective mechanism to prevent starvation. The need for such a mechanism is suggested by the rapid weight loss (Table III) in the absence of food or water in this species. Discovery of the two torpid animals in traps in late May and early July demonstrated that when temperature is cool enough to permit it (below 22C) stress, probably in the form of a diminished supply of food or water, will induce torpor in *Z. princeps* in the field. Since unseasonable snowcover is the most likely natural stress, it is likely that the associated temperatures would be low enough to allow torpor. Although arousal from induced torpor could never be invoked in the laboratory under cold conditions, such an arousal would be unnecessary in nature. Montane snowcover in June or July is usually short-lived and followed by temperatures high enough to aid arousal from torpor.

Inducing torpor by lack of food or lack of food and water is common among many hibernators (Davis, 1967: Johansen and Krog, 1957; Bartholomew and Cade, 1959). The entrance of *Z. princeps* into torpor upon removal of water alone has apparently not been reported previously for hibernators. This unique dependence upon water shown by *Zapus* points up the importance water must play in the life of this small mammal.

Around mid-August, after maximum weight had been attained, jumping mice appeared to begin final preparations for torpor. Lethargic behavior was accompanied by a sharp weight loss as the animal appeared to be gradually shutting down its body mechanisms in preparation for the long torpid period ahead. Animals under natural conditions, controlled conditions and stress conditions (no food and/or no water) all demonstrated a lethargic period of this type prior to torpor. The amount of weight loss and the length of this final preparatory period probably depended upon temperature, disturbance, and the physiological state of the animal upon entrance into the period (Pengelley, 1967). High ambient temperatures or a great deal of disturbance would probably increase the length of final preparations, as would a degree of sexual activity or any other physiological block to torpor. Ambient conditions probably play the dominant role in increasing the length of the lethargic period under natural situations, whereas physiological state is probably dominant in cases of induced torpor out of season. It is interesting to note that mice lose less weight per day in cases where the length of this period is increased.

An F test of the means (Lentner, 1968) from Tables II and III indicates that animals under field conditions lose significantly less weight prior to initial torpor than animals deprived of food and water ($p \leqslant .01$). This comparison and observations of food and water suggest that some eating and drinking occur under natural conditions during this final preparatory period. Some of the food disturbance might have been associated with storage.

A decline in the number of Z. *princeps* trapped in late August and early September might be expected in association with the short periods of torpor occurring during this time. During these initial short periods of torpor movement to a more well constructed nest was also noted in both high and low-area cages. Thus *Zapus* could occupy two nests during its yearly cycle in its natural habitat also. An active animal might occupy a shallow accessible nest whose temperature is related to the air temperature in such a way as to aid arousal from induced torpor, should it be necessary. The second nest, used for final torpor, would be located deeper so as to facilitate hibernation.

Strumwasser (1967) believes the test drop period of occasional periods of torpor in *Citellus lateralis* functions in allowing the hibernator to gradually slow down the body functions as it approaches prolonged torpor. The period of lethargy and the period of occasional torpor could both have this function in Z. *princeps*.

A drop in temperature to near 0C at night seems to usually be responsible for the end of the test drop phase and the beginning of long periods of torpor. This was the case in the high-area cage. In the two low-area cages nightly lows of near 0C nearly always invoked torpor and several times apparently induced movement of nesting place. The cold dependent induction of extended torpor might explain why Brown

(1967) was able to trap no *Zapus* following a short cold spell which began on August 31, at 10.500 feet, even after the weather became warm again. He found the same occurrence at 8500 and 9500 feet after a snowstorm on September 17 at these altitudes. Thus weather may act to trigger the final stage of the hibernation cycle in a prepared *Z. princeps*. This mechanism would allow the animal to maintain its weight for as long as possible by use of lethargy and some food consumption.

From the experiments in constant temperature and 24 hours of light it appears that hibernation was controlled by a temperature independent clock mechanism similar to the type found by Pengelley (1966) in *C. lateralis*. The role of photoperiod in such a clock is questionable. Jumping mice kept in 24 hours of light began gaining weight and entered torpor two-three weeks later than animals caged in the field, however, a normal preparatory period and normal torpor did occur close to schedule. Experiments inducing torpor in constant light showed no delay. Possibilities for a Zeitgeber in the clock of *Z. princeps* include geophysical forces or a clock mechanism set by photoperiod before this experiment began (Pengelley, 1967).

Characteristics of Torpidity in *Zapus princeps*

The phenomenon of hibernation in *Z. princeps* has several characteristics which set it apart from many hibernators.

Brown (1967) theorized that an upper critical limit for torpidity existed in *Z. princeps* near 10C. The present study showed that a threshold, beyond which torpor cannot occur, does exist in this species, but is closer to 22C. This complements similar findings by Strumwasser (1967) in *C. lateralis*. Periodic arousals occur at less frequent intervals as hibernation progresses in *Z. princeps*, agreeing with the findings of both Twente and Twente (1965) and Strumwasser (1967) for other hibernators. The maximum period between arousals was not determined due to termination of this experiment on October 28, when arousals were occurring 11 days apart. Studies of other hibernators make the statement of Brown (1967), that periods between arousals in *Z. princeps* average 37 days and can last as long as five months, seem very unlikely. Periodic arousals in this species are very short (less than 12 hours) and were detected in the present study only by Pengelley's (1961) sawdust technique in one case and disturbance of carefully arranged nest materials in the other. The short duration of these arousals may have led Brown (1967) to his conclusions. There was also strong evidence here that some eating did occur during such periodic arousals by at least one animal.

The invariable presence of distended urinary bladders in animals after they had been torpid for over 24 hours extending up to the time of death suggested that the kidneys of *Z. princeps* can function at low body temperatures. Pengelley (1966) stated that it was improbable that kidneys of hibernators did function at low temperatures and that the urinary bladder was probably filled after arousal. Willis (1967) stated that filtration did occur in the kidneys but not reabsorption at low body temperatures.

The presence of food and other material in the stomach and intestine of torpid animals does not appear to have been recorded in other animals. The lowest body temperature at which *Zapus* hibernates, 1.6C, is lower than recorded for any other species.

Direct measurements showed that *Z. princeps* is capable of using anterior-posterior temperature differentiation during arousal from torpor. Blanching of the hind feet indicates that posterior vasoconstriction plays a role in this differentiation. Rapid heart rate acceleration during low rectal temperatures indicates the effect that differentiation has on vital organ functions. The important role that brown fat plays in a differential arousal in *Z. princeps* is indicated by the absence of differential arousals during the early weight gaining phase when brown fat is scarce. Smalley and Dryer (1963) agree that at higher temperatures arousal can occur without brown fat, allowing for arousal from induced torpor in *Z. princeps*.

Evolutionary Relationships of Hibernation

There is general agreement among the majority of workers that hibernation is a primitive feature of polyphylogenetic origin, the behavior and physiology of the present forms being the result of convergent evolution (Pengelley, 1967). It has been generally agreed that in the class Rodentia there has been a progressive loss of hibernating mechanisms with a tendency toward increased homeothermy (Pengelley, 1967). It appears that on the basis of characteristics of hibernation, hibernating rodents can be divided into two very general groups. The first and most primitive group consists mostly of smaller rodents and is characterized by a labile body temperature. Although extreme diurnal fluctuations of active body temperature do occur in some species of this group (Johansen and Krog, 1959) moderate body temperature lability (active range of 31-39C) is more often the rule (Cade, 1963; Bartholomew and Cade, 1957). Torpor is easily induced by removal of food and entrance into torpor occurs in a straight drop, without a period of test drops. Entrance into dormancy seldom seems to be controlled by a clock mechanism (Bartholomew and Cade, 1957; Hudson, 1967) and winter stress is met by torpor and food storage, but seldom fat accumulation. Species typical of this group include *Baiomys taylori*, several species of *Perognathus*, *Sicista betulina*, *Microdipodops pallidus*, and several species of *Eutamias* (Hudson, 1967).

The second group consists mainly of the sciurids and is characterized by relatively stable active body temperatures, fat accumulation prior to torpidity, in most cases control of yearly cycle by a biological clock (Pengelley, 1966), entrance into torpor through a period of test drops (Strumwasser, 1959), and physiological specializations necessary to produce differential anterior-posterior temperatures during arousal from torpor (Lyman, 1965). Hudson, 1967, claims there is no evidence that small hibernators possess the latter "physiological specializations".

The hibernation characteristics of *Z. princeps* appear to lie between these two major groups of hibernators. Throughout the weight gaining phase of preparation for torpor body temperature is stable. Temperature lability, however, is present in the period of lethargy immediately prior to initial torpidity. A straight drop into torpor can easily be induced in *Zapus* by removing either food or water, yet under natural conditions a test drop period occurs before extended periods of torpor begin. Both fat accumulation and food storage occur. A controlling clock mechanism and the physiological equipment necessary for anterior-posterior temperature differentiation are present, but *Z. princeps* is still capable of entering torpor out of season and arousing from torpor with a minimum of temperature differentiation. All these characteristics indicate that hibernation in *Zapus princeps* has evolved to a point midway between the two major groups of rodent hibernators.

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