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Influence of energy stores on activation of reproductive function in male golden-mantled ground squirrels

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Summary. Male golden-mantled ground squirrels were captured in the field in late summer and placed on either unlimited or restricted (80% normal) food rations in the laboratory until each animal began to hibernate. At entrance into hibernation mean body mass was 274.5 g for the unlimited group and 224.5 g for the restricted group. Only six of 21 males subsequently underwent reproductive maturation during winter. Each of these six received the unlimited ration prior to hibernation, and these six included the five heaviest animals in the experiment. In addition to activating their reproductive systems, these heavier squirrels began hibernation later and ended hibernation earlier than the lighter squirrels. Squirrels that remained reproductively quiescent averaged 25.5% body mass as fat in early spring and thus were not severely limited in energy stores during winter.

In another experiment a group of 13 male and female squirrels were housed together in a common outdoor enclosure in order to examine the possible relationship between reproductive condition and social standing of males. Although there appeared to be a social hierarchy among males, each male fattened substantially in fall and became reproductively active during winter.

These results suggest that the level of energy stores accumulated as fat prior to hibernation in fall affects the potential of male ground squirrels to breed in spring. This dependence of reproductive development on energy stores may reflect the high energetic costs associated with breeding in males when they emerge from hibernation in spring when food availability is low.

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Introduction

Golden-mantled ground squirrels have a single, brief mating season that begins each year directly after females emerge from hibernation in spring (McKeever 1964; Bronson 1979). A significant proportion of males, however, do not attempt to reproduce each year. Field studies on golden-mantled ground squirrels in the eastern Cascade Mountains of Washington (Kenagy and Barnes, unpublished) and the Sierra Nevada of California (Bronson 1979) have shown that one-quarter to one-third of all males do not undergo testicular development by the time of emergence from hibernation. Such males remain reproductively quiescent throughout spring and do not participate in breeding.

I undertook to investigate whether activation of reproductive function in male golden-mantled ground squirrels is dependent on the amount of energy stored as fat prior to entrance into hibernation. Male golden-mantled ground squirrels, like those of many other species of hibernating ground squirrels, terminate hibernation before females in early spring when snow is still present and before growth of food plants has begun (Davis 1976). Under these conditions males must meet the energetic demands of activity and preparation for the breeding season through the use of fat stored the previous year. Should a male not accumulate sufficient energy stores in fall, it might forego reproductive opportunities and instead continue to hibernate until food becomes abundant.

To determine the relation between pre-hibernatory body mass and spring reproductive status I restricted the food available to captive male golden-mantled ground squirrels in late summer prior to the hibernation season. I then examined condition of the reproductive system at the end of hibernation in spring by measuring size of the testes and plasma levels of gonadal steroid hormones.

In addition to the influence of energy stores, social interactions may affect the reproductive status of males. Male juvenile Uinta ground squirrels appear to be inhibited from developing reproductively as a result of aggression by dominant individuals (Slade and Balph 1974). Therefore I also examined the influence of social interactions on the development of reproductive function in male golden-mantled ground squirrels.

Materials and methods

Food restriction

Male *Spermophilus saturatus* were captured in the eastern Cascades, Chelan County, Washington during the last week of August 1981. *S. saturatus*, found in Washington and British Columbia, is closely related and possibly conspecific to the more familiar golden-mantled ground squirrel *S. lateralis* which is common to the mountains of much of western North America (Hall 1981). Without dissection it is difficult to distinguish juvenile from older animals at this time of year (Kenagy and Barnes, unpublished); but, based on the late date in the season, it is likely that all of the animals captured for this experiment were juveniles. Squirrels were separated into two groups of equal distribution according to body mass and housed separately in metal cages with burlap cloth for bedding. One group ($n=10$) received Purina Rodent Laboratory Chow *ad libitum*, the other ($n=15$) received limited amounts of lab chow every three days such that the group mean of body mass remained at 80% that of the group with unlimited food. This percent was chosen because it represents the difference between the mean spring weights of reproductive and non-reproductive squirrels under natural conditions (Bronson 1979). Both groups were exposed to changes in daylength that approximated natural daylengths at the area of capture (47°N. Lat.) and to an ambient temperature of 20 °C which was lowered weekly by two or three degrees until 4 °C was reached on 1 November 1981. Each animal was examined daily for torpor, and sawdust was placed on hibernating squirrels to indicate by its subsequent presence or absence whether an arousal had occurred between observations. Food was removed from each animal's cage on the second consecutive day of torpor. In spring food was replaced on the third day of continuous activity or on 5 May, whichever came first. Animals were weighed twice per week until 1 November and thereafter every three weeks. Blood samples (0.8 ml) were taken by cardiac puncture at three week intervals between 1400 and 1600 hrs PST after exposure to ether. In all cases blood samples were obtained within two min after each animal was picked up.

Four hibernating males died on 24 March 1982 during a mechanical failure that led to a temporary excursion of ambient temperature below 0 °C. The carcass of each was dried and extracted with chloroform in a Soxhlet apparatus for an analysis of total body fat.

On 18 May each male was removed from the cold, anesthetized with ether, and, upon laparotomy, the length and width of one testis was measured from which mass of both testes was estimated (Kenagy 1979). At the same time the scrotal skin was scored as being either pigmented or nonpigmented.

Radioimmunoassays. Concentration of testosterone was measured in plasma by RIA using procedures similar to those of Wingfield and Farner (1975). 100–200 µl of plasma were equilibrated with approximately 2000 cpm of ^{3}H-testosterone and extracted with 10 volumes of dichloromethane. Extracts were dried, resuspended in 10% ethyl acetate in isooctane and transferred to columns of Celite:ethylene glycol:propylene glycol (6:1.5:1.5, w:v:v) with Celite:water (3:1, w:v) "glycol traps". Testosterone was eluted with 20% ethyl acetate in isooctane after elution of dihydrotestosterone with 10% ethyl acetate in isooctane. The dried extracts were then assayed for testosterone in duplicate and individual values corrected for recovery. Minimum detectable levels of hormone for an average plasma volume ranged from 40–50 pg/ml. The hormone values compared were measured within one assay.

Social behavior. Nine males and four females were housed together on the roof of Kincaid Hall in Seattle in an outdoor enclosure (2 × 3 × 2.5 m) with wire screen sides and a translucent roof. Four males and four females were captured in May 1981 and placed in the enclosure; the remaining 5 males were captured and put into the enclosure in August 1981. Each squirrel was toe clipped and distinctively marked by black hair dye. Lab chow and water were provided *ad libitum*. Nine wooden nest boxes with burlap were provided, and a series of runways allowed squirrels to move throughout the height of the cage. From September to December, 35 half-hour observation sessions were conducted from a blind on separate days. At the beginning of each session 50–100 g of sunflower seeds (a highly preferred food) were scattered over the floor of the enclosure. The order in which individuals emerged from nest boxes to feed and the identity of squirrels involved in agonistic behaviors (chases and fights) were recorded. Each month animals were weighed and the reproductive condition of males was determined by the externally apparent size of the testes and the presence or absence of black pigment in the scrotal skin.

Results

Body mass and reproduction

At the end of the hibernation season only six of the 21 squirrels housed individually had become reproductively active, and all of these were from the group with unrestricted food. Mean testis size, plasma testosterone levels, and scrotal skin pigmentation in these six males (Table 1) were similar to those in reproductively active males at a similar date in the field (Kenagy and Barnes, unpublished; Barnes 1983) or in the laboratory (Licht et al. 1982; Barnes 1983). Testes in the other squirrels remained undeveloped, plasma testosterone at low levels, and scrotal skin unpigmented (Table 1).

Mean body mass of the six males that became reproductively mature measured either at the seasonal maximum in early fall or on the first day of hibernation was significantly greater than that of the non-reproductive males at the same times (Fig. 1; Table 1). At the time of capture mean body masses of the subsequently reproductive and non-reproductive groups were not significantly differ-

Table 1. Body mass, dates of hibernation, and reproductive condition at end of hibernation of male golden-mantled ground squirrels that underwent testicular development during winter ('Reproductives') or remained reproductively immature ('Non-reproductives'). The beginning of hibernation was defined as the first day of a torpor bout lasting at least two successive days

	Body mass (g)				Dates of hibernation		Testes mass (g) 18 May	Percent of males with pig-scrota 18 May	Testos-terone in plasma (ng/ml) 28 May
	26 Aug (capture)	At maxi-mum	At start of hiber-nation	18 May	Begin	End			
Reproductives ($n=6$)									
Mean	180.3	311.2	274.5	245.7	9 Dec	12 Apr	1.736	100%	4.016
SD	±41.2	±32.9	±37.4	±20.8	±14 days	±19 days	±0.801		±8.053
Non-reproductives ($n=14$)									
Mean	173.9	254.8[a]	224.5[a]	168.5[a]	2 Nov[a]	17 May[a]	0.303[a]	0%	0.584
SD	±45.3	±18.9	±18.2	±24.1	± 9 days	±5 days	±0.071		±0.794

[a] Significantly different from the Reproductive group; $P<0.001$, t-test

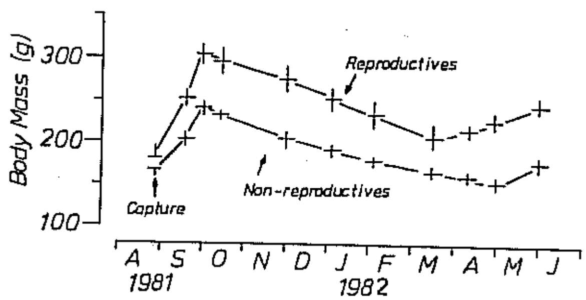

Fig. 1. Means (±SE) of body mass for golden-mantled ground squirrels that underwent gonadal maturation during winter ('Reproductives'; $n=6$) or remained reproductively immature ('Non-reproductives'; $n=15$). Capture represents the time all animals were trapped in the field

ent (Table 1). Table 2 lists the body mass of individual squirrels at four times during the experiment. As a result of restricting food some heavy males at capture entered hibernation at reduced weights and subsequently failed to become reproductive (e.g. the first and second heaviest animals at capture). Conversely, relatively light males at capture that were able to fatten substantially before hibernation did mature reproductively during winter.

The males that became reproductively mature entered hibernation later, terminated hibernation earlier, and thus had a shorter dormancy season than males that remained reproductively quiescent (Table 1).

Body fat in hibernating squirrels was not depleted by early spring. Animals analyzed for body fat content on 24 March averaged 164.0 g (range 149–185) in total body mass of which an average of 25.5% (range 21–32%) was fat.

Table 2. Body mass (rank in parentheses) of individual male golden-mantled ground squirrels as determined at capture, seasonal maximum, entrance into hibernation, and at the time of testis measurement, 18 May. Reproductive and non-reproductive categories are as in Table 1

	Body mass (g)			
	Capture 25 Aug	Maxi-mum	Hiber-nation begin	18 May
Reproductives	230(3)	346(1)	331(1)	267(2)
	229(4)	332(2)	274(4)	236(4)
	160(12.5)	330(3)	283(3)	272(1)
	161(11)	306(4)	291(2)	224(6)
	175(7)	299(5)	243(8)	250(3)
	127(21)	254(14)	225(14)	225(5)
Non-reproductives	290(1)	290(6)	206(19)	219(7)
	164(10)	280(7)	234(10)	165(14)
	141(17.5)	275(8)	255(5)	195(8)
	232(2)	274(9)	228(12)	182(9.5)
	227(5)	264(10)	232(11)	182(9.5)
	143(16)	260(11)	219(15.5)	157(16)
	141(17.5)	257(12)	237(9)	161(15)
	209(6)	255(13)	219(15.5)	179(11)
	168(9)	250(15)	213(17)	130(21)
	145(14.5)	249(16.5)	249(6)	149(17.5)
	135(20)	249(16.5)	227(13)	149(17.5)
	160(12.5)	247(18)	247(7)	175(13)
	139(19)	229(19)	207(8)	176(12)
	169(8)	227(20)	198(20)	130(20)
	145(14.5)	222(21)	197(21)	145(19)

Social interactions

The nine males and four females housed together within the outdoor enclosure interacted aggressively immediately after presentation of sunflower seeds. Four of the males consistently emerged last from nest boxes to feed and three of these did not eat sunflower seeds when other animals were present. These four animals were also the subordinate individuals in 16 of 19 observed chases. Despite this apparent social stratification in the presence of sunflower seeds, all squirrels fed freely on laboratory chow, each male reached a body mass greater than 300 g during winter, and all males came into reproductive condition (each with enlarged, scrotal testes and pigmented scrotal skin) by mid April.

Discussion

The magnitude of the gain in body mass in late summer and early fall appears to influence the reproductive status of male golden-mantled ground squirrels in spring. In general, only males that fattened above 300 g before hibernating underwent testicular development during winter. One exceptional male weighed only 127 g at capture and increased to twice that mass before hibernating, a proportional change that was surpassed by only one other, also reproductive male. Thus male golden-mantled ground squirrels that become reproductively active either surpass a threshold in body mass or greatly increase their proportional body mass while preparing for hibernation in the fall. These conclusions are supported by similar observations in another hibernating sciurid rodent *S. beldingi* (Morton and Gallup 1975; French 1982).

Because the seasonal gain in body mass in hibernating squirrels is related primarily to the accumulation of fat (Morton 1975), activation of reproductive function may be related to the amount of fat stored at the beginning of hibernation. Golden-mantled ground squirrels generally are not known to store food in their hibernacula (Mullally 1953; McKeever 1964), and thus body reserves of fat and protein at the start of hibernation represent the sources of nutrition that must suffice until food becomes available in spring. These stores may be important not only in winter survival but also for costs associated with emerging early from hibernation.

In the present study and in that of French (1982) on *S. beldingi*, males that underwent gonadal recrudescence terminated hibernation early in spring and prior to the replacement of food in their cages. Males that remained reproductively quiescent, however, continued to hibernate late into spring, and only remained normothermic following an arousal after food was returned. An early emergence from hibernation would benefit reproductive males by increasing their likelihood of encountering sexually receptive females. Since female ground squirrels generally mate shortly after emergence from hibernation (Michener 1983), a late emerging male would find females past sexual receptivity. Because dates of female emergence can vary from year to year (Shaw 1925; Michener 1983; Bronson 1979), the best strategy for a reproductive male should be to become active on a date that precedes the earliest appearance of females. It is the ability of a male to sustain himself at this time in an active metabolic condition that may determine whether he becomes reproductive each year.

Males that remained reproductively quiescent were not severely limited in energy reserves. Body fat content of squirrels analyzed in late March whose weights then were representative of subsequently non-reproductive squirrels (Fig. 1) was well above the seasonal minimum of 2.2% body fat in this species (Kenagy and Barnes, unpublished). Therefore the subsequently nonreproductive males were not being 'starved', but perhaps instead were responding to levels of energy stores that were inadequate to risk emergence from hibernation before food was available. Males that defer reproduction in years of low energy stores presumedly will gain in reproductive opportunities by increasing survivorship.

The earlier mean date of entry into hibernation for the nonreproductive groups (Table 1) may be because most of this group were on the restricted ration. An early entrance into hibernation would minimize metabolic expenditures for an animal with a limited food supply.

A correlation between energetic state and reproductive condition implies a mechanism of assessing nutritional and energetic reserves in golden-mantled ground squirrels that influences reproductive development during winter and, in turn, the timing of emergence from hibernation in spring. How energetic state influences reproductive maturation is unknown; the regulation of hibernation, however, is affected by circulating levels of gonadal androgens. Implantation of testosterone terminates hibernation in Turkish hamsters (Hall and Goldman 1980), and arousals and emergence from hibernation are associated with elevated plasma levels of testosterone in golden-mantled ground squirrels (Barnes 1983). These studies suggest a regulating influence of the testes on hibernation.

Factors affecting body mass

Age and social status might be expected to influence the amount of fat that a squirrel gains prior to hibernation, and thereby its subsequent reproductive status.

Age. Because juvenile golden-mantled ground squirrels do not appear above ground until early July and then must complete their basic growth (Phillips 1981), juveniles have less time in summer and fall to accumulate fat stores than do older adults. Thus yearlings should be the least likely to become reproductive. In fact, for populations of golden-mantled ground squirrels in the Sierra Nevada (Bronson 1977) and the Cascades (Kenagy and Barnes, unpublished), yearlings comprise 80–90% of the reproductively quiescent males. Importantly, however, as many as one-third of the yearlings in these populations do become reproductive. Therefore age at one or two years does not exclusively determine the timing of reproductive maturation. All three or more year old male *S. lateralis* in Bronson's (1977) populations were reproductive. This suggests that size dependency of reproductive maturation may not hold for old individuals in these populations in which the average life expectancy is less than two years (Bronson 1979).

Social conditions. Whether a male develops reproductively each year may depend upon the density of conspecifics encountered or the amount of aggression received (Slade and Balph 1974). These social factors, however, could act either directly in preventing reproductive maturation or indirectly by decreasing a subordinate animal's access to food and thereby its ability to fatten. The test involving social interactions reported here sought to distinguish between direct and indirect effects of social inhibition by determining whether subordinate males that had the opportunity to fatten would still be prevented from developing reproductively. Males housed together in the present experiment were subjected to varying amounts of aggression but each individual fattened substantially and all came into reproductive condition by spring. Golden-mantled ground squirrels generally are thought to be less social than other ground squirrel species (Michener 1983), but they are known to form dominance hierarchies (Wirtz 1967) and appeared to do so in this experiment. However if social factors affect timing of reproductive maturation, they may act in an indirect fashion by influencing a subordinate's ability to accumulate fat.

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