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**Relationships between hibernation and reproduction in male ground squirrels**

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The reduction of body temperature and metabolic rate common to all mammalian hibernators is generally accepted as a physiological strategy for reducing energy needs during seasonal food shortages, which in high latitude and high altitude environments can extend for 7-9 months. To optimise adult survivorship, hibernation might be expected to be prolonged throughout this time until food is once again available. However, the conversely short growing seasons in these environments also select for early starts to reproduction, to time lactation to when foraging costs are affordable and to maximise available time for offspring growth and fattening (Kenagy *et al.* 1989; Michener 1992). Therefore, in hibernating animals there is the potential for conflict between maximising the length of dormancy yet advancing the timing of breeding (Wimsatt 1969). This paper discusses how conflicts between winter dormancy and reproductive development and breeding have been met in male hibernating ground squirrels and describes patterns of reproductive hormone and testis growth during the active season of two species sampled in the field.

A constraint on reproductive development by hibernation is especially apparent in hibernating male ground squirrels, which must end the heterothermic portion of hibernation and resume 2-4 weeks of high, euthermic body temperatures before being physiologically able to breed. In four species in which the relationships between hibernation and male reproductive maturation have been investigated, testes remain undeveloped during the heterothermic portion of hibernation. Males then end torpor earlier than females and undergo gonadal growth and development during the intervening period. For example, during heterothermy in captive golden-mantled ground squirrels, *Spermophilus lateralis*, there is little testicular growth and no advancement of spermatogenic stage; instead, sequential testicular biopsies have shown that only over the first three weeks after the end of torpor do testes actively enlarge, seminiferous tubules expand, and primary spermatocytes divide and mature into elongating spermatids (Fig. 1). Motile spermatozoa first appear in the epididymides only after one month of sustained euthermy after hibernation (Barnes *et al.* 1986). In this species torpor and testicular development and function appear to be completely incompatible, for testes are endocrinologically unresponsive at low tissue temperatures (Barnes *et al.* 1987), and if males return to hibernation before testes have matured, spermatogenesis stops and gonadal involution and regression begins (Barnes *et al.* 1986); reciprocally, males forced to prematurely end hibernation advance the timing of their gonadal development (Barnes & York 1990).
Fig. 1. Change in estimated paired testis mass and plasma testosterone levels in golden-mantled ground squirrel males during and after hibernation. Data are means of 6-12 animals shown relative to when each individual ended hibernation. Redrawn from Barnes et al. (1988).

A similar relationship between the end of hibernation and the completion of gonadal growth and development has been shown in three additional species of ground squirrels. Holekamp and Talamantes (1991) found in a population of California ground squirrels (S. beecheyi) that 2-3 months after emergence from aestivation is required for adult males to become 100% spermatogenic. Using temperature-sensitive radiotelemetry collars on Richardson's ground squirrels (S. richardsoni) overwintering in natural burrows, Michener (1992) demonstrated that males end hibernation on average 27 days before females and complete spermatogenesis during that interval. We have followed the establishment of spermatogenesis before and after hibernation in arctic ground squirrels (S. parryii) living in outdoor enclosures in Fairbanks, Alaska. Males end torpor 2-6 weeks before females (Barnes & Ritter 1993), and at that time have testes containing only spermatogonia and spermatocytes, an initial stage of maturation. In both Richardson's and arctic ground squirrels, subsequent testicular biopsies show spermatogenesis proceeding to the stage of motile spermatozoa within seminiferous tubules in as little as 9-10 days after hibernation ended. Including the time it takes for sperm to transit to and mature within the epididymis, however, adds on an additional 2.5-5 days. Thus, at least 11.5 days in these species and 31 days or longer in S. lateralis and S. beecheyi are required after resumption of post-torpor euthermia for the development of male reproductive competence.

Male ground squirrels spend much of this period of reproductive development remaining sequestered within their hibernacula during a pre-emergence euthermic interval (PEI) (Barnes et al. 1986; Michener 1993). Telemetry studies have shown that, after ending torpor, males may remain sequestered underground at sustained high body temperatures for up to 25 days in S. richardsoni (Michener 1992), 21 days in S. parryii (Barnes & Ritter 1993), and 12 days in S. colombianus (Young 1990) (Table 1). Combining
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The laboratory data of 31 days required for maturation in golden-mantled ground squirrels (Fig. 1) with field examinations of newly emerged males in fully mature reproductive condition (Bronson 1979; Kenagy & Barnes 1988) suggests male golden-mantled ground squirrels spend a month-long PEI before resuming above ground activity.

<table>
<thead>
<tr>
<th>Species</th>
<th>Average</th>
<th>Range</th>
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<tbody>
<tr>
<td>S. colombianus (Young 1990)</td>
<td>8.0 days</td>
<td>3–12 days</td>
</tr>
<tr>
<td>S. parryi (Barnes &amp; Ritter 1993)</td>
<td>15.2 days</td>
<td>10–21 days</td>
</tr>
<tr>
<td>S. richardsoni (Michener 1992)</td>
<td>15.8 days</td>
<td>2.5–25 days</td>
</tr>
<tr>
<td>S. lateralis (*)</td>
<td>31 days</td>
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* Assumed from laboratory studies. Data for other species are from telemetry studies under field or semi-natural conditions (S. parryi).

Yet, among and within each of these species there is a wide variation in the length of the PEI (Table 1), with some individuals spending only 2-3 days euthermic before coming above ground. Variation in the length of PEI may be due to intrinsic differences in rates of reproductive maturation between species and to differences in the relative costs and benefits for males of being above or below ground before females emerge. Benefits to remaining underground would include maximising the rate at which males replenish winter weight loss by feeding from caches of food in the hibernacula (Michener 1999). Male S. saturatus and S. parryi are capable of emerging in spring at body weights exceeding those at immergeace the previous fall (Kenagy et al. 1989; Buck & Barnes, unpublished). This recovery, with its consequences for improved body condition in spring, is presumed to occur during the PEI, and thus the size and quality of a male's cache should play an important role in determining the size and competitiveness of males during the ensuing mating season. Advantages of coming above ground soon after ending torpor might relate to new foraging opportunities in the absence of a cache or after its depletion and to the importance of social and exploratory behaviours to different individuals and species at this time of year. In arctic ground squirrels in Alaska, foraging opportunities in early spring are nil, as males usually must tunnel through decimeters of snow to emerge (Hock 1960), but bare ground and greenery exist prior to first emergence of male golden-mantled ground squirrels in the Cascade Mountains of Washington State (Kenagy & Barnes 1988). On this basis relatively long and short PEIs could be predicted for these two species. But the importance and degree of general sociality varies widely among ground squirrel species with arctic ground squirrels ranked among the most social and golden-mantled ground squirrels ranked the least social (Michener 1984). To the degree that they may reflect the relative importance of male–male interactions in early spring, these rankings would result in the opposite prediction of comparative PEI lengths in these two species. Costs of appearing above ground early would relate to potential for increased risk from predators and, due to increased activity and exposure, increases to daily existence metabolism (Chappell 1981). Within species differences in cache size, the importance of social interactions, and the need for above ground assessment of environmental conditions
may also contribute to individual differences in the length of the PEI. More investigations of these relative risks and benefits to males in early spring will be required to increase understanding of comparative PEI lengths in different ground squirrel populations.

On a mechanistic level the rate of reproductive development and pattern of accompanying changes in circulating levels of reproductive hormones during the PEI may determine when individual males first are stimulated to resume above ground behaviour. This hypothesis is supported by the pattern of circulating testosterone concentrations in captive golden-mantled ground squirrels during their post-heterothermic period of gonadal growth and maturation. In the first 3 weeks of euthermy, testosterone levels are significantly elevated over levels measured during torpor, but at <5ng/mL levels are low relative to the increase to the seasonal peak of 10-20ng/mL in week 4-5 post torpor (Fig. 1). This increase in testosterone occurs after testis development is near complete, and high levels are sustained after testes begin to regress, therefore testosterone concentrations are not merely directly correlated with testis size. Instead plasma testosterone levels may be regulated to remain at moderate levels compatible with the establishment of spermatogenesis during the interval males remain sequestered in their burrows, but then increase to high levels at the end of the PEI, stimulating emergence and the beginning of aggressive and reproductive behaviours associated with the mating season.

Elevating testosterone levels inhibits hibernation in ground squirrels (Lee et al. 1990), and a sustained increase in circulating testosterone in males is what endocrinologically distinguishes terminal arousals from previous, periodic arousals from torpor (Barnes et al. 1988). Thus, an additional function of the moderate rise in testosterone after the end of heterothermy may be to prevent males from returning to torpor, thereby ensuring sustained euthermy and active reproductive maturation.

To further characterise relationships between reproductive function and hibernation in the annual cycle of hibernating ground squirrels, patterns of gonadal size and reproductive hormones during the active season of free-living male ground squirrels of two species are presented.

**Methods**

Golden-mantled ground squirrels (S. saturatus) were captured on the east slope of the Cascade Mountains near Fish Lake, Chelan County, Washington (47°49' elevation 600-675m). Animals were live trapped along logging roads during monthly or semi-monthly trapping sessions between March and September 1981 (Kenagy & Barnes 1988). Captured animals were driven to a cabin near the field site and, after 1-2 min of ether anaesthesia, sampled for 2mL of blood by cardiac puncture. Time from capture to processing varied 2-10 hours. Plasma was separated and kept at -5°C for 1-2 days and then at -20°C until assayed within 6 months. Testosterone concentration was measured in plasma by RIA after extraction with dichloromethane and celite chromatography as detailed in Barnes (1986). Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in plasma from S. saturatus were measured by RIA using anti-rat antisera as described in Barnes (1986). Data on mass of testes are from Kenagy and Barnes (1988).

Arctic ground squirrels (S. parryii) were captured on the north slope of Alaska along the northern and eastern shores of Toolik Lake (68°38' elevation 809m). Animals were live trapped in weekly to monthly intervals from late April to early September 1989-1991. Traps were opened throughout the day and checked each 1-3 hours. Animals were brought to a trailer near the field site, and after 3-5min of methoxyflurane anaesthesia, sampled exposure estimates with abs to recover varied fr year. Tes Barnes e compare multiple p<0.05 l

**Results**

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sampled for 1mL of blood by cardiac puncture. Each 2-4 weeks males, with continued exposure to the anaesthetic, were laparotomized and one testis exposed and its mass estimated from linear dimensions (Barnes et al. 1986). After underlying tissue was stitched with absorbable sutures and skin closed with sterile cotton thread, animals were allowed to recover for 1-2 hours and released at the site of capture. Time from capture to processing varied from 1-4 hours. Plasma was stored below -20°C before being assayed within one year. Testosterone was measured by RIA after extraction with ethyl ether as detailed in Barnes et al. (1988). Seasonal changes in hormone concentrations and testis size were compared by Kruskal-Wallis one way ANOVA on ranks; when significant, Dunn's pairwise multiple comparison was used to determine which groups differed from others at the p<0.05 level of significance.

Results
When first trapped after emergence from hibernation the first week of April in the eastern Cascades Mountains, male golden-mantled ground squirrels had fully enlarged and spermatogenic testes (Fig. 2). Testis size significantly declined in May from its seasonal peak in late April, and by late June testes were at their minimum size and non-spermatogenic (Kenagy & Barnes 1988). Testosterone in plasma was slightly elevated in the first males caught and increased to the seasonal high in adults two weeks later, after females had emerged and the time most representative of when copulations occurred that year (Kenagy & Barnes 1988). Among individuals testosterone concentrations were highly variable, and means did not differ statistically in April and May before reaching basal levels beginning in June. Juvenile males appeared in early July and were the only males caught in late August. Testes in juveniles remained small, undeveloped, and aspermatogenic until animals could no longer be trapped above ground in fall (Kenagy & Barnes 1988). However, plasma testosterone levels in juvenile males increased significantly after July, with individual values as high as 5.0ng/mL in August. In adults and juveniles plasma LH levels paralleled changes in testosterone levels and FSH levels remained unchanged and basal throughout the active season. In captive golden-mantled ground squirrels, blood FSH levels show an annual peak that parallels but precedes testis growth (Barnes 1986); any similar peak in field animals must have occurred before males emerged.

Arctic ground squirrels were already active above ground when trapping began on 24 April 1989 at Toolik Lake, Alaska. Since first emergence of males in this population occurs as early as 12 April in other years (Buck & Barnes, unpublished), males may have been active above ground for about 2 weeks before first being sampled in this study. In late April males had fully enlarged and spermatogenic testes. Testes significantly regressed in late May and remained small and undeveloped July-September. Plasma testosterone levels were elevated during spring in adult males and basically remained elevated throughout their active season. A statistically significant peak in mean testosterone concentration occurred in mid-August in adult males. Mid-August levels were greater than levels in late July and early September (p<0.001), whereas other means were statistically indistinguishable. In juveniles, testosterone levels, minimal when pups first appeared above ground in July, also rose to a seasonally significant peak (p<0.001) in mid-August and decreased to low levels by early September before animals entered hibernation. Testes in juveniles remained small, undeveloped, and aspermatogenic throughout their first summer.
Fig. 2. Mean (± SE) plasma concentrations of A luteinizing hormone (LH), follicle-stimulating hormone (FSH) and B testosterone and testis size during the active seasons of adult and juvenile male golden-maniled ground squirrels living in the Cascade Mountains, Washington and, C for testosterone and testes only, of adult and juvenile male arctic ground squirrels living in the northern foothills of the Brooks Range, Alaska. Number of animals sampled is shown next to each mean; sample numbers in A are the same as shown for testosterone in B.
Discussion

Because of the incompatibility of hibernation and active gonadal growth and the length of time required for testis maturation, male golden-mantled and arctic ground squirrels trapped with large, spermatogenic testes in April must have been euthermic for 2-4 weeks before being sampled. Most or all of this interval is likely spent underground in golden-mantled ground squirrels, where first emergence of males is early to mid April (Kenagy & Barnes 1988; Kenagy et al. 1989), but telemetry studies of animals in enclosures (Barnes & Ritter 1993) and observance of males above ground at the field site as early as 12 April (Buck and Barnes, unpublished), suggest male arctic ground squirrels emerge part way during their interval of reproductive development. This conclusion is supported by Hock's (1960) description of male S. parryii living south of the Alaska Range first appearing through the snow in spring with only partly developed testes.

The spring pattern of plasma concentrations of testosterone in male golden-mantled ground squirrels is very similar to that described in free-living California ground squirrels (Holekamp & Talamantes 1991, 1992), with levels peaking before and during the mating season and declining before matings were over. Holekamp & Talamantes (1992) attribute this pattern to a hypothesised importance for direct stimulation by testosterone of behaviours related to space use and territoriality in males before females appear and to a temporal lag between blood levels of testosterone and effects on agonistic and sexual behaviours. No specific information on the occurrence and timing of these behaviours is available for golden-mantled ground squirrels to correlate with the hormone levels reported in this study, but Kenagy et al. (1989) report a seasonal high (8.2% of above ground time) in April in time spent in running and chasing behaviours for golden-mantled males living in the same study area. In male arctic ground squirrels testosterone levels did not decrease before or after matings, but instead remained elevated to mid-May and were still elevated in early July. Lack of sampling in June and low sample sizes in late July prevent characterising whether testosterone levels ever reach baseline before September in male arctic ground squirrels.

In August plasma testosterone levels significantly increased in adult and juvenile arctic ground squirrels and in juvenile golden-mantled ground squirrels; in arctic males testosterone levels reached their seasonal peak at this time. A late summer increase in testosterone was also seen in free-living juvenile California ground squirrels. Holekamp & Talamantes (1991) correlate this “pulse” to the initiation of testicular and penile development which began 1-2 months later in these nonhibernating juveniles, and they compare it to the brief increases in testosterone that are functionally related to the activation of puberty in other mammals. A very slight increase in testis size accompanied the late summer rise of testosterone in arctic and golden-mantled males, but active maturation in these species does not occur until 7-8 months later. Late summer increases in circulating testosterone may potentiate such delayed gonadal development; they may also be causally related to late summer changes and intensification of behaviours in male arctic and golden-mantled ground squirrels. Males in late summer disperse and increase foraging and burrow guarding behaviours (Kenagy et al. 1988; Buck & Barnes, unpublished). Territoriality and male-male aggression in arctic ground squirrels can reach seasonal highs at this time of year (Carl 1971). Further discussion of functional links between August changes in testosterone and behaviour in male ground squirrels await manipulative experiments in the field.
Fig. 3. Chronologies of hibernation and reproduction for adult males (M) and females (F) of 5 species of North American ground squirrel living at the indicated latitudes. Shown are the average dates for the end of heterothermy, the average length of the pre-emergence euthermic interval (PEI), range of mating dates, average gestation and lactation intervals, range of immurgence dates, and average length of pre-immurrence euthermic interval (PIEI) and date of beginning of heterothermy. Data are compiled from Barnes & Ritter (1993) and Buck & Barnes (unpublished) for S. parrtyii, Murie & Harris (1982) and Young (1990) for S. colombianus, Kenagy et al. (1989) for S. saturatus, Michener (1992) for S. richardsoni, and Bronson (1979) and Phillips (1984) for S. lateralis.

The elevated but, on a seasonal basis, moderate concentrations of testosterone in newly emerged golden-mantled ground squirrels do not support the hypothesis that maximal levels of testosterone are required to stimulate emergence of males from their hibernacula. Testosterone levels were also low in 3 of 5 male California ground squirrels sampled soon after emergence from estivation (Holekamp & Talamantes 1992), although in this population there is a 2-3 month interval between emergence of males and mating, which suggests these males need not maximise their season of hibernation. Spring-time measurements of hormone levels in arctic ground squirrels came too late to examine relationships between plasma testosterone and emergence from the hibernacula in these males. Rejection of the hypothesis linking testosterone levels with emergence timing leaves the pattern of testosterone rising 3-4 weeks after the end of heterothermy in golden-mantled ground squirrels (Fig. 1) unexplained, though its persistence in captive males, isolated from females and in constant environmental conditions, is part of a robust and endogenously generated annual cycle of reproductive function (Barnes 1986).

Finally, though compared in different years, the timing of mating and testis regression in S. parrtyii and S. saturatus were remarkably similar, despite 20 degrees of latitude and the arctic circle separating the two populations. To further characterise the
timing of reproduction and hibernation in North American ground squirrel species, Fig. 3 compares these events in 5 species separated by over 6000km from north to south. Emergence times and the short (<3 week) mating periods are overlapping in 4 of 5 species, and active season lengths are similar in all 5, again emphasizing the importance in hibernating ground squirrels of early starts to reproduction that provide long growing and fattening seasons for young. The climate and food availability experienced by these animals at emergence, however, differ greatly among populations.

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References


