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Original Investigation

Age and sex differences in hibernation patterns in free-living Anatolian ground squirrels

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ABSTRACT

Anatolian ground squirrels, *Spermophilus xanthoprymnus* (Bennett, 1835), are nearly endemic to Turkey. Various aspects of the biology of Anatolian ground squirrels have been studied. However, a detailed description of hibernation, with special attention to age and sex differences, has not previously been reported. Thus, in this study, we aimed to present a detailed description of hibernation in a population of free-living Anatolian ground squirrels, with special attention to age and sex differences, in relation to soil temperature (Tsoil) at the expected depth of the hibernacula. This study is the first such study for Old World ground squirrels (the genus *Spermophilus* sensu stricto) and incorporates an additional species from a different environment (e.g. warmer than those of others) into comparative studies of hibernation patterns in Holarctic ground squirrels (the tribe Marmotini) under natural conditions. Body temperature (Tb) was continuously recorded from late summer to spring by intraperitoneally implanted temperature loggers and Tsoil by a temperature logger at a depth of 1 m in the field. Anatolian ground squirrels spent about half of the year in hibernation. However, differences in timing of the beginning and end of hibernation resulted in differences in the duration of hibernation among age-sex classes. The duration of torpor bouts was short at the beginning of hibernation, increased as Tsoil decreased, reached its maximum toward the end of hibernation, and decreased thereafter. Adult males exhibited hibernation characteristics rather different from those of other age-sex classes, spending less time in hibernation, exhibiting shorter torpor bouts and longer interbout arousals, especially toward the end of hibernation, and spending proportionally less time in torpor. In conclusion, we observed that hibernation patterns in free-living Anatolian ground squirrels were influenced by age-sex classes, as well as environmental conditions.

Introduction

From an ecophysiological point of view, animals must maintain a long-term balance between energy income and energy expenditure (McNab, 1974). This balance is particularly challenging for small mammals that require large amounts of energy to maintain their high metabolism (Aschoff, 1981). Moreover, especially in small mammals of the temperate zone, the energetic cost of maintaining high metabolism is exacerbated during winter months when both ambient temperature (Ta) and the quantity and/or quality of food resources are low (Bartholomew, 1982; Speakman, 2000). Many small mammals overcome these energetic constraints by employing torpor, a temporary and controlled reduction of metabolism, body temperature (Tb), and other physiological functions, as an energy-saving strategy (Lyman, 1982; Heldmaier and Ruf, 1992). Expression of torpor can be highly flexible among species and even among individuals of a single species, although two common patterns of torpor have been most commonly recognized: daily torpor (lasting < 24 hours) and hibernation (Geiser and Ruf, 1995; Ruf and Geiser, 2014). Hibernation consists of a sequence of multiday torpor bouts, during which metabolism decreases significantly below basal metabolic rate and Tb is often lowered, universally interrupted by brief interbout arousals (Pengelley and Fisher, 1961; Pohl, 1961; Wang, 1979; Geiser, 1988; Ruf and Geiser, 2014).

Anatolian ground squirrels, *Spermophilus xanthoprymnus* (Bennett, 1835), are group-living, diurnal, hibernating, and predominantly herbivorous, burrowing ground-dwelling squirrels (Kryštufek and Vohralík, 2005; Kart Gür and Gür, 2010). They are nearly endemic to Turkey, present in central and eastern (especially northeastern) Anatolia, with minor range extensions into Armenia and northwestern Iran. Anatolian ground squirrels associate with a continental climate, i.e. a drier climate with greater seasonality, and inhabit short-grass steppes (Kryštufek and Vohralík, 2005,

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2012; Kart Gür and Gür, 2010; Gür, 2013). In central Anatolia, they hibernate individually in underground burrows, from about late summer or early autumn (August or September) to late winter or early spring (February or March). Females mate shortly after emergence from the hibernacula and wean only one litter each year. Parturition occurs in April and juveniles appear aboveground in May. Anatolian ground squirrels appear to rely mainly on fat reserves stored during the active season as the source of energy overwinter and therefore dramatically lose body mass during hibernation (Gür and Kart Gür, 2005; Kart Gür and Gür, 2010).

Various aspects of the biology of Anatolian ground squirrels have been studied (Yiğit et al., 2000; Kart Gür and Gür, 2010 and references therein). However, although daily rhythmicity of Tb before and during hibernation under both natural and laboratory conditions was examined using intraperitoneally implanted temperature loggers (Kart Gür et al., 2009), a detailed description of hibernation, with special attention to age and sex differences, has not previously been reported. Thus, in this study, we aimed to present a detailed description of hibernation in a population of free-living Anatolian ground squirrels, with special attention to age and sex differences, in relation to soil temperature (Tsoil) at the expected depth of the hibernacula. This study is the first such study for Old World ground squirrels (the genus *Spermophilus* sensu stricto; Helgen et al., 2009), because previous studies were performed under either semi-natural (*Spermophilus citellus*; Hut et al., 2002 – a sister species of Anatolian ground squirrels) or laboratory conditions (*S. dauricus*; Yang et al., 2011). Moreover, this study incorporates an additional species from a different environment (e.g. warmer than those of others; see Fig. 5) into comparative studies of hibernation patterns in Holarctic ground squirrels (the tribe Marmotini; Thorington et al., 2012) under natural conditions and therefore extends the understanding of how hibernating mammals respond to the climatic and ecological conditions of the environment in which they live. Owing to previous findings on differences in autumnal and spring behaviors and requirements for reproductive maturation of males and females in hibernating species of ground squirrels (Williams et al., 2014 and references therein; Gür and Kart Gür, 2005; Kart Gür and Gür, 2010), we hypothesized that hibernation characteristics would vary substantially among age-sex classes.

Material and methods

Ethics statements

This study was carried out according to the "Guidelines for the Capture, Handling, and Care of Mammals as Approved by the American Society of Mammalogists" (Animal Care and Use Committee, 1998) and the "Guide for the Care and Use of Laboratory Animals" (Institute for Laboratory Animal Research-ILAR, 1996), and approved by the Local Ethical Committee for Animal Care and Use of Hacettepe University School of Medicine, Ankara, Turkey.

Study area

The study area is a short-grass steppe landscape located in the dry forest-anthropogene steppe subregion of the central Anatolian continental ecoregion (Atalay and Mortan, 2006), about 50 km south of Ankara, Turkey (39.48 N, 32.85 E, 1 190–1 205 m altitude). In the study area, mean annual air temperature is 10.0 °C, with the warmest quarter from June to August (mean = 20.2 °C) and the coldest quarter from December to February (mean = −0.4 °C). Mean annual precipitation is 399 mm, with the wettest quarter from March to May (mean = 134 mm) and the driest quarter from July to September (mean = 44 mm).

Field studies

The field studies were conducted from April 2005 to May 2007 in a population of free-living Anatolian ground squirrels. Individuals were trapped using treadle-style, wire-mesh live traps (Collapsible Traps with One Trap Door, Code: 202; Tomahawk Live Trap Co., Tomahawk, WI, USA) baited with peanut butter, placed at burrow entrances. At first capture, Anatolian ground squirrels were marked permanently with a numbered metal tag in each ear (National Band and Tag Co., Newport, KY, USA) and their fur was marked with a commercial hair dye in individually recognizable patterns. At each capture, date, location, tag number, age, sex, body mass (±5 g, Pesola spring scale; Pesola AG, Rebmattli, Baar, Switzerland), and reproductive status were recorded. Individuals were identified as juvenile if trapped during the active season of their birth year and as adult after their first hibernation. Males were defined as reproductive if they had testes descended into a pigmented scrotum. Females were defined as pregnant if they had enlarged, darkly pigmented teats and as lactating if they had large, lightly pigmented teats with surrounding fur flattened (Gür and Kart Gür, 2005; Kart Gür and Gür, 2010).

Towards the end of the active season (on multiple dates from 13 June to 22 August of 2005 and 2006), 37 free-living Anatolian ground squirrels chosen for implanting temperature loggers were trapped and then released to the field after implantation of the loggers. At the beginning of the active season (on multiple dates from 21 February to 26 April of 2006 and 2007), 23 (62%) of 37 free-living Anatolian ground squirrels (three adult males, nine adult females, four juvenile males, and seven juvenile females) implanted with temperature loggers were re-trapped and then released to the field after removal of the loggers. Because of the risk of predation in the field (Gür and Barlas, 2006), these individuals were trapped within a few days after emergence from the hibernacula.

Tsoil was recorded by a temperature logger (DS1922L; for specifications, see below) at a depth of 1 m in the field. This depth was chosen, because it is the expected depth of the hibernacula of Anatolian ground squirrels (Karabağ, 1953; Kart Gür et al., 2009).

Temperature loggers

Tb of free-living Anatolian ground squirrels was recorded by two types of temperature loggers: Thermochron iButtons (DS1922L, ~3 g, range −40 to 85 °C, resolution 0.0625 or 0.5 °C; Maxim Integrated Products, Inc., Sunnyvale, CA, USA) or StowAway Tidbits (customized temperature data logger, 8.7 g, range −4 to 44 °C, resolution 0.16 °C; Onset Computer Corporation, Bourne, MA, USA). Adults were surgically implanted with Thermochron iButtons and juveniles with StowAway Tidbits. The loggers were programmed to record Tb at 15-min (StowAway Tidbits) or 50-min (with a resolution of 0.5 °C; Thermochron iButtons) intervals. All other details of preparation of the loggers were described by Kart Gür et al. (2009).

Implantation and removal of temperature loggers

Free-living Anatolian ground squirrels were transported from the field to the laboratory at Hacettepe University, Ankara, Turkey, at a distance of approximately 60 km. Temperature loggers were surgically implanted into or removed from the peritoneal cavity of individuals under sterile conditions and general anesthesia. After recovery, individuals were released to the field at the site where they were trapped. All other details of implantation and removal of the loggers were described by Kart Gür et al. (2009).