Why do Anatolian ground squirrels exhibit a Bergmannian size pattern? A phylogenetic comparative analysis of geographic variation in body size

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A phylogenetic comparative analysis of geographic variation in body size of an obligately hibernating marmotine species (Anatolian ground squirrels, *Spermophilus xanthoprymnus*) is presented in relation to environmental variables that pertain to four principal hypotheses (heat conservation, heat dissipation, primary productivity, and seasonality hypotheses). Adult Anatolian ground squirrels (78 males and 90 females) were collected from ten geographic localities in Anatolia for use in morphometric analyses. First, the study tested whether significant variation in body size occurs over the geographic range of *S. xanthoprymnus*. Then, to understand the possible cause(s) of the observed pattern of geographic variation in body size of Anatolian ground squirrels, four hypotheses were tested, separately and simultaneously, using a phylogenetic comparative method. Overall, food availability (primary productivity hypothesis) and, especially in males, over-winter fasting endurance (seasonality hypothesis) are likely the primary underlying mechanisms generating the observed pattern of increasing body size towards colder, more seasonal environments, with higher summer precipitation and productivity (or a Bergmannian size pattern). © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **100**, 695–710.

ADDITIONAL KEYWORDS: Bergmann's rule – ecogeographic rules – evolution – hibernation – marmotine squirrels – phylogenetic generalized least-squares – subspecies.

INTRODUCTION

Because body size has important physiological, ecological, and evolutionary consequences, it is one of the most important characteristics of an animal (Calder, 1984; Schmidt-Nielsen, 1984). Moreover, body size is one of the most prominent organismal characteristics that respond to local environmental factors and therefore show geographic variation. Variation in body size along spatial and environmental gradients within species of birds and mammals has been documented in many studies (Ashton, Tracy & de Queiroz, 2000; Ashton, 2002; Meiri & Dayan, 2003). Four principal hypotheses have been proposed to explain these patterns of geographic variation (Wigginton & Dobson, 1999): (1) heat conservation (Bergmann, 1847; James, 1970; traditionally appended to Rensch's (1938) and Mayr's

Mayr's (1956, 1963) intraspecific formulation of Bergmann's rule; (2) heat dissipation (James' intraspecific formulation of Bergmann's rule; James, 1970, 1991; Aldrich & James, 1991; see also Hamilton, 1961); (3) primary productivity (Rosenzweig, 1968a); and (4) seasonality (Boyce, 1978; Lindstedt & Boyce, 1985; Millar & Hickling, 1990). These explanations all assume that patterns of geographic variation in body size are the result of natural selection (Ashton *et al.*, 2000).

The best known of the above-mentioned hypotheses is heat conservation. This hypothesis suggests that, within species of endothermic animals, individuals should be larger in colder than in warmer environments. The lower surface-to-volume ratio of larger individuals serves heat conservation in colder environments (Bergmann, 1847; James, 1970; but see also McNab, 1971, 1999, 2002). This mechanism traditionally is appended to Rensch's (1938) and Mayr's

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(1956, 1963) intraspecific formulation of Bergmann's rule (an increase in body size of a species with decreasing ambient temperature; this is frequently referred to as Bergmann's rule; Ashton *et al*., 2000; Ashton, 2002; Meiri & Dayan, 2003; de Queiroz & Ashton, 2004; but see also Blackburn, Gaston & Loder, 1999). However, Bergmann's rule is purely an empirical pattern and makes no statement about the possible hypotheses (Mayr, 1956, 1963); for mechanisms other than heat conservation, see below.

James (1970, 1991) and Aldrich & James (1991) suggested that intraspecific variation in body size of endothermic animals is related to a combination of ambient temperature and humidity. Consequently, heat dissipation hypothesis states that individuals living in warm humid environments should be smaller than individuals living in either colder or drier environments. The higher surface-to-volume ratio of smaller individuals facilitates heat dissipation in warm humid environments.

Body size must be maintained by a sufficient food supply. Natural selection should adapt animals to various energy flow rates by modifying their body size in accord with productivity (Rosenzweig, 1968a; Wigginton & Dobson, 1999). Thus, primary productivity hypothesis suggests that body size should be positively correlated with actual evapotranspiration (Rosenzweig, 1968a), a significant predictor of primary productivity (Rosenzweig, 1968b).

One of the most important aspects of seasonal environments is variation in the availability of an essential resource (e.g. food, water, nutrients, or energy; Boyce, 1979). Energy (fat) reserves increase with body size faster than metabolic rate and therefore larger individuals have greater fasting endurance or longer survival time during periods of food shortage in seasonal environments. Consequently, seasonality hypothesis states that body size should be larger in more seasonal than in less seasonal environments (Boyce, 1978; Lindstedt & Boyce, 1985; Millar & Hickling, 1990; but see also Dunbrack & Ramsay, 1993). This empirical pattern 'is possible only if food availability permits the higher cost of maintenance inherent in a large size' (McNab, 2002).

As understood from these hypotheses, environmental variables that have been proposed to influence geographic variation in body size include ambient temperature (heat conservation hypothesis), a combination of ambient temperature and humidity (heat dissipation hypothesis), primary productivity (primary productivity hypothesis), and seasonality (seasonality hypothesis). One way to understand the mechanisms generating patterns of geographic variation in body size within species (e.g. Bergmannian size patterns) is to test different environmental variables separately and, because these variables are usually intercorrelated, especially simultaneously (Wigginton & Dobson, 1999). Few studies exist in this respect and therefore more studies are clearly needed (Ashton, 2004a).

Ground squirrels (*Spermophilus*), prairie dogs (*Cynomys*), and marmots (*Marmota*) are among the most intensively studied groups of mammals with respect to their ecology and behaviour. Although obligate seasonal hibernation is characteristic of many species in these genera (*Marmota*: Armitage, 2003; *Cynomys*: Hoogland, 2003; *Spermophilus*: Yensen & Sherman, 2003; see also Michener, 1983, 1984), patterns of geographic variation in body size and their relationship to a suite of interacting environmental variables have not been well documented in hibernating species of marmotine squirrels. Such burrowing mammals, however, may be treated as special cases in studies of Bergmann's rule because they are expected to avoid harsh environmental conditions and therefore should not exhibit Bergmannian size patterns (despite some preliminary evidence to the contrary; Panteleev *et al*., 1998). This makes hibernating species of marmotine squirrels an interesting system in which to examine the association between body size and environment.

Anatolian ground squirrels, *Spermophilus xanthoprymnus* (Bennett, 1835), are group-living, diurnal, obligately hibernating marmotine rodents inhabiting steppe areas throughout central and eastern (especially northeastern) Anatolia and adjacent Armenia and northwestern Iran (Fig. 1). Anatolian ground squirrels are mainly active from March to September and hibernate during the remaining months (Gür & Kart Gür, 2005; Kart Gür, 2008). *Spermophilus xanthoprymnus* exhibits an extensive degree of geographic variation in body size (Mursaloğlu, 1965; Kryštufek & Vohralík, 2005), although the nature and geographic pattern of this variation has not been well investigated. The present study reports a phylogenetic comparative analysis of geographic variation in body size of Anatolian ground squirrels in relation to environmental variables that pertain to the four hypotheses (heat conservation, heat dissipation, primary productivity, and seasonality hypotheses). First, *sensu* Wigginton & Dobson (1999), the study tested whether significant variation in body size occurs over the geographic range of *S. xanthoprymnus*. Then, these hypotheses were tested, separately and simultaneously, to understand the possible cause(s) of the observed pattern of geographic variation in body size of Anatolian ground squirrels. Because populations of *S. xanthoprymnus* show clear phylogeographic structuring, with subdivision into five cytochrome *b* (cyt *b*) lineages (Gündüz *et al*., 2007) and may not represent independent samples, the effects of explanatory variables on body size were
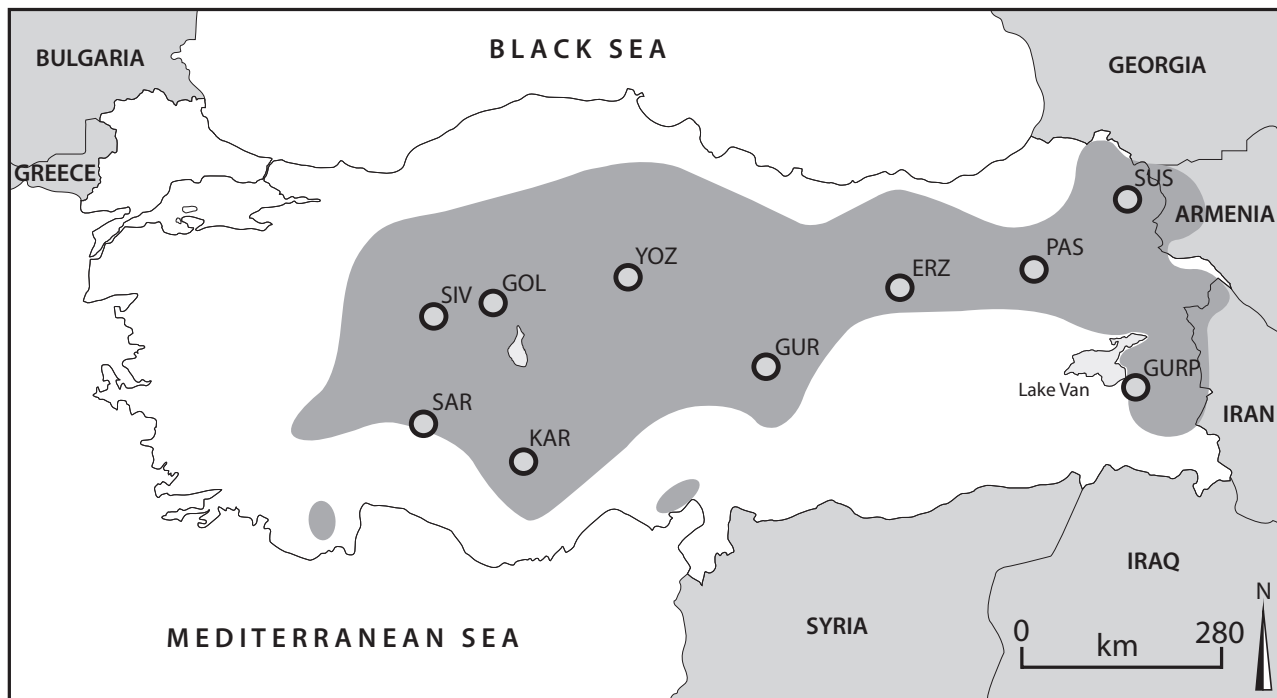
Figure 1. Geographic range of Anatolian ground squirrel (*Spermophilus xanthoprymnus*; Kryštufek & Vohralík, 2005; Demirsoy, 2006; Gündüz *et al.*, 2007; Gür, 2007; Özkurt *et al.*, 2007). Abbreviations correspond to geographic localities where Anatolian ground squirrels were collected. Abbreviations and names (nearest province, district) of the collection localities (arranged in the order of longitude), sample sizes (male, female), geographic coordinates, and elevation: SAR, Isparta, Şarkikaraağaç (3, 10; 37.999°N, 31.469°E; 1245 m); SIV, Eskişehir, Sivrihisar (5, 5; 39.191°N, 31.624°E; 806 m); GOL, Ankara, Gölbaşı (4, 4; 39.478°N, 32.849°E; 1185 m); KAR, Konya, Karapınar (8, 13; 37.713°N, 33.314°E; 1005 m); YOZ, Yozgat, Merkez (10, 10; 39.853°N, 34.959°E; 1182 m); GUR, Sivas, Gürün (10, 10; 38.782°N, 37.102°E; 1691 m); ERZ, Erzincan, Merkez (10, 10; 39.823°N, 39.396°E; 1489 m); PAS, Erzurum, Pasinler (10, 9; 39.983°N, 41.594°E; 1700 m); SUS, Kars, Susuz (10, 10; 40.726°N, 43.157°E; 1687 m); GURP, Van, Gürpınar (8, 9; 38.293°N, 43.373°E; 1752 m).

analysed using a phylogenetic comparative method. To date, phylogenetic comparative analyses have seldom been used in testing for intraspecific patterns (Gaston, Chown & Evans, 2008; but see Ashton, 2001; Niewiarowski, Angilletta & Leaché, 2004). This is the first intraspecific phylogenetic comparative analysis of geographic variation in body size in any mammal.

The analysis of size variation in Anatolian ground squirrels permits a comprehensive evaluation of whether a Bergmannian size pattern is exhibited by a hibernating, burrowing mammal, as well as environmental variables that influence geographic variation in body size. A number of features in the annual cycle of Anatolian ground squirrels are shared with most hibernating species of marmotine squirrels (Gür & Kart Gür, 2005), so the findings obtained with respect to *S. xanthoprymnus* may also apply to hibernating marmotine squirrels and therefore presumably to other fat-storing hibernators.

MATERIAL AND METHODS

During June and July 2005 and 2006, 168 adult (≥ 1-year-old) Anatolian ground squirrels (*S. xanthoprymnus*; 78 males and 90 females) were collected from ten geographic localities in Anatolia (Fig. 1) for use in morphometric analyses. These localities range over approximately 3° latitude (335 km), approximately 12° longitude (1025 km), and approximately 1000 m of elevation. Ground squirrels were trapped using live traps (Tomahawk Live Trap Co.) baited with peanut butter. At capture, age, sex, body mass, and reproductive condition were recorded. Adults were distinguished from juveniles especially by body mass (there was no overlap in body mass between juveniles and adults during the collection period when juveniles were approximately 2–3 months old; Gür & Kart Gür, 2005; Gür & Barlas, 2006), and sex was determined from ano–genital distance (considerably longer in males than in females; Karabağ, 1953).

Thus, body mass and ano–genital distance are highly reliable characters to determine age (juvenile or adult) and sex, respectively. Body mass was measured with a spring scale (Pesola AG) to the nearest 5 g.

Live-trapped adult Anatolian ground squirrels were euthanized in the field, in accordance with guidelines for the capture, handling, and care of mammals as approved by American Society of Mammalogists (Animal Care and Use Committee, 1998). Skulls were prepared and measured with digital callipers to the nearest 0.01 mm in the laboratory. Four measurements were chosen that jointly summarize body size of this species: condylobasal length (CBL; Mursaloğlu, 1965), zygomatic width (ZW; Mursaloğlu, 1965), length of mandible (ML; from the plane formed by posterior points of angular and condyloid processes to anteromedial margin of incisor's entry into mandible), and height of mandible (MH; Barnett, 1977). These measurements were made twice, with an interval of at least 1 week between the measurement sessions. Univariate one-way analysis of variance (ANOVA) was used to determine measurement error associated with the four skull characters in each sex. Individual was included as a random-effect factor (Model II ANOVA; Sokal & Rohlf, 1995). Consequently, within- and among-individual components of variance of these characters were estimated for each sex. Measurement error (%ME) was given as the percent of the total variance attributable to within-individual variance component (Gür, 2004).

Before statistical analyses, skull characters were transformed to $\log_{10}$ values and screened for univariate and multivariate outliers and normality, equality of variances, and equality of variance–covariance matrices.

Body size was estimated from a principal components analysis (PCA) on the variance–covariance matrix of the four skull characters (CBL, ZW, ML, and MH; James & McCulloch, 1990). This was performed for each sex separately. The first principal component (PC1) can be interpreted as a measure of body size, if the different measurements load strongly and evenly on PC1 (James & McCulloch, 1990). The standardized PC1 was preferred to the unstandardized PC1 because of insensitivity of the standardized PC1 to the effects of measurement error (Gür, 2004). Body size (note that it refers only to PC1 of skull characters) was also tested to confirm that it met the assumptions of parametric tests (normality and equality of variances).

Variation both among geographic localities and between sexes in the four skull characters (CBL, ZW, ML, and MH) was examined by means of univariate and multivariate two-way analyses of variance (ANOVA and MANOVA). Geographic locality and sex were included as fixed-effect factors

(Model I ANOVA and MANOVA; Sokal & Rohlf, 1995). Body size was analysed separately from skull characters. The geographic pattern of size variation among geographic localities was examined separately in males and females using univariate one-way ANOVA. Hochberg's GT2-method, based on unequal sample sizes (Sokal & Rohlf, 1995), was used for multiple comparisons. The degree of geographic variation in body size was computed for each sex using added variance components (% interlocality variation; Sokal & Rinkel, 1963). These components, however, should be taken as an approximation, because geographic locality was more properly considered a fixed-effect rather than a random-effect factor (Eger, 1990).

Spatial and environmental variables were recorded for each locality: latitude, longitude, elevation, and mean monthly and annual values for dry-bulb and soil (100-cm depth) temperature, precipitation, and relative humidity. Latitude and longitude were converted to decimal degrees. Means of long-term (15–31 years) environmental data were obtained from the nearest meteorological station (mean ± SD distance = 24.60 ± 16.57 km, range = 5.86 –61.35 km) to each locality (Turkish State Meteorological Service; http://meteoroloji.gov.tr). Mean monthly and annual values for wet-bulb temperature were estimated from mean monthly dry-bulb temperature and relative humidity (Psychrometric Calculator PsyCalc® 98 Software; http://linric.com/psyc_98.htm). Mean monthly and annual values for actual evapotranspiration, a significant predictor of primary productivity (Rosenzweig, 1968b), were computed from latitude and from mean monthly dry-bulb temperature and precipitation (Thornthwaite & Mather, 1957; Thornthwaite and Mather Water Budget Software; http://watershedhydrology.com/html/WHsoftware.html). Environmental data were transformed to $\log_{10}$ values before statistical analyses. All temperature data were also transformed to degrees Fahrenheit to allow logarithmic transformation and calculation of coefficient of variation (CV). The CVs of mean monthly air (dry- and wet-bulb) and soil temperature, precipitation, and primary productivity were used as measures of seasonality (i.e. seasonality increases with increasing CV). General seasonality was estimated from a PCA on the correlation matrix of the five measures of seasonality. Fifteen of the above spatial and environmental variables were selected as explanatory variables: latitude, longitude, elevation, mean monthly (coldest and warmest month) and annual values for air (dry- and wet-bulb) and soil temperature, March to September (summer) precipitation and primary productivity, and general seasonality (for spatial variables, see Fig. 1; environmental variables are provided in the Supporting information, Appendix S1). These variables were

screened for univariate outliers and normality before statistical analyses.

To represent the environmental relationships among geographic localities, an unweighted pair-group method with arithmetic mean (UPGMA) dendogram was constructed based on Euclidean distances. Euclidean distances among geographic localities were calculated from the environmental data.

The relationships among explanatory variables were examined using correlation analysis. Only significant relevant correlations are reported in the Results. The correlation between general seasonality and length of the growing season was also estimated to determine whether a period of food shortage actually lasts longer in more seasonal environments. Length of the growing season (based on temperature data of ≥ 5 °C) was obtained from Karaoğlu (2002).

The effects of explanatory variables on body size were examined for each sex by using Martins & Hansen's (1997) phylogenetic generalized least-squares analysis (PGLS), as implemented in COMPARE 4.6b: Phylogenetic Comparative Methods (Martins, 2004). PGLS estimates a single parameter (α; estimated range = 0–15.5) that represents the magnitude of the evolutionary constraint acting on a phenotype (e.g. as a result of stabilizing selection). When the parameter α is zero, it indicates unconstrained evolution similar to Brownian motion (i.e. random genetic drift and/or selection, in which the direction of selection varies randomly over the long periods of time represented by the phylogeny), so PGLS produces results identical to those of Felsenstein's (1985) phylogenetic independent contrasts analysis. When the parameter α is large, it indicates strong constrained evolution (e.g. stabilizing selection with a negligible influence of random genetic drift), so PGLS produces results identical to those of a nonphylogenetic analysis (TIPS, refering to tips of a phylogenetic tree; Martins & Hansen, 1997; Ord & Martins, 2006).

Phylogenetic comparative analyses were used because intraspecific comparative datasets, similar to interspecific ones, may contain significant amounts of phylogenetic signal, suggesting that intraspecific comparative studies should also incorporate phylogenetic information (Ashton, 2004b), to take into account phylogenetic nonindependence of samples and then to estimate correctly the type I error of statistical analyses (Diniz-Filho et al., 2007). Moreover, the high degree of phylogeographic structuring in S. xanthoprymnus (Gündüz et al., 2007) indicates that gene flow is minor especially among lineages (most of the study populations were from different lineages), thereby justifying using phylogenetic comparative analyses for this dataset (for

intraspecific studies using phylogenetic comparative analyses; Ashton, 2001; Niewiarowski et al., 2004).

Despite the potential limitations of using a single locus as a source of molecular data, the phylogenetic relationships among sampled populations of S. xanthoprymnus were based on variation in the cyt b mitochondrial (mt)DNA, the only available locus in this respect. The cyt b mtDNA sequences were obtained from Harrison et al. (2003) and Gündüz et al. (2007). The sequences were matched to the study populations by choosing the sequence from the population that was geographically closest to the study populations (mean ± SD distance = 31.89 ± 22.41 km, range = 2.11–71.43 km). A neighbour-joining phylogenetic tree with bootstrap support values (1000 replicates; Fig. 2) was constructed using the maximum composite likelihood evolutionary distance in MEGA4, version 4.0 (Tamura et al., 2007). Even when the sequence from the population that was geographically second closest to the study populations was chosen if available, the topology of the phylogenetic tree was not changed (note that most of the study populations were from different lineages), suggesting that, although molecular data were not directly obtained for this study, the phylogenetic
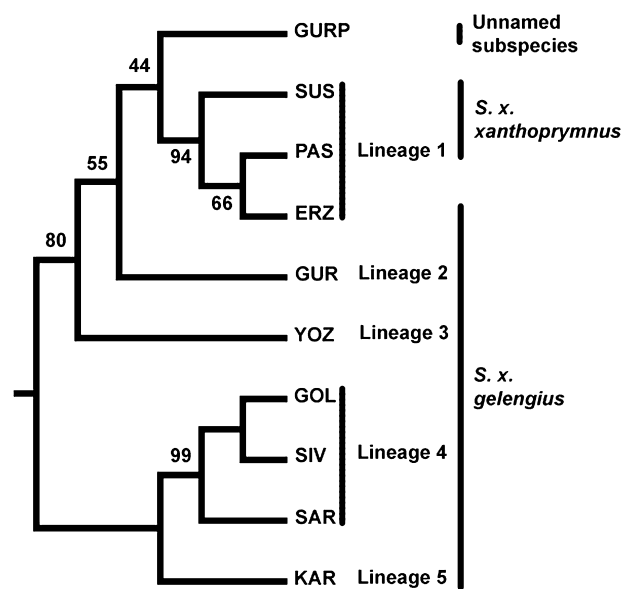

Figure 2. Phylogenetic relationships among sampled populations of Anatolian ground squirrels (*Spermophilus xanthoprymnus*), with bootstrap support values at the internal nodes. Terminal populations are named with abbreviations that correspond to geographic localities (Fig. 1). Cytochrome b lineages (Gündüz et al., 2007) and subspecies (Mursaloğlu, 1965) to which the populations belong are also shown. The haplotype that is geographically closest to GURP does not fall clearly within one of the five cytochrome b lineages (Gündüz et al., 2007).

relationships were conclusive. For PGLS, population means and standard errors of body size in males and females were used (see Supporting information, Appendix S2). All phylogenetic comparative analyses were performed on the phylogenetic tree with all branch lengths held constant. PGLS uses the comparative data themselves to estimate best-fit branch lengths and therefore is robust to inaccuracy in the initially specified branch lengths (Martins, Diniz-Filho & Housworth, 2002; Turner *et al.*, 2007).

COMPARE reports the 95% confidence interval (CI = slope ± 1.96 SE), but not the probability, of a given PGLS regression slope (Martins, 2004). Consequently, regression slopes were considered statistically significant if their CIs did not include zero (Ord & Martins, 2006). *Sensu* Ord & Martins (2006), these significance tests were performed only for PGLS with estimated α to avoid the complications of multiple comparisons and Bonferroni corrections (Nakagawa, 2004). Note that, for visualization, the raw data were plotted in figures (results using PGLS with estimated α were almost the same under TIPS).

Adjusted (type III) sum of squares was used in all *F*-tests. All correlation analyses were performed using Pearson's product-moment correlation coefficient. In statistical analyses other than phylogenetic comparative analyses, $P \leq 0.05$ (two-tailed) was considered statistically significant.

RESULTS

Measurement errors for the four skull characters of Anatolian ground squirrels (*S. xanthoprymnus*) were uniformly low in each sex (%ME ≤ 0.22), indicating their suitability for further analyses.

GEOGRAPHIC VARIATION

Two-way MANOVA of the four skull characters (CBL, ZW, ML, and MH) revealed a strongly significant main effect of both sex (Pillai's Trace = 0.519, $F_{4,145} = 39.164$, $P < 0.001$) and geographic locality (Pillai's Trace = 1.393, $F_{36,592} = 8.792$, $P < 0.001$). This analysis also showed a significant interaction between sex and geographic locality (Pillai's Trace = 0.403, $F_{36,592} = 1.844$, $P = 0.002$). Two-way ANOVA of each of these characters also revealed a strongly significant main effect of both sex (all $P < 0.001$) and geographic locality (all $P < 0.001$). A significant interaction between sex and geographic locality was observed for only MH ($P = 0.005$). Males and females were treated separately in subsequent analyses.

In both sexes, the first principal component (PC1) was interpreted as a measure of body size. All characters in each sex loaded strongly and evenly on PC1

Table 1. Factor loadings of the four skull characters on the first principal component (PC1) computed separately for male and female Anatolian ground squirrels (*Spermophilus xanthoprymnus*)

Characters	Males (N = 78) PC1	Females (N = 90) PC1
Condylobasal length (CBL)	0.904	0.845
Zygomatic width (ZW)	0.902	0.817
Length of mandible (ML)	0.960	0.922
Height of mandible (MH)	0.918	0.901
Variance explained (%)	85.030	77.534
Eigenvalue	0.0012	0.0007

(Table 1). One-way ANOVA of body size revealed a strongly significant main effect of geographic locality for males ($F_{9,68} = 15.637$, $P < 0.001$) and females ($F_{9,80} = 16.191$, $P < 0.001$). Interlocality differences accounted for 65.53% and 63.00% of the variation in body size of males and females, respectively. In both sexes, the largest individuals were found in two localities (PAS and SUS) from northeastern Anatolia and the smallest individuals in one locality (KAR) from south-central Anatolia. However, multiple comparisons using Hochberg's GT2 method revealed that males and females exhibited slightly different patterns of geographic variation in body size. In males, two localities (PAS and SUS) from northeastern Anatolia formed a distinct homogeneous subset (all $P \leq 0.011$ in pairwise comparisons with other localities). The remaining localities were grouped into two broadly overlapping homogeneous subsets. In females, a distinct homogeneous subset was not identified. All localities were grouped into four homogeneous subsets, all of which were broadly overlapping. These subsets in each sex did not reflect cyt *b* lineages or groups of lineages within *S. xanthoprymnus*.

ENVIRONMENTAL INFLUENCES ON BODY SIZE

Major climatic differences occur between two localities (PAS and SUS) from northeastern Anatolia and all other localities (UPGMA dendogram of ten geographic localities based on Euclidean (climate) distances; the results are not presented graphically). These two localities are colder, more seasonal, and also tend to have higher summer precipitation and productivity than the other eight localities (see Supporting information, Appendix S1).

The traditional explanation for Bergmann's rule (heat conservation) suggests that spatial variables (especially latitude) influence body size indirectly
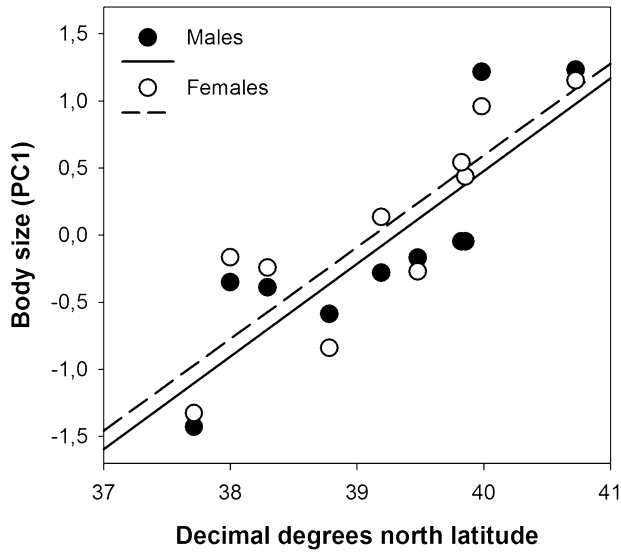
Figure 3. The association of latitude with body size (PC1) for male and female Anatolian ground squirrels (*Spermophilus xanthoprymnus*) from ten geographic localities.

through their effects on ambient temperature. At the collection localities, longitude and elevation, but not latitude, were usually negatively correlated with temperature variables because Anatolia becomes more mountainous towards the east (Atalay, 2002). In both sexes, however, body size was not usually associated with longitude and elevation, but positively with latitude (Fig. 3, Table 2). For this reason, the correlations of latitude with environmental variables were examined further. At the collection localities, latitude showed the strongest significant correlations with summer precipitation ($r = 0.858$, $N = 10$, $P = 0.002$) and productivity ($r = 0.789$, $N = 10$, $P = 0.007$). It is important to note that, in both sexes, body size was negatively associated with ambient (air and soil) temperature (see below), thereby indicating that Anatolian ground squirrels exhibited a Bergmannian size pattern.

Heat conservation hypothesis
Soil temperature was included in addition to dry-bulb temperature, because, as is typical of hibernating species of *Spermophilus* (Michener, 2002), Anatolian ground squirrels spend the majority of their lives sleeping and hibernating in underground burrows. Only mean monthly (coldest and warmest month) and annual values for these temperature variables were considered. In both sexes, the strongest significant negative associations observed were those between body size and mean temperatures of the coldest months (January and February for dry-bulb and soil temperature, respectively; Fig. 4A, B, Table 2).
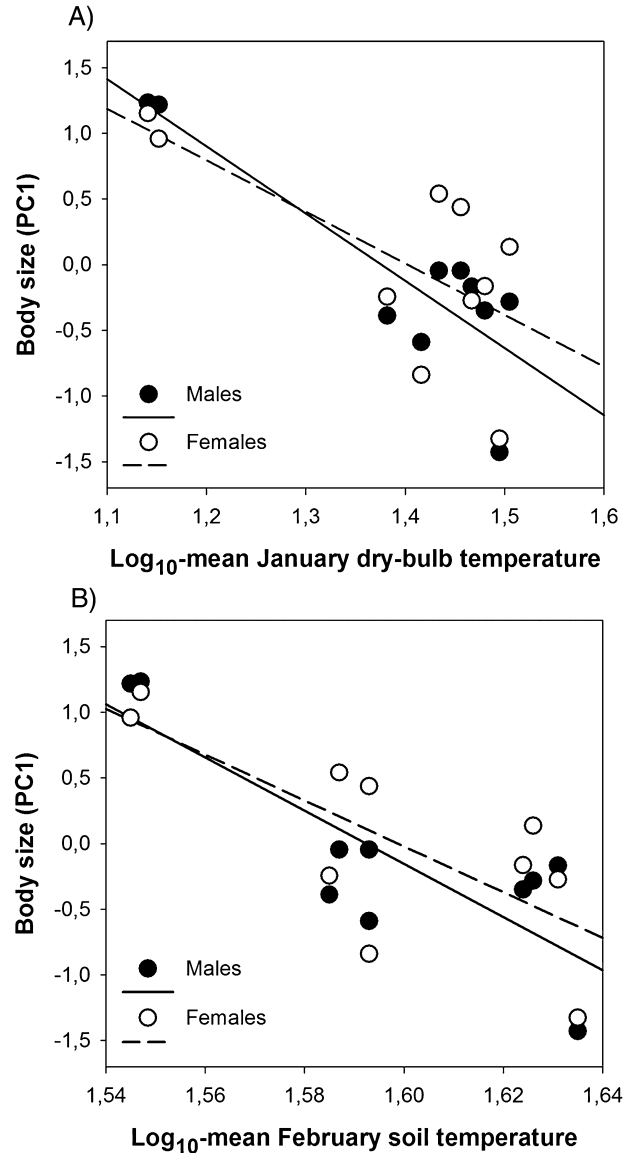

Figure 4. The association of (A) mean January dry-bulb temperature and (B) mean February soil temperature with body size (PC1) for male and female Anatolian ground squirrels (*Spermophilus xanthoprymnus*) from ten geographic localities.

Heat dissipation hypothesis
The association of the combination of ambient temperature and humidity with body size was examined using wet-bulb temperature. Only mean monthly (coldest and warmest month) and annual values for this temperature variable were considered. In both sexes, the strongest significant negative association observed was that between body size and mean temperature of the coldest month (January; Table 2). However, this hypothesis may be best tested by examining the association of mean temperature of the

Table 2. The univariate effects of three spatial and 12 environmental variables on body size (PC1) for male and female Anatolian ground squirrels (*Spermophilus xanthoprymnus*) from ten geographic localities

Spatial and environmental variables	Males				Females			
	Model α	Model % r^2	Slope	Slope SE	Model α	Model % r^2	Slope	Slope SE
Spatial variables								
Latitude	15.50	70.99	**0.69**	**0.16**	15.50	74.04	**0.68**	**0.14**
Longitude	15.50	34.92	**0.10**	**0.05**	9.63	24.87	0.08	0.05
Elevation	12.66	25.66	0.00	0.00	7.41	9.66	0.00	0.00
Environmental variables								
Heat conservation hypothesis								
Dry-bulb temperature								
January	8.23	75.10	**-5.12**	**1.04**	15.50	47.01	**-3.92**	**1.47**
July	1.16	61.98	**-26.01**	**7.20**	2.54	32.98	**-19.22**	**9.69**
Annual	3.59	66.22	**-18.15**	**4.58**	7.27	39.16	**-13.58**	**5.98**
Soil temperature								
February	1.16	74.13	**-27.68**	**5.78**	6.56	53.95	**-17.80**	**5.82**
August	2.63	37.21	**-14.93**	**6.86**	3.87	16.77	-9.88	7.79
Annual	3.22	57.90	**-20.81**	**6.27**	6.40	34.75	**-15.31**	**7.42**
Heat dissipation hypothesis								
Wet-bulb temperature								
January	8.26	74.33	**-4.90**	**1.02**	15.50	45.88	**-3.73**	**1.43**
July	2.92	9.16	-11.56	12.87	3.40	0.57	-2.82	13.14
Annual	4.25	50.91	**-16.52**	**5.74**	6.34	25.28	-11.21	6.81
Primary productivity hypothesis								
Summer								
Precipitation	2.73	69.38	**4.99**	**1.17**	1.67	81.38	**5.02**	**0.85**
Productivity	2.19	58.10	**5.03**	**1.51**	1.85	66.66	**5.11**	**1.28**
Seasonality hypothesis								
Seasonality	7.42	75.05	**0.70**	**0.14**	15.50	52.15	**0.56**	**0.19**

Model α was estimated using a maximum-likelihood grid search. Model % r^2 and slope (±SE) are based on phylogenetic generalized least-squares analysis with estimated α. Significant results are shown in bold.
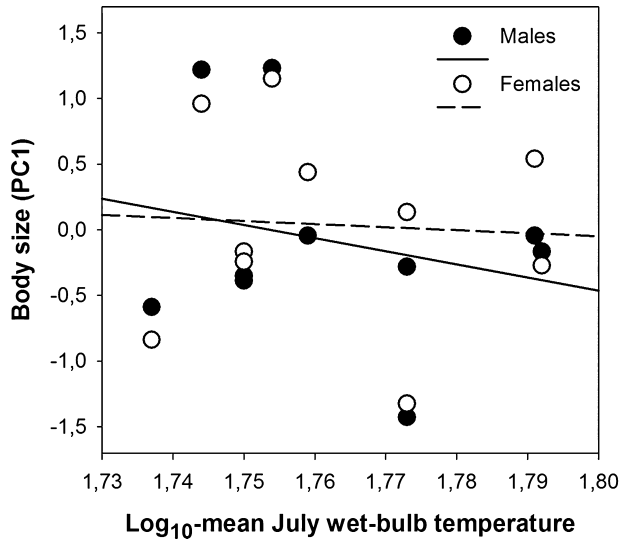
Figure 5. The association of mean July wet-bulb temperature with body size (PC1) for male and female Anatolian ground squirrels (*Spermophilus xanthoprymnus*) from ten geographic localities.

warmest month (July) with body size because overheating is not a critical problem during winter when Anatolian ground squirrels hibernate. This association was not significant for either sex (Fig. 5, Table 2).

Mean temperatures of the coldest months (January and February for air (dry- and wet-bulb) and soil temperature, respectively) were strongly and positively correlated with each other ($r \geq 0.918$, $N = 10$, $P < 0.001$).

Primary productivity hypothesis
The associations of precipitation and primary productivity with body size were examined using total precipitation and primary productivity for the months of March to September (referred to as summer precipitation and productivity, respectively), the months when Anatolian ground squirrels are active above ground. Anatolian ground squirrels spend the remaining months hibernating in underground burrows (depth 100 cm; Kart Gür, 2008; Kart Gür, Refinetti & Gür, 2009). Summer precipitation and productivity were positively associated with body size of both sexes (Fig. 6A, B, Table 2).

Summer precipitation and productivity were strongly and positively correlated with each other ($r = 0.963$, $N = 10$, $P < 0.001$).

Seasonality hypothesis
The first principal component (PC1) of a PCA on the five measures of seasonality accounted for 66% of the variation. The correlations between the original variables and PC1 were significantly positive (the CVs of
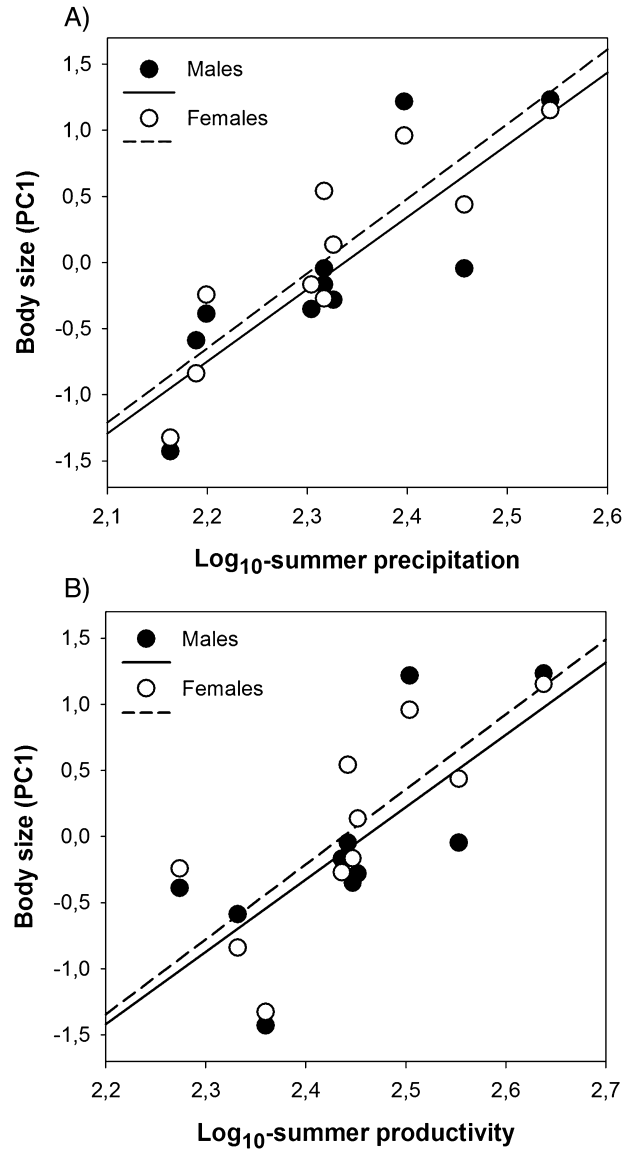

Figure 6. The association of (A) summer precipitation and (B) productivity with body size (PC1) for male and female Anatolian ground squirrels (*Spermophilus xanthoprymnus*) from ten geographic localities.

air (dry- and wet-bulb) and soil temperature and primary productivity: $r = 0.981$, 0.982, 0.729, and 0.900, respectively, $N = 10$, $P \leq 0.017$), except for the CV of precipitation that showed a nonsignificant negative correlation ($r = -0.206$, $N = 10$, $P = 0.568$). PC1 was interpreted as an index of general seasonality (referred to as seasonality). Seasonality was positively associated with body size of both sexes (Fig. 7, Table 2).

Seasonality was strongly and negatively correlated with mean temperatures of the coldest months ($r \leq -0.954$, $N = 10$, $P < 0.001$). A significant negative

correlation was also found between seasonality and length of the growing season ($r = -0.809$, $N = 10$, $P = 0.005$), suggesting that a period of food shortage lasts longer in more seasonal environments.

A simultaneous test of competing hypotheses

A priori selection of the environmental variables to be subjected to further testing in the multivariate model was performed by choosing only the variable for each hypothesis most strongly associated with body size of both sexes and more meaningful for evaluating that hypothesis. Heat dissipation hypothesis was not considered because the above-mentioned analyses did not support this as a viable explanation of the observed pattern of geographic variation in body size.
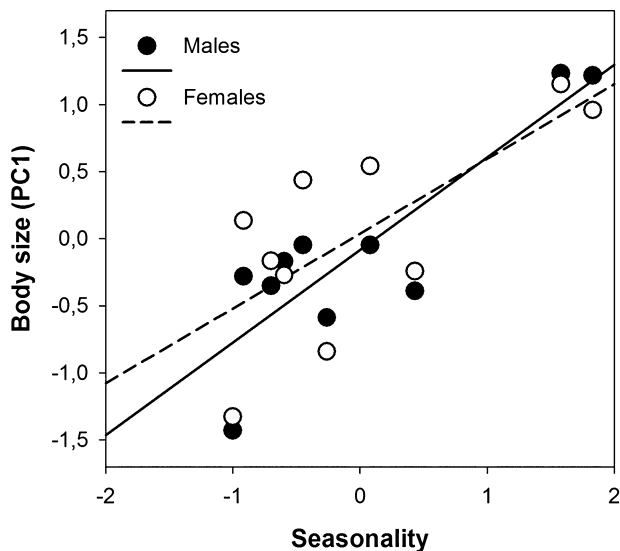

Figure 7. The association of seasonality with body size (PC1) for male and female Anatolian ground squirrels (*Spermophilus xanthoprymnus*) from ten geographic localities.

Consequently, the environmental variables selected were mean February soil temperature (heat conservation hypothesis), summer precipitation (primary productivity hypothesis), and seasonality (seasonality hypothesis).

Collinearity analysis showed that variance inflation factors were just slightly greater than 10 (a value suggested as the limit to maintain a variable; Quinn & Keough, 2002) for mean February soil temperature (11.690) and seasonality (11.145), whereas condition indexes (derived from the eigenvalues of centered correlations) were less than 30 (a value suggested as the limit to maintain a variable; Quinn & Keough, 2002) for all variables. Thus, although some collinearity of mean February soil temperature and seasonality occurred, it was not a critical issue.

For males, body size increased with summer precipitation and seasonality, as predicted by primary productivity and seasonality hypotheses, respectively. For females, body size increased with summer precipitation, as predicted by primary productivity hypothesis (Table 3).

DISCUSSION

Anatolian ground squirrels (*S. xanthoprymnus*) exhibit a Bergmannian size pattern: increasing body size with decreasing ambient (air and soil) temperature. Thus, the present study represents one of the first comprehensively documented examples of Bergmann's rule in an obligately hibernating marmotine species (see also Panteleev *et al*., 1998). To date, however, little evidence of Bergmannian size patterns has come from hibernating, burrowing mammals (Ashton *et al*., 2000; Meiri & Dayan, 2003).

Of the four principal hypotheses proposed to explain patterns of geographic variation in body size (Wigginton & Dobson, 1999; see also Introduction), heat conservation is the best known. Three others (heat dissipation, primary productivity, and seasonal-

Table 3. The multivariate effects of a priori selected three environmental variables on body size (PC1) for male and female Anatolian ground squirrels (*Spermophilus xanthoprymnus*) from ten geographic localities

Sex	Model α	Model % r^2	Environmental variables	Slope	Slope SE
Males	4.73	93.92	Soil temperature (February)	–	–
			Summer precipitation	3.29	0.69
			Seasonality	0.49	0.09
Females	1.67	81.38	Soil temperature (February)	–	–
			Summer precipitation	5.02	0.85
			Seasonality	–	–

PGLS was re-run sequentially removing variables that did not contribute significantly. Model α was estimated using a maximum-likelihood grid search. Model % r^2 and slope (± SE) are based on phylogenetic generalized least-squares analysis with estimated α.

ity hypotheses) suggest that environmental variables other than (or in addition to) ambient temperature may generate Bergmannian size patterns. These additional hypotheses may also explain examples of reversed Bergmannian size patterns. To understand the possible cause(s) of the observed pattern of geographic variation in body size of Anatolian ground squirrels, these hypotheses were tested, separately and simultaneously. *Spermophilus xanthoprymnus* displays extensive sexual dimorphism, with males being considerably larger than females (Mursaloğlu, 1964; Gür & Kart Gür, 2005; Kryštufek & Vohralík, 2005), so that males and females were treated separately.

An approach using separate tests supported three (heat conservation, primary productivity, and seasonality hypotheses) of the four hypotheses in each sex. No support was found for the heat dissipation hypothesis in either sex. However, environmental variables that are invoked by each of the three supported hypotheses are significantly intercorrelated. Colder environments (PAS and SUS) are more seasonal, and also tend to have higher summer precipitation and productivity than warmer environments (e.g. KAR; see Supporting information, Appendix S1). It is therefore not surprising that three of the four hypotheses were supported separately. The present study is also a clear example both of why 'examinations of intraspecific variation in body size should investigate the relationship between size and several environmental parameters, not just latitude or mean temperature' (Ashton *et al*., 2000) and of why these parameters should also be tested simultaneously (Millien *et al*., 2006). A simultaneous test of the three supported hypotheses suggested that, in Anatolian ground squirrels, the most important factors driving geographic size variation are summer precipitation and seasonality for males (as predicted by primary productivity and seasonality hypotheses, respectively) and summer precipitation for females (as predicted by primary productivity hypothesis). At the collection localities, the latitudinal cline (an increase in body size with latitude) for both sexes appears to be the result of a positive correlation between latitude and summer precipitation. Similarly, Kryštufek (1996) reported that, in both sexes of European ground squirrels (*Spermophilus citellus*), the most important factors driving geographic size variation are the standard deviation of the mean monthly temperature (likely reflecting the seasonality of the environment) and summer precipitation, both of which are positively associated with body size, but did not discuss what the underlying cause(s) of these associations may be (see below). Additionally, in Columbian ground squirrels (*Spermophilus columbianus*), body size is not associated with elevation (Dobson, 1992)

and probably not with latitude, supporting the argument that ambient temperature alone is not a primary influence on geographic variation in body size of hibernating species of marmotine squirrels.

Shared evolutionary history (i.e. phylogeny) can generate spurious associations between phenotype and environment (Felsenstein, 1985). A high phylogenetic constraint (i.e. low values of α; Table 3) suggests the data to be non-independent of the phylogeny. However, when the phylogenetic relationships among populations were taken into account, the above-mentioned environmental associations were found for body size, thereby justifying that some evolutionary mechanism (e.g. selection; see below) other than phylogeny is required to explain the evolution of body size.

Anatolian ground squirrels are mainly active from March to September and hibernate during the remaining months (Gür & Kart Gür, 2005; Kart Gür, 2008). Many species of marmotine squirrels hibernate for as many as 8–9 months of the year. They rely on fat reserves as the source of energy during hibernation. Consequently, over-winter survival is positively correlated with pre-hibernation fat reserves (*Marmota*: Armitage, 2003; *Cynomys*: Hoogland, 2003; *Spermophilus*: Yensen & Sherman, 2003). However, for fat-storing hibernators, because of both morphological constraints and costs associated with fat storage, the maximum size of fat reserves is consistently 40–50% of total body mass, and therefore the capacity to store fat increases proportionally with body mass (Humphries, Thomas & Kramer, 2003; Humphries, Umbanhowar & McCann, 2004). For example, in Belding's ground squirrels (*Spermophilus beldingi*), fat reserves were positively associated with body mass (Morton & Tung, 1971). Because large individuals store more fat prior to hibernation and deplete their fat reserves less rapidly (French, 1988), they may be more likely to survive over-winter (including immediately after hibernation) in seasonal environments where severe winters and long periods of food shortage occur. In other words, over-winter fasting endurance may favour large body size (Boyce, 1978; Lindstedt & Boyce, 1985; Millar & Hickling, 1990). However, large body size is possible only if food availability during the growing season is sufficiently high to meet the energetic costs of that size (McNab, 2002). Golden-mantled ground squirrels (*Spermophilus lateralis*) from a high-altitude environment are larger and store more fat than those from a low-altitude environment (Blake, 1972). Thus, greater over-winter fasting endurance of ground squirrels from a high-altitude environment allows them to survive more severe winters and longer periods of food shortage. In California ground squirrels (*Spermophilus beecheyi*), precipitation is the most important factor driving geographic size variation, as

occurs in European (Kryštufek, 1996) and Anatolian (present study) ground squirrels, and increased food availability may lead to greater over-winter fasting endurance in the form of large body size (Blois, Feranec & Hadly, 2008). Because food availability during the growing season may influence over-winter fasting endurance through its effect on body size, especially in hibernating species of marmotine squirrels and other fat-storing hibernators, food availability (primary productivity hypothesis) and over-winter fasting endurance (seasonality hypothesis) may not be mutually exclusive. In Anatolian ground squirrels, food availability and, especially in males, over-winter fasting endurance are therefore likely to comprise the primary underlying mechanisms generating the observed pattern of increasing body size towards colder, more seasonal environments with higher summer precipitation and productivity (or a Bergmannian size pattern). The above arguments may also explain, at least in part, the geographic pattern of size variation in European ground squirrels (see Kryštufek, 1996). These explanations, however, do not exclude the possibility that other mechanisms (e.g. selection acting on life-history traits that may be correlated with body size) may also contribute to the observed pattern of geographic variation in body size.

In Anatolian ground squirrels, adult males have shorter hibernation seasons than do adult females (mean, 168 days versus 212 days), beginning hibernation later (mean, 31 August versus 11 August), and ending earlier (mean, 14 February versus 10 March). Torpor bouts account for a smaller proportion of the hibernation season in adult males than in adult females (mean, 86% versus 91%). Obviously, adult males remain euthermic longer during winter, especially during the late winter when soil temperature at the depth of the hibernaculum, and presumably also sleeping chambers, is at or near the lowest value and food is still scarce (Kart Gür, 2008; Kart Gür et al., 2009). They therefore run more risk of starving, as is the case, for example, in adult male Belding's ground squirrels (*S. beldingi*; French, 1982, 1988), especially in more seasonal environments. This may explain why males and females respond differently to the seasonality of the environment (note that seasonality hypothesis received further support in males only) and therefore exhibit slightly different patterns of geographic variation in body size. Multiple comparisons revealed that, in males, but not in females, two localities (PAS and SUS) from more seasonal northeastern Anatolia showed the greatest degree of differentiation. It is important to note that major climatic differences occur between these two localities and all other localities. As expected, sexual size dimorphism, too, varies geographically and appears to increase towards northeastern Anatolia (Gür, 2007).

Gündüz et al. (2007) reported that *S. xanthoprymnus* is subdivided into five cyt *b* lineages that likely diverged 0.30–0.75 Mya, with the majority of population splits taking place 0.50–0.65 Mya. These cyt *b* lineages have signals of recent range expansion. Consequently, Gündüz et al. (2007) claimed that Anatolian ground squirrels survived the last glacial maximum (LGM) in small suitable habitats (refugia), and that the postglacial recolonization of Anatolia arouse from these LGM refugia. In the LGM and perhaps in other glacial maxima, when the climate is considered to have been drier, colder, and more seasonal in Anatolia (Atalay, 1992, 1996), it is reasonable to consider that Anatolian ground squirrels shifted their range towards small suitable habitats (refugia) where summer precipitation, one of the most important factors driving geographic size variation, was sufficiently high to allow the accumulation of fat reserves required to survive the prolonged winters under more extreme glacial conditions. According to Gündüz et al. (2007), mountain ranges may have been refugia during the LGM because of the higher precipitation than in lower altitude areas.

Spermophilus xanthoprymnus exhibits an extensive degree of geographic variation in body size (Mursaloğlu, 1965; Kryštufek & Vohralík, 2005). Mursaloğlu (1965) distinguished two named and one unnamed subspecific taxa based on body size: the largest (nominate) *Spermophilus xanthoprymnus xanthoprymnus* from northeastern Anatolia (in the present study, PAS and SUS), the intermediate *Spermophilus xanthoprymnus gelengius* from central Anatolia (all other localities except GURP), and the smallest (unnamed) subspecies from the east of Lake Van (GURP) (Fig. 2). Kryštufek & Vohralík (2005) reluctantly accepted the taxonomic validity of the two named subspecies, but considered the smallest (unnamed) subspecies to be anomalous. In the present study, the smallest individuals were not in the east of Lake Van (GURP), but in warmer, less seasonal and less productive south-central Anatolia (KAR). Moreover, much of the morphometric variation occurs along environmental gradients. Gündüz et al. (2007), sensu Mursaloğlu (1965) and Kryštufek & Vohralík (2005), claimed that, contrary to the findings regarding skull or mandible shape, size may distinguish cyt *b* lineages or groups of lineages within *S. xanthoprymnus*. The results obtained in the present study do not confirm Gündüz et al.'s (2007) claim because multiple comparisons revealed that statistically homogeneous subsets did not reflect cyt *b* lineages or groups of lineages. Obviously, either *S. x. xanthoprymnus* or *S. x. gelengius* does not yield a monophyletic set of the cyt *b* mtDNA sequences (Fig. 2), as occurs in, for example, some species of birds examined in this respect (Zink, 2004; Phillimore & Owens, 2006). Indeed, two sister

populations (ERZ and PAS) on the phylogenetic tree are put into different subspecies (*S. x. gelengius* and *S. x. xanthoprymnus*, respectively) on the basis of body size (Fig. 2). In my opinion, these subspecies are not supported well by geographic patterns of morphometric (i.e. body size) and mtDNA variation.

In conclusion, Anatolian ground squirrels, an obligately hibernating marmotine species, exhibit a Bergmannian size pattern, although the mechanism generating this pattern does not appear to be heat conservation, as the traditional explanation would imply.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Values of 12 environmental variables for the nearest meteorological stations to ten geographic localities from which Anatolian ground squirrels (*Spermophilus xanthoprymnus*) were collected. These variables (except seasonality) were transformed to $\log_{10}$ values before statistical analyses. Temperature variables were also transformed to degrees Fahrenheit to allow logarithmic transformation and calculation of coefficient of variation. For abbreviations of geographic localities, see Fig. 1.

Appendix S2. Population mean ± SE of body size (PC1) for male and female Anatolian ground squirrels (*Spermophilus xanthoprymnus*) from ten geographic localities. Note that the values of body size for males and females cannot be compared with one another because principal components analysis (PCA) was performed for each sex separately. For abbreviations of geographic localities, see Fig. 1.

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