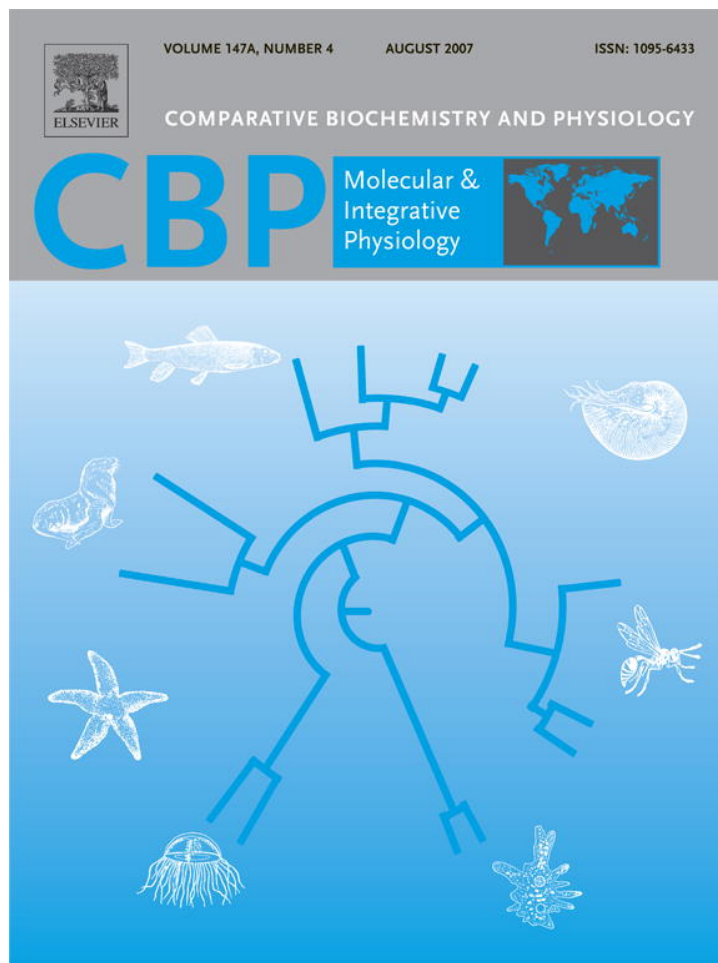


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Energy and distribution in subterranean rodents: Sympatry between two species of the genus *Ctenomys*

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Abstract

The low basal metabolic rate (BMR) observed in subterranean rodents, compared to that of surface-dwelling species of comparable size, has been proposed to be an adaptation to underground life. Two main hypotheses have been proposed to explain this finding, the cost of burrowing and the thermal stress. The former states that the low BMR is due to the high cost of extending the tunnel system whereas the other relates it to the possibility of overheating in burrows where evaporative and convective heat exchange are restricted. Additionally, both hypotheses related the energetics of subterranean rodent with spatial distribution. The genus *Ctenomys* is an excellent model to evaluate the cost of burrowing or thermal stress, since they are widely distributed, with members differing markedly in body mass. The aim of this study was to assess digging and basal energetics in two *Ctenomys* species that live in sympatry in a coastal grassland, but differ in their microspatial distribution by soil preference. We used the obtained energetic data to test both energy–distribution hypotheses. We measured BMR and digging metabolic rate (DMR) through open flow respirometry in two species exposed to soft and hard soils. In brief, DMR in *Ctenomys talarum* (100–170 g), as in *Ctenomys australis* (250–600 g), was unaffected by soil hardness. Within thermoneutral zone of each species, DMR/RMR quotient was lower in the smaller species. Our data did not support the thermal stress hypothesis, but the cost of burrowing hypothesis was not rejected. Other alternative hypotheses are proposed to explain the distribution of *C. talarum* and *C. australis*.

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Keywords: Cost of burrowing hypothesis; Thermal stress hypothesis; *Ctenomys*; Digging energetics; Subterranean mammals distribution

1. Introduction

Subterranean rodents show morpho-physiological features that allow them to face the high cost of extending their tunnel systems. In particular, the low basal metabolic rate (BMR) observed in these rodents, compared with surface-dwelling rodents of the same body size, have been proposed as an adaptation to living underground (McNab, 1966; Vleck, 1979). Two hypotheses have been proposed to explain the low mass-specific BMR observed in subterranean rodents. The thermal stress hypothesis (McNab, 1966, 1979) proposes that low BMR is related to the possibility of overheating in an ambient with

restricted convective and evaporative heat loss. The cost of burrowing hypothesis (Vleck, 1979, 1981), states that individuals must have a sufficient energy intake to balance the high cost of burrowing. These hypotheses have in common that they both proposed a relationship between BMR, body size and distribution of subterranean rodents (see Lovegrove and Wissel, 1988; for an alternative hypothesis, such as food–aridity distribution). In some cases, either the cost of burrowing hypothesis or the thermal stress hypothesis could drive similar interpretations between physiological and ambient interactions. For example, low primary productivity, or low porosity and soil transmittance, both related to soil hardness, might determine the exclusion of bigger subterranean rodents from arid deserts (Vleck, 1981). Thus, the bigger species occurs in soft soils with high primary productivity, as a strategy to lower the cost of digging in such soils (see Vleck, 1979; Kennerly, 1959) or

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because the possibility to dig to a deeper layer of the soil, and avoid overheating (see McNab, 1966; Nevo and Shkolnik, 1974). Thus, the main factors affecting distribution include soil type, ambient temperature, and primary productivity.

Different approaches have been carried out to test both hypotheses. Allometric comparisons of BMR were made among semifossorial and strictly subterranean species (White, 2003), while intraspecific studies were conducted to show how BMR and digging metabolic rate (DMR) are affected by environmental factors (Bozinovic et al., 2005). From both studies no unequivocal results were obtained, therefore both hypotheses may not be mutually exclusive (White, 2003; Bozinovic et al., 2005).

The genus *Ctenomys* is an excellent model to evaluate the hypotheses cost of burrowing and thermal stress, since this genus is wide distributed, occurring from coastal grassland to mountain hillslopes (Contreras, 1973; Reig et al., 1990). In such wide distribution, the genus *Ctenomys*, exhibit a broad range of body sizes, with body masses from 100 g (*Ctenomys pumoti*) to more than 1000 g (*Ctenomys conoveri*). Two species of the genus are of particular interest in the analysis of energy–distribution hypotheses: *Ctenomys talarum* (100–170 g) and *Ctenomys australis* (250–600 g). Both species live sympatrically in a restricted area of coastal grassland of the Buenos Aires Province, Argentina, from Necochea (38°36'S, 58°48'W) to Bahía Blanca (38°45'S, 62°13'W; Contreras and Reig, 1965; Comparatore et al., 1992). Although, these species occur in sympatry they are microspatially segregated by different soil preferences (Vassallo, 1998), and the observed soil preference is related with the quality and quantity of food in the inhabited area. The bigger species inhabits soft soils, with low and poor primary productivity; in contrast, the smaller one occurs in hard soils with dense and diverse plant cover (Malizia et al., 1991). Because they occur in a coastal environment, the relationship between soil hardness and plant biomass is opposite to other examples described in the literature (Du Toit et al., 1985; Lovegrove, 1989). Also, this study model has inverse features to those proposed in the cost of burrowing hypothesis (Vleck, 1979). Regarding their energetics, *C. talarum* and *C. australis* show lower BMR (Busch, 1989) than those expected by the allometric equation stated by Kleiber (1961), with the BMR of *C. australis* being particularly low (46% of the expected value; Busch, 1989). In addition, *C. australis* show better thermoregulatory capacity than *C. talarum* (Busch, 1989).

Therefore, the analysis of digging metabolic rate in both species, in relation to soil type, provides a way to assess how different factors are related in the interaction among BMR, body size, and distribution. The aim of this study is to evaluate the influence of soil type on digging energetics in natural soils inhabited by *C. talarum* and *C. australis*. The results are used to evaluate the cost of burrowing or thermal stress hypotheses by comparing basal and digging energetics in relation to microspatial distribution.

According to the thermal stress hypothesis, the bigger species (*C. australis*) is expected to overheat when digging in hard soil and, consequently, an increment in the DMR due to an increase in the cost of dissipation is also expected. This result is not expected in the smaller species digging in soft soils.

According to the cost of burrowing hypothesis a high DMR/BMR is expected when a low BMR and limited resources are observed. Given that in this system the bigger species occupies soft but unproductive soils, we predict that, at thermoneutrality, *C. australis* will show higher DMR/BMR than that observed in the smaller *C. talarum*. Contrary to the thermal stress hypothesis, no thermal effects are expected when the bigger species digs in hard soils.

2. Materials and methods

2.1. Study animals and soil extraction

Animals (12 *C. talarum* and 23 *C. australis*) were live-trapped in Necochea (38°36'S, 58°48'W, Buenos Aires Province, Argentina). Captured animals were carried to the laboratory and housed individually in cages (0.30×0.40×0.25 m³). Wood shavings were provided as nesting material. The animal room temperature was kept at 24±1 °C, and photoperiod was LD 12:12 (lights on at 7:00 a.m.). Animals were fed mixed grasses, carrots, lettuce, corn, alfalfa and sunflower seeds *ad lib*. Soil samples were collected in Necochea using a steel tube. We dug down to the mean burrow depth, and then ran the steel tube horizontally to extract soil. Each cylinder of soil was transposed to a PVC tube of the same diameter, and carried to the laboratory. This procedure allowed us to maintain the same properties of the intact soil verified in the natural habitat (Antinuchi and Busch, 1992; Luna et al., 2002).

2.2. Oxygen consumption

Oxygen consumption was measured using a computerized positive pressure open-flow respirometry system (Sable System, Henderson, NV, USA). The digging chamber system consisted of a cube-shaped acrylic soil collector attached to an acrylic tube. A perforated tube containing the soil extracted in the field was placed inside the acrylic tube, leaving an air space between the perforated tube and the outer tube (0.01 m) to permit airflow through the system (total volume 19.17 l). A metallic perforated door separated the cube-shaped soil collector and the acrylic tube, allowing free airflow between both. A cylindrical chamber (volume 1.84 l) was used to estimate BMR of *C. talarum*. Digging chambers were comparable to those described in Luna et al. (2002) and Luna and Antinuchi (2007).

Flow rate used in the digging chamber was 3 l min⁻¹ calibrated with a flowmeter (Cole-Parmer Instrument Company, Vernon Hills, IL, USA). To estimate BMR, the cylindrical chamber received air at 1.5 l min⁻¹ from a mass flow controller (Sierra Instruments, Monterey, CA, USA). Both chambers received dry and CO₂ free air. Air passed through a CO₂-absorbent (IQB®) and water scrubber (Silica Gel) before and after passing through both chambers. Excurrent air was subsampled at 180±10 ml min⁻¹ and oxygen consumption was obtained from an Oxygen Analyzer FC-1B every 0.5 s by a Datacan V-PC program (Sable System).

We introduced the animal in the cube-shaped soil collector, closed the metallic perforated door, and connected it to the tube containing the soil, closing the digging chamber system. After

that we connected the air pump to the system, to allow the complete turnover of the chamber air. The digging chamber was left for 35 min to achieve equilibration (Lasiewski et al., 1966; Withers, 1977). During this period animals were quiet. After equilibration period, we opened the metallic perforated door, and the tuco-tucos began to burrow within 10 min and continued until they reached the opposite end of the chamber. Data were discarded if the animals stopped digging, or remained inactive in the digging chamber system. Oxygen consumption values were calculated using Eq. (4a) of Withers (1977). Digging metabolic rate (DMR) was estimated for each individual as the average during the plateau in oxygen consumption in the digging trial. Minimal metabolism was measured as the 5-min lowest steady-state values of the last 30 min of a 90-min trial. BMR was calculated using the lowest steady-state metabolic rate values of tuco-tucos within the thermoneutral zone (TNZ). RMR was calculated from the lowest steady-state oxygen consumption values in resting, below the TNZ. *C. talarum* have a wide TNZ (T_a from 25 °C to 30 °C; Busch, 1989) whereas *C. australis* have a narrow one (T_a of 30 °C; Busch, 1989). Because MR was also measured in *C. australis* individuals below TNZ (25 °C), we report minimal MR as RMR to avoid confusions. Notwithstanding, we use the term BMR in the discussion to refer to the theoretical framework. In all cases we used mass-specific metabolic rate to report the data, unless noted otherwise. Oxygen consumption of soil microorganisms was negligible, so the baseline of the digging chamber system was set at 20.95% O₂ at the beginning of each experiment.

To assess the effect of soil hardness on DMR, two soil types were extracted in Necochea, the first one was extracted in the area where *C. talarum* lives (hard soil, 36.79±2.17 kg cm⁻²), and the second one where *C. australis* occur (soft soil, 6.9±1.6 kg cm⁻²). To determine the relationship between body size and soil occupancy, individuals of both species were randomly selected to establish four groups, regarding species and soil type (6 individuals of *C. talarum* digging in hard soil, and 6 in soft soil, 9 individuals of *C. australis* digging in hard soil, and 9 in soft soil). Since mean spring–summer T_a in the capture site is near 24 °C (Argentine National Weather Forecast Service, <http://www.meteofa.mil.ar>), and because this T_a is the near to the TNZ of *C. talarum*, in the first set of experiments, the digging chamber was maintained at T_a of 25 °C. After that, for another set of individuals of *C. australis* ($n=5$), DMR and BMR at T_a of 30 °C were measured. Again, *C. talarum* have a wider TNZ than *C. australis* and the TNZ of the latter species overlaps that of the former species.

Thermal conductance after digging of *C. australis* at T_a of 25 °C was estimated in both soil types. After each burrowing trial, T_b was measured using an electronic digital thermometer (MediLife, accuracy: ±0.1 °C), inserted 2 cm into the rectum. Thermal conductance of burrowing individuals was estimated according to Seymour et al. (1998), as $C=MR/(T_b - T_a)$, where MR is the metabolic rate, and T_b is the final body temperature. After each burrowing trial, burrowing speed (BS) was calculated as total length burrowed divided by the time used to reach the opposite side of the digging tube.

2.3. Statistical analysis

All values are expressed as mean±S.E.M. Because the same individuals were used to estimate both DMR and RMR, a two-way repeated-measures ANCOVA was used to test the null hypothesis that there was no difference in whole-animal DMR and whole-animal RMR between soil type and species. Body mass was used as a covariate in the ANCOVA analysis. Between-subject factors were soil type and species, and within-treatment factors were metabolic rates (digging and resting). A one-way ANOVA was used to evaluate differences in burrowing speed between soil types in each species. Additionally, a two-way repeated-measures ANOVA was used to evaluate differences in mass-specific DMR and mass-specific RMR between species and soil type. A two-way ANOVA was used to detect differences in DMR/RMR between species and soil type. Finally, Student's *t* test was used to evaluate differences in thermal conductance in *C. australis* digging at T_a of 25 °C in both soil conditions.

A one-way repeated-measures ANCOVA was used to detect differences in whole-animal DMR and whole-animal RMR of *C. australis* between T_a of 25 °C and 30 °C, using body mass as covariate. No data transformation was used to obtain metabolic ratios. Student's *t* test was used to establish differences in DMR/RMR of *C. australis* between T_a of 25 °C and 30 °C, and between the same quotient (in TNZ) between *C. talarum* and *C. australis*.

3. Results

When assumptions of the two-way repeated-measures ANCOVA (species and soil type) were tested, no parallelism was found in the total DMR and total RMR regression lines between species (Wilks' test, $F_{2,13}=4.95$, $P=0.02$; Fig. 1). So, repeated-measures ANCOVA was performed in each species separately.

In *C. talarum*, whole-animal DMR differed from whole-animal RMR in both soil conditions ($F_{1,10}=92.58$, $P<0.001$); however, no differences were found in whole-animal DMR or RMR between soft and hard soils ($F_{1,10}=0.89$, $P=0.38$). The same pattern was observed in *C. australis* (DMR vs. RMR, $F_{1,16}=303.92$, $P<0.001$, soft vs. hard soil, $F_{1,16}=0.36$, $P=0.56$).

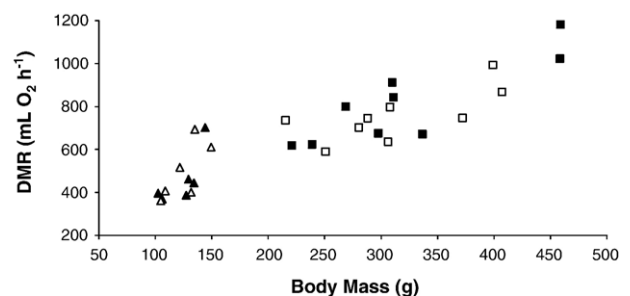


Fig. 1. Whole-animal digging metabolic rate (DMR) and whole-animal resting metabolic rate (RMR) in relationship to body mass in *Ctenomys talarum* and *Ctenomys australis* digging in soft and hard soils. Triangles: *C. talarum*, squares: *C. australis*, filled: soft soil friable, empty: hard soil.

Table 1
Digging metabolic rate (DMR), resting metabolic rate (RMR), and quotient between these variables of *Ctenomys talarum* (Ct) and *Ctenomys australis* (Ca) digging in soft and hard soil at T_a of 25 °C

		DMR (ml O ₂ g ⁻¹ h ⁻¹)	RMR (ml O ₂ g ⁻¹ h ⁻¹)	DMR/RMR (ml O ₂ g ⁻¹ h ⁻¹)
Ct	Soft	3.69±0.26 ^a	1.03±0.04 ^c	3.58 ¹
	Hard	3.95±0.30 ^a	0.95±0.05 ^c	4.15 ¹
Ca	Soft	2.57±0.11 ^b	0.70±0.02 ^d	3.67 ¹
	Hard	2.46±0.14 ^b	0.66±0.04 ^d	3.73 ¹

Superscript letters indicate statistical differences in DMR and RMR between species and soil type (two-way repeated-measures ANOVA, DMR vs. RMR, $F_{1,26}=3.54$, soft vs. hard, $F_{1,26}=0.53$).

Superscript number indicate statistical differences in DMR/RMR between variables (two-way ANOVA, $F_{1,26}=1.03$).

No differences were found in burrowing speed in both *C. talarum* ($5.14±0.66$ m h⁻¹, $F_{1,10}=1.24$, $P=0.29$), or *C. australis* ($6.40±0.58$ m h⁻¹, $F_{1,16}=0.03$, $P=0.86$).

When the interspecific analysis of the mass-specific DMR and RMR at T_a of 25 °C was performed (Table 1), differences in both variables between species were observed ($P<0.001$), but not between soil types ($P=0.47$). DMR of *C. talarum* was higher than that in *C. australis* (Tukey's test, $P<0.0001$). RMR of *C. talarum* was also higher (Tukey's test, $P<0.001$). No differences were found in DMR/RMR between species and soil type ($F_{1,26}=1.03$, $P=0.32$; Table 1). Thermal conductance of *C. australis* after digging was similar between soil conditions at T_a of 25 °C ($0.21±0.01$ ml O₂ g⁻¹ h⁻¹, $t=0.12$, $n=15$, $P=0.91$).

When the effect of T_a on whole-animal DMR and whole-animal RMR in *C. australis* was evaluated, whole-animal DMR was different from whole-animal RMR ($P<0.001$; Table 2). No differences in whole-animal DMR between 25 °C and 30 °C were observed in *C. australis* (Scheffé's test, $P=0.46$), on the contrary, whole-animal RMR at 30 °C was lower than those observed at 25 °C (Scheffé's test, $P=0.03$).

The quotient DMR/RMR in *C. australis* was different between T_a of 25 °C and 30 °C (Table 2). Within TNZ of each species, DMR/RMR of *C. talarum* was lower than those observed for *C. australis* (Table 2).

4. Discussion

4.1. Energetics

Digging cost is one of the main factors shaping morphology and physiology of subterranean species (Luna et al., 2002). It

represents, at least, 2.5 times the cost of maintenance and varies depending on soil type (hardness, porosity, heat dissipation capacity), and primary productivity (McNab, 1966; Vleck, 1979; Luna and Antinuchi, 2006).

Regarding the effect of soil type and primary productivity on BMR, different studies have failed to support either of the proposed energy–distribution hypotheses (see White, 2003; Bozinovic et al., 2005). This study provides the first evidence that digging energetics remains similar in two sympatric subterranean rodents of different size (*C. talarum* and *C. australis*) when digging in different soil types (Fig. 1).

Different factors might explain the lack of differences in digging cost. On one hand, digging energetics appears not to be the main cause influencing soil type selection in *C. talarum* and *C. australis*. Supporting this idea, Bozinovic et al. (2005) proposed that similarity in digging cost observed in *Spalacopus cyanus*, occurring in different soil types in central Chile, was due to the Andean origin of this specie. It is possible that *S. cyanus* that originated in Andean places were adapted to relatively hard soils. Later, when populations moved to coastal soft-soils locations, they could have retained the value of the digging metabolic trait.

In a similar way, *C. australis* belongs to the Mendocinus group (Ortells, 1990). This subclade, shows genetic homogeneity but a great variation in body size. Most species of the Mendocinus subclade live in hard soils, only *C. australis* and *Ctenomys flamarioni*, the bigger members of the group, occur in soft soils. This feature has been proposed as causation of the speciation, at least in *C. australis* (Ortells, 1990; Vassallo, 1998). Thus, digging in hard soil without an increment in DMR might be the result of the retention of the ancestral morphophysiological adaptations to this condition of species of the Mendocinus group. However, if it would be the case, hard selection pressure should have affected the entire genus *Ctenomys*. Another explanation could be that, in the bigger species, digging energetic is not affected in hard soil because their body size, and so, it has more than enough muscular machinery to dig in such soils.

Regarding *C. talarum*, the lack of differences in DMR between individuals digging in hard and soft soils could be explained because individuals develop ontogenetically in hard soils, would extend higher body structural components (muscular mass and bones densities) than in soft soils or because are plastically adjusted to live in it. This increase in work machinery would need a basal energy supply which cannot be lowered when they dig in soft soils. Yet, it should be

Table 2
Effect of ambient temperature on whole-animal digging metabolic rate (DMR), whole-animal resting metabolic rate (RMR), and DMR/RMR ratio in *Ctenomys australis* digging in soft soils

	Body mass (g)	DMR (ml O ₂ g ⁻¹ h ⁻¹)	Body mass (g)	RMR (ml O ₂ g ⁻¹ h ⁻¹)	DMR/RMR (ml O ₂ g ⁻¹ h ⁻¹)
25 °C	311.45±22.91	792.22±51.86 ^a	311.60±22.77	215.86±14.00 ^b (85.30)	3.71 ¹
30 °C	294.12±43.09	729.43±25.10 ^a	293.84±42.57	146.53±32.57 ^c (57.98)	6.14 ^{2*}

Superscript letters indicate statistical differences between variables (MR, one-way repeated-measures ANCOVA, $F_{1,15}=279.02$).

Superscript number indicate statistical differences in DMR/RMR between variables (Student's test, $t=2.43$, $n=15$, $P=0.03$).

In parenthesis, percentage of RMR expected by the allometric equation of Kleiber (1961) for surface-dwelling mammals.

* Thermoneutral zone (TNZ) of *C. australis*, DMR/RMR at 30 °C was higher than the ratio observed in *C. talarum* within TNZ (Student's test, $t=2.17$, $n=17$, $P=0.05$).

noted that, for this species, a temporal variation in BMR and body temperature when individuals were acclimatized to extreme hard soil condition has been reported (Antinuchi et al., in press).

Opposite to the experiment carried out in this study, individuals from Mar de Cobo, that permanently inhabited soft soil when transferred to a hard soil condition (soil from Necochea), for testing DMR, show differences in such variable (Luna and Antinuchi, 2006). So, irrespective of the genetic differences observed between populations from Mar de Cobo and Necochea (Cutrera et al., 2006) or morphological characters used in digging activity can show phenotypic plasticity, the increase in DMR reported for *C. talarum* from the Mar de Cobo population (Luna and Antinuchi, 2006) does not contradict our present findings.

As an alternative explanation, members of the genus *Ctenomys* tend to use surface intensively (Busch et al., 2000; Luna and Antinuchi, 2003), so locomotor performance could be the result of interplay of opposite selection pressures. Lesser commitments to subterranean life, associated with aboveground food habits and increasing predation pressures, tend to favor morphologies more suited for traveling performance, at the expense of exaggerated morphological adaptations for digging.

Even that maximum metabolic rate during running is similar to DMR, at least in *C. talarum* ($3.06 \pm 0.17 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$), the relationship between net metabolic rate and speed during terrestrial locomotion (net cost of transport; Taylor et al., 1970) have an increment of 14.8 times during digging (Luna, personal observation). In that sense, muscles of *Ctenomys* species, contrary to specialized diggers, have a heterogeneous composition of types of muscle fibers, which would allow them to face different functional demands (digging, locomotion, and handling food; Alvarez et al., 2004). In fact, observed maximum natural locomotor speed within *Ctenomys* is greater compared to those observed in more specialized digger species, such as *Thomomys bottae* and *Tachyoryctes splendens* (Luna and Antinuchi, 2003). So the lack of capacity to adjust their energetic performance when individuals are changed from hard to soft soil, and vice versa, might be the result of higher selection pressures acting on the interplay between subterranean and surface locomotion more than on digging performance. Thus, phylogenetic origin of species, local adaptations (genetic variability), phenotypic plasticity related to digging or the commitment between subterranean and surface locomotion, might account for the observed DMR values in individuals of *C. talarum* and *C. australis*.

4.2. Energy–distribution hypotheses

In light of energy–distribution hypotheses, the lack of differences in both, DMR, and thermal conductance, between soil types, in the bigger species, are not in agreement with the predictions based on thermal stress hypothesis (McNab, 1966, 1979). If overheating had existed, not only differences with *C. talarum* would have been expected, but also differences in DMR between soil types (Table 1). Harder soil would increase DMR, due to an increment of dissipative costs. No such

relationship could be verified in our study. In contrast, thermal stress hypothesis neglects any thermoregulatory adjustments through behavior (White, 2003). Conduction through the soil, has been proposed to be an important behavior to dissipate heat, not only in subterranean rodents (see Luna and Antinuchi, 2007), but also in surface-dwelling species when returned to the burrow after surface foraging (Chappell and Bartholomew, 1981). Moreover, *C. australis* has a better thermoregulatory capacity (maintain their T_b constant even at T_a of 10 °C) than the smaller species (Busch, 1989).

In terms of the cost of burrowing hypothesis a high DMR/BMR would be expected, when a low BMR and limited resources are observed. DMR/BMR at thermoneutrality in *C. australis* was higher than those found in *C. talarum*. In this system, the bigger species shows a lower BMR than the smaller one and lives in a low productive environment. This fact is in accordance with the cost of burrowing hypothesis and it could account for differences between species.

Notwithstanding, other factors might be responsible for soil preferences of each species. Since, low soil porosity precludes passive O_2 diffusion (Kennerly, 1964; Withers, 1978), the low BMR might be seen as an adaptation to the low $p\text{O}_2$ underground (Arieli and Nevo, 1991). To face the hypoxic characteristics of burrow environments, subterranean mammals have acquired mechanisms that improve oxygen transport capacity of the blood (Ar et al., 1977; Nevo, 1999 and references herein). Similar to other subterranean rodents (Shams et al., 2005; van Aardt et al., 2007; Wei et al., 2006), blood parameters of *C. talarum* and *C. australis*, such as high O_2 capacity values (19.2% higher than surface-dwelling mammals), high hemoglobin content (170 g l^{-1}), and low hematocrit (39.1%), have been related to oxygen transport, and hence, to face hypoxia (Busch, 1987).

Since, *C. australis* inhabit more permeable soils than *C. talarum* (Luna, personal observation), there are no reasons to suspect O_2 limitation on BMR. In the same way, it would be expected that *Ctenomys* species that live at high altitude show allometrically lower BMR than other species of the genus. However, species inhabiting high altitude (i.e. *Ctenomys tuconax*) apparently show similar BMR compared with those from low altitudes (Luna, personal observation). Similar findings were obtained by Bozinovic et al. (2005) for *S. cyanus*, and by Wei et al. (2006) for *Myospalax baileyi*.

At the intraspecific level, if there are no digging energetic restrictions intrinsically to determine the soil occupancy in both species, which are the factors that might determine that the smaller *C. talarum* did not occur in soft soil inhabited by *C. australis*? First, the areas of *C. australis* are relatively poor in vegetation (Malizia et al., 1991; Vassallo, 1993, 1998), leading a wide amplitude of T_a on the surface. For example, *S. cyanus* limit and modify their surface activity due to variations of environmental temperatures (Rezende et al., 2003). Therefore, *C. talarum* could be affected by thermal restrictions during locomotion outside the burrow at high T_a values. Second, *C. talarum* fur color is conspicuous on top of soft soil occupied by *C. australis*. Symmetrically, fur color of *C. australis* differs from top color in hard soils inhabited by *C. talarum*. In accordance, Wlasiuk et al.

(2003) have reported for *Ctenomys rionegrensis* that these features have a heritable basis. Thus, in addition to thermal restrictions on surface, both species might be exposed to intense predation risk (Vassallo et al., 1994).

As mentioned before, the bigger *C. australis* restricts the soil preferences to soft soils, and does not overlap its microspatial distribution with *C. talarum* (Malizia et al., 1991). A factor limiting *C. australis* distribution could be their spatial requirements. Soil depth in areas of hard soil is restricted by a calcrete layer at ~35 cm under surface (Malizia et al., 1991), which is impossible to dig, either by *C. australis* or *C. talarum*. Maximum burrow depth of the tunnels systems of the bigger species is ~120 cm (Luna, personal observation), so calcrete layer might restrict their vertical movements.

4.3. Conclusion

Soil hardness did not affect DMR, in both *C. talarum* and *C. australis*. So, the outstanding hypothesis that digging cost influences the anatomy, physiology and behavior of subterranean rodent remains under discussion (see Bozinovic et al., 2005). In that sense, different adaptations and/or evolutionary constraints, in relation to microspatial distribution, could be important factors determining the observed energetic pattern. Thus, microspatial distribution of both species could be related to thermal restrictions, when individuals gather food outside the burrow, to predation risk when fur color contrast with soil, and/or to macro-structure of soil related to morphological restrictions when individuals cannot extend their burrows by geological barriers.

At interspecific level, thermal stress hypothesis (McNab, 1966, 1979) is not supported by the observed results, at least in this example, and cost of burrowing hypothesis (Vleck, 1979, 1981) can not be rejected. Finally, other hypotheses, such as the consequences of food patches distribution or predation risk, should be evaluated with more emphasis.

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