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Bioenergetic Characteristics of Genus *Mus* (Mammalia: Rodentia) from South Europe

Roumiana Metcheva, Michaela Beltcheva

Institute of Biodiversity an Ecosystem Research, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel Blvd., 1000 Sofia, Bulgaria; E-mails: rummech@yahoo.com; mnedialkova@gmail.com.

Abstract: Thermoregulation characteristics of genus Mus from South Europe were studied as follows: *Mus musculus* musculus (Linaeus, 1758), *Mus musculus domesticus* (Schwarz & Schwarz 1943), *Mus spicilegus* (Petenyi, 1882), *Mus macedonicus* (Petrov & Ruzic 1983) and *Mus spretus* (Lataste 1883). The thermoregulation curves in these species were significantly different. The lowest value of metabolism rates in thermo-neutral zone, assumed to approximate BMR values fell in range from 25 ^{0}C to 30 ^{0}C. The body temperature in mice at 20 ^{0}C remained fairly similar. The lowest intensity of thermoregulation was found in M. spicilegus, and the lowest insulation index was found in M. m. musculus. The results indicate certain adaptations in metabolism rate of the rodent species studied, relevant to the conditions in the habitats where rodents live.

Key words: thermoregulation, metabolism rate, oxygen consumption, *Mus* sp.

Introduction

In homeothermic organisms minimal metabolism is most often measured as basal or resting metabolic rate (BMR, RMR) which sets the pace of life and continues to be of paramount importance for a number of evolutionary and ecological investigations.

Both BMR and RMR are standardised measures that represent the minimal rate of metabolism necessary to maintain basic physiological processes in the organism. RMR differs from BMR that it allows violation of certain standard conditions; usually the food intake is not excluded, while still requiring the animal to rest in thermo-neutrality. Values of RMR vary widely among, and within species. After accounting for the large proportion of variation in RMR explained by body mass and different level taxonomic affiliation, comparative studies have linked with variation to numerous abiotic and biotic factors including climate, altitude, and environmental productivity etc. (Larive´e *et al.* 2010).

Genus *Mus* (Rodentia, Mammalia) consists of ubiquitous species, which forms many subspecies by crossing synantropic species with original wild ones. The house mouse (*Mus musculus* (Linnaeus, 1758) is a well-known inhabitant of human settlements, however, only some of it populations experience this commensally way of life inside buildings. Under favourable climatic conditions others of its populations may return to the wild, away from human settlements. In South Europe, two synantropic subspecies of the genus *Mus* occurs - *Mus musculus musculus* (Linnaeus 1758) – semi-synantropic and *Mus musculus domesticus* (Shvartz, Shvartz 1983) entirely synantropic. The closest relatives to them are three aboriginal species - *Mus spretus* (Lataste 1883)*, Mus spicilegus* (Peteniy 1882) and *Mus macedonicus* (Petrov, Ruzic 1983). They are strictly free ranging, avoid human settlements, and exhibit some peculiar features of ecology and behavior (Auffray

et al. 1990, Cassaing, Croset 1985, Gorecki *at al.* 1990, Metcheva, Gerasimov 1994, Sokolov *et al.* 1990).

In regards to European *Mus* species, Bulgarian territory is too interesting, especially from the evolutionary view point. North and South natural borders of distribution of all *Mus* species, excepting *M. spretus* overlap here. Stara Planina Mountain simultaneously is a south border of distribution for *M. m. musculus* and *M. spicilegus* and North for *M. m. domesticus* and *M. macedonicus* (Gerasimov *et al.* 1990). On the other hand the region of South France belongs to the north natural distribution border for *M. spretus* (Orsini *et al.* 1983). It is possible to assume that the environmental conditions for each one of the investigated species are near to the pessimum.

The aim of the investigation is to determine basic physiological parameters as metabolic rate and thermoregulation in all five representatives of genus *Mus* in South Europe and to establish the differences among them.

Material and Methods

The study of thermoregulation and resting metabolism rate was carried out on *M. m. musculus* (L. 1758), *M. m. domesticus* (Schwarz, Schwarz 1943), *M. spicilegus* (Petenyi 1882), *M. macedonicus* (Petrov, Ruzic 1983) and *M. spretus* (Lataste 1883). The animals were collected in species-specific locations in South Europe (Fig. 1). *M. spretus* (31 individuals) were caught on cultivated fields near Montpellier (Southern France). *M. m. musculus* (16 individuals) were captured in Sofia region (West Bulgaria), *M. m. domesticus* (17 individuals) on warehouses from Haskovo region (South Bulgaria), *M. spicilegus* (18 individuals) on farmlands from Pleven region (North Bulgaria) and *M. macedonicus* (27 individuals) on vegetable gardens from Plovdiv region (South Bulgaria). Mice were raised in vivarium under natural light regime, conventional food for small rodents and fresh water and *ad libitum*. To preserve species-specific characteristics of the testing mice species, the metabolism rate, thermopreferendum and all other studies was measured during the autumn-winter period, immediately after catching the animals. Environmental thermopreferendum was determined on 31 animals by open type thermogradient device. The oxygen consumption was measured by standard closed system respirometer. To restrict the locomotor activity mice were put into individual metal cages and placed in glass chambers of 0.5 L capacity, dunked into temperate water nearest of ± 0.2 ^{0}C. The measurements were performed at 0^0, 5^0, 10^0, 15^0, 20^0, 25^0 and 30 ^{0}C ambient temperatures. The duration of each experimental run was 20 min, with an acclimatization period of 30 min for each one test animal.

The rectal temperature was measured individually only for *M. spicilegus, M. macedonicus* and *M. spretus* at depth of about 1.5-2 cm by an electronic thermistor thermometer with accuracy nearest to 0.1 ^{0}C.

All statistical analyses were performed using either SPSS software or version 6.0 (Norusis 1992) or MINITAB 13.31. Values are given as mean ± standard deviation (SD).

Results and Discussion

The values of the environmental thermopreferendum successfully characterized physiological response of the animals to maintain the optimal temperature with minimum energy losses and their adaptations to the changing environmental conditions during different seasons. These species possess wide scope of specific variability depending on the particularities of the environmental conditions, animals' body mass and size of the heat exchange organs (Bashenina 1977).

Results show that the mice body mass does not differ significantly among species. The average values of the investigated animals are as follows: *M. m. musculus* 14.2±3.0 g; *M. m. domesticus* – 14.9±2.0 g; *M. spicilegus* - 14.7±2.1 g; *M. macedonicus* – 14.9±3.4 g and *M. spretus* – 14.1±2.1 g. During the experiments mice were not sexually active and statistically significant differences between males and females were not established and the results are presented totally for the each one species group.

The results of the temperature preferences during autumn - winter period are presented on FIg. 2.

The most frequently visited zone for *M. m. musculus* was between 26 ^{0}C and 36 ^{0}C, where animals spent about 58% of the time of experiment. For *M. m. domesticus* the preferred temperature zone was within the interval 28 ^{0}C - 39^0 C, which corresponds to 75% of the time. *M. spicilegus* preferred temperature ranges from 27 ^{0}C to 36 ^{0}C (62% of the time), *M. macedonicus* from 26 ^{0}C to 33^{0}C (48% of the time), while *M. spretus* preferred lower temperature inter-

Fig. 1. Collecting species specific locations in South Europe.

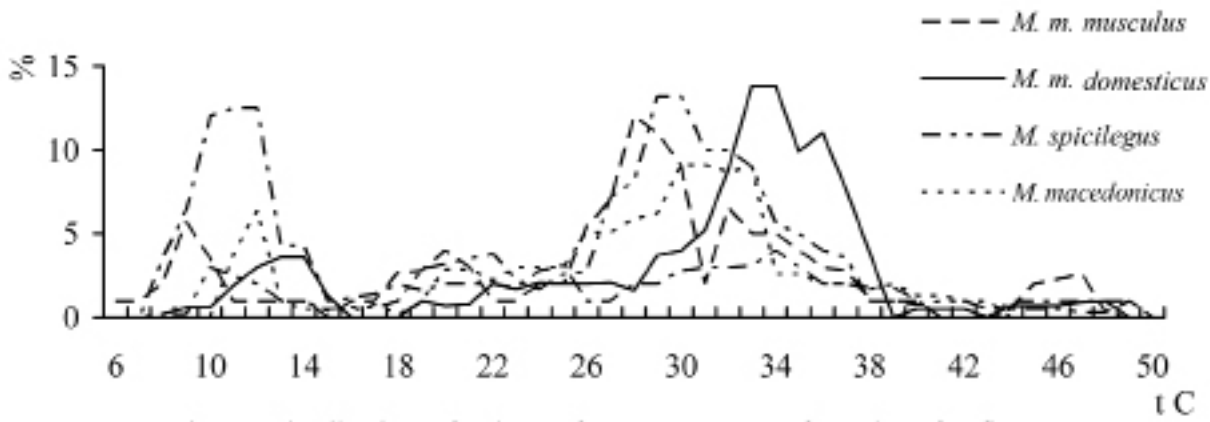

Fig. 2. Distribution of values of temperature preferendum for five representatives of genus Mus from South Europe.

vals between 8 ^{0}C and 14 ^{0}C where it spent about 67% of the experimental time.

A comparison of the above data for five investigated house mice species show that for the autumn-winter period *M. m. domesticus* preferred highest temperatures but *M. spretus* – the lowest. Statistically significant differences (at p = 0.01) were obtained between *M. spretus* and the other four mice species. Those between *M. spicilegus* and *M. macedonicus* were on the border of the statistical significance and between *M. m. musculus* and *M. m. domesticus* they were not substantial.

The thermopreferendum is a behavioural reaction, towards to the temperature sector of most comfortable conditions. It depends on preceding adaptations and can easy be changed experimentally and hence under natural conditions. According Panteleev (1983) as a rule house mouse is one of the species where thermopreferendum is decreasing by moving to the north. Such relationship was established also by Metcheva, Gerasimov (1994). In the present study most of the obtained results are generally similar but closely relates to the physiology and life mode of each one of the investigated species. The lowest temperature preferences of southernmost species *M. spretus* are a result of its entirely natural live mode all over the year, it spent the winter singly, inhabiting shallow holes and actively looking for food (Orsini *et al.* 1983). Comparatively smaller body size also is a proof supporting the proposition exposed (Hart 1971). The obtained statistically significant differences in thermopreferendum values between *M. spretus* and the other entirely wild species *M. spicilegus* are not only due to the differences in their body size but to the species specific way of wintering of the steppe form. Life mode in groups and building the 'hillocks' reduces the need for adaptation to very low temperatures (Orsini *et*

Table 1. Changes of the body temperature under different environmental temperature conditions for three entirely wild mice species.

t^0 C	*Mus spicilegus* n=11	*M. macedonicus* n=29	*Mus spretus* n=35
0	32.4±5.2	34.9±3.7	33.7±2.5
5	34.7±0.8	35.9±1.9	34.7±1.3
10	36.4±0.6	36.9±1.0	35.7±0.7
15	36.4±0.6	36.5±0.9	35.9±1.2
20	37.1±0.7	36.4±1.0	36.8±0.8
25	37.4±1.0	37.0±0.7	37.0±0.8
30	37.1±1.1	37.1±0.5	37.2±0.7

al. 1983). The other three European mice species are evolutionarily adapted to a semi or entirely *(M. m. domesticus)* synantropic life at relatively high temperatures and high caloric diet mainly with anthropogenic origin.

Chemical thermoregulation constitutes a reflectory strengthening of heat generation as an answer to the decrease of the environmental temperature. It is a specific adaptive reaction of the homeothermic organisms, which is not affected by the functioning of physiological systems ensuring the basic vital processes. Imperfect thermoregulation mechanisms in mice and the dynamic changes in body temperature to changes of environmental temperature also relates to metabolic intensity. Changes of the body temperature under different environmental temperature conditions for three entirely wild mice species – *M. spicilegus, M. macedonicus* and *M. spretus* are presented in Table 1.

Results show that the decreasing of the surrounding temperature leads to dropping down the animal's body temperature. For *M. spicilegus* statistically significant differences were obtained between of 0^0 and 5^0 C (at p=0.02) and among 0^0 - 15^0 C, 0^0 - 20^0 C, 0^0 - 25^0 C and 0^0 - 30^0 C (at p=0.01). For *M. macedonicus* statistically significant are differences among 0^0 - 10^0 C, 0^0 - 25^0 C and 0^0 – 30^0 C (at p=0.02). Hence the thermoregulation potential of *M. spicilegus* was relatively greater. The body temperature of *M. spretus* at 10^0 and 15^0 C of the environment show significant differences (at p=0.01) in comparison to the others two investigated species at the same temperatures.

An important adaptive feature of small rodents is the ability for enduring considerable changes in body temperature and at the same time retaining vital functions (Panteleev 1983). In conditions of rapid changes of the environment they possess good adaptive ability to respond quickly by changing intensity of the physiological processes (Grodzinski 1975).

The values of chemical thermoregulation may vary considerably. The basal metabolic rate (BMR) has the highest values, which considered as good indicator of the main energy losses of the organism – metabolism, measured at physiologically low temperatures of the environment and shows the highest values of heat generation (Hayssen, Lacy 1985; McNab 1987).

The present work discusses the results concerning the resting metabolism rate (RMR) (higher then BMR), because it did not exclude the 'specific dynamic action' of food (Bashenina 1977). Not only among but within species, animals resting metabolic rates vary considerably (Hayes *et al.* 1992, Speakman 2000) and, in general, not all this variations are due simply to body mass (Speakman 2000). Studies of the variation in RMR and active daily energy metabolism (ADMR) have generated the hypothesis that variability in RMR may be positively associated with maximum sustainable metabolic rate (Selman *et al*. 2001).

RMR calculations for the five European mice species includes a broad range of temperatures from 0 ^{0}C to 30 ^{0}C. Results are present in Table 2.

Mice body mass does not differ significantly among species as mentioned above (Table 1). The lowest body weight was established for *M. m. domesticus* (14.15 ± 3.0 g) and the highest for *M. macedonicus* (14.88 ± 3.44 g).

The lowest value of oxygen consumption in thermoneutral zone assumes to approximate for resting metabolism rate (RMR) (Fig. 2). The lowest val-

Table 2. Body weight (g) and oxygen consumption ($cm^3 O_2/g/h$) at different ambient temperatures in the five *Mus* species.

Body weight (g)		Temperature (^{0}C)						
	n	0^0	5^0	10^0	15^0	20^0	25^0	30^0
Mus spretus								
14.06 ± 2.10	31	7.74 ± 1.34	6.41± 1.32	6.58 ± 1.19	5.82 ± 1.72	5.21 ± 1.91	4.15 ± 1.67	3.79 ± 2.23
Mus m. musculus								
14.15 ± 3.00	16	8.35± 0.97	8.20 ± 1.12	7.10 ± 1.07	5.90 ± 0.87	5.00 ± 0.97	3.50 ± 0.54	3.60 ± 0.69
Mus m. domesticus								
14.86 ± 2.00	17	9.39± 0.23	7.80 ± 1.42	6.90 ± 0.81	6.50 ± 1.08	6.00 ± 0.89	4.80 ± 0.90	3.70 ± 0.77
Mus spicilegus								
14.71 ± 2.12	18	8.56 ± 1.46	7.91 ± 1.22	7.34 ± 2.67	6.19 ± 0.72	5.66 ± 1.16	4.56 ± 0.64	3.20 ± 0.71
Mus macedonicus								
14.88 ± 3.44	27	8.17 ± 1.53	7.66 ± 1.14	6.91 ± 1.63	6.17 ± 1.20	4.89 ± 0.98	4.09 ± 0.71	3.60 ± 0.71

ues of oxygen consumption were register in 30 ^{0}C as follows: 3.79 ± 2.23 $cm^3 O_2/g/h$ for *M. spretus*, 3.70 ± 0.77 $cm^3 O_2/g/h$ for *M. m. domesticus*, 3.20 ±0.71$cm^3 O_2/g/h$ for *M. spicilegus* and 3.60 ± 0.71 $cm^3 O_2/g/h$ *M. macedonicus*. Only for *M. m. musculus* the lowest value of oxygen consumption was obtained at 25 ^{0}C - 3.50 ± 0.54 $cm^3 O_2/g/h$ without statistically significant differences among species so as between values of oxygen consumption for *M m. musculus* at 25 and in 30 ^{0}C. The highest tolerance of thermoregulation zone proceeds from the adaptation to wider spectrum of ecological conditions of *M m. musculus* (Kotenkova, Bulatova 1994) due to its wide area of distribution.

The oxygen consumption level sharply increases in physiologically low environmental temperatures (0-5 ^{0}C). The highest values per g body weight were obtained for *M. m. domesticus* (9.3 ± 0.30 $cm^3 O_2/g/h$). For *M. macedonicus, M. m. musculus* and *M. spicilegus* these levels varied from 8.17 ± 1.53 $cm^3 O_2/g/h$ to 8.56 ± 1.46 $cm^3 O_2/g/h$. Only the oxygen consumption for *M. spretus* varied statistically ($p<0.001$) with comparison to the other discussed *Mus* species. The lowest level of oxygen consumption was registered for *M. spretus* and the highest for *M. m. domesticus*. A lower metabolism levels allows maintaining life at the minimum possible metabolic costs (Hayssen 1984) and may represent an adaptive feature in habitats or seasons where productivity is low and foraging cost energetically very expensive (Hayssen 1984 Armitage *et al.* 1990; Bozinovic *et al.* 2000; Lovegrove 2000; Simeonovska-Nikolova 2012; Simeonovska-Nikolova, Lomlieva 2012).

The metabolism rate increases linear (from 25 ^{0}C to 0 ^{0}C) for each one species and was described by a regression equation. The obtained results were as follows:

Mus m. musculus	M = 8.85 - t. 0.20
Mus spicilegus	M = 8.69 - t. 0.16
Mus m. domesticus	M = 8.90 - t. 0.16
Mus macedonicus	M = 8.42 - t. 0.17
Mus spretus	M = 7.58 - t. 0.13

The range of the obtained RMR was slightly higher than those reported by Pearson (1947) and Mokrievich (1966) and overlaps with results obtained by Gorecki, Krzanowska (1970, 1971) for laboratory mice.

The rectal temperature determined by 20 ^{0}C in *M. m. domesticus* was by 0.5 ^{0}C higher than this for the other investigated *Mus* species. The specific habitat conditions - predominantly human settlements are the probable explanation for the difference in the temperature preferences (Kotenkova, Bulatova 1994).

The insulation indexes (I_i) (Table 3) closely relates to the habitat that rodents occupied. It was

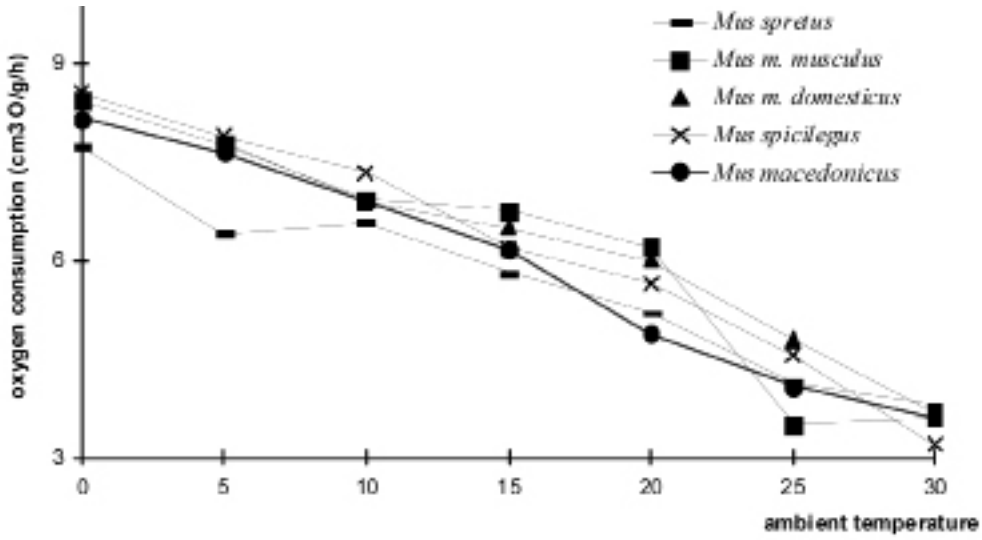

Fig. 5. Thermoregulation curves of the five *Mus* species.

Table 3. Insulation index for the five investigated *Mus* species.

species	n	Body temperature at 20 ^{0}C	Oxygen consumption cm^3 O_2/g/h at 20 ^{0}C	Insulation index I_i
Mus spretus	10	36.66 ± 0.90	5.21 ± 1.91	3.22 ± 1.40
Mus m. musculus	14	36.96 ± 0.98	5.00 ± 0.97	2.84 ± 0.98
Mus spicilegus	11	36.90 ± 0.70	5.66 ± 1.16	2.98 ± 0.93
Mus macedonicus	20	36.80 ± 0.80	4.89 ± 0.98	3.43 ± 0.90
Mus m. domesticus	20	37.41 ± 0.52	6.00 ± 0.89	3.50 ± 0.70

calculated according formula suggested by Hart, Heroux (1955).

The results indicate certain adaptations in metabolism rate of these rodent species, relevant to the conditions in the habitats where mice lived. The lowest value of I_i at 20 ^{0}C was found for *Mus m. musculus* and *M. spicilegus* which occupied North Bulgaria.

For the other species, these values were comparatively similar. *M. m. domesticus* occurs in the warmest situation, followed by the southern *M. spretus* without statistically significant differences.

The obtained results confirmed data presented by Gorecki *et al.* (1990) for *M. spretus* and *M. m. musculus* about the insulation index and temperature conditions of the environment where animals live.

The intensity of metabolism is one of the ways to determine the animal ecological adaptations and to compare species-specific requirements to different environmental conditions among investigated *Mus* species. Results determine the entirely synantropic *M. m. domesticus* as mostly temperate and adapted to the warmest conditions which is due to the specific temperature and food conditions in human settlements.

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