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Reproduction in *Mus macedonicus* (Mammalia: Rodentia) in the Balkans

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Abstract. The material of 538 specimens of *Mus macedonicus* was collected during 1983–1994 in 64 localities in northern Greece, southern Bulgaria and former Yugoslav Macedonia. Duration of the breeding period, sexual maturation in relation to the body weight, proportion of sexually active individuals in the population, intensity of reproduction, litter size, and embryonal resorption were evaluated. Reproduction had distinctly seasonal character. Litter size was between 4 and 10, $\bar{x} = 6.73$ ($n = 45$). Our results in *Mus macedonicus* were compared with the published data on other free living mice species of the genus *Mus* in the western part of the Palaearctic region.

Key words. *Mus macedonicus*, wild mice, reproduction, litter size.

Introduction

Mus macedonicus Petrov & Ružič, 1983, is a strictly free living mouse species inhabiting the south of the Balkan Peninsula (Orsini et al. 1983, Vohralík & Sofianidou 1987, 1992), as well as the large territories of the Middle East extending from Turkey, to Armenia and Azerbaijan in the north, to Iran in the east, and to Israel in the south (Kratochvíl 1986, Auffray et al. 1990b, Orlov et al. 1992). Despite its large distribution area it was recognized as a distinct species only in the 1980s, when a considerable research effort was devoted to the taxonomy of *Mus musculus* sensu lato. Due to this fact, hitherto studies dealing with *Mus macedonicus* were confined mostly to problems associated with systematics and taxonomy, e. g., biochemical systematics (Bonhomme et al. 1984), karyology (Orlov et al. 1992), morphology (Orsini et al. 1983, Kratochvíl 1986, Gerasimov et al. 1990, etc.), and behaviour (Frynta et al. 1992).

Despite that its specific status was accepted universally, there is no agreement about its valid name. So far, the following names were suggested successively: *Mus abbotti* Waterhouse, 1837, *Mus tataricus* Satunin, 1908, *Mus spretoides* Thaler, 1983 (nomen dubium, suggested by Bonhomme et al. 1984), *Mus macedonicus* Petrov & Ružič, 1983. Recently, the validity of the three former names has been cast into doubt (cf. Marshall 1986, Auffray et al. 1990a) and the later one was suggested by Musser & Carleton (1993) as currently valid name. Although it is much probable that the taxonomic revision of all *Mus* taxa described from the distribution range of the species will reveal older valid names, we follow the above authors and use the name *Mus macedonicus* here.

There is fairly good information on the reproduction in at least two free living mice species of western Palaearctic. The most extensive data are available about the Mound building mouse, *Mus spicilegus*. From the 1940s it was studied by various authors in south Ukraine and Moldavia, and the results were summarized in the

book by Sokolov et al. (1990). Valuable information is also contained in the monography by Mikeš (1971) who studied in detail its ecology in the Vojvodina region, Yugoslavia. The other, thoroughly studied species was *Mus spretus* that received considerable attention only recently, in Spain and France (e. g., Vargas et al. 1984, 1986, 1991, Cassaing & Croset 1985, Durán & Sans-Coma 1986, Durán et al. 1987). Other free living mice populations the taxonomic status of which, unfortunately, remains unclear were studied extensively in Kazakhstan, Central Asia (Borisenko 1977). Also *Mus musculus* populations collected in fields in former Czechoslovakia by Pelikán (1974) could be included among free living mice. In the south of the Balkans, the only comprehensive study on the biology of free living mice populations (Straka 1966) suffers from the unclear specific status of the used material, that may be composed from three mouse species, at least.

According to our knowledge, the only data on the biology of reproduction in *Mus macedonicus* are few records on litter size from southern Bulgaria by Markov (1964) who referred this mouse to *Mus musculus hispanicus*. Therefore, the aim of this study is to provide a basic information about the reproductive biology in *Mus macedonicus* and to compare it with that in other free living mice species from the western part of the Palaearctic region.

Material and methods

Altogether, 538 specimens of *Mus macedonicus* captured during the period 1983–1994 in 64 localities in the south of the Balkan Peninsula, were used in our analysis. Most of the material was collected in northern Greece: 34 localities in Macedonia (275 specimens), 17 localities in Thrace (186 specimens) and 3 localities in Epeirus (9 specimens). For details about localities, date and number of specimens collected in most of the above material see Vohralík & Sofianidou (1987, 1992) and Sofianidou & Vohralík (1991). Additional 30 specimens were collected in 4 localities of southeastern Bulgaria (Krumovo, district Jambol; Dâbovec and Knižovník, district Chaskovo; Sozopol, district Burgas), 27 specimens in 3 localities of south-western Bulgaria (Sandanski, Strumjani and Baldevo, district Blagoevgrad) and 11 specimens in 3 localities (Star Dojran, Pretor and Vozarci) in former Yugoslav Macedonia. All the material is deposited in collections of the Department of Zoology, Charles University, Prague.

Mice were obtained mostly by snap traps. Body weight was recorded to the nearest gram. Next, mice were dissected and the condition of their reproductive organs was ascertained.

Embryos were counted macroscopically and their length (in the longitudinal axis) was measured. The presence of placental scars was also recorded. Embryos which were conspicuously smaller than the remaining ones in the set, were considered to be resorbed. Weight of embryos in the set was estimated according to Zejda (1968). Net body weight of pregnant females (i. e., without weight of embryos) was used in further analysis.

The term “mature” females is used for specimens which already have taken part in the reproduction, being either actually pregnant, lactant, or with placental scars. Females not participated in reproduction as yet, were considered to be “immature”.

Testis (length and width) were measured with the precision to the nearest 0.1 mm, and length of vesicular glands (from the point of their fusion to their outmost margin) to the nearest millimetre. Area of cross-section of the testes (AC-ST) was used as a criterion of sexual activity. It was computed using formula: $AC-ST [mm^2] = testis\ length [mm] * testis\ width [mm] * 3.1415 * 1/4$. We considered males showing values of $AC-ST > 25\ mm^2$ to be sexually active. This arbitrary criterion was determined on the basis of relationships between AC-ST and the length of vesicular glands in specimens collected in May. In spring and summer there is a full correspondence between the terms sexually mature and sexually active males. However, in autumn and winter samples mature males affected by testes regression are sexually inactive.

Results

Sexual maturation in relation to body weight

Intensity of sexual maturation process was expressed as increase in the proportion of mature specimens within subsequent weight categories in the material collected during the period of intensive reproduction (i. e., May to August period in our material).

Lightest mature males weight 12 g. Four of nine specimens (44 %) belonging to weight category 11–12 g were mature (Table 1). In the total material of the following three weight categories (13–18 g) only a small proportion of males (7 %, n = 55) remained immature. No immature specimens were found among males exceeding the weight of 18 g (n = 25).

Maturation process in females seems to start earlier than that in males (Table 1). The lightest sexually mature female had 10 g. For subsequent weight categories an intensive maturation was typical. As many as 65 % of 20 females between 11 and 16 g were mature. Within weight categories above 16 g, proportion of mature specimens attained the maximal level, with only 8 % (n = 60) remaining immature. However, it is most probable that the real percentage of mature females in our material was slightly underestimated due to following reasons. Among immatures were included also primiparous females during the first five days of their pregnancy, when it is not possible to simply recognize gravidity by means of the macroscopical examination of the uterus (cf. Pelikán 1974). Also, mature females which had participated in reproduction several months before, but had remained sexually inactive in the last months could have been exceptionally scored as immature.

Proportion of sexually active males

The proportion of sexually active males gradually decreased during the course of the period May to December (Fig.1). The highest percentages of sexually active males were found in May (87 %, n = 37) and June (91 %, n = 11), i. e., in the period in which overwintered, sexually active males highly predominated over males born in

Table 1: Proportion of sexually mature males and females within the individual weight categories during the period of intensive reproduction (May to August).

Body weight	Males (n = 100)			Females (n = 90)		
	n	mature	%	n	mature	%
5–6	2	0	0	1	0	0
7–8	3	0	0	1	0	0
9–10	6	0	0	8	1	13
11–12	9	4	44	4	2	50
13–14	10	9	90	7	6	86
15–16	11	9	82	9	5	56
17–18	34	33	97	11	10	91
19–20	17	17	100	19	16	84
21–22	8	8	100	18	18	100
23–24	0	—	—	9	8	89
25–26	0	—	—	3	3	100

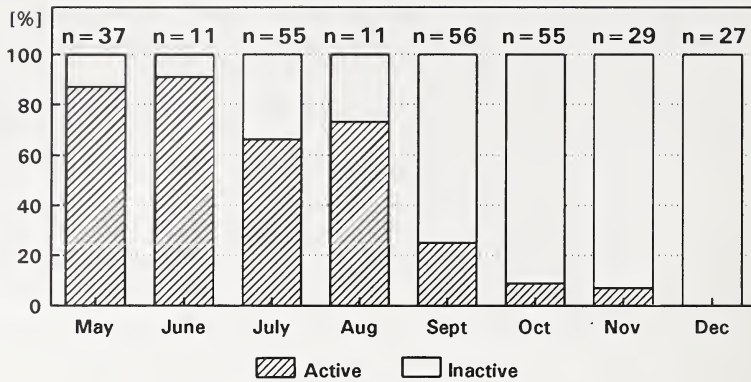


Fig. 1: Variation in the proportion of sexually active males in *Mus macedonicus* during the period May to December.

the current year. As a result of the continual appearance of young, immature individuals, the proportion of sexually active males decreased in July and August to 66 % (n = 55) and 73 % (n = 11), respectively. In the following months the decrease continued owing to mass occurrence of young animals, decreased intensity of maturation, and testes regression in mature males. Corresponding values in September, October and November were 25 % (n = 56), 9 % (n = 55) and 7 % (n = 29), respectively. All males captured in December (n = 27) were already inactive.

Proportion of sexually mature females

Proportion of mature females (Fig. 2) was highest in May 95 % (n = 21). Distinct decrease in the following months was a result of mass occurrence of young animals during the summer (June 64 %, n = 11, July 70 %, n = 57, August 75 %, n = 8). Autumnal drop (September 42 %, n = 62, October 40 %, n = 38, November 22 %, n = 23, December 21 %, n = 24) can be attributed in addition to the above factor also to the selective mortality of mature animals and the termination of the maturation process at the end of the breeding season.

Duration of breeding season

Due to the lack of the material from the January to April period, the only information available on the beginning of the breeding season can be based on the age estimation in current year specimens collected in May. The best for this purpose seems to be a sample of 48 animals collected during the period 3–11 May 1994 in three localities of Macedonia, Greece. The young, in the current year born individuals were identified among them according to their lower body weight, small body dimensions, fur coloration, and in males also according to considerably smaller length of vesicular glands. Altogether, we found five current year specimens in this sample. The biggest among them were a male (body weight 16 g) and a female (14 g) which was already pregnant (length of embryos 3 mm). According to our knowledge on postnatal growth in laboratory born *Mus macedonicus* (D. F., unpublished data), the birth date of these two specimens can be estimated approxi-

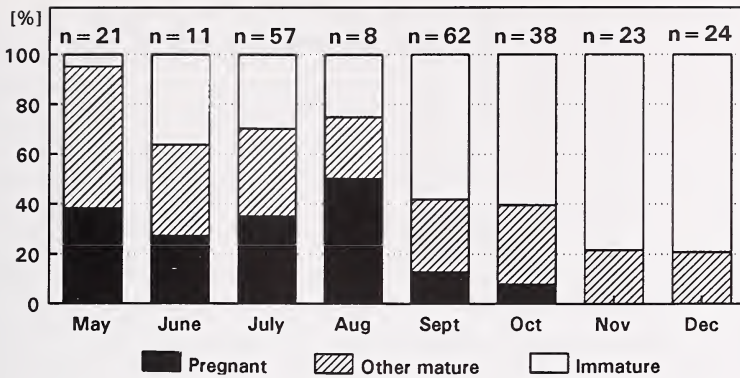


Fig. 2: Variation in the proportion of mature and pregnant females in *Mus macedonicus* during the period May to December.

mately to the beginning of March and consequently, their conception to mid February.

The end of the breeding season was put to October when last pregnant females were collected (Fig.2). They composed small proportion only among mature (20 %, $n = 15$) as well as among all captured females (8 %, $n = 38$). Last pregnant females were from the 10th, 18th and 20th October, and their embryos measured 11, 15 and 16 mm, respectively. These data suggest that parturitions take place till the end of October. Therefore, the duration of the breeding season could be estimated at about 8½ months (i. e., mid February — late October). However, we ought to keep in mind that the length of the breeding period can vary between years and that our estimation of its beginning is based on data obtained in a single year only.

Breeding intensity

The reproduction in Balkan populations of *Mus macedonicus* has distinctly seasonal character. The breeding intensity during the year was expressed as proportion of pregnant among all the females in the sample. As evident from Fig.2, this proportion was fairly high during the spring—summer period (May 38 %, $n = 21$; June 27 %, $n = 11$; July 35 %, $n = 57$; August 50 %, $n = 8$). Marked drop in breeding intensity was recorded in September (13 %, $n = 62$) and October (8 %, $n = 38$). Pregnant females were not present in November ($n = 23$) and December ($n = 24$) samples.

Breeding intensity can also be given as percentage of pregnant among mature females. This percentage was also high throughout the entire spring—summer period (May 40 %, $n = 20$; June 43 %, $n = 7$; July 50 %, $n = 40$; August 67 %, $n = 6$). Its rapid decrease was observed from September (31 %, $n = 26$) till the end of the breeding season (October 20 %, $n = 15$).

Litter size

In 45 visibly pregnant females, the litter size (i. e., all visible embryos in the set, including the resorbed ones) varied within the range 4–10, $\bar{x} = 6.73$ embryos (S. E. = 0.23). The value represents the annual mean of all females, irrespective of

Table 2: Variation in litter size during the period May to October including embryos affected by resorption (mean indicated by asteric was computed for unresorbed embryos only).

Month	n	4	5	6	7	8	9	10	Mean	Mean*
May	8	0	0	0	3	3	0	2	8.13	8.00
June	3	0	0	1	1	1	0	0	7.00	7.00
July	20	2	4	6	5	1	1	1	6.30	6.10
August	4	1	0	0	2	1	0	0	6.50	6.50
September	7	1	0	4	0	1	0	1	6.57	6.29
October	3	0	0	2	1	0	0	0	6.33	5.00
Total	45	4	4	13	12	7	1	4	6.73	6.49
% (S. E.)	—	9	9	29	27	16	2	9	(0.23)	(0.26)

Table 3: Variation in resorption rate during the period May to October. Explanations: SLA = size of litters affected (numbers of resorbed embryos are given in parentheses). Percents were computed for two-months periods.

Month	sets	affected	%	embryos	affected	%	SLA
May	8	1		65	1		7(1)
June	3	0	9.1	21	0	1.16	
July	20	2		126	4		10(2) 5(2)
August	4	0	8.3	26	0	2.63	
September	7	1		46	2		6(2)
October	3	2	30.0	19	4	9.23	7(1) 6(3)
Total	45	6	13.3	303	11	3.63	

date and the locality of capture. If only unresorbed embryos were counted, the corresponding mean litter size was 6.49 (S. E. = 0.26).

Variation in litter size during the year is given in Table 2. Despite very low sample sizes, one-way analysis of variance revealed significant variation of litter size between the two months periods ($F = 4.505$; $P = 0.0169$, non-resorbed embryos only). Tukey test confirmed that the mean litter size in May to June (7.73, $n = 11$) is significantly higher than the corresponding values both in July to August (6.17, $n = 24$) and in September to October (5.90, $n = 10$). It is to be remarked here, that the mean net body weight of pregnant females was almost identical in all the above periods ($F = 0.198$; $P = 0.8213$) and the effect of season on litter size even increased when the body weight was introduced as covariate into ANOVA model ($F = 4.758$; $P = 0.0149$).

In the total of 45 visibly pregnant females, 6 embryo sets (13.3 %) were affected by resorption. In two cases, only one embryo was affected, in three cases, two embryos. In one case, even three of six embryos in the set were resorbed. In total, only 11 of 303 embryos examined were affected (3.63 %). Resorption rate showed slightly higher values at the end of the breeding season (Table 3).

Discussion

There is a general agreement between our findings in *Mus macedonicus* and observations reported by most authors studying free living mice populations (*Mus spicilegus*: southern Ukraine, Naumov 1940, Vojvodina, Mikeš 1971; *Mus* sp.: Kazakhstan, Borisenko 1977; *Mus musculus*: field populations in Czechoslovakia, Pelikán 1974, 1981) that the majority of individuals of both sexes attain their sexual maturity in the size ranging between 10 and 14 grams. Also our estimation concerning the duration of the breeding season in *Mus macedonicus* (mid February to late October) corresponds fairly with data available on other free living mice species. In various populations of *Mus spicilegus* in southern Ukraine the following breeding periods were found: mid March to October (Sokolov et al. 1990), early February to October (Pisareva 1948) and mid April to October (Naumov 1940). For the same species from Vojvodina Mikeš (1971) reported the period March to November. Similarly, a breeding season lasting from February to October was reported in *M. spretus* from southern Spain (Vargas et al. 1991). In general, we can conclude that a seasonal reproduction period with an interruption during winter months appears to be a common phenomenon in all free living mice populations of the western Palaearctic, studied so far.

Comparing the proportion of pregnant among all the females collected during the period of most intensive reproduction (May to August), it is evident that the value found in our material of *Mus macedonicus* (36.1 %, $n = 97$) is closest to those in *Mus spicilegus* from Vojvodina (36.7 %, $n = 120$, Mikeš 1971), while the value reported in *Mus spicilegus* from southern Ukraine (50.0 %, $n = 204$, Sokolov et al. 1990) is considerably higher.

Mean litter size in our total material of *Mus macedonicus* (6.73) is practically the same as the value computed from the data published by Markov (1964), who had at his disposal a sample collected during 1960–1962 in southern Bulgaria (mean = 6.75, range 3–9, $n = 16$). Fairly close values were reported in populations of *Mus spicilegus* from S Ukraine (6.7, $n = 189$, Sokolov et al. 1990; 6.9, Naumov 1940), as well as in free living mice captured in Bulgaria (6.53, range 3–10, $n = 132$, Straka 1966). Slightly higher values were reported in *Mus spicilegus* from Vojvodina (7.30, range 2–15, $n = 56$, Mikeš 1971) and in field populations of *Mus musculus* from Czechoslovakia (7.85, range 4–12, $n = 67$, Pelikán 1974). Similar span of the average litter size was found in free living mice populations collected in different parts of Kazakhstan. Corresponding values in the populations from districts Aktjubinsk and Ural (Borisenko 1977), Kustanaj and Turgajsk (Borisenko 1964) and Alma-Ata (Machmutov 1970) were 6.8 ($n = 17$, range 5–10), 7.7 ($n = 68$, range 1–14) and 7.0 ($n = 65$, range 5–11), respectively. On the other hand, mean values of net litter size (i. e., without resorbed embryos) reported in *Mus spretus*, 5.0 (Durán et al. 1987) and 5.53 ($n = 193$, range 2–10; Vargas et al. 1991) are distinctly smaller than average net litter size 6.49 found in our material of *Mus macedonicus*. Comparing above differences we ought to keep in mind the general tendency in rodents to decrease their litter size in east-west, conceivably also in north-south directions in Europe. For example, in the Wood mouse, *Apodemus sylvaticus* the differences in mean litter size between populations from the Iberian Peninsula and the Balkans (Frynta & Vohralík

1992) are nearly the same as those given above in free living mice. However, it has to be mentioned here that Pelikán (1974) found similar differences also between field (7.85, $n = 67$) and commensal populations (5.58, $n = 183$) of *Mus musculus* in Czechoslovakia, which fact suggests that not only latitude and longitude, but also habitat can considerably affect the litter size in murine rodents.

It is of interest that the May—June peak in mean litter size found in our material of *Mus macedonicus* has a parallel in an April—May peak reported by Vargas et al. (1991) in *Mus spretus*. They attributed this phenomenon to the higher age of overwintered females prevailing in the population during this period. On the contrary, highest mean litter size values were found during the June—August period in *Mus spicilegus* of southern Ukraine (Sokolov et al. 1990).

Although the percentage of resorbed embryos in our material of *Mus macedonicus* (3.63 %, $n = 303$) is twice as high as corresponding values 1.57 % ($n = 1068$) reported by Vargas et al. (1991) in *Mus spretus* or 1.87 % ($n = 536$) reported by Pelikán (1974) in field populations of *Mus musculus* in Czechoslovakia, the sample size in our material is too small and seasonal variation too high for any further conclusion.

Basing on above comparisons we can conclude that, in general, the reproduction pattern in *Mus macedonicus* resembles that reported in other free living mice species of the western Palaearctic.

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Zusammenfassung

Die Fortpflanzung von *Mus macedonicus* wurde an 538 Exemplaren dieser Art von 64 Standorten in Nordgriechenland, Südbulgarien und Mazedonien untersucht. Gegenstände der Untersuchung waren: Länge der Fortpflanzungszeit, Geschlechtsreife im Bezug zum Körpergewicht, Anteil geschlechtsreifer Individuen in der Population, Fortpflanzungsintensität, Wurfgröße, Embryonalresorption. Die Fortpflanzung von *Mus macedonicus* trägt ausgeprägten Saisoncharakter, die Wurfgröße beträgt 4–10, $\bar{x} = 6.73$ ($n = 45$). Die gewonnenen Erkenntnisse wurden mit Literaturangaben über andere freilebende Mäuse der Gattung *Mus* in Europa und Mittelasien verglichen.

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