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Reproduction of the Algerian mouse (*Mus spretus* Lataste, 1883) in the south of the Iberian Peninsula

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Abstract. The reproduction characteristics of *Mus spretus* in the south of the Iberian Peninsula were determined by analyzing 2067 specimens (1186 ♂, 881 ♀) trapped at monthly intervals during six years: March 1982—February 1988. *M. spretus* exhibits, in the study area, seasonal reproductive cycles with two well marked phases: a period of sexual inactivity in winter (November to January), with reduction in the size of testicles and seminal vesicles, and a period of sexual activity during the remaining months, with two phases of maximum activity: April—May and August—September. The females reach sexual maturity between 6—7 weeks, while the males mature from 8 weeks onwards. However, the season of birth seems to influence the age of maturity in both sexes. The average litter size was 5.3 ± 1.4 embryos. Moreover 1.57 % of embryos in different phases of reabsorption were found. Male sexual activity is positively correlated with both temperature and length of photoperiod. Female sexual activity is similarly modulated.

Key words. *Mus spretus*, reproduction, sexual activity, southern Spain.

Introduction

Research publications about the reproduction of *Mus spretus* in the wild are quite common, but most of them only refer to specific localities and to certain limited aspects of reproductive activity in the context of other material. Because of this, the information in the bibliography is fragmented (Bernard 1965; Fayard & Erome 1976; Sage 1981; Orsini et al. 1982; Carrascosa 1982; Alcover 1983). The lack of more complete information is due, in part, to the fact that for a long time, *M. spretus* was considered to be a subspecies of *Mus musculus* (L., 1758) (Thaler et al. 1981).

At the present time, only three works, Vargas et al. (1984, 1986) and Durán et al. (1987) describe the reproductive biology of males and females of *M. spretus* for at least one annual cycle. These authors studied several wild populations in different parts of the Iberian Peninsula. The present work reports and discusses the results of a six-year study in which a series of specimens of both sexes of *M. spretus* were captured monthly from a population in the south of Spain. The data collected was processed to generate a model which damps-out/smooths the observed interannual fluctuations displayed by some reproductive parameters; it also takes into account their range of variation.

While the results of this present work should be interpreted, in principle, as being linked to geographical distribution and although the populations of *M. spretus* are quite uniform phenetically and morphometrically (Palomo et al. 1983, 1985), biochemically (Bonhomme et al. 1984), or caryologically (Cano et al. 1984), this does not mean that the observed uniformity of these biological parameters necessarily extends to all the characteristics of the reproduction dynamics of a species (Durán et al. 1987).

Material and Methods

a) Study area. All the specimens of *M. spretus* analyzed were captured in the sugar cane fields at the mouth of the Guadalhorce river (36° 40' N and 4° 27' W) in the Málaga province (southern Spain). The bioclimate is medium-dry, Warm-Mediterranean (Rivas-Martínez 1984); rainfall is 469 mm and mean annual temperature, 18.5 °C. Information about the sugar cane plantations and the agricultural dynamics can be found in Grana & Olalla (1976).
b) Material analyzed. A total of 2067 specimens (1186 males and 881 females) were captured between March 1982 and February 1988 (Table 1).
c) Assessment of relative age. The relative ages of the animals were determined by inspecting the successive coats and moults displayed by the animals throughout their life and the images of the upper molar wear, using the criteria of España et al. (1985) to establish eight relative-age classes (A-H).
d) Assessment of sexual state. The criteria of sexual state of the male animals were: the presence or absence of external, descended testicles, testicular length (TL) and the length of seminal vesicle (VL), the finding of spermatozoa in the reproductive tract following staining with Diff-Quick (Gosálbez et al. 1979) and also histological analysis of the gonads. For the females, the criteria were: presence or absence of embryos and placental scars, the degree of uterine development and irrigation, the development of mammary tissue and the histology of the ovaries.
e) Statistical analysis. Student's test and X^2 test were used to compare mean values and percentages; in one case, variance analysis was employed. Correlations were calculated from Pearson's correlation coefficient (Sokal & Rohlf 1969).

An analysis of autocorrelation (Davis 1973) was employed to determine whether the observed temporal variations of reproductive activity were periodical. A multiple regression analysis program from the S.P.S.S. statistical packet (Nie et al. 1975) was used to determine how much these variations might be due to climatic fluctuations occurring during the study period. The considered variables were: monthly average temperature and monthly average relative moisture at 7.00, 13.00 and 18.00 h., total monthly rainfall in mm, number of rainy days per month and total monthly evaporation.

Table 1: Monthly distribution of *Mus spretus* captured during the six years of study: March 1982 — February 1988.

	1982		1983		1984		1985		1986		1987		1988		T	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
J			47	32	27	19	18	23	24	18	11	8	11	5	138	105
F			28	15	15	19	17	14	15	7	20	14	14	3	109	72
M	13	9	22	9	13	3	15	12	16	9	9	9			88	51
A	22	13	9	11	13	12	18	17	35	22	5	5			102	80
M	13	6	16	13	13	6	14	10	20	18	6	12			82	65
J	2	2	9	9	12	8	31	24	15	11	13	12			82	66
J	18	11	8	12	8	12	25	23	13	12	14	11			86	81
A	16	19	12	8	13	12	13	6	10	11	19	9			83	55
S	25	11	9	14	11	8	11	13	3	3	12	9			71	58
O	59	27	13	9	13	20	10	11	9	7	11	14			115	88
N	37	17	13	17	9	21	39	17	14	5	10	9			122	86
D	33	23	19	9	10	11	20	14	9	7	17	10			108	74
T	238	128	205	158	157	151	231	184	183	130	147	122	25	8	1186	881

Results

The macro- and microscopic analyses of the gonads of both sexes revealed that the youngest males with demonstrable reproductive capacity belonged to the relative-age class C (TL $\geqq$ 6.0 mm; VL $\geqq$ 4.5 mm; with mature spermatozoa). On the other hand, females developed reproductive capacity much earlier, as is shown by the capture of 2 pregnant animals and another 2 with uteri showing placental scars, all in relative-age class B. However, in class C, the percentage of sexually active captured females (18.2 %; n = 88) is smaller than that of sexually active males (49.0 %; n = 108) (t = 6.39; p <0.001) because the males, once sexually mature, are constantly active during the breeding season, while the females are subject to the œstrus cycle and have alternate periods of sexual activity and inactivity. Both sexes reach puberty from age B, this period is shorter in the females, however whether birth occurs early or late in the breeding season appears to be an important factor.

Throughout the six years of the survey 193 gestating females with 1068 visible embryos were examined. The average litter size was 5.53 ±1.37 embryos (range: 2 — 10; mode: 5) excluding reabsorbed embryos. The interannual variations of the average litter size are of overall significance (F = 18.13; p <0.01). The annual averages are shown in Table 2.

It was shown that there is a correlation between litter size and weight of the female (r = 0.35; p <0.001) and between litter size and the size (head and body length) of the female (r = 0,26; p <0.01). At the same time, the average number of embryos per litter increased with the age of the females, as shown in Table 3, because it appears to depend, in part, on the number of previous litters, that is to say, that the multiparas tend to produce larger sized litters than primiparas and litter-size increases with the number of previous gestations. The decrease observed for relative-age class H was attributed to the senility of these females. Inspection of figure 1 shows that the largest sized litters were produced in April and May and correspond largely to the F, G and H relative-age classes. From August to October, inclusive, a considerable part of the reproductive females are animals born that year and are producing litters for the first time; this explains the smaller litter sizes in comparison with those of the April—May period. In all cases, the implantation of embryos was equally distributed between both uterine horns ($\overline{x}$ left uterine horn = 2.8; $\overline{x}$ right uterine horn = 2.7; X^2 = 0.18; p >0.05).

Table 2: Interannual variations of the mean litter size.

Cycle	n	$\overline{x}$	s
1	26	5,00	0.98
2	30	6,30	1,26
3	31	5,70	1,65
4	39	5,60	1,18
5	39	5,40	1,44
6	28	5,10	1,27

Table 3: Variation in mean litter size in relation to female age class.

Age class	n	$\overline{x}$	s
B	2	4,50	0,71
C	2	4,50	0,71
D	22	5,18	0,90
E	61	5,36	1,21
F	57	5,53	1,36
G	34	6,50	1,54
H	10	5,70	1,49

Seventeen embryos were observed in different stages of reabsorption; this represents 1.57 % of the total embryos studied. In 13 of these cases, the reabsorption was simple, however, one animal presented multiple reabsorptions (2 embryos in the left uterine horn and 2 in the right). The embryos being reabsorbed were equally distributed between the two uterine horns (X^2 = 0.52; p >0.05) and the smallest litters appeared to be affected as much as the largest and both more affected than intermediated ones (Table 4). This variation in reabsorption rate can suggest that females with very small litters are physiologically affected individuals, as indicated also by the small numbers of embryos implanted, while females with a large number of implants are apparently incapable of nourishing such a large set. These results agree with those found by Pelikan (1981) in *Mus musculus.* The average number of embryos in those litters showing reabsorption is 6.00 ±1.52, a value which was not significantly different from that already mentioned for normal litters (t = 0.48; p >0.05). The females with embryonic reabsorption showed the following distribution by relative-age classes: D (n=2); E (n=4); F (n=3) and G (n=4).

Table 4: Reabsorption rate in relation to number of implanted embryos.

Number of implanted embryos	2	3	4	5	6	7	8	9	10
Sets examined	1	8	33	62	45	27	13	3	1
Sets affected of reabsorption	0	1	1	3	3	5	0	1	0
% sets affected	0	12,5	3,0	4,8	6,6	18,5	0	33,3	0
Embryos examined	2	24	132	310	270	189	104	27	10
% embryos resorbed	0	4,2	0,8	1,0	1,1	4,2	0	3,7	0

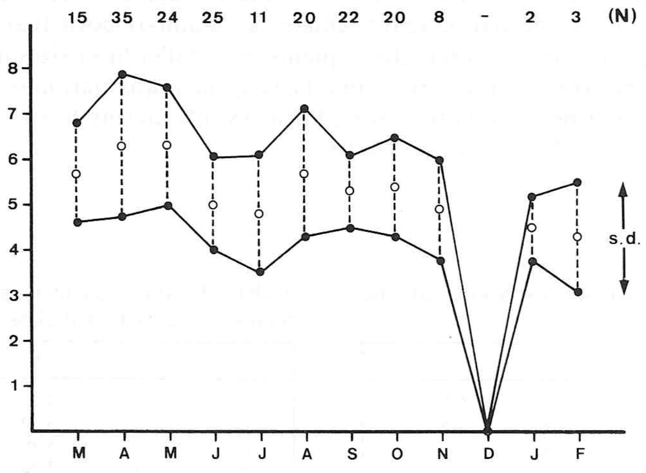

Fig. 1: Monthly variation of the mean size of the litters throughout the annual cycle. N = number of gestating females analyzed; s. d. = standard deviation.

The analysis of autocorrelation of the monthly percentages of the sexually active males and females confirmed the cyclic character of the reproductive activity of *M. spretus*. The values obtained in the autocorrelogram show a succession of reproductive cycles for both sexes with an annual rhythm that is maintained for 3 years in the males, and for 4 years in the females ($p < 0.05$; Fig. 2). Other significant values of autocorrelation, although of negative sign, and 6 months out-of-phase with the previous ones, were detected for the first 4 cycles in males, and only in the first cycle in females (see Fig. 2). This reflects the existence of an annual phase of generalized sexual activity and another of rest. It should be mentioned that the percentages of active males and females throughout the year are closely correlated ($r = 0.93$; $p < 0.001$). Figure 3 displays the duration and intensity of the reproductive period throughout the annual cycle.

The multiple regression function between the percentages of sexually active males and the climatic variables is statistically significant ($R = 0.74$; $p < 0.001$). Inspection of the magnitude and sign of the standardized partial regression coefficients for the climatic variables shows that the mean monthly temperatures at 18.00 and 13.00 hours are the most important values for predicting the monthly percentage of sexually active males. In the first case, the coefficient is positive; in the second, it is negative. This result is a mathematical contradiction (the sexual activity of the males is positively correlated with both temperatures) and should be interpreted as being due to the fact that the sexual activity of the males increases with the quotient

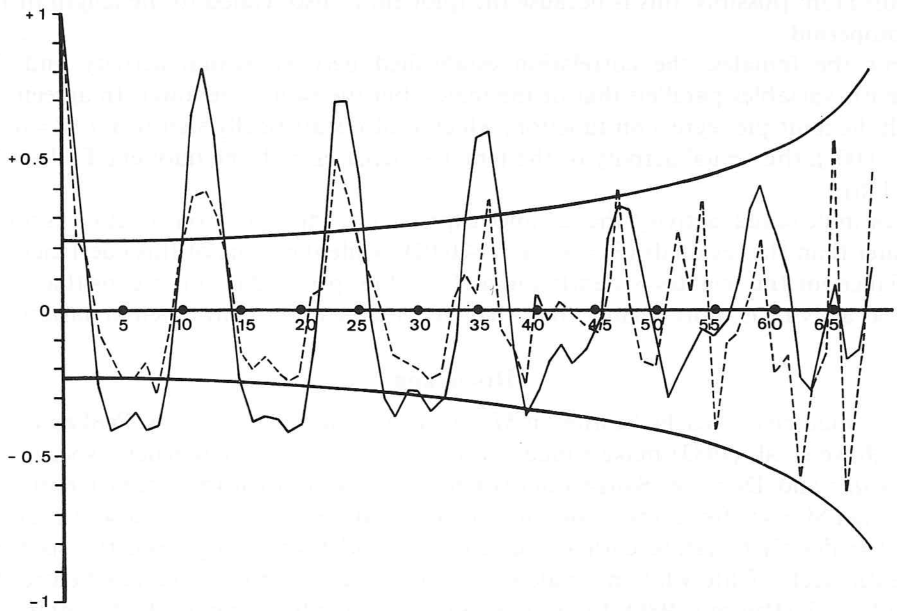

Fig. 2: Autocorrelogram of the relative monthly frequencies of the sexual active ♂ (continuous line) and ♀ (dotted line). The symmetrical curve indicates the confidence level $p < 0.05$.

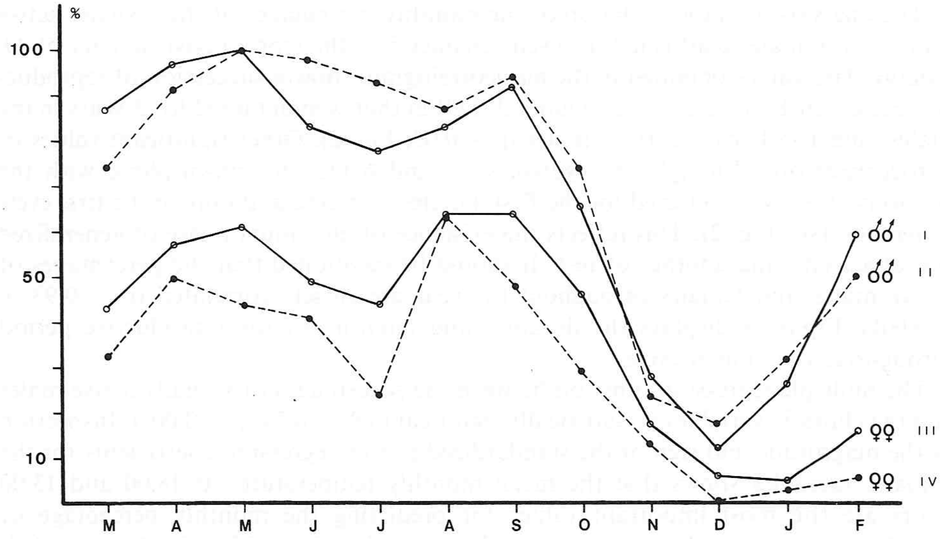

Fig. 3: Characterization of the sexual state of the ♂ and ♀ throughout the annual cycle. I: sexually active ♂; II: ♂ with descended testicles; III: sexually active ♀; IV: gestating ♀.

T18h/T13h; possibly, this is because the quotient is also related to the length of the photoperiod.

For the females, the correlation established between sexual activity and the climatic variables parallels that of the males, but the values are lower. In agreement with the multiple regression function, which is also statistically significant ($R=0.53$; $p<0.001$), the sexual activity of the females increases with the quotient T18h/(T7h x T13h).

For male sexual activity, the variance expressed by the regression function is much greater than the residuals ($F = 6.74$; $p<0.01$), while the value of this coefficient of variance for the females is clearly lower ($F = 2.18$; $p<0.05$). This means that male sexual activity is more related than that of females with environmental variations.

Discussion

Sexual maturity is reached earlier in *M. spretus* females than in males. Pelikan (1981) and Rowe et al. (1983) make similar observations about the congeneric species *M. musculus* and Durán & Sans-Coma (1986) partially confirm this characteristic for Algerian Mouse after carrying out histological analyses of ovaries. In absolute terms, the females that mature earliest can conceive and bear young from the sixth or seventh week of life while no male of the species can fertilize a female before the eighth week (Palomo 1986). In any case, in each individual animal, the beginning of sexual maturity appears to depend on a complex of unique characteristics such as environmental and populational factors (Bronson & Rissman 1986). In fact, Vargas et al. (1984, 1986) studying *M. spretus*, show that the main determinant of the beginning of sexual activity in each animal is whether it was born early or late in the breeding season, as shown by Breakey (1963) for *M. musculus*.

The changes in litter size observed in different populations of *M. spretus*: $\overline{x} = 5.6$, $\overline{x} = 5{,}8$ (Sage 1981); $\overline{x} = 5.0$ (Durán et al. 1987) fall within the ranges of annual means described in this present work, in which the interannual variability showed significant differences that suggest they are widespread. It was not possible to compare these differences between different populations because only insufficient data about other populations in other areas was available. However, there have been many other attempts to determine if the litter-sizes of other rodents vary and why; a number of researchers (Niethammer 1972; Innes 1978; Bronson 1979; Pelikan 1981; Cassaing 1982; Rowe et al. 1983; Zejda & Pelikan 1984; Myers et al. 1985; Perrin 1986) have suggested multi-factorial influence.

In the five-month period October—February the testicles and seminal vesicles of the males decrease in size ($r = 0.97$; $p < 0.001$) (for comparison with other rodent species see Saint-Girons 1967; Huminski 1969; Claude 1970; Martinet 1972; Gosálbez & Sans-Coma 1976; Sans-Coma & Gosálbez 1976; Craven & Clarke 1982; Conte 1986; Zamorano et al. 1987; Ventura 1988). However, in this same period, the numbers of spermatozoa, even in the reduced-size testicles, did not vary significantly with the numbers found during the other months of the year. This finding coincides with that of Durán et al. (1987) for *M. spretus* and those of Berry (1970), López-Fuster (1978) and Ventura (1988) for other species of rodents, however, this should not be taken to be a general rule (Claude 1970; Conte 1986; Zamorano et al. 1987). It is highly probable, at least in the case of *M. spretus*, that the reduction in size of the testicles diminishes the amount of spermatozoa produced and the secretion of the hormones involved in spermatogenesis but does not necessarily imply a complete interruption of spermatogenesis.

Taken together, the relative characteristics of the reproductive biology of *M. spretus* in the south of the Iberian Peninsula may be considered to be a typical model of an essentially surface-living, non-commensal, nocturnal rodent of the Mediterranean region (Vargas et al. 1987). The reproductive cycle is markedly seasonal with an approximately three-month winter rest period (November—January) and two phases of maximum sexual activity spread over the remaining months, similar to the pattern described for other species of wild rodents (Laurie 1946; Breakey 1963; Saint-Girons 1967; Pelikan 1972, 1981; López-Fuster 1978; Conte 1986; Perrin 1986; Zamorano et al. 1987; Ventura 1988; Palomo et al. 1989.).

The interannual variations detected in the durations and intensities of the reproductive cycles appear to be attributable mainly to the environmental conditions, in particular to the temperature. Sexual activity increases with the mean monthly temperature. For both sexes, although much more obvious for males, an absolute general increase of temperature during the summer months is not as essential as the elevation of evening-day to mid-day temperature in summer.

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Zusammenfassung

Es wurde eine Untersuchung über die Fortpflanzung der Heckenhausmaus, *Mus spretus*, im Süden der Iberischen Halbinsel durchgeführt. Insgesamt wurden 2067 (1186 ♂, 881 ♀) Exemplare analysiert. Die Mäuse wurden von März 1982 bis Februar 1988 in monatlichen Abständen gefangen. In der untersuchten *spretus*-Population dauert die Fortpflanzung meistens von Ende Februar bis Anfang November. Die Fortpflanzungsintensität erreicht die höchsten Werte im April—Mai und August—September. Im Winter gibt es eine Vermehrungspause, während der sogar die Hoden- aber auch die Samenbläschen-Länge zurückgeht. Die ♀ erreichen die Geschlechtsreife schon im Alter von 6—7 Wochen, die ♂ etwas später, d. h. ab der 8. Lebenswoche. Jedoch hängt die Erreichung der Fortpflanzungsfähigkeit bei ♀ und ♂ von der Jahreszeit der Geburt ab. Die durchschnittliche Embryonenzahl je Wurf beträgt 5.53 $\pm$1.37. Es wurden 17 Embryonen-Resorptionsfälle festgestellt, d. h. 1.54 % in bezug auf die Gesamtanzahl der gefundenen Embryonen. Die Geschlechtstätigkeit der ♂ läßt sich gut sowohl mit der Temperatur als auch mit der Länge der Photoperiode korrelieren; die der ♀ scheint auch in Zusammenhang mit diesen Umweltfaktoren zu stehen.

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