SEXUAL MATURATION AND REPRODUCTIVE ACTIVITY OF SPRING-BORN FEMALE CORN MICE, *Calomys musculinus*, IN ABSENCE OF ADULTS

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ABSTRACT: The aim of this study was to analyze the effects of parents on maturation and reproductive activity of spring-born females in fenced populations of *Calomys musculinus*, at the beginning of the breeding period. The field study was carried out in four 0.25-ha enclosures (two control and two experimental), each situated on natural pasture. This study had two periods: 1) Absence of fathers (AF) (from September 2003 to January 2004), and 2) Absence of mothers (AM) (from October 2004 to February 2005). In both periods, in control enclosures both parents remained with the offspring. During AF period, in experimental enclosures only the mothers remained with their offspring. In AM period, only the adult males remained with their offspring in experimental enclosures. Three trapping sessions of 8 successive nights every fortnight were carried out. Sexual maturation and reproductive activity of spring-born females were compared between treatments using repeated-measures ANOVA. The repeated measures factor was the age of young females. In both periods the number of spring-born females in each reproductive condition was independent of treatments. The females matured according to their physiological times (30-40 days of age). The removal of fathers and mothers did not affect either the timing of sexual maturation or the reproduction of *C. musculinus* spring-born females. Future research to test the effect of adult female density on juveniles should be done to test the same parameters.

RESUMEN: Maduración sexual y actividad reproductiva en ausencia de adultos en hembras de *Calomys musculinus* nacidas en primavera. El objetivo de este estudio fue analizar los efectos de los padres sobre la maduración y la actividad reproductiva de hembras nacidas en primavera, en poblaciones de clausura de *Calomys musculinus* al comienzo del periodo reproductivo. El estudio fue llevado a cabo en cuatro clausuras de 0.25-ha cada una (dos controles y dos experimentales), ubicadas en una pastura natural. El estudio estuvo dividido en 2 etapas: 1) Ausencia de padres (AP) (desde septiembre de 2003 a enero de 2004), y 2) Ausencia de madres (AM) (desde octubre de 2004 a febrero de 2005). En AP y AM, en las clausuras controles ambos padres permanecieron con sus crías. Durante AP, en las clausuras experimentales solo las madres permanecieron con sus crías. Por otro lado, durante AM solo los padres permanecieron con sus crías en las clausuras experimentales. Se realizaron tres sesiones de muestreo de 8 noches consecutivas de duración cada quince días. La maduración sexual y la actividad reproductiva de las hembras nacidas en primavera fueron comparadas entre tratamientos mediante un ANOVA de medidas repetidas. El factor de medidas repetidas fue la edad de las hembras. En ambas etapas el número de hembras de cada condición reproductiva fue independiente del tratamiento. La maduración de las hembras se correspondió con el tiempo fisiológico (30-40 días de edad). La remoción de los padres y las madres no afectó ni el tiempo de
INTRODUCTION

*Calomys musculinus* is the dominant rodent species of central and north-western Argentina, and the reservoir of Junin virus, the etiological agent of Argentine hemorrhagic fever (AHF) (Mills and Childs, 1998). It inhabits Pampean agrarian ecosystems and is found in a variety of habitats including natural pastures, crop-field edges, cultivated fields undisturbed after harvest, border areas protected by wire enclosures with little agricultural disturbance, roadsides, and railway banks (Busch et al., 2000). *C. musculinus* populations are characterized by seasonal density changes, with low density during winter (16 mice/ha) and peaks during summer or early autumn (260 mice/ha) (Mills et al., 1991, 1992).

The reproductive period of this species begins in mid-September and finishes at the end of April (Mills et al., 1998). *C. musculinus* has a short gestation length (21 days) and each female can produce many pups in her lifetime (6 pups per litter). Females show a high frequency of postpartum estrus, which implies that a new pregnancy may overlap with the lactation of the previously produced litter (Mills et al., 1992; Buzzio and Castro-Vasquez, 2002). Juveniles of *C. musculinus* reach sexual maturity between 30 and 40 days of age, with a mean weight close to 16.5g. (de Villafañe, 1981). In the laboratory, *C. musculinus* females typically build covered nests, males do not contribute to the construction of the nest, and there is no nest co-habitation by a male-female pair (Yunes et al., 1991). Lactating females display much more aggression towards sexually mature females than towards stranger or familiar males, and the presence of another female near the nest is deleterious for litter survival (Laconi, 1998; Laconi et al., 2000). During the breeding period, females are territorial and their home ranges are both crossed by transient and by resident males but never by breeding females (Steinmann et al., 2008). On the other hand, males have home ranges that are twice as large as those of females and they fully share them with both sexes and their spatial distribution is strongly influenced by searching for mates (Steinmann et al., 2005, 2008). This spacing behaviour pattern of *C. musculinus* agrees with a promiscuous mating system (Steinmann et al., 2008). In promiscuous species, in which females typically mate with more than one male during each estrous period, males are expected to evolve large testes relative to their body size (Heske and Ostfeld, 1990). *C. musculinus* male’s testis present an unusual development of Leydig cells, and testosterone levels in plasma higher than monogamous mouse and vole species (Castro-Vázquez et al., 1987; Buzzio and Castro-Vázquez, 2002).

The age at which sexual maturation is reached, fecundity and the duration of breeding season are the most important reproductive variables in the determination of rodent population abundances (Dapson, 1979; Mihok et al., 1985; Rodd and Boonstra, 1988). In rodent populations the sexual maturation of juvenile cohorts can be related to the presence of adult animals (Wasser and Barash, 1983; Wolff et al., 2001; Wolff et al., 2002). Thus, the removal of specific segments of rodent populations affects reproduction in younger cohorts (Saitoh, 1981; Gilbert et al., 1986; Rodd and Boonstra, 1988; Pusenius and Viitala, 1993). In promiscuous species, territorial females are generally assumed to have a greater impact on inhibiting juveniles than males, due to the fact that females typically
Sexual maturation and reproductive activity in Calomys compete for exclusive offspring-rearing space (Wolff, 1993; Bond and Wolff, 1999). Thus, young females that cannot acquire an exclusive breeding site may delay sexual maturation until space becomes available (Wolff, 1997). Delayed sexual maturation or reproductive suppression of juveniles may result from either direct contact with adults generally through their intrasexual intolerant behaviour, or from chemical signals from urine of related or grouped females (Getz et al., 1983; Heise and Rozenfeld, 1999). However, Wolff et al. (2001) did not observe that the presence of mothers suppressed reproduction in their daughters for Microtus ochrogaster and M. pennsylvanicus. Besides, in Calomys venustus (a promiscuous-polygynous South American rodent) Priotto et al. (2006) observed that both juvenile females and males matured in relation to physiological times independently of the presence of adults.

The aim of this study was to test the hypothesis that the presence of parents causes a delay in maturation and reproductive activity of spring-born females in fenced populations of C. musculinus, at the beginning of the breeding period.

**METHODS**

**Field procedures**

This study was carried out on Espinal Reservation of the campus of the National University of Río Cuarto (33° 07’S, 64° 14’W). The study area was a natural pasture interspersed with bushy and weedy species, and it had high vegetal cover (about 100% throughout the year) being similar to natural habitats of C. musculinus. We set up four 0.25 ha enclosures (two control and two experimental) made of galvanized iron sheets extending 0.3 m underground and 0.7 m above ground. In each enclosure, six reproductive shelters were enclosed with a concrete circle of 1 m diameter and 0.7 m height and were covered by iron mesh. On the inner margin of each enclosure, a 1 m-wide grass strip was devegeted with herbicide. For a detailed description of the study area and enclosure construction see Priotto and Polop (2003), and Priotto et al. (2004).

The study was carried out between September 2003 and February 2005. It had two periods: 1) Absence of fathers (AF) (from September 2003 to January 2004), and 2) Absence of mothers (AM) (from October 2004 to February 2005). Between AF and AM periods (February-September 2004) all animals captured in the enclosures were removed. The rodent populations were from an area located 30 km away from the place of study. In September and October 2003 (AF), and October and November 2004 (AM), 24 and 16 mates respectively were preserved in the laboratory in individual reproductive cages. In both periods, adults and their offspring were weighed and ear-tagged for permanent identification. Both, sex and birth date of the offspring were also recorded. In both periods, after the offspring were weaned in the laboratory, adults and their offspring were carried to the enclosures and then each family group was located in a reproductive shelter. After three days the reproductive shelters were opened and the animals dispersed into the enclosures. During AF period, in control enclosures both parents remained with the offspring (12 mates and 58 juveniles). In experimental enclosures only the adult females remained with their offspring (12 adult females and 45 juveniles). In AM period, there were 8 positive mating less than in AF period. In control enclosures both parents remained with their offspring (8 mates and 47 juveniles) whereas, in experimental enclosures only the adult males remained with their offspring (8 adult males and 49 juveniles).

In each enclosure there was a CMR grid of 6 x 10 traps with an interstation interval of 6 m. One Sherman live-trap was placed at each station and baited with a mixture of peanut butter and cow fat. Three trapping sessions of 8 successive nights every fortnight were carried out from November to January in AF and from December to February in AM. In each devegetated edge, in order to detect those voles that were not able to settle in the habitat area of the plot, 28 Sherman live traps were placed at 6 m intervals. Animals that were trapped three consecutive times in devegetated edge areas within each trapping sessions were removed from the population since we assumed that they were not able to settle within the enclosures. Traps were checked each morning and trapped animals were weighed, and sex and reproductive state were recorded. New animals were individually marked with a numerical code in their ears and released in the site of capture.
Reproductive condition of spring-born females was judged from vagina perforation (perforate or imperforate vagina), pregnancy and size of nipples. When reproductive condition of female could not be judged by external characters, the condition of vaginal smears was analysed. Vaginal smears were taken with a small Pasteur pipette and then a smear was made on a glass slide and observed under a microscope. Vaginal smears were classified as estrous smears when the number of cornified cells was greater than epithelial cells and leucocytes (Buzzio and Castro-Vazquez, 2002). Spring-born females were classified as immature if they had imperforate vagina or no estrous vaginal smear, mature but non-active if they had an estrous vaginal smear or perforate vagina but non-evidence of pregnancy or suckling, or mature and active if they were pregnant or suckling.

Data analyses

The population size for each trapping session was based on the minimum number of animals known to be alive (MNKA), expressed as the number of animals per hectare. The population density was compared among treatments using repeated-measures ANOVA. Each trapping session was the repeated-measure factor. To analyze the number of cohort 1 females in relation to their reproductive condition (immature, mature non-active and mature active females) and treatment (AF and AM), repeated-measures ANOVA was also used (Von Ende, 2001). The repeated-measure factor was the age range at which reproduction condition was measured in each enclosure (e.g.: <20, 20-30, 30-40, 40-50, 50-60, 60-70, and >70 days). Because the F statistics for within-subject factors (and their interactions) are inflated in repeated-measures ANOVA when the sphericity assumption is not met (Von Ende, 2001), Greenhouse–Geisser corrected probability was used when interactions were statistically significant.

RESULTS

AF period

From November 2003 to January 2004 a total of 1267 captures were recorded in 5760 night traps. A total of 384 individuals (187 males and 197 females) were ear-tagged. The population density did not vary between control and experimental enclosures (F = 15.212; d.f: 1.2; P = 0.599), but varied in relation to the trapping session (F = 26.642; d.f: 1.2; P = 0.004). In November, the mean population densities were 35 mice per ha in the control enclosures and 28 in experimental enclosures, and towards January they decreased to 25 and 22 in control and experimental enclosures respectively. During this study, no animal was trapped three consecutive times in live traps placed in the devegetated edge areas within each weekly census, therefore all studied animals were able to settle in the plots.

At the beginning of the breeding period there were not significant differences in the number of cohort 1 females in relation to treatment (F = 3.116; d.f: 1, 6; P = 0.123), but there were significant differences in relation to reproductive condition (F = 16.337; d.f: 2, 6; P = 0.004) and age (F = 4.152; d.f: 6, 36; P = 0.003). However, the interaction between reproductive condition and age was statistically significant (F = 25.903; d.f: 12, 36; P = 0.000). The number of mature non-active females was higher than the number of immature and mature active females. In control and experimental enclosures most females were mature between 30 and 50 days of age, and the reproductive activity started between 50 and 70 days of age (Fig. 1). The other first order interactions (treatment \times reproductive condition; treatment \times age) and the second order interaction (treatment \times reproductive condition \times age) were not statistically significant (P values > 0.05).

AM period

From the beginning of December 2004 to February 2005 a total of 1335 captures were recorded in 5760 night traps. A total of 422 C. musculinus individuals were ear-tagged (232 males and 190 females). Population density did not vary either between control and experimental enclosures (F = 13.4102; d.f: 1.2; P = 0.6148) or in relation to the trapping session (F = 14.2312; d.f: 1.2; P = 0.6201). In December, the mean population densities were 22 mice per ha in control enclosures and 23 in experimental enclosures, and towards February they increased to 28 and 29 in control and experimental enclosures, respectively. During this study, only ten animals were removed of
the enclosure populations because they were trapped three consecutive times in live traps placed in the devegetated edge areas. We assumed that the rest of the animals were able to settle within the enclosures.

At the beginning of the breeding period there were not significant differences in the number of cohort 1 females in relation to treatment ($F = 0.1608; \ d.f. \ 1, \ 6; \ P = 0.7023$), but there were significant differences in the number of cohort 1 females in each reproductive condition ($F = 6.859; \ d.f. \ 2, \ 6; \ P = 0.028$), and first order interaction (age $\times$ reproductive condition) was observed ($F = 26.520; \ d.f. \ 12, \ 36; \ P = 0.000$). In this study period, the number of mature active females was higher than the number of immature and mature non-active females. In control and experimental enclosures the majority of females were mature between 20 and 40 days of age, and the reproductive activity started between 30 and 40 days of age (Fig. 2). The other first order interac-

Fig. 1. AF period. Average numbers of spring-born females of *Calomys musculinus* in relation to age, treatment and reproductive condition. (i) Immature females, (mna) mature but non active females and (ma) active females. Control: presence of both fathers and mothers. Experimental: absence of fathers.

Fig. 2. AM period. Average numbers of spring-born females of *Calomys musculinus* in relation to age, treatment and reproductive condition. (i) Immature females, (mna) mature but non active females and (ma) active females. Control: presence of both fathers and mothers. Experimental: absence of mothers.
tions (treatment × reproductive condition; treatment × age) and the second order interaction (treatment × reproductive condition × age) were not statistically significant (P values > 0.05).

DISCUSSION

In this field study we achieved that the control and experimental populations comprised individuals with known relatedness and early life history. In addition, our approach allowed us to control the age distribution of the population and to standardize the post-weaning social environment. These manipulations might have caused some obscure side effects and thus reduced the compatibility of the result with respect to a completely natural situation. Nevertheless, due to the specific nature of the aim of this study, the requirement of strict control was given priority.

In the present study the absence of a relationship between population densities and treatment (father or mother removal) allowed us to analyse the reproduction of young females assuming independence of population densities.

In our study young females reached maturation between 20 and 50 days of age, independently of treatment. These physiological times were similar to those reported by de Villafañe (1981) and Buzzio and Casto-Vazquez (2002). In both control and experimental enclosures, the reproductive activity started between 30 and 50 days of age.

A lot of studies have suggested that adult females regulate sexual maturation of juvenile females (Batzli et al., 1977; Redfield et al., 1978; Saitoh, 1981; Getz et al., 1983; Haigh, 1987; Bondrup-Nielsen, 1986; Carter et al., 1986; Gilbert et al., 1986; Rodd and Boonstra, 1988; Schadler, 1990). Because females typically compete for territories (Wolff, 1993; Wolff and Peterson, 1998), young females that cannot acquire an exclusive breeding site may delay sexual maturation until density decline or space becomes available (Wolff, 1997). Thus, adult female would affect sexual maturation of young females but not young males (Wolff et al., 2002). C. musculinus is a promiscuous species where females are territorial and do not share their home range with other females, whereas male home ranges overlap with both sexes (Steinmann et al., 2008). These characteristics suggest that mother removal (AM) more than father removal (AF) could affect the timing of sexual maturation of their daughters at the beginning of the breeding season. However, neither the absence of mothers nor fathers in C. musculinus populations affected the timing of sexual maturation and the proportion of young that become reproductive. This finding does agree with those registered in field by Priotto et al. (2006) in C. venustus and in laboratory by Wolff et al. (2001) in M. ochrogaster and M. pennsylvanicus. In our study, the absence of an inhibition effect of adult females on sexual maturation of juveniles could be due to the fact that: a) the initial density of adult females in each enclosure (0.25ha) was lower than a threshold density of twelve females per 0.25ha, considering the average home range size reported by Steinmann et al. (2005); b) adult females do not have any effect on sexual maturation of juveniles independently of their numbers. In this study we were able to test if adult female or male absence affected reproductive parameters of young females at low and constant number of adult rodents. Therefore, considering the space use of C. musculinus females, and the availability of exclusive breeding sites, future research to test the effect of adult female density on juveniles should be done to prove if the spacing behaviour of females limit the number of breeding females and therefore it acts as regulation factor of population density.

ACKNOWLEDGEMENTS

We thank Susana Vilor for the English version. This research was made possible by grants from the Consejo Nacional de Investigación Científica y Tecnológica (CONICET) and Secretaría de Ciencia y Técnica (SECyT), Universidad Nacional de Río Cuarto.
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