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Nota

REPRODUCTIVE SUCCESS IN *Mus musculus* (RODENTIA) EXPOSED TO CONSPECIFIC'S ODORS AND OVERCROWDING IN LABORATORY CONDITIONS

Nora Burroni, María V. Loetti, and María Busch

Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires - IEGEBA (CONICET-UBA), Intendente Güiraldes 2160, Ciudad Universitaria, C1428EGA Buenos Aires, Argentina [correspondence: Nora Burroni <nburroni@ege.fcen.uba.ar>].

ABSTRACT. To assess the use of conspecific's odors for controlling commensal *Mus musculus*, we studied the effect of overcrowding on the reproductive success of females in laboratory conditions. While pregnancy rates, litter size, the weight at birth and survival until weaning did not differ between overcrowding and control groups, the time elapsed until pregnancy was lower for the control group, while the mean weight and the total number of offspring surviving at weaning were higher in the control group. Control females produced a mean of 1.25 offspring, while overcrowded females produced 0.6. A lower weight of the offspring of crowded females may affect their future chances of survival and reproduction.

RESUMEN. Éxito reproductivo en *Mus musculus* (Rodentia) expuesto a olores de conespecíficos y hacinamiento bajo condiciones de laboratorio. Para evaluar el uso de olores de conespecíficos para el control de *M. musculus* comensales, estudiamos el efecto del hacinamiento sobre el éxito reproductivo de hembras en condiciones de laboratorio. La tasa de preñez, el tamaño de camada, el peso al nacer y la supervivencia al destete no difirieron significativamente entre hembras hacinadas y controles. El tiempo transcurrido hasta la preñez fue menor en el grupo control, mientras que el peso promedio y el número de crías sobrevivientes al destete fueron mayores. Las hembras control produjeron un promedio de 1,25 crías y las hacinadas 0,6. El menor peso de las crías de las hembras hacinadas podría afectar su supervivencia y futura reproducción.

Key words: House mice. Pests-control. Reproduction.

Palabras clave: Control de plagas. Ratón doméstico. Reproducción.

Mus musculus Linnaeus, 1758 (Rodentia, Muridae) is one of the most widespread mammalian species in the world. From its origin in the grasslands of Central Asia, it followed humans around the world. This mouse is frequently restricted to commensal habitats (Elias, 1988; Singleton et al., 2005), but in many parts of the world it also inhabits many other habitats such as cropfields, pastures, and natural habitats with low human intervention (Rowe et al., 1983; Pocock et al., 2005; Witmer and Jojola, 2006; McAllan et al., 2008). Although there is extensive information about reproductive characteristics of laboratory strains of *M. musculus* (Bruce, 1960; Benavides and Guénet, 2003), there is scarce information about wild popu-

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lations, both in field and laboratory. Sexual maturity is attained at about 10 g (Rowe et al., 1983), adult weights are higher than 10.5 g for females and 11 g for males (Drickamer et al., 1999). Although in natural habitats this species usually breeds seasonally (Lund, 1994) in commensal environments with mild temperature and high food availability it can reproduce throughout the year (Laurie, 1946; Chou et al., 1998; Miño et al., 2007; Vadell et al., 2010). Pregnancy lasts about 21 days (Benavides and Guénet, 2003); mean litter size is about 5 offspring (Rowe et al., 1983; McAllan et al., 2008), and there is post partum oestrous. These characteristics allow for high population increase when conditions are favorable.

Mus musculus is considered a pest in urban, suburban, and rural areas where it causes food losses and contamination, damages building structures by gnawing and burrowing and is a potential threat to both human and animal health through transmission of diseases (Timm, 1987). Its control includes environmental sanitation, exclusion by physical barriers, and the application of rodenticides, especially in grain or food stores (Myllymäki, 1987). One of the main problems for the long term success of rodent control is caused by the recolonization of vacant areas from the surroundings (Ryan et al., 1993), by the passive recolonization of individuals carried out by man with breeding animals or food (Pelz, 2003; Brown and Tuan, 2005), and by the population recovery after control through an increase in the reproductive rate of survivors (Brown and Tuan, 2005). After a population decrease caused by control, a newly founded mouse population may grow rapidly (Chou et al., 1998), because reproductive success depends on social conditions, and is restricted in crowded populations. Population recovery implies the need of new rodenticide applications, but the effectiveness of the application of anticoagulants to rodent control decreases after some time of use because of the development of resistant populations and aversion behaviors in animals that ingest non lethal doses (Hussain and Prescott, 2003).

An alternative to increase the mortality rate of a pest is to decrease its birth rate (Smith, 1994), which can be accomplished by different approaches: to use odors to disrupt reproductive behavior (Bruce, 1960), to use chemical inhibitors, or biological sterilants (Singleton and Redhead, 1990; Smith, 1994). Odors play a role in chemical communication both between conspecifics and heterospecifics, and bring a tool for more effective management of pest species (Volfová et al., 2010). Studies on house mice have shown that odors allow recognitions of kins, social status of males and oestrous states of females (Koyama, 2004). In females of house mice, the odor of males induces oestrous (Whitten, 1956), while the odor of an unfamiliar male causes pregnancy block in recently inseminated individuals (Bruce effect, Bruce, 1960). In addition, olfactory stimuli can cause a decrease in the reproductive success of house mice in crowding conditions (Parkes and Bruce, 1961; Feoktistova et al., 2003). On the other hand, predator odors can have effects in rodent reproduction that are similar to those produced by crowding (Feoktistova et al., 2003), resulting in smaller litters and hindering normal development of juveniles (Apfelbach et al., 2005).

In Argentina, *M. musculus* frequently uses different habitats in cities, as human houses, especially those of low income people, parks or vacant areas with weedy vegetation (Castillo et al., 2003; Cavia et al., 2009; Vadell et al., 2010;). In rural areas of the Buenos Aires province, it is mainly present in poultry farms. Its control in farms is mainly conducted by rodenticide application; however, more than 90% of the farms are infested with rodents (Gómez Villafañe et al., 2001). In poultry farms, *M. musculus* reproduces throughout the year (León et al., 2007; Miño et al., 2007), and can reach high densities without control (León et al., 2007). Guidobono et al. (2010) found evidences of resistance to anticoagulant rodenticides in *M. musculus* populations inhabiting poultry farms, which may favor the recovery of populations through reproduction after rodenticide applications. The failure in *M. musculus* control through traditional methods reinforces the need of alternative methods, such as the utilization of chemical signals to reduce reproductive success. Another advantage of control with specific chemical signals as odors is the decrease in the effect on non target species, and reduction of environmental pollution with rodenticide. As a first step to assess the use of conspecific odors to *M. musculus* control in field conditions, we studied the effect of overcrowding on wild *M. musculus* females: pregnancy rates, birth success, litter size, offspring weight at birth and at weaning, survival until weaning and the number of offspring surviving at weaning.

Between May 2009 and November 2010 mice were captured with Sherman live traps around the chicken breeding sheds of poultry farms in Exaltación de la Cruz, Buenos Aires province, Argentina (34° 19'S; 59° 14'W). The climate of the area is temperate and wet with a mean annual temperature of 16°C and an annual rainfall average of 1000 mm (Cabrera and Willink, 1980).

Animals were kept for at least 10 days after capture for acclimatization (range 10-20 days) in individual metal cages (18 x 25 x 15 cm) with softwood shavings, cotton and cardboard tubes as nesting material. Food and water were provided ad libitum, and the photoperiod was set at 12 Light: 12 Dark. Twenty four hours before the introduction of a male in a female's cage we added bedding from the male's cage to induce oestrous (Whitten effect, Chipman and Fox, 1966). We conducted 24 mating encounters in control conditions and 25 in overcrowding conditions; pairs were assigned randomly to the treatments. For both treatments, pairs were maintained together during 16 days (four estrous cycles).

The control group was maintained with food ad libitum in a ventilated cabinet of 0.7 m^3 and bedding was renewed every three days. A maximum of 4 pairs were placed simultaneously, but after the detection of pregnancy, the males were removed and placed in the overcrowded group.

The overcrowded group was maintained in a non-ventilated room (2 m^3), and bedding was renewed weekly. During the experiment there were more than 15 animals in the non ventilated room, in addition to bedding with urine and faeces from other animals. Food and water were provided ad libitum.

Animals in both treatments were adults; mean weights of females at the beginning of the experiment were 16.92 g and 17.82 g, for control and overcrowded conditions, respectively. For males, mean weights were 17.92 g and 18.24 g, for control and overcrowded conditions, respectively.

Males were removed from the cages after mating and females' pregnancy was assessed approximately one week later through visual inspection and palpation. The time elapsed between mating and pregnancy was estimated by subtracting pregnancy duration (21 days) to the date of parturition. Litter size, weight at birth and at weaning (with a digital scale at ± 0.01 g), and survival at weaning were recorded for each litter.

Pregnancy success was compared between groups using a test of difference between proportions for independent samples. The number of days elapsed between mating and pregnancy, litter size and the total number of offspring per female surviving until weaning were compared between groups by a one sided t test for independent samples. Offspring survival at weaning were compared between groups by means of a U test. The mean offspring weight at birth and at weaning were compared between groups by a one sided t test for independent samples (Zar, 1999). The proportion of cannibalism between overcrowded and control groups were compared using the test of proportions for independent samples (One sided test).

The mean time elapsed until pregnancy was lower in the control group (7.43 days) than in the overcrowded group (9.25 days), ($t_{(13)} = -1.773$; $p = 0.0498$). In 2 out of 25 pairings the female was found dead, while in two other occasions both individuals died. Pregnancy success did not differ ($p = 0.8200$) between control (7 pregnancies, 29.2%) and overcrowded groups (8 pregnancies, 32%).

Mean litter size was higher in the control (5.5) than in the overcrowded group (4.66), but the difference was not statistically significant ($t_{(10)} = 1.274$, $p = 0.1157$).

The mean percentage of offspring survival at weaning was higher for the control group (77.1 versus 35.7%, **Table 1**), but the difference was not significant ($U_{(7,8)} = 13.50$, $p = 0.0930$). The proportion of females that killed all the offspring of the litter was significantly lower

Table 1

Litter size, mean offspring weight at birth and at weaning, and number surviving at weaning, for the control and overcrowding conditions.

	Litter size	Mean litter weight (g)		Number surviving (%)
		at birth	at weaning	
Control group	5	1.34	7.92	5 (100)
	5	1.39	7.97	5 (100)
	*	*	*	0 (0)
	6	1.36	7.35	6 (100)
	5	1.34	7.69	2 (40)
	5	1.37	7.49	5 (100)
	7	1.46	7.56	7 (100)
Mean	5.5	1.38	7.66	4.29 (77.1)
Overcrowding group	7	1.57	5.67	6 (85.7)
	3	1.3	*	0
	*	*	*	0
	5	1.29	6.60	5 (100)
	4	1.26	*	0
	*	*	*	0
	5	1.34	*	0
	4	1.22	6.73	4 (100)
Mean	4.66	1.33	6.33	1.88 (35.7)

*Missing data because of cannibalism by the female

($p = 0.0395$) in the control (0.142, n= 7) than in the overcrowded group (0.625, n=8).

Females in the control group produced a total of 33 offsprings, with a mean success of 1.25 offspring per female, while those in the overcrowded group produced only 15 offspring at weaning with a mean success of 0.6 offspring per female (**Table 1**). This difference was not statistically significant ($t_{(47)} = -1.181$, $p = 0.1218$). When considering only those females that became pregnant, control females were significantly more successful than overcrowded females ($t_{(13)} = 1.83$, $P = 0.0452$), the mean number of offspring surviving at weaning were 4.29 and 1.88 for control and experimental groups, respectively (**Table 1**).

Mean offspring weight of litter at birth did not differ between groups ($t_{(10)} = 0.866$, $p = 0.4067$), but at weaning litter mean weight in the control group was higher than in the overcrowded group ($t_{(7)} = 5.067$, $p = 0.0010$) (**Table 1**).

Pregnancy success was low in both groups (29 and 32%) in comparison with laboratory strains (47 - 92%; Zacharias et al., 2000; Benavides and Guénet, 2003; Beaton and De Catanzaro, 2005), and in comparison with wild *M. musculus* under laboratory conditions (76%, Chipman and Fox, 1996). This result may have been the consequence of stress caused by confined conditions that affected in a similar way both groups (De Catanzaro et al., 1995; Alvarez, 2008), or the effect of the presence of other conspecifics, specially males (although in low numbers) in the control group. During the acclimation period, however, females were in contact with different males, and it has been shown that memory of odors of surrounding males at the time of copulation are tentatively imprinted on females and may be involved in suppressing block of pregnancies by odors of unfamiliar males ("Bruce effect", Koyama, 2004).

Despite the low number of successful pregnancies in both groups, our results suggest that crowding conditions affected the reproductive success of *M. musculus*. Overcrowded females showed a longer time until pregnancy (that may affect the number of litters produced along their life), and they produced a lower number of offspring surviving at weaning. This result may have been the consequence of cumulative differences at the various stages of reproduction (litter size and survival at weaning), as was reported by Singleton et al. (2001) for different life-history traits in field *M. musculus* populations.

On the other hand, the low weight of the offspring of overcrowded females may affect their chances of survival independently of the mother and the future reproductive success in natural conditions (Stearns, 1995).

To sum up, the effect of overcrowding on reproductive success must be confirmed with more test individuals and the use of isolated cages in the control group.

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