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GENERAL BIOLOGY

Effect of Odor of Commensal House Mice on the Reproduction of the Pine Vole *Microtus rossiaemeridionalis*

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The superspecific complex *Mus musculus* s. l. consists of two different groups, which include commensal species. The first group comprises *M. musculus, M. domesticus*, and *M. castaneus*, and the second group includes the wild species *M. spretus, M. macedonicus*, and *M. spicilegus* [1]. Formation of the commensal life style during evolution is one of the most interesting but poorly studied problems of the phylogeny of this group. Different authors believe that the evolution resulted in independent formation of commensalism in different species and, possibly, subspecies of house mice [2, 3]. On the basis of literature and our data, it has been concluded that a unique combination of ecological, behavioral, and physiological characteristics provides comfortable coexistence of house mice and humans [4]. Small rodents predisposed to facultative commensalism may be found very rarely in the human constructions together with house mice. One of the reasons is a high aggressiveness of the commensal species *M. musculus* and *M. domesticus* with respect to other small rodents. As a result of aggressive interactions, house mice displace other species from human constructions [5–7]. In the absence of house mice, various types of constructions are actively inhabited by other rodents [8, 9]. We hypothesized that olfactory signals of commensal house mice may suppress reproduction of other rodents predisposed to commensalism. Along with the high level of aggressiveness, the negative effect of their smell on the reproduction of other species predisposed to commensalism may determine success in the competition between these species and house mice in the human constructs, as a specific ecological niche. We used the pine vole (*Microtus rossiaemeridionalis*), a species predisposed to facultative commensalism, as an object of our studies. Preliminary experiments showed that our hypothesis may be correct for females involved in the reproduction for the first time [10]. In this work, we evaluated the effect of the odor of urine of commensal house mice on the reproduction of males and females of pine voles.

The experiments were performed in the laboratory of the Chernogolovka Research Experimental Station of the Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences in spring of 2007. Young mice (male and females) aged 20 days were divided into groups of five animals of the same sex. The mice were kept in standard plastic cages for rodents with sizes of $43 \times 28 \times 15$ cm. We used two groups of females and four groups of males (the number of animals in the group varied from 9 to 15). Females at an age from 30 to 50 days were exposed to urine of laboratory mice or water (control). Males at an age from 30 to 50 days and males at an age from 40 to 60 days were exposed to urine of laboratory mice or water. The control and experimental groups were kept in different rooms. To collect urine of laboratory mice (groups of five animals of the same sex), the animals were placed into small cages. The collected urine from five males and five females was mixed and frozen. The voles were exposed to this mixture. Before exposure, the urine was unfrozen and applied with a pipette into each cage containing experimental animals two times a day (12:00 a.m. and 12:00 p.m.) in a volume of 0.3 ml. A similar procedure was performed with control animals but, instead of urine, we used water.

To evaluate the fertility, each female aged 70 days was kept for three days together with a male that previously gave more than two litters. Then, the females were separated from the males and kept singly. To detect pregnancy, the females were weighed twice: before coupling and 16 days after coupling. The weight of pregnant females increased by 20–35 g, which served as additional indicator of pregnancy. From the 18th to the 23rd day after coupling, the cages containing females were examined twice a day for the presence of pups. At the day of birth, we calculated the number

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of newborn pups, and the sex of these pups was determined at an age of 18 days.

At the end of the exposure period, the males were decapitated. The testicles were isolated, weighed, placed in 0.5 ml of phosphate buffer solution, homogenized, and centrifuged for 30 min at 4°C; the supernatant was stored at –20°C. Peripheral blood was collected and centrifuged for 20 min at 4°C; the serum was stored at –20°C. Testosterone in the serum and homogenates of the testicles was measured with the use of ELISA using a Steroid IFA-testosteron-01 kit (Al'kar-Bio, Russia). The measurements were performed in accordance with the manufacturer's protocols. The calibration curves were constructed using the standard testosterone preparation dissolved in phosphate buffer solution (for thtesticles) or steroid-free mouse serum (for blood). The number of spermatozoa in both epididymides was calculated in a hemocytometer. To this aim, the caudal parts of both epididymides obtained from each male were minced in 2000 μl of phosphate buffer solution and left for 30 min at periodical stirring for isolation of spermatozoa. After that, the solution was filtered using a capronic filter, and an aliquot of ≈0.02 ml was placed into a hemocytometer. The number of spermatozoa was counted under a light microscope at a magnification of ×200.

Only one female had offspring in the experimental group (n = 12), the litter consisting of five pups (two females and three males). In the control group (n = 15), we obtained five litters, each litter containing five pups, i.e., a total of 25 pups (13 females and 12 males). The experiment and control groups did not differ significantly in the number of females that had or had no offspring ($\chi^2 = 2.32$, $p > 0.05$); however, with respect to the number of pups born, the differences between the experimental and control groups of females were significant ($\chi^2 = 80.75$, $p < 0.001$). Thus, the results obtained suggest that the fertility of female pine voles was suppressed by the odor of commensal house mice.

In male voles, we observed considerable individual differences in the level of testosterone in the blood serum and testicles. We did not find significant differences between the control and experimental groups in the level of testosterone in the blood serum or testicles or the number of spermatozoa in either epididymides or testicles (table). Thus, pheromones of urine of commensal mice do not affect the testicular function of voles. However, this does not exclude the possibility of

Tables 1. The level of testosterone (T) in the blood serum and testicles, number of spermatozoa in both epididymides, and weight of testicles of experimental and control male pine voles

Indices studied	Treatment	Number of voles	Mean	Maximum	Minimum	Z	Significance (Mann–Whitney test)
T level in blood serum, ng/ml	Exposure to urine at age from 30 to 50 days	10	1.43 ± 0.43	4.86	0.32	0.411	>0.05
	Control	9	0.58 ± 0.14	1.47	0.14		
	Exposure to urine at an age from 40 to 60 days	10	0.89 ± 0.20	2.13	0.15	0.449	>0.05
	Control	10	1.07 ± 0.22	3.38	0.33		
T content in the testicles, ng/both testicles	Exposure to urine at age from 30 to 50 days	10	14.67 ± 5.84	48.87	2.28	0.573	>0.05
	Control	9	8.55 ± 2.90	30.75	2.39		
	Exposure to urine at an age from 40 to 60 days	10	6.70 ± 1.57	18.53	2.51	1.398	>0.05
	Control	10	10.98 ± 2.92	32.13	3.18		
Number of spermatozoids, 10^6/both epididymides	Exposure to urine at age from 30 to 50 days	10	5.53 ± 2.67	23.46	0.47	0.367	>0.05
	Control	9	2.77 ± 0.85	8.76	1.02		
	Exposure to urine at an age from 40 to 60 days	10	11.97 ± 1.93	23.38	3.49	0.367	>0.05
	Control	10	10.93 ± 2.04	25.46	2.38		
Weight of testicles, mg	Exposure to urine at age from 30 to 50 days	10	200.2 ± 23.41	360.0	90.0	0.816	>0.05
	Control	9	205.1 ± 10.77	262.0	164.0		
	Exposure to urine at an age from 40 to 60 days	10	223.3 ± 10.03	272.0	176.0	0.735	>0.05
	Control	10	211.0 ± 7.71	248.0	174.0		

a suppression effect of odor signals of mouse urine on the sexual motivation or elements of sexual behavior in voles that may rapidly and effectively exclude them from reproduction. These effects were found in laboratory mice during the formation of social hierarchy in group [11].

The strong odor typical of commensal house mice has no explanation as yet and, according to some researchers, contradicts to the rules of adaptation, because it clearly signals about the presence of mice. Wild species of the superspecific complex *M. musculus* s. l. does not have this smell. It is possible that the strong smell acquired by commensal mice during evolution is a way of suppression of reproduction of other rodents, which may promote elimination of these rodents from human constructions as a specific ecological niche.

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