Table 2. Data and analyses for the autogrooming behavioral response

		Number autogrooming Control	Treatment
o-Aminoacetophenone			
	Before	5	7
	After	15	42
$\chi^2 = 1.13$, $p > 0.05$, df = 1			
Octanoic acid			
	Before	6	14
	After	28	22
$\chi^2 = 3.87$, $p \approx 0.05$, df = 1			
1-Dodecanol			
	Before	25	39
	After	21	39
$\chi^2 = 0.22$, $p > 0.05$, df = 1			

Discussion. *o*-Aminoacetophenone is the first repellent pheromone reported for honeybees. The effectiveness of this compound in small social groups is now well documented; its function in the more complex social environment of a colony still needs to be investigated. In small social groups, queens release large quantities of fecal material under conditions of agonistic social interactions with workers and other queens; however, small quantities are continuously released and sometimes appear to be attractive to workers (personal observations). The higher molecular weight hydrocarbons could serve as carriers for *o*-AAP as well as releasers of autogrooming.

o-Aminoacetophenone is also produced by the sawfly, *Cephalcia lariciphila*[6], and the primitive fungus-growing ant, *Mycocepurus goeldii*[7]. As with worker honeybees, worker ants are attracted to this compound in low concentrations and repelled at higher concentrations. These are the only three arthropods known to produce this compound[8].

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Ultrasonic vocalizations by adult prairie voles, *Microtus ochrogaster*

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Summary. Male and female *Microtus ochrogaster* were presented with anesthetized and awake conspecifics while ultrasonic vocalizations (USVs) were monitored. Males produced significantly more USVs than females during 5-min testing sessions. Males tended to produce more USVs to unfamiliar females than to familiar female siblings. Sexual experience led to increased USV scores by males. These results suggest that USVs by male prairie voles communicate to females the male's gender and his availability for reproductive behavior.
Key words. Prairie voles; *Microtus ochrogaster*; ultrasonic vocalizations; reproductive behavior; sexual experience.

Ultrasonic vocalizations (USVs) occur above the normal range of human hearing and are produced by a variety of mammalian species. Many insectivore species use these vocalizations for prey-finding and navigational purposes (e.g., bats), whereas many rodent species appear to use them as communication signals. Both immature and adult rodents emit USVs; pups emit them as distress signals to attract parental care[1] and adults emit them during social interactions[2], suggesting that they are components of a communication system. Demonstrations of the true functional significance of USVs by adults have been few, but some general parameters concerning the behavioral contexts in which they are emitted have emerged. For example, in pine voles, *Microtus pinetorum,* males emit USVs much more frequently than females, and the emission of USVs is regulated by gonadal androgens[3], as in many other species of muroid rodents[4]. In golden hamsters, *Mesocricetus auratus,* Floody et al.[5] demonstrated that following brief exposure to males, tape-recorded playbacks of USVs by males prolong lordosis in estrous females. Furthermore, sexual experience increases rates of USV and facilitates reproductive behavior in male house mice[6]. These findings support the hypothesis that USVs are important components of male reproductive behavior.

Prairie voles, *Microtus ochrogaster,* have attracted research attention in an effort to determine the factors which influence the striking population fluctuations shown by this and many other species of voles and lemmings. Particular attention has been focused on reproductive biology. Like most species of voles that have been studied, prairie voles are induced ovulators. The reproductive system of female prairie voles is generally quiescent in the absence of stimulation from males. Following introduction to unfamiliar conspecifics of the opposite sex, male and females prairie voles immediately engage in extensive investigation of each other[7], resulting in the exchange of sensory information, especially chemosignals, that stimulates reproduction. Mating usually initiates 24–48 h after pair formation[8] and copulation induces ovulation[9].

The reproductive unit of prairie voles appears to be the monogamous pair. Field studies have demonstrated that a particular pair of breeding prairie voles can be consistently found together in the same nest[10]. Following the successful production of a litter, laboratory-housed males and females show aspects of mate-fidelity, including aggression to unfamiliar animals and increased latency to engage in sexual behavior with them[11]. Furthermore, inbreeding avoidance between familiar siblings is profound in laboratory set-

tings [12]. We were interested in determining how these characteristics of prairie vole social organization and reproductive biology correlate with the social uses of USVs by adults. We predicted that males would produce more USVs than females, in accordance with earlier findings for pine voles [3]. We also predicted that males would preferentially direct more USVs to unfamiliar females than to familiar female siblings, and that sexual experience would facilitate the production of USVs by males.

The prairie voles used in these experiments were laboratory descendants of wild voles trapped in Missouri [9] and subsequently bred at the Monell Center. All subject and stimulus animals were between 3 and 12 months of age. The voles were housed in polypropylene cages fitted with wire lids. Pine shavings were provided for bedding and cages were cleaned weekly. The animals were housed and tested in the same colony room where they had lived since weaning. Photoperiod was 14L : 10D and temperature was maintained at 23 ± 3 °C. Water and Agway rabbit chow were available ad libitum.

General testing procedures consisted of placing the home cage of the subject approximately 10 cm under the microphone of a QMC S-100 bat detector set to 40 kHz. A stimulus animal, either awake or anesthetized with ketamine-acepromazine (100 mg/kg and 1 mg/kg, respectively), was placed into the subject's cage, and the presence or absence of ultrasonic vocalizations during each of the ensuing 60 5-s blocks of time (5 min total) were recorded. Vocalizations recorded when an awake stimulus animal was present could have been produced by either the subject or the stimulus. However, vocalizations recorded when an anesthetized stimulus animal was present must have been emitted by the subject. We also monitored duration of sniffing (subject or stimulus animal's nose within 1 cm of the other animal) and frequency of activity (one crossing of the midline across the short axis of the cage was counted as one activity unit). All testing was done during the final 3 h of the lights-on portion of the light cycle. Statistical analysis consisted of repeated measures analysis of variance (ANOVA) followed by Duncan's new multiple range test [13] in the first and second experiments and paired t-tests in the third experiment.

In the first experiment we tested 5 males and 10 female subjects that had been individually housed for at least 3 weeks before testing. Each of these subjects was tested on 4 consecutive days, with counterbalanced presentations of the following classes of stimulus animals: a) awake male; b) anesthetized male; c) awake female; d) anesthetized female. The results are presented in figure 1: in all cases in which at least one male – whether subject or stimulus – was awake, USVs were abundant. Statistically, there were no significant differences in rates of USV production in all of the testing sessions in which at least one male was awake. Notably, USV scores were statistically lower when only awake female were present compared to sessions in which at least one awake male was present (interaction between subject gender and stimulus anesthesia state, $F_{1,13} = 9.48$, $p < 0.01$; Duncan's test, $p < 0.05$). These results suggest that under these testing conditions USV response to conspecifics is primarily a characteristic of male, rather than female, prairie voles. It is entirely possible that all of the USVs produced when an awake female was tested with an awake male were in fact produced by the female. In these tests, however, subject males presented with anesthetized conspecifics always produced more USVs than did subject females presented with anesthetized conspecifics. Females may produce more USVs under other testing circumstances [14].

The subject's activity levels were decreased when the stimulus animal was anesthetized relative to those observed with awake stimuli ($F_{1,13} = 5.2$, $p < 0.04$), with mean (± SEM) activity scores of 7.6 (± 2.0) versus 14.3 (± 1.4) line crossings, for anesthetized versus awake stimuli, respectively. Conversely, the duration of sniffing the stimulus animals by the subjects was increased when the stimulus animal was anesthetized relative to those observed with awake stimuli ($F_{1,13} = 10.85$, $p < 0.006$), with mean (± SEM) sniffing durations 122.6 (± 12.0) versus 70.1 (± 14.8) seconds, for anesthetized versus awake stimuli, respectively. There were no significant differences attributed to subject or stimulus gender.

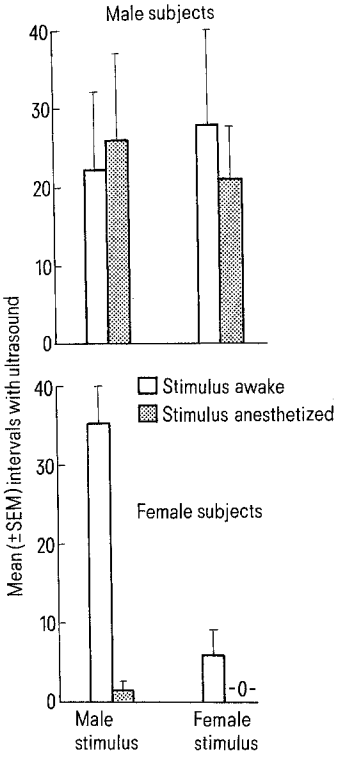

Figure 1. Mean (± SEM) 5-s intervals with ultrasonic vocalizations (USVs) recorded when male and female prairie voles were presented with anesthetized and awake conspecifics. Note that substantial amounts of USVs were observed only when at least one awake male was present.

In the second experiment 10 male subjects that had been housed with a single female sibling since weaning were observed for USV responses to awake and anesthetized unfamiliar, unrelated females versus awake and anesthetized familiar female siblings. Statistically indistinguishable amounts of USVs were obtained for all treatment groups (fig. 2), however, there was a nearly significant trend for lower USV scores when the stimulus was the female sibling (stimulus familiarity effect, $F_{1,18} = 4.07$, $p < 0.06$). As reported for experiment 1, duration of sniffing increased and activity decreased when the stimulus animal was anesthetized rather than awake. These data suggest that males tend to direct USVs to potential mates rather than to familiar siblings, corroborating reports of inbreeding avoidance between sibling prairie voles [12].

In the final experiment, we tested 24 sexually-inexperienced males for their USV response to an anesthetized, unfamiliar female (pre-treatment tests). We then divided the 24 males into 2 groups that were equated for pre-treatment USV scores: one group remained isolated in their cages, and males in the other group were paired for 60 h with an estrogen-primed, sexually-receptive female (daily s.c. injections of 5 µg estradiol benzoate in sesame oil for 2 days prior to, and once during pairing). Most of the paired males successfully mated with the females as microscopically verified by the presence of sperm in vaginal contents. Following the sex

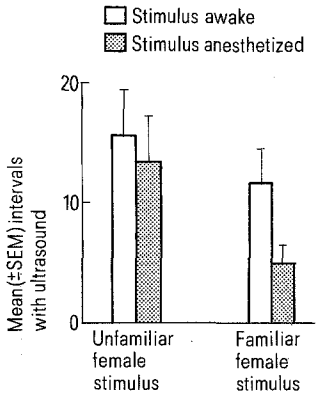

Figure 2. Mean (± SEM) 5-s intervals with ultrasonic vocalizations (USVs) recorded when male prairie voles were presented with anesthetized and awake familiar female siblings or unfamiliar females. There is a trend for higher amounts of USVs when the stimulus was an unfamiliar female.

Mean (± SEM) 5-s intervals with ultrasonic vocalizations (USV) by male prairie voles that were presented with an anesthetized female. Half of the males received sexual experience and the other half remained in social isolation following the pre-treatment test. Only those males that received sexual experience (group 1) showed significantly elevated USV scores in the post-treatment test.

		Mean (± SEM) USV scores	
Group 1 (n = 12)	pre-treatment	4.2 (± 1.3)	
	↓ sexual experience		$p < 0.04$*
	↓ post-treatment	17.0 (± 5.5)	
Group 2 (n = 12)	pre-treatment	3.7 (± 1.8)	
	↓ social isolation		Not significant
	↓ post-treatment	6.7 (± 2.8)	

* Paired t-test.

experience, both groups were retested with unfamiliar anesthetized females (post-treatment tests). Data were analyzed with paired t-tests comparing each group's pre- and post-treatment scores. It is clear that sexual experience resulted in higher USV scores (table; $p < 0.04$). There were no statistically significant differences in activity or duration of sniffing in either group of males.

Taken together, these results support the hypothesis that male prairie voles use USVs during social interactions as part of a reproductive strategy. Generalizations must be tempered by limitations imposed by the testing circumstances, but we speculate that USVs from males may communicate to unfamiliar, viz unrelated, females that the vocalizer is male, unfamiliar and available as a mate. As such, USVs may be a component in a mutual positive feed-back system, originally proposed for the exchange of stimulatory chemosignals [15], in which males and females physiologically and behaviorally stimulate each other toward reproduction. This explanatory hypothesis interdigitates knowledge of the reproductive biology of these rodents. To females prairie voles, USVs may reiterate tactile and chemosensory cues from males. These cues may stimulate the females' hypothalamic-pituitary axis, resulting in the secretion of gonadotropins and the subsequent activation of the ovaries. This in turn may cause the potentiation of the female's ability to stimulate the male. Gonadotropin and androgen responses to females are well established for males in many species of rodents. In male house mice, endocrine responsiveness to female cues appears to be partially mediated by social experience and vomeronasal chemoreception [16, 17]. We are currently investigating the roles of sexual experience and vomeronasal chemoreception in the reproductive behavior and physiology of male prairie voles.

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