

ULTRASONIC VOCALIZATION IN PRAIRIE VOLES (*MICROTUS OCHROGASTER*):
EVIDENCE FOR BEGGING BEHAVIOR IN INFANT MAMMALS?

Brian N. Lea

A Thesis Submitted to the
University of North Carolina Wilmington in Partial Fulfillment
of the Requirements for the Degree of
Master of Arts

Department of Psychology

University of North Carolina Wilmington

2006

Approved by

Advisory Committee

Dr. Katherine E. Bruce

Dr. Carol A. Pilgrim

Dr. D. Kim Sawrey
Chair

Accepted by

Dean, Graduate School

TABLE OF CONTENTS

ABSTRACT	iv
ACKNOWLEDGMENTS	vi
LIST OF TABLES	vii
LIST OF FIGURES	viii
INTRODUCTION	1
Ultrasonic Vocalizations.....	1
Begging.....	6
Prairie Voles	8
Comparison of the Montane Vole with the Prairie Vole	10
Related Research.....	11
Hypotheses.....	13
METHOD	13
Subjects.....	13
Apparatus	14
General Procedure.....	14
Statistical Analysis.....	15
RESULTS	16
USV Production	16
Weight Differences	17
Subpopulation Differences in USV Production.....	19
DISCUSSION.....	24
USV Production and the Effects of Litter Size and Pup Deprivation.....	24

General USV Characterization	26
Subpopulation Differences	26
Analysis of the Current Research and Future Directions	27
REFERENCES	30

ABSTRACT

This research examines ultrasonic vocalization (USV) emission by infant prairie voles (*Microtus ochrogaster*). Rodent pups of many species emit USVs (Anderson, 1954), commonly in response to stressors such as isolation and hypothermic conditions (Allin & Banks, 1971; Oswalt & Meier, 1975). Much research has been conducted to examine the use of these ultrasounds as the basis of a communication system between offspring and parents (Zippelius & Schleidt, 1956), and the malleability of USV production by infant rodents (Bell, *et al.*, 1972; Blake, 1992) suggests high susceptibility of ultrasounds to ontogenetic selection. USV production by prairie voles has been compared to a sympatric species, the montane vole (*Microtus montanus*), in several studies (Blake, 2002; Rabon, *et al.*, 2001; Shapiro & Insel, 1990) and prairie voles have been found to produce ultrasounds at a higher rate, a finding which has been attributed to the different mating systems of the two species. Prairie voles exhibit a monogamous mating system, biparental care of pups, tenacious nipple attachment by pups, and litter overlap, all contributing to sibling competition among the young of this species (Gilbert (1995). In contrast, montane voles mate polygynously, only the dam cares for the pups, and there is no tenacious nipple attachment or litter overlap, suggesting reduced sibling competition. In the current study, data indicate that prairie voles show no differences in ultrasound production by the heaviest and lightest pups in a small litter. However, in large litters, where pup number may exceed the number of functional nipples the dam provides, the lightest pup produces USVs at a much higher rate than the heaviest pup. It is suggested that this difference is reflective of the relatively large difference in deprivation level in large litters between the pups at the two weight extremes. This finding is related to the avian begging literature, which also includes some examples of mammalian begging. It is hypothesized that prairie voles may beg by using

ultrasounds as an honest signal of need to the dam and then by engaging in a scramble competition with littermates for access to nipples when the dam nurses.

ACKNOWLEDGMENTS

I would like to thank my mother Susan, my father Mebane, and my step mother Barbara, as well as the rest of my family for their patience, support, love, and patience in seeing me through this process. I would also like to thank Amy Williams for her support and encouragement.

I would like to extend my deepest thanks to my advisor and committee chair, Dr. Kim Sawrey. His guidance and wisdom in the ways of animal behavior have been an invaluable resource to me, and I appreciate all he has done to help me in my graduate education.

I would like to recognize the Graduate School of the University of North Carolina at Wilmington for their generous fellowship to fund this research. My appreciation is also extended to the faculty of the Department of Psychology at the University of North Carolina at Wilmington for all of the knowledge and skills they have imparted to me.

Finally, I would like to thank the members of my thesis committee, Dr. Sawrey, Dr. Kate Bruce, and Dr. Carol Pilgrim, as well as Dr. James Johnson, for their guidance in completing this research.

LIST OF TABLES

Table	Page
1. Previous research on USV production by prairie and montane voles	4
2. Life-history characteristics of prairie and montane voles	12

LIST OF FIGURES

Figure	Page
1. USV Production by Litter Size and Pup Size	18
2. Pup Weight by Litter Size and Pup Size.....	20
3. USV Production by Litter Size and Pup Size Among Illinois Voles.....	22
4. USV Production by Litter Size and Pup Size Among Missouri Voles.....	23

INTRODUCTION

Ultrasonic Vocalizations

Since the first reporting of the production of ultrasonic vocalizations (USVs) by infants of many species of rodents (Anderson, 1954; Zippelius & Schleidt, 1956 as cited in Hofer, 1996) the causes and functions of these high frequency emissions have been studied broadly and debated extensively. Stress has been shown to induce USV production in the infants of many rodent species (Amsel, Radek, Graham & Letz, 1977). In rats (*Rattus norvegicus*), as well as other species, isolation from the dam is a very effective elicitor of USVs. However, when isolated from the dam, the presence of a littermate (even if anesthetized) will reduce these isolation calls in an unfamiliar environment (Hofer & Shair, 1978). Much evidence has shown that thermal stress to the infant in the form of body temperature reduction can elicit USVs (Allin & Banks, 1971; Oswald & Meier, 1975). Oswald and Meier (1975) identified olfactory correlates of USV production observing that the presence of bedding from the home cage reduced isolation calling in rat pups, while clean bedding did not. In an attempt to examine the role of tactile stimulation on USV production, these researchers also found that placing the rat pup in a dish with no bedding resulted in higher USV production than placing it in a dish with either clean bedding or soiled bedding from the home cage. Meanwhile, Hofer and Shair (1980) examined the effects of specific sensory stimuli on USV production in infant rats and found that the tactile stimulation of fur was very important to the reduction of USVs. However, they reported that if the pup was made anosmic so it could not smell a littermate which was present, the typical reduction of USVs would not occur. All of these different sensory modalities seem to have independent and additive effects on USV production by the infant (Hofer, 1996).

Early in the study of USVs, it was hypothesized that they were part of a system of communication between infants and parents (Zippelius & Schleidt, 1956 as cited in Hofer, 1996). For a brief time, researchers even explored the possibility that USVs were a form of rodent echolocation (Rosenzweig, Riley, & Krech, 1955). However, there are those who dismiss the idea that USV emission evolved as, for example, a distress signal to parents. Fearing that researchers were engaging in anthropomorphism by suggesting that infant rodent USVs may be analogous to human infant crying, Blumberg and colleagues advanced another theory (Blumberg & Alberts, 1990). They suggested that USVs were merely a byproduct of a thermogenic process that the infant can engage in when thermally stressed. This abdominal compression reaction (ACR) is hypothesized to assist the transport of venous blood back to the heart, resulting in an increase in body temperature. To increase intraabdominal pressure, the larynx is proposed to constrict (acting as a brake) during expiration resulting in an ultrasonic byproduct. These researchers assert that while data show that USV production by infants may facilitate searching and retrieval behavior by parents (Zippelius & Schleidt, 1956 as cited in Hofer, 1996), it cannot necessarily be inferred that rodent pups' USV emissions evolved for their communicative function. Their alternative explanation is that the adults may simply be taking advantage of an acoustical byproduct of the ACR process. These researchers have found some correlative evidence to support their hypothesis (Blumberg & Alberts, 1990, 1991; Blumberg & Sokoloff, 2001; Blumberg, Sokoloff, Kirby, & Kent, 2000).

However, this theory does not account for all the observed data on infant rodent USV emission. Shapiro and Insel (1990) compared USVs in infants of two different species of voles, the monogamous prairie vole (*Microtus ochrogaster*) and the polygynous montane vole (*Microtus montanus*). The data showed an obvious disparity between the two species in USV

production by infants, with prairie voles peaking at an average of 601 calls during a five-minute test between eight and ten days of age versus an average of sixteen calls by eight-to-ten-day-old montane voles over the same time period. A similarly large difference in USV production was observed by Rabon and colleagues (2001), who recorded a peak at six to eight days of age for isolated prairie voles of over 500 USVs during a three-minute test, while montane voles averaged less than twenty-five USVs during the test.

Finally, Blake (2002) observed an average of 954 USVs produced by infant prairie voles across a twenty-minute test period compared to an average of seventeen USVs produced by montane voles. Prairie vole infants in this study, despite their higher rate of calling, showed a much smaller degree of variance in calling than montane vole infants, suggesting that prairie vole infants may be ultrasounding at or near their maximum level (results of these studies are summarized in Table 1). Blake also explored the effects of ambient temperature on USV production in the two species, finding that the overwhelming difference in USV production between prairie and montane vole infants was unaffected when they were cooled. The large gap in USV production between these two morphologically similar species in a cold environment runs counter to the arguments of Blumberg and colleagues. It is likely that infants of the two similar species do not differ greatly in their respective abilities to thermoregulate. Thus, if USV production were indicative of the employment of a thermoregulatory process, we should not expect to observe a difference in USV production when the infants are cooled, let alone the large difference actually observed between the two species.

Blake (2002) suggested that a key difference between these species may involve the divergence of their mating systems. Whereas prairie vole pups are typically raised by both parents, montane vole pups are only cared for by their dam (McGuire & Novak, 1984, 1986).

Table 1.

Previous research on USV production by prairie and montane voles.

	Test Length	Pup Age	Average USV production	
			<i>M. ochrogaster</i>	<i>M. montanus</i>
Shapiro & Insel (1990)	5 minutes	8-10 days peak	601 calls	16 calls
Rabon <i>et al.</i> (2001)	3 minutes	6-8 days peak	> 500 calls	< 25 calls
Blake (2002)	20 minutes	0-10 days	954 calls	17 calls

The biparental caregiving of prairie vole pups would most likely result in the pups not being left unsupervised very often. In fact, evidence has been found to suggest that prairie vole sires may coordinate their returns to the nest with the departure of the dam (McGuire & Novak, 1984). Meanwhile, the montane dam must forage for food and leave her litter unattended for long periods of time, thus her pups' calls may be just as likely to attract a predator as a parent. This differential selection pressure on pup USV production could be the driving force behind the observed differences between these species in pup calling.

Prairie and montane voles are hardly unique species regarding the disparity of USV emission. Several studies have focused on the variability of USV production by infant rodents. Motomura (2002) and colleagues, for example, compared USV rates across a diverse array of rodent species, finding species-specific variations in the sound frequencies of USVs as well as emission rates. Blake (1992) examined differences within the rodent Family Arvidcolidae, finding that field vole (*Microtus agrestis*) infants emitted the most USVs while water vole (*Arvicola terrestris*) infants produced the fewest, with bank vole (*Clethrionomys glareolus*) infants responding intermediately. Within species differences have also been observed in USV production; three inbred strains of infant mice (C57B1/6/J, BALB/c/J, and C3H/He/J) were compared and found to have different patterning of USV production across their development (Bell, Nitschke, & Zachman, 1972). Finally, even within strains of species, rates of USV production show great malleability. Rats of the N:NIH strain that have been selectively bred for high and low USV emission show breeding line differences within a small number of generations (Brunelli, Vinocur, Soo-Hoo, & Hofer, 1997; Hofer, Shair, Masmela, & Brunelli, 2001). The selected low-USV line diverged significantly from a randomly-bred line in just two generations, while the selected high-USV line diverged significantly from the random control

line by the third generation. Such flexibility of this trait suggests that it would lend itself readily to ontogenetic selection.

Begging

If rodents are using this system as a form of communication between offspring and parents, it might be considered analogous to another well-researched communication system. Begging by juvenile birds has been explored in great detail, and recently has been studied using mathematical models to examine what types of variables influence the dynamics of begging. Some recent simple mathematical models (Godfray, 1995; Godfray & Johnstone, 2000; Johnstone, 2004) have suggested that parent-offspring begging systems may require that the begging behavior exhibited by the young be costly, either in terms of energy required to produce the signal or by attracting predators to the nest. Simulations show that only honest signaling would result in a system that would not be susceptible to invasion by individuals who misrepresent their need, rendering the system an evolutionarily stable strategy. In a system in which the signals were not costly, an individual who signaled more regardless of need would induce provisioning of larger amounts of food at the detriment of the hard-working parent, which would then be selected to ignore the signal. However, if the signal is costly, any gains due to misrepresentation of need would be offset by the costliness of the signal, making the system very stable.

Analogs of begging in mammalian species have been investigated to a much lesser extent than in avian species. When discussed in the avian begging literature, nursing in mammals is typically offered as an example of a scramble competition, where there is competition within the litter to find a nipple to suckle, and thus gain nourishment (Parker, Royle, & Hartley, 2002a; 2002b). In an honest signaling system of infant-parent communication, for example, the infant

displays a signal to which the parent responds by providing an appropriate (for the signal given, and relative to the signals of siblings) amount of food to the individual; thus, the parent controls allocation of resources. In contrast, scramble competitions are not regulated by the parent; rather food allocation is determined by the scrambling ability of each individual pup relative to its littermates, and the parent is not as active in the process. An example of scramble competition from the avian literature comes from starlings (*Sturnus vulgaris*), some of which make nests in small holes in trees. Due to the limiting nature of the nest opening, the parent starling returning to the nest is forced to allocate resources only to the chick that has successfully barred access to the nest opening from its broodmates; the parent has no choice of which offspring to feed (Kacelnik, Cotton, Stirling, & Wright, 1995). A mammalian species exhibiting scramble competition, the European rabbit, *Oryctolagus cuniculus*, only nurses her infants once per day for three to four minutes, and leaves them unattended in a burrow the rest of the day. No evidence has been found that the young attempt to harm each other or physically block or remove each other from a nipple during the scramble before nursing. While the dam passively stands over the nest, the young simply try to find an available nipple as quickly as possible and wait for milk letdown (Bautista, Mendoza-Degante, Coureaud, Martinez-Gomez, & Hudson, 2005). Meanwhile, some evidence has been found to suggest that domestic piglets (*Sus scrofa*) both call more and call differently when they are undernourished, either long-term in the case of runts, or short-term in the case of piglets who have missed a nursing bout (Weary & Fraser, 1995). This latter situation may be an example of honest signaling by infant mammals to their caregivers. To regard rodent USVs as a form of begging is a relatively unexplored area of research.

Prairie Voles

Prairie voles (*Microtus ochrogaster*) are a frequently-studied species of rodent because, as mentioned above, adult prairie voles display characteristics of behavioral monogamy (Carter & Getz, 1993; Getz & Hoffman, 1986). Adults of this species typically exhibit pair-bond formation (Shapiro, Austin, Ward, & Dewsbury, 1986; Shapiro & Dewsbury, 1990), display biparental care of pups (McGurie & Novak, 1984; Thomas & Birney, 1979), are not sexually dimorphic (Hoffmeister & Getz, 1968), and pair dissolution is most often realized through the death of one of the members of the pair (Getz & Hofmann, 1986; Getz, McGuire, Pizzuto, Hofmann, & Frase, 1993). In addition, pairs tend to demonstrate nearly complete home range overlap and both males and females aggressively defend their territory from both male and female intruders (Carter, DeVries, & Getz, 1995; DeVries, Johnson, & Carter, 1997; Getz, Carter, & Gavish, 1981; Getz, *et al.*, 1993; Insel, 1997; Williams, Catania, & Carter, 1992). However, while once widely thought to be strictly sexually monogamous, research on prairie voles enclosed in a semi-natural habitat has demonstrated multiple paternity in five out of nine litters sampled (Solomon, Keane, Knoch, & Hogan, 2004). So, while not technically sexually monogamous, prairie voles do display many of the commonly associated behavioral characteristics of pair bonding.

Another interesting characteristic exhibited by prairie voles is tenacious nipple attachment by infants. A prairie vole pup attaches to the dam's nipples so powerfully that oftentimes in the laboratory she can be seen leaving the nest dragging her pups behind her, still attached to her nipples. Several hypotheses of the adaptive value of this behavior have been offered and were reviewed by Gilbert (1995). One hypothesis is that a tenaciously-attached infant may be less likely to be caught by predators because if the dam is startled from the nest,

she can drag the pup and its siblings to safety. Not much evidence has been found to support this hypothesis and there are issues with face validity (e.g., the weight of the pups may slow the dam down and make her more likely to be caught by the predator). It has also been suggested that tenacious attachment may make the pup less likely to fall from a high nest, though tenacious nipple attachment has not been documented in arboreal rodents (such as the squirrel Family Sciuridae), where this selection pressure would be most likely to shape the evolution of the behavior, and is rather found mostly in ground-dwelling rodents. Another suggestion is that the pups of these species may be less likely to drown, though it is much more common in terrestrial than in aquatic species of rodents, so this hypothesis does not completely account for the behavior either.

The sibling competition hypothesis of tenacious nipple attachment (Gilbert, 1995) suggests that in situations where sibling competition for limited resources is intense natural selection will favor extreme responses which secure those resources, such as tenacious nipple attachment which results in the monopolization of one nipple by a given pup. One reason for increased sibling competition in prairie voles which may presage tenacious nipple attachment involves viability of the dam's nipples as a nutritional source. While prairie vole dams have posterior, middle, and anterior pairs of nipples, six nipples altogether, there is a reliable preference for the posterior over the middle nipples and middle over the anterior nipples (McGuire, 1998, 2001). Furthermore, some evidence suggests that the anterior pair of nipples may have such a low milk yield (L. D. Hayes, personal communication) that pups show no preference between attaching to an anterior nipple and being unattached from any nipples. In the laboratory, prairie vole litter size across studies is approximately 3.9 pups (Stalling, 1990), suggesting that the litter size would exceed the functional nipple number a large proportion of

the time. Thus, extreme competition for the best nipples may exert sizable selection pressure on this species.

Overlap of litters may also contribute to sibling competition and thus to the evolution of tenacious nipple attachment (Gilbert, 1995). When females of a species experience a post-partum estrous, as prairie vole females do, the subsequent close spacing of litters may result in a litter being born as the dam's previous litter is being weaned, resulting in more competition for resources. In addition, there have been numerous studies on nesting habits that report communal breeding in prairie voles (Getz & Hofmann, 1986; Getz, *et al.*, 1993), and in some laboratory studies, more than one breeding female may share a nest and the dams have been found to be nursing each other's offspring at least part of the time (Hayes & Solomon, 2004). With the increased number of pups there would be a proportional increase in dams; however, during the absence at the nest of one dam, with two litters and only one dam present, the pup to nipple ratio would be more unfavorable to the pups in the nest.

Comparison of the Montane Vole with the Prairie Vole

Montane voles (*Microtus montanus*), a species sympatric in parts of their geographic range with prairie voles, provide an interesting contrast to the prairie vole. As previously mentioned, *M. montanus* pups do not nearly approximate the level of USV production of *M. ochrogaster* pups. Montane voles differ from prairie voles on several dimensions related to the level of sibling competition the young of each species are expected to experience. No evidence has been reported suggesting that any of the eight mammae (Sera & Early, 2003) of the montane vole are less functional than the others or that any are preferred, though this information is difficult to assess in rodent species that do not exhibit tenacious nipple attachment, as montane voles do not. Thus, the average laboratory litter size of six pups (Sera & Early, 2003) yields a

more favorable pups:nipples ratio in the montane vole (3:4) than in prairie voles (almost 1:1) when the two less-functional anterior nipples of prairie voles are factored out. Also in contrast to prairie voles, montane voles do not show litter overlap. Montane vole young are actually abandoned by the dam approximately fifteen days after birth (Jannett, 1978), the time of weaning, as the dam moves on to construct a new nest in which to rear her next litter (McGuire & Novak, 1986). Among montane vole young, these differences may be enough to alleviate the sibling competition that prairie voles experience. The key differences between prairie voles and montane voles as they may be related to USV production by infants are outlined in Table 2.

Related Research

Maulsby (2003) examined the possibility that USVs produced by prairie vole pups were signals of need. A pup was removed from the dam for either five or 55 minutes and USVs were recorded while the pup was alone and then again after reintroduction of the dam. Pups isolated from the dam for 55 minutes, presumed to be in a more deprived state due to less recent feeding, emitted more USVs than those isolated from the dam for only five minutes. These findings support the hypothesis that USVs may be used as a distress signal when the pup is hungry.

The current study sought to extend the findings of Maulsby (2003) by examining USV production of the heaviest pup, presumably the least needy, and the lightest pup, presumably the most needy, of prairie vole litters in the absence of the dam. While Maulsby examined deprivation on a more temporary or local scale, the present study sought to demonstrate the effects of more permanent states of deprivation on USV production. This expansion on the influence of the condition of pups on USV production should provide evidence to determine

Table 2.

Life-history characteristics of prairie and montane voles.

Species-Typical Characteristic	Prairie Vole	Montane Vole
Infant USV Production	High Rate	Low Rate
Mating System	Monogamous	Polygynous
Tenacious Nipple Attachment	Yes	No
Litter Size:Nipples (Functional Nipples)	3.9:6(4)	6:8(8)
Litter Overlap	Yes	No
Communal Rearing of Pups	Possibly	No

Note: Prairie vole dams may only have four nipples (labeled “Functional Nipples” above) that produce enough milk to sustain a pup.

whether USVs can be considered distress calls that evolved due to their value as a form of communication between offspring and parents.

Hypotheses

It was hypothesized that the lightest pup from litters of five or more pups, in which litter size exceeded the number of acceptable (posterior and middle) nipples on the dam, would produce USVs at a high rate. Greater sibling competition in larger litters and poorer competitive ability of the lightest sibling should result in pups in an extremely deprived condition which emit many USVs in response to this condition. Conversely, it was hypothesized that the heaviest pup in a litter and/or pups from litters of four or fewer pups, in which there are enough acceptable nipples for each pup, would produce USVs at a lower rate. The potentially better competitive ability of the heaviest pup in a litter and the lower amount of sibling competition in small litters should result in pups in at least a moderate condition which, therefore, do not signal as much.

METHOD

Subjects

Data were collected between August of 2003 and September of 2004. Subjects were pups from prairie vole (*Microtus ochrogaster*) litters born to multiparous females descended from stock originally trapped in either southern Illinois ($n = 17$ litters) or Missouri ($n = 15$ litters). Subjects were maintained in a windowless vivarium (20-25°C) on a reversed 14L:10D light-dark schedule with dark onset at 12:00 noon. Animals were housed in clear, polycarbonate cages (48 by 27 by 16 cm) with wood shavings as bedding, and Purina® Rabbit Chow and water were available ad lib.

Apparatus

Ultrasounds were converted to audible sounds using an Ultra Sound Advice Mini-3 bat detector (range 15-160 kHz \pm 1.5 kHz; bandwidth \pm 4 kHz). Headphones were used to minimize disturbance of the subject. The bat detector was positioned approximately 30 cm above the testing (home) cage. The frequency of the device was set at approximately 36-37 kHz to best contain the frequency of prairie vole infants' ultrasounds (Rabon, *et al.*, 2001).

General Procedure

Subjects were tested on day five (the day of parturition being designated day zero) early in the dark phase of the day. Gender of subjects was not assessed due to the unreliability of gender judgment at the early age of testing. The impracticality of marking pups until gender determination was possible also prevented gender analysis.

Subjects were moved from the colony room into a separate testing room, removed from the dam's nipples if necessary, and weighed to the nearest 0.1 g. Removal of the pup from the dam's nipple was accomplished by placing a finger over the nares of the pup until it was forced to inhale through its mouth, thus causing it to release from the nipple. A small amount of bedding was removed from the home cage, though none from the nest, and placed in a small holding cage (29 by 19 by 13 cm) to cover the floor. Once all pups were weighed, the heaviest and lightest pups were placed in the holding cage with the sire, while the dam and any other pups were housed in a third (small) bedded cage, leaving the home cage empty. From this point until the end of the test, the only light in the room was provided by a red, 25-watt incandescent light mounted approximately 40 cm above the home cage.

Fifty minutes after all animals had been moved to holding cages, testing was initiated. The subject animals were the heaviest and lightest pups from the litter. Since the room was

dimly lit, it was difficult to distinguish between the two pups in the holding cage when there was a small weight disparity between them; in this case, testing order was randomly chosen. When there was a great enough weight disparity, the experimenter could differentiate the pups and could attempt to counterbalance for order of testing. Perfect counterbalancing of order of testing according to Pup Size could have been achieved by weighing pups immediately prior to testing, but this excessive handling may have artificially inflated USVs, so pups were not weighed at testing initiation.

After the first test subject was selected, it was removed from the holding cage (housed with the sire) and placed in the nest of the home cage. No attempts were made to keep the pup inside the nest during testing. The number of ultrasounds produced by the first subject was counted by hand for each minute of a ten-minute test. At the end of the test, the first subject was placed back in the holding cage with the sire, and the second subject was placed in the nest of the home cage for testing under the same conditions. At the end of the test, the subjects were weighed again to determine which pup was the heaviest and which was the lightest. After testing, the sire, dam, and all pups were then returned to the home cage which was returned to the vivarium.

Statistical Analysis

Considering the infrequent use of the anterior nipples by prairie vole infants, functional nipple number of prairie vole dams is considered to be four for the purposes of the current study. This definition creates a natural dichotomy along the litter size dimension. A litter size of four or fewer pups, in which nipple number equals or exceeds the number of pups, should result in lower amounts of sibling competition between pups in the litter. Meanwhile, in litters of five or more pups, where the supply of nipples does not meet the demand created by the number of pups

present, sibling competition is predicted to be higher. Many of the analyses conducted will consider the effect of these two Litter Sizes as well as the Pup Size (Heaviest vs. Lightest in the litter) on USV production.

RESULTS

The attempt to counterbalance was reasonably successful, resulting in eighteen litters in which the lightest pup was tested first, and fourteen litters in which the heaviest pup was tested first. There were no effects due to order of testing on the number of ultrasonic vocalizations produced by either the heaviest ($t(30) = 1.46, p = .15$) or the lightest ($t(30) = 1.07, p = .29$) pup. A within-litter analysis (removing the between-litter variance) also suggested no evidence of any order effects on the total difference in USV production between the heaviest and lightest pups of a litter ($t(30) = 0.35, p = .73$). All further analyses are collapsed across testing order.

USV Production

Overall, pups produced an average of 724.3 ($SE = 68.5$) USVs over the course of the ten-minute testing period, well above one call per second. Dividing the test period into ten one-minute segments, a 2 Litter Size (between-subjects 2-4 vs. 5-6 pups) X 10 Test Minute (within-litters) ANOVA was conducted on the dependent variable Total USVs produced in the one-minute test segment. The main effect of Test Minute was significant, $F(9,558) = 6.97, p < .0001, MSE = 1555.3$. Tukey's HSD post hoc test revealed that pups made more calls during the first minute of the test ($M = 105.6, SD = 78.9$) than minutes two through ten, which were not significantly different from each other (overall $M = 68.7, SD = 64.6$). Neither the main effect of Litter Size, $F(1,62) = 1.95, p = .17, MSE = 29579.9$, nor the Litter Size by Test Minute interaction, $F(9,558) < 1, p = .55, MSE = 1555.3$, was significant.

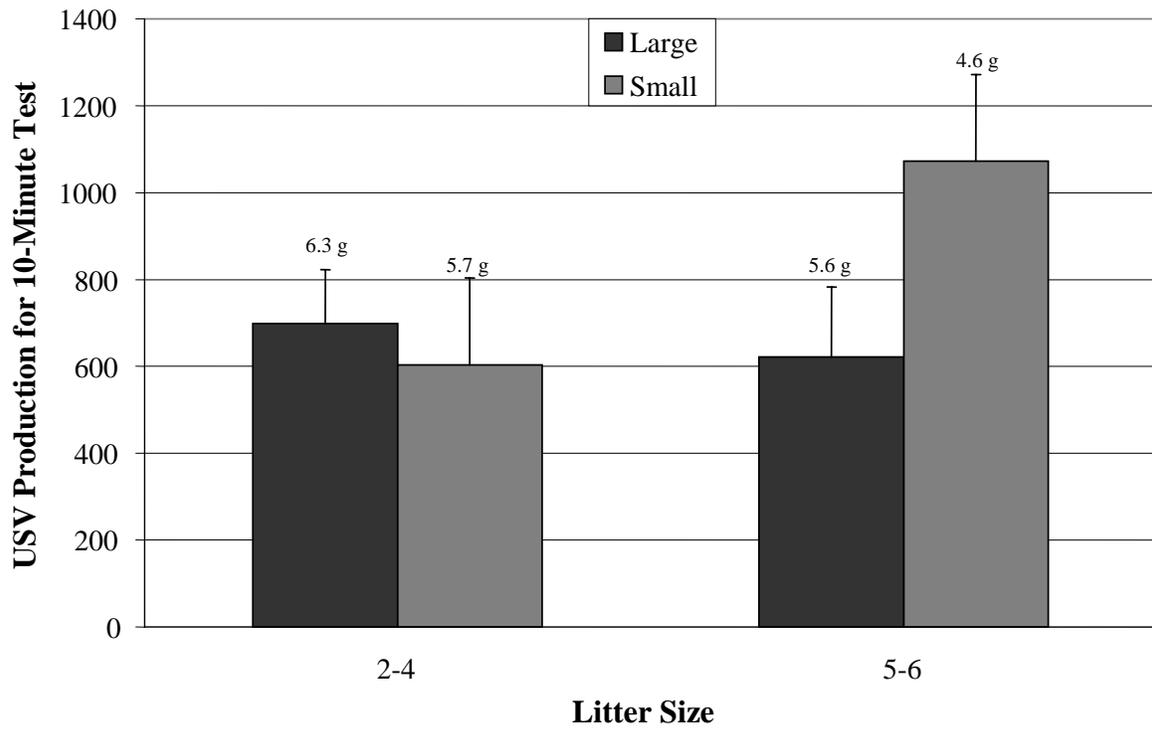
A 2 Litter Size (between-subjects 2-4 vs. 5-6 pups) X 2 Pup Size (within-litters heaviest vs. lightest) ANOVA was conducted on the dependent variable Total USVs emitted in the ten-minute test period. The Litter Size by Pup Size interaction was significant, $F(1,30) = 8.56, p = .007, MSE = 130300.2$. In small litters (two to four pups), the mean number of ultrasounds produced by the heaviest pup ($M = 698.0, SD = 558.3$) and lightest pup ($M = 603.6, SD = 365.4$) during the ten-minute testing period were not significantly different, $t(19) = 0.79, p = 0.44$. However, in large litters (five to six pups), the mean number of USVs produced by the lightest pup ($M = 1072.3, SD = 684.5$) was substantially greater than the numbers produced by the heaviest pup ($M = 621.3, SD = 557.2$), $t(11) = -3.32, p = .007$ (see Figure 1) Neither the main effect of Litter Size, $F(1,30) = 1.32, p = .26, MSE = 437370.6$, nor Pup Size, $F(1,30) = 3.51, p = .07, MSE = 130300.2$, was significant.

Weight Differences

Weights for pups from Illinois and Missouri were very similar, and were grouped for weight analysis. A 2 Litter Size (between-subjects 2-4 vs. 5-6 pups) X 2 Pup Size (within-litters heaviest vs. lightest) ANOVA was conducted on the dependent variable Pup Weight. Since the Pup Size variable was analyzed within litters, the main effect of Pup Size was significant by definition, with the heaviest pup ($M = 6.0g, SE = 0.16g$) weighing more than the lightest pup ($M = 5.3g, SE = 0.17g$), $F(1,30) = 97.9, p < .0001, MSE = 0.0941$. The main effect of Litter Size was significant, $F(1,30) = 10.0, p = .004, MSE = 1.22$, such that pups from small litters ($M = 6.0g, SE = 0.16g$) weighed more than pups from large litters ($M = 5.1g, SE = 0.14g$). The Litter Size by Pup Size interaction was also significant, $F(1,30) = 7.51, p = .01, MSE = 0.0941$. In small litters (two to four pups), the heaviest pup ($M = 6.3g, SE = 0.22g$) outweighed the lightest pup ($M = 5.7g, SE = 0.21g$) by an average of 0.6g, while in large litters (five or six pups), the

Figure 1.

USV Production by Litter Size and Pup Size.



Note: The error bars are the standard error of the mean. The average weight of each group is listed above its bar.

heaviest pup ($M = 5.6\text{g}$, $SE = 0.12\text{g}$) outweighed the lightest pup ($M = 4.6\text{g}$, $SE = 0.13\text{g}$) by a full gram (see Figure 2). Simple linear regressions were conducted to highlight the importance of the effect of litter size on the weight of both the heaviest (Adjusted $R^2 = .181$) and lightest (Adjusted $R^2 = .422$) pup in the litter, as well as the weight difference between the two extremes (Adjusted $R^2 = .208$). The average weight of both the heaviest and lightest pup decreased as litter size increased, while the weight differential between the heaviest and lightest pup increased with increasing litter size.

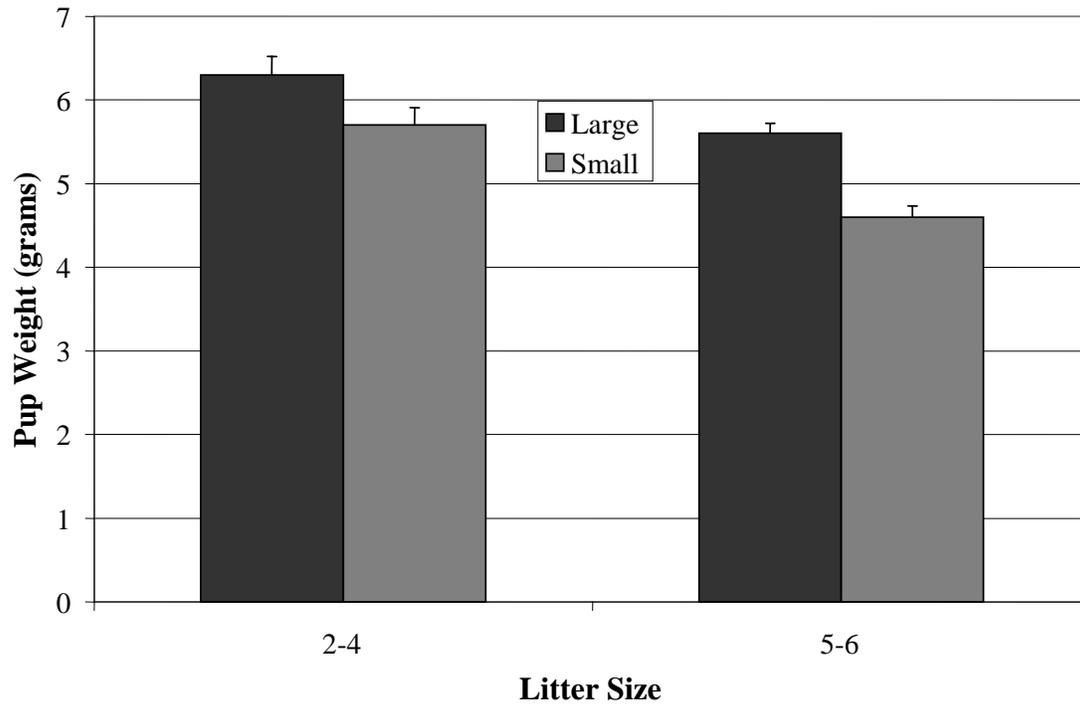
Pups were grouped by two Litter Size categories (2-4 pups and 5-6 pups) and two pup size categories (heaviest pup and lightest pup in the litter), yielding 4 combinations of pup characteristics. A one-way ANOVA was conducted on the dependent variable Pup Weight to eliminate the automatic main effect of pup size in the analysis above, and evidenced a significant effect of Category, $F(3,60) = 10.86$, $p < .0001$, $MSE = 0.658$. Tukey's HSD post hoc analysis revealed that the lightest pup in a large litter ($M = 4.6\text{g}$, $SE = 0.13$) weighed significantly less on average than pups from any of the other three groups, which did not differ significantly from each other ($M = 5.9\text{g}$, $SE = 0.13$).

Subpopulation Differences in USV Production

For Illinois voles, a 2 Litter Size (between-subjects 2-4 vs. 5-6 pups) X 2 Pup Size (within-litters heaviest vs. lightest) ANOVA was conducted on the dependent variable Total USVs produced in the ten-minute test period. The Litter Size by Pup Size interaction was significant, $F(1,15) = 12.32$, $p = .003$, $MSE = 118148.8$. In small litters (two to four pups), there was a nonsignificant trend toward the heaviest pup ($M = 1138.6$, $SD = 504.4$) producing more USVs than the lightest pup ($M = 788.0$, $SD = 295.6$) during the ten-minute testing period, $t(8) = 1.99$, $p = 0.081$. However, the opposite pattern was found for large litters (five to six pups),

Figure 2.

Pup Weight by Litter Size and Pup Size.



Note: The error bars are the standard error of the mean.

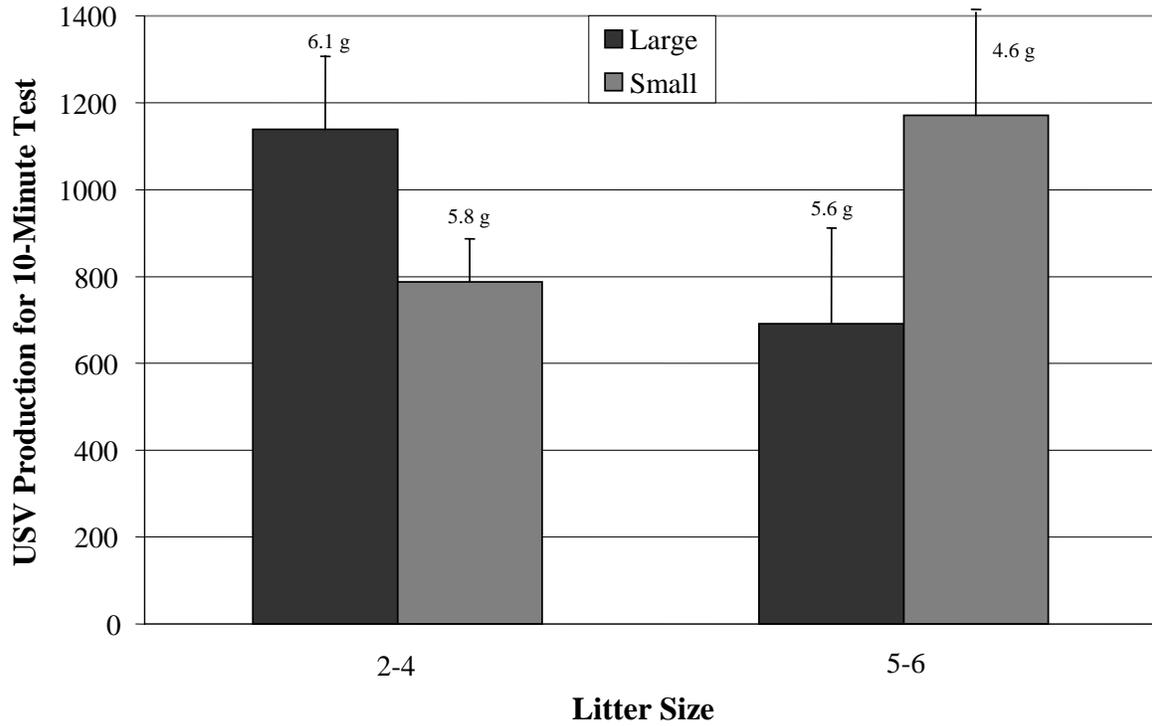
where the lightest pup ($M = 1170.4$, $SD = 736.8$) produced significantly more USVs than the heaviest pup ($M = 691.8$, $SD = 620.9$), $t(7) = -3.12$, $p = 0.017$ (see Figure 3). The main effects of both Litter Size, $F(1,15) < 1$, $p = .90$, $MSE = 497435.4$, and Pup Size, $F(1,15) < 1$, $p = .61$, $MSE = 118148.8$, were not significant.

For Missouri voles, a similar ANOVA was performed yielding no significant main effects of Litter Size ($F(1,12) = 2.88$, $p = .11$, $MSE = 163384.0$) or Pup Size ($F(1,12) = 3.08$, $p = .10$, $MSE = 122350.0$), nor was the Litter Size by Pup Size interaction significant, $F(1,12) < 1$, $p = .35$, $MSE = 122350.0$. This test was underpowered with $n = 11$ small litters and only $n = 4$ large litters. However, the observed trend for large litters was consistent with that found in both the overall analysis and among Illinois voles, with the lightest pup ($M = 876.2$, $SD = 611.9$) producing more USVs than the heaviest pup ($M = 480.5$, $SD = 446.3$) ($t(3) = -1.31$, $p = 0.28$), though not significantly so. In contrast to the Illinois voles, in small Missouri litters, the lightest pups ($M = 452.8$, $SD = 358.1$) also were observed to produce slightly, though again not significantly, more USVs than their heavier counterparts ($M = 337.5$, $SD = 266.7$) ($t(10) = -0.84$, $p = 0.42$). The lightest pup in large litters produced USVs at a rate almost double that of any of the other three groups, yet the small sample size prevents drawing conclusions about this subpopulation (see Figure 4).

Illinois voles produced more USVs than Missouri voles. The heaviest pup in litters from Illinois ($M = 590.5$, $SE = 143.2$) produced more USVs than the heaviest pup in litters from Missouri ($M = 312.7$, $SE = 80.7$), $t(24.9) = 3.36$, $p = .0029$, while the lightest pups from Illinois ($M = 565.6$, $SE = 137.2$) and Missouri ($M = 457.6$, $SE = 118.1$) performed more similarly, but still significantly differently from each other, $t(30) = 2.19$, $p = .0363$.

Figure 3.

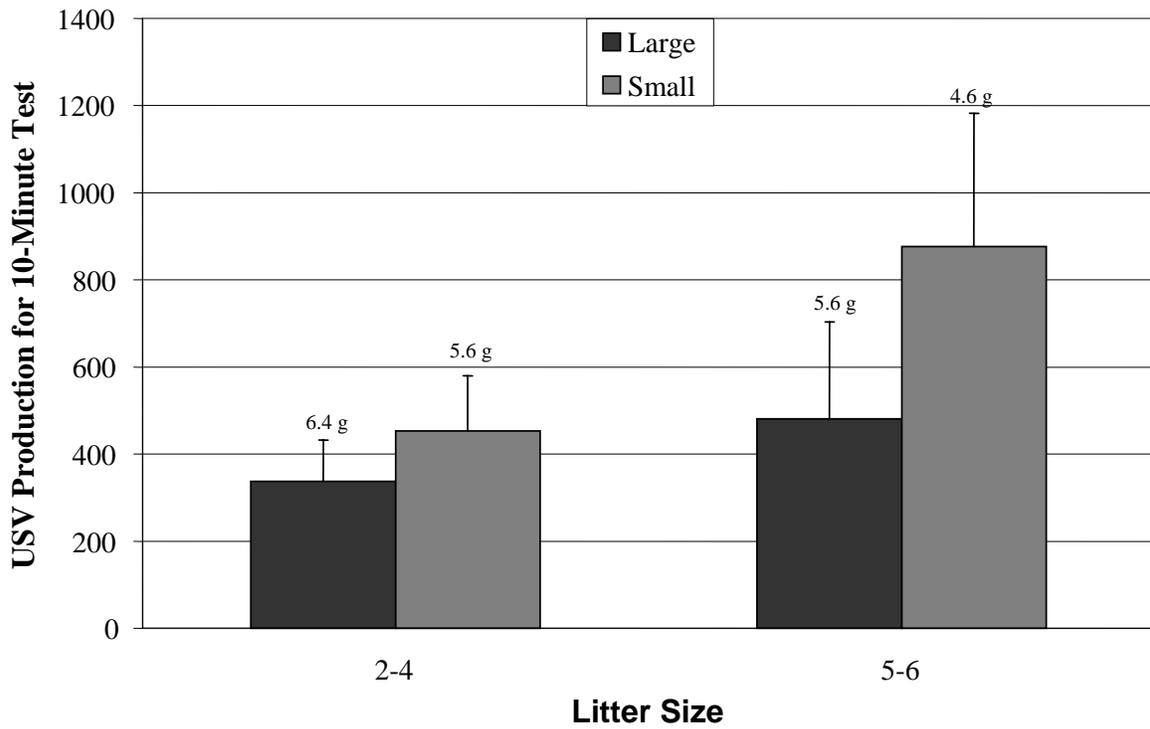
USV Production by Litter Size and Pup Size Among Illinois Voles.



Note: The error bars are the standard error of the mean. The average weight of each group is listed above its bar.

Figure 4.

USV Production by Litter Size and Pup Size Among Missouri Voles.



Note: The error bars are the standard error of the mean. The average weight of each group is listed above its bar.

DISCUSSION

USV Production and the Effects of Litter Size and Pup Deprivation

In litters of two to four pups, there was a trend toward the heaviest pup in the litter producing more USVs than the lightest pup in the litter. This result might be expected, and such an outcome could be due to several factors. One potential explanation would seem to be that the lightest pup simply may not be able to physically match the pace of the heaviest individual, who presumably has a larger energy supply to draw from. However, this interpretation of the pattern of USV production, coupled with the weight differences between pups from small and large litters, makes the data from large litters much more intriguing.

A comparison of the weights of the lightest pups in large and small litters suggests that in large litters the lightest pup may have lower fitness (due to lower absolute weight) than in small litters. However, the lightest pups outperformed the heaviest pups in terms of USV production in litters of five or six pups, despite their even greater weight disadvantage in these large litters. If one grants that USVs of prairie vole young may be used to signal deprivation level to their parents, several explanations for this finding present themselves. First, in a large litter where the four most productive nipples are always being used, the lightest pup might more often be the pup finding itself unable to attach to one of the most productive food sources (or not attached to a food source at all), possibly resulting in poorer nutrition, and thus its lower weight. Whether the size disadvantage leads to an inability to compete in the scramble competition or the inability to compete in the scramble competition leads to the size disadvantage is irrelevant to the effect observed. The pup may simply be at a higher, relative to its siblings, threshold level of need at which the pup calls at a higher rate, or as often as possible. The lightest pup in large litters both weighed less than and emitted more USVs than the other three combinations of Pup Size and

Litter Size, supporting this hypothesized threshold. Overall, the lightest pup in large litters averaged 4.6 grams, while the other three combinations of pup size and litter size averaged between 5.6 and 6.3 grams, suggesting that this hypothesized threshold would be somewhere between 4.6-5.6 grams. More research would need to be conducted to examine this hypothesis more fully.

The use of pup weight rank, which tends to be a stable feature of prairie vole litters, allows for another possible explanation. Perhaps the lightest pup has learned that it needs to call to let the dam know that it is unattached, which may be due to lack of availability of acceptable nipples due to its siblings occupying them. Moreover, when the dam removes all of the pups, its high calling rate may attract the dam to the lightest pup, instead of its less needy siblings, which should be calling at a lower rate. Further research will be required to test these hypotheses against each other and other hypotheses about the governance of USV emission by prairie vole young.

However, this research provides evidence that prairie vole infants may be engaged in begging behavior. While the race to attach to a nipple may be a pure scramble competition, USV production prior to the dam nursing may be comparable to the honest signaling of infant birds. Whereas in the scramble competition it appears that the young are in control of the division of resources, signaling may be necessary to prompt the delivery of food, and it is possible that the dam adjusts the total amount of nursing based on the begging level (ultrasounding rate). Prairie vole pups' USVs may simply be part of a two-tiered begging system, where 1) a pup (honestly) signals to the dam that it needs a chance to nurse, and 2) when given that chance it will be up to that individual pup to compete with its siblings to jostle (scramble) into position to do so. Pigs may use the same type of system, grunting more when food deprived, possibly to signal to the

sow that it needs to nurse, followed by a scramble against its littermates for nipple-occupancy (Weary & Fraser, 1995). We would expect in this case that, if we were able to hold the total amount of food delivered constant, as litter size increased, USV production would increase due to the proportionally higher level of deprivation of the young. Thus, in larger litters, and especially among the offspring that are the worst-equipped for scrambling, high USV emission should be expected because the dam would most likely not be able to provide enough food to maintain a large litter at an optimal (from the viewpoint of the pups) energy level.

General USV Characterization

The overall average of 724.3 USV emissions during the ten-minute testing period is in good agreement with the results of other studies on prairie vole USV production. One would expect to find the mean in a ten-minute test to be between Shapiro and Insel's (1990) observation of 601 calls in five minutes and Blake's (2002) report of 954 calls in twenty minutes, and the data do fall into that range. The main effect of Test Minute was due to pups calling at a very high rate during the first minute of the test and subjects producing USVs at a lower and similar rate during all subsequent minutes. Most likely, this effect can be attributed to the handling of pups immediately prior to data recording. The lower rate of 68.7 calls per minute for minutes two through ten is probably closer to the actual average rate at which pups of this species produce ultrasounds when the dam separates herself from them.

Subpopulation Differences

With the relatively low number of litters tested in the Missouri population, it is difficult to draw conclusions about differences between the two populations of prairie voles. However, some evidence has been found that populations of prairie voles from Illinois and Kansas show different social systems. Kansas prairie voles seem to exhibit a more polygynous mating system,

with sexual dimorphism, home-range overlap of one male with several females, and no evidence of pair-bond formation, while Illinois voles exhibit the more species-typical monogamous mating system (Roberts, Williams, Wang, & Carter, 1998). While the authors of that study did not measure USV rates in the two populations, as mentioned above, several researchers have recorded USVs of young of the monogamous prairie vole and polygynous montane vole (*Microtus montanus*), and attributed the large difference in infant USV production in favor of prairie voles to the mating system (Blake, 2002; Rabon, *et al.*, 2001, Shapiro & Insel, 1990). There may be a greater likelihood that the prairie vole pups (whose parents both tend to provide care for infants) will have calls answered by a parent than the montane vole pups, whose calling may be as likely to attract a predator as its mother. Meanwhile, in the current study, the Missouri subpopulation was found to emit USVs at a lower rate than the Illinois subpopulation. While the difference was not as great as the difference between prairie and montane voles, this could be interpreted as some preliminary evidence that the Missouri subpopulation is less monogamous than the Illinois subpopulation. More research would need to be undertaken before any conclusions should be drawn.

Analysis of the Current Research and Future Directions

In Maultsby's (2003) study of parent-offspring interactions and its relation to ultrasounding, as mentioned above, temperature was a potential confounding variable. Pups removed from the home cage and placed with the sire, typically a very attentive caregiver, for five minutes before testing would likely not lose heat very quickly, even if the sire were not particularly skilled at huddling to keep them warm without the dam's assistance. However, when the pups were removed for 55 minutes before testing, any deficiencies of the sire in providing thermoregulatory support for the offspring would surely have materialized in the form

of a lower body temperature of pups in the 55-minute group at testing time compared to the five-minute group. While this seems an unlikely scenario, the current study eliminates that confound, since there were only two pups from each litter being tested, and initiation of testing for the siblings was only ten minutes apart. However, a new potential confound arises in that the lightest pup would most likely lose heat more quickly than the heaviest pup, which could explain the difference in USV emission between the heaviest and lightest pup. However, the likely small difference in thermoregulatory ability seems insufficient to solely explain the observed difference in ultrasounding.

Another advantage of the current research is the examination of the subpopulation differences. This area of research deserves more attention, as some subpopulation differences within *M. ochrogaster* have already been documented (Roberts, *et al.*, 1998). While the lack of power in this study prevents drawing conclusions about the behavioral differences the Missouri subpopulation displays compared to the Illinois subpopulation, the data suggest there could be differences in USV production, and the factors that affect these differences should be examined much more closely. Meanwhile, it is fortunate that the Illinois subpopulation was the adequately-powered group, as much of the literature on prairie vole behavior is drawn from this subpopulation. Thus, the data presented here can be easily incorporated into the existing prairie vole literature without having to qualify it due to possible geographic variations.

A limitation of the current study is the lack of power to adequately analyze the behavior of the Missouri population. A larger sample size may have revealed a rich data set for comparison with the Illinois voles to further examine the effects of geographical variation on the species-typical behavior of prairie voles. Unfortunately, large litters are not born often in the population used in this study, and attempts at cross-fostering to increase sample size have not

been successful. Additionally, some researchers have reported no effects of gender on USV production (Motomura, *et al.*, 2002; Oswalt & Meier, 1975). However, given the results of this study, the effect of gender merits further research since the gender of the subject may mediate USV production through systematic weight differences during development between males and females.

REFERENCES

- Allin, J. T. & Banks, E. M. (1971). Effects of temperature on ultrasound production by infant albino rats. *Developmental Psychobiology*, 4, 149-156.
- Amsel, A., Radek, C. C., Graham, M., & Letz, R. (1977). Ultrasound emission in infant rats as an indicant of arousal during appetitive learning and extinction. *Science*, 197, 786-788.
- Anderson, J. W. (1954). The production of ultrasonic sounds by laboratory rats and other mammals. *Science (Washington, D.C.)*, 119, 808-809.
- Bautista, A., Mendoza-Degante, M., Coureaud, G., Martinez-Gomez, M., & Hudson, R. (2005). Scramble competition in newborn domestic rabbits for an unusually restricted milk supply. *Animal Behaviour*, 70, 1011-1021.
- Bell, R. W., Nitschke, W., & Zachman, T. A. (1972). Ultra-sounds in three inbred strains of young mice. *Behavioral Biology*, 7, 805-814.
- Blake, B. H. (1992). Ultrasonic vocalization and body temperature maintenance in infant voles of three species (*Rodentia: Arvicolidae*). *Developmental Psychobiology*, 25, 581-596.
- Blake, B. H. (2002). Ultrasonic calling in isolated infant prairie voles (*Microtus ochrogaster*) and montane voles (*M. montanus*). *Journal of Mammology*, 83, 536-545.
- Blumberg, M. S. & Alberts, J. R. (1990). Ultrasonic vocalizations by rat pups in the cold: An acoustic by-product of laryngeal braking? *Behavioral Neuroscience*, 104, 808-817.
- Blumberg, M. S. & Alberts, J. R. (1991). Both hypoxia and milk deprivation diminish metabolic heat production and ultrasound emission by rat pups during cold exposure. *Behavioral Neuroscience*, 105, 1030-1037.
- Blumberg, M. S. & Sokoloff, G. (2001). Do infants rats cry? *Psychological Review*, 108, 83-95.
- Blumberg, M. S., Sokoloff, G., Kirby, R. F., & Kent, K. J. (2000). Distress vocalizations in infant rats: What's all the fuss about? *Psychological Science*, 11, 78-81.
- Brunelli, S. A., Vinocur, D. D., Soo-Hoo, D., & Hofer, M. A. (1997). Five generations of selective breeding for ultrasonic vocalization (USV) responses in N:NIH strain rats. *Developmental Psychobiology*, 31, 255-265.
- Carter, C. S., DeVries, A. C., & Getz, L. L. (1995). Physiological substrates of mammalian monogamy: The prairie vole model. *Neuroscience and Biobehavioral Reviews*, 19, 303-314.
- Carter, C. S. & Getz, L. L. (1993). Monogamy and the prairie vole. *Scientific American*, 268, 100-106.

- DeVries, A. C., Johnson, C. L., & Carter, C. S. (1997). Familiarity and gender influence social preferences in prairie voles (*Microtus ochrogaster*). *Canadian Journal of Zoology*, *75*, 295-301.
- Getz, L. L., Carter, C. S., & Gavish, L. (1981). The mating system of the prairie vole, *Microtus ochrogaster*: Field and laboratory evidence for pair-bonding. *Behavioral Ecology and Sociobiology*, *8*, 189-194.
- Getz, L. L. & Hofmann, J. E. (1986). Social organization in free-living prairie voles, *Microtus ochrogaster*. *Behavioral Ecology and Sociobiology*, *18*, 275-282.
- Getz, L. L., McGuire, B., Pizzuto, T., Hofmann, J. E., & Frase, B. (1993). Social organization of the prairie vole (*Microtus ochrogaster*). *Journal of Mammology*, *74*, 44-58.
- Gilbert, A. N. (1995). Tenacious nipple attachment in rodents: The sibling competition hypothesis. *Animal Behaviour*, *50*, 881-891.
- Godfray, H. C. J. (1995). Signaling of need between parents and young: Parent-offspring conflict and sibling rivalry. *The American Naturalist*, *146*, 1-24.
- Godfray, H. C. J., & Johnstone, R. A. (2000). Beffing and bleating: The evolution of parent-offspring signaling. *Philosophical Transactions of the Royal Society of London Series B*, *355*, 1581-1591.
- Hayes, L. D. & Solomon, N. G. (2004). Costs and benefits of communal rearing to female prairie voles (*Microtus ochrogaster*). *Behavioral Ecology and Sociobiology*, *56*, 585-593.
- Hofer, M. A. (1996). Multiple regulators of ultrasonic vocalization in the infant rat. *Psychoneuroendocrinology*, *21*, 203-217.
- Hofer, M. S. & Shair, H. N. (1978). Ultrasonic vocalization during social interaction and isolation in 2-week-old rats. *Developmental Psychobiology*, *11*, 495-504.
- Hofer, M. A. & Shair, H. N. (1980). Sensory processes in the control of isolation-induced ultrasonic vocalization by 2 week old rats. *Journal of Comparative Physiological Psychology*, *94*, 271-279.
- Hofer, M. A., Shair, H. N., Masmela, J. R., & Brunelli, S. A. (2001). Developmental effects of selective breeding for an infantile trait: The rat pup ultrasonic isolation call. *Developmental Psychobiology*, *39*, 231-246.
- Hoffmeister, D. F. & Getz, L. L. (1968). Growth and age-classes in the prairie vole, *Microtus ochrogaster*. *Growth*, *32*, 57-69.

- Insel, T. R. (1997). A neurobiological basis of social attachment. *American Journal of Psychiatry*, 154, 726-735).
- Jannett, F. J. (1978). The density-dependent formation of extended maternal families of the montane vole, *Microtus montanus nanus*. *Behavioral Ecology and Sociobiology*, 3, 245-263.
- Johnstone, R. A. (2004). Begging and sibling competition: How should offspring respond to their rivals? *The American Naturalist*, 163, 388-406.
- Kacelnik, A., Cotton, P. A., Stirling, L., & Wright, J. (1995). Food allocation among nestling starlings: Sibling competition and the scope of parental choice. *Proceeding of the Royal Society of London Series B*, 259, 259-263.
- Maultsby, C. L. (2003). The effects of food deprivation on ultrasonic vocalization (USV) production on infant prairie voles. Unpublished undergraduate honors thesis: University of North Carolina at Wilmington.
- McGuire, B. (1998). Suckling behavior of prairie voles (*Microtus ochrogaster*). *Journal of Mammology*, 79, 1184-1190.
- McGuire, B. (2001). A possible function of the preference for hind nipples in prairie voles (*Microtus ochrogaster*). *Journal of Comparative Psychology*, 115, 439-443.
- McGuire, B. & Novak, M. (1984). A comparison of maternal behaviour in the meadow vole (*Microtus pennsylvanicus*), prairie vole (*M. ochrogaster*) and pine vole (*M. pinetorum*). *Animal Behaviour*, 32, 1132-1141.
- McGuire, B. & Novak, M. (1986). Parental care and its relationship to social organization in the montane vole (*Microtus montanus*). *Journal of Mammology*, 67, 305-311.
- Motomura, N., Shimizu, K., Shimizu, M., Aoki-Komori, S., Taniguchi, K., Serizawa, I., & Saito, T. R. (2002). A comparative study of isolation-induced ultrasonic vocalization in rodent pups. *Experimental Animals*, 51, 187-190.
- Oswalt, G. L. & Meier, G. W. (1975). Olfactory, thermal, and tactual influences on infantile ultrasonic vocalization in rats. *Developmental Psychobiology*, 8, 129-135.
- Parker, G. A., Royle, N. J., & Hartley, I. R. (2002a). Begging scrambles with unequal chicks: Interactions between need and competitive ability. *Ecology Letters*, 5, 206-215.
- Parker, G. A., Royle, N. J., & Hartley, I. R. (2002b). Intrafamilial conflict and parental investment: A synthesis. *Philosophical Transactions of the Royal Society of London Series B*, 357, 295-307.

- Rabon, D. R., Sawrey, D. K., & Webster, W. D. (2001). Infant ultrasonic vocalizations and parental responses in two species of voles (*Microtus*). *Canadian Journal of Zoology*, *79*, 830-837.
- Roberts, R. L., Williams, J. R., Wang, A. K., & Carter, C. S. (1998). Cooperative breeding and monogamy in prairie voles: Influence of the sire and geographical variation. *Animal Behaviour*, *55*, 1131-1140.
- Rosenzweig, M. R., Riley, D. A., & Krech, D. (1955). Evidence for echolocation in the rat. *Science (Washington, D.C.)*, *121*, 600.
- Sera, W. E. & Early, C. N. (2003). *Microtus mantanus*. *Mammalian Species*, *716*, 1-10.
- Shapiro, L. E., Austin, D., Ward, S. E., & Dewsbury, D. A. (1986). Familiarity and female mate choice in two species of voles (*Microtus ochrogaster* and *Microtus montanus*). *Animal Behaviour*, *34*, 90-97.
- Shapiro, L. E. & Dewsbury, D. A. (1990). Differences in affiliative behavior, pair bonding, and vaginal cytology in two species of vole (*Microtus ochrogaster* and *M. montanus*). *Journal of Comparative Psychology*, *104*, 268-274.
- Shapiro, L. E. & Insel, T. R. (1990). Infant's response to social separation reflects adult differences in affiliative behavior: A comparative developmental study in prairie and montane voles. *Developmental Psychobiology*, *23*, 375-393.
- Solomon, N. G., Keane, B., Knoch, L. R., & Hogan, Paula J. (2004). Multiple paternity in socially monogamous prairie voles (*Microtus ochrogaster*). *Canadian Journal of Zoology*, *82*, 1667-1671.
- Stalling, R. T. (1990). *Microtus ochrogaster*. *Mammalian Species*, *355*, 1-9.
- Thomas, J. A. & Birney, E. C. (1979). Parental care and mating system of the prairie vole, *Microtus ochrogaster*. *Behavioral Ecology & Sociobiology*, *5*, 171-186.
- Weary, D. M. & Fraser, D. (1995). Calling by domestic piglets: Reliable signals of need? *Animal Behaviour*, *50*, 1047-1055.
- Williams, J. R., Catania, K. C., & Carter, C. S. (1992). Development of partner preferences in female prairie voles (*Microtus ochrogaster*): The role of social and sexual experience. *Hormones and Behavior*, *26*, 339-349.