# Ultrasonic calling in 2 species of voles, *Microtus pinetorum* and *M. pennsylvanicus*, with different social systems

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When isolated, pups of muroid rodents emit ultrasonic vocalizations, an indication that they are stressed by being alone and exposed to cooling temperatures. Rate of vocalizations is greatest at the end of their 1st week and beginning of the 2nd week, declines in the 2nd week as eyes open and thermoregulation becomes fully established, and ceases in the 3rd week. Young of 1 vole species, the prairie vole (*Microtus ochrogaster*), vocalize significantly more than those of other species, which has been attributed to the social structure of the species, because they have monogamous mating behavior and high level of biparental care. To determine whether this vocalizing behavior is typical of vole species with monogamous social systems, I examined calling behavior in another monogamous species with biparental care, the pine (or woodland) vole (*M. pinetorum*), and I compared it to that of a polygynous species that has only maternal care, the meadow vole (*M. pennsylvanicus*). Pups were isolated for 20 min at 22°C ± 2°C and the number of ultrasonic calls they emitted was monitored. Pine vole pups vocalized at high rates, similar to prairie voles, emitting a mean of 47 calls/min at the ages when calling was greatest (8–16 days). In contrast, meadow vole pups vocalized significantly less, emitting a mean of 9 calls/min at the age of greatest calling (5–9 days). Pine voles continued vocalizing to 23 days, like prairie voles but different from other vole species, including meadow voles, which cease calling earlier. I conclude that young voles of monogamous species, which are highly affiliative and have paternal as well as maternal care, respond to the stress of isolation more strongly than do other vole species and emit more ultrasonic vocalizations than species that are less social and have less parental care.

Key words: communication, isolation calling, meadow vole, monogamous mating system, parental care, pine vole, polygynous mating system, thermoregulation, ultrasonic vocalizations, woodland vole

Nestling muroid rodents emit ultrasonic vocalizations (USVs) when separated from the mother and nest, and parents retrieve the young (Costantini and D'Amato 2006; Okon 1970, 1971, 1972; Portfors 2007; Sales and Pye 1974). Young in the superfamily Muroidea are altricial—at birth they are small and hairless, unable to see, hear, or move about on their own. Parents respond to the ultrasonic calls by approaching the pup and retrieving it, usually carrying it back to the nest. Ultrasonic calls by rodent pups have been seen as distress calls (Hahn and Lavooy 2005; Noirot 1972; Portfors 2007). Drugs that reduce distress in humans reduce the rate of USVs emitted by mice and rat pups, whereas drugs that enhance distress increase their rate of vocalizing (Brain et al. 1991; Costantini and D'Amato 2006; Hahn and Lavooy 2005). The calling response to isolation is closely tied to development of thermoregulation. Most young nestling muroids are not able to maintain a homeothermic body temperature when away from nest and

family members, and when alone in a cooling environment their body temperature decreases. Retrieval to the warmth of the nest restores body temperature. Okon (1970, 1971, 1972) showed that calling occurs at ages when pups cool, and the lower the ambient temperature, the more they call.

Although it has long been assumed that the vocalizations are distress calls, Blumberg and Alberts (1990) argue that they are not communication signals but simply a by-product of a respiratory mechanism. They contend that the mechanism, laryngeal braking, serves to increase oxygen pressure in the lungs and blood and facilitates nonshivering thermogenesis (Blumberg and Alberts 1990; Blumberg et al. 1992). Despite this physiological explanation there is strong support for the

view that infant USVs are a form of communication (Costantini and D'Amato 2006; Hofer 2002; Hofer and Shair 1993; Portfors 2007). Additionally, the calls begin within minutes of a vole pup being placed in a cooling environment, before a marked drop in body temperature, suggesting that the sensation of cool air rather than physiological warming initiates calling (Blake 1992). Kölliker (2005) points out that infant isolation calls fit the definition of solicitation behavior—behavior that solicits parental care, which complies with the definition of a signal. Additionally, the parental response—approach and retrieval—is a strong response and not readily extinguished (Sales and Pye 1974). The mother not only responds to the pup's needs by retrieving it, but D'Amato et al. (2005, 2006) have shown that her behavior also affects calling behavior of the pup. The physiological mechanism at the basis of isolation calling, laryngeal braking, could very likely be the origin of the signal that evolved into a vocalization communicating to parents that a pup is out of the nest and should be retrieved—an evolutionary step that has been suggested (Blake 2002; Hofer 2002).

Throughout various muroid groups, the calling response of isolated pups changes with age, but not in a simple way. Rate of ultrasonic calling increases with age in young nestlings, reaches a peak, and then decreases: a graph of calling rate with age is in the shape of an inverted U (Blake 1992; Branchi et al. 2001; De Ghett 1974; Geyer 1979; Okon 1970, 1971, 1972; Sales and Pye 1974). Typically, pups call little or not at all on the day of birth but emit more and more calls in the following days; the rate reaches a peak at about 6–8 days postpartum, and then declines as pups mature. As their eyes open and they develop the ability to hear, move about, and thermoregulate well, they call less and less and finally stop calling by about 16–19 days.

Prairie voles (*Microtus ochrogaster*), however, do not show this developmental profile of rising and falling calling rates during development. Rather, isolated infants emit USVs at high rates from the 1st day postpartum (Blake 2002; Rabon et al. 2001; Shapiro and Insel 1990). They continue to vocalize at rates that are considerably higher than for other vole species in the 1st week after birth, averaging >50 calls/min when isolated at room temperature, compared to 1–10 calls/min in montane voles (*M. montanus*) and field voles (*M. agrestis*—Blake 1992, 2002; Rabon et al. 2001; Shapiro and Insel 1990). Additionally, young prairie voles continue calling past 20 days, considerably longer than other vole species. These differences in calling, however, do not appear to be linked to the development of thermoregulation. In their first 10 days, as prairie and montane voles grow from 2–3 g to about 5–7 g, their ability to maintain body temperature during isolation improved at the same rate, but their rates of isolation calling differed significantly (Blake 2002).

Prairie voles are socially monogamous (Getz et al. 1993). Monogamy is not common in mammals, with ≤3% of mammals exhibiting some form of monogamy (Kleiman 1977). Like most other mammalian species, montane voles and meadow voles (*M. pennsylvanicus*) are polygynous

(Boonstra et al. 1993; Jannett 1980; Madison 1980a, 1980b). In these 2 species in the wild, the female rears the young alone, nursing them periodically and leaving them alone in the nest while she forages, and she deserts them, thus weaning them, when they are about 14 days old (Jannett 1980, 1982; Madison 1980a, 1981). Similarly, in the laboratory only mothers provide parental care for pups (McGuire and Novak 1984, 1986; Oliveras and Novak 1986). In contrast, prairie voles establish strong male–female pair-bonds (Getz et al. 1993). They provide biparental care of young with both parents spending considerable time in the nest with the litter; pups are rarely left alone in the nest without at least 1 parent present (McGuire and Novak 1984). Although prairie vole pups can be separated from parents without losing body mass at 13 days (Blake 2002), they are not weaned at that age but continue to suckle until 19–20 days, and they tend to delay dispersal and remain in the natal nest after the next litter is born, helping to care for younger siblings (McGuire and Novak 1984; Wang and Novak 1992, 1994). Prairie voles are also highly affiliative, with more physical contact between siblings and between parents and young than in meadow voles (Wilson 1982a, 1982b).

It has been assumed that the high calling rate of isolated prairie vole young is associated with their social system, in particular with the strong tendency toward affiliation and biparental care (Blake 2002; Rabon et al. 2001; Shapiro and Insel 1990). It seems reasonable to assume that the more social a species is, the more need it has for communication within the social group. Alternatively, the high calling rate of prairie voles may simply be a unique characteristic of the species and independent of their social system. For example, differences in calling rates might be due to allometry if pups of different species differ in size (although they do not differ in size in prairie and montane voles). If high rates of ultrasonic calling are related to monogamy and intensive biparental care, the high rates also should be seen in other monogamous and biparental vole species (although size also could be a factor if there is a size discrepancy in pups).

My objective was to determine whether high calling rates of pups relate to the social structure of the species in voles. I examined calling rates of another pair of species that are closely related (Conroy and Cook 2000) but differ in mating systems, the pine (or woodland) vole (*M. pinetorum*), which is monogamous (FitzGerald and Madison 1983; McGuire and Novak 1984), and the meadow vole, which is not. Like prairie voles, young pine voles suckle until at least 21 days of age and juveniles stay in the nest after the next litter is born and help care for it (Geyer 1979; McGuire and Novak 1984; Powell and Fried 1992). I used the same protocol and equipment as in my previous study (Blake 2002), which allowed me to compare rates of isolation calling of pine and meadow voles and to determine if the monogamous species, the pine vole, vocalized at higher rates than the polygynous species, the meadow vole, as occurs in prairie and montane voles. This study is the 1st to monitor ultrasonic calling rates in pine voles in conjunction with development of thermoregulation and to relate calling

rates to social structure of the species. It also is the 1st study of USVs or of development of thermoregulation in meadow voles.

I predicted that pine voles, like prairie voles, would have a high calling rate from day of birth with rates significantly higher than those of the polygynous species, the meadow vole. Because adults of these 2 species also differ in body size I compared calling rates and body mass of pups to expected expiratory rates (because calling occurs during expiration—Roberts 1972) to determine if body size explained differences in calling rate. I also monitored development of thermoregulation, and I predicted that it would not differ markedly between pine voles and meadow voles.

## MATERIALS AND METHODS

*Experimental animals.*—The study was performed on captive nestling pine voles and meadow voles. A pine vole colony was begun with stock from a colony at Smith College, Northampton, Massachusetts, which had been started with descendants of voles originally captured in an orchard in New Paltz, New York, in 1997 (McGuire and Sullivan 2001). The meadow vole stock was begun with voles captured in Fletcher, Henderson County, North Carolina, in 1995; pups used in the study were progeny of females that were born in captivity. Pine voles were tested from September 1999 to July 2000 and meadow voles from October 1995 to December 1997.

Pine voles are smaller in body size than meadow voles (Nadeau 1985). Breeding adults in the study colony had the following mean body mass: nonpregnant females, 26.6 g ($SD =$ 4.5, $n = 17$) for pine voles and 39.8 g ($SD = 7.7$, $n = 15$) for meadow voles; and males, 25.4 g ($SD = 5.2$, $n = 13$) for pine voles and 51.1 g ($SD = 10.9$, $n = 11$) for meadow voles.

Voles were housed in the animal facility at Bennett College in a small building adjacent to the laboratory building. They were held in polycarbonate or polyacrylic cages, $48 \times 27 \times 20$ cm. Ambient temperature in the animal room fluctuated somewhat with outdoor temperatures, ranging from 12–21°C in winter to 18–26°C in summer. Voles were provided rabbit pellets and laboratory mouse chow (Purina Rabbit Chow or Advanced Nutrition Rabbit Feed Professional Formula and Purina Mouse Diet 5015 Lab Diet; Purina Mills, St. Louis, Missouri), occasionally apples or fresh greens, and water ad libitum. A mixture of wood shavings and peat moss was placed in cages, as were strips of newspapers that the voles used for making nests. Cardboard tubes and glass jars or plastic tubes also were present in cages. Pine voles were given a layer of hay or dried grass about 10 cm deep in which to burrow and build nests. Photoperiod was 14L:10D with lights on at 0900 h. Tests were performed between 1300 and 1800 h in a laboratory separate from the animal facility. Adults were weighed prior to pairing and females also were weighed subsequently to check progress of pregnancies.

*Vocalizations and body temperature during isolation.*—I used the same protocol and equipment with pine and meadow voles as I had used in the previous study on prairie and montane voles and the same protocol as for field voles (Blake

1992, 2002). This allowed me to compare results directly between these species.

To determine ultrasonic calling response, individual vole pups were isolated and cooled for 20 min and their USVs were monitored. I selected a 20-min observation time because I judged this to be long enough for pups to recover from the stimulus of being moved into a cool environment and for their reaction to isolation and cooling to stabilize. This is longer than in many studies—Hahn and Lavooy (2005) found that the mean observation time was about 8 min in 67 studies of infant isolation calls that they surveyed.

Pups were selected arbitrarily from their home cage; they were not marked for individual identification nor was sex determined. A single pup was placed in a plastic container (13 $\times 6 \times 5$ cm) on a clean cotton ball and the container was put in an incubator at nest temperature (32–33°C) for 2–5 min to allow the infant to rest after being handled. The container was then placed in a constant-temperature cabinet (Lab Line Ambi Hi-Lo Chamber; Lab Line Instruments, Melrose Park, Illinois) maintained at a cooling temperature of 22°C ± 2°C. Fresh air was passed through the container holding the pup at a flow rate of 50 ml/min. Vocalizations were counted for 20 min, after which the pup was removed from the cabinet, its body temperature was measured as described below, and it was weighed. Infants were held in an incubator at nest temperature until tests on the litter were completed, then all littermates were returned to the home cage. Test containers were washed with hot water and detergent after each test.

Ultrasonic calls were monitored in isolated pine voles at ages 1–24 days and meadow voles at 1–14 days (with 0 days being day of birth). Meadow voles were not studied beyond 14 days because the colony stopped reproducing before tests at the later ages had been conducted. Monitoring was done on 13 litters of pine voles and 10 litters of meadow voles, with a total of 37 tests on individual pine vole pups and 21 on individual meadow vole pups. Some litters were sampled at several ages (1–6 different ages for pine voles and 1–5 ages for meadow voles), although never on 2 days in succession. The number of pups in a litter that were tested at a given age was limited by litter size. Litters were small in pine voles ($\bar{X} = 1.8 \pm 0.6 \ SD$, $n = 13$ litters); most (31 of 37) tests were done on 2 pups, and in 6 tests only 1 pup was available. Meadow voles had larger litters ($\bar{X} = 3.9 \pm 1.4$, $n = 33$ litters); 3 pups were used in 13 of 21 tests, 2 pups in 6 of 21 tests, and the 1 available pup in 2 tests.

Tests also were done on 4 litters born to the 4 wild-caught females that made up the original stock. Twenty-six tests were performed on these pups at ages 3–18 days, with 1–3 tests per litter. Data for these litters were not included in statistical analyses because rearing of young by wild-caught females may not be comparable to rearing by laboratory-born females; data from the 2 groups were not combined. Mean data for these 4 litters are reported for comparison with data on pups of laboratory-born females.

Days on which pups were tested depended on litter availability and on my schedule and those of students working

with me. Because emission of USVs is quite variable, with some pups calling little or not at all and their littermates emitting many calls, I averaged number of calls for all pups in a litter tested on a given day (after log-transformation—see below).

Ultrasonic calls were monitored with a bat detector (model S-25; Ultra Sound Advice, London, United Kingdom). The detector was set to its "divide-by-N" mode in which any instantaneously dominant frequency in the range 15–200 kHz is divided by 10 to produce a frequency that is audible to humans. The microphone was suspended vertically within the constant-temperature cabinet about 10 cm above the infant vole.

Calls were monitored with headphones and counted with a tally counter. The cumulative number of vocalizations was noted for each minute of the 20-min test. I use the term "call" or USV to refer to individual pulses of sound emitted at ultrasonic frequency.

The nestling period in voles is brief, ≤3 weeks in most vole species. Development consists of 3 stages. In the 1st stage, about 1 week long, pups are very immature, cannot see or hear, and their thermoregulatory response is so limited that they are completely dependent on maternal care for thermoregulation. In the 2nd stage, also about 1 week in length, development is rapid as pups acquire hearing, sight, locomotor skills, and the ability to thermoregulate independently; in this stage pups become able to survive without their mother. In the 3rd stage, thermoregulation and locomotion are well developed and pups may disperse from the nest (Blake 1992; Hill 1976; Pépin and Baron 1978). Isolation calling in voles is greatest in the first 2 stages and declines and ceases in the 3rd stage (Blake 1992, 2002).

I compared calling rates of the 2 species at 3 periods: week 1, week 2, and the period of peak calling. I defined week 1 as 1–6 days (because no data were taken at 0 days), week 2 as 7–14 days, and the peak period as the 5–9 days when calling rates were highest. I determined peak calling period by inspection of age-group means (as calculated from antilog of mean log-transformed number of calls in 20 min), with peak period being the time when mean calling rates were at least twice as high as before and after the peak.

In addition to number of calls emitted, the tendency to vocalize when isolated also may be seen in latency to begin calling and in proportion of pups that emit calls when isolated (both of which differ between prairie and montane voles [Blake 2002]). Cumulative number of USVs emitted was noted after each minute of the test, and I determined latency to call as the first 1-min interval in which a call was detected. I calculated proportion of pups calling from number of individuals (1–14 days of age) that emitted any USVs during the 20-min isolation test.

Body temperature was measured after the 20-min isolation test to determine how well pups maintained their body temperature when exposed to a cooling environment. Body temperature was measured to the nearest 0.1°C with an electronic thermometer (NIST; Fisher Scientific, Atlanta,

Georgia) using a thin probe (YSI 402, YSI 511, or YSI 554; Yellow Springs Instruments, Yellow Springs, Ohio). To minimize invasive procedures, the probe was placed in the axilla or, for very small infants, on the thorax with the foreleg folded over it, for 45 s (as described in Blake 1992). Gębczyński (1975) showed that axillary measurements of the bank vole (*Myodes glareolus*) were within 0.1°C of deep-body temperature. Body mass was measured to the nearest 0.1 g on an electronic balance (Ohaus model CT600S or Ohaus Scout model SC 4010; Ohaus Corporation, Florham Park, New Jersey).

All procedures conformed to guidelines of the Animal Care and Use Committee of the American Society of Mammalogists (Sikes et al. 2011). Procedures were approved by the Institutional Animal Care and Use Committee of Bennett College.

*Statistical analyses.*—Values for number of USVs in 20 min were not distributed normally (i.e., means and variances for each species were positively correlated), so data were normalized by logarithmic transformation (Martin and Bateson 1993). This allowed direct comparison to calling data for prairie, montane, and field voles, which also were log-transformed. Inferential statistics were calculated on transformed data. Descriptive statistics are presented as logarithms and also converted to number of calls per minute for ease of interpretation. Log-transformed data are presented as mean ± 1 *SD*.

For graphical representation, data were reduced by calculating means for 3-day age groups, beginning with ages 1–3 days (tests were not done on day of birth, 0 days). Group means were calculated from litter means. Sample size for number of calls and body mass was 3–6 litters in each age group for pine voles and 2–6 litters for meadow voles; for body temperature it was 2–6 litters per age group for each species.

To avoid pseudoreplication from using data for litters that had been tested repeatedly, statistical tests were performed on subsets of data consisting of 1 test per litter, comparing calling rates at the 3 periods described above. Subsets for each species were selected so there was an approximately equal distribution of ages throughout the selected period, with no repeated measures on any litter (i.e., independent observations). For all statistical comparison, $\alpha = 0.05$.

To compare number of USVs between the 2 species, permutation tests with 1,000 permutations were performed on the subsets of data for number of log-transformed calls in 20 min (Manly 1998). To compare latency between species, a median sign test was used on subsets.

To examine whether calling rates vary with body mass of pups, I compared rate of calling during the peak calling period in the 2 species to the expected rate of respiration, because USVs are produced during the expiratory phase of respiration (Roberts 1972). I calculated expected respiration rate for pups from their body mass, using the equation for rate of respiration of mammals in general, which scales with body mass as $53.5M^{-0.26}$ (in breaths per minute), where M is body mass in kilograms (Stahl 1967). Comparison between species was
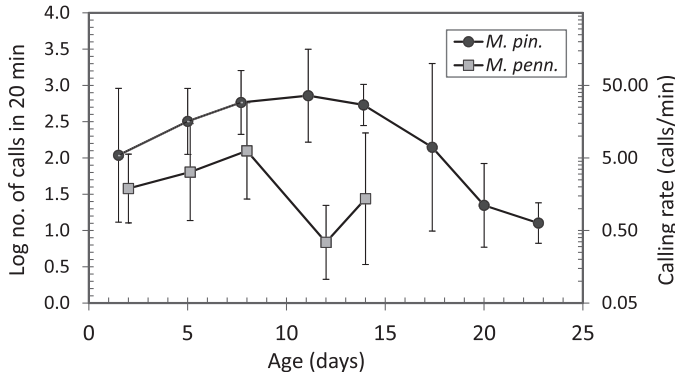
FIG. 1.—Rate of ultrasonic calling by isolated vole pups showing the higher rate for the monogamous species, the pine vole (*Microtus pinetorum* [*M. pin.*]), than the polygynous species, the meadow vole (*M. pennsylvanicus* [*M. penn.*]). Statistics were calculated on log-transformed number of calls in 20-min tests; calling rate shows values as number of calls per minute. Symbols indicate mean for pups in each 3-day age group; vertical lines indicate $\pm$ 1 *SD*. Sample size is 3–6 litters per age group for pine voles and 2–6 litters per group for meadow voles.

made using mean body mass of pups in each litter (at age tested) for the subsets of data with no repeated measures at age of peak calling ($n = 8$ litters for each species).

## RESULTS

*Ultrasonic calling.*—Pine vole pups emitted more USVs than did meadow vole pups, as predicted (Fig. 1; Table 1). Pine voles showed the typical developmental profile of muroid rodents, emitting fewer vocalizations in the 1st few days than later in the nestling period, reaching a peak of calling, then declining (Fig. 1). Meadow voles showed a similar rise and then decline in calling rates (Fig. 1). During the 1st week postpartum, pine voles did not differ significantly from meadow voles in number of isolation calls emitted, although they called at a slightly higher average rate (7 calls/min, compared to 3 calls/min for meadow voles [Table 1]). During their 2nd week, though, pine voles emitted significantly more USVs than did meadow voles (mean of 23 calls/min compared

to 3 calls/min [Table 1]). Time of peak calling was later in pine voles than in meadow voles (Fig. 1; Table 1), and calling rate at the peak period was significantly greater in pine voles ($\bar{X} = 47$ calls/min) than in meadow voles ($\bar{X} = 9$ calls/min [Table 1]).

Pine voles were still vocalizing at 23 days, the last age tested, although at low rates ($\bar{X} = 0.6$ calls/min, $n = 4$ litters; Fig. 1). Meadow voles had a low mean rate of calling at 12 days ($\bar{X} = 0.3$ calls/min, $n = 2$ litters) and a slightly higher rate at 14 days ($\bar{X} = 1.3$ calls/min, $n = 3$ litters; Fig. 1), but they were not tested beyond that age.

Results for meadow voles born to the 4 wild-caught females confirm that meadow vole pups essentially cease calling by 12 days, much earlier than pine voles. Mean rates for pups of wild-caught mothers were similar to rates for pups of laboratory-born mothers at 3, 6, and 10 days (6, 18, and 1.5 calls/min, respectively), with a peak at 6 days, but by 12 days only an occasional call was emitted ($\bar{X} \leq 0.05$ call/min at 12, 14, and 18 days).

Pine and meadow voles had similar tendencies to vocalize when isolated, as indicated by latency to call and proportion of pups tested that emitted USVs. Pine and meadow voles did not differ in latency to begin vocalizing—pups of both species began calling at the beginning of the isolation test. Median latency for both pine and meadow voles was min 1 at all 3 periods examined, 1–6 days, 7–14 days, and peak calling ages ($P > 0.10$ for each age group; median sign test). The 2 species did not differ in proportion of pups calling. Most pups called when isolated in weeks 1 and 2: at these ages calls were emitted in 43 (98%) of 44 tests on pine voles and in 51 (96%) of 53 tests on meadow voles ($P > 0.50$, *d.f.* = 1; chi-square test).

Pups from litters of 1 tended to call less than pups of similar ages from litters of 2 or more, in both pine and meadow voles, but differences were not statistically significant ($P > 0.10$ in each comparison; median sign tests). However, the study was not designed to detect differences in calling rates between litters of different sizes, and samples in each age group were small.

*Growth and development.*—Meadow vole pups were larger than pine vole pups from birth, as would be expected from the larger body size of meadow vole adults. Meadow voles

TABLE 1.—Number of calls emitted by isolated pine voles (*Microtus pinetorum*) and meadow voles (*M. pennsylvanicus*) for 3 time periods, 1st week postpartum, 2nd week postpartum, and age of peak calling, showing that number of calls in 20 min was significantly greater in the monogamous species (the pine vole) than in the polygynous species (the meadow vole) during the 2nd week and at peak calling. Data are based on subsets with no repeated measures. Mean and *SD* are based on log-transformed number of calls in 20 min; *P* (probability) is for comparison of log-transformed number of calls between species, using a permutation test. Number of calls and rate were determined from antilogs of mean number of calls.

| Comparison | Species | Ages (days) | N (litters) | Log no. calls in 20 min ($\bar{X} \pm SD$) | No. calls in 20 min | Rate (calls/min) | P (permutation test) |
|---|---|---|---|---|---|---|---|
| First week | *M. pinetorum* | 1–6 | 8 | 2.151 ± 0.868 | 141 | 7 | |
| | *M. pennsylvanicus* | 1–6 | 7 | 1.783 ± 0.548 | 60 | 3 | 0.19 |
| Second week | *M. pinetorum* | 7–14 | 7 | 2.665 ± 0.530 | 462 | 23 | |
| | *M. pennsylvanicus* | 7–14 | 8 | 1.724 ± 0.795 | 52 | 3 | 0.013 |
| Peak calling | *M. pinetorum* | 8–16 | 8 | 2.976 ± 0.346 | 945 | 47 | |
| | *M. pennsylvanicus* | 5–9 | 8 | 2.236 ± 0.401 | 171 | 9 | 0.002 |

A



B
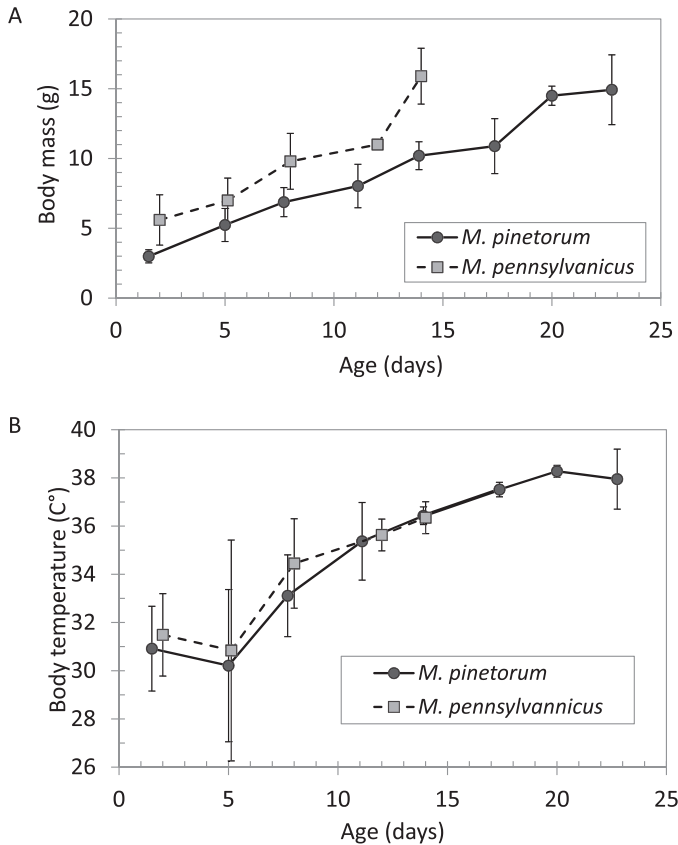


**Fig. 2.**—Growth and development of homeothermy in pine voles (*Microtus pinetorum*)and meadow voles (*M. pennsylvanicus*) during the nestling period. A) Body mass, showing larger size of meadow voles. B) Body temperature after isolation and cooling for 20 min at 22°C, showing similarity in maintaining body temperature by 11 days and full homeothermy by 13 days (mean) and 14 days (all litters) in both species, despite size differences. Symbols indicate mean for pups in each 3-day age group; vertical lines indicate ± 1 *SD*. Sample size is 3–6 litters per age group for body mass of pine voles and 2–6 litters per group for body mass of meadow voles and for body temperatures of both species.

weighed more at 14 days ($\bar{X}$ =15.9 g) than pine voles weighed at 23 days ($\bar{X}$ = 14.9 g; Fig. 2A).

These differences in body size between pine and meadow voles did not explain differences in calling rates. At the time of peak calling, when calling rates were significantly different, pups of the 2 species had almost the same body mass—for pine voles (with peak rates later, at 8–16 days) mean body mass was 8.7 g, and for meadow voles (with peak rates at 5–9 days) it was 8.6 g (Fig. 2A). Expected respiratory rates for these body masses, calculated from the relationship x = 53.5M$^{-0.26}$ (with mass in kilograms—Stahl 1967), are thus almost the same: 183.7 breaths/min for pine voles and 184.2 breaths/min for meadow voles. Yet pine voles had significantly higher calling rates during the age of peak calling than did meadow voles (Table 1).

Despite being smaller, pine vole pups developed independent thermoregulation at almost the same age as meadow voles

(Fig. 2B). (Here I am defining "independent thermoregulation" or "full homeothermy" as a pup maintaining body temperature ≥ 36°C when isolated at 22°C for 20 min.) Although meadow voles held body temperature above a mean of 35°C when cooled at 9 days and pine voles not until 11 days, by 14 days all litters of each species maintained homeothermic temperatures with mean body temperatures > 36°C (means of 3 litters for meadow voles at both ages and for 3 litters of pine voles at 14 days and 2 litters at 11 days).

## DISCUSSION

As predicted, infant pine voles vocalized at higher rates than meadow voles when isolated, a difference much like that between prairie vole and montane vole young (Blake 2002; Rabon et al. 2001; Shapiro and Insel 1990). Pine vole pups vocalized at a very high rate during their peak calling period, similar to prairie voles—means of 47 calls/min in pine voles (Table 1) compared to 54 calls/min in prairie voles (age 0–8 days—Blake 2002). By contrast, mean peak rates in the 2 polygynous species were 9 calls/min in meadow voles (Table 1) and 4 calls/min in montane voles (age 4–10 days—Blake 2002). Pine and prairie voles also were similar in that young continued emitting isolation calls to about 23 days (Fig. 1), later than pups of most muroid species, which cease calling by 19 days (Blake 1992, 2002; De Ghett 1974, 1977; Okon 1970, 1971, 1972; Smith and Sales 1980; Yoshinaga et al. 1997).

One result I had not expected was that pine vole pups did not call at peak rates from day of birth as prairie voles do (Blake 2002). In this respect the developmental profile of pine voles resembled that of other muroids, with less calling in their 1st few days followed by a peak of calling, not the early peak seen in prairie voles. It also is not clear why age of peak calling differs in prairie and pine voles.

In other vole species, peak calling period ends a few days before independent thermoregulation is established, and then rates decline (Okon 1970, 1971, 1972). Most muroids, including voles, have peak calling at about 5–10 days of age (Blake 1992, 2002; Okon 1970, 1971; Rabon et al. 2001), and montane, meadow, and field voles achieve full homeothermy soon after, at 11–13 days. By contrast, in prairie and pine voles, peak calling seems to be dissociated from development of thermoregulation; prairie voles call at peak rates at 0–8 days but achieve homeothermy later, at 16–18 days (Blake 2002), whereas pine voles have peak rates at 8–14 days and achieve homeothermy during that period, at 13 days (Figs. 1 and 2B). Apparently factors in addition to isolation and cooling influence calling rates in prairie and pine voles. It is known that vocalizing behavior in muroids is altered by social and environmental factors such as odors or presence of the mother (Costantini and D'Amato 2006; Geyer 1979, 1981; Hofer 2002; Kapusta et al. 1995). More study is needed on additional factors that may affect calling rates in monogamous vole species. Nonetheless, the overall picture of a stronger vocal response by the 2 monogamous species supports my hypothesis that the rate of infant vocalizing during isolation

is related to social structure in voles, with infants of monogamous species vocalizing more than infants of polygynous species when isolated.

Results suggest that pups from litters of 1 call less when isolated than pups from larger litters. A potential explanation for this could be that pups that have no littermates are less stressed by being isolated. Because this trend was seen in both pine and meadow voles, there is no indication that it was related to social system. Future studies could explore the effect of isolation on pups from litters of varying sizes.

It would be reasonable to ask whether the differences in vocalization rates are due to body mass, because adults of the 2 monogamous species are smaller (<40 g) than those of the 2 polygynous species (>40g). USVs are elicited by cooling, and pups vocalize more when exposed to lower cooling temperatures (Blake 1992; Okon 1971). Because small pups cool faster than large ones, one might predict that they vocalize at higher rates. Vocalizations also are related to respiration in that they are produced during exhalation (Roberts 1972), and respiratory rate is inversely related to body mass in mammals (Stahl 1967). If body mass alone determined vocalization rate, smaller pups would vocalize at higher rates than larger ones. However, examination of vocalizing rates and body mass of pups shows that this does not explain the difference between pine and meadow voles. During the peak calling period pups of the 2 species had almost the same body mass, but calling rate was 26% of expected respiratory rate for pine voles and only 5% of expected for meadow voles. A similar difference is seen between prairie and montane voles: peak calling rates are 29% of expected respiratory rate in prairie vole pups but only 3% in montane vole pups (calculated from mean calling rates and mean body mass of pups during peak periods [Blake 2002]). Thus, differences in body mass do not explain differences in calling rate between the monogamous and polygynous vole species.

The high rate of calling of prairie vole pups has been attributed to the high level of sociality in the species (Rabon et al. 2001; Shapiro and Insel 1990). Like prairie voles, pine voles have a high degree of sociality. Prairie and pine voles live in extended family groups, whereas meadow and montane voles tend to be solitary (FitzGerald and Madison 1983; Getz et al. 1993; Jannett 1980, 1982; Madison 1980a, 1980b; Webster and Brooks 1981). Laboratory studies also show that prairie and pine voles are prone to social contact with conspecifics; parents and young spend significantly more time sitting or lying in bodily contact with one another than do meadow and montane voles (Salo et al. 1993; Shapiro et al. 1989; Wilson 1982a, 1982b).

Several studies indicate that young prairie voles are more stressed than young of other species when isolated from parents and littermates. Most studies on USVs of infant rodents elicit calls by isolating the pup at cooling temperatures (≤25°C—Hahn and Lavooy 2005). Although sometimes referred to as isolation calls, vocalizations can be elicited by a number of stressors, including cooling. In studies on 5 muroid species, Okon (1970, 1971, 1972) found that pups

vocalized little or not at all when isolated at nest temperatures (31–35°C), which suggests that the USVs they emitted at ≤22°C were mostly in response to being cooled; Blumberg et al. (1992) found similar results—rat pups (*Rattus norvegicus*) do not vocalize when isolated without tactile stimulation at 35°C. Prairie voles (4–7 days old), however, do vocalize when isolated at nest temperature (34°C), calling at a mean rate of 12 calls/min. This is not significantly different from the rate for montane voles (2.2 calls/min) that were isolated and also cooled (at 22°C—Blake 2002). Unfortunately, similar studies have not been reported for pine voles.

Studies of stress hormones also support the conclusion that social isolation is stressful to prairie voles, because plasma corticosterone levels are higher in prairie voles isolated at 4–10 days than in montane voles (Shapiro and Insel 1990). Isolation alone, without cooling, therefore seems to be more stressful to prairie vole pups than to montane voles or other muroid species.

Calling patterns of young deermice also differ between species that have different social systems. The California deermouse (*Peromyscus californicus*) is considerably more social than many of its congeners, with a monogamous mating system and paternal care of the young (Ribble 1991; Wright and Brown 2002). Isolated California deermouse pups emit many USVs from ages 1 to 15 days (at a mean rate of ≥100 calls/min—Vieira and Brown 2002). By contrast, pups of the North American deermouse (*P. maniculatus*), a polygynous species, emit fewer USVs when isolated: they mostly emit audible calls in their first 4 days, and their peak of ultrasonic calling, in the middle of the 2nd week, is at a mean rate of 52 calls/min (Smith 1972).

Several aspects of social structure of monogamous voles may explain why pups of these species vocalize more than those of polygynous species when isolated. One aspect is that it can be risky for a pup that is outside the nest to vocalize extensively if it is not retrieved quickly. USVs of vole pups tend to be in the range 30–60 kHz (Colvin 1973; Mandelli and Sales 2004; Motomura et al. 2002), and small carnivores can hear frequencies in this range (Heffner and Heffner 1985a, 1985b; Powell and Zielinski 1989). Nearby predators, or even conspecific adult males, may be attracted to the calls and attack the pup. Pups of prairie and pine voles (monogamous species) are rarely left unattended. In addition to attentive parents, there are often older siblings living in the nest that help care for and retrieve young pups (Gruder-Adams and Getz 1985; McGuire and Novak 1984, 1986; Powell and Fried 1992; Wang and Novak 1992, 1994). Thus, family members are near and can respond quickly to retrieve a calling pup. In contrast, females of meadow and montane voles (polygynous species) are the only ones nesting with the pups and they leave pups alone for extended periods (Madison 1981; McGuire and Novak 1984, 1986; Wang and Novak 1992), so mothers often would not hear pups calling. Thus, the benefit (retrieval) of continued and extensive calling would be greater for prairie and pine vole pups and the risk (predation) less than for meadow and montane vole pups.

In addition to presence of both parents and older siblings in the nest with pups, there are a number of other traits that characterize monogamous species. Dewsbury (1981) devised a monogamy scale based on 9 characters in muroid rodents that vary with social system, including copulatory behaviors, pair-bonding, and joint parental care. Of 8 species of *Microtus*, prairie and pine voles scored the highest for monogamy and meadow and montane voles scored the lowest. McGuire et al. (2011) identified 11 characters including suckling behaviors, parental presence in the nest, and a number of pup behaviors and found a distinct difference between monogamous and polygynous vole species. Prairie voles exhibit all 11 of the characters and pine voles 10 of the 11, but montane voles show only 1 (and possibly 2 others) and meadow voles only 1. Another trait, not included in the characters examined by Dewsbury (1981) or McGuire et al. (2011) but doubtless related to them, is affiliative behavior. Monogamous vole species are significantly more prone to bodily contact than are polygynous species (Salo et al. 1993; Wilson 1982a, 1982b). Taken together, these studies show there is a large suite of characters that clearly distinguish the 2 monogamous vole species from the polygynous ones.

I conclude that the tendency for pups to emit ultrasonic vocalizations at a high rate when isolated is another character in this suite. As with the other reproductive and behavioral traits, high calling rates occur in 2 monogamous vole species, prairie and pine voles, but not in 2 polygynous species, montane and meadow voles. Future studies should reveal whether these differences in rates of isolation calling between monogamous and polygynous species are seen in other vole species as well as in other muroid groups.

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