

Female and male adult brush mice (*Peromyscus boylii*) use ultrasonic vocalizations in the wild

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Abstract:

We examined the individual context of ultrasonic vocalizations (USVs) produced by free-living wild male and female adult brush mice (*Peromyscus boylii*). We tested the hypothesis that USV production is dependent on behavioral context, and is important during both adult male and female interactions. Our methods included a 12-channel microphone array, radio-telemetry and thermal imaging that allowed us to determine: (1) who produced USVs, (2) characteristics of USVs, (3) type of USVs, (4) behavioral context of USVs and (5) the identity of the second mouse if an individual was not alone when a USV was produced. Females vocalized as much as males and produced the same types of USVs as males. There were no differences between spectral characteristics of male and female USVs. Females and males vocalized in the presence of one another. Importantly, when females were together they vocalized more than expected based on the proportion of time they spent together. Our results suggest that, in addition to facilitating courtship and mating, USVs are general territorial calls for neighbors because females vocalized in the presence of their neighbors. Despite a large literature on laboratory mouse (*Mus musculus*) USVs, studies are heavily biased towards males. Our results on brush mice, a species with a similar breeding system to the lab mouse and other rodents, suggest that female-female communication is an important and underappreciated component of the evolution and maintenance of mouse USVs.

Keywords: USV | wild | nocturnal | mouse | field

Article:

1. Introduction

Laboratory-based research on the laboratory mouse (*Mus musculus*) demonstrates that adult mice emit ultrasonic vocalizations (USVs) to both facilitate and inhibit social interaction (Portfors, 2007). However, debate exists on the adaptive function of adult USVs. One hypothesis suggests that adult males use USVs to facilitate courtship and mating (Holy & Guo, 2005) whereas an

alternative hypothesis suggests that USVs are general territorial calls directed at neighbors or intruders (Hammerschmidt et al., 2012). These two hypotheses are not mutually exclusive; rather they may be context dependent, whereby the use of USVs will depend on whether a male or female is interacting with a putative mate or territorial neighbor. In addition, these two hypotheses may be very difficult to tease apart using the standard 'resident-intruder' paradigm in the laboratory because it is an artificial construct that may have little relevance to the context in which USVs evolved. Wild mice of many species, including species of *Mus* (e.g., Wolff, 1985) and *Peromyscus* (Ribble & Stanley, 1998), establish territories that are adjacent to others' territories. Thus, individuals are not faced with intruders, but with neighbors, for the majority of their lives. As a result, it is important to examine the social context under which USVs are being produced in the wild.

The majority of laboratory research has focused on male laboratory mouse USV production in the context of male-female interactions. Very generally, male laboratory mice use USVs before copulation and after ejaculation (Whitney et al., 1973; Nyby et al., 1981), to attract females (Hammerschmidt et al., 2009; Musolf et al., 2010), to retain females in close proximity (Pomerantz et al., 1983; Hammerschmidt et al., 2009), to convey social status (Nyby et al., 1976), to reduce female aggression (Costantini & D' Amato, 2006), and as an index of social recognition (Musolf et al., 2010). Male laboratory mice emit different types of USVs, produce more complex syllables than females (Gourbal et al., 2004; Holy & Guo, 2005), and produce USVs when presented with novel females (Musolf et al., 2010). Individual males have different USVs (Holy & Guo, 2005) that may influence female mate choice (Musolf et al., 2010). However, in response to female intruders as opposed to mates, males produce syllables that are similar to those of females responding to female intruders (Hammerschmidt et al., 2012), suggesting that context (i.e., the vocalization is directed toward an intruder vs a potential mate) is important for the type of USV produced.

Less is known about vocalizations during female-female interactions. In the laboratory mouse, female USVs are associated with social contexts involving non-mate mice. Resident female laboratory mice produce USVs in the presence of female intruders (Maggio & Whitney, 1985; Hammerschmidt et al., 2012), serving an affiliative function for the establishment of a dominance hierarchy (Maggio & Whitney, 1985; Moles et al., 2007). Female laboratory mice are responsive to intruders of both sexes, suggesting that female-female interactions have been important for the evolution of USVs (Hammerschmidt et al., 2012).

Here, we report on the individual context of USVs produced by free-living adult male and female brush mice (*Peromyscus boylii*) in the wild. We used the brush mouse as a model because it produces USVs in the wild (Kalcounis-Riippell et al., 2006) and has a life history and mating behaviors similar to the majority of rodents, including *M. musculus* (Wolff, 1985), ranging from polygynous to promiscuous (Ribble & Stanley, 1998; Kalcounis-Rueppell & Spoon, 2009). We tested the hypothesis that USV production is dependent on the behavioral context during which a USV was produced, and is important during both adult male and female interactions. The behavioral contexts we examined were whether a mouse was alone or not alone during the production of a USV, and when not alone, whether a mouse produced the USV in the presence of a male or a female. We also determined whether particular USV motifs were associated with

males or females, and behavioral context. Lastly, we determined if there were spectral and temporal differences between USVs produced by males and females.

2. Materials and methods

Fieldwork was conducted at the Hastings Natural History Reservation (HNHR), in Monterey County, CA, USA, from February-June 2008 and in January 2009. We used remote sensing techniques, as fully described in Briggs & Kalcounis-Rueppell (2011), to record USVs from individuals. Our methods included a 12-microphone-array set in brush mouse habitat (an approx. 10 x 10 m area termed focal area (FA)), individual brush mice radio-tagged in the FA, and 4 radio-antennae to receive radio-signals from those radio-tagged individuals. We mounted a thermal camera above the FA to visualize the mice in the focal area as they interacted and as they vocalized. Using our methods we were able to determine in free living individuals: (1) who produced USVs, (2) spectral and temporal characteristics of USVs, (3) type of USVs produced, (4) behavioral context of USVs produced and (5) the identity of the second mouse if an individual was not alone when producing a USV.

2.1. Determining resident individuals

Live trapping was conducted to determine the residency of individual mice. Trap stations were approximately 10 m apart, and at each trap station there were three live traps: one Longworth (Rogers Manufacturing, Peachland, BC, Canada; box 14 x 16.5 cm; tunnel 4.5 x 4.5 cm) and two Sherman (AB Sherman Traps, Tallahassee, FL, USA; 7.6 x 8.9 x 23.3 cm) traps. Traps were baited with a rolled oat and sunflower seed mixture, and provided with a small piece of cotton. Trapping sessions were conducted throughout the field season. Traps were opened one and a half hours before sunset, and checked and deactivated three hours before sunrise. Traps remained on the grid throughout the field season. Upon capture, the species, sex, age and reproductive condition (scrotal or abdominal testis for males; pregnant, lactating or perforate vagina for females) of each mouse were assessed. Newly captured mice were tagged with uniquely numbered metal ear tags (Monel 1005 numeric, National Band and Tag, Newport, KY, USA).

We determined home ranges of resident mice using Animal Movement software in ArcView 3.2 (ESRI, Redlands, CA, USA). While in the field, we generated 50% contours of the fixed-kernel home range estimator with a smoothing factor of 5. Area within the 50% contour represented the core area of the home range. We defined resident individuals as those individuals captured more than 3 times over the trapping season within a 30 m buffer of a single trap station. We further defined residents of an FA as individuals whose 50% core home range was encompassed by the FA. All other resident mice whose core home ranges were not encompassed by the FA, but were captured around the FAs, were considered neighbors of residents.

2.2. Remote sensing at focal areas

Remote sensing equipment was deployed at each FA for about 14 days, and then the equipment was moved to a new FA location. The FA was selected based on the number of resident *P. boylii* in that area and the feasibility of assembling the remote sensing equipment (microphone array, telemetry system, thermal camera). We looked for FAs with approximately 4 resident

individuals. Prior to setting-up the remote sensing equipment, live trapping of the FA (with approximately 15 extra traps) and a buffer of three trap stations in every direction around the FA was conducted for three consecutive nights to ensure all known resident mice were captured. Upon capture, FA residents and their neighbors were each outfitted with a 0.55 g M1450 mouse-style transmitter (ATS, Isanti, MN, USA). After FA mice were outfitted with transmitters, the remote sensing equipment began recording. While remote sensing equipment was recording, there was no trapping in the FA or in the surrounding 30 m.

Assigning USVs to individual mice is fully described, with examples, in Briggs & Kalcounis-Rueppell (2011). Briefly, clearly recorded sound files of mouse USVs were analyzed using Avisoft SASLab Pro (Avisoft Bioacoustics, Berlin, Germany). Spectrographs were generated using a fast Fourier transform (FFt) length of 512, and 100% frame size with Hamming window. Window overlap was 50%. Frequency range was 5-125 kHz with a frequency resolution of 488 Hz and a temporal resolution of 1.024 ms. Sound files with mouse USVs were examined at 12 channels of the microphone array to determine the order at which the sound arrived at each of the microphones. Using a map of the focal area in which the USV was recorded and the time delay of arrival at each microphone, we determined the approximate location at which the USV was emitted. To assign a USV to a mouse, we examined telemetry data for one minute before and one minute after the USV was produced. We determined if any of the mouse locations detected by transmitters matched USV locations detected by the microphone array. If a transmitter location matched the location of the USV, the USV was assigned to the mouse carrying the transmitter. If the mouse produced a vocalization in the presence of another mouse, we used the telemetry data to determine the identity of the second mouse. Identification of the second mouse was only possible if the second mouse was wearing a working transmitter (i.e., if the second mouse was a resident of the focal area). When two mice were present, we were able to determine who produced the USV based on the position of the USV from time delay of arrival of the USV to the microphone array, and the position of the two mice based on the telemetry data.

For each USV that could be assigned to an individual, we analyzed spectral and temporal characteristics of the USV using Avisoft SASLab Pro. For the spectral and temporal analysis of USVs, we selected from the 12 channels the recording with a clear waveform and high amplitude. We measured parameters described in Table 1 for each syllable of recorded USVs. Motif types were described by the number of syllables they contained, and by the time between syllables. In general, *Peromyscus* in the wild produce USVs as single- or multi-syllable vocalizations with less than 500 ms between syllables in multi-syllable vocalizations. The single- or multi-syllable vocalization can be classified as a particular type, or motif. Individual wild *Peromyscus* rarely produce more than one USV at a time. Moreover, individual wild *Peromyscus* do not normally duet USVs, so it is rare that two individuals would produce USV s at the same time, or even within the span of 1 min. Thus, it is relatively easy to ascribe USVs to individuals because USVs are not produced in rapid succession, and they are only produced by one individual, even when more than one individual is present. Description and identification of wild *Peromyscus* USV motifs are available in Kalcounis-Rueppell et al. (2006, 2010) and Briggs & Kalcounis-Rueppell (2011).

Table 1. Spectral and temporal variables measured for each USV and used in statistical comparisons between male and female *Peromyscus boylii*.

Variable	Description	Units
gr duration	duration of entire phrase (identical to duration for a ISV)	s
duration	duration of syllable	s
disttomax	duration from start to peak freq (max)	s
start time	syllable start time	s
end time	syllable end time	s
peak freq (start)	*frequency at start of syllable	Hz
peak freq (end)	*frequency at end of syllable	Hz
peak freq (max)	*frequency at maximum amplitude of syllable	Hz
peak freq (minentire)	*minimum frequency of syllable	Hz
peak freq (tminentire)	time of minimum frequency of syllable	s
peak freq (maxentire)	*maximum frequency of syllable	Hz
peak freq (tmaxentire)	time of maximum frequency of syllable	s
Bandwidth	peak freq (maxentire) – peak freq (minentire)	Hz
Modulation	bandwidth/duration	Hz/s
Comp 1	first principal component axis reducing the five original (*) frequency variables	n/a

All original variables were extracted from recordings using Avison SASLab Pro (Berlin, Germany). 'Comp 1' is the first principal component axis derived from all original frequency variables on all syllables included in the Supplementary Material in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/1568539x>. 'Comp 1' explained 88.4% of variation in the data set with loadings as follows: peak freq (start) = -0.421, peak freq (end) = -0.446, peak freq (max) = -0.457, peak freq (maxentire) = -0.458, peak freq (minentire) = -0.454.

Our recordings and video data were examined in two ways to determine behavioral context of USV production by *P. boylii* (in addition to using the camera for remote sensing to assign USVs to individual mice; see above and Briggs & Kalcounis-Rueppell (2011)). First, we examined the minute surrounding the high quality USVs (1 Minute Video Analysis (IMVA)) that we also used for our motif and spectral comparisons. USVs in this analysis were selected based on methods fully described in Briggs & Kalcounis-Rueppell (2011) and include only high quality USVs that were unanimously assigned to individuals. The minute flanking the USV was examined, and behavioral context was determined. The behavioral context of each USV was classified as the vocalizing mouse being alone or not alone (i.e., 'female alone' vs 'female not alone', and 'male alone' vs 'male not alone'). For those mice that were not alone when vocalizing, the behavioral context was further classified as vocalizing mouse being with a male or a female (i.e., 'female with female', 'male with male', 'female with male', and 'male with female'). Further classification was only possible when the identity of the second mouse could be determined, because that was the only way to know if the second mouse was a male or a female. Where a second mouse was present, radio-telemetry data were used to determine the identity of the second mouse if the second mouse was wearing a transmitter. Trapping records were used to determine the phenology of the mouse. For lactating females, we determined the age of her litter by backdating parturition dates from trapping records. For lactating females that had pups in the nest, we did not consider their pups when defining their behavioral context because they were vocalizing in the FA, outside of their nest. Thus, a female that had pups and vocalized in the FA with no other mouse present was classified as 'female alone' because there was no other adult mouse present in the FA and she was physically separated from her pups during the time of vocalization.

Second, we examined 1-h segments of video to quantify the proportion of time that individuals spent alone or not alone, and when not alone, how much time in the presence of a male or a

female. This allowed us to determine when USVs were produced in relation to particular behavioral contexts (1-h video analysis (IHVA)). For the IHVA, we sampled one-hour of video from each of two days from each of 9 focal areas (total of 18 1-h video segments). Selection of IHVA segments was random but restricted to days (1) for which we were sure that all resident mice had working transmitters and (2) that had more than 1 USV recorded. We watched each video and recorded each enter time and exit time for mice. During the interval between entry and exit, we coded whether the mouse was alone or not alone. Total on-camera time for each context was calculated by adding all interval times, rounded to the nearest minute. Telemetry data (as above and Briggs & Kalcounis-Rueppell (2011)) were used to determine the identity of individual mice viewed during an interval, and acoustic data were used to determine if any USVs were produced during the time on camera. As above, mice identified in the IHVA were characterized based on phenology from trapping records.

Thus, the IMVA described the behavioral context during high quality recordings in the field, whereas the IHVA provided information about which behavioral contexts were common in the field, and how USV production was associated with the behavioral contexts. Not all USVs from the IHVA are represented by USVs in the IMVA because there were USVs detected in the IHVA that did not meet the strict criteria of inclusion for the initial analysis (i.e., high quality recording of the USV for spectral analysis).

2.3. Statistical analysis

We used chi-square and contingency tests to examine observed distributions of USVs, and time spent, in particular behavioral contexts. We analyzed behavioral context data using both USVs and individuals as our unit of replication. Although analysis at the individual level was preferred because there was no pseudoreplication, the nature of our data was such that 'per individual' sample sizes precluded statistical analyses, in some cases. However, where we analyze the number of USVs, we always present the number of individuals that produced the USVs to show that USVs were not recorded from a single individual. To determine if males and females differed in spectral and temporal characters, we compared each syllable of each motif type, with individuals as the unit of replication, using Wilcoxon Rank Sum tests in R Commander (Fox, 2005). We applied a conservative rejection criterion of 0.005 because of our relatively small number of individuals and multiple tests. We conducted all other statistical analyses using R (R Development Core Team, 2008), with a rejection criterion of 0.05 for all tests.

3. Results

Data were collected on 131 nights at 11 FAs (13.6 ± 4.8 nights/FA; mean \pm 1 SD). We attached 36 transmitters to 33 individual adults (21 females, 12 males; 3.3 ± 1.9 individuals/FA). Three mice received transmitters in > 1 FA. We recorded 198 high quality USVs, of which 170 USVs (Figure 1) were assigned to 24 individual brush mice with unanimity (as in Briggs & Kalcounis-Rueppell, 2011). A single female made 8 'bark' calls, and the remaining 162 USVs were single syllable and multi-syllable vocalizations (SVs). The remainder of the results focus on the 162 SVs. Nine scrotal males produced 86 USVs with 9.6 ± 10.6 USVs/male. Fifteen reproductively active females produced 76 USVs with 5.1 ± 4.2 USVs/female ($t_{9.5} = -1.21$, $p = 0.25$; all data are in the Supplementary Material that can be found in the online edition of this journal, which can

be accessed via <http://booksandjournals.brillonline.com/content/1568539x>). We were able to ascribe behavioral context to a subset of the total USV s produced by males and females as described in Figure 2.

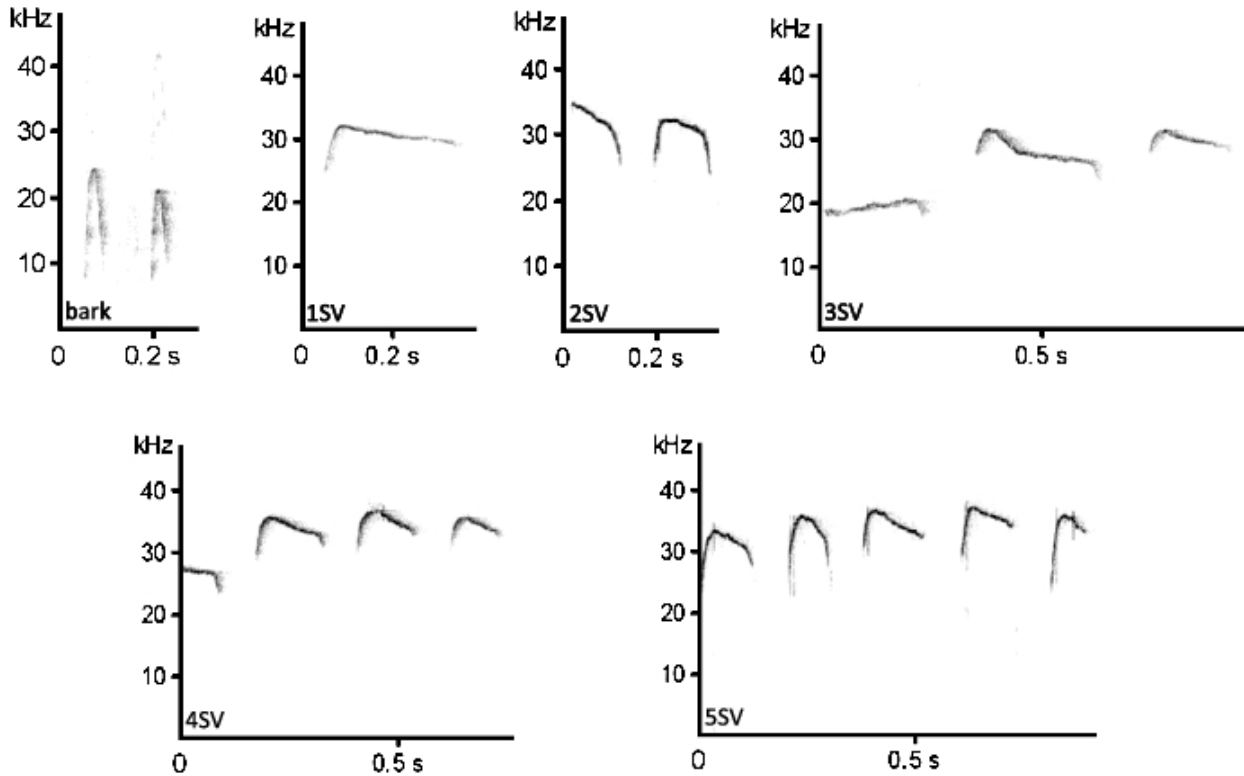


Figure 1. Spectrographs of examples of the 6 ultrasonic vocalization (USV) motifs produced by brush mice (*Peromyscus boylii*) recorded at the HNHR. Spectrograph parameters: FFT length 512, 100% frame size with Hamming window, 50% overlap. Sample sizes for individuals are: 1SV, 6 females, 6 males; 2SV, 12 females, 7 males; 3SV, 9 females, 6 males; 4SV, 4 females, 2 males; 5SV, 1 female, 1 male. Sample sizes for USVs are: 1SV, 16 female, 24 male; 2SV, 37 female, 34 male; 3SV, 18 female, 18 male; 4SV, 4 female, 9 male; 5SV, 1 female, 1 male.

Whether a mouse was alone or not alone while producing a USV during the IMVA could be determined for 52 of 86 male produced, and 58 of 76 female produced, USVs. Males produced more USVs when not alone (16 USVs when alone vs 36 USVs when not alone: $\chi^2 = 7.69$, $df = 1$, $p = 0.006$; Figure 3a). However, there was no significant difference in the proportion of individual males who produced USV s when alone or not alone (5 individuals when alone vs 6 individuals when not alone, out of 8 individual males that produced USVs where the alone vs not alone behavioral context could be determined: $\chi^2 = 0.09$, $df = 1$, $p = 0.76$; Figure 2). When males were not alone, USVs were produced in the presence of a female more often than in the presence of a male (4 USVs in the presence of a male vs 14 USVs in the presence of a female, out of 18 male-produced USVs total where the second mouse could be identified: $\chi^2 = 5.56$, $df = 1$, $p = 0.018$; Figure 3a). For 11 of the 14 male USV s produced in the presence of a female, the female was in postpartum estrus. Two individual males vocalized in the presence of a male, whereas 3 individual males vocalized in the presence of a female, out of 3 total individual males who produced USV s where the second mouse could be identified (Figure 2).

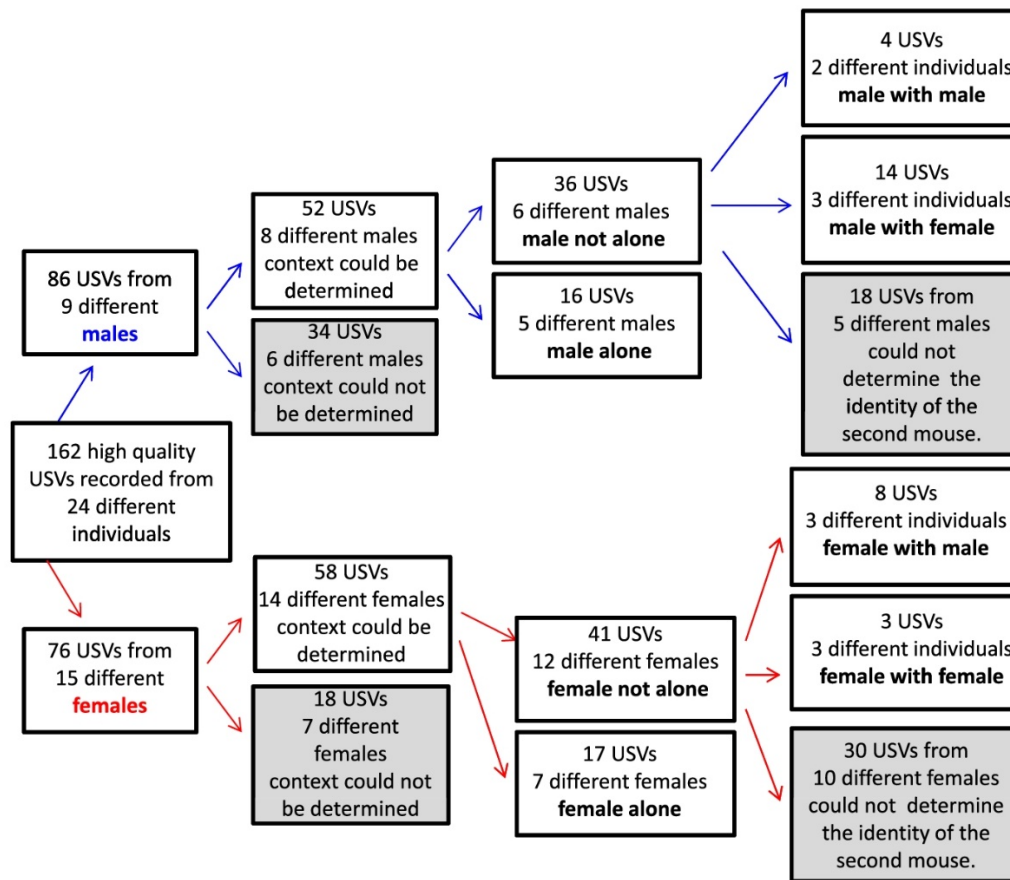


Figure 2. Flow chart showing the sample breakdown of ultrasonic vocalization (USV) and individuals that produced USVs from the high quality calls that were included in the 1 Minute Video Analysis (IMVA). Male produced USVs are on top and follow blue arrows. Female produced USVs are on bottom and follow red arrows. In each box both number of USVs and different individuals producing USVs are reported. Grey boxes indicate samples that were dropped from the IMVA because behavioral context could not be determined. Bold text indicates category of behavioral context for analyses. AU video and USVs recorded at the HNHR. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.briUonline.com/content/1568539x>.

As with males, females from the IMVA analysis produced more USVs when not alone (17 USVs when alone vs 41 USVs when not alone; $\chi^2 = 9.93$, $df = 1$, $p = 0.002$; Figure 3a). However, there was no significant difference in the proportion of individual females that produced USVs while alone or not alone (7 individuals when alone vs 12 individuals when not alone, out of 14 individual females that produced USVs where alone vs not alone context could be determined: $\chi^2 = 0.32$, $df = 1$, $p = 0.25$; Figure 2). When females were not alone, they vocalized in the presence of both sexes (3 USVs between females vs 8 USVs between a female and a male, of 11 female-produced USVs where the second mouse could be identified; $\chi^2 = 2.27$, $df = 1$, $p = 0.13$; Figure 3a). The same number of individual females produced USVs in the presence of each sex (3 individual females vocalized in the presence of a female and 3 individual females vocalized in the presence of a male, out of 6 total individual females where the second mouse could be identified; Figure 2). The 8 USVs produced by females in the presence of a male were made by 3 different reproductively active females who each had pups in the nest. The 3 USVs produced by females in the presence of a female were made by 3 different females to their neighbor, as determined from the home range mapping.

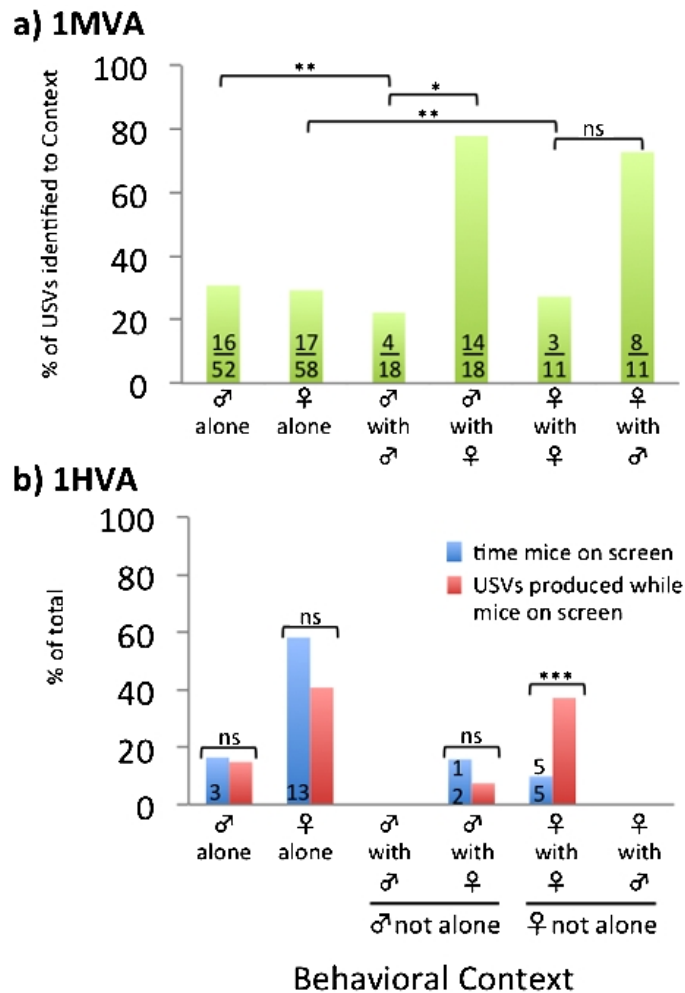


Figure 3. (a) 1 Minute Video Analysis (1MVA). Proportion of high quality USVs (total recorded= 162) from males (total= 86) and females (total= 76) where behavioral context could be determined (sample size of USVs for each category shown in bar over sample size from which it is drawn - see Figure 2). For individuals represented by each bar see Figure 2. (b) 1 Hour Video Analysis (1HVA). Proportion of total time on screen for each behavioral context (total time mice on screen = 3.39 h) and USVs (total = 27) produced during 18 h of IHVA. Numbers in, or on top of, bars represent number of individual mice represented by the bar (number of vocalizer(s) on top when vocalizer is not alone). Asterisks indicate significance in chi-square comparisons between bars indicated (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). All video and USVs recorded at the HNHR. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/1568539x>.

Of the 18 h of video examined in the IHVA, mice were on screen for 3.39 h. During the 3.39 h of video, 27 USVs were produced. Percentage of time spent in each of the 6 behavioral contexts was not equally distributed across all 6 behavioral contexts ($\chi^2 = 138.56$, $df = 5$, $p < 0.0001$; Figure 3b). The most common behavioral context from the IHVA was 'female alone', representing over 50% of the total time we viewed mice in the FA (Figure 3b). Individual mice we observed in the IHVA analysis were more likely to be females in the 'female alone' category than any other behavioral context ($\chi^2 = 33.63$, $df = 5$, $p < 0.0001$; Figure 3b). The majority of USVs were produced by females when alone or with other females ($\chi^2 = 26.56$, $df = 5$, $p < 0.0001$; Figure 3b). The distribution of time spent in a particular behavioral context differed from the distribution of USVs produced in a particular behavioral context ($\chi^2 = 21.95$, $df = 3$, $p < 0.0001$; Figure 3b). There were more USVs produced, than expected based on the time that mice

spent in particular behavioral contexts, when two females were together ('female to female': $\chi^2 = 15.81$, $df = 1$, $p < 0.0001$) but not when a male was alone ($\chi^2 = 0.08$, $df = 1$, $p = 0.08$), a female was alone ($\chi^2 = 3.06$, $df = 1$, $p = 0.08$) or a male was vocalizing to a female ($\chi^2 = 2.99$, $df = 1$, $p = 0.08$; Figure 3b).

The most common motif type produced was 2SVs (Figure 4). Motif type was not dependent on whether a mouse was 'alone' or 'not alone' for either males ($\chi^2 = 3.21$, $df = 4$, $p = 0.52$) or females ($\chi^2 = 3.26$, $df = 4$, $p = 0.51$; Figure 4). When considering motifs within the 'not alone' classifications, sample sizes become too small for statistical analyses, with some motifs not being represented at all (Figure 4).

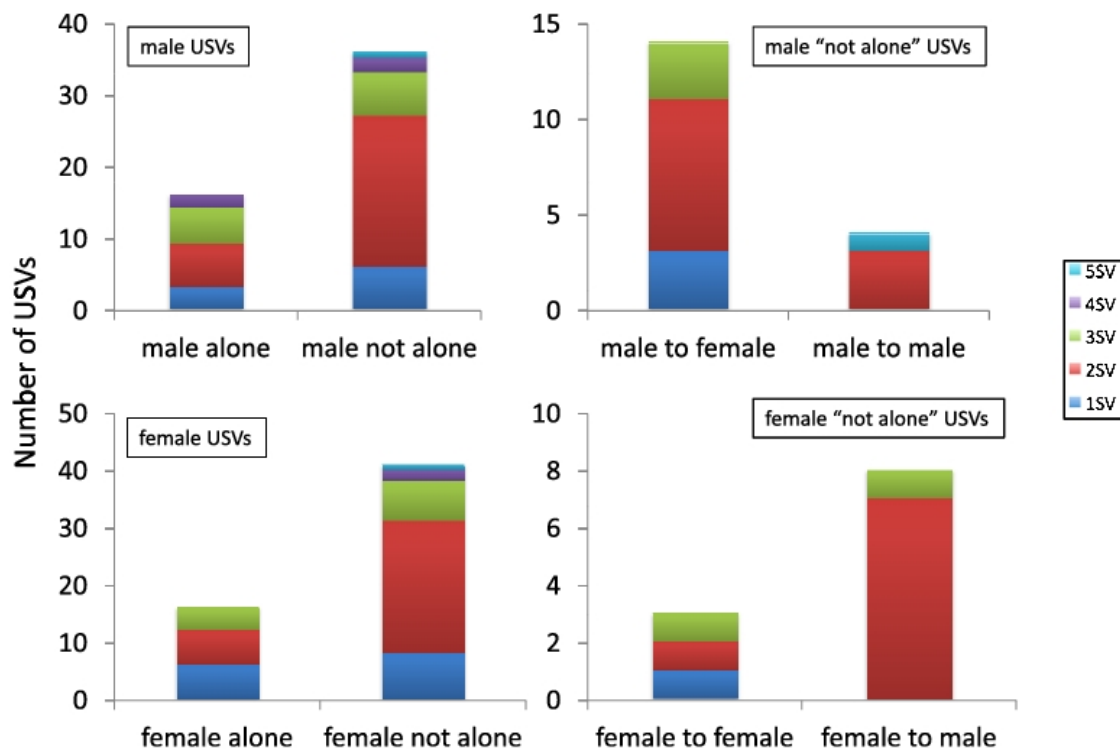


Figure 4. Distribution of motif types across behavioral contexts. Distributions are shown for male and female USVs that were produced when 'alone' vs 'not alone' in the left panels. Distributions for male and female 'not alone' USVs produced in the presence of a male or a female are shown in the right panels. Sample sizes for male and female USVs are as follows (1SV, 2SV, 3SV, 4SV, 5SV): 'male alone', 3, 6, 5, 2, 0; 'male not alone', 6, 21, 6, 2, 1; 'female alone', 6, 7, 4, 0, 0; 'female not alone', 8, 23, 7, 2, 1. Sample sizes for male and female 'not alone' USVs are as follows in the order shown in the legend: 'male with female', 3, 8, 3, 0, 0; 'male with male', 0, 3, 0, 0, 0; 'female with female', 1, 1, 1, 0, 0; 'female with male', 0, 7, 1, 0, 0. All USVs recorded from brush mice (*Peromyscus boylii*) at the HNHR. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/1568539x>.

Motif type was not dependent on sex when considering total USVs produced in each motif type ($\chi^2 = 3.04$, $df = 4$, $p = 0.551$; sample sizes presented in the legend to Figure 1). In addition, motif type was not dependent on sex when considering total individuals that produced at least one of a particular motif type ($\chi^2 = 0.76$, $df = 4$, $p = 0.94$; sample sizes presented in the legend to Figure 1). There were no differences in spectral and temporal characteristics of any syllables of any USV motifs between males and females (Table 2).

Table 2. Comparison of spectral and temporal variables of ISV (syllable vocalizations), 2SV, 3SV and 4SV motifs among female and male *Peromyscus boylii*.

Acoustic variable	Median		Wilcoxon	
	♀	♂	<i>W</i>	<i>p</i>
1SV				
<i>N</i>	6	6		
Bandwidth	5325.00	4108.33	28.0	0.132
Comp 1	-0.20	-0.53	17.0	0.937
Disttomax	0.05	0.04	27.0	0.183
Duration	0.19	0.13	28.0	0.132
Modulation	30587.29	26846.89	21.0	0.699
2SV				
<i>N</i>	12	7		
Syllable 1				
Bandwidth	6166.67	4566.67	54.0	0.331
Comp 1	0.56	-0.15	53.0	0.385
Disttomax	0.03	0.03	40.0	0.899
Duration	0.15	0.16	31.0	0.385
Modulation	31314.64	33356.76	34.0	0.536
Syllable 2				
Bandwidth	6366.67	4337.50	66.0	0.047
Comp 1	-0.38	-0.73	52.0	0.432
Disttomax	0.04	0.05	34.5	0.554
Duration	0.14	0.16	38.0	0.773
Modulation	46394.55	30475.00	55.0	0.229
3SV				
<i>N</i>	9	6		
Syllable 1				
Bandwidth	3400.00	4435.00	27.5	1.000
Comp 1	1.85	3.51	17.0	0.272
Disttomax	0.04	0.04	25.0	0.864
Duration	0.10	0.13	19.0	0.388
Modulation	24527.83	37781.34	11.0	0.066
Syllable 2				
Bandwidth	6333.33	6060.00	32.0	0.685
Comp 1	0.93	0.43	32.0	0.694
Disttomax	0.05	0.05	32.0	0.694
Duration	0.16	0.16	30.0	0.867
Modulation	37511.86	34026.95	31.0	0.779
Syllable 3				
Bandwidth	4400.00	3450.00	40.5	0.125
Comp 1	0.29	-0.32	30.0	0.776
Disttomax	0.24	0.25	28.0	0.955
Duration	0.13	0.13	24.0	0.776
Modulation	42391.30	37526.88	33.0	0.529
4SV				
<i>N</i>	4	2		
Syllable 1				
Bandwidth	5800.00	4606.25	5.5	0.639
Comp 1	0.06	1.06	4.0	1.000
Disttomax	0.03	0.04	2.0	0.533
Duration	0.12	0.14	3.0	0.800
Modulation	33834.26	28346.35	4.0	1.000
Syllable 2				
Bandwidth	5800.00	6300.00	3.0	0.700
Comp 1	-2.39	-0.01	2.0	0.400
Disttomax	0.02	0.05	3.0	0.700
Duration	0.15	0.15	5.0	1.000
Modulation	39189.19	47891.80	2.0	0.400
Syllable 3				
Bandwidth	5400.00	5550.00	3.0	0.700
Comp 1	-1.07	-0.45	1.0	0.200
Disttomax	0.24	0.23	7.0	0.400
Duration	0.12	0.15	2.0	0.376
Modulation	43902.44	47804.90	4.0	1.000
Syllable 4				
Bandwidth	5400.00	6287.50	4.0	1.000
Comp 1	-1.24	-0.38	3.0	0.800
Disttomax	0.22	0.25	3.0	0.800
Duration	0.11	0.10	5.0	0.800
Modulation	60549.60	66065.72	4.0	1.000

The Wilcoxon Rank Sum test is significant at $p < 0.005$. Sample size refers to number of individuals. All comparisons were also made with parametric two sample tests and the results do not change. Vocalizations were recorded from a wild population of *P. boylii* at Hastings Natural History Reserve (Carmel Valley, CA, USA), from February-June 2008 and January 2009. Duration and Disttomax are in ms, Bandwidth is in Hz and Modulation is in Hz/s.

4. Discussion

This is the only study to examine the behavioral context of adult USV production by a polygynous/promiscuous mouse species in the wild. We demonstrated that males and females both vocalized to the same extent in the presence of other mice and that there were no differences, either at the motif level or the spectral and temporal characteristics of USV level, between male and female vocalizations. Importantly, we found that adult females, in the wild, spent most of their time alone. However, on the rare occasion when they encountered another female, they vocalized more than expected based on the time they spent in that behavioral context (i.e., 'female with female'). When females vocalized with other females, they vocalized with their resident neighbors. Taken together, our results suggest that, in addition to facilitating courtship and mating (e.g., Whitney et al., 1973; Nyby et al., 1976, 1981; Pomerantz et al., 1983; Hammerschmidt et al., 2009; Musolf et al., 2010) between males and females, USVs are general territorial calls for neighbors, especially female-female neighbors. Thus, USVs are context dependent whereby the use of USVs, for example by males or females, depends on whether he/she is interacting with a putative mate or territorial neighbor. Because we found that males and females produced the same USV motifs and motifs did not differ in USV spectral characteristics, our results suggest that USVs are not communicating gender in wild brush mice.

In the wild, male brush mice vocalized alone and in the presence of an estrous female, consistent with USVs facilitating mate attraction and mating. As with *M. musculus* in the laboratory, USVs by lone brush mouse males in the wild may inform the resident females, who are in nearby, or overlapping, territories, of male presence and sexual arousal (Hammerschmidt et al., 2009; Musolf et al., 2010). When produced in the presence of a resident female, USVs may serve to coordinate reproductive behavior (Costantini & D' Amato, 2006), reduce female aggression (White et al., 1998; Costantini & D' Amato, 2006), and retain the female in close proximity to facilitate copulation (Pomerantz et al., 1983; Hammerschmidt et al., 2009).

Brush mice in the wild vocalized in the presence of another scrotal male based on our IMVA. However, this behavioral context was rare, as we never observed two males together in our IHVA. Although the results from our IMVA and IHVA appear contradictory, the discrepancy is an artifact of our two approaches to examining behavioral context. In the wild, interactions between males should be rare because each resident male has a relatively large territory that is exclusive of other males, but overlaps multiple females (Ribble & Stanley, 1998; Kalcounis-Rueppell & Spoon, 2009). Interactions between males would not likely occur in the core of a male's territory, but at the edge. We set up our FAs to maximize the number of resident brush mice that we could observe, and to do this we focused on the core of the territory. The scale of our FAs allowed us to capture interactions between neighboring females in the IHVA that have small territories relative to males, but not neighboring males. Regardless, we captured in our IMVA, males vocalizing in the presence of males. In *M. musculus* in the laboratory, when males vocalize in the presence of another male, the USVs convey the male's presence and social status to the second male (Nyby et al., 1976). Thus, on the rare occasion when brush mouse males come into contact with one another, USVs likely indicate presence and communicate social (i.e., territory holding neighbor) status.

We found that female brush mice vocalized in the presence of their female neighbors. This result supports the hypothesis that females vocalize to mediate social interactions with other females (Maggio & Whitney, 1985; Moles et al., 2007). More importantly, females vocalized more often than expected based on the proportion of time they spent together. It is rare to find two females together in a FA because female brush mice, like females of other rodent species, are territorial (Ribble & Stanley, 1998; Kalcounis-Rueppell & Spoon, 2009). However, our data show that when they do come together, they are likely to vocalize. Of all four 'not alone' behavioral contexts (i.e., 'female with female', 'male with male', 'female with male', and 'male with female'), only the 'female with female' behavioral context experienced USVs out of proportion with the time that mice spent in particular behavioral contexts. This result strongly suggests that female to female vocalizations may be as important as male to female vocalizations in the evolution and maintenance of USVs. As with *M. musculus*, it is likely that females produce USVs during encounters with other females to affiliate and establish the dominance hierarchy (Maggio & Whitney, 1985; Moles et al., 2007), a function that would be very important given the likelihood of encountering a territorial female neighbor and the importance of territory maintenance in the wild. Regardless, our results demonstrate that USVs are important for communication between neighboring females, as opposed to being a chiefly male trait (White et al., 1998).

Females also vocalized in the presence of a male and, in all cases (except for one that we could not determine) had pups. Moreover, females produced vocalizations when alone in the FA, and in all cases vocalizing females had pups in the nest. Female *M. musculus* vocalize when they have pups emerging from the nest (Costantini & D' Amato, 2006), and USVs can impart information about motivational state (Moles & D' Amato, 2000). Thus, we speculate that USVs produced by lone brush mouse females may serve as warning signals for pups emerging from the nest, and predict that warning signals may be mediated by pup age. Alternatively, USVs produced by lone females could be unrelated to pups and serve some other communication function. Without careful experiments, these alternatives will be difficult to test because brush mice have a postpartum estrus (Kalcounis-Rueppell & Spoon, 2009) and most females have pups in the nest throughout the breeding season.

Our results show that, despite a large literature on mouse USVs that is heavily biased towards males (e.g., Whitney et al., 1973; Nyby et al., 1976, 1981; White et al., 1998; Holy & Guo, 2005), female-female communication is an important component of the evolution and maintenance of rodent USVs (see Maggio & Whitney, 1985; Moles et al., 2007). We show that in the wild, female brush mice vocalized as much as males and females produced the same types of vocalizations as males. Importantly, female brush mice vocalized more than would be expected in the presence of other females based on the proportion of time they spent together. Our results suggest that, in addition to facilitating courtship and mating, USVs are general territorial calls for female-female neighbors. However, our data are limited to descriptions of behavioral contexts and cannot be used to test the adaptive functions of USVs. Field experiments, for example with removals, introductions, or playbacks, would be necessary to test hypotheses about function. Our results underscore the need to include female function within those tests.

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