Similar Acoustic Structure and Behavioural Context of Vocalizations Produced by Male and Female California Mice in the Wild

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Abstract:

Ultrasonic vocalizations (USV) are an important part of multimodal communication in mice; however, nothing is known about the behavioural context of USV production by individual mice in the wild. Using remote-sensing methods we recorded USVs from individual adult free-living *Peromyscus californicus*. Because adult male and female *P. californicus* share duties in rearing offspring and defending territories, we predicted that male and female *P. californicus* would produce USVs in similar behavioural contexts and with similar spectral and temporal characteristics. We found that adult male and female *P. californicus* produced USVs, with the most common motifs being one-, two- and three-syllable vocalizations. USVs of males and females did not differ significantly in type or number, or in spectral or temporal characteristics. *Peromyscus californicus* produced USVs when alone and when they were with another mouse, and the three-syllable vocalization (3SV) motif, which has a relatively long first syllable, was more likely to be produced in the presence of another mouse than when a mouse was alone. The likelihood of vocalizing and the spectral and temporal characteristics of vocalizations did not differ when an individual was producing a USV in the presence of a mate or nonmate. Males and females produced USVs in the same behavioural contexts. Thus, as with other behaviours associated with parenting and territorial defence in *P. californicus*, USVs of males and females are produced in similar behavioural contexts and have similar spectral and temporal characteristics.

Keywords: California mouse | microphone array | multimodal communication | noctural parental care | Peromyscus californicus | telemetry | territorial defence | thermal video | ultrasonic vocalization | wild

Article:

Rodents are the most speciose and behaviourally diverse group of mammals (Kay & Hoekstra 2008) and have a great potential as a comparative model for studying acoustic communication, in addition to birds and anurans. In particular, they provide study systems of acoustic communication as a secondary modality to olfaction. Ultrasonic vocalizations (USVs) are an important component of multimodal communication in rodents (Sales, 1999, Costantini and D'Amato, 2006, Brudzynski, 2007, Portfors, 2007, Scattoni et al., 2009 and Takahashi et al., 2010). However, what we know about the context and function of adult rodent USVs is limited and based on evidence mainly from laboratory mice (*Mus musculus*-derived strains) and rats (*Rattus norvegicus*-derived strains) in the context of mating (but see Pasch et al. 2011). Evidence suggests that USVs indicate an individual's affective state, rank or status and/or facilitate social interactions, including reproduction. Laboratory rats produce USVs to establish dominant-subordinate relationships (Inagaki et al. 2005), convey an individual's affective state and coordinate reproductive behaviour (Brudzynski, 2007 and Portfors, 2007). Laboratory mice produce USVs to coordinate reproductive behaviour and reduce aggression (Sales, 1972 and Costantini and D'Amato, 2006), attract mates (Hammerschmidt et al., 2009 and Musolf et al., 2010), retain conspecifics in close proximity (Pomerantz et al., 1983 and Hammerschmidt et al., 2009), convey social status (Nyby et al. 1976) and facilitate social recognition (D'Amato, 1997, D'Amato and Moles, 2001, Moles et al., 2007 and Musolf et al., 2010). Work with laboratory mice suggests that there is individual variation in USVs (Holy and Guo, 2005 and Musolf et al., 2010) that may reflect individual quality. Despite what we have learned about rodent USVs from laboratory studies, we have no information about whether rodents use USVs as part of their behavioural repertoire in the wild.

In addition to laboratory mice and rats, other muroid rodents in the genus *Peromyscus* also produce USVs as adults (Pomerantz and Clemens, 1981, Nunez et al., 1985, Kalcounis-Rueppell et al., 2006 and Kalcounis-Rueppell et al., 2010). A particularly well-studied species of *Peromyscus* is the California mouse,*Peromyscus californicus*, because it is a model for monogamy and parental care in mammals (e.g. Dudley, 1974a, Dudley, 1974b, Gubernick, 1990, Gubernick and Laskin, 1994, Gubernick et al., 1994, Vieira and Brown, 2002, Vieira and Brown, 2003, Wright and Brown, 2004, Bester-Meredith et al., 2005, Lee and Brown, 2007, Trainor et al., 2008a and Trainor et al., 2008b) where monogamy is rare (Mock & Fujioka 1990). The California mouse is an obligate behaviourally and genetically monogamous mouse (Ribble 1991) that displays high levels of parental care (Dudley, 1974a, Dudley, 1974b, Gubernick and Alberts, 1987,Gubernick and Nordby, 1993, Ribble and Salvioni, 1990, Ribble, 1991 and Bester-Meredith et al., 1999) and territoriality (Ribble,

1992a, Gubernick and Nordby, 1993, Bester-Meredith and Marler, 2001, Bester-Meredith and Marler, 2003, Bester-Meredith and Marler, 2007 and Davis and Marler, 2003). Individuals establish a pair bond with another individual and mate for life unless their mate dies or disappears (Ribble 1992b). All of a female's offspring are sired by her mate (Ribble 1991). Mated pairs nest together during breeding and nonbreeding seasons and maintain an exclusive territory (Ribble & Salvioni 1990). Ribble & Salvioni (1990) and Ribble (1991) demonstrated that genetically monogamous pairs are consistent in their home range use. The territory is protected year round by both the male and female, which both display aggression towards any intruder. Males participate in all aspects of parental care except for nursing (Dudley, 1974a, Gubernick and Alberts, 1987 and Gubernick and Teferi, 2000). Male care is needed for offspring survival in the wild (Dudley, 1974b, Gubernick and Alberts, 1987 and Gubernick and Teferi, 2000) and is mediated by chemosignals in the female's urine and by copulation (Gubernick, 1990 and Gubernick et al., 1994). Dispersal of subadults occurs 60 days postpartum and is female biased; males either disperse a distance equivalent to one home range (1161 m^2), or inherit their parent's home range, whereas females disperse at least two home ranges from their natal home range (Ribble and Salvioni, 1990 and Ribble, 1992a).

As with other muroid rodents, USVs in *P. californicus* are an integral component of their behaviour (Scattoni et al. 2009). In *P. californicus,* USVs are produced by both neonates and adults (Vieira and Brown, 2002,Wright and Brown, 2004, Kalcounis-Rueppell et al., 2006 and Kalcounis-Rueppell et al., 2010). Previously reported ultrasonic vocalizations from *P. californicus* in the wild (Kalcounis-Rueppell et al., 2006 and Kalcounis-Rueppell et al., 2010) were not attributed to individuals because these studies sought to eavesdrop on, and examine USVs from, groups of mice. Therefore, it is not clear which individuals (i.e. male or female) produce USVs, whether USVs vary in spectral and temporal characters (i.e. between males and females), or whether USVs are produced in multiple behavioural contexts (i.e. when alone or not alone).

In *P. californicus,* USVs may not function in the same way as in laboratory rats and mice because of their monogamous mating system. Neither *R. norvegicus* nor *M. musculus* are monogamous, nor do they form strong pair bonds with their mates as is seen in *P. californicus* (Ribble & Salvioni 1990). Rather than mediating relationships among unrelated individuals and potential mates, as is the pattern with *R. norvegicus*and *M. musculus*, USVs in *P. californicus* probably function to facilitate maintenance of an established pair. For example, USVs could facilitate pair bond maintenance as in other vocalizing animals with strong bonds between individuals (Ford, 1989, Sugiura, 1998, Kazial et al., 2001 and Hall and Peters, 2008). Alternatively, or in addition, USVs may be important for the coordination of territorial defence, as is seen in pair-bonded birds (e.g. Payne, 1971 and Harcus, 1977).

Here, for the first time, we determine the context of USV production by free-living adult *P. californicus*individuals in the wild. Using remote-sensing methods we recorded USVs in the wild and assigned them to the individuals that produced them. We describe (1) the

individuals that produced USVs, (2) the spectral and temporal characteristics of USVs from individual mice and (3) the behavioural contexts in which USVs were produced. Our remote-sensing methods focused on individuals moving about their home ranges during nightly activities, rather than on individuals at the nest. Because adult male and female *P. californicus* share similar duties in rearing offspring and defending territories, we predicted that they would produce USVs in similar behavioural contexts and with similar spectral and temporal characteristics.

Methods

Fieldwork took place at The Hastings Natural History Reservation (HNHR) in upper Carmel Valley, California, U.S.A. (Monterey Co: 36° 22′ N, 121° 33′ W). Details of the study site and live-trapping grids can be found inKalcounis-Rüppell & Millar (2002). Our study occurred on the Lower Robertson Creek grid (Grid LRC), which consists of a 4 × 34 configuration of trap stations encompassing 2.2 ha. Our study took place during December 2007–June 2008 and January 2009, with focal areas (see below) covered during February–June 2008 and January 2009. All animal capture, handling and recording methods were approved by the Institutional Animal Care and Use Committees of the University of North Carolina at Greensboro (UNCG IACUC Protocol No. 07-05) and the University of California Berkeley (approval of UNCG IACUC Protocol No. 07-05) and were authorized by the California Department of Fish and Game through Scientific Collecting Permits (SC-001358, SC-9663, SC-9661, SC-9806).

Establishment of Focal Areas

Eleven 10 m^2 sections of the grid were designated as focal areas for the purpose of recording USVs from individual mice. Focal areas were chosen sequentially because it was only possible to collect data from a single focal area at a time. Focal areas were placed along the grid based on two main considerations. First, there needed to be relatively high densities of resident *P. californicus* and *Peromyscus boylii* (for a companion study) to maximize the probability of recording USVs. Second, the forest canopy had to accommodate our pulley system (see below). Details on placement of focal areas relative to mouse densities and home ranges are described in Supplementary Information A. At each focal area, we set up a microphone array, a radiotelemetry system and a thermal-imaging camera to record USVs from individual mice. Briefly, the 12 microphones recorded broadband sound (including mouse USVs) from within the focal area and were set out in a 4 × 3 configuration approximately 1–2 m apart. The telemetry system surveyed all resident mice in the focal area so that we could localize individuals that were producing USVs. The thermal camera system surveyed the focal area from the canopy of the forest and recorded all mammal activity in the focal area. The thermal images were used, where possible, to ensure that only known, resident mice were present when a particular USV was recorded. A schematic diagram of our remote-sensing equipment on a focal area, with two focal areas shown as examples, can be found in Supplementary Information B.

Live Trapping

We used live trapping to determine where to establish focal areas based on density of resident*P. californicus*. Sections of the entire trapping grid were consecutively trapped for three nights throughout the entire field season using standard live-trapping techniques. Mice were captured using Sherman and Longworth traps provisioned with oats and bedding. Two Sherman traps and one Longworth trap were set at each station at sunset and checked approximately 4 h prior to sunrise. Upon capture of a new individual, a single eartag (Monel Numeric, National Band and Tag Co., Newport, KY, U.S.A.) with a unique number was attached. Standard measures including mass, sex, age and reproductive status were recorded for every individual upon every capture. Mice were released at the site of capture. An individual that was captured at least three times between two trapping sessions was classified as a resident. Trapping data and coordinates of trap sites were uploaded into the Animal Movement Extension (Hooge & Eichenlaub 1997) for Arcview 3.2 to map individual home ranges and to examine areas of high density of *P. californicus*.

Sound Recording in a Focal Area

The microphone array consisted of 12 Emkay FG Series microphones capable of recording broadband sound (10–120 kHz; Avisoft Bioacoustics, Berlin). All microphones were wired to a 1216H UltraSoundGate system (Avisoft Bioacoustics) attached via a 2.0 USB interface to a laptop (Dell Latitude D410). The system was powered by a 12 V dry cell battery using a 150 W inverter. The microphones sampled at 250 kHz with a 16-bit resolution. Vocalizations were recorded using Recorder software (Avisoft Bioacoustics). It was possible to determine the location at which the vocalization was recorded by examining the times that the sound was intercepted by all 12 microphones. Prior to the start of recording, an ultrasonic whistle was used to verify proper function of all microphones.

Radiotelemetry of Resident Mice in the Focal Area

Captured resident mice at each focal area were fitted with a mouse-style 0.55 g M1450 transmitter with a unique frequency (Advanced Telemetry Systems, ATS, Isanti, MN, U.S.A.). To receive the radiotransmitter signals, small antennas (Sigflex 15 cm omni-directional) were set in each corner of the focal area and wired to a central receiver (4 MHz R4000), an antenna switch box and a data logger (DSU D50410; all from ATS). The receiver was programmed to search for all mouse transmitter frequencies in the focal area so that each mouse could be monitored. When a frequency was detected, the receiver recorded the signal strength at each antenna and then moved onto the next frequency. Because focal areas were small, transmitters were custom-made by ATS to produce a very weak signal to allow us to discriminate amongst signal strengths at the four antennas.

A few days prior to putting a transmitter on a mouse, the transmitter was measured on the focal area at each microphone to generate a reference grid of signal strengths. The transmitters were

placed at each microphone site for 3 min while the signal strength at each antenna was recorded. Generating a reference grid for each transmitter before it was placed on a mouse ensured that the system and transmitters were working properly and provided a pattern of signal reception of each transmitter in a given focal area. Although we created reference grids for each transmitter, the signal strengths of the transmitters were sufficiently low so as to be detected by only one or two antennas in any given focal area (see Supplementary Information B, C for details).

Thermal Imagery of the Focal Area

A thermal-imaging lens (Photon 320 14.25 mm; Flir/Core by Indigo) was used to document the presence of mice (and other homeothermic mammals) in the focal area. The lens was suspended by a simple pulley system in the tree canopy and was approximately 10 m above the focal area, allowing us to film the entire microphone array space. The thermal-imaging lens fed real-time images directly to a ground-based digital video recorder (JVC Everio DVR).

All equipment was set at approximately dusk and turned off at approximately dawn, with hard disk capability of storing all data collected through the night. At the start of recording each evening, time on all equipment was synchronized. In summary, the 12 microphones recorded sound continuously (with the capability of localizing the origin of the sound), the radio receiver continuously scanned for the transmitter frequencies of each radiocollared mouse, and the thermal-imaging camera filmed activity of mammals in the focal area. Every morning, sound files and video files were uploaded to an external hard drive and reviewed to ensure that the system was working. In addition, the radiotelemetry data were uploaded and reviewed each morning to determine whether any radiocollars were no longer working. When recording equipment was running in a focal area, all trapping in that section of the grid ceased so as not to influence the behaviour of mice in the focal area.

Integration of Field Data to Assign USVs to Individual Mice

To assign an individual to each USV recorded in a focal area, we integrated the field data as follows. First, all sound files recorded were played back at 11.025 kHz to determine whether the sound was a USV from a mouse. Second, sound files that contained mouse USVs were spectrographically examined in SASLab Pro (Avisoft Bioacoustics). In SASLab Pro we used the time and order of arrival of the USV at each of the 12 microphones in the array to determine where on the focal area the mouse was located during the production of the vocalization. Third, telemetry data were used to determine which individual mouse was at the position of the USV in the focal area when the USV occurred. The signal strength recorded at each of the four antennas was used to determine whether any detected transmitter matched the location of the USV. Lastly, where possible, thermal images were used to determine (1) whether there was a mouse present during the production of the USV, (2) whether there was another untransmittered mouse on the focal area that could be responsible for the production of the USV, and (3) to examine the position of all mice on the focal area when a USV was produced. The integration described

above was done manually for every USV recorded. Three researchers independently assigned individuals to USVs. For the USV to be included in the analyses presented herein, at least two researchers had to independently assign the same individual mouse to the USV. Examples of how we used our methods to assign USVs to individuals can be found in Supplementary Information C, along with companion videos.

Ultrasonic vocalizations produced by *Peromyscus* can be categorized into distinct phrase types or motifs that have been described elsewhere (Kalcounis-Rueppell et al., 2006 and Kalcounis-Rueppell et al., 2010) using terminology defined in Holy & Guo (2005).
Briefly, *Peromyscus* vocalizations occur in single motifs (with multiple syllables up to a maximum of approximately 6). Common motifs for *P. californicus* are presented in Kalcounis-Rueppell et al. (2010) and include one- to five-syllable vocalizations (1SV, 2SV, 3SV, 4SV and 5SV, respectively). These motifs are statistically distinct (Kalcounis-Rueppell et al. 2006). Moreover, time between motifs is much longer than the time between syllables within a phrase and ranges from 10 s of seconds to hours or days (as opposed to approximately 100 ms between syllables). Thus, motifs are easily distinguished from one another in a recording. When we use the term USV herein, we are referring to a single motif that could comprise one to five syllables.

Ultrasonic vocalizations assigned to an individual were analysed spectrographically using SASLab Pro (FFT length 512; Hamming window; 100% frame size; 50% resolution overlap). Any given USV was analysed from one microphone in the array and was chosen based on the amplitude of the waveform. Using the automated detection feature in SASLab Pro, we measured the following parameters: group duration (duration of the entire USV from the start of the first syllable to the end of the last syllable), syllable duration (duration of each syllable in the USV), minimum frequency of each syllable (Min F), maximum frequency of each syllable (Max F), peak frequency at the start (Start F) and end (End F) of each syllable, and frequency at the time point of maximum amplitude of each syllable (F Max Amp). To quantify modulation of each syllable, we calculated the slope of each syllable from start point to end point (overall modulation), and from the point of maximum frequency to minimum frequency (internal modulation; for details and an annotated spectrograph of measurements see Kalcounis-Rueppell et al. 2010). As in Kalcounis-Rueppell et al. (2010), each USV was categorized by the number of syllables it contained (1SV, 2SV, 3SV, 4SV or 5SV).

To examine the context of USV production, we examined thermal-imaging video within the minute that the USV was produced. We determined whether the mouse was alone or not alone during the minute that the USV was produced. When the mouse was not alone, we determined through our telemetry methods (described above) the identity of the second mouse. This contextual analysis was only available for a subset of USVs because in some cases, the mouse producing the USV was at the edge of the focal area (determined through acoustic localization and telemetry) and off camera, or the second mouse could not be identified to individual (for example, because of a failing transmitter where it could not be determined whether the second mouse was a mate or nonmate to the calling mouse). Because Ribble & Salvioni (1990) and

Ribble (1991) demonstrated that genetically monogamous pairs are consistent in their home range use, shared home ranges of reproductively active males and females were used to determine monogamous pairs.

Statistical Analysis

To determine (1) whether both males and females produce USVs, (2) whether USVs are produced when members of a pair are not in direct contact, (3) whether USVs are more frequently produced when the vocalizer is alone or with another individual, and (4) to examine whether USVs are more frequently produced in the presence of a nonmate, we used chi-square tests of independence. For these analyses, we used total number of USVs recorded as our dependent variable. Although our data were not strictly independent, because some individuals contributed multiple USVs to the totals, we used the chi-square approach because of the nature of our data, whereby a relatively limited number of individuals produced multiple USVs of different motifs at a relatively low rate. Thus, we considered each USV to be an independent behavioural event. Sample sizes of individuals relative to USVs precluded statistical analysis of USVs nested within individuals.

The five frequency variables (start frequency, end frequency, minimum frequency, maximum frequency and frequency at maximum amplitude) for each syllable were subjected to a principal components (PC) analysis. For statistics comparing spectral characters between sexes, we took mean values of USVs from the same individual to avoid pseudoreplication. We compared spectral characters between sexes, PC scores, internal and external modulation and duration of USVs using Kruskal–Wallis tests (a two-group generalized form of the Mann–Whitney *U* test with a chi-square test statistic) because assumptions of normality were violated based on Kolmogorov–Smirnov tests. We used the same analyses to compare spectral characters between behavioural contexts except that we did not remove an outlier (Female 1065) from these analyses and we did not take mean values of individuals (because sample sizes were small and there was an equitable distribution of individuals across behavioural contexts; see Results). All statistical tests were completed using base package in R (R Development Core Team 2010). All results are presented as mean $\pm$ 1 SD or mean rank. A probability criterion of α = 0.05 was used for all statistical tests.

Because we predicted no differences between males and females, we present power estimates for all statistical tests to determine the likelihood of not finding effects that may have existed. Power estimates were calculated using G*Power 3.1.3 (Faul et al. 2007). We assumed large effect sizes (ρ) for each class of test used. For chi-square comparisons, we used the post hoc chi-square family estimator with ρ set to 0.50. For our Kruskal–Wallis two-group comparisons, we used the post hoc *t* test family estimator with ρ set to 0.80. We used the *t* test family estimator for these Kruskall-Wallis comparisons because no estimator was available for Kruskal–Wallis tests

with two independent mean group comparisons. *P* values obtained for these data sets were similar to those obtained using Kruskal–Wallis tests (data not shown).

Results

Remote-sensing equipment was successfully deployed, with no equipment failure at 11 focal areas, for a total of 131 nights during February 2008–June 2008 and January 2009 (see Supplementary Information A for map). Equipment was deployed at focal areas for an average of 13.6 ± 4.8 days. Forty-three transmitters were placed on resident *P. californicus*. Eleven mice received transmitters at more than one focal area. Overall, 28 individual mice (17 females, 11 males) received transmitters. Of these, four were subadults and 24 were adults. On average, 3.8 ± 1.7 individuals carried transmitters at each focal area.

In total, 1090 audio files from *P. californicus* or the syntopic *P. boylii* (as part of companion studies: Carney, 2009 and Petric, 2010) were recorded. We were able to assign 226 USVs to 18 *P. californicus* residents (13 females, 5 males; 17 adults, 1 subadult; 5 monogamous pairs) recorded over 39 nights. A subset of these recordings (24 of 226) were compared with USVs produced by laboratory-reared *P. californicus* (Kalcounis-Rueppell et al. 2010). Although we were able to assign only 226 of these 1090 USVs to individual*P. californicus* that produced them, separate analyses of known differences in spectral characters of USVs between *P. californicus* and *P. boylii* revealed that approximately 500 of 1090 USVs were produced by*P. californicus* (Carney 2009).

Fourteen of the 18 individuals produced one to six USVs each. Four of the 18 individuals produced more than six USVs; Male 1131 produced 34, Female 1151 produced 12, Female 1229 produced 9 and Female 1065 produced 129. All four individuals that produced more than six USVs were members of a monogamous pair (but not necessarily partners). Female 1065 produced 129 USVs in only three nights of recording and was considered to be an outlier. She was removed from some analyses where we examined total numbers of USVs because she was responsible for over 50% of the total number of USVs, and our results on total number of USVs (e.g. examining differences in total number of USVs by motif type between males and females) would have been biased towards her behaviour. We describe instances in which she was included in our analyses below. We recorded an unusually high number of USVs from Female 1065 because her nest site was within one of the focal areas, and thus, USVs that she produced were more likely to be assigned to her. Female 1065's circumstances were unique in relation to our other pairs (see Discussion).

On average, we recorded 5.71 ± 7.96 USVs per individual when Female 1065 was excluded and 12.56 ± 30.07 USVs per individual when Female 1065 was included. Males and females did not differ in the number of calls produced regardless of Female 1065's inclusion (males: N = 5, mean = 10.40 ± 13.28, mean rank = 12.00; females: N = 13, mean = 13.38 ± 34.92, mean

rank = 8.54; Kruskal–Wallis chi-square test: $x^2_1 = 1.57$, $N = 18$, $P = 0.21$) or exclusion (males: $N = 5$, mean = 10.40 ± 13.28, mean rank = 12.00; females:$N = 12$, mean = 3.74 ± 3.70, mean rank = 7.75; Kruskal–Wallis chi-square test: $x^2_1 = 1.57$, $N = 17$, $P = 0.21$). Although the four mice that produced the highest numbers of USVs were part of a monogamous pair, a mouse that was within a pair did not produce more USVs than a mouse that was single, regardless of Female 1065's inclusion (paired: $N = 9$, mean = 22.33 ± 41.23, mean rank = 11.78; single: $N = 9$, mean = 2.78 ± 1.99, mean rank = 7.22; Kruskal–Wallis chi-square test: $x^2_1 = 3.40$, $N = 18$, $P = 0.10$, power = 0.30) or exclusion (paired: $N = 8$, mean = 9.00 ± 10.77, mean rank = 7.22; single: $N = 9$, mean = 2.78 ± 1.99, mean rank = 11.00; Kruskal–Wallis chi-square test: $x^2_1 = 2.48$, $N = 18$, $P = 0.12$, power = 0.30).

All of the USVs assigned to individuals contained one to five syllables (1SV–5SV; Kalcounis-Rueppell et al., 2006 and Kalcounis-Rueppell et al., 2010). One-syllable vocalizations (1SV, $N = 20$), two-syllable vocalizations (2SV, $N = 41$), three-syllable vocalizations (3SV, $N = 45$) and four-syllable vocalizations (4SV,$N = 11$) were recorded from multiple *P. californicus*. We recorded six 5SVs from Female 1065 but did not analyse these further because she was the only mouse from whom we recorded 5SVs. In addition, we recorded 1SV ($N = 21$), 2SV ($N = 37$), 3SV ($N = 47$) and 4SV ($N = 18$) from Female 1065. Unless otherwise noted, the following descriptive and inferential statistics exclude Female 1065.

The 1SVs consisted of one long syllable with a mean frequency of 28.37 ± 5.53 kHz and a mean duration 142.00 ± 41.8 ms (Fig. 1a). The 2SVs consisted of two syllables with a mean frequency of 26.78 ± 6.5 kHz and a mean duration of 180.96 ± 36.5 ms for syllable 1 and a mean frequency of 28.42 ± 6.05 kHz and a mean duration of 145.44 ± 51.9 ms for syllable 2 separated by a 321.32 ± 92.37 ms interval (Fig. 1b). The 3SVs consisted of three syllables with a mean frequency of 24.64 ± 6.48 kHz and a duration of 157.30 ± 56.26 ms for syllable 1, a mean frequency of 28.38 ± 5.52 kHz and a duration of 186.57 ± 64.62 ms for syllable 2, and a mean frequency of 27.33 ± 5.31 kHz and a duration of 102.67 ± 42.44 ms for syllable 3 (Fig. 1c). Mean interval between syllables 1 and 2 was 310.67 ± 103.75 ms, and that between syllables 2 and 3 was 331.73 ± 114.18 ms (Fig. 1c). The 4SVs consisted of four syllables with a mean frequency of 25.31 ± 4.73 kHz and a duration of 126.67 ± 78.34 ms for syllable 1, a mean frequency of 31.35 ± 3.57 kHz and a duration of 160.67 ± 41.97 ms for syllable 2, a mean frequency of 31.64 ± 3.76 kHz and a duration of 149.17 ± 32.01 ms for syllable 3, and a mean frequency of 30.60 ± 3.75 kHz and a duration of 102.29 ± 36.70 ms for syllable 4 (Fig. 1d). Mean intervals between syllables 1 and 2, syllables 2 and 3, and syllables 3 and 4 were 233.75 ± 141.01 ms, 254.58 ± 68.36 ms and 246.25 ± 49.55 ms, respectively (Fig. 1d).

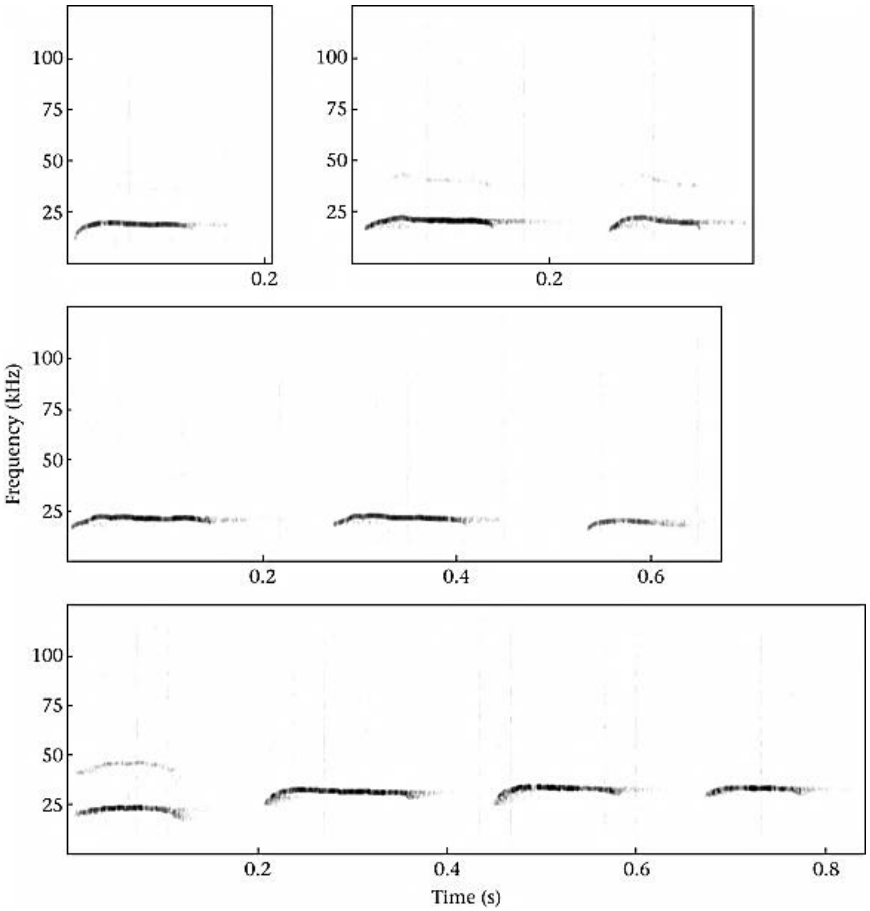

Figure 1. Representative spectrographs of (a) one-syllable vocalizations (1SV), (b) two-syllable vocalizations (2SV), (c) three-syllable vocalizations (3SV) and (d) four-syllable vocalizations (4SV) produced by *Peromyscus californicus*. Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, U.S.A., during February 2008–June 2008 and January 2009. Parameters of the spectrograph included: FFT length of 526, and a 100% frame size with a flat top window. Window overlap was 50%.

Both males and females produced all common motif types (males = 52 USVs, females = 45 USVs; Fig. 2). Motif produced was independent of sex (chi-square test: $x^2_3 = 7.06$, $N = 97$, $P = 0.07$, power = 0.99; Fig. 2). The five variables that were used to measure frequency were represented by the first PC axis (PC1), which explained most of the variation for all four motifs (Table 1). For all motifs, there was no significant difference in spectral characteristics of USVs between males and females (power range 0.31–0.75; Table 2). These results were consistent whether syllables were pooled within each motif type (Table 2) or compared independently (data not shown).

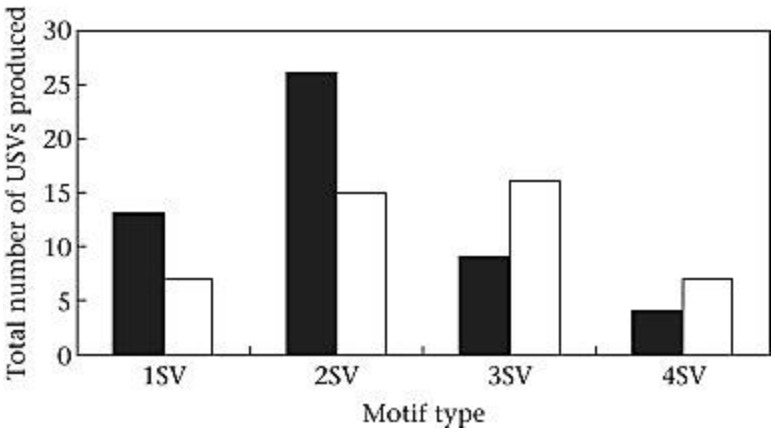

Figure 2. Total number of ultrasonic vocalizations (USVs) of each common motif type produced by male (solid bars) and female (open bars) *Peromyscus californicus*. USVs are from 12 females and 5 males and do not include Female 1065 (see text). USVs were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, U.S.A., during February 2008–June 2008 and January 2009.

Table 1. Analysis of spectral characters of ultrasonic vocalizations (USVs) of male and female California mice containing one to four syllables (1SV–4SV)*, and of USVs containing one to three syllables (1SV–3SV) when the behavioural context was known

Acoustic variable	1SV	2SV	3SV	4SV
Males and females	**(93.26%)**	**(97.14%)**	**(91.96%)**	**(95.58%)**
Start F (kHz)	−0.95	−0.98	0.92	0.87
End F (kHz)	−0.97	−0.98	0.96	0.95
F Max Amp (kHz)	−0.98	−0.99	0.98	0.96
Min F (kHz)	−0.97	−0.99	0.97	0.97
Max F (kHz)	−0.96	−0.99	0.97	0.97
Context known	**(93.94%)**	**(95.60%)**	**(90.95%)**	
Start F (kHz)	−0.95	−0.97	0.93	
End F (kHz)	−0.96	−0.97	0.95	
F Max Amp (kHz)	−0.98	−0.99	0.97	
Min F (kHz)	−0.97	−0.99	0.96	
Max F (kHz)	−0.98	−0.98	0.97	

Eigenvalues (in parentheses) and factor coordinates for frequency variables of principal component 1 are given for each USV motif. Frequency variables are labelled as follows: minimum frequency of syllable (Min F), maximum frequency of syllable (Max F), peak frequency at the start (Start F) and end (End F) of the syllable, and frequency at the time point of maximum amplitude of the syllable (F Max Amp).∗Excludes Female 1065.

Table 2. Spectral characters of ultrasonic vocalizations (USVs) of male and female *Peromyscus californicus*

Motif		Number of USVs	Duration (ms)	Overall modulation	Internal modulation	PC1
1SV	Males	4	132.50±27.53	27952.34±10261.40	20927.36±23969.09	−8134.47±10101.02
	Females	6	148.33±50.76	31282.53±18722.03	20937.17±13954.26	−2681.08±13980.86
	χ^2		0.93	0.00	0.00	0.73
	P		0.33	1.00	1.00	0.39
2SV	Males	4	175.42±34.45	26533.08±2848.81	9616.43±14849.87	−8026.81±10849.36
	Females	9	157.78±52.33	31517.47±14029.75	15330.63±39745.22	−1873.95±13577.36
	χ^2		0.61	0.44	1.63	1.11
	P		0.44	0.51	0.20	0.29
3SV	Males	4	153.27±61.75	41435.19±26882.36	17099.10±25864.72	4105.10±13381.01
	Females	6	144.99±67.14	36014.10±19791.03	13129.10±23666.44	−537.37±11588.62
	χ^2		0.22	0.30	0.11	0.95
	P		0.64	0.58	0.73	0.33
4SV	Males	4	121.25±37.22	55206.47±28606.28	23143.96±39683.32	−3048.71±8778.03
	Females	5	147.92±63.89	38289.51±14863.13	9001.28±22646.88	379.65±12712.15
	χ^2		0.93	3.27	1.45	0.17
	P		0.33	0.07	0.77	0.68

1SV, 2SV, 3SV and 4SV refer to one-, two-, three- and four-syllable vocalizations, respectively. Each motif was tested independently on mean values of USV parameters for each individual. Statistical comparisons were made with a two-group Kruskal–Wallis test (a two-group generalized form of the Mann–Whitney *U* test with a chi-square test statistic) because of violations of parametric statistics. Power range 0.31–0.75.

We were able to determine the behavioural context of 64 USVs (28.3% of all USVs assigned to individuals). In the following description we include USVs from Female 1065 because her contribution did not overwhelm the sample (she was responsible for 8 of the 65 USVs) and because almost every individual was represented (15 of 18 individuals were represented in the behavioural context data with an average of 4.27 ± 4.20 USVs per individual, range 1–15). Of the 64 USVs where behavioural context was determined, 33 USVs were produced when the vocalizer was alone on the focal area, 20 USVs were produced when a nonmate was present with the vocalizer on the focal area, and 11 were produced (from 2 males and 3 females) when the vocalizer's mate was present on the focal area (Fig. 3a–c). In all 11 of these latter cases, the mate was more than 1 m away from the vocalizing animal; of the 64 USVs where behavioural context was determined, USVs were never recorded from an individual when its mate was in close proximity (i.e. within 1 m of the vocalizer;Fig. 3d).

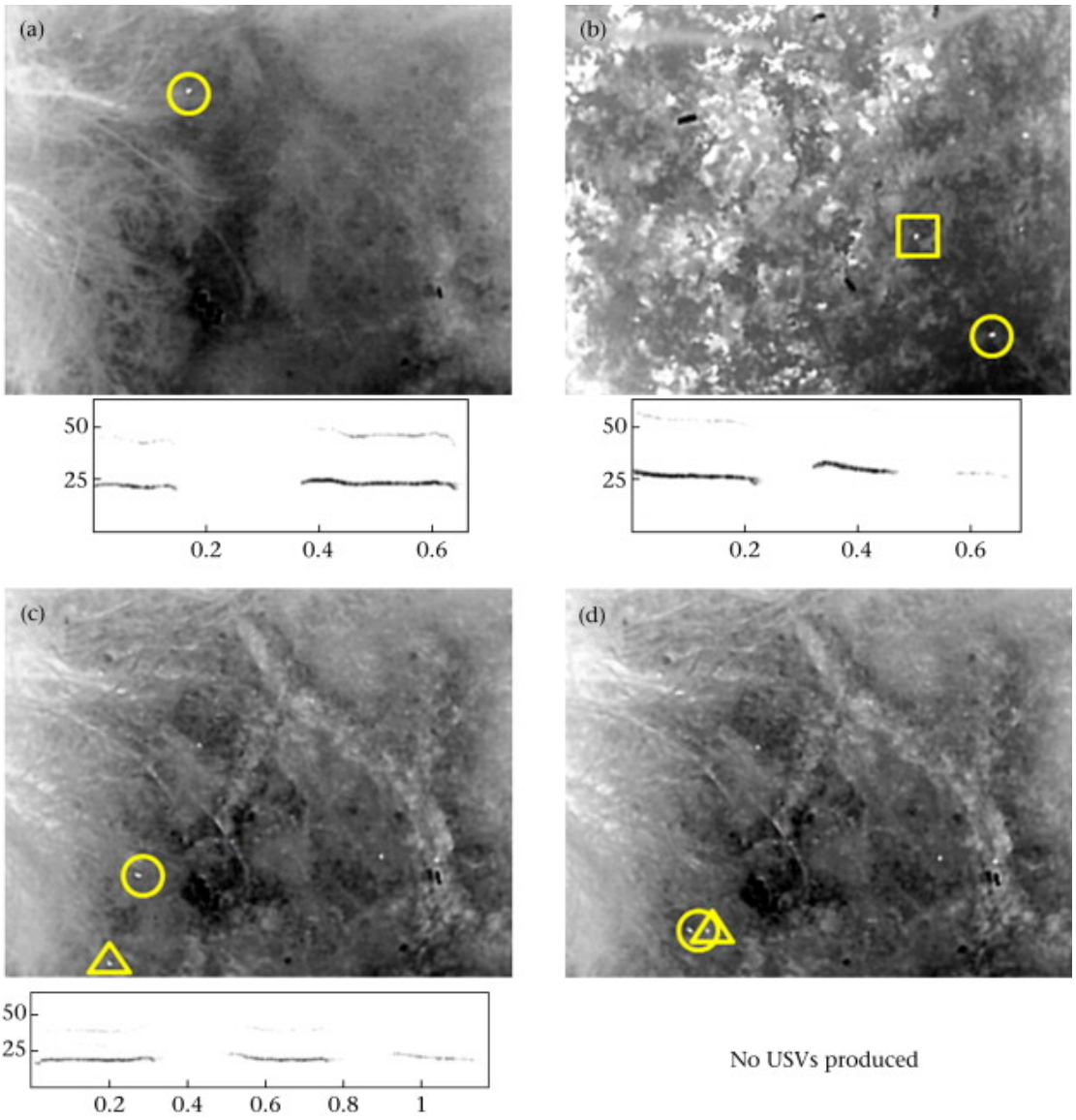

Figure 3. Single frames of thermal video images of the behavioural contexts and corresponding ultrasonic vocalizations of *Peromyscus californicus*. Vocalizations and images were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, U.S.A., during February 2008–June 2008 and January 2009. (a) Vocalizing mouse (circle) alone on the focal area, (b) vocalizing mouse (circle) with a nonmate present (square), (c) vocalizing mouse (circle) with a mate present (triangle) and (d) mated mouse pair (circle and triangle) within 1 m of one another. Parameters of the spectrograph included: FFT length of 256, and a 100% frame size with a Hamming window. Window overlap was 50%. Frequency (kHz) is on the *Y* axis, and time (s) is on the *X* axis.

Motif type was dependent on whether the vocalizer was alone or in the presence of another individual (chi-square test: $\chi^2_3 = 12.13$, $N = 64$, $P = 0.01$; Fig. 4a). In particular, 3SVs were produced more often when the vocalizer was in the presence of another individual, and 1SVs were produced more often when the vocalizer was alone. However, the distribution of

behavioural context was equitable between males and females for 1SVs (chi-square test: $\chi^2_1 = 0.19$, $N = 17$, $P = 0.66$, power = 0.54; Fig. 4b), 2SVs ($\chi^2_1 = 0.006$, $N = 25$, $P = 0.93$, power = 0.71; Fig. 4b) and 3SVs ($\chi^2_1 = 0.35$, $N = 17$, $P = 0.66$, power = 0.54; Fig. 4b), although there was a tendency for 3SVs to be produced by females that were not alone more often than by males that were not alone.

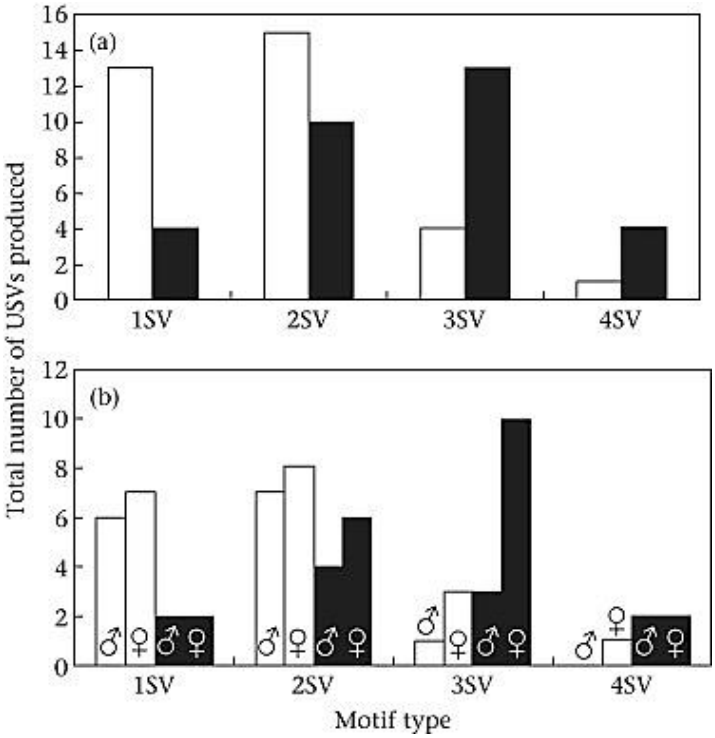

Figure 4. (a) Total number of ultrasonic vocalizations (USVs) containing one to four syllables (1SV–4SV) that were produced by vocalizing mice that were alone (open bar) or not alone (closed bar). The number of different individual mice contributing to these data included all 5 of the males and 11 of the 13 females (1SV: 3 males, 5 females; 2SV: 4 males, 8 females; 3SV: 2 males, 5 females; 4SV: 2 males, 2 females). (b) Total number of USVs of each common motif type produced by vocalizing male and female mice that were alone (open bars) or not alone (solid bars). Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, U.S.A., during February 2008–June 2008 and January 2009.

The duration of the first syllable of the 3SV was the only acoustic characteristic that significantly differed when produced while the vocalizer was alone or in the presence of another individual (Kruskal–Wallis test: $\chi^2_1 = 5.13$, $N = 64$, $P = 0.02$). These results were consistent whether syllables were pooled within each motif type (power range 0.38–0.61; Table 3) or compared independently (data not shown). When the vocalizer was alone on the focal area, the average duration of the first syllable of 3SVs was 76.38 $\pm$ 32.68 ms; however, when the vocalizer was in the presence of another individual, the average duration was 199.85 $\pm$ 87.01 ms. Three of four

4SVs were produced when the mouse was not alone, but sample sizes precluded statistical comparison of spectral characters.

Table 3. Spectral characters of ultrasonic vocalizations (USVs) of *Peromyscus californicus* that were either alone or not alone on the focal area when the USV was produced

Motif		Number of USVs	Duration (ms)	Overall modulation	Internal modulation	PC1
1SV	Alone	13	137.96±61.33	45358.69±37230.91	8628.43±30605.76	−2458.03±12959.37
	Not alone	4	144.63±55.67	28287.65±20875.17	23515.44±19987.51	2235.06±14463.30
	χ^2		1.85	1.04	1.85	0.46
	P		0.17	0.31	0.17	0.50
2SV	Alone	15	182.89±75.99	35018.25±29672.00	7578.17±28937.66	−2948.94±16343.67
	Not alone	10	165.04±79.40	33870.92±22036.49	15787.04±25739.60	−4299.80±12285.10
	χ^2		0.59	0.18	0.46	0.15
	P		0.44	0.67	0.50	0.70
3SV	Alone	4	139.55±74.07	50153.29±44863.52	8895.01±53209.87	−7262.91±12706.70
	Not alone	13	174.68±74.42	40684.54±33316.62	16821.24±35516.31	−4235.75±9732.40
	χ^2		1.83	0.54	1.21	0.91
	P		0.18	0.46	0.27	0.33

1SV, 2SV and 3SV refer to one-, two- and three-syllable vocalizations, respectively. Small sample size of four-syllable vocalizations, 4SVs (alone: $N = 1$ USV; not alone: $N = 3$ USVs) precluded statistical analysis. Statistical comparisons were made with a two-group Kruskal‑Wallis test (a two-group generalized form of the Mann‑Whitney *U* test with a chi-square test statistic) because of violations of parametric statistics. Syllables within 2SV and 3SV motifs are pooled. Power range 0.38‑0.61.

Individuals were just as likely to vocalize in the presence of a nonmate or a mate (chi-square test: $\chi^2_3 = 3.35$,$N = 41$, $P = 0.34$, power = 0.77; Fig. 5a). Although sample sizes precluded statistical analyses, there was a tendency for females and males to produce similar amounts of vocalizations in the presence of mates (Fig. 5b), and for females to vocalize more than males, using 2SVs and 3SVs, in the presence of nonmates. There were no significant differences in the acoustic structure of 1SVs, 2SVs and 3SVs produced in the presence of a mate or nonmate (power range 0.47‑0.79; Table 4). These results were consistent whether syllables were pooled

within each motif type (Table 4) or compared independently (data not shown). All three 4SVs produced when a mouse was not alone were produced in the presence of a nonmate. In cases where the nonmate could be determined through radiotelemetry data (*N* = 15 USVs from 7 *P. californicus*individuals), the nonmate was either a neighbouring *P. californicus* (*N* = 8), a *P. boylii* (*N* = 4), a neighbouring*P. californicus* and a *P. boylii* (i.e. two nonmates in the focal area at the time of the USV; *N* = 2), or a nestmate (*N* = 1; the vocalizing animal and the nonmate were subadults).

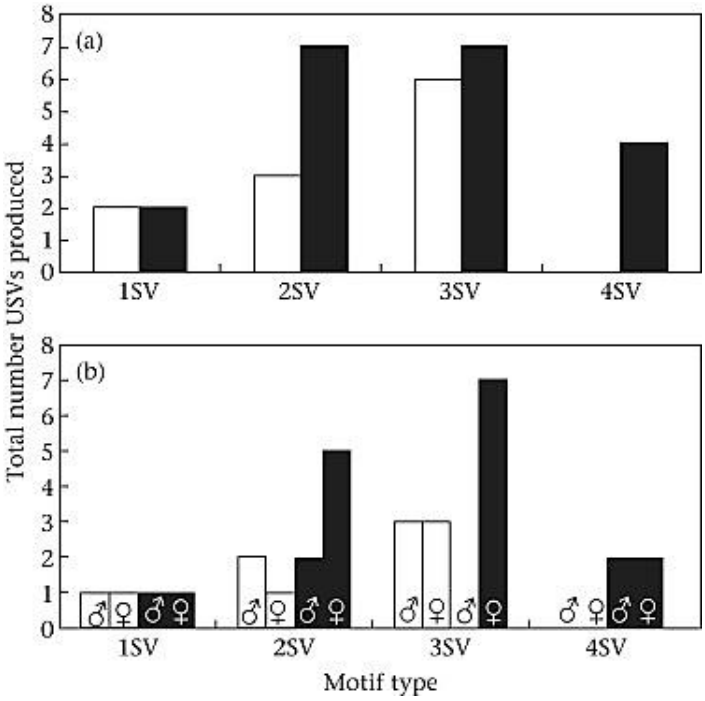

Figure 5. (a) Total number of ultrasonic vocalizations (USVs) containing one to four syllables (1SV–4SV) that were produced by vocalizing mice when their mate (open bars) or a nonmate (solid bars) was present. The number of different individual mice contributing to these data included 4 of the 5 males and 8 of the 13 females (1SV: 3 males, 5 females; 2SV: 4 males, 8 females; 3SV: 2 males, 5 females; 4SV: 2 males, 2 females). (b) Total number of USVs of each common motif type produced by vocalizing male and female mice when their mate (open bars) or a nonmate (solid bars) was present. Vocalizations were recorded from a wild population of *P. californicus* at Hastings Natural History Reserve, Carmel Valley, CA, U.S.A. during February 2008–June 2008 and January 2009.

Table 4. Spectral characters of ultrasonic vocalizations (USVs) of *Peromyscus californicus* that were vocalizing when their mate or a nonmate was present

Motif		Number of USVs	Duration (ms)	Overall modulation	Internal modulation	PC1
2SV	Mate	3	160.90±74.41	24104.81±13692.40	6419.09±16031.84	−6345.53±13049.37
	Nonmat	7	167.10±84.9	38753.97±24232.8	20471.02±28901.1	−3276.94±12345.8

	e		3	1	4	0
	χ^2		0.08	2.25	2.54	0.14
	P		0.78	0.13	0.11	0.71
3SV	Mate	6	174.74±71.47	44828.57±31138.11	14865.60±37168.32	−6318.31±9030.76
	Nonmate	7	174.62±78.61	37132.51±35442.17	18497.51±34871.50	−2450.69±10168.42
	χ^2		0.003	1.91	0.05	3.25
	P		0.96	0.17	0.82	0.07

2SV and 3SV refer to two- and three-syllable vocalizations, respectively. Small sample sizes of one-syllable vocalizations, 1SVs (mate: $N = 2$; nonmate: $N = 2$) and four-syllable vocalizations, 4SVs (mate: $N = 0$; nonmate: $N = 3$) precluded statistical analyses. Statistical comparisons were made with a two-group Kruskal–Wallis test (a two-group generalized form of the Mann–Whitney *U* test with a chi-square test statistic) because of violations of parametric statistics. Power range 0.47–0.79.

Discussion

This is the first description of individual USVs produced by free-living nocturnal rodents. The male and female members of *P. californicus* mated pairs share similar duties in parental care (Dudley, 1974a, Dudley, 1974b, Gubernick and Alberts, 1987, Ribble and Salvioni, 1990, Ribble, 1991, Gubernick and Nordby, 1993 and Bester-Meredith et al., 1999) and territoriality (Ribble, 1992a, Gubernick and Nordby, 1993, Bester-Meredith and Marler, 2001, Bester-Meredith and Marler, 2003, Bester-Meredith and Marler, 2007 and Davis and Marler, 2003). Therefore, we predicted that both male and female *P. californicus* would produce USVs with similar spectral and temporal characteristics and in similar behavioural contexts. We found support for our prediction. Free-living adult male and female *P. californicus* in their native habitat produced ultrasonic vocalizations, with the most common motifs being one-, two- and three-syllable vocalizations. Males and females produced the same type and number of USVs, and their USVs did not differ in spectral or temporal characteristics. *Peromyscus californicus* produced USVs when they were alone and when they were with another mouse, but they were more likely to produce the 3SV motif, with a relatively long first syllable, in the presence of another mouse. Individuals were just as likely to vocalize in the presence of a nonmate or a mate. Spectral and temporal USV characteristics were not dependent on whether an individual produced the USV in the presence of a mate or nonmate. Males and females produced USVs in the same behavioural contexts. Individuals did not vocalize to their mates when their mates were within 1 m. Taken together, our results show that, as with other behaviours associated with parenting and territorial defence in*P. californicus*, the USVs of adult males and females are used similarly and have similar spectral and temporal characteristics. Overall, our power estimates were low to moderate, especially given our assumptions of large effect sizes. However, we are confident in our conclusions because our results were consistent across multiple approaches of examining our data to look for differences between males and females, and larger sample sizes were simply not possible given the density of animals and the effort required to collect these data.

The 226 USVs we recorded and assigned to individuals were of the same common motif types previously reported for group recordings (1SV–5SV; Kalcounis-Rueppell et al. 2006). In addition, the 226 USVs recorded and assigned to individuals were reflective of the subset of 24 USVs featured in Kalcounis-Rueppell et al. (2010). The similarity of these 226 USVs with earlier recordings (Kalcounis-Rueppell et al. 2006) and laboratory recordings (Kalcounis-Rueppell et al. 2010) demonstrates that these single and multisyllabic vocalization motifs are commonly produced by *P. californicus* and are an important part of their behavioural repertoire in the wild.

Male and female *P. californicus* show the same behaviours with respect to parenting and territorial defence, and our study suggests that USVs should be added to this list of behaviours because USVs of males and females did not differ in type or context. Male *P. californicus* share all aspects of parental care except for nursing. Both males and females are aggressive in defending their territories. This similarity in behaviours has to do with the exclusive monogamous relationship, including long-term pair bonds, among mates. Not only did we find that both male and female *P. californicus* produced equivalent proportions of the USV motifs, but we also found that the USVs of males and females were not distinguishable based on common spectral and temporal characteristics, and that both males and females produced the same motif types in the same behavioural contexts.

As with parental and territorial defence behaviour, the similarity in USV production probably reflects similarity in behavioural function of USVs between males and females and could reflect the monogamous social structure with long-standing pair bonds. Our objective was to describe which individuals produce USVs, the spectral and temporal characteristics of USVs from individual mice, and the behavioural contexts in which USVs are produced. Our study was not designed to test hypotheses about the biological function of USVs in adult male and female *P. californicus*, but our results on the behavioural contexts in which USVs were produced lays the ground work for future experiments to determine biological function. We suggest, based on our results, at least two functions of USV production by male and female *P. californicus*. First, because males and females produced USVs in the presence of a nonmate, there is probably a territorial defence function to the USVs we recorded in this study. Second, males and females produced USVs in the presence of a mate that was not nearby (i.e. within 1 m), so they probably function in pair bond maintenance as well. USVs may also be produced to coordinate territorial defence between members of a pair, rather than in direct defence of the territory. Hall (2000) hypothesized that birds produce duets to coordinate territorial defence, allowing them to locate each other on their home range. When *P. californicus* pairs are foraging together, they remain close to each other and may not need vocalizations to coordinate territorial defence, and this may be why we never recorded vocalizations when members of a pair were close to each other.

However, although we suggest these potential functions, distinguishing among these functions of USVs, which are probably not mutually exclusive, would require rigorous experiments in the field using methods similar to those of the present study. For example, a test of a territorial function of USVs would be to introduce a strange individual into a mated pair's territory. If USVs serve a territorial function, both male and female residents should vocalize in response to an intruder relative to a control (i.e. an offspring). A test of a contact call function would be to remove a member of a pair from the pair's territory. Pre-recorded vocalizations from the removed individual could be played back while the partner is active on the territory. If the vocalizations function as contact calls, each partner tested would respond more to the playback of the missing partner's vocalizations (relative to a control) by vocalizing and/or approaching the playback speaker. Our results open up exciting potential for experimental tests of USV function in wild *P. californicus*.

An observation supporting the production of USVs as pair bond maintenance through contact calling comes from Female 1065 (who produced 129 of the 226 USVs assigned to individuals). She was a female for whom a microphone happened to be placed near her nest (without our knowledge, while the focal area, FA2, was being set up; see Supplementary Information A). All of her USVs were produced over a 3-night period at the beginning of a new focal area when she was lactating. Female 1065 and her mate (Male 1206) were both outfitted with transmitters prior to the start of the focal area recording, and over the 3 nights that she produced all of her USVs, her partner's transmitter was not recorded by the telemetry system even though he was observed through telemetry and was captured multiple times subsequent to the dates corresponding to this focal area. Pairs usually forage together except during the first 15 days postpartum when the pups are incapable of thermoregulation and one parent is needed in the nest (Gubernick & Teferi 2000). Female 1065 may have been producing USVs during this critical pup development period in order to establish contact with her mate while he was not near the nest. Moreover, of the five pairs in the study, the one containing Female 1065 was the only pair that did not last for the duration of our observations; Female 1065's mate moved from focal area FA2 to approximately 220 m away (focal area FA9) and paired with another female. Additional support for USVs being important for pairs of mice, whether in territory defence or pair bond maintenance, comes from the observation that the four mice that produced the highest number of USVs belonged to a pair.

Independent of function, our results suggest that the vocalizer's sex is not being communicated through USVs. Spectral and temporal characteristics of USVs did not differ between male and female *P. californicus*. For rodents that regularly search for mates, such as polygamous or promiscuous mice, there may be a benefit to advertising both sex and individual characteristics through acoustic signals if vocalizations are used for courtship behaviours. For example, male laboratory mice vocalize via USVs while approaching females, a behaviour that reduces aggressive behaviour from females (Whitney et al. 1973). Because of their long-term pair bonds, *P. californicus* males and females are in regular contact with their mates and would be expected to advertise sex only when initially searching for a mate. If *P. californicus* produces

USVs to attract a mate, then we would predict a divergence in USVs by sex in subadults, since subadults would be unlikely to have a partner and would therefore be more likely produce courtship vocalizations.

Male and female laboratory-bred strains of rats also produce the same motifs during copulation, play and aggressive interactions (Thomas & Barfield 1985). In contrast, although both male and female laboratory-bred strains of mice produce USVs, the number of USVs of particular motifs produced by each sex varies depending on the individual with whom they are interacting; the male produces the majority of USVs during male–female interactions (Warburton et al. 1989) whereas resident females produce USVs during female–female interactions (Moles et al. 2007). Sex differences in USV production between laboratory-bred strains of rats and mice can be explained by their different social systems (Costantini & D'Amato 2006), whereby rats are more colonial, with more extensive pair bonds, than mice.

The lack of differences between males and females may only pertain to motifs 1SV–4SV, which were recorded while individuals were moving through their home range. Note that all vocalizations assigned to*P. californicus* in this study were recorded while individuals were moving through their home range. We did not record vocalizations produced by adults while in the nest site (except for Female 1065), or while in close contact (i.e. within 1 m) with another individual. Laboratory evidence (J. D. Pultorak, C. A. Marler & M. C. Kalcounis-Rueppell, unpublished data) suggests that when *P. californicus* are within centimetres (as opposed to metres) of one another, they produce very quiet USVs that would not have been recorded unless the pair was within a few centimetres of the microphones. For example, in big brown bats, *Eptesicus fuscus*, vocalizations by males and females differ when they are roosting but not when they are foraging (Grilliot et al. 2009). Thus, although we found no sex differences in acoustic structure of USVs containing one to four syllables, this does not preclude sex differences in other types of vocalizations or in other situational contexts. While moving within their territories, the vocalizations of *P. californicus* may not benefit from differences between males and females; however, in the nest or in direct contact, USVs may serve different functions that benefit from a divergence in acoustic characteristics between males and females.

Like pups of many muroid rodents, *P. californicus* pups produce USVs. *Peromyscus californicus* pups vocalize from age 2 to 30 days, with female pups vocalizing more than male pups (Vieira and Brown, 2002 and Wright and Brown, 2004). The function of USVs by *P. californicus* pups is not entirely clear, but there is some evidence that they may elicit parental retrieval of pups (Wright & Brown 2004). Spectral characteristics of *P. californicus* pup vocalizations have not been described. Although all vocalizations assigned to *P. californicus* in this study were recorded while individuals were moving through their home range, we cannot unequivocally exclude the possibility that the USVs we recorded were from pups in nest sites. However, this is unlikely for the following reasons. First, when nests occurred in our focal area, we could often see consistent heat in our thermal video from the nest and from the pups as they

moved in and around the nest, and we did not record USVs from these nests. Second, if we were recording USVs from nest sites that were not apparent to us on our thermal video (e.g. they were too deep in the ground), we would have expected USVs to come consistently from the same location without an adult mouse present over multiple days, and this did not happen. Lastly, we knew where the nest sites of our radiocollared mice were and we placed independent ultrasound recording systems near those sites to examine the types of sounds being produced from the nest as a separate study. Nest sites of our focal animals were often not on focal areas (because nest site habitat differed from optimal focal area placement habitat), and although there was some overlap in USV motifs recorded at nest sites, there were at least three motif types recorded at nest sites that were never recorded in focal areas (M. C. Kalcounis-Rueppell, unpublished data). Additionally, it is not likely that our vocalizing adults were vocalizing towards pups in the nest because of the distance from focal areas to nests. However, we cannot and do not exclude the possibility that some adult vocalizations may have been directed to pups when nests were in focal areas. Whether USVs from adults function in parent-offspring communication towards pups has not been well explored, even in the laboratory.

We have shown for the first time in a wild rodent that USVs are an important component in the behavioural repertoire of adult male and female *P. californicus* in different behavioural contexts. Consistent with other behaviours associated with the rare monogamous mating system of *P. californicus*, males and females did not differ in USV behaviours. Further experimental work with *P. californicus* USVs in the wild will allow us to determine the role that USVs play in mediating and maintaining long-term pair bonds, territorial defence and biparental care.

Finally, although USVs appear to be an important part of the behavioural repertoire of *P. californicus* in the wild, *P. californicus* do not produce USVs very often when compared to other vocalizing vertebrates (i.e. birds, primates, frogs). Our sample size of 226 USVs represents a low number of vocalizations given our sampling effort (131 nights) and a low number of vocalizing animals (28 individuals). Even when taking into considering the methodological limitations that reduced our sample, based on our ability to assign USVs to the individuals that produced them, *P. californicus* did not produce USVs very often. However, this level of vocalization is a genuine component of *P. californicus* behaviour: these mice do not call repeatedly under normal circumstances. Nevertheless, a low rate of USV production does not preclude USVs from being biologically relevant. While the biological function remains to be determined, it is important to consider the differences between *Peromyscus* (or other muroid rodents) and other vertebrates that vocalize when considering their acoustic communication patterns in a comparative framework. Namely, *Peromyscus* is completely nocturnal and relies heavily on olfactory communication.

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Supplementary Material

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