

## The function of ultrasonic vocalizations during territorial defence by pair-bonded male and female California mice

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Acoustic communication is vital to complex social behaviours such as territorial defence. The use of ultrasonic vocalizations, particularly in territorial defence by monogamous species and females, remains understudied. We studied ultrasonic vocalization production and associated aggression in the monogamous, biparental and territorial California mouse, *Peromyscus californicus*, in which both males and females were found to display similar levels of physical aggression against same-sex intruders. We identified specific ultrasonic vocalization calls that are modulated based on social context: (1) sustained vocalizations, which are long, low-bandwidth calls ranging from 22 to 25 kHz, and (2) barks, which are short, high-intensity calls beginning and ending in the audible range. Despite similarities in physical aggression, sex differences emerged in vocal communication. Only resident males, and not females, produced sustained vocalizations prior to the onset of physical aggression, and were found to shorten the duration of individual sustained vocalization calls over both the course of the pre-encounter phase and from the pre-encounter to encounter phase. In addition, the degree of sustained vocalization shortening in males predicted offensive aggression of the resident. Males exhibited shorter sustained vocalization calls during encounters than females. Barks occurred more frequently during female–female physical aggression than in male–male encounters, and correlated highly with defensive aggression by intruders. Finally, a newly identified highly complex call, sweep phrases, was recorded in a subset of both sexes in the pre- and post-encounter phases. The overall results indicate that ultrasonic vocalizations may play an important role in territorial defence during both territorial advertisement and aggression in a monogamous rodent. Overall, this monogamous species showed sex similarities in physical aggression but sex differences in vocal communication and a more sophisticated function for sustained vocalizations than previously recognized.

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Vocalizations play a distinct role in the expression and escalation of aggressive behaviours in many species (van Staaden, Searcy & Hanlon, 2011). These signals can provide information to conspecifics about the resource-holding potential (RHP) and/or fighting ability of the signaller, thus determining whether contests will escalate (Maynard Smith, Price, Smith, & Parker, 1973). Vocal signals during aggression can differ or be modulated in several ways including their amplitude, frequency, complexity and duration (Seyfarth & Cheney, 2010; van Staaden et al., 2011). High-amplitude calls are indicators of aggression in many species (Brumm, 2004; Brumm & Ritschard, 2011; but see ; Maddison, Anderson, Prior, Taves, & Soma, 2012), and low-frequency calls

often signal larger body size and greater fighting ability (Reby et al., 2005; Reichert & Gerhardt, 2014). Complexity of calls, such as bird trills, indicate fighting quality, and playback studies reveal that producing more trills by an “intruder” reduces the aggressive response of territory holders (Cramer & Jordan Price, 2007; Illes, Hall, & Vehrencamp, 2006). Alterations in call duration can signal aggression in some species; for example, black redstarts, *Phoenicurus ochruros*, shorten call duration to produce more calls in the same time frame (Apfelbeck, Kiefer, Mortega, Goymann, & Kipper, 2012), while others such as European starlings, *Sturnus vulgaris*, increase song length during territorial defence (Alger, Larget, & Ritters, 2016). Taken together, this information on non-rodent species indicates that modulation of call types and spectral properties is of particular importance to aggression.

In rodents, the role of vocalizations in both aggressive behaviour and territorial defence remains understudied with limited overlap

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with the species just described. Generally, rodents produce ultrasonic vocalizations at frequencies >22 kHz as a means of vocal communication during social behaviour (Arriaga et al., 2012; Holy & Guo, 2005; Sales, 2010). Rats, for instance, produce ultrasonic vocalization calls in two major frequency bands, 22 kHz and 50 kHz (Burgdorf et al., 2008; Kroes, Burgdorf, Otto, Panksepp, & Moskal, 2007; Sales, 1972; Sirotin, Costa, & Laplagne, 2014), whereas house mice, *Mus musculus*, produce a variety of ultrasonic vocalization calls at a frequency of >45 kHz (Hoffman, Musolf, & Penn, 2012; Portfors, 2007; von Merten et al., 2014). However, lower-frequency calls do not uniformly indicate increased aggression or body size across rodent species (Hoffman et al., 2012). Furthermore, increased call amplitude, a signal of aggression or territoriality in many nonrodent species, has instead been linked to submissive behaviour in rodents (Constantini & D'amato, 2006; Portfors, 2007). Other call attributes, therefore, may be more important to rodent aggression. Calls are altered during aggression in species such as the Turkish spiny mouse, *Acomys cilicicus*, in which males increase the number of ultrasonic vocalizations produced and alter the proportion of call types in response to agonistic encounters (Griffiths, Dow, & Burman, 2010). In contrast, house mice and Syrian hamsters, *Mesocricetus auratus*, decrease ultrasonic vocalization calling and increase lower-frequency broadband calls (<20 kHz) during aggression (Arriaga et al., 2012; Fernández-Vargas & Johnston, 2015; Keesom, Rendon, Demas, & Hurley, 2015). Call duration and complexity have been tied to aggressive output in rodents. For example, castrated Alston's singing mice, *Scotinomys teguina*, produce fewer trills with shorter durations while expressing less aggression compared to testosterone-implanted controls (Alger et al., 2016; Pasch, George, Hamlin, Guillette, & Phelps, 2011). Further exploration of the role of ultrasonic vocalizations in aggression in rodents is warranted, particularly in females and monogamous species, which to this point remain understudied.

The vast majority of studies of vocal communication within aggressive contexts have focused on males (Cain & Langmore, 2015; Cain, Cockburn, & Langmore, 2015). In many rodent and nonrodent species, males are both more aggressive and more vocal than females (Reby et al., 2005; Reichert & Gerhardt, 2013). However, across taxa, in species where females are territorial and in monogamous species, aggressive behaviour is more similar between sexes, with both males and females displaying aggression towards novel intruders (Langmore, 1998). In monogamous species, pairs that share territorial defence responsibilities often do so in a sex-specific manner, defending territories against same-sex intruders as a form of mate guarding, with both males and females using similar vocal repertoires during aggression (Cross, Zedrosser, Nevin, & Rosell, 2014; Fedy & Stutchbury, 2005; Koloff & Mennill, 2011; Levin, 1996; Levin & Wingfield, 1992; but see Yang, Zhang, Cai, Stokke, & Liang, 2011). While there is evidence in both duetting birds (Colombelli-Négrel, 2016; Quinard & Cézilly, 2012) and California mice, *Peromyscus californicus* (Rieger & Marler, n.d.) for coordinated territorial defence that is not sex specific, same-sex intruders were used to induce reliable aggression. Here we expand our understanding of the role of vocal communication, particularly ultrasonic vocalizations, across different phases of an aggressive encounter by studying male–male and female–female aggression and the associated vocalizations of the monogamous California mouse.

We worked with the California mouse, a strictly monogamous biparental species that forms lifelong pair bonds (Gubernick, 1988; Ribble, 1991). Both male and female California mice exhibit reliable aggression against intruders, especially in the context of territorial defence (Ribble & Salvioni, 1990). California mice have a rich and distinct set of ultrasonic vocalization call

types that have been categorized both in the field and laboratory (Kalcounis-Rueppell, Metheny, & Vonhof, 2006; Kalcounis-Rueppell et al., 2010), that vary based on social context (Pultorak, Fuxjager, Kalcounis-Rueppell, & Marler, 2015; Pultorak, Matusinec, Miller, & Marler, 2017) and do not appear to differ spectrally between sexes (Briggs & Kalcounis-Rueppell, 2011). In this study, we focused on three major call types: sustained vocalizations, barks and 'complex sweep phrases' (which are described here for the first time; see Methods). Briefly, sustained vocalizations have been hypothesized to act as long-distance communication between individuals and can be modulated both in duration and total number of calls produced (previously referred to as syllables) as well as the number of calls produced within a bout (previously referred to as a phrase) (Kalcounis-Rueppell, Pultorak, & Marler, in press). Barks are hypothesized to be aggressive in nature. The function of sweep phrases is to this point unknown. Individual sweeps that make up these phrases, however, are simple frequency-modulated calls whose production can be altered in response to changing social context (Kalcounis-Rueppell et al., in press).

To understand the role of ultrasonic vocalizations in intra-sexual territorial defence of monogamous pairs we used a resident–intruder paradigm encompassing three main objectives. The first was to directly compare the aggressive behaviours and territorial defence of male and female California mice using same-sex encounters. We hypothesized that, as a monogamous species in which both sexes display aggression (Davis & Marler, 2004, 2003; Fuxjager, Oyegbile, & Marler, 2011; Oyegbile & Marler, 2005; Trainor et al., 2011), males and females would exhibit few sex differences in aggression when compared directly. Second, we sought to characterize the ultrasonic vocalization call types produced by male and female residents at different stages of a territorial intrusion: baseline (prior to the introduction of an intruder), pre-encounter (before any physical aggression but after initial visual, olfactory and auditory contact), encounter and post-encounter (after contest resolution). We were able to isolate the vocalizations of individuals both prior to and after physical aggression but not during physical encounters. We predicted that sustained vocalizations would be the predominant vocalization for males and females prior to physical aggression as previous field research suggested that these calls function in long-distance communication (i.e. communication with the mate; Kalcounis-Rueppell et al., 2006). We further explored both the duration of single sustained vocalization calls and the number of sustained vocalization calls in a bout across social contexts to determine whether sustained vocalization characteristics were modulated based on physical aggression. We also predicted that barks would be the predominant call during aggression due to their role in male–female interactions, where after a period of separation, reunited mates produced barks only when aggression also occurred (Pultorak et al., 2017). Finally, we tested whether these vocalizations were predictive of aggressive behaviour. Overall, we sought to bring a greater understanding of the role of ultrasonic vocalizations in the dynamic aggressive interactions of rodents.

## METHODS

### Animals

Twenty-four male (12 bonded and 12 sexually naïve, age 4–6 months) and 24 female California mice (12 bonded and 12 sexually naïve, age 4–6 months) were obtained from a laboratory colony at the University of Wisconsin-Madison. Mice were housed either in opposite-sex pairs or with one or two same-sex conspecifics in

standard cages (48 × 27 × 16 cm) lined with aspen bedding, a nestlet and water and food (Purina 5015™ mouse chow) available ad libitum. The colony room was maintained at 20–23 °C on a 14:10 h light:dark cycle (lights on at 21:00 hours Central Standard Time), with behavioural testing occurring 1–4 h after the onset of the dark cycle under dim red light. Animals used in dyads (either for pairing or aggressive encounters) were unrelated for at least two generations.

#### *Ethical Note*

Animals were maintained in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals*. Animal treatment and research protocols were approved by the University of Wisconsin, Madison College of Letters and Sciences Institutional Animal Care and Use Committee (IACUC-L00547). No animals were injured by any of the behavioural manipulations and/or assays.

#### *Testing Apparatus*

Testing occurred in a glass aquarium (50 × 30 × 30 cm) retrofitted with metal tracks to allow for the introduction and removal of a Plexiglas divider. The Plexiglas divider included two mesh cutouts that allowed for visual, olfactory and acoustic interactions between conspecifics but prevented physical contact. With the divider present, the testing chamber was split into two even compartments (25 × 30 × 30 cm). The lid of the chamber included 5 cm diameter holes placed 2.5 cm from the corners of the arena for the placement of microphones into opposite sides of the chamber to record ultrasonic vocalizations. With the divider in place, we recorded ultrasonic vocalizations in the early stages of an aggressive encounter prior to physical contact and were able to assign ultrasonic vocalizations to a single individual.

#### *Experimental Procedure*

Males and females were randomly assigned to be either bonded residents or sexually naïve intruders, with residents cohabitating for 2 weeks with their pair-bond mate prior to testing, while intruders remained with their original same-sex cage-mates. Bonding was verified by the observation of side-by-side contact between mates during cohabitation as seen in other monogamous rodents (Insel, Preston, & Winslow, 1995; Williams, Catania, & Carter, 1992). Twenty-four hours prior to aggression trials, residents were moved from their standard cage to the testing chamber (divider not present) lined with aspen bedding, a nestlet and containing food and water ad libitum. This 24 h period allowed for the formation of a residency effect in which the arena became the individuals' territory, creating a home field advantage and increasing their likelihood of winning against intruders (Bester-Meredith & Marler, 2001; Fuxjager & Marler, 2009; Fuxjager, Mast, Becker, & Marler, 2009; Fuxjager, Xhao, Rieger, & Marler, 2017). Intruders were randomly assigned to a same-sex resident and had a small patch of fur shaved from their right flank to allow for experimenters to recognize individuals. All intruders were socially naïve except for exposure to cage-mates with no sexual or aggression testing experience.

We used a resident–intruder paradigm divided into four encounter phases to characterize territorial defence and the accompanying ultrasonic vocalizations. The four encounter phases were (1) baseline (focal animal was isolated with no divider present), (2) pre-encounter (a same-sex intruder was introduced to the arena behind a Plexiglas divider with mesh cutouts), (3) encounter (the divider was removed and physical contact was

made possible) and (4) post-encounter (the resident and intruder were once again separated behind the divider).

The focal resident remained in the testing chamber, while the resident's mate was removed, and returned to the original standard cage. The focal resident was isolated in the testing chamber for 10 min prior to baseline testing. During the baseline phase, we made 1 min acoustic and video recordings of the isolated resident to obtain a baseline level of vocalizations. The divider was introduced and the pre-encounter phase occurred with the resident on the nest side of the testing chamber and the intruder on the opposite side of the divider. Vocalizations and behaviour were recorded for 4 min. The divider was then removed so the mice could interact in the encounter phase for 8 min, which has previously been shown to be a sufficient time for California mice to win encounters and produce a winner effect (Fuxjager et al., 2011). During this interaction phase, however, calls could not be conclusively localized to an individual and, therefore, resident and intruder calls were combined for analysis. The mice were then separated by the divider on their original sides and the post-encounter phase occurred for 4 min, allowing for discrimination of each individual's calls. Differences in phase length were based on pilot studies used to determine how long individuals were likely to call. Following testing, animals were removed from the arenas and returned to their standard cage.

#### *Behaviour Analysis*

Video recordings were scored for aggressive behaviours by an observer blind to the sex of the animal. The number of aggressive behaviours were counted and parsed out into either offensive (bites to the flank, chases and wrestling bouts), or defensive (bites to the neck, rearing up and boxing/jabbing) behaviours (Blanchard, Wall, & Blanchard, 2003) and scored as frequency of occurrence for each specific behaviour. Submissive behaviours (retreats, jumps away and freezes) were also counted as frequency of occurrence (Pultorak et al., 2015). A winner was designated as a mouse that conducted three consecutive attacks that elicited losing behaviours by its opponent (Fuxjager & Marler, 2009; Fuxjager, Montgomery, Becker, & Marler, 2010; Oyegbile & Marler, 2006). A winner index was also calculated to assess the difference between aggressive and submissive behaviours as a function of total behaviour for each individual:  $\frac{\text{aggressive behaviour} - \text{submissive behaviour}}{\text{aggressive behaviour} + \text{submissive behaviour}}$  (Fuxjager et al., 2010). The winner index allows for a finer-grain analysis of the efficiency by which an individual wins an aggressive encounter and better reveals individual variation across winners.

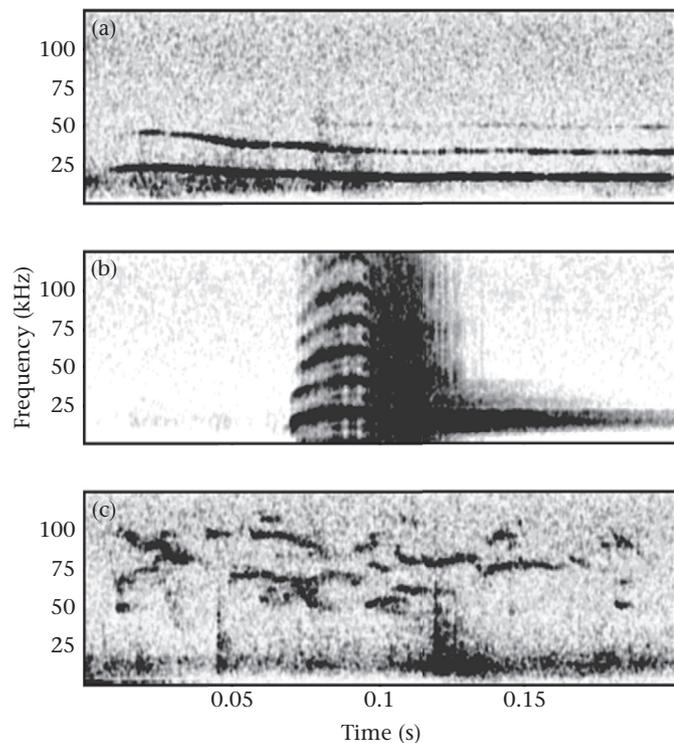
#### *Ultrasonic Vocalization Analysis*

Ultrasonic vocalizations were recorded using two Emkay/Knowles FG series microphones (detection range 10–120 kHz) placed in opposite corners of the arena 55 cm apart and 20 cm from the arena floor, with one microphone in each of the resident and intruder compartments. To control for potential differences in microphone sensitivity, microphone placement was randomized across trials between the resident and intruder sides of the arena. Microphone channels were calibrated to equal gain (–60 dB noise floor). Recorder software (Avisoft Bioacoustics, Berlin, Germany) was used to produce WAV file recordings for each of the four encounter stages in all trials. Recordings were made using a 250 kHz sampling rate with 16-bit resolution. Spectrograms were produced with a 512 fast Fourier transform (FFT) using Avisoft-SASLab Pro sound analysis software (Avisoft Bioacoustics).

Ultrasonic vocalizations were differentiated by visual and auditory inspection of spectrograms and WAV files with the sampling rate reduced to 4% of real-time speed for auditory inspection (11025 kHz). All calls were attributed to either the resident or the intruder based on visual amplitude differences seen in the spectrogram during phases when the divider was in place. In the rare case that there was ambiguity in call origin, time of arrival of the start of the ultrasonic vocalization was compared between channels (accuracy up to 0.0001 s); this occurred in <4% of all calls analysed. In cases where no divider was present (encounter phase) and interacting animals were close together (generally in the centre of the arena) calls were analysed as a dyad. All calls were analysed by an experienced observer blind to the sex and residency status of the focal mouse.

#### Ultrasonic Vocalization Terminology and Structure

Three categories of ultrasonic vocalization calls were analysed in this study (Fig. 1), two of which, sustained vocalizations and barks, were defined based on previous work focusing on California mouse ultrasonic vocalizations (Kalcounis-Rueppell et al., 2010; Pultorak et al., 2015). Sustained vocalizations are low-bandwidth calls that feature low modulation with a peak frequency around 20 kHz and a duration of 100–500 ms for each individual syllable. In our laboratory setting, a singular bout of sustained vocalizations included up to 13 calls, with an intersyllable interval of 150 ms (intersyllable intervals >150 ms denote the start of a new bout; Kalcounis-Rueppell et al., in press). In this study, we analysed sustained vocalizations in two ways, first by counting each individual call and second by counting the number of calls per bout produced by an individual. The length of individual calls was measured to determine whether differences correspond with different aspects of social behaviour as described



**Figure 1.** Representative spectrograms of three ultrasonic vocalization calls: (a) sustained vocalization (SV); (b) bark; (c) sweep phrase.

in both field and laboratory studies (Kalcounis-Rueppell et al., 2010; Pultorak et al., 2015). Barks are relatively short, high-amplitude calls, with an upside down chevron shape that begins and ends in the audible range, usually occurring in phrases of more than one bark (Kalcounis-Rueppell et al., 2006, in press). Sweep phrase calls are previously undescribed calls with durations of 30–100 ms, made up of continuous and overlapping frequency-modulated calls, with sweeps modulated upward and downward, similar to frequency jump calls in mice (Arriaga & Jarvis, 2013; Chabout, Sarkar, Dunson, & Jarvis, 2015; Hoffman et al., 2012; Kalcounis-Rueppell et al., in press). Compared to other California mouse calls sweep phrases are highly complex with a wide bandwidth from 25 to 100 kHz, with multiple inflection points over a short duration and periods of silence of at least 30 ms both before and after the call (for examples of each call type, see [Supplementary Audios S1–S3](#)).

#### Statistics

All analyses were performed using SPSS (SPSS version 22, IBM Corp., Armonk, NY, U.S.A.). One male and two females were excluded from analyses because they did not perform any aggressive or submissive behaviours and produced no vocalizations throughout the paradigm, giving a final sample size of 11 male and 10 female resident–intruder dyads. Aggressive and submissive behaviours and ultrasonic vocalization calls per minute were  $\log(x + 1)$ -transformed to correct for non-normal data. We analysed offensive and defensive aggression (measured as frequencies) and attack latencies using two-way ANOVA with sex and residency as factors, and we compared winner indices between males and females using a Student's *t* test. We first analysed sustained vocalizations and bark calls across phases at the level of the dyad (to control for the inability to triangulate calls during the encounter phase as calls produced per minute) using a repeated measures ANOVA with sex and phase as factors. Where calls could be assigned to an individual, we then compared call production between resident and intruder males and females using a two-way repeated measures ANOVA with sex and residency as between-subject factors and phase as a within-subject factor. Sustained vocalization calls per bout were analysed using a chi-square test on pooled calls within a dyad. Sweep phrases were compared both within and between sexes as proportions of total calls produced during each phase using chi-square and Fisher's exact tests because of the small sample sizes. Tukey post hoc tests were used to correct for multiple statistical tests. Correlations between ultrasonic vocalization calls and aggressive behaviours were calculated using linear regression on  $\log(x + 1)$ -normalized data. The alpha level for all analyses was set at 0.05.

## RESULTS

#### Male and Female Resident Aggressive Behaviour

Overall, male ( $N = 11$ ) and female ( $N = 10$ ) residents were remarkably similar in their aggression levels. Resident males and females were equally likely to win a given same-sex encounter (11 of 11 males, 9 of 10 females; Fisher's exact test:  $P = 0.48$ ) and showed no difference in overall winner index (males:  $0.88 \pm 0.08$ ;  $P = 0.35$ ), offensive aggression (males:  $10.0 \pm 2.47$ ; females:  $6.50 \pm 1.21$ ; ANOVA:  $F_{1, 20} = 0.973$ ,  $P = 0.33$ ) or defensive aggression (males:  $0.09 \pm 0.08$ ; females:  $0.40 \pm 0.18$ ; ANOVA:  $F_{1, 20} = 1.32$ ,  $P = 0.26$ ) towards same-sex conspecifics. There was also no

significant sex difference in residents' submissive behaviours (males:  $0.36 \pm 0.28$ ; females:  $1.40 \pm 0.50$ ; ANOVA:  $F_{1, 20} = 0.02$ ,  $P = 0.89$ ). Moreover, there were no size differences between male ( $40.65 \pm 1.71$  g) and female ( $44.87 \pm 3.42$  g) residents (ANOVA:  $F_{3, 34} = 1.08$ ,  $P = 0.29$ ) or between residents ( $N = 21$ ,  $42.76 \pm 1.93$  g) and intruders ( $N = 21$ ,  $43.49 \pm 2.46$  g) (ANOVA:  $F_{1, 41} = 0.22$ ,  $P = 0.83$ ).

There were no sex differences exhibited by intruder males ( $N = 11$ ) or females ( $N = 10$ ) in the display of offensive aggression (males:  $2.91 \pm 0.65$ ; females:  $3.5 \pm 0.54$ ; ANOVA:  $F_{3, 34} = 0.01$ ,  $P = 0.9$ ), defensive aggression (males:  $6.45 \pm 2.88$ ; females:  $13.7 \pm 5.23$ ;  $F_{3, 34} = 0.87$ ,  $P = 0.39$ ) or submissive behaviours (males:  $11.75 \pm 3.53$ ; females:  $8.50 \pm 1.95$ ; ANOVA:  $F_{3, 34} = 0.37$ ,  $P = 0.72$ ) during the encounter.

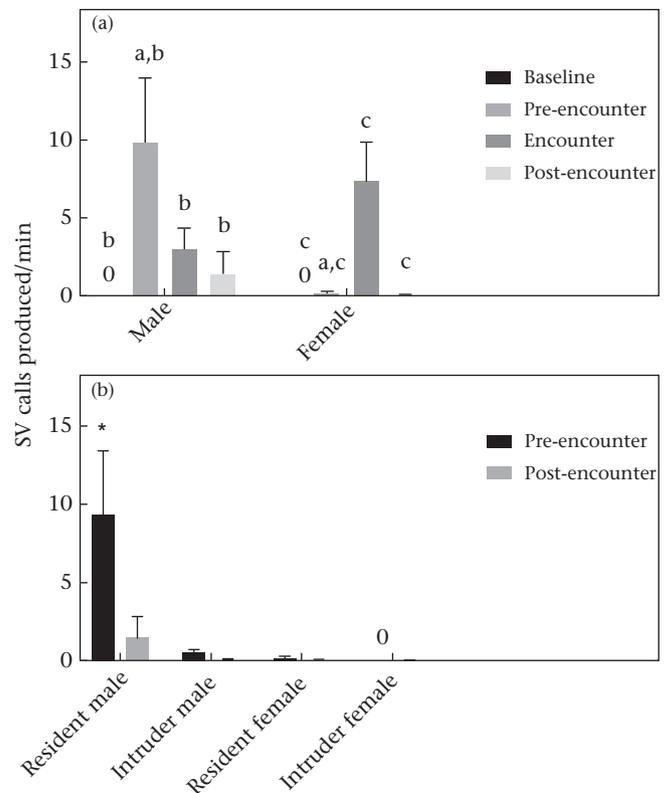
When comparing all residents ( $N = 21$ ) and intruders ( $N = 21$ ) and controlling for sex, residents displayed significantly more offensive aggression than intruders (residents:  $8.33 \pm 1.40$ ; intruders:  $3.19 \pm 0.42$ ; ANOVA:  $F_{1, 41} = 11.70$ ,  $P < 0.01$ ), while intruders displayed significantly more defensive aggression (residents:  $0.24 \pm 0.11$ ; intruders:  $9.90 \pm 3.06$ ;  $F_{1, 41} = 8.96$ ,  $P < 0.01$ ) and more submissive behaviours than residents (residents:  $0.86 \pm 0.25$ ; intruders:  $9.24 \pm 1.73$ ;  $F_{1,41} = 35.99$ ,  $P < 0.01$ ).

### Ultrasonic Vocalization Analysis

#### Sustained vocalizations

When analysing calls at the level of the dyad, we found changes in sustained vocalization production both between and within sexes over the four encounter phases: baseline, pre-encounter, encounter and post-encounter. There was a significant change in sustained vocalization production across phases by sex (ANOVA:  $F_{1, 17} = 5.26$ ,  $P < 0.01$ , partial-eta squared = 0.454). Specifically, post hoc tests showed that male dyads ( $N = 11$ ) produced significantly more sustained vocalizations than female dyads ( $N = 10$ ) in the pre-encounter stage (males:  $9.84 \pm 4.15$ ; females:  $0.15 \pm 0.15$ ; ANOVA:  $F_{1, 18} = 5.26$ ,  $P < 0.01$ ). This sex difference disappeared in the encounter phase when female sustained vocalization production increased to male levels (males:  $3.01 \pm 1.35$ ; females:  $7.36 \pm 2.5$ ; ANOVA:  $F_{1, 18} = 0.18$ ,  $P = 0.976$ ).

Within sexes, both males and females showed changes across the four phases ( $F_{1,18} = 9.15$ ,  $P < 0.01$ , partial eta<sup>2</sup> = 0.591; Fig. 2a). Using post hoc tests we found that, for male dyads ( $N = 11$  pairs), the presence of a same-sex intruder behind a divider elicited a significant increase in sustained vocalizations compared to baseline (baseline:  $0 \pm 0$ ; pre-encounter:  $9.84 \pm 4.15$ ;  $P = 0.004$ ). Male sustained vocalization production remained at pre-encounter levels during the encounter phase (pre-encounter:  $9.84 \pm 4.15$ ; encounter:  $3.01 \pm 1.35$ ;  $P = 0.266$ ). In contrast, female dyads ( $N = 10$ ) did not differ from baseline in pre-encounter sustained vocalization production, with only one female producing sustained vocalization calls during this phase (baseline:  $0 \pm 0$ ; pre-encounter:  $0.15 \pm 0.15$ ;  $P = 0.339$ ). Females significantly increased sustained vocalization production during the encounter phase compared to the pre-encounter phase (encounter:  $7.36 \pm 2.50$ ; pre-encounter:  $0.54 \pm 0.50$ ;  $P = 0.015$ ), equalling the sustained vocalization production of males. During the post-encounter stage, male sustained vocalization production decreased to baseline levels (post-encounter:  $0.05 \pm 0.05$ ;  $P = 0.31$ ). Vocalizations were then tested at the level of the individual for the two phases, pre-encounter and post-encounter in which both individuals were present and calls could be assigned to a specific individual. Resident males ( $N = 7$  of 11) were significantly more likely to produce sustained vocalization calls during the pre-encounter stage than resident females ( $N = 1$  of 10) (Fisher's exact test:  $P < 0.01$ ). Resident males also produced

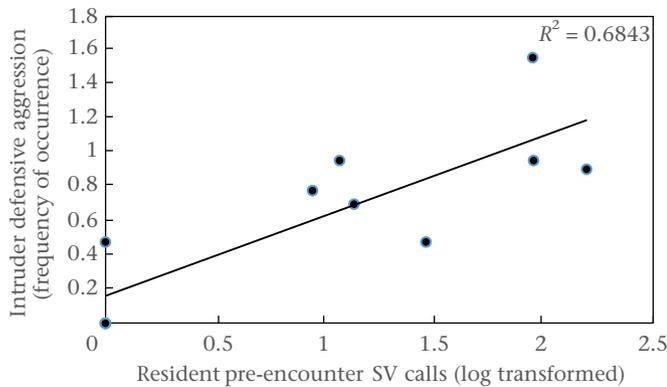


**Figure 2.** All analyses were completed on  $\log(x + 1)$ -transformed calls/min to control for unequal testing times. Bars represent raw means and SEM. (a) Production of sustained vocalization (SV) calls across phases within a dyad. SV calls within each phase were pooled by dyad ( $N = 11$  male dyads,  $N = 10$  female dyads). (b) Production of SV calls by male residents ( $N = 11$ ), intruder males ( $N = 11$ ), resident females ( $N = 10$ ) and intruder females ( $N = 10$ ) during pre-encounter and post-encounter phases. Statistical significance ( $P < 0.01$ ) is denoted by letters above bars in (a) and by an asterisk in (b).

significantly more sustained vocalizations per minute than any other group at either time point (resident males: pre-encounter:  $9.31 \pm 4.12$ ; post-encounter:  $1.43 \pm 1.41$ ; intruder males ( $N = 11$ ): pre-encounter:  $0.53 \pm 0.21$ ; post-encounter:  $0.05 \pm 0.05$ ; resident females: pre-encounter:  $0.15 \pm 0.15$ ; post-encounter:  $0.05 \pm 0.05$ ; intruder females ( $N = 10$ ): pre-encounter:  $0.00 \pm 0.00$ ; post-encounter:  $0.03 \pm 0.03$ ; Fig. 2b).

#### Sustained vocalization calls and aggressive behaviour

Several lines of evidence suggest that the number of sustained vocalization calls produced by male residents during the pre-encounter stage was associated with greater aggression during the encounter stage. Most notably, the number of pre-encounter sustained vocalization calls produced by resident males was positively correlated with increased defensive aggression (rearing and boxing) in intruders (linear regression:  $F_{1, 9} = 19.5$ ,  $R^2 = 0.68$ ,  $P < 0.01$ ; Fig. 3) but not with resident offensive aggression ( $F_{1,9} = 0.31$ ,  $R^2 = 0.04$ ,  $P = 0.59$ ). Intruders who were not exposed to sustained vocalization calls during the pre-encounter stage ( $N = 4$ ) did not display any defensive aggression (i.e. no boxing or rearing). There was a nonsignificant tendency for a weak correlation between pre-encounter sustained vocalization calling and a faster latency to aggression in males (linear regression:  $F_{1, 7} = 2.04$ ,  $R^2 = 0.17$ ,  $P = 0.15$ ), hinting at a role for sustained vocalization calling in territorial advertisement or in the resident's motivation to fight. Finally, pre-encounter sustained vocalization calls were correlated with number of barks produced



**Figure 3.** Relationship between frequency of intruder defensive aggression and residents' production of ultrasonic vocalization (SV) calls (linear regression:  $P < 0.01$ ). Log( $x + 1$ )-transformed data points are displayed for male resident SV calls and subsequent defensive behaviour.

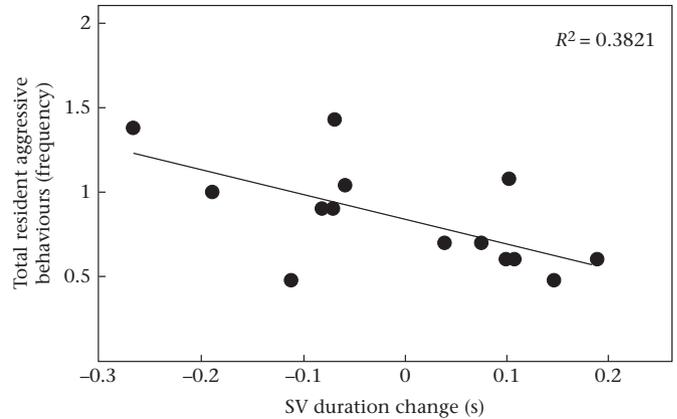
during the subsequent encounter phase (linear regression:  $F_{1,10} = 5.097$ ,  $R^2 = 0.384$ ,  $P = 0.04$ ). In summary, pre-encounter sustained vocalization calling behaviour by residents was associated with greater levels of defensive behaviour in the intruders.

#### Sustained vocalization call duration

Significant changes in sustained vocalization call duration were found both between phases and between sexes. In resident males, pre-encounter sustained vocalization call duration was analysed within subjects such that the first 50% of calls produced by the resident were compared to the second 50%. The magnitude of durational change was correlated with total aggression displayed by residents (linear regression:  $F_{1,5} = 12.95$ ,  $N = 7$ ,  $R^2 = 0.7215$ ,  $P = 0.012$ ). Pre-encounter sustained vocalization call duration did not differ between male residents and intruders ( $N = 7$  residents, 403 calls:  $184.83 \pm 18.5$  ms;  $N = 5$  intruders, 30 calls:  $166.3 \pm 72.0$  ms; Student's  $t$  test:  $t_{430} = 0.29$ ,  $P = 0.81$ ). Only one female resident produced sustained vocalization calls during the pre-encounter stage of similar average duration as male residents' calls (males:  $N = 403$  calls,  $184.83 \pm 18.5$  ms; female:  $N = 6$  calls,  $137.3 \pm 56.1$  ms; Student's  $t$  test:  $t_{407} = 0.90$ ,  $P = 0.71$ ). Sustained vocalization call durations were significantly shorter during the encounter phase than during the pre-encounter phase in male dyads (pre-encounter:  $N = 433$  calls,  $183.54 \pm 20.5$  ms; encounter:  $N = 282$  calls,  $104 \pm 4.10$  ms; Student's  $t$  test:  $t_{713} = 4.85$ ,  $P < 0.01$ ). Males dyads also had significantly shorter sustained vocalization call durations during encounters than female dyads (males:  $N = 282$  calls,  $104 \pm 4$  ms; females:  $N = 587$  calls,  $125 \pm 3$  ms; Student's  $t$  test:  $t_{867} = 4.07$ ,  $P = 0.013$ ). Furthermore, a linear regression showed that the magnitude of the change in sustained vocalization call duration in dyads, pre-encounter to encounter, predicted total aggression during the encounter phase ( $F_{1,12} = 6.794$ ,  $R^2 = 0.3818$ ,  $P = 0.024$ ; Fig. 4).

#### Sustained vocalization bouts

Analysis of sustained vocalization bout length across phases at the level of the dyad found that phrases of up to 11 syllables were produced in the pre-encounter phase with the most common bout length being one-call bouts (33%; chi-square test:  $\chi^2_{10} = 26.65$ ,  $P = 0.03$ ) followed by two- and three-call bouts (21% each) and four-call bouts (10%), with all other bouts comprising less than 15% of the total bouts. Sex differences could not be examined for the pre-encounter phase because only one female produced any sustained vocalization calls.

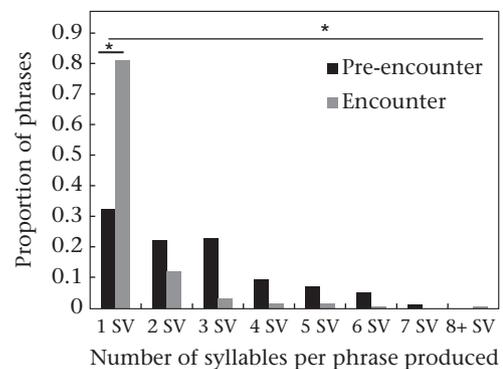


**Figure 4.** Change in duration of sustained vocalization (SV) calls from the pre-encounter phase to the encounter phase within a dyad ( $N = 14$ ) relative to total aggressive behaviour shown by residents (linear regression:  $P = 0.024$ ). Shorter SVs during the encounter stage, as denoted by negative values, indicate a higher frequency of aggressive behaviours by residents, whereas longer SV calls indicate lowered aggression.

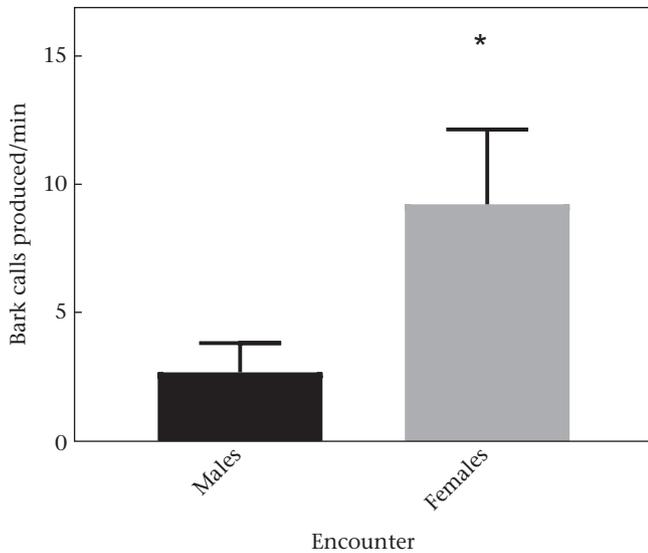
During the encounter phase, one-call bouts were also the most common bout length for both sexes (81% of bouts; Fisher's exact test:  $P < 0.01$ ). However, a significantly greater percentage of one-call bouts were produced by dyads during the encounter phase (81%) than during the pre-encounter phase (33%) (Fisher's exact test:  $P < 0.01$ ; Fig. 5). Two-call bouts were the next most common bout length (12%), with all other bout lengths making up only 7% of the total sustained vocalization vocalizations. The maximum sustained vocalization bout length observed in both the pre-encounter and encounter phase was 11 calls, and no sex differences were detected in either length or proportion of sustained vocalization bouts during encounters (chi-square test:  $\chi^2_7 = 4.0$ ,  $P = 0.41$ ).

#### Barks

A sex difference was found in the production of barks during the encounter phase such that female dyads ( $N = 10$ ) produced more barks than male dyads ( $N = 11$ ) (females:  $9.22 \pm 3.47$ ; males:  $2.68 \pm 1.15$ ;  $F_{1,21} = 1.79$ ,  $P = 0.046$ , partial  $\eta^2 = 0.194$ ; Fig. 6). Controlling for sex, bark production changed across phases for both male and female dyads such that barks were produced most often during the encounter phase than during all other phases (encounter:  $N = 21$ ,  $5.79 \pm 1.26$ ; all other phases:  $0.02 \pm 0.02$ ; ANOVA:  $F_{1,21} = 32.127$ ,  $P < 0.001$ , partial  $\eta^2 = 0.594$ ).



**Figure 5.** Proportion of sustained vocalization (SV) bout lengths across phases. Data within dyads were pooled across sexes for pre-encounter ( $N = 8$  dyads) and encounter ( $N = 18$  dyads) bout lengths. Bars represent the proportion of bout lengths compared to the whole. \* $P < 0.05$ .



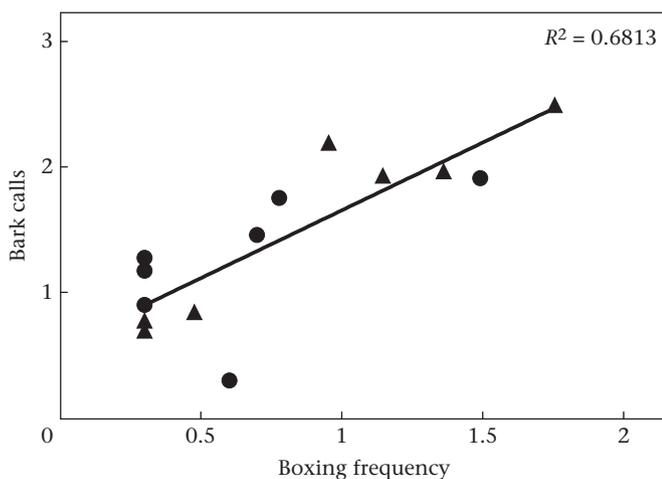
**Figure 6.** Number of bark calls produced/min by female dyads ( $N = 10$ ) and male dyads ( $N = 11$ ). Analyses were done on  $\log(x + 1)$ -transformed data. Raw means and SEM are shown. \* $P = 0.046$ .

#### Barks and aggressive behaviour

Barks only occurred following the onset of physical aggression, indicating that barks are intrinsically tied to physical aggression. Barks correlated strongly with frequency of occurrence of intruder boxing behaviour, a form of defensive aggression (linear regression:  $F_{1,21} = 48.07$ ,  $R^2 = 0.6923$ ,  $P < 0.01$ ; Fig. 7) occurring most often when intruder mice were in the reared-up position and boxing at the resident. We confirmed that barks were produced by intruders in this position in 8 of 17 dyads from video recordings, by hearing the bark while being able to observe the mouse opening its mouth while expelling air (see [Supplementary Video S1](#)). In the other cases, animals were generally blocked from view by the resident mouse or too far away from the camera to see this behaviour clearly.

#### Sweep phrases

Sweep phrases were produced by 6 of 11 male residents and 3 of 10 female residents. Sweep phrases occurred as stand-alone



**Figure 7.** Relationship between boxing frequency and bark call production (linear regression:  $P < 0.01$ ).  $\log(x + 1)$  data points are displayed for males (circles) and females (triangles).

calls with distinct periods of silence both before and after the call for a minimum of 30 ms. The frequency range for these calls was between 25 kHz and 100 kHz and had a duration of at least 30 ms. Over this time course, sweep phrases included a minimum of three inflection points compared to individual sweeps, which include no more than one inflection point (Kalcounis-Rueppell, Pultorak, & Marler, in press). Because of the small sample size, we calculated statistics for sweep phrases based on their relative proportion of total calls and calls per phase (see [Supplementary Table S1](#)). Likelihood of producing sweep phrases did not differ between resident male and female mice (6 of 11 males, 3 of 10 females; Fisher's exact test:  $P = 0.21$ ). When calculated across all phases at the level of the dyad, males produced a significantly greater proportion of sweep phrases than females (males: 6.24% of all calls; females: 1.24% of all calls; Fisher's exact test:  $P < 0.01$ ). However, females produced a greater proportion of sweep phrases than males within the pre-encounter (males: 9.03% of calls; females: 66.66% of calls; Fisher's exact test:  $P < 0.01$ ) and post-encounter phases (males: 26.14% of calls; females: 62.5% of calls; Fisher's exact test:  $P < 0.01$ ). The number of sweep phrases produced during pre-encounter and post-encounter phases were highly correlated (Pearson's correlation:  $r_8 = 0.718$ ,  $P = 0.012$ ). There were no significant differences in either offensive or defensive aggression or submissive behaviours based on whether residents produced sweep phrase calls (ANOVA:  $F_{1, 21} = 0.29$ ,  $P > 0.81$ ).

## DISCUSSION

Our findings reveal the repertoire of ultrasonic vocalizations used by the monogamous California mouse during same-sex resident–intruder encounters including sustained vocalizations, barks and a newly identified complex call type, sweep phrases. In addition, relatively similar levels of aggressive behaviour were expressed between the sexes as would be predicted in a monogamous species. In contrast, however, sex differences were revealed in detailed analyses of vocalizations. Only males gave sustained vocalizations earlier during the encounters prior to physical aggression, and they produced shorter sustained vocalizations than females during physical encounters. Females on the other hand, produced more barks during physical aggression than males. Finally, our results suggest that modulation of the duration of sustained vocalization calls is predictive of increased escalation of aggression in subsequent male–male encounters, while barks appear to play a role in the defensive behaviours of intruders during same-sex encounters (Fig. 7) and infrequent sweep phrases occur primarily during pre-encounters.

#### Vocalizations Across the Different Phases of a Same-sex Encounter

All ultrasonic vocalization calls were produced in greater numbers when a conspecific was present. Sustained vocalization calls increased in males when acoustic, chemical and visual contact were possible during the pre-encounter. With the addition of physical contact, sustained vocalization call production remained steady in males and was increased in females. Sustained vocalization calls then dropped to baseline levels during the post-encounter phase. For barks, almost no calls were produced until physical contact had been made during the encounter period, and bark calling ceased during the post-encounter phase. Sweep phrases were present in a subset of males and females during the pre-encounter phase, were reduced as a proportion of total calls during the encounter phase, and then increased as a proportion of total calls during the post-encounter phase ([Supplementary Table S1](#)).

Of these calls, sustained vocalizations (Briggs & Kalcounis-Rueppell, 2011; Kalcounis-Rueppell et al., 2006; Pultorak et al., 2015) and barks (Briggs & Kalcounis-Rueppell, 2011; Kalcounis-Rueppell et al., 2010; Pultorak et al., 2015) have previously been identified in both the laboratory and field. Sustained vocalizations have been recorded from isolated individuals in the field (Briggs & Kalcounis-Rueppell, 2011; Petric & Kalcounis-Rueppell, 2013) and also during social interactions in the field (Kalcounis-Rueppell, Metheny, & Vonhof, 2006) and laboratory, indicating that these calls likely have multiple functions. Specifically, this laboratory study found that sustained vocalizations are used in aggression while previous laboratory studies have shown that sustained vocalizations also occur frequently in affiliative interactions and when a pair is briefly separated and then reunited (Pultorak et al., 2015; Pultorak, Alger, Loria, Johnson, & Marler, n.d.). Barks have been limited to aggressive encounters in laboratory studies, including the current study and others (Pultorak et al., 2015, 2017). The function of barks appears to be restricted to aggressive interactions, and here we find that these calls are related to defensive rather than offensive aggression. Finally, the production of sweep phrases before and after encounters may suggest a function associated with either general territorial advertisement, but they are only produced by a subset of individuals.

#### *Function of Vocalizations and Levels of Aggression*

We can add to our speculation about the function of these different calls by examining physical interactions. Both male and female residents showed a similar aptitude for winning fights against same-sex intruders as would be expected of a strictly monogamous species (Colombelli-Négrel, 2016; Fedy & Stutchbury, 2005; Koloff & Mennill, 2011). The number of aggressive behaviours produced by males and females were not significantly different, suggesting a general similarity in aggression levels.

There were also no sex differences between the social interactions of residents and intruders, but there were differences in types of physical aggression between residents and intruders. Residents displayed more overall aggression and, specifically, more offensive attacks than intruders, including chasing, wrestling and bites to the flank or belly (Blanchard et al., 2003). In male dyads, sustained vocalization duration shortened during the pre-encounter stage, and the change in duration from the pre-encounter stage to the encounter stage correlated with increased offensive aggression by residents. This provides evidence that modulation of sustained vocalization calls may play a role in the escalation of aggression, or territorial or motivational displays prior to fighting. In contrast, intruders showed little overall aggression, mainly using defensive aggression including rearing up and boxing, which act to protect the flank and other vital areas of an individual being aggressed against (Blanchard & Blanchard, 1977). Barks seem to play an important role in defensive aggression as these vocalizations were highly correlated with boxing, were audible during the display of defensive behaviours during which the mouth was visibly open.

#### *Sustained Vocalization Calls: Sex Differences, Modulation and Function*

Neither male nor female residents produced sustained vocalization calls while isolated in this paradigm, indicating that, similar to previous findings in the laboratory (Pultorak et al., 2015), the presence of another individual helps to elicit sustained vocalization calls. Additional functions of sustained vocalization calls are likely to be established because male and

female residents in the field produce calls in isolation (Kalcounis-Rueppell, Petric, Briggs, & Carney, Marshall, & Willse, 2010). The onset of sustained vocalization calling differed based on sex, with males producing sustained vocalization calls in the pre-encounter phase, while females did not begin producing sustained vocalization calls until physical interaction occurred. We speculate that overall pre-encounter sustained vocalization calling acts as a territorial advertisement or as a signal of motivation to fight an intruder. This would indicate a sex difference similar to aggressive advertisement calls seen in other species such as red deer, *Cervus elaphus* (Reby et al., 2005) and frogs and toads (Reichert & Gerhardt, 2013). Alternatively, pre-encounter sustained vocalization calls could be a signal to the nonpresent mate that an intruder is present. As in primates (Gouzoules, Gouzoules, & Marler, 1984; Slocombe & Zuberbühler, 2007), these calls could recruit the mate to help defend the territory or act as a warning for the mate to stay in the nest. Like birds, pre-encounter sustained vocalization calls would then be expected to increase as intruders move closer to the nest (Hau, Wikelski, Soma, & Wingfield, 2000; Levin & Wingfield, 1992); however, we did not test for this function.

These changes in sustained vocalization duration and bout length prior to and during aggression indicates that, like other species (Alger et al., 2016; Pasch, George, Hamlin, et al., 2011; Weerts, Miczek, & Miczek, 1996), male California mice modulate the duration of their calls based on social context. Previously it has been shown that sustained vocalization duration in male California mice is positively correlated with female approach behaviour (Pultorak et al., 2017). In the current study, the shortening of sustained vocalization calls within male dyads correlated with increased resident aggression. This shortening of sustained vocalization calls in male dyads may indicate greater motivation to aggress if these calls are being produced by residents, as greater shortening of calls indicated both increased offensive aggression by residents and increased defensive behaviours by intruders. Similarly, squirrel monkeys, *Saimiri sciureus*, shorten the duration of their peep calls to display dominance (Weerts et al., 1996). Conversely, neotropical singing mice add trills to their song and produce longer overall songs that correlate with greater aggression. This is controlled by testosterone, with castrated mice producing shorter calls and displaying less aggression than testosterone-implanted controls (Pasch, George, Campbell, & Phelps, 2011; Pasch, George, Hamlin, et al., 2011). We speculate, but have not tested, whether testosterone may also shorten sustained vocalization call duration since it increases aggression in California mice (Fuxjager et al., 2011; Oyegbile & Marler, 2005; Trainor, Bird, & Marler, 2004). Overall, we have evidence that duration of individual calls and bout length are important spectral parameters in California mouse behaviour, likely more so than total calls alone.

#### *Barks Act as Aggressive Signals*

Barks are the lowest-frequency call produced by California mice and they start in the audible range, making them similar to broadband calls produced by Siberian hamsters during aggression (Keesom et al., 2015). Like barks in California mice, Siberian hamsters increase their proportion of broadband calls during aggression, but, unlike hamsters, female California mouse dyads showed an increase in bark calls during aggressive encounters compared to males. The reason for this sex difference remains unknown, as females did not show increased defensive aggression compared to males. Similar to rats (Knutson, Burgdorf, & Panksepp, 2002; Riede, 2013), we speculate that low-frequency barks of California mice are produced more by intruders than by

residents because intruders performed more defensive aggression than residents and defensive aggression correlated highly with barks (Fig. 7). Moreover, anecdotal visual and auditory inspection of dyads during aggressive encounters showed intruders producing barks while engaged in boxing behaviour (see [Supplementary Video S1](#)). This likely indicates a stress response or subordinate behaviour to being aggressed against, which would be consistent with the calls only occurring after the onset of physical aggression. Overall, this corroborates previous findings that low-frequency calls in rodents are closely tied to aggressive behaviours.

### Sweep Phrases

We discovered a new, complex call type for California mice that we termed sweep phrases. These calls show a level of complexity in frequency modulation and structure that has yet to be seen in this species. Sweep phrases were mostly seen in the pre- and post-encounter phases making up 9–67% of the total calls produced by males and females within the pre-encounter and post-encounter phases, but were produced less during aggressive interactions and not at all in isolation. Females produced a greater proportion of sweep phrases in the pre-encounter and post-encounter phases than males. As such, sweep phrases appear to be induced by the presence of a conspecific. These calls may function as advertisements of motivation to defend territory by a resident, similar to songbirds (Cain & Langmore, 2015; Wacker, Coverdill, Bauer, & Wingfield, 2010). This is further supported by the fact that only resident mice produced these calls during the pre- and post-encounter phases. Thus, this may indicate that life history (i.e. territory ownership or bonding) is important to the production of these calls.

These calls seem to be similar in complexity to those produced by songbirds (Nowicki & Searcy, 2004), the trill-based calls of rats (Riede, 2013), trilled songs of neotropical singing mice (Pasch, George, Campbell, et al., 2011) and frequency jump calls of house mice (Hammerschmidt, Radyushkin, Ehrenreich, & Fischer, 2012; Hoffman et al., 2012; von Merten et al., 2014). Similar to the 22 kHz trill calls of rats and the songs of neotropical singing mice, sweep phrases show constant upward and downward modulation, however the duration of rat trills are much shorter than sweep phrases and both rat trills and singing mouse songs are of a much lower frequency (Pasch, George, Hamlin, et al., 2011; Riede, 2013). Sweep phrases are more similar in frequency to mouse frequency jumps that occur at frequencies greater than 45 kHz (von Merten et al., 2014). However, much still remains unknown about California mouse sweep phrases (as with sustained vocalizations) including whether sweep phrases include repeatable motifs or whether there is a learning component of these calls as in birdsong (Arriaga & Jarvis, 2013; Chabout et al., 2015). Currently, similar to mice, it appears that these high-frequency ultrasonic vocalization calls do not play a major role in aggression. However, further study in multiple social contexts is ongoing to elucidate the function of these calls as well as their importance to factors such as individual recognition, territorial defence and mate acquisition.

### Integration of Sustained Vocalizations and Barks with Rodent Aggressive Vocalizations

In the rat literature there has been an emphasis placed on interpreting 22 kHz calls as signifying a negative affect (Brudzynski & Holland, 2005; Burgdorf et al., 2008; Kim, Kim, Covey, & Kim, 2010; Knutson et al., 2002). In particular, 22 kHz calls are produced by rats in response to aversive stressful events,

including male–male aggression (Burgdorf et al., 2008; Sales, 1972), social defeat (Kroes et al., 2007), predator exposure (Blanchard, Blanchard, Agullana, & Weiss, 1991; Litvin, Blanchard, & Blanchard, 2007) and fear response (Choi & Brown, 2003; Kim et al., 2010). While California mice have calls within their vocal repertoire that are similar to calls of both rats and house mice, the majority of call types described to this point are more similar to rat vocalizations. This vocal similarity is consistent with the knowledge that *Peromyscus maniculatus* share more similarities at the genome level to rats than to mice (Ramsdell et al., 2008). This is particularly true of sustained vocalization calls and barks, which play a role during aggressive encounters and are more similar to the 22 kHz calls in rats than to the ultrasonic vocalization calls in mice (Briggs & Kalcounis-Rueppell, 2011; Brudzynski, 2013).

Sustained vocalization calls in particular share similar spectral properties with 22 kHz rat calls, including a long duration and low modulation (Briggs & Kalcounis-Rueppell, 2011; Brudzynski & Holland, 2005). Rat calls exhibit only slight variations in frequency (Brudzynski & Holland, 2005; Wöhr & Schwarting, 2013), and call duration is bimodally distributed between short and long 22 kHz calls (Vivian & Miczek, 1993). Similarly, California mice in the present study showed little variation in frequency or duration of sustained vocalization calls. While sustained vocalizations of California mice are similar to 22 kHz rat calls during encounters (likely indicating stress or fear), prior to an interaction, changes in sustained vocalization duration are predictive of future aggressive behaviour. This greater complexity in function is further illustrated by longer-duration sustained vocalizations being produced during nonaversive social contexts including courtship (Pultorak et al., 2015) and reunion (Pultorak et al., 2017). As such, sustained vocalizations likely convey more information to the receiver than simply the affective state of the signaller as they occur prior to and predict aggressive behaviour.

Barks have similarities with both hamster broadband calls and 22 kHz rat calls. Functionally, broadband calls and barks both increase during aggression. In California mice, barks were produced more by females than by males but only occurred after the onset of physical aggression and were closely tied to defensive behaviour. This is similar to broadband call production in hamsters, which increases during aggressive encounters but does not show a sex difference (Keesom et al., 2015). In rats, intruders that have been previously aggressed against will increase 22 kHz calls, resulting in a decrease in aggressive behaviours expressed by the resident (Kroes et al., 2007; Lore, Flannelly, & Farina, 1976; Wöhr & Schwarting, 2013). We therefore speculate that coupling barks with jabbing may help the intruder prevent the resident from attacking. This indicates that barks occur as a reaction to an aversive stimulus and therefore may, as in rats (Portfors, 2007), indicate a negative affective state of the signaller.

Overall, we studied the production and function of ultrasonic vocalizations during territorial defence in a monogamous species. We saw considerable similarities between the sexes in both the expression of aggression towards a same-sex intruder and the overall vocal repertoire; however, several sex differences were found in vocal communication. Females produced more barks that were tightly associated with defensive aggression for both sexes (see Results, Fig. 7). Furthermore, females produced a greater proportion of sweep phrases in the pre-encounter and post-encounter stages. Males produce more sustained vocalization calls than females prior to physical aggression, which may indicate that males play a greater role in territorial advertisement than females. Moreover, males have shorter sustained vocalization calls than females during physical encounters, and the shortening of sustained vocalization calls predicts future

escalation of aggression. This study expands the hypothesized functions for sustained vocalizations beyond long-distance communication and courtship to include aggression (Briggs & Kalcounis-Rueppell, 2011; Pultorak et al., 2015). It is, as yet, unknown what factors may control sustained vocalization plasticity, but testosterone (Inagaki & Mori, 2014) and the ascending mesolimbic cholinergic system (Brudzynski, 2014) play important roles in 22 kHz call production and in conveying affective state, making them likely candidates. Further exploring this plasticity in other social contexts such as male–female parent–offspring interactions is warranted.

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## Supplementary material

Supplementary material associated with this article is available in the online version, at <https://doi.org/10.1016/j.anbehav.2017.11.008>.

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