

RESEARCH ARTICLE

Production and Perception of Sex Differences in Vocalizations of Wied's Black-Tufted-Ear Marmosets (*Callithrix kuhlii*)

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Males and females from many species produce distinct acoustic variations of functionally identical call types. Social behavior may be primed by sex-specific variation in acoustic features of calls. We present a series of acoustic analyses and playback experiments as methods for investigating this subject. Acoustic parameters of phee calls produced by Wied's black-tufted-ear marmosets (*Callithrix kuhlii*) were analyzed for sex differences. Discriminant function analyses showed that calls contained sufficient acoustic variation to predict the sex of the caller. Several frequency variables differed significantly between the sexes. Natural and synthesized calls were presented to male–female pairs. Calls elicited differential behavioral responses based on the sex of the caller. Marmosets became significantly more vigilant following the playback of male phee calls (both natural and synthetic) than following female phee calls. In a second playback experiment, synthesized calls were modified by independently manipulating three parameters that were known to differ between the sexes (low-, peak-, and end-frequency). When end-frequency-modified calls were presented, responsiveness was differentiable by sex of caller but did not differ from responses to natural calls. This suggests that marmosets did not use end-frequency to determine the sex of the caller. Manipulation of peak-and low-frequency parameters eliminated the discrete behavioral responses to male and female calls. Together, these parameters may be important features that encode for the sex-specific signal. Recognition of sex by acoustic cues seems to be a multivariate process that depends on the congruency of acoustic features. Am. J. Primatol. 71:324–332, 2009. © 2008 Wiley-Liss, Inc.

Key words: marmoset; signal production; vocal behavior; perceptual mechanism; long-distance vocalizations; sex differences

INTRODUCTION

In many species, the vocal repertoires of males and females possess functionally identical call types that are acoustically divergent. Traditionally, structural analyses of these calls are used to identify sex-specific acoustic parameters [e.g. Benz et al., 1990; Robinson, 1979a]. However, structural analysis alone does not tell us whether conspecific listeners make use of this information. Playback experiments are necessary to understand the biological relevance of these acoustic differences. Using playback experiments, animals have discriminated between vocal signals emitted by each sex by exhibiting differential behavioral responses [e.g. Raemaekers & Raemaekers, 1985; Ryan, 1998]. Recognition of sex from vocalizations is critical during within-group interactions and between-group encounters. This suggests that there should be features within the structure of animal vocalizations that allow for identification of specific features of the caller. It is likely that one or several acoustic parameters are used to encode and decode the sex of the caller, and recent studies have begun to

experimentally address this possibility in primate species [Miller et al., 2004].

When dealing with a sexually dimorphic vocalization, each signal has multiple potential receivers, males and females. In intergroup encounters, long-distance vocalizations are often critical, initial interactions [Robinson, 1979b]. In monogamous and territorial species, same-sex interactions result in aggressive, territorial behavior and increased cardio-physiological activity [Anzenberger et al., 1986; Garber et al., 1993; Ross et al., 2004; Saltzman,

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2003]. In kind, behavioral responses between individuals during opposite-sex interactions are many times context-specific [Gerber & Schnell, 2004]. In the presence of a pairmate, tamarins rebuff opposite-sex strangers, but in the absence of the pairmate, opposite-sex strangers elicit courtship behaviors [Inglett et al., 1990]. The nature of an interaction and the willingness of two animals to interact often depend on the sex of those animals.

In the monogamous Neotropical marmosets and tamarins, long-distance calls are emitted in three contexts: territorial encounters, social isolation, and mate attraction [Cleveland & Snowdon, 1982; Halloy & Kleiman, 1994; Miller & Hauser, 2004; Miller et al., 2001]. During intergroup encounters, conspecifics respond territorially to long-distance calls [Lazaro-Perea, 2001]. There is sexual dimorphism in both the acoustic structure [e.g. Benz et al., 1990; Norcross & Newman, 1993] of and behavioral and neural response [Masataka, 1987; Miller et al., 2004; Norcross et al., 1994; Pistorio et al., 2006] to long-distance calls of marmosets and tamarins. From this sex-specific signal-receiver interaction, the production and perception of this signal can be investigated.

We conducted a series of three experiments with marmosets (*Callithrix kuhlii*) that: (1) examined the acoustic morphology of phee calls to determine sex differences; (2) investigated behavioral responses by males and females to playbacks of same- and opposite-sex callers; and (3) evaluated sexually dimorphic acoustic features of phee calls for functional significance. The principal goal of this study was to test whether an individual acoustic feature, within the context of a call, was sufficient to convey the sex of the caller to the listener. In reaching this goal, two steps needed to be confirmed in the signal-receiver process. First, the signal had to provide sex-specific information (experiment I). Second, conspecifics needed to show discrimination of the sex-specific signal via differential behavioral responses (experiment II). With support from the first two experiments, the final experiment tested the principle goal of the study. If a specific acoustic feature could convey the sex of the caller, conspecifics should respond appropriately to calls with an acoustic feature modified to represent sex-typical values of the opposite sex.

EXPERIMENT I: STRUCTURAL ANALYSES

Methods

Subjects

Subjects were ten adult male and nine adult female Wied's black-tufted-ear marmosets (*C. kuhlii*) housed as male-female pairs (one female was sequentially housed with one of two males). Colony rooms contained two or three pairs at the Callitrichid Research Center (CRC) at the University of Nebraska at Omaha (UNO). Housing enclosures for

each pair were wired mesh about $0.9 \times 0.8 \times 2.0$ m and equipped with branches, nest boxes, and various enrichment items. The rooms were maintained at $19.1\text{--}21.9^\circ\text{C}$ and a 12:12 light-dark cycle. Partitions prevented visual contact between pairs, but olfactory and vocal communications were possible. Additional details for housing, diet, and husbandry can be found in Schaffner et al. [1995]. This study was reviewed and approved by Institutional Animal Care and Use Committee (Protocol#: 07-031-05-FC). The CRC is registered with the U.S.D.A. and accredited by the Association of Zoos and Aquariums. All guidelines for housing and research were followed.

Recording vocalizations

Spontaneously occurring phee calls from males and females were recorded from the colony. All recordings were collected on a Marantz model PMD 201 portable analogue cassette recorder on high-output tape cassettes with a Sennheiser ME 80 directional microphone (Sennheiser, Old Lyme, CT; frequency range 50–15,000 Hz), and completed between 0930 and 1400 hr. Animals were allowed 2 min to habituate to the presence of the observer before the 30 min recording sessions. During sessions, doors between colony rooms were slightly ajar to stimulate vocal interaction [McConnell & Snowdon, 1986]. Subsequent to each phee call, the identification of the caller was spoken into the microphone [Jorgensen & French, 1998].

Structural analysis

Ten phee calls recorded from each individual were digitized onto Raven Pro version 1.3 bioacoustics software (Cornell Lab of Ornithology, Ithaca, NY). Sound spectrograms were produced from each vocalization. Frequency and temporal variables were measured to the nearest Hz and ms, respectively, using tools from the bioacoustics software (see Table I for a list of all variables). The acoustic parameters chosen for analyses in this study were

TABLE I. Structural Analysis

Acoustic parameter	Male ($n = 10$)	Female ($n = 9$)	P
Start frequency ^a	6.40 ± 0.62	5.88 ± 0.44	n.s.
End frequency	7.76 ± 0.58	6.98 ± 0.43	**
Peak frequency	8.60 ± 0.68	7.91 ± 0.35	*
Low frequency	6.18 ± 0.40	5.70 ± 0.34	*
Frequency range	2.48 ± 0.32	2.24 ± 0.24	n.s.
Call duration ^b	2.73 ± 0.79	2.82 ± 0.85	n.s.
Syllable duration	0.57 ± 0.11	0.56 ± 0.16	n.s.
Inter-syllable interval	0.21 ± 0.08	0.19 ± 0.06	n.s.
Number of syllables	3.82 ± 1.11	4.17 ± 1.48	n.s.

Note: Values indicate mean $\pm$ s.d. * $P < 0.05$, ** $P < 0.005$, n.s. $P > .05$.

^aFrequency values expressed in kHz.

^bDuration values expressed in seconds (sec).

similar to those used in prior studies that demonstrated sexual and individual acoustic variation [Benz et al., 1990; Rukstalis et al., 2003]. The start, end, peak, and low frequencies and the number of syllables of the entire call were recorded. The frequency range was computed by subtracting the lowest frequency of the call from the peak frequency. Intervals between syllables of a call were recorded and averaged to compute the inter-syllable interval of a phee call. Syllable duration was an average of the durations for each syllable of a call, and total call duration included both syllable durations and inter-syllable intervals.

Data analysis

Previous research has successfully used discriminant function analyses (DFA) to investigate sexual dimorphism and acoustic variation in several species of callitrichine primates [Benz et al., 1990; Rukstalis et al., 2003]. In this study, predictive DFA was used to evaluate whether the acoustic variance of phee calls produced by male and female marmosets differed. Using ten phee calls per individual, mean values were generated for each acoustic parameter for those individuals. Discriminant functions for classifying individuals based on sex were calculated on SPSS 14.0.2 (SPSS Inc, Chicago, IL) using both temporal and frequency parameters from calls. Phee calls were categorized by the sex of the caller, and the proportion of correctly classified phee calls produced by each sex was tested against the expected value of 50% males and 50% females, using a χ^2 test. One-way analysis of variances (ANOVAs) were conducted for each acoustic variable to evaluate parameter differences between sexes. All α levels were set at $P < 0.05$.

Results

Marmoset phee calls were statistically discriminable by the sex of the caller. Phee calls were correctly classified to the sex of the caller at high rates (94.7%). This classification differed significantly from the expected 50% for both males ($\chi^2_1 = 10.00$, $P < 0.05$) and females ($\chi^2_1 = 5.44$, $P < 0.05$). In both multivariate and univariate analyses, frequency parameters were critical in statistically differentiating male and female phee calls. The standardized canonical discriminant function coefficients (indicating the relative importance of each measure in discriminating cases by sex) for frequency variables were large (2.11–5.03). No coefficients for temporal or other variables were larger than 1.02. Table I presents the means of males and females for all acoustic parameters. Univariate ANOVAs showed that only frequency values (peak-, end-, and low-frequency) were significantly different between males and females, $F_{S1,18} > 6.46$, $P < 0.02$.

EXPERIMENT II: BEHAVIORAL RESPONSE TO NATURAL AND SYNTHESIZED CALLS

The first experiment clearly indicated that phee calls from male and female black-tufted-ear marmosets were statistically discriminable, and that frequency parameters contribute the most to the distinction. This experiment determined whether marmosets responded to calls from each sex differently, hence providing evidence that sex-specific information encoded in calls is detected and functional. We also synthesized digital copies of normal marmoset phee calls to ascertain whether listeners would respond to synthesized calls in a manner similar to natural exemplars.

Methods

Subjects

We utilized a subset of the sample (five male–female pairs housed in separate home enclosures) in experiment I. Housing and husbandry conditions were the same as experiment I.

Stimulus generation

Calls from three different males and females were randomly selected and used as natural exemplars (see Fig. 1 for examples of calls). Calls were either two or three syllables in length (males: $X = 2.6$; females: $X = 2.3$). Synthetic phee calls were then generated using the natural phee call exemplars as templates measuring values of frequency (Hz), temporal (ms), and amplitude (dB) variables using Avisoft-SAS Lab Pro (Sound Analysis and Synthesis Laboratory, Avisoft Bioacoustics, Germany). Six synthetic calls were generated using three natural phee calls from each sex as templates. The protocol to generate stimuli was as follows. Using the vocalizations digitized on Raven Pro bioacoustics software, a spectrogram and waveform was generated. From the spectrogram, the frequency contour for each syllable of a call was traced. Both frequency and temporal values for the entire call were recorded, including intersyllable intervals. Using the waveform, the amplitude form was also traced, and both amplitude and temporal values were recorded. Synthetic replicas were then generated on Avisoft-SASLab Pro sound analysis and synthesis software. Frequency and amplitude values were entered into a graphic synthesizer workspace to recreate contours that mirrored the natural exemplars over the duration of the call.

Stimulus presentation

Six natural and six synthetic stimuli were stored as digital files and played back, in a randomized order. A single speaker was fixed on the left side of each colony room (ranging 3–5 m from cages). There were four classes of stimuli presented to each male–female

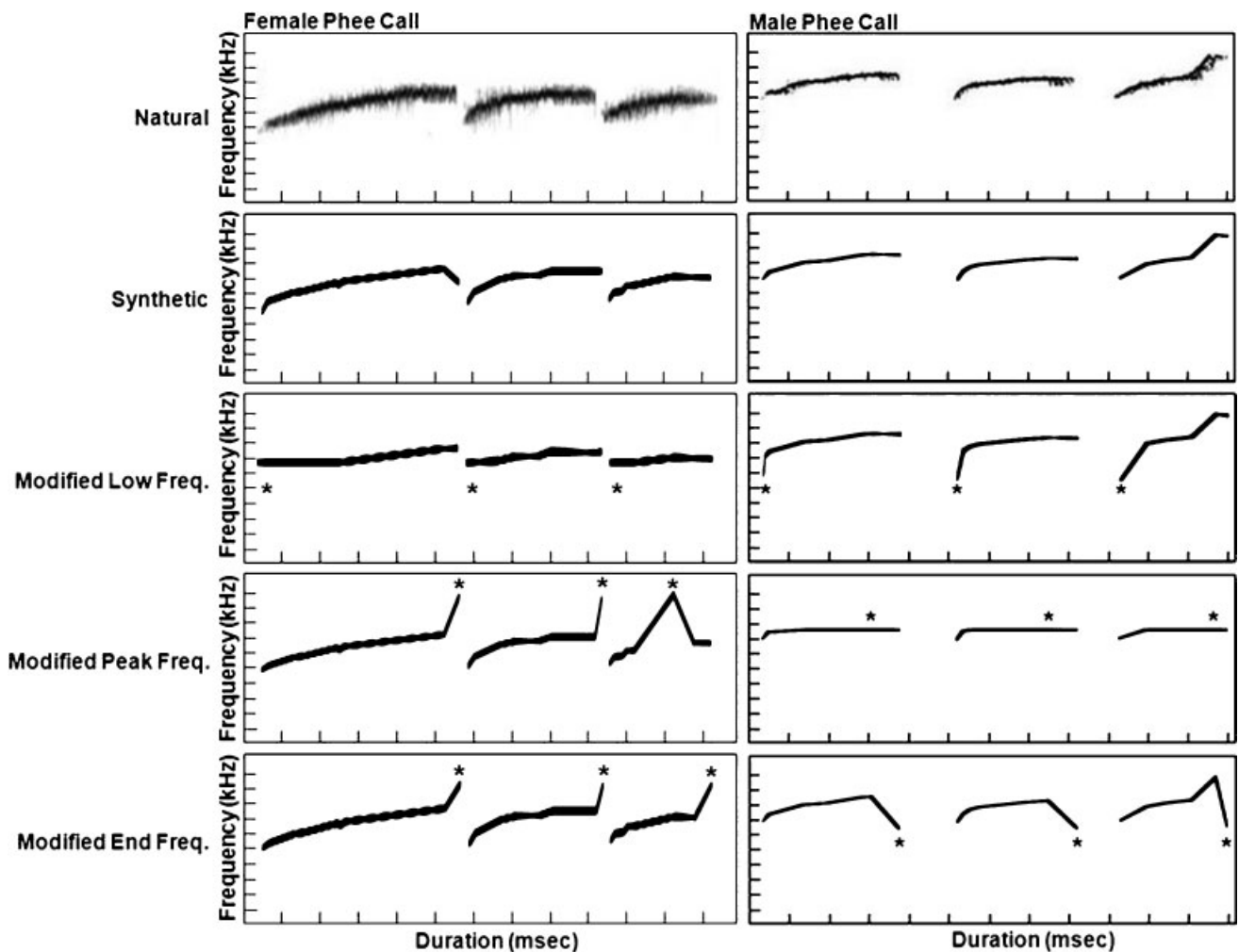


Fig. 1. Sonograms illustrating the modified synthetic phee calls, by sex of template caller and modified acoustic parameter. Female and male templates were synthetic phee calls using natural female and male phee calls as templates measuring values of frequency, temporal, and amplitude variables, respectively. End-, low-, and peak-frequency were the acoustic parameters that were modified (an asterisk denotes modified acoustic parameter) to hold sex-typical values of a standard deviation from the mean of the opposite sex from the rest of the acoustic parameters within the synthetic phee call. Frequency calibration marks are 1 kHz apart, and time marks are 100 msec apart for female calls and 200 msec apart for male calls.

pair: natural male call, natural female call, synthesized male call, and synthesized female call. Three calls from each stimulus type were presented to all male–female pairs, with the caveat that no pair heard a call recorded or derived from either marmoset in that pair. Each pair was presented with a single call at 1145 hr. A minimum of 48 hr elapsed between successive playback trials. Playback amplitude ranged between 60–75 dB SPL, typical for vocalizations between groups in adjacent rooms.

Behavioral observations

Behaviors were selected from previous field [Lazaro-Perea, 2001] and laboratory research [Norcross et al., 1994] as well as from personal observations. Observed behaviors were typical behaviors during intergroup interactions and can be found in Table II. Behaviors (i.e. approach partner,

leave partner, proximity to partner, and phee calls) were observed using “all occurrences” sampling during a 5 min precondition period before playback presentation (for baseline behavior) and a 1 min postcondition period immediately following the stimulus presentation (for behavioral response). Vigilance was a behavioral term derived from grouping of head scanning and directionality, both only observed during the stimulus. Vigilance was scored as an “all-or-nothing” sampling. Observers were blind to the sex of the caller and the stimulus type presented. Behavioral observations were recorded simultaneously from all home cages by different observers. Reliability ratings between all observers were > 95%.

Data analysis

Behavioral responses to phee calls were compared with three-way mixed-design ANOVAs across

TABLE II. Ethogram

Behavioral category	Behaviour	Definition	Scoring method	Duration (pre/post)
Regulation of spatial proximity	Approach	moving to a distance of < 10 cm from partner	frequency	5 min/1 min
	Leave	moving to a distance of > 10 cm from partner	frequency	5 min/1 min
Vocalization	Proximity	duration that male–female pair spend with < 10 cm apart	duration	5 min/1 min
	Phee call	loud, single or multiple syllable whistle	frequency	5 min/1 min
Vigilance	Head scan	side to side movement of head while at approximately 45° from shoulders and must occur during another's phee call	frequency	0 min/during stimulus presentation
	Directionality	turning head into the direction of speaker during production of stimulus	frequency	0 min/during stimulus presentation

within-subject variables (pre- and postcondition interval and sex of caller) and between-subject variable (sex of responder). All of the behavior during the 5 minutes before the stimulus was averaged across a single minute and then was compared with the behavior during the first minute after the stimulus presentation. For vigilance, a two-way mixed-design ANOVA was conducted using both a within-subject (sex of caller) and between-subject (sex of responder) variable. Any significant differences within behavioral responses were analyzed using Tukey's HSD post hoc test. All α levels were set at $P < 0.05$.

Results

Behavioral responses were differentiable by the sex of caller but not by the sex of the responder. When marmosets heard a natural phee call from a male, they became more vigilant than when a natural female phee call was presented (see Fig. 2), $F_{1,8} = 7.71$, $P < 0.05$. When synthetic phee calls were played, behavioral responses by marmosets tended to differ by the sex of the caller. Similar to responses to natural phee calls, marmosets became more vigilant after hearing synthesized male calls than synthesized female calls, $F_{1,8} = 4.82$, $P < 0.06$. Vigilance responses by males and females to phee calls, regardless of the sex of the caller, did not differ significantly between natural ($X = 0.48$, $SE = 0.10$) and synthetic ($X = 0.41$, $SE = 0.07$) call types. The sex of the caller was not a factor in the response rate for any of the other behaviors observed. In addition, there was no detectable sex difference in the response rate or proportion of responses for any behavior.

EXPERIMENT III: FUNCTION OF INDIVIDUAL ACOUSTIC PARAMETERS

Marmoset phee calls have been established to be sexually dimorphic in experiment I and prior research [Benz et al., 1990; Norcross & Newman,

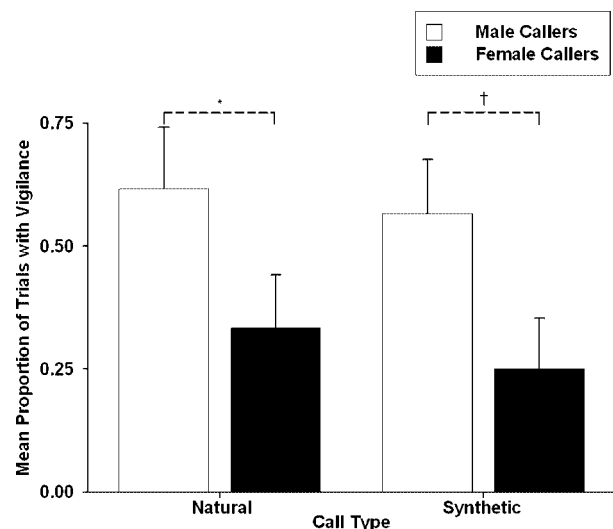


Fig. 2. Proportion of trials with vigilance responses for natural and synthetic phee calls comparing the sex of the caller. An asterisk indicates $P < 0.05$, and a cross indicates $P < 0.06$.

1993]. Presentation of these phee calls evoke differential behavioral responses based on sex of caller in experiment II and Norcross et al. [1994]. Experiment II also established synthetic phee calls as appropriate, comparable stimuli to natural phee calls as both stimulus types evoked similar behavioral responses by males and females. In light of these results, the manipulation of the acoustic properties of synthesized calls can be an important experimental tool to determine the role of individual acoustic parameters in encoding the sex of the signaler. In this final experiment, individual acoustic parameters in synthetic calls were modified to represent sex-typical values opposite from all other parameters of that synthetic call. These sex-modified synthetic calls were presented to male–female pairs. If an individual acoustic parameter sufficiently conveys the sex of the caller, then by modifying that structure to match the

acoustic value of the opposite sex, conspecifics should respond as if the call were emitted by the opposite sex.

Methods

Subjects

Subjects were the same five male–female pairs tested in experiment II, and housing conditions were identical to those in experiment I.

Stimulus generation

The same three natural call exemplars from each sex selected as templates to generate synthetic calls in experiment II served as templates for generating modified synthetic calls in the current experiment. The protocol for stimulus generation was identical to experiment II. After synthetic templates were generated, individual acoustic parameters were modified. Acoustic parameters in experiment I that contained significant acoustic variation between sexes (i.e. peak, low-, and end-frequency) were selected as parameters to be modified. For all frequency measures that differed significantly, females had lower mean frequencies than males. For synthetic calls that were generated from a natural male exemplar, the frequency value of the modified acoustic parameter represented one standard deviation below the female population mean calculated from experiment I. For example, in the condition in which we manipulated a synthesized male call that contained a female-typical end-frequency (FEF), the synthesized male call retained all male-typical acoustic parameters, except that the end-frequency was one standard deviation lower than the mean for all females. When natural female exemplars were used as templates to generate synthetic calls, modified acoustic parameters were modified such that the frequency values represented one standard deviation above the male population mean calculated from experiment I. Only one acoustic parameter was modified for each synthetic call. Modified synthetic calls represented one of six conditions (see Fig. 1 for examples).

Stimulus presentation, behavioral observations, and data analysis

Three synthetic calls per condition were stored as digital files and presented to the five male–female pairs of marmosets. Protocols for stimulus presentation, behavioral response collection, and data analysis were identical to experiment II. However, only vigilance behaviors were analyzed, as they were the only behaviors found to differ significantly in experiment II. A one-way-nested ANOVA was completed to test the effect of vigilance response to modified calls as a function of the sex of the caller. To test whether a specific acoustic parameter was critical in the recognition of the sex of the caller, a two-way-nested ANOVA was conducted controlling

for the sex of the caller (male vs. female) and stimulus type (natural, unmodified calls vs. one of the three modified call types). If there is an interaction between the sex of the caller and stimulus type, then the modified acoustic parameter is critical in the sex recognition process.

Results

Responsiveness, as measured by vigilance, to modified phee calls did not differ from responses elicited by natural phee calls. No statistical difference was found in the mean proportion of trials that elicited vigilance responses for each modified call type (end frequency: $X = 0.49$, $SE = 0.09$; peak frequency: $X = 0.37$, $SE = 0.08$; low frequency: $X = 0.48$, $SE = 0.09$) compared with natural calls ($X = 0.48$, $SE = 0.10$) from experiment II. Thus, marmosets responded to modified and natural calls similarly and seemed to recognize the modified signals as phee calls.

Marmosets responded differently to the presentation of male and female end-frequency-modified synthetic phee calls, see Fig. 3. They became more vigilant following the presentation of FEF-modified male synthetic calls than following MEF-modified female synthetic calls, $F_{1,8} = 12.76$, $P < 0.01$. Behavioral responses by marmosets to end-frequency-modified synthetic calls modeled responses evoked by natural and synthetic phee calls in experiment II. Although structural analysis suggested end-frequency was critical in conveying sex of the caller, behavioral response did not depend on the sex-typical value of end-frequency. This acoustic parameter was not critical in conveying the sex of the caller to conspecifics.

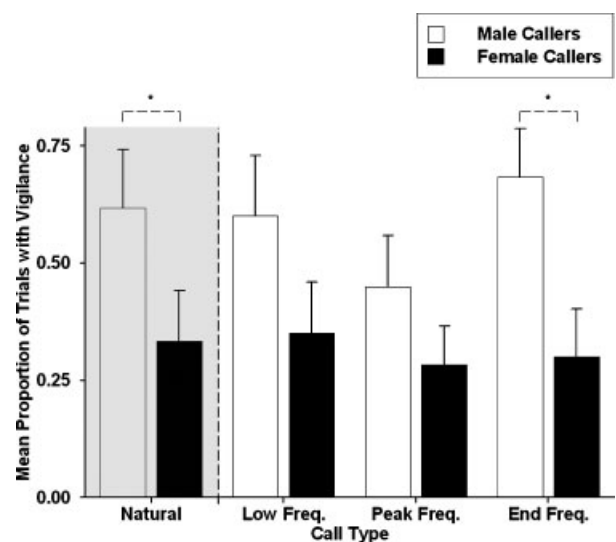


Fig. 3. Proportion of trials with vigilance responses for natural phee calls vs. low-, peak-, and end-frequency-modified synthetic phee calls comparing the sex of the caller. Significant P values are indicated by an asterisk.

When low- and peak-modified synthetic phee calls were presented to male–female pairs, no sex differences in behavioral responses were observed, see Fig. 3. Vigilance responses to these two modified call types seem to diverge from responses elicited by natural calls; however, the change in behavior was not a significant difference.

DISCUSSION

The vocal repertoires of many species contain long calls such that both sexes produce acoustic variations. Even though the acoustic differences between males and females are recognized, little is known about the perceptual mechanism, more specifically the behavioral meaningfulness of individual acoustic cues. In experiment I, natural phee calls were clearly discriminable by sex using structural analysis. Conspecifics recognized the sex-specific signal in the natural and synthetic phee calls and displayed discriminating behavioral patterns in playback trials during experiment II. In experiment III, the perceptual mechanism was evaluated by modifying sex-typical values in individual acoustic features. When several parameters were individually modified and calls were presented to male–female pairs, behavioral responses toward some of the modified call types deviated from responses elicited by natural calls. Marmosets did not recognize the sex-specific signal within the context of a single acoustic feature. Thus, the perception of the caller's sex from phee calls may be a multivariate process and depended on the congruency of sex-typical values from several features.

Many different types of encoded biological and social information have been investigated in the calls of both marmosets and tamarins. This study evaluated the production of sex differences in marmoset phee calls. Using structural analysis, the basic structure of a sex-specific signal was identified, and estimations of typical values of acoustic units were computed. Two main findings were illuminated regarding the sex differences in the acoustic structure of the vocalizations that were consistent with other primate literature [Heymann, 1987; Norcross & Newman, 1993; Weiss et al., 2001]. First, multiple features were integrated in the sex-specific signal of marmosets. Second, when measured for sex-typical variation, key features (i.e. low-, peak-, and end-frequency) were weighted differently.

Sex differences of phee calls from Wied's black-tufted-ear marmosets were similar, but not identical, to reported results from other marmoset and tamarin monkeys. Depending on the species, a range of spectrotemporal features of long-distance contact calls have been reported as sex-specific. In general, sex-specificity has been based on temporal features in tamarin long calls and frequency features in marmoset phee calls. The exemption to this

oversimplification has been presented in moustached tamarins [Heymann, 1987] and golden lion tamarins [Benz et al., 1990] where several frequency features of long calls have been identified as being sex-specific. Again, a relatively clear distinction is made between the sex-specificity of long-distance calls of tamarins and marmosets. When frequency features of long-distance contact calls produced by tamarins differed between sex, males produced calls with lower frequencies than females. However, this trend is in the opposite direction for marmosets such that females produce calls with lower frequencies than males [Norcross & Newman 1993; this study]. This comparative difference may be the result of delineation in the evolution of these two groups. Regardless of the species, structural analysis of long-distance contact calls always results in multiple sex-specific features [see above references and Masataka, 1987; Weiss et al., 2001].

Natural phee calls were clearly discriminable by sex, and frequency variables held the greatest weight in the acoustic variation between the sexes. In a discussion of the evolution of anuran communication, Ryan [1988] considered the differences in the vocal anatomy as a physical explanation for the variation of vocal production in male frogs. Ryan [1988] noted that as airflow from the lungs passes through the larynx before it vibrates the vocal cords and fibrous masses, it is this region where differences in anatomy could result in variation in frequency parameters in vocal production. Despite the overall absence of sexual dimorphism in marmosets and tamarins, Hershkovitz 1977, p 852] reported that there is sexual dimorphism in the median ventral laryngeal sac of tamarins. Further research in the variation of anatomy in this region may contribute in the understanding of acoustic variation between sexes.

Numerous species use multiple acoustic cues for vocal recognition and other encoded signals [see discussion in Ghazanfar et al., 2002]. Although multiple acoustic features result in vocal recognition, individual cues tend to be weighted differently [Nelson, 1988; Ghazanfar et al., 2002]. In this study, acoustic variance in the frequency parameters of males and females also seemed to be weighted differently in signal recognition. Although male and female marmosets produced phee calls with variations of end-frequency, behavioral responses were not altered when frequency values of this parameter were modified to represent a sex-typical value of the opposite sex. Hence, albeit the signal exists, there was no perceptual bias. Behavioral responses to playbacks of low- and peak-frequency-modified synthetic signals diverged from typical response patterns elicited by natural exemplars. Manipulation of peak- and low-frequency parameters eliminated the differential behavioral responses to male and female calls.

Two conclusions are conceivable for the process of sex recognition from phee calls based on the results from the three experiments. First, the recognition process may be determined by a single acoustic parameter that was not tested in experiment III. As the acoustic parameters modified in experiment III were the only parameters that had sex-typical values as indicated by the structural analysis of male and female phee calls in experiment I, this interpretation is unlikely. However, a more exhaustive analysis of the structures of male and female phee calls may derive additional parameters with sex-typical values. The second, and more probable conclusion, is that the sex-specific signal encoded in phee calls is based on multiple acoustic parameters. The proportion of calls that elicited vigilance responses were the same between natural and modified synthetic calls, but distinct from natural calls, responses to low- and peak-modified synthetic calls were not sexually dimorphic. Modifying a single parameter (i.e. low- and peak-frequency) may have caused inconsistencies in the sex signal leading to difficulty in recognition and appropriate response.

Sex differences exist in primate vocal behavior in both acoustic structure and responsiveness to this sex-specific signal. Moving beyond production of this signal, the new focus becomes the perceptual mechanism used by conspecific receivers that elicit behavioral responses. Several studies have evaluated the responsiveness of conspecifics to the sex-specific signal [Masataka, 1987; Norcross et al., 1994]. However, these studies have limited the analysis of sex recognition by evaluating behavioral responses to the call as a whole. In this study, sex recognition was examined using signal modification in order to assess acoustic features that may be important in the perception of the sex-specific signal. Miller et al. [2004] also modified the sex-specific signal of long calls produced by cotton-top tamarins. The research by Miller et al. [2004] was limited to structural modification of a single acoustic cue, even though structural analysis by Weiss et al. [2001] found sex differences in multiple acoustic features. Despite this limitation, results from Miller et al. [2004], similar to this study, suggest that the responsiveness of receivers to this sex-specific signal depends on the perceptual salience of that signal as a function of the variation of sex-typical values of key acoustic features.

Marmoset phee calls are characterized by relatively high frequency (5.7–8.6 kHz in *C. kuhlii*), and high frequency sounds are more susceptible to degradation over long distances than are lower frequency sounds [Wiley & Richards, 1978]. It is possible, therefore, that acoustic information regarding the sex of the calling marmoset may not be available to potential receivers. However, given the close proximity of group members during foraging, travel, and rest in *Callithrix* [Digby, 1995], it is likely

that little degradation of acoustic signals would occur in an intragroup context. In wild *C. kuhlii*, intergroup encounters occur at very close ranges, so that visual displays, chases, and even fights with contact between individuals in adjacent groups occur frequently [Raboy et al., 2008]. Notably, these long intergroup encounters in the wild (average encounter length is 62 min) are characterized by phee calls along with other vocalizations. Under these conditions, then, it is unlikely that signals would degrade sufficiently to mask sex-specific cues associated with phee calls in an intergroup context.

Phee calls may be the mediators of behavioral interaction during between-group encounters. Behavioral responses of marmosets toward natural phee calls are similar to responses elicited from physical presentation of unfamiliar conspecifics [French & Snowdon, 1981; Gerber & Schnell, 2004; Gerber et al., 2002; Lazaro-Perea, 2001]. Vocal encounters between groups of captive cotton-top tamarins increased overt territorial behaviors (i.e. scent marking and piloerection) [McConnell & Snowdon, 1986]. Sex differences exist in both the acoustic structure of phee calls and territorial behavior elicited by these vocalizations. Owing to poor visibility and functional use of olfactory cues in dense forests, vocal communication is extremely important in arboreal species [Epple, 1968]. Therefore, it is functionally beneficial to be able to perceive the sex of a caller before behavioral or visual interactions. Further research assessing the perceptual salience of sex differences and behavioral responses to phee calls may provide insight to intergroup interactions.

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