Do sexual calls in female black capuchin monkeys (*Sapajus nigritus*) vary with fertility? An acoustic analysis

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Running title: Sexual calls and fertility in female black capuchin monkeys (*Sapajus nigritus*)

Abstract

Females across a range of animal taxa produce vocalizations and signals uniquely associated with periods of mating. While such signals may ultimately function to increase female attractivity to males, conflicting findings challenge the extent to which these signals co-vary in accordance with the probability of conception. Female black capuchin monkeys (*Sapajus nigritus*) display an elaborate repertoire of both vocal and visual components as part of their socio-sexual behavior, and previous analyses have shown that the rates of production of visual, but not vocal, components provide graded information on female ovulation. It remains possible, however, that the acoustic parameters of these sexual calls, rather than their rate of productions, co-vary with female fertility. To test this, we analyzed structural and temporal call parameters from estrous calls and post-copulatory calls recorded over five consecutive mating seasons in 12 sexually mature females at Iguazú National Park, Argentina. Calls given during the fertile phase of the female ovarian cycle were compared with those given during the non-fertile phase, as determined by profiles of female reproductive hormones. Similarly, within the fertile phase, we tested whether temporal or spectral acoustic parameters of calls gradually change with the approach of ovulation. We did not find any significant relationship between call parameters and the two measures of female fertility in either female estrous calls or post-copulatory calls. However, some differences between pre- and post-copulatory calls were apparent. Overall, our results indicate that sexual calls in black capuchin females do not provide precise information about the timing of ovulation, but may allow listeners to make probabilistic inferences about whether copulations have taken place. This, combined with previous findings, suggests that females in our study may use signals in different modalities to convey information about their fertility and sexual behavior with varying degrees of precision.

Keywords: Bioacoustics, vocal communication, sexual signals, female reproductive hormones, *Sapajus nigritus.*

Introduction

Primate females, in contrast to most mammals, have developed a large variety of behavioral and morphological signals that are produced in association with increased sexual activity (Dixson, 2012). Many of these are assumed to be sexually selected traits, and appear to function within the contexts of inter-sexual conflict and mate choice (Andersson, 1994). Female sexual signals are especially common in catarrhines, particularly in those species characterized by multimale-multifemale social organization and promiscuous mating systems (Dixson, 2012). In some species, females produce multiple sexual signals in different modalities (e.g., production of both visual morphological signals and acoustic vocal signals: Nunn, 1999; Pradhan, Engelhardt, van Schaik, & Maestripieri, 2006). In such cases, it is necessary to conduct detailed analysis of all signals in order to understand whether the signals potentially provide receivers with redundant or distinct information (Liebal, Waller, Slocombe, & Burrows, 2013; Partan & Marler, 1999), and to better understand female mating strategies (see Kappeler & van Schaik, 2004).

Previous studies on sexual signals in female primates have paid particular attention to the extent to which they have the potential to inform receivers about the female's fertility state (Brauch et al., 2007; Deschner, Heistermann, Hodges, & Boesch, 2004; Engelhardt, Hodges, Niemitz, & Heistermann, 2005). While particular attention focused on exaggerated sexual swellings in this regard (Brauch et al., 2007; Engelhardt et al., 2005), it has been suggested that female sexual calls, in most studies referred to as copulation calls, may also provide information regarding the timing of ovulation (e.g., Aujard, Heistermann, Thierry, & Hodges, 1998; Pfefferle, Brauch, Heistermann, Hodges, & Fischer, 2008). However, there is considerable variation across primate species in the extent and the precision to which short-term hormonal changes in female ovarian cycles associate with changes in both the production and acoustic structure of sexual calls (Aujard et al., 1998; Pfefferle, Heistermann, Pirow, Hodges, & Fischer, 2011). In human females, for instance, voice characteristics such as frequency and amplitude can change during the menstrual cycle (Abitbol, Abitbol, & Abitbol, 1999; Brodnitz, 1979), including differences between high- and low-fertility days (Bryant & Haselton, 2009; but see Fischer et al., 2011). In non-human primates, initial evidence supported a link between sexual calls and female fertility (Tonkean macaques, *Macaca tonkeana*: Aujard et al., 1998; chacma baboons, *Papio ursinus*: O'Connell & Cowlishaw, 1994; Barbary macaques, *Macaca sylvanus*: Semple & McComb, 2000; yellow baboons, *Papio cynocephalus*: Semple, McComb, Alberts, & Altmann, 2002). However, more recent studies, often based on more detailed acoustic analysis of call structure and precise determination of the timing of ovulation through analysis of female reproductive hormones, have found that these calls do not reliably reflect female fertility and ovulation (bonobos, *Pan paniscus*: Clay & Zuberbühler, 2011; long-tailed macaques, *Macaca fascicularis*: Engelhardt, Fischer, Neumann, Pfeifer, & Heistermann, 2012; Barbary macaques: Pfefferle et al., 2008; chimpanzees, *Pan troglodytes schweinfurthii*: Townsend, Deschner, & Zuberbühler, 2011). While sexual swellings in these species tend to be graded signals of fertility that provide probabilistic information regarding the likelihood of conception, it seems that in at least some cases sexual calls are less reliable in this regard. That these signals are produced in different modalities suggests that they may function to provide different audiences with different information (Liebal et al., 2013) regarding female mating behavior and fertility. However, in addition to being limited to catarrhines, these studies on sexual calls have largely focused on copulation calls. Little is known about the function of other kinds of sexual calls that are uttered during pre- and post-copulatory contexts (but see Aujard et al., 1998), and little work has been done on sexual signals in other primate taxa.

Robust (or tufted) capuchin monkeys (genus *Sapajus*) represent an ideal study system to explore the adaptive significance of sexual calls because females in this genus signal sexual proceptivity via a rich repertoire of visual sexual signals, as well as through distinct vocalizations uttered almost exclusively around the period of female behavioral proceptivity (including estrous calls produced while females closely follow a target male, during post-copulatory displays, and during copulation) (Carosi & Visalberghi, 2002; Di Bitetti & Wheeler, 2017). Interestingly, the production of both visual and vocal components of these proceptive displays in black capuchin females (*Sapajus nigritus*) have been shown to be significantly higher during female periovulatory periods than pre- or post-periovulatory periods (Tiddi, Wheeler, & Heistermann, 2015). However, while the rate of production of the visual components of the proceptive displays have been demonstrated to be a probabilistic signal of ovulation to males (i.e. proceptive displays gradually increase in frequency with the approach of ovulation), the rate of production of non-copulatory sexual calls did not raise accordingly with the approach of ovulation (Tiddi et al., 2015). It remains unclear, however, whether their acoustic structure could change in relation to the timing of ovulation, thus potentially providing listeners with information about the timing of the female fertile phase.

The aim of the present study was to explore the association between female fertility and the acoustic parameters of female sexual calls in wild black capuchin monkeys (*Sapajus nigritus*). Firstly, we describe the repertoire of sexual calls given by females, and test whether there are differences between call bouts given in pre- and post-copulatory contexts. Then, we assess whether changes in female reproductive state, as determined by analysis of female ovarian hormones, are associated with changes in the acoustic parameters of sexual calls by comparing calls given during the periovulatory phase (fertile phase) with calls given during non-periovulatory phase (non-fertile phase). Finally, we tested whether, within the periovulatory phase, the temporal or spectral acoustic parameters of the calls varied based on the timing of ovulation (i.e., days relative to ovulation).

Methods

Study site and subjects

The data for this study were collected in Iguazú National Park, Argentina (25°40′ S, 54° 30′W). The study area comprises a protected area of 60000 ha in the Upper Parana Atlantic Forest, constituted by semi-deciduous secondary forest with a humid subtropical climate (Di Bitetti, Placci, & Dietz, 2003).

Black capuchin monkeys are medium-sized (2.5-3.5kg), diurnal, arboreal and omnivorous primates (Brown & Zunino, 1990; Smith & Jungers, 1997). Black capuchins at the field site live in stable multimale-multifemale groups ranging from 7 to 44 individuals (Janson, Baldovino, & Di Bitetti, 2012). Each group has a clear alpha male that is spatially and socially central in the group and holds feeding and mating priority (Janson, 1990; Tiddi, Aureli, Schino, & Voelkl, 2011; Tiddi et al., 2015). Females remain in their natal groups, whereas males disperse upon reaching sexual maturity (Janson et al., 2012). Females in this population show strong mating seasonality (from May to August) with births occurring from the beginning of October to the end of February (Di Bitetti & Janson, 2001).

Robust capuchin females (S*apajus spp.*) lack any external morphological sexual signals. However, during their proceptive periods (average length: 4-5 days), they display a rich and elaborate repertoire of sexual behaviors that consists of both visual (postures, gestures and facial expressions) and vocal components (Carosi, Heistermann, & Visalberghi, 1999; Carosi & Visalberghi, 2002; Di Bitetti & Wheeler, 2017; Matthews, 2012; Tiddi et al., 2015). Although detailed acoustic analysis has not been conducted on these vocalizations, a recent analysis of most of the calls in the vocal repertoire of black capuchins noted that these sexual calls are easily distinguishable by ear from other call types (Di Bitetti & Wheeler, 2017). When females are proceptive, they actively initiate sexual interactions and persistently solicit a male; while this is most frequently the alpha male, females mate promiscuously and copulations with lower-ranking males are common (Janson, 1984; Lynch Alfaro, 2005). After ejaculation has occurred, males and females can display intense reciprocal post-copulatory courtship in which both individuals produce vocal and visual signals like those produced by proceptive females (Carosi, Linn, & Visalberghi, 2005; Carosi & Visalberghi, 2002).

Fecal sample collection, hormone analysis and definition of female fertile phase

During the entire study, we observed 72 periods of female proceptivity, and we were able to match acoustic data with hormonal profiles in 26 periods from 12 females. Fecal samples for monitoring female reproductive state were collected opportunistically (between 7:00 and 18:00) and with sufficient regularity to allow a reliable determination of the timing of ovulation. Fecal sample collection and extraction of hormone metabolites in the field laboratory was performed following the procedure described in Wheeler et al. (2013; 2014). As in Tiddi et al. (2015), the assessment of female fertile phase and the timing of ovulation were based on the pattern of progesterone metabolites excreted in feces (N=409 fecal samples). Fecal progestogen levels were determined using a validated enzyme immunoassay as described in detail in Tiddi et al. (2015). Intra- and inter-assay coefficients of variation of high- and low-value quality controls measured within and between assays were 6.9% and 14.8% (high) and 7.7% and 16.0% (low), respectively. Based on a short excretion time of fecal hormone metabolites more typical of urinary hormone metabolites (Carosi et al., 1999; Wheeler et al., 2013), we defined a 2-day window in which ovulation was most likely to occur, consisting of the day of the defined rise in fecal progestogen levels and the day before (Tiddi et al., 2015). The fertile phase (peri-ovulatory period, POP) of each ovarian cycle was then defined as the period comprising the 2 days of the ovulation window plus two preceding days (from day -2 to day 0 from ovulation), in order to account for sperm life span in the female reproductive tract (Brauch et al., 2007). In addition, the 3-day periods preceding and following the fertile phase were defined as the pre-fertile phase (pre-POP) and post-fertile phase (post-POP), respectively.

Sexual calls, collection of recordings and acoustic analysis

For the purpose of this study, we divided sexual calls into three contextual types according to the temporal occurrence of the specific call in relation to mating. “Estrous calls” were uttered before copulation while females solicit and follow a target male, “copulation calls” were given during mating events, and “post-copulatory calls” were emitted during post-copulatory courtship when both the female and the male reciprocally produce similar vocal and visual signals while oriented toward one another (Carosi et al., 2005; Janson, 1984). Females often reinitiated following and soliciting the male after the post-copulatory display, at which point calls were again considered “estrous calls”.

Acoustic recordings were collected opportunistically over 5 consecutive mating seasons (2010 to 2014) from early May until late August, on 12 sexually mature, individually-recognized females belonging to three well-habituated groups (Macuco, Rita and Spot groups). Female sexual calls were audio recorded at a 16-bit resolution and a 44.1 kHz sampling frequency using a Sennheiser ME-67 or ME-66 directional microphone coupled with a K6 amplifier and connected to a Marantz PMD-660 or PMD-661 audio recorder, generally at a distance of 3-10 m from the caller.

We collected over 500 recordings, but only a subgroup of these could be matched with hormonal data (26 ovarian cycles in total) and therefore used for the main analysis. Accordingly, we included 154 recordings of estrous calls from 12 study females and 56 recordings of post-copulatory calls from 9 females.

Sexual call recordings were imported into Avisoft SASLab Pro 5 (R. Specht, Berlin, Germany), where the sampling frequency was converted to 22.05 kHz at 16-bit resolution. We then generated frequency-time spectrograms using a fast Fourier transformation (512-pt FFT, Hamming window with 87.5% overlap and time resolution of 2.9ms). To carry out the acoustic analysis, we started by sampling single 30-sec fragments from each recording available, and only when a recording was longer than 5 minutes we additionally selected 30 seconds at the end of it. Spectrograms from these 30-sec fragments were visually inspected, and any fragments that showed interference of calls from conspecifics or heterospecifics was not further considered. These fragments (including both estrous calls and post-copulatory calls) were composed of multiple bouts, which in turn consisted of several call units (mean ± SE = 52.7 ± 11.7 call units per fragment), elsewhere termed ‘‘notes’’ (Geissmann, 2002) or ‘‘syllables” (Robinson, 1984; Wheeler & Hammerschmidt, 2013). Different call units were visually inspected and categorized based on the frequency contours of the first harmonic on the spectrogram (see Results for further details). Each call unit was generally clearly distinct from others based on visual inspection of spectrograms. We defined a bout as a vocal utterance consisting of either a single unit or a temporal sequence of several units. The delay between two consecutive call units within a bout was by definition shorter that the delay between two consecutive bouts (see Figure S1 in the ESM). In those cases in which visual identification of call units was complicated by the scatter structure of the recording (i.e., the interval between two consecutive calls consisted of several seconds), we applied a 0.20 sec interval between two consecutive units in order to define a call bout. This interval was chosen as appropriate temporal threshold when visually inspecting our spectrograms (the typical interval separating units within clearly-identifiable bouts was < 0.10 sec) and at the same time measuring the delay between some obvious bouts. For every 30-sec fragment, we measured a total of 22 structural/temporal call parameters (e.g., unit type, unit delivery rate; unit interval, unit duration) in our three types of calls using Avisoft SASLab Pro 5 (see Table 1 for a complete description of these parameters).

Data analysis

To characterize the repertoire of sexual calls and determine if estrous calls could be distinguished from post-copulatory calls, we performed a 2-step cluster analysis (using IBM SPSS 24) based on call type, call duration, number of calls per bout, and number of different calls per bout. We included 1373 calls of 627 call bouts from nine subjects in the cluster analysis. To test if the use of bouts belonging to the three resultant clusters (see Results) differs across our *a priori* context-based call categories (i.e., estrous calls versus post-copulatory calls), we first calculated the percent of call bouts from each context that fall into each cluster for each of the nine subjects, and then conducted three Wilcoxon signed ranks tests (one for each cluster) comparing the proportion of estrous and post-copulatory calls that fall into each cluster (using IBM SPSS 25).

To test if the variables we measured on call bouts co-varied with female fertility, we first reduced the number and correlation between the different acoustic measurements by performing a principal component analysis (PCA) for estrous calls as well as for post-copulatory calls. We included in the subsequent analysis all factors with an eigenvalue above 1. To get a better interpretation of factor loadings, we did a subsequent Varimax rotation. We then tested for a relation between these principal component values and female fertile phase using within-subject linear regressions. These fixed effect models were based on a within-subject centering approach which allow one to assess whether the between-subject effect differs from the between-subject effect of the covariate, while accounting for unequal sample sizes and repeated observations of the same subjects (van de Pol & Wright, 2009). Using these models, we analyzed the effect of female fertile phase on acoustic parameters characterizing female estrous and post-copulatory calls. To do so, we ran different within-subject linear regressions where components extracted from the PCA were entered as the response variable and female fertile phase as the main explanatory variable, with the latter considered in two different ways. First, we ran an initial set of models in which the response variable was the selected factors from the PCA for estrous calls (7 in total, see Table S1 in the ESM) and the independent variable was a categorical variable representing days within the periovulatory period (POP, fertile phase) or within the non-periovulatory period (non-POP, non-fertile phase). Non-periovulatory periods included both the 3-day period preceding the fertile phase (pre-POP) and the 3-day period following it (post-POP) in which females showed sexual displays. Because only a small number of post-copulatory calls were recorded during non-POP periods, it was not possible to compare the acoustic structure of these calls between fertile and non-fertile periods. Female identity was entered as a fixed effect in all models.

As second step, in order to test whether any parameters of the calls gradually changed within the periovulatory period (POP) with the approach of the ovulation, we ran addition within-subject linear regressions in which the response variables were the selected factors extracted by the PCA, and the independent continuous variable was the number of days to ovulation within the POP. In this case, given the sufficient number of recordings of both estrous calls and post-copulatory calls within the fertile period, we ran these models separately for the two call types (estrous calls: 7 extracted factors; post-copulatory calls: 6 extracted factors; see Tables S1 and S2 in the ESM, respectively). As above, female identity was entered as a fixed effect.

Principal component analysis was performed using IBM SPSS 21 and within-subject linear regressions were run in R version 3.2.1. All analyses were two-tailed and the significance level was set at $P<0.05$. We applied a Bonferroni correction for multiple testing, therefore setting the significance at 0.007 on the models for the effect of cycle stage (POP/non-POP) on estrous calls, as well as on the models for the effect of the day to ovulation on estrous calls, and at 0.008 on the models for the effect of day to ovulation on post-copulatory calls.

Ethics Guidelines

This study was approved by the Animal Welfare Officer at the German Primate Center (DPZ) and by the Argentine Administration of National Parks (permit number: NEA 158 bis Rnv 5), and adhered to the legal requirements of Argentina. Finally, this research adhered to the American Society of Primatologists' Principles for the Ethical Treatment of Primates.

Results

Description of repertoire of sexual calls

Based on visual inspection of spectrograms, we identified seven acoustically distinct call units that were produced during bouts of estrus calling (labelled “A” calls, “B” calls, and so on through “G” calls; see Figure 1 for an example of visual identification of the different call units). Some of these seven call types show some acoustic similarities to other call types in the black capuchin vocal repertoire previously described for non-sexual contexts (Di Bitetti & Wheeler, 2017). “A” calls show structural similarities to some variants of calls termed “whistle series”, particularly the “infant whistle series” and the “intergroup encounter whistle series,” but has shorter and more widely spaced call units than does the either, is of lower frequency than the former, and is of far lower amplitude than the latter. “B” calls are most similar to what Di Bitetti and Wheeler (2017) referred to as “piripipi estrous calls”, which they noted share some similarities to calls (“sirenas”) given by same-group males reuniting after periods of separation (Lynch Alfaro, 2008). The concave and upward sweeping frequency contours of “C” and “D” calls, respectively, are unique compared to other calls from the repertoire that have been described. Individual units of the “E” call, characterized by a convex frequency contour, are similar to what has been called the “contact note”, although “E” calls appear to be higher in frequency and are given in bouts with rapid repetition of the unit, whereas repeated contact notes are much more widely spaced; the rapid repetition of these units within bouts is similar the “aggressive contact note” given in agonistic contexts, although “E” calls are again higher in frequency. The waveform contour of “F” calls are similar to “squeals”, and this call may grade into the “sirena” reunion calls mentioned above. Finally, “G” calls are the noisiest calls in the repertoire of sexual calls, and are most similar to “screams”, but appear to be lower amplitude and are usually characterized by shorter unit durations than is typical of screams, although unit duration varies and may be longer immediately before and during copulation, although further study is needed to confirm this (see also Di Bitetti & Wheeler, 2017)

Running a 2-step cluster analysis based on call type, call duration, number of calls per bout, and number of different calls per bout, we found support for a three cluster solution, indicating that calls could be considered to fall into one of three categories. Calls belonging to the first cluster (30.3%) were mostly single calls or bouts of the same call type and comprised the call types "A", "B", "D", "E" and "F". The second cluster (20%) consisted nearly exclusively of "F" and/or "G" call types (only a small proportion of "G" calls did not fall in this cluster"). The third cluster (49.7%) consisted of bouts characterized by different call types.

Contextual variation in call production

In testing whether estrous and post-copulatory calls differed in terms of cluster usage, we found no difference between these calls in terms of the proportion of each that belonged to cluster 3 (two-tailed Wilcoxon Signed Ranks Test: $T^- = 19$, $N = 9$, $p = 0.734$; Figure 2). In contrast, for all nine subjects, a greater proportion of their estrous calls than their post-copulatory calls fell into cluster 1 ($T^+ = 0$, $N=9$, $p > 0.01$; Figure 2), while for 7 of 9 subjects a greater proportion of their post-copulatory calls than their estrous calls fell into cluster 2 ($T^- = 3$, $N = 9$, $p = 0.02$; Figure 2). Spectrograms of typical estrous and post-copulatory calls, together with a typical copulation call, can be found in Figures 3a-d.

Estrous calls in relation to the ovarian cycle phase and the timing of ovulation

Principal components analysis gave seven axes for estrous calls, accounting for 84.2% of the variability in the data. The factor loadings (Table S1 in the ESM) indicated that the different factors mainly described: (1) unit delivery rate, (2) unit duration, (3) noisiness, (4) upward sweeping units, (5) convex frequency modulated units, (6) concave frequency modulated units, and (7) flat frequency units. Results show that whether a female was in her fertile phase (POP) or not (non-POP) did not significantly affect any of the PCA components (Table 2a; Fig. 4a). Similarly, when considering only data collected within the periovulatory period (POP), none of the PCA components varied significantly with the approach of ovulation (Table 2b; Fig. 4b).

Post-copulatory calls in relation to the timing of ovulation

Six factors were extracted from the principal components analysis for post-copulatory calls (Table S2), accounting for 85.4% of the variability. Factors mainly represent: (1) unit delivery rate, (2) unit duration, (3) noisiness and flat frequency units, (4) upward sweeping and convex frequency modulated units, (5) concave frequency modulated units and (6) bout delivery rate. When considering the day to ovulation within the fertile phase (POP), our results indicated that none of the PCA components were associated with the approach of ovulation (Table 3; Fig. 5).

Discussion

The results of the present study provide details on the sexual call repertoire of black capuchin monkey females and show that patterns of estrous and post-copulatory calls form three distinct clusters, with calls falling into one of these clusters being more associated with pre-copulatory contexts, the second being more associated with post-copulatory contexts, and the third being associated with both contexts. However, our results indicate that there is no relationship between female fertile phase and the characteristics of sexual calls. Specifically, although mainly produced around fertile periods (Tiddi et al., 2015), we did not find any significant difference between acoustic parameters of estrous and post-copulatory calls given during the periovulatory (i.e., fertile) period (POP) and those given during the non-periovulatory period (non-POP). Furthermore, when only sexual calls occurring within the periovulatory period were considered, none of the acoustic parameters changed in relation to the timing of ovulation. Thus, the acoustic structure of estrous and post-copulatory calls do not appear to have the potential to provide receivers with information regarding either the caller's fertility state or the timing of ovulation within the fertile period.

The results of this study indicating that the use of sexual calls varies probabilistically based on context (i.e., between pre-copulatory estrous calls and post-copulatory displays) but not on female fertility corroborates previous work indicating that these signals contrast with visual signals in the extent to which they provide reliable information on black capuchin females' states of fertility. More broadly, our results also contrast with early evidence indicating that sexual calls function as signals of female fertility in primates (e.g., Aujard et al., 1998), and add to the evidence that covariation of the acoustic structure of sexual calls with female fertility might be less common than initially thought among primates (Brauch et al., 2007; Clay & Zuberbühler, 2011; Engelhardt et al., 2012; Townsend et al., 2011). The lack of such covariation has been argued to be related to the fact that primate females are usually characterized by elevated levels of estrogens for only a few days during the ovulatory cycle, and this brief rise in estrogens may be sufficient to induce the production of sexual calls, but not sufficient to cause an up-regulation of hormone receptors in the sound production organs (Pfefferle et al., 2011). This may be the case here, as the occurrence of estrous calls in black capuchin females is much more common during a female's fertile period (Tiddi et al., 2015), but we did not find any fine-tuned differences in the acoustic structure of these calls with the approach of ovulation. In other words, levels of estrogens above a given threshold may stimulate the production of estrous calls, but variation above the threshold may not cause additional changes in the acoustic characteristics of these calls. It should be noted, however, that the production of visual signals of fertility (facial expressions and gestures) by female black capuchins do show variation in relation to the timing of ovulation and thus variation above this threshold of estrogen levels (Tiddi et al., 2015). Future hormonal analysis focused on detailed changes in estrogens during the female ovarian cycle may provide insight into these different responses of signal modalities to the approach of ovulation.

Although our study could not assess the function of copulation calls, the absence of precise cues of fertility in black capuchin female estrous and post-copulatory calls indicate that these vocalizations may serve to advertise mating to other group males, and thus motivate the target male to invest in mate-guarding. Within this context, capuchin females may benefit by gaining repeated mating with the target male and, therefore, increase the probability of fertilization by males with good genes (Maestripieri & Roney, 2004). Alternatively, given the prevalence of sexually selected infanticide in our study population (Ramirez-Llorens, Di Bitetti, Baldovino, & Janson, 2008), advertising mating may provide females with the advantage of increasing the confidence of paternity in alpha males, who in turn may be more motivated to engage in infanticide deterrence behaviors (Henzi, 1996). The lack of fertility cues in sexual calls may also be explained by considering a more active role of females in choosing their mates (O'Connell & Cowlishaw, 1994). In this scenario, where females and males face a conflict of reproductive interest, female vocalizations may incite male sperm competition by eliciting the interest of other group males, and mating males may respond by mate-guarding females. Indeed, previous work on robust capuchins has suggested that male post-copulation courtship may represent a form of mate guarding (Carosi et al., 2005; Lynch Alfaro, 2005). It is important to note, however, that overall these different functions of copulation calls are not mutually exclusive and that females may fully benefit by combining behaving in a way that both increases confidence of paternity in dominant males and at the same time confuses paternity across multiple males (Zinner, Nunn, van Schaik, & Kappeler, 2004). The current findings, combined with previous results on visual signals and call production patterns, suggest that black capuchin females may in fact apply such combined behavior; on the one hand, they increase paternity confidence in the alpha male via visually conveying to him graded signals of fertility, while on the other hand confusing paternity by encouraging promiscuous mating by varying the structure of their calls only according to pre- and post-copulatory contexts, but not indiscriminately broadcasting fertility via vocal signals. This scenario seems plausible in our study population, wherein females show a high preference for the alpha male in their group, but still mate promiscuously with other group males, and face a relatively high risk of infanticide during within-group male take-overs (Janson et al., 2012).

Further support for the idea that females may be balancing paternity confusion across multiple males while concentrating paternity certainty in the alpha male is provided by the evidence that capuchin females may use multimodal sexual signals to advertise their fertility with different degrees of accuracy (Higham et al., 2012; Rigaill, Higham, Lee, Blin, & Garcia, 2013; Tiddi et al., 2015). As sexual calls are almost exclusively produced in association to the fertile period (Carosi et al., 1999; Tiddi et al., 2015), females may use them to advertise their general fertility state to all group males, without conveying precise information regarding the timing of ovulation. Such precise information is provided, instead, by facial expressions and gestures (Tiddi et al., 2015), which are typically visible only to neighboring individuals due to vegetation density (see Liebal et al., 2013). Such non-redundant multi-modal signaling (see

Partan & Marler, 1999) via visual and vocal components may enable females to provide only a general and less accurate signal of their fertility to subordinate peripheral males via sexual calls, while more precise signals related to the timing of ovulation is conveyed mainly via visual proceptive displays which are only received by the target males in close proximity. Such non-redundant multimodal signals may facilitate mating with multiple males when conception is less likely, while also concentrating paternity certainty in the dominant male by encouraging monopolization by them during the days in which mating is most likely to lead to conception (Tiddi et al., 2015).

Future work is needed to better understand the acoustic variation that exists within and between estrous calls, copulation calls, and post-copulatory calls, and the causes of this variation. Although systematic acoustic analysis is lacking, previous work has described black capuchin copulation calls as being acoustically similar to the noisy estrous calls given in conjunction with higher intensity visual displays, but distinct from those given nearly continuously by estrous females when following males (Di Bitetti & Wheeler, 2017). Anecdotal observations in the current study suggest that such noisy pre-copulatory calls are relatively rare, and it remains unclear the extent to which listeners can reliably determine the occurrence of a copulation based solely on these calls. Given the lack of an association between female fertile state and acoustic parameters in the current study, together with the anecdotal observation that call structure seems to vary with both the occurrence of copulations and the intensity of the visual displays, it seems likely that acoustic differences in these calls may be proximately related to female arousal. Additional research is needed to test this hypothesis, and the factors other than copulation that contribute to variation in female arousal.

In conclusion, our results indicate that black capuchin females do not provide precise information about their fertile period or the timing of ovulation via changes in the acoustic structures of their sexual calls. However, as these calls are almost exclusively produced during or immediately before or after a fertile period (Tiddi et al., 2015), females may produce them to advertise their general fertility to group males, without more providing precise information about the likelihood of conception. This, combined with previous findings, suggests that black capuchin females may use different modalities, such as vocal and visual displays to convey information about their fertility with different degrees of accuracy. Similar studies of multimodal sexual signaling across taxa may allow a better understanding of the ecological and social pressures under that shape mating strategies in primates.

Conflict of Interest

The authors declare no conflicts of interest.

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616 **Table 1.** Descriptions of the 22 parameters used in the analysis of estrous and post-copulatory calls.

Parameters	**Description**
Unit delivery rate	Number of calls units uttered per second
Unit interval	Mean interval between one call unit and the next one [s]
Unit duration	Mean duration from the beginning to the end of the call unit [s]
Flat frequency units delivery rate	Mean number of flat frequency units uttered per second
Proportion of flat frequency units	Mean proportion of flat frequency units in comparison to the total number of call units given.
Downward sweeping units delivery rate	Mean number of downward sweeping units uttered per second
Proportion of downward sweeping units	Mean proportion of downward sweeping frequency units in comparison to the total number of call units given.
Concave units delivery rate	Mean number of concave units uttered per second
Proportion of concave units	Mean proportion of concave units in comparison to the total number of call units given.
Upward sweeping units delivery rate	Mean number of upward sweeping units uttered per second
Proportion of upward sweeping units	Mean proportion of upward sweeping units in comparison to the total number of call units given.
Convex units delivery rate	Mean number of convex units uttered per second
Proportion of convex units	Mean proportion of convex units in comparison to the total number of call units given.
Waveform units delivery rate	Mean number of waveform units uttered per second
Proportion of waveform units	Mean proportion of waveform units in comparison to the total number of call units given.
Noisy units delivery rate	Mean number of noisy units uttered per second
Proportion of noisy units	Mean proportion of noisy units in comparison to the total number of call units given.
Bout rate	Number of bouts uttered per second
Single units/sequences	Ratio of the number of single call units to the number of sequences
Sequence length	Mean duration from the beginning to the end of the sequence [s]

Units per sequence	Mean number of call units in a sequence
Different units per sequence	Mean number of different call units in a sequence

Table 2. Results of the fixed effect models testing the association between sexual hormones and acoustic parameters of estrous calls in black capuchin females. The factors extracted by the Principal Components Analysis (PCA) are used as the dependent variable in the models and either a) cycle stage (periovulatory period or non-periovulatory period) or b) day to ovulation as the independent variable. Female identity was entered as a fixed factor.

	Estimate	SE	t value	Pr(>|t|)
a)				
FAC1 “unit delivery rate”	0.095	0.086	1.109	0.270
FAC2 “unit duration”	0.144	0.101	1.430	0.156
FAC3 “noisiness”	0.129	0.108	1.185	0.239
FAC4 “upward sweeping units”	-0.023	0.100	-0.231	0.818
FAC5 “convex units”	0.164	0.110	1.492	0.139
FAC6 “concave units”	-0.127	0.106	-1.205	0.231
FAC7 “flat frequency units”	-0.118	0.107	-1.109	0.270

N = 154 recordings from 12 females. Female identity was entered as a fixed factor.

b)				
FAC1 “unit delivery rate”	0.512	0.260	1.973	0.051
FAC2 “unit duration”	-0.335	0.291	-1.152	0.251
FAC3 “noisiness”	-0.387	0.297	-1.301	0.196
FAC4 “upward sweeping units”	-0.113	0.270	-0.417	0.677
FAC5 “convex units”	0.301	0.294	1.027	0.306
FAC6 “concave units”	-0.393	0.318	-1.236	0.219
FAC7 “flat frequency units”	0.394	0.295	1.334	0.184

N = 124 recordings from 12 females. Female identity was entered as a fixed factor.

Table 3. Results of the fixed effect models assessing the effect of sexual hormones on acoustic parameters of post-copulatory calls in black capuchin females. The factors extracted by the Principal Components Analysis (PCA) are used as the dependent variable in the models and day to ovulation as the independent variable.

	Estimate	SE	t value	Pr(>|t|)
FAC1 “unit delivery rate”	-0.183	0.120	-1.521	0.135
FAC2 “unit duration”	0.231	0.115	2.007	0.051
FAC3 “noisiness and flat frequency units”	-0.028	0.135	-0.206	0.838
FAC4 “upward sweeping and convex units”	-0.281	0.132	-2.123	0.039
FAC5 “concave units”	0.015	0.154	0.100	0.921
FAC6 “bout delivery rate”	0.141	0.145	0.970	0.337

N = 56 recordings from 9 females. Female identity was entered as a fixed factor.

Figure Legends

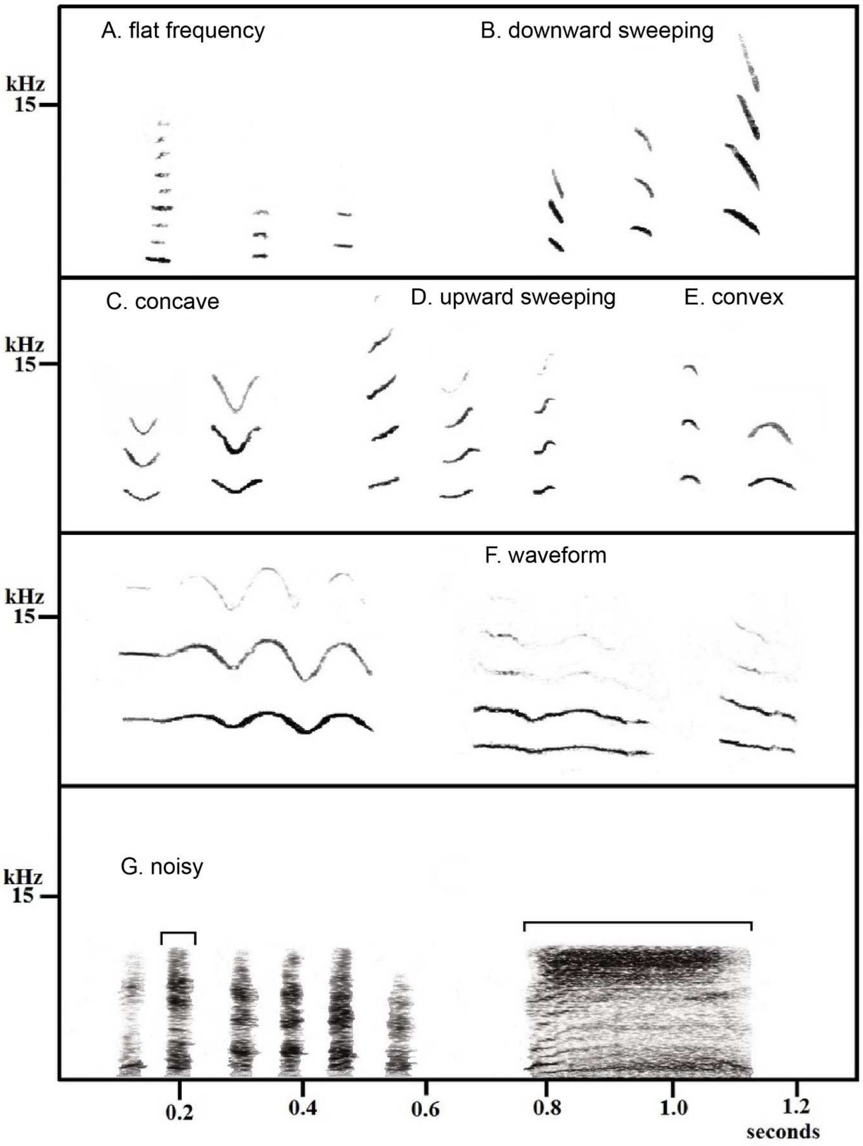
Figure 1. Spectrograms showing visual identification and categorization of the 7 acoustically-distinct units that characterize sexual calls ("A" calls through "G" calls) based on their frequency contours. See Methods for spectrogram parameters.

Figure 2. Bar chart showing mean (± SE) proportion of estrous and post-copulatory calls that fell into each of the three clusters for nine females.

Figure 3. Time-frequency spectrograms illustrating typical female sexual calls. (a) Estrous calls, (b) Copulation calls, and two Post-copulatory calls given (c) immediately after copulation and (d) around the middle/end part of the courtship (bottom), when they become similar estrous calls. See Methods for spectrogram parameters.

Figure 4. Boxplots showing Factor 1 ("unit delivery rate") for estrous calls: a) during non-fertile phase (non-POP) and fertile phase (POP), and b) with respect to the day relative to ovulation within the fertile phase. Boxplots show median (dark line), 1st and 3rd quartiles (box) and range (whiskers).

Figure 5. Boxplots showing Factor 1 ("unit delivery rate") for post-copulatory calls in relation to the day relative to ovulation within the fertile phase. Boxplots show median (dark line), 1st and 3rd quartiles (box) and range (whiskers).



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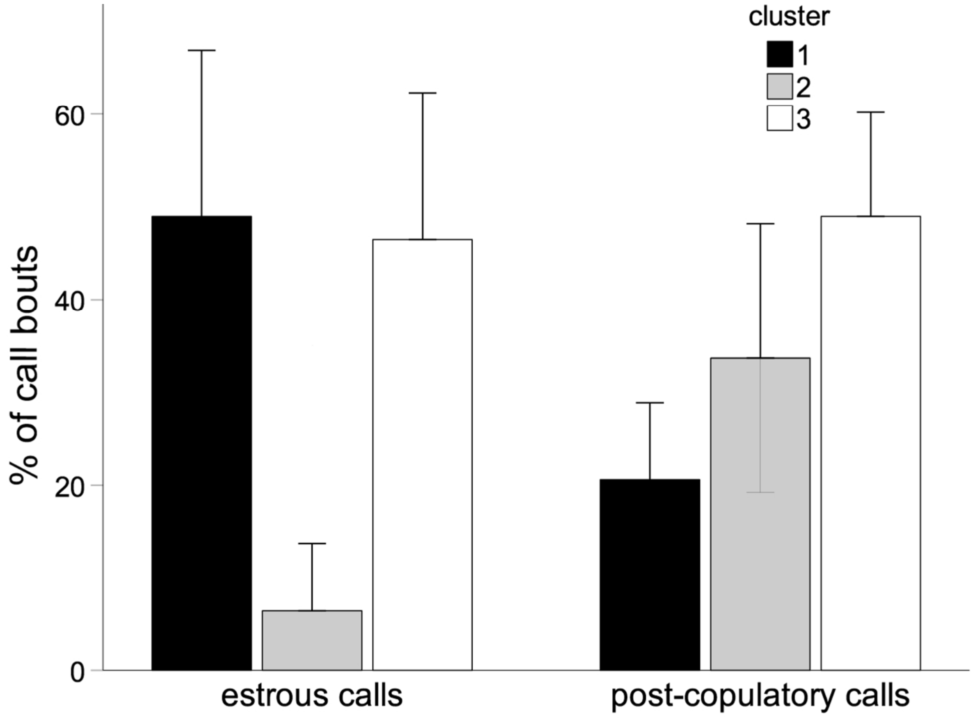

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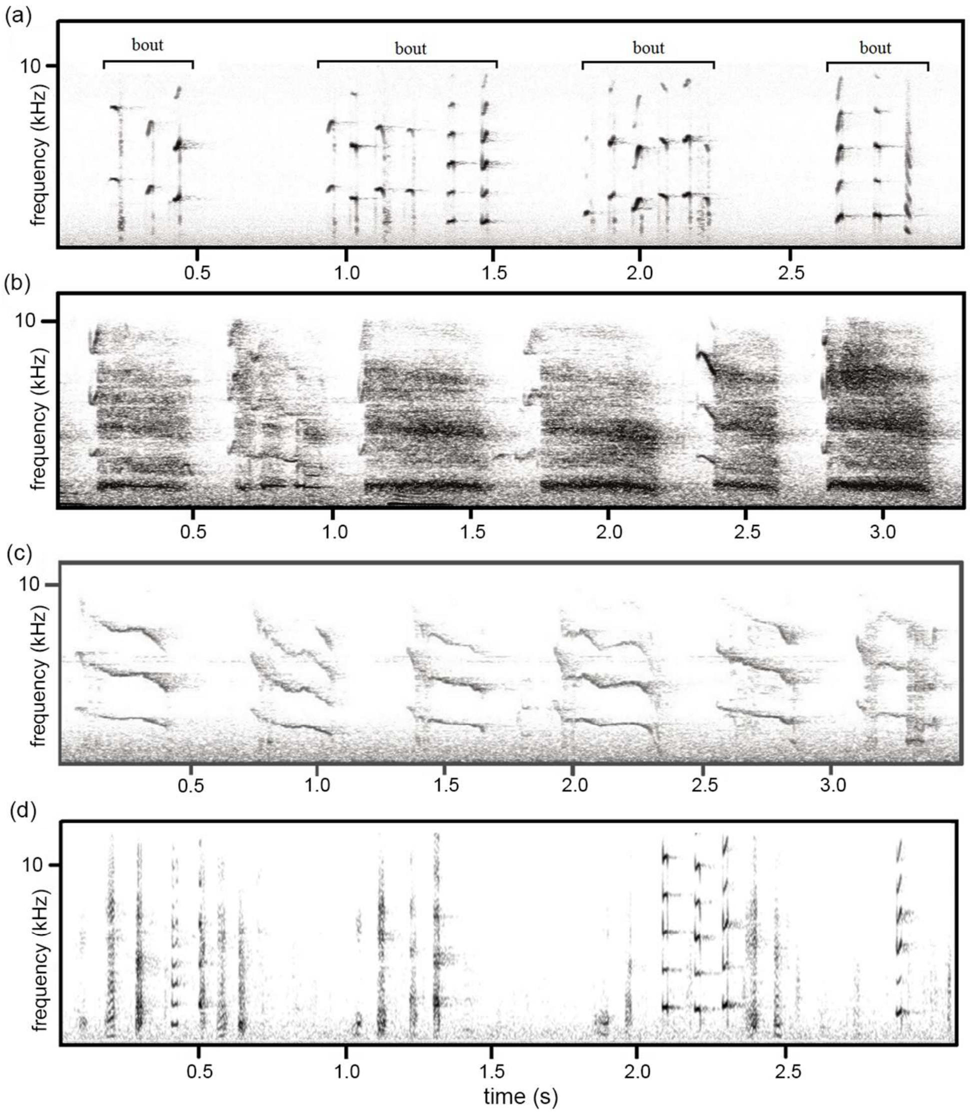

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Fig.3

a)

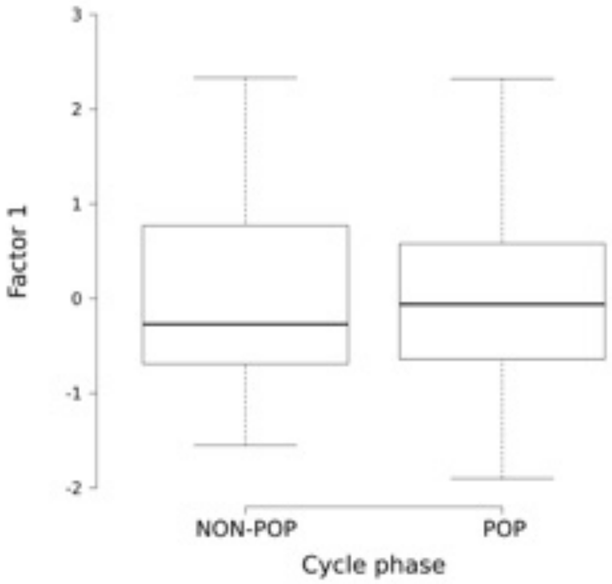


Fig.3
b)

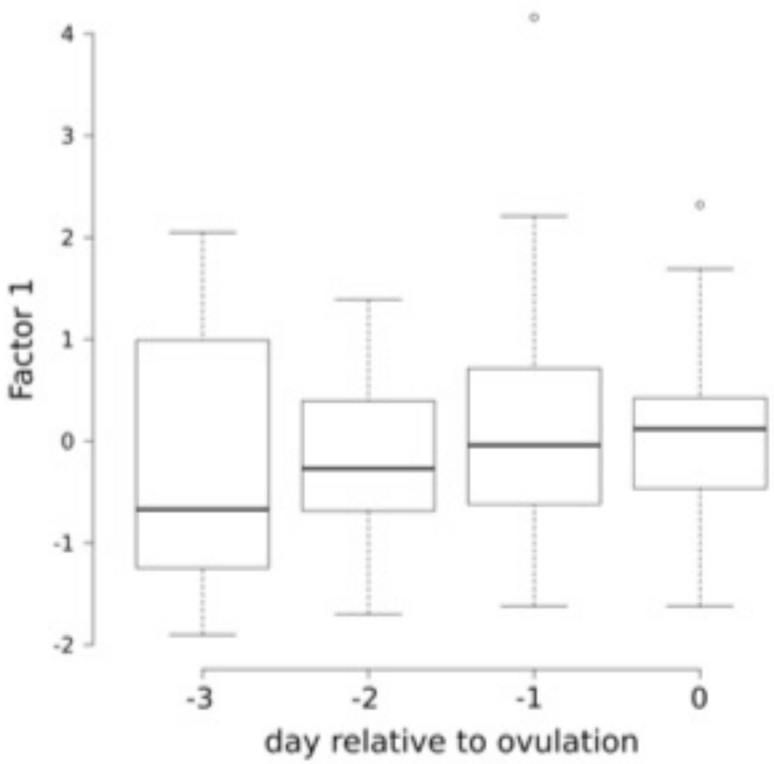
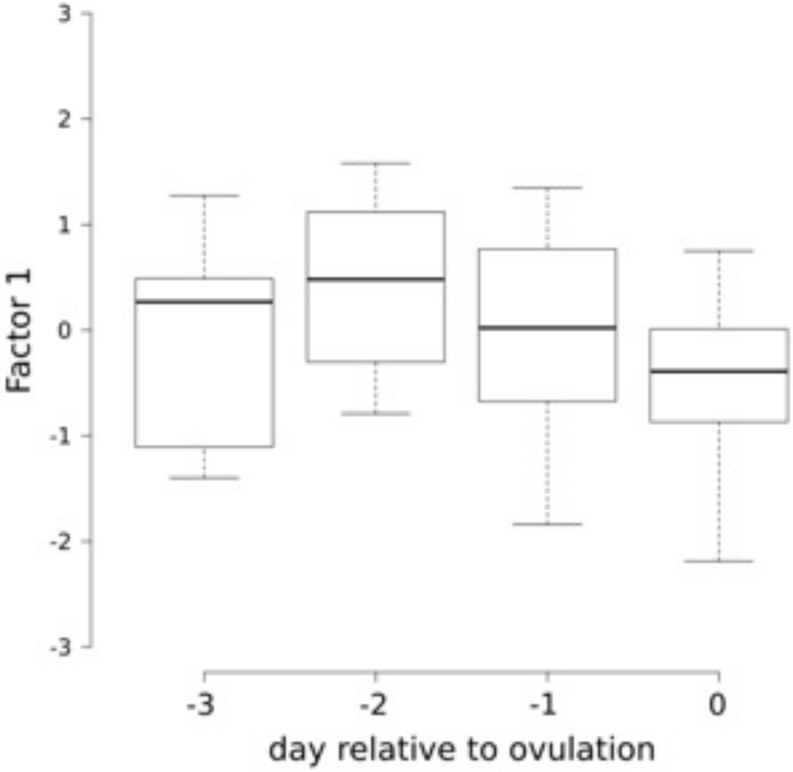


Fig. 4



Supplementary Materials for

Do sexual calls in female black capuchin monkeys (*Sapajus nigritus*) vary with fertility? An acoustic analysis

Ester Bernaldo de Quirós, Brandon C. Wheeler, Kurt Hammerschmidt, Michael Heistermann, Barbara Tiddi

Table S1. Factor loadings from the Principal Component Analysis (PCA) for estrous calls after Varimax rotation. Only factor loadings higher than 0.4 are shown.

	Factors						
Acoustic parameters	1	2	3	4	5	6	7
Unit delivery rate	0.701						
Unit interval	-0.759						
Unit duration		0.931					
Flat frequency units delivery rate	0.701						0.558
Proportion of flat frequency units							0.948
Downward sweeping units delivery rate	0.902						
Proportion of downward sweeping units			-0.483	-0.533			-0.479
Concave units delivery rate						0.915	
Proportion of concave units						0.946	
Upward sweeping units delivery rate				0.668			
Proportion of upward sweeping units				0.902			
Convex units delivery rate	0.479				0.776		
Proportion of convex units					0.889		
Waveform units delivery rate		0.867					
Proportion of waveform units		0.937					
Noisy units delivery rate			0.915				
Proportion of noisy units			0.961				
Bout rate	0.714						
Single units/sequences	-0.579						
Sequence length	0.733						
Units per sequence	0.797						
Different units per sequence	0.485			0.541			
% of total variance	26.357	12.934	10.953	10.010	8.579	8.335	7.031
% cumulative variance	26.357	39.291	50.245	60.255	68.834	77.169	84.200

Table S2. Factor loadings from the Principal Component Analysis (PCA) for post-copulatory calls after Varimax rotation. Only factor loadings higher than 0.4 are shown.

Acoustic parameters	Factors					
	1	2	3	4	5	6
Unit delivery rate	0.866					
Unit interval	-0.765					
Unit duration		0.882				
Flat frequency units delivery rate	0.445		0.648			
Proportion of flat frequency units			0.679			
Downward sweeping units delivery rate	0.707	-0.545				
Proportion of downward sweeping units		-0.685	-0.651			
Concave units delivery rate					0.917	
Proportion of concave units					0.915	
Upward sweeping units delivery rate				0.758		
Proportion of upward sweeping units				0.825		
Convex units delivery rate				0.804		
Proportion of convex units				0.859		
Waveform units delivery rate		0.916				
Proportion of waveform units		0.911				
Noisy units delivery rate			0.784			
Proportion of noisy units			0.782			
Bout rate						0.901
Single units/sequences	-0.592					
Sequence length	0.602	0.513				-0.427
Units per sequence	0.857					
Different units per sequence	0.491		0.491	0.441		
% of total variance	19.240	18.735	15.239	14.890	10.561	6.729
% cumulative variance	19.240	37.975	53.214	68.104	78.665	85.394

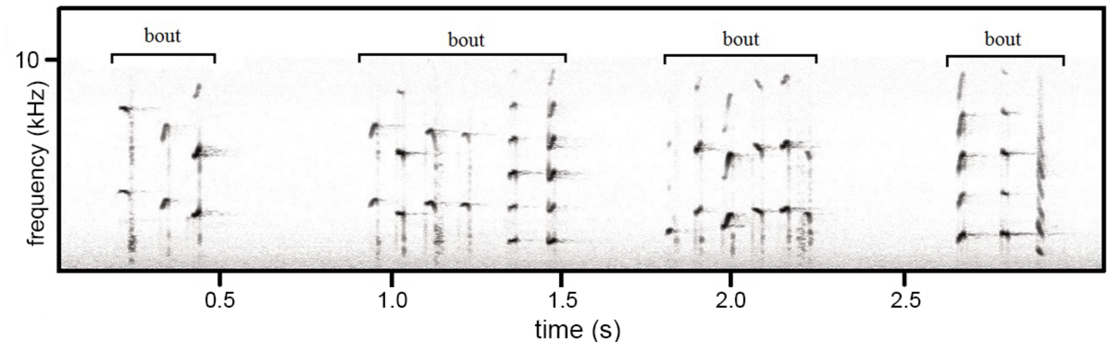


Figure S1. An example estrous call showing how bouts were defined by inter-unit intervals greater than those between units within bouts.