



The Structure of Bonobo Copulation Calls During Reproductive and Non-Reproductive Sex

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Abstract

Copulation calls in primates are usually identified as sexually selected signals that promote the reproductive success of the caller. In this study, we investigated the acoustic structure of copulation calls in bonobos (*Pan paniscus*), a great ape known for its heightened socio-sexuality. Throughout their cycles, females engage in sexual relations with both males and other females and produce copulation calls with both partners. We found that calls produced during sexual interactions with male and female partners could not be reliably distinguished in terms of their acoustic structure, despite major differences in mating behaviour and social context. Call structure was equally unaffected by the size of a female's sexual swelling and by the rank of her mating partner. Rank of the partner did affect call delivery although only with male, but not female partners. The only strong effect on call structure was because of caller identity, suggesting that these signals primarily function to broadcast individual identity during sexual interactions. This primarily social use of an evolved reproductive signal is consistent with a broader trend seen in this species, namely a transition of sexual behaviour to social functions.

Introduction

During mating events, numerous mammals and birds produce loud and acoustically distinct vocalisations, usually referred to as 'copulation calls'. In some species, males are the main producers (e.g. little brown bats, *Myotis lucifugus*, Barclay & Thomas 1979), but generally they are given by females, sometimes accompanied by their male partner (e.g. elephant seals, *Mirounga angustirostris*, Cox & Le Boeuf 1977; African elephants, *Loxodonta africana*, Poole et al. 1988). Copulation calls are widespread among primates, particularly in multi-male multi-female Old World species, in which females mate promiscuously and advertise their receptivity with pronounced sexual swellings (Dixson 1998).

The prevalence and conspicuous nature of female copulation calls has evoked considerable interest and a broad array of adaptive hypotheses has been put

forward (Pradhan et al. 2006). Essentially, all converge on the theme that copulation calls are sexually selected signals that promote the caller's reproductive success (e.g. Maestriperi & Roney 2005). For example, copulation calls may advertise sexual receptivity (e.g. Semple 1998, 2001), allowing a female to accrue mate choice benefits by inciting male-male competition, either directly (Cox & Le Boeuf 1977) or indirectly via sperm competition (O'Connell & Cowlishaw 1994). Copulation calls may also reduce threats of infanticide (Pradhan et al. 2006) by promoting paternity uncertainty or enhancing mate guarding (O'Connell & Cowlishaw 1994). In chimpanzees (*Pan troglodytes*), females are vulnerable to infanticide by both males (e.g. Muller et al. 2007) and other females (Townsend et al. 2007). In a recent study, it was found that chimpanzee females called most when mating with high-ranked males, possibly as a means to confuse paternity and enlist their future

support (Townsend et al. 2008). At the same time, calling was inhibited in the presence of high-ranked females, who also are the most likely perpetrators of female-led infanticides (e.g. Pusey et al. 2008). Overall, this suggests that copulation calls in primates can be part of a strategy used by females to maximise reproductive success while minimising social competition.

Examining the acoustic structure of copulation calls can provide information relating to their adaptive significance and communicative potential. In yellow baboons (*Papio cynocephalus*), female copulation calls convey a range of information, including caller identity, partner rank and swelling size (Semple 2001; Semple et al. 2002). In chimpanzees, copulation call structure is a reliable indicator of female identity (Townsend et al. 2011). In Barbary macaques (*Macaca sylvanus*), copulation calls co-varied acoustically with the occurrence of ejaculation (Pfefferle et al. 2008a), and this variation was perceived by listeners (Pfefferle et al. 2008b). Providing information about identity, receptivity and ejaculations may enable females to influence the behavioural decisions of potential mating partners in ways that are beneficial to them (Semple & McComb 2000; Pfefferle et al. 2008b).

Bonobo females (*Pan paniscus*) also produce copulation calls during mating events (Thompson-Handler et al. 1984; Kano 1992). This is consistent with Dixon's (1998) predictions that emphasise multi-male, multi-female social structure, fission–fusion dynamics and the presence of promiscuous females, who exhibit pronounced sexual swellings (Furuichi 1989; Kano 1992). For a number of reasons, bonobos represent an intriguing species to study copulation calls. Females are the sex more likely to emigrate (e.g. Kano 1992) but, despite a relatively low degree of genetic relatedness among them (e.g. Gerloff et al. 1999), they form strong intra-sexual affiliations and are highly gregarious (e.g. Furuichi 1989; Kano 1992; Hohmann et al. 1999). By developing enduring intra-sexual affiliations and coalitions, bonobo females can exert considerable social power by dominating males, defending resources and avoiding infanticide (e.g. Furuichi 1989; Parish 1994; White & Wood 2007).

In bonobos, sex is incorporated into daily life, with individuals engaging in sexual interactions in all age and sex combinations, across their sexual cycles, and in a multitude of positions (e.g. Thompson-Handler et al. 1984; de Waal 1987; Paoli et al. 2006). Partly divorced from reproduction, sex is thought to have numerous social functions, such as reducing social

tension, facilitating reconciliation, enabling affiliation and expressing social status (Thompson-Handler et al. 1984; de Waal 1987; Hohmann & Fruth 2000). Sex appears to have particular relevance for newly arriving immigrant females, who use sexual interactions to facilitate their integration into a new community and to allow subsequent peaceful coexistence and affiliation with non-related group members (e.g. Furuichi 1989; Idani 1991). The performance of homosexual socio-sexual behaviours is thought to assist in facilitating affiliations between unrelated females (e.g. Fruth & Hohmann 2006).

Sexual interactions between females are known as 'genital contacts', whereby two females embrace ventro-ventrally, swinging their hips laterally while keeping their vulvae in contact (Hohmann & Fruth 2000). Intriguingly, during genital contacts, females sometimes give calls that sound identical to the ones they produce when copulating with males (Thompson-Handler et al. 1984; Kano 1992), something that is not explained by current functional hypotheses (e.g. Pradhan et al. 2006). Owing to this acoustic similarity and to remain consistent with the established terminology, we term these vocalisations 'copulation calls'.

In a previous study on call use, we found that call production was less common during homo- than heterosexual interactions. In particular, when mating with a male partner the average likelihood of a female giving copulation calls was about twice as high as when mating with a female partner (32.9% vs. 16.4%; Clay et al. 2011). Despite this difference, the general patterns of call production were very similar, regardless of the partner's sex, which suggested that these calls have a broader social function (Clay et al. 2011). In particular, the average call production was highly dependent on partner rank with females most likely to call during sexual interactions with high-ranked partners, regardless of their sex (high-ranked male partners = 59.9%, high-ranked female partners = 28.2%; low-ranked male partners = 9.2%, low-ranked female partners = 3.0%; Clay et al. 2011). These results thus suggest that, although this signal may have originally evolved within the reproductive context, it has become ritualised to function as a more general social signal, with the potential of being used in flexible and strategic ways.

The aim of the current study was to conduct an acoustic analysis of female bonobo copulation calls to investigate the kinds of information conveyed by these signals. If sexual behaviour in bonobos has a social function, as suggested by previous research,

then call morphology should be less affected by partner's sex and more affected by the partner's social status, a factor that has been shown to influence call rates (Clay et al. 2011). We investigated whether information on partner rank was potentially conveyed by the acoustic structure of copulation calls, in addition to caller identity and swelling size, two variables that have had demonstrable effects on the acoustic structure in other species (e.g. Deputte & Goustand 1980; Semple 2001; Townsend et al. 2011).

Bonobos live in low-visibility forest habitats, suggesting that the ability to provide identity cues to out-of-sight group members is beneficial, especially because females sometimes overlap in their oestrous cycles. However, we expected sexual selection to act against accurate signalling of the fertile period, mainly because this would interfere with a female's strategy to confuse paternity. Concealing ovulation may be a more successful strategy, if this allows females to reduce the risk of being monopolised by the most dominant male, with an associated decrease of paternity certainty of others and corresponding loss of future support (e.g. Pradhan & van Schaik 2008).

Methods

Study Group

We conducted observations of three bonobo groups at Lola Ya Bonobo Sanctuary, Kinshasa, DR Congo, between September and November 2008 and between August and November 2009. Individuals spent their days free-ranging outdoors in one of three naturalistic forest enclosures (size ranging 20–15 ha), which were comprised of a natural rainforest, lake, swamp, streams and open grass areas. At night, individuals slept together inside a dormitory (approx. 75 m², divided into open sub-rooms). The daily routine at the sanctuary remained the same throughout observation periods (see Supporting information). In 2008, we observed individuals in enclosure 1, henceforth 'Group 1a'. In 2009, we collected data from two groups housed in the same and the adjacent enclosure; henceforth, 'Group 1b' and 'Group 2' (Table S1). Group composition in the first enclosure changed between the two study periods owing to transfers of individuals between groups. Group 1a was composed of 22 individuals (n = 9 females, n = 9 males and n = 4 infants). Group 1b was composed of 20 individuals (n = 7 females, n = 9 males and n = 4 infants). Group 2 was composed of 19 individuals (n = 5 females, n = 11 males and n = 3 infants; see

Table S1). To maximise sample size, we pooled data sets from across the three groups and combined data for dyads that met each other again in the second year (n = 9 female–female dyads and n = 19 male–female dyads). This resulted in a sample size of n = 14 females in total.

Data Collection

In bonobos, sexual behaviour can take a variety of forms, such as heterosexual copulations (with pelvic thrusts and intromission), mountings, homosexual genital contacts and genital stimulation using an object or body part. Here, we recorded behaviours and vocalisations of females engaging in copulations with males and engaging in genital contacts with females. We conducted observations (approx. 1093 h) using *ad libitum* and all-day focal sampling, balanced across individuals (Altmann 1974).

We use the term 'copulation calls' to refer to calls given during sexual interactions with male or female partners. With male partners, we collected copulation calls made during intromissions and with female partners, during genital contacts with the partner. Copulation calls were acoustically distinct and never observed in contexts other than sexual interactions. Bonobo copulation calls typically consist of single or a succession of high-frequency squeaks and screams that usually begin during the copulation (Thompson-Handler et al. 1984; Kano 1992; Fig. 1). We recorded vocalisations at distances of 3–20 m using a SENNHEISER MKH816T directional microphone (www.sennheiser.com, Old Lyme, CT, USA) and MARANTZ PMD660 solid-state recorder (www.marantz.com, Mahwah, NJ, USA) (sampling rate: 44.1 kHz, 16 bits accuracy). Verbal comments made by the observer were later transcribed. In addition, we also coded the females' reproductive states according to veterinary assessments that were based on daily checks of swelling sizes and lactation behaviour and regular pregnancy tests. We collected daily records of swelling sizes, using Furuichi's (1987) four-point scale based on degree of wrinkling. Ideally, the females' fertile states would have been assessed by monitoring hormonal changes (e.g. Townsend et al. 2011). Unfortunately, this was not possible at the time of the study because of the reasons beyond our control.

Dominance

To assess the effect of dominance status on calling, we created dominance hierarchies based on the outcome of agonistic interactions between individuals

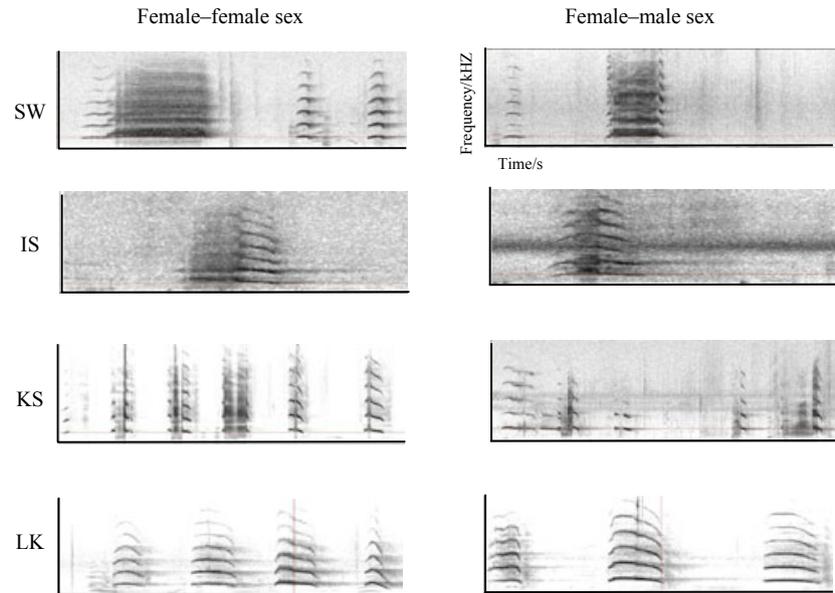


Fig. 1: Time–frequency spectrograms illustrating copulation calls produced by four female bonobos (SW, IS, KS, LK) during mating interactions with female partners and with male partners.

(e.g. Stevens et al. 2006). We used ‘fleeing upon aggression’ as a behavioural marker for dominance, following previous work showing this to be a reliable measure of dominance in bonobos (e.g. Vervaecke et al. 2000; Stevens et al. 2006). We collected all-occurrence data on agonistic interactions during focal sampling periods complemented by *ad libitum* data.

We used the Matman analysis programme (Noldus, version 1.1 (www.Noldus.com, Wageningen, The Netherlands)) to examine hierarchy linearity in the three groups separately. Following earlier studies (e.g. Stevens et al. 2006; de Vries et al. 2006), we investigated dominance linearity by calculating the adjusted linearity index h' , corrected for the number of unknown relationships (de Vries 1998; de Vries et al. 2006). We calculated the directional consistency index, a measure for assessing how frequent a type of behaviour occurred in its more frequent direction, relative to the total number of times it occurred. For significantly linear hierarchies, we calculated rank scores for each individual, using David’s Scores (DS) (e.g. Stevens et al. 2006). DS are cardinal measures, which use dyadic dominance proportions to provide a measure of dominance for a given individual. DS are based on the individual’s proportions of wins and losses in agonistic encounters, taking into account the relative strengths of opponents (e.g. de Vries et al. 2006). The full procedure is described in the Supporting information.

For females, we demonstrated significantly linear hierarchies in all three groups, and thus calculated individual ranks scores using normalised DS (corrected for chance). Using regression plots of the rank

scores, we divided the females into either high- or low-rank clusters, based on their position in the hierarchies (Fig. S1). For males, our analyses revealed an absence of significant linearity for two of the three groups (see Results and Supporting information). Despite this, we also noted the presence of several high-ranking males in each group who consistently elicited submission from others. Thus, to account for this dominance distinction, we assigned males to a high- and low-rank category based upon the number of agonistic interactions in which an individual dominated their partner. We assigned ‘high-rank’ status to any male who dominated at least 50% of the other males in the group (see Supporting information).

Acoustic Analyses

For acoustic analyses, we took a balanced and randomised sample of copulation calls produced by seven females during interactions with male and female partners. We analysed eight calling episodes per female in both homo- and heterosexual contexts, except for one female (LI), who only contributed seven female–male copulation calls. We were unable to include the other females ($n = 7$) because of low sample sizes. This was mostly due to the fact that copulation calls were generally less common during homosexual interactions and rarely given by high-ranked females (see Clay et al. 2011).

Bonobo copulation calls consist of either a single call or a call sequence (Thompson-Handler et al. 1984; Figs 1 and 2). We carried out quantitative acoustic analyses of the call units and call sequences,

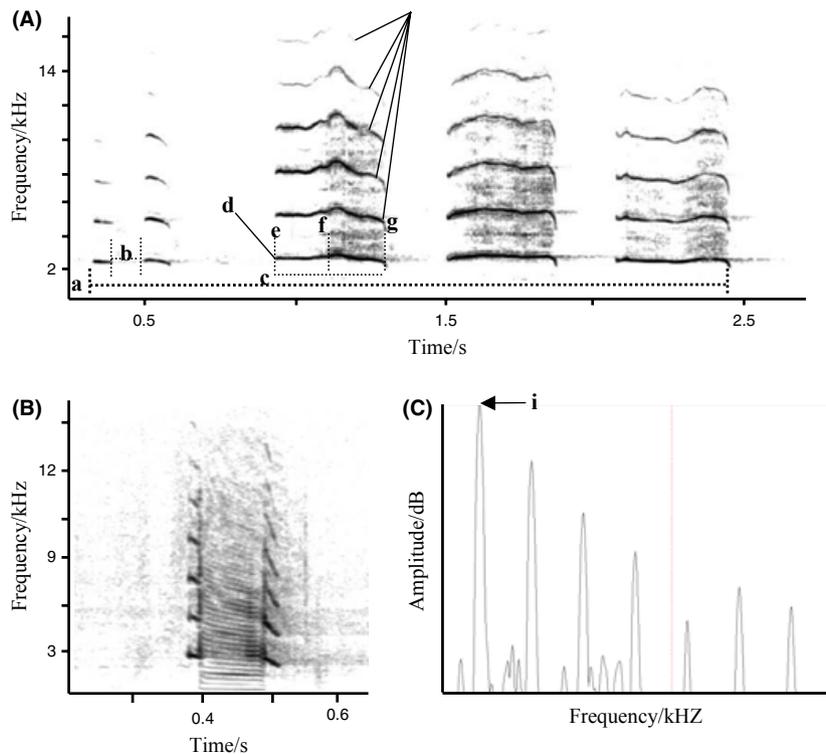


Fig. 2: (A) Example of a time–frequency spectrogram of a female bonobo copulation call illustrating how some of the acoustic parameters were measured. Copulation call sequence duration = duration of (a) (from start of first call to end of final call). Inter-call interval = duration of (b) between subsequent calls. Call duration = c (g–e). Fundamental frequency (F_0) upon which all spectral variables were measured, Hz = d. Transition onset (Δ Hz) = Frequency of maximum energy at call onset (e) minus at call middle (f) Transition offset (Δ Hz) = frequency of maximum energy at call middle (f) at call end (g) Overall transition (Δ Hz) = frequency of maximum energy at call end (g) minus at call beginning (e) Number of harmonics = h (e.g. $n = 5$). (B) Time–frequency spectrogram of a copulation call illustrating non-linear properties. (C) Example of spectral slice of a copulation call showing how peak frequency was calculated. Peak frequency = (i) (kHz).

using PRAAT 4.3.17 (<http://www.Praat.org>) with the following settings: pitch range: 1500–4500 Hz, optimised for voice analysis; spectrogram settings: analysis window length: 0.025 s, dynamic range: 70 dB, spectrogram view range: 0–20 kHz (to determine the number of harmonics). We performed pitch analysis using a script written by Michael Owren (pers. commun.). Overall, we analysed 22 acoustic parameters (Table 1, Fig. 2).

To standardise the varying number of calls per calling event, we calculated \bar{x} scores per call sequence. First, we screened the data for outliers by producing standardised Z scores, rejecting any calls with a Z score >3.29 in one or more parameters (Tabachnick & Fidell 2001). Next, we regressed all parameters to check for multi co-linearity and singularity, removing any parameters with a variance inflation factor >10 (Belsey et al. 1980). Variance inflation factors measure the degree to which the variance of one parameter is inflated by the existence of linear and higher order correlation among other parameters in the model.

Sexual Interaction Type

We used the discriminant function analysis (DFA) procedure to investigate whether, when combined in one model, the acoustic variables could generate discriminant functions that correctly discriminated

copulation calls produced with male and female partners. Following a multi-colinearity screening procedure, we entered 17 of the original 22 acoustic variables into the DFA for $n = 111$ calls [eight calls per female per context, except for one female (LI) who only contributed seven male–female calls]. We used the leave-one-out classification procedure to cross-validate the discriminant functions. Because the data were two-factorial (individual identity; sexual interaction type) and comprised of eight calls per combination of the two factors, it has been argued that conventional DFA does not allow for a valid estimation of the significance of discriminability (Mundry & Sommer 2007). Thus, to estimate the significance of the number of correctly classified calls (cross-validated), while controlling for female identity, we conducted a permuted DFA (pDFA) with caller identity as a controlled factor. Additionally, we conducted one-way analysis of variance tests (ANOVAs) on each of the non-correlated acoustic parameters to investigate whether specific parameters varied statistically with sexual interaction type (sex = fixed factor; identity = random factor).

Caller Identity

We used the DFA (cross-validated) procedure to test whether the copulation calls could be acoustically

Table 1: Acoustic parameters used in the acoustic analysis of female bonobo copulation calls

Parameter	Description
Episode duration (s)	Length of total episode from the start of first call to end of last call
No. of calls	No. of calls within the episode
% of calls with noisy or non-linear properties	See Fig. 2b
Mean intercall interval (s)	Time between subsequent calls, taken from point of call offset of first call to onset of next call
Call duration (s)	Duration of call from point of onset to offset
Peak time	Temporal location where maximum acoustic energy occurs, expressed as a proportion of call duration
No. of harmonics	No. of visible harmonic bands visible above the fundamental frequency
Mean fundamental frequency (Hz)	Average F_0 across the entire call
Minimum fundamental frequency (Hz)	Minimum frequency of F_0 across entire call
Maximum fundamental frequency (Hz)*	Maximum frequency of F_0 across entire call
Mean amplitude (dB)	Mean acoustic energy of the call
Peak position	Temporal position of max F_0 divided by call duration
% voiced	% of the call that was voiced
Jitter	Measure of pitch stability or short-term perturbation in the F_0 (voice roughness)
Shimmer	Measure of sound pressure level perturbation caused by vibratory variations from one vocal fold cycle to the next (voice hoarseness)
Peak frequency at call onset*; middle; offset *(Hz)	Frequency of maximum energy, as indicated using a spectral slice (see Fig. 2b)
Transition onset (Δ Hz)	Frequency of maximum energy at call onset, minus frequency of maximum energy at call middle
Transition offset (Δ Hz)*	Frequency of maximum energy at call middle minus frequency of maximum energy at call offset
Overall transition (Δ Hz)	Frequency of maximum energy at call end minus frequency of maximum energy at call beginning

Asterisks indicate those parameters excluded in acoustic analyses owing to multi-collinearity with another variable.

discriminated on the basis of caller identity ($n = 7$ females). As outlined before, we entered 17 variables into the DFA for $n = 111$ calls. We conducted two separate DFAs to investigate copulation calls produced in homo- vs. heterosexual contexts. Following this, we analysed whether female identity was encoded to the same degree in mating events with males vs. other females, using a matched-pairs t -test.

Swelling Size

We investigated whether the acoustic structure of calls co-varied with swelling size, an approximate indicator of fertility in bonobos (Heistermann et al. 1996). Accurate estimation of ovulation requires hormonal data. In chimpanzees, swelling size only provides a rough indication of the time of ovulation, which usually takes place during the period of maximum tumescence (e.g. Townsend et al. 2008). However, because of local restrictions we were limited to rely on tumescence to assess a female's fertility state. In our sample, the majority of calls were produced by females during their mid or maximum swelling states, with very few calls produced at low-swelling sizes. Thus, we could only analyse a limited data set, comparing copulation calls produced by four cycling females (LI, IS, KS, LK) with low (size zero or one) vs. high (size two to three) swelling sizes. Each

female contributed a minimum of one call during high-swelling stages (3–8 calls) and low-swelling stages (1–8 calls) with both male and female partners (female–female: $n = 12$ low-swelling calls and $n = 24$ high-swelling calls; female–male: $n = 11$ low-swelling calls and $n = 23$ high-swelling calls). We conducted separate pDFAs for homo- and heterosexual contexts to examine whether copulation calls provided information about swelling state.

Partner Rank

We investigated whether copulation calls provided acoustic cues to partner dominance rank. For male partners, we analysed 54 copulation calls from all females for which we had matched samples ($n = 5$), with each female contributing an equal number of calls with high- and low-ranked partners (IS, KL, SW = 12 calls, LK = 10 calls and KS = 8 calls). We conducted a pDFA on the 17 non-correlated parameters (controlling for female identity) and conducted finer-scaled analyses using one-way ANOVA tests for each acoustic parameter (controlling for female identity). For female–female interactions, sample sizes were low as calling was very rare with low-ranked female partners (see Clay et al. 2011). Therefore, we conducted Wilcoxon matched-pairs signed-ranks tests for $n = 5$ females on their \bar{x} values per acoustic

parameter ($n = 17$). These values were extracted from a sample of 55 calls, with each female contributing a minimum of three calls for the high-ranked partner condition (range 3–7) and one call for low-ranked partner condition (range 1–5).

Statistical Analyses

We conducted all statistical analyses using SPSS version 17.0 (IBM Corporation, NY, USA), except for the pDFAs, which were computed using R (version 2.5.11; R Core Development Team (www.r-project.org, UK)). Unless mentioned, tests were two-tailed and significance levels were set to $\alpha = 0.05$. Parametric analyses were conducted wherever possible, that is, following successful checks for normality and equal variance. Otherwise, non-parametric statistics were used. For small sample sizes, we calculated exact p -values (Mundry & Fischer 1998).

Results

Dominance

For females, we found significantly linear dominance hierarchies in all three groups with a clear alpha female (Fig. S1, Table S2). To facilitate analyses, we assigned individuals to 'high' and 'low' dominance classes, following their natural clustering into low and high DS (corrected for chance), which resulted

in $n = 7$ high-ranked and $n = 8$ low-ranked females (Fig. S1, Table S1). For males, we found a significant linearity in only one of the three groups (Table S2). Thus, we assigned males to high- and low-rank classes based on whether or not they dominated more than 50% of other males. This generated four high-ranking males in Group 1a, one high-ranking male in Group 1b and three high-ranking males in Group 2 (details in Supporting information).

Sexual Interaction Type

Results from a pDFA (controlling for caller identity) revealed that calls produced during homosexual genital contacts could not be reliably discriminated from those produced during heterosexual copulations (cross-validated classification after $n = 1000$ permutations = 7.82, $p > 0.05$; Table S3). On a finer scale, analyses of each acoustic parameter continued to reveal considerable acoustic overlap for homo- and heterosexual copulation calls, with no statistical differences for the majority of acoustic parameters (15/17 variables, all $p > 0.05$) and only marginal significance at the level of the call sequence (sequence duration: $F_{1,6} = 6.50$, $p = 0.043$; inter-call intervals: $F_{1,6} = 4.07$, $p = 0.090$). Overall, results suggested that females essentially produced the same copulation calls with male and female partners, although this interpretation was compromised by sample size.

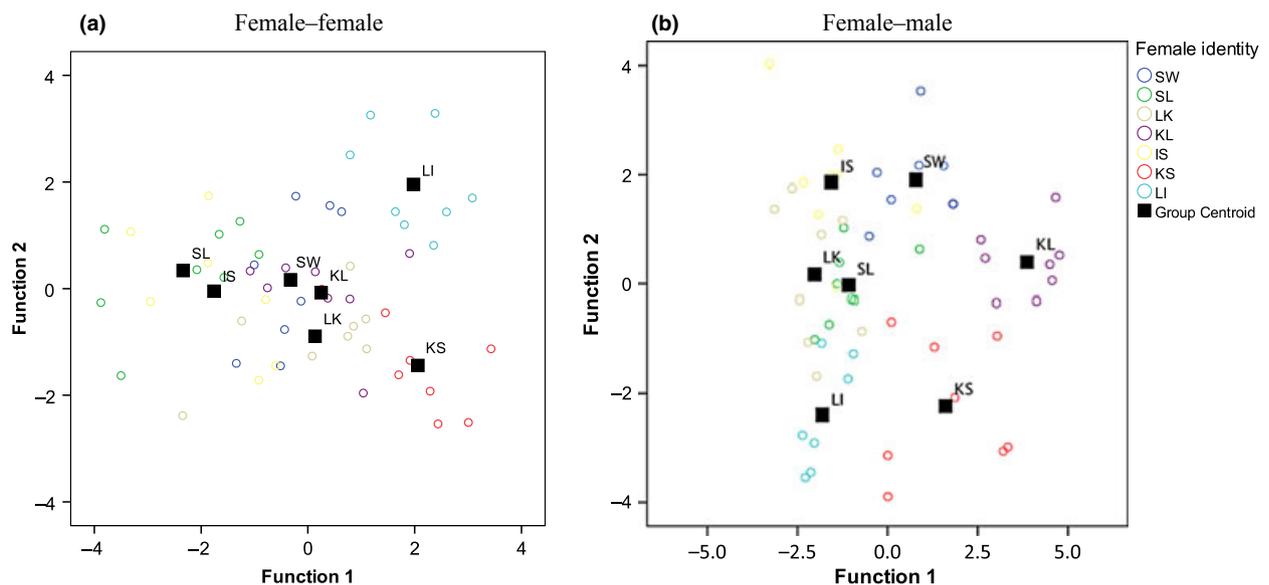


Fig. 3: Distribution of the discriminant scores for copulation calls produced by $n = 7$ different female bonobos during (a) female–female and (b) female–male sexual interactions. The discriminant scores lie along two discriminant functions established to discriminate female identity in both mating contexts. Group centroids per female are indicated with black squares (two letter code per individual).

Caller Identity

Results from DFAs indicated that caller identity was reliably encoded into copulation calls in both contexts, with both models generating seven significant discriminant functions (heterosexual copulation calls: Wilks' lambda: 0.006, $\chi^2_{102} = 213.224$, $p < 0.001$; homosexual copulation calls: Wilks' lambda = 0.020, $\chi^2_{102} = 168.248$, $p < 0.001$; Fig. 3). Cross-validated analysis indicated that both homo- and heterosexual copulation calls could be significantly discriminated on the basis of caller identity [cross-validated correct classification: heterosexual: 50.9%, Binomial test (0.14) $p < 0.001$; homosexual: 37.5%, Binomial test (0.14) $p = 0.001$]. A comparison of the per cent correct classification between contexts for each female demonstrated that cues to caller identity were encoded more strongly in heterosexual copulation calls compared with homosexual ones (paired *t*-test, two-tailed: $t_6 = 2.838$, $p = 0.030$). Group membership was unlikely to have influenced calling behaviour as several females (SW, SL KL IS) participated in both groups during the two data collection periods.

Swelling Size

Results from pDFAs on the acoustic structure of calls during homo- and heterosexual interactions revealed that calls produced during low- and high-swelling phases could not be statistically discriminated from

one another (cross-validated classification after 1000 permutations: homosexual context = 12.3, $p > 0.05$; heterosexual context = 15.0, $p > 0.05$).

Partner Rank

A pDFA on the factor of the dominance rank of male partners (high or low) failed to classify calls given to high- and low-ranked males with a significant level of accuracy ($n = 54$ calls for five females; cross-validated classification after $n = 1000$ permutations = 19.85, $p > 0.05$). However, results from one-way ANOVA tests (with caller identity as a random factor) revealed that, at the level of call delivery, females produced significantly longer copulation call bouts during copulations with high-ranked males compared with low-ranked males ($F_{1,4} = 36.30$, $p = 0.001$; standard Bonferroni corrections for multiple comparisons; Fig. 4) and also produced a greater number of calls ($F_{1,4} = 13.46$, $p = 0.018$). For high- compared with low-rank male partners, the inter-call intervals tended to be shorter although not significantly so ($F_{1,4} = 4.55$, $p = 0.095$).

For female–female interactions, we carried out Wilcoxon paired tests, comparing copulation calls for $n = 5$ females with high- and low-ranked partners, which revealed no statistical effect of partner rank on any of the acoustic parameters (all tests: $p > 0.05$). However, low sample sizes caused by low call production rates with low-ranked partners prevented meaningful conclusions.

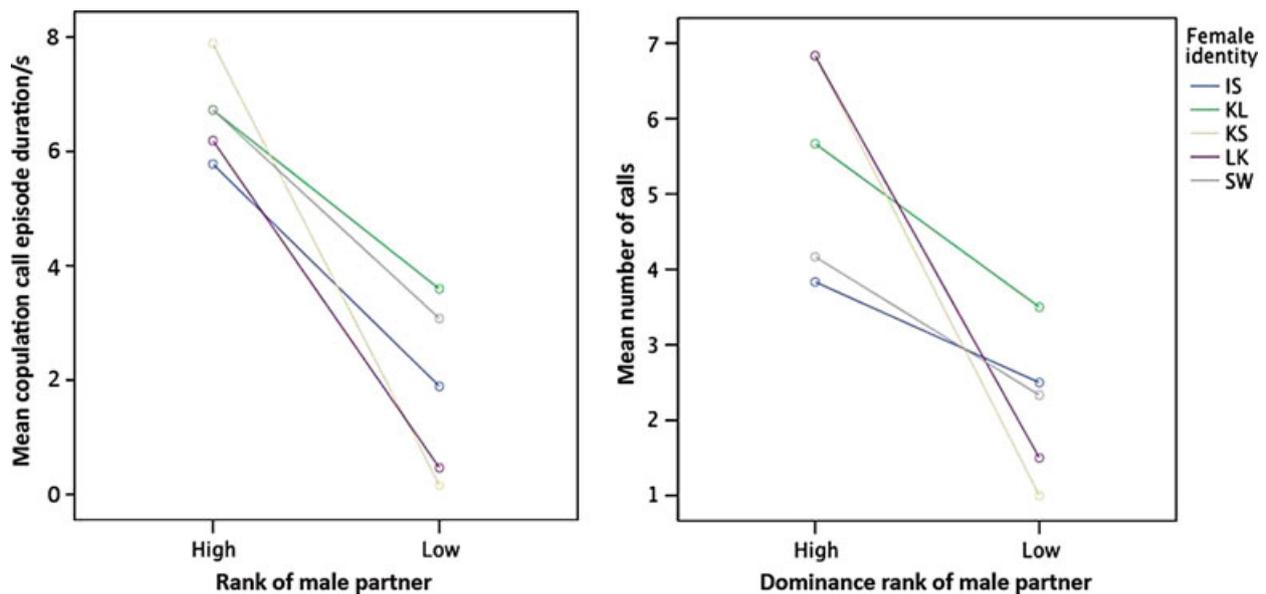


Fig. 4: Line graphs showing the values obtained for the parameters of mean copulation call episode and mean number of call units as a function of male partner rank for $n = 5$ female bonobos engaging in mating interactions with male partners.

Discussion

Bonobos are well known among the primates for their heightened levels of socio-sexuality, with individuals engaging in sexual interactions with both male and female partners (e.g. de Waal 1987; Kano 1992; Hohmann & Fruth 2000). We investigated the acoustic structure of female copulation calls produced during their sexual interactions with males and females. The key finding was that calls produced with male partners could not be reliably distinguished from those produced with female partners. To our knowledge, this is the only known example where copulation calls do not differ in reproductive vs. non-reproductive contexts. For instance, in Barbary macaques, females occasionally give calls similar to copulation calls during female–male agonistic encounters. However, acoustic analyses revealed that these calls differed from copulation calls produced during sexual interactions (Todt et al. 1995).

Previous studies of copulation calls have typically focussed on the reproductive significance of these calls, by identifying them as sexually selected signals that promote the caller's reproductive success (e.g. Maestriperi & Roney 2005; Pradhan et al. 2006). While our results indicate that these calls in bonobos have retained some features relating to reproductive function, their broader use within the homosexual interactions indicate that, like the sexual behaviour they accompany, copulation calls have become ritualised in ways that go significantly beyond pure reproduction. They are used as more general social signals in flexible and seemingly strategic ways.

What underlying mechanisms could explain the observed acoustic similarities of copulation calls? One reasonable prediction was that heterosexual mating events caused higher levels of arousal than homosexual genital contacts, because of the proprioceptive differences caused by penile intromission, thrusting movements and so on. However, despite these considerable physical differences, we found that females produced the same acoustic signals in both contexts, indicating that the type of sexual stimulation experienced had no relevant effect on their calling behaviour, apart from slight differences in the duration of call episodes and inter-call intervals (although this effect was driven by high- vs. low-ranked male partners).

Bonobo copulation calls convey significant cues to caller identity in both homo- and heterosexual contexts, although identity was more strongly encoded during interactions with males, the putative ancestral function of the call. Alerting other males to both

the calling female's presence and her sexual receptivity could maximise the benefits received from indirect mate choice, either by inciting male–male competition or by increasing the quality and number of partners (e.g. O'Connell & Cowlshaw 1994; Semple 1998, 2001). Such a strategy may be especially important for species in which females regularly overlap in their oestrous cycle, breed non-seasonally (e.g. Altmann 1996) and compete for mating opportunities (Vervaecke & van Elsacker 2000; Hohmann & Fruth 2003), as it is the case for bonobos. Consistent with this explanation is the evidence of individually distinctive copulation calls in numerous other primate species that share similar reproductive ecologies (e.g. chimpanzees, *P. troglodytes*; Townsend et al. 2008; baboon species: e.g. *Papio cynocephalus ursinus*, Semple 2001; and macaque species, e.g. *Macaca fascicularis*, Deputte & Goustard 1980).

Providing cues to identity in same-sex interactions may, on the one hand, represent a functionless by-product of a call that originally evolved as reproductive signal. Alternatively, it may help a female to advertise her presence and sexual activity to other group members. As highlighted before, sexual interactions in bonobos play an important role in the regulation and formation of social relationships between individuals that are only distantly related (de Waal 1987; Hohmann & Fruth 2000; Fruth & Hohmann 2006; Paoli et al. 2006). Vocal advertisement of such interactions may be especially relevant during immigration, when newly arriving females try to integrate and develop affiliations with other group members, with whom they lack genetic ties (Idani 1991; Hohmann et al. 1999). Consistent with this is the evidence that, during immigration, newly arriving females engage in frequent sexual interactions with group members and often focus their sexual efforts towards the high-ranking females (Idani 1991). Furthermore, results from our previous study revealed that, during genital contacts, callers were generally low-ranking females and regardless of partner sex, females were most likely to produce copulation calls during interactions with high-ranked partners (Clay et al. 2011).

Analyses of partner rank revealed that, similar to mating type, dominance status of male partners influenced call delivery, but not acoustic morphology. While we found no corresponding acoustic differences for female partner rank, small sample sizes and low rates of interactions may not have been sufficient to detect context-specific variation in sound structure, preventing strong conclusions for this analysis. Nevertheless, these results and our pre-

vious findings (Clay et al. 2011) indicate that females advertise their sexual interactions with high-ranked partners on the basis of call delivery and overall call use (i.e. calling or remaining silent), rather than encoding such information within the acoustic signals themselves. At this stage, the adaptive significance of this phenomenon still requires further investigation, although it is likely that the encoding of information about partner rank may enable a female to influence the behavioural decisions of potential mates and promote sperm competition, a hypothesis which has been put forward in previous studies of other primates which report similar rank effects (e.g. *Papio cynocephalus*; Semple et al. 2002; *P. troglodytes*: Townsend et al. 2008; *Macaca sylvanus*, Pfefferle et al. 2008b). Further work using playback experiments is required to examine what information receivers' extract from copulation calls in bonobos and to investigate call function.

In terms of reproductive state, we found no evidence that swelling size (a rough indicator of fertility; Heistermann et al. 1996) is reflected in the calls' acoustic structure. However, further studies based on more accurate hormonal analyses of ovulation and fertility states are required before more certain conclusions can be drawn. However, our negative result is consistent with previous findings from studies of other Old World primates, such as Barbary macaques and chimpanzees (Pfefferle et al. 2008a; Townsend et al. 2011; but see Semple & McComb 2000). The evolution of concealed ovulation in primates is thought to represent an adaptive strategy for promoting paternity confusion among males, especially high-ranking ones (e.g. Pradhan & van Schaik 2008). By concealing ovulation, female bonobos can reduce the risk of monopolisation by the most dominant male and thus promote sperm competition as well as paternity confusion among males, a strategy that may promote their future support (e.g. Pradhan & van Schaik 2008). While infanticide has not been directly observed in bonobos, significant levels of aggression in the context of mating, particularly from other females, suggests that copulation calls may represent one potential mechanism to cement the support of high-ranked allies against the threats of female–female competition. For example, there have been numerous cases of females mishandling, abducting and aggressing other females' infants in both the wild and captivity, as well as indirect cases of infanticide (e.g. Vervaecke & van Elsacker 2000; Hohmann & Fruth 2003).

In sum, our results show that copulation calls in bonobos have retained some features pertaining to their evolved reproductive function. At the same

time, they have also become ritualised signals used in broader social contexts that have nothing to do with the caller's direct reproductive interests, suggesting a 'duality of function' for bonobo copulation calls. More generally, the study demonstrates how vocal behaviour that has evolved for a specific biological function (i.e. reproduction) can undergo an evolutionary transition towards broader and more flexible use. The transition of copulation calls from a reproductive into a social signal is relevant for future debates on the function of copulation calls specifically, and the role that social life plays in the evolution of animal vocal communication more generally.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1: Results of simple linear regressions showing the linear dominance hierarchies of all females in the three study groups (Group 1a: N = 9; Group 1b: N = 7, Group 2: N = 5), calculated using normalized David's scores based on the dyadic dominance index, corrected for chance. Dashed circles indicate the separation of dominance classes, with individuals in the left hand circle being 'high' rank and individuals in the right-hand circle being 'low' rank.

Table S1: Information about group composition for the three study groups at Lola Ya Bonobo Sanctuary. Information about their identity code, name, age and dominance rank is indicated. Individuals arrived as wild juveniles/infants, thus age ranges are approximate estimations based on veterinary assessment. Dependent infants are indicated in superscript next to their mother's name, with their sex in brackets.

Table S2: Results from Matman tests for linearity of dominance hierarchies calculated for the three bonobo groups in this study.

Table S3: Mean values (+SDs) for acoustic parameters for analysis of homosexual versus heterosexual copulation calls for 7 bonobo females.

Data S1: Supporting Methodology on Study Population, Data Collection and Dominance Analyses.

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