The role of acoustic signaling for spacing and group coordination in a nocturnal, pair-living primate, the western woolly lemur (Avahi occidentalis)

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ABSTRACT

Objectives

How social groups govern their distribution in time and space is a central question in socioecology. The aim of this study is to explore the role of acoustic signaling for spacing and cohesiveness in a nocturnal, cohesive, pair-living strepsirrhine.

Material and Methods

The study was conducted in northwestern Madagascar. Six pairs of *Avahi occidentalis* were radio-collared and home range usage, vocalizations and call-associated behavior recorded using GPS-based focal animal sampling. Home range size was analyzed using ArcView GIS 3.3. Calls were characterized by a multiparametric sound analysis.

Results

Three frequently used, acoustically distinct call types were identified: the avahee call, the whistle call, and the growling call, the latter is a soft; the two others are loud calls. Call types are given by both sexes and convey individually-specific signatures. Call types are used primarily in the locomotion context in the non-core-area of home ranges. The least common avahee call is responded by the avahee call from farther away. The more common whistle call, given when partners become visually isolated, and the growling call emitted at close distances, were answered by the whistle and the growling call. Results suggest a spacing function for the avahee call and group coordination functions for the other call types.

Discussion

Our study provides first empirical evidence for a nocturnal, cohesive pair-living strepsirrhine that vocal signaling represents an important mechanism for spacing, group coordination and decision making. Findings contribute to a better understanding of the evolutionary roots of primate vocal communication.
INTRODUCTION

A major question in socio-ecology is how pairs coordinate group movement and maintain group cohesiveness (Boinski and Garber, 2000; Couzin and Krause, 2003; de Waal et al., 2003; King and Sueur, 2011). Haplorrhine primates (except the orangutan) and diurnal Malagasy lemurs live in permanent social groups in which group members cooperate in foraging, predator detection and defense, offspring rearing or resource defense by using elaborate sets of visual, tactile, olfactory and auditory signals for social communication (Cheney and Seyfarth, 1990; Zimmermann, 1992; Hauser, 1996; Clarke et al., 2006; Röper et al., 2014). In the auditory domain, loud calls (Marten and Marler, 1977; Waser and Waser, 1977; Waser and Brown, 1984), choruses (Hall and DeVore, 1965; Tenaza, 1976; Geissmann and Mutschler, 2006), songs and duets (Pollock, 1986; Cowlishaw, 1992; Geissmann and Mutschler, 2006) are often used for inter-group spacing and group reunion, whereas soft vocalizations are given to regulate group coordination and intra-group cohesion (e.g. African elephant: Poole et al., 1988; Golden brown mouse lemur: Braune et al., 2005; redfronted lemur: Pflüger & Fichtel, 2012; Black howler monkey: da Cunha and Byrne, 2006; Spider monkey: Ramos-Fernández, 2005; Spehar and Di Fiore, 2013; gibbon: Clarke et al., 2015).

Malagasy lemurs represent a monophyletic group including nocturnal, cathemeral and diurnal species living in highly diverse social systems (e.g. Müller and Thalmann, 2000), making them an ideal model group for understanding the impact of phylogeny, social complexity and activity on the evolution of acoustic mechanisms regulating inter-group spacing and group coordination in primates (Braune et al., 2005). To date, data on the role of vocal communication for inter-group spacing and intra-group coordination are available for diurnal group- and pair-living as well as for nocturnal, solitary-foraging lemur species living in dispersed social systems or in pairs. Thus, diurnal Indriidae, such as the pair-living indris or group-living sifakas are known to use contagious calling, loud duetting or chorusing for
territory demarcation and loud alarm calls (roars) for advertising the detection of disturbances or predators (Maretti et al., 2010; Fichtel and Kappeler, 2011). Soft vocalizations (e.g. hums in indris; grumbles calls in sifakas) are described regulating contact with other group members at short distances (Pollock, 1975; Petter and Charles-Dominique, 1979; Macedonia and Stanger, 1994; Maretti et al., 2010; Patel and Owren, 2012). Nocturnal lemurs which form permanent sleeping groups, but forage solitarily during the night, such as mouse lemurs, use acoustic signals in the context of mating, intra-group cohesion (mother–infant, sleeping group reunions) and group coordination (Hafen et al., 1998; Braune et al., 2005; Scheumann and Zimmermann, 2007b), as well as during conflicts with con- and heterospecifics (Zimmermann, 2010; Zimmermann, 2013; Zimmermann et al., 2013). Olfactory mechanisms seem to be important for regulating the distribution of groups in space (Braune et al., 2005) and avoiding predators (Sündermann et al., 2008; Kappel et al., 2011). For nocturnal lemurs with a dispersed pair system, such as sportive lemurs, a broad variety of different loud call types are described, which are arranged in complex series to regulate space use and cohesiveness between pair partners and neighbors (e.g. Rasoloharijaona et al., 2006; Méndez-Cárdenas and Zimmermann, 2009; Rasoloharijaona et al., 2010).

Until now, empirically based bioacoustic studies of nocturnal cohesive pair-living primates have been lacking. To collect first empirical data and embed them into the evolution of mechanisms governing primate group coordination in time and space, we studied the western woolly lemur (Avahi occidentalis), distributed in the dry deciduous forests of northwestern Madagascar. These weasel-sized lemurs that weigh approximately 1 kg (Ramanankirahina et al., 2011), form permanent pairs in which pair partners forage together during the night and sleep together at safe sleeping sites in trees with dense foliage during the day (Albignac, 1981; Warren and Crompton, 1997; Ramanankirahina et al., 2012). As do sportive lemurs, woolly lemurs belong to the so-called clingers and leapers (Warren, 1997; Warren and Crompton, 1997), and are able to move rapidly through dense forests. In contrast to sportive
lemurs, woolly lemurs are folivorous specialists, selecting underrepresented food resources for which pairs seem to compete (Thalmann, 2001). The sexes are monomorphic and do not differ in body mass and size (Ramanankirahina et al., 2011). Pair partners interact extremely peacefully, although females are dominant in the feeding context (Ramanankirahina et al., 2011). The mean home range size of pairs was estimated to be about 1.59 ha (Warren and Crompton, 1997). Woolly lemurs are the only nocturnal representative of the family Indriidae. Information on their vocalizations is limited so far. Petter and Charles-Dominique (1979) were the first and only authors displaying spectrographic representations of the calls. However, they did not provide empirical information on the callers and call usage, nor on the geographical origin of the callers (Table 1). Harcourt (1991), Warren and Crompton (1997), and Thalmann (2003), studying feeding ecology, locomotion energetics and social behavior of woolly lemurs described some frequently heard calls as avahee call, whistle call and growling call, and speculated on their function (Table 1), but neither presented empirical data on their acoustic structure nor on their usage.

Table 1

Thus, our study aims i. to acoustically characterize these qualitatively described call types in woolly lemurs, and ii. To explore for the first time their suggested functions. For the latter analysis we linked the structure of the acoustically characterized call types to the sound-associated context. Furthermore, by performing a multivariate acoustic analysis, we tested for individuality in call structure. Likewise, by relating caller positions for the respective call types to the home range of the respective pair, we explored the spatial distribution of calling positions.
MATERIALS AND METHODS

Study area and animals

The study was carried out in the research area JBA (Jardin Botanique A, 30.6 ha; 16°19'S, 46°48'E) of the Ankarafantsika National Park in northwestern Madagascar. The vegetation consists of dry deciduous forests, characteristic of the western lowlands of Madagascar. Six pairs of western woolly lemurs were studied. For one pair the female changed during the observation period.

Data collection

Each animal was darted, weighed and morphometrically characterized and fitted with a radiotrigger on a backpack (for more details on darting focal animals, radiotelemetric methods, group sizes and focal animal contact times, see Ramanankirahina et al., 2011; Ramanankirahina et al., 2012). The median body length was 20 cm for males and females, the mean body mass was 825 g for males and 999 g for females with no significant difference between sexes (see Ramanankirahina et al., 2011). GPS-based radio telemetry was used to monitor behavioral activity and habitat usage of focal animals. Using a portable receiver and an antenna, a radio-collared individual was followed between May to November 2008. Each focal animal was observed with the aid of headlamps during two half nights (6-12 p.m. and 12-6 a.m.) every month except for May and August (one half night 6-12 p.m.). Unfortunately, not more than one focal animal could be observed in one night because of budget limitations. The total contact time was 667 hours (Ramanankirahina et al., 2011). One female could only be followed for October and November for a total of 19 hours contact time since she had been caught first in October. For the pair where the female changed during observation, the first female was observed during May and June and the second female from August to November. Since both females were associated with the same male, data of both females were treated as
one female pair partner for further analyses, as done in two previous papers (Ramanankirahina et al., 2011; Ramanankirahina et al., 2012).

Focal animal sampling with continuous recording was conducted (Altmann, 1974; Martin and Bateson, 1993). The movement pattern of a focal animal was noted by taking the geographical positions every 10min (GPSMAP 60CSx, Garmin, mean error < 10 m). Calls were classified into three major call types: avahee call, whistle call and growling call. We collected information regarding the identity of the caller, the time, the type of the tree where the focal individual vocalized (resource site=site used for feeding or sleeping, or non-resource site=all other sites, except feeding and sleeping sites). Calling context (events inducing a call) was recorded, as well as the focal animal’s activity, e.g. resting, feeding or locomotion, according to Méndez-Cárdenas and Zimmermann (2009). If a call was responded by another individual (meaning immediately after the focal animal gave its call), the call type of the response call was recorded. If the focal animal gave a call immediately after we had heard another animal calling, the call type of its response was recorded as a response call to another individual’s call.

**Determination of home range size and overlapping area**

GPS coordinates per half night were entered immediately after a focal animal night into ArcView GIS 3.3 (ESRI). Home range size was assessed according to common methods (Ganas and Robbins, 2005; Harris, 2006) and corrected for obvious outliers: The size was determined by using GPS positions (Male: median, 532 points/individual, range 427-661 points, N=6; Female: median, 503 points/individual, range 186-645 points, N=6) collected every 10min during the nocturnal focal animal sampling. To be comparable with most other lemur studies, we estimated home range sizes and overlaps between partners and pairs by using the 100% Minimum Convex Polygon (MCP). To determine if there was a sex difference in home range size, the home range sizes of males and females were compared.
using the Mann-Whitney U test. Additionally, the proportions of home range overlaps between the different focal individuals were calculated by an overlay procedure of the geoprocessing extension in ArcView 3.3. Mann-Whitney U tests were used to examine whether the proportion of overlap between home ranges of same sex neighbors (male/male, female/female), opposite sex neighbors (male/female) or pair partners differed.

**Acoustic recording and analyses**

Vocalizations were recorded using a Sennheiser directional microphone (ME67, Sennheiser, Wedemark, Germany: frequency range: 40 – 20 000 Hz) with a windshield, linked to a stereo cassette recorder (Sony Professional WM-D6C; Sony, Japan) onto audio recording cassettes (Sony super chrome class UX-S IECII/Type II). All vocalizations were digitized with a sample frequency of 44.1 kHz and a sample size of 16 bit using Batsound Pro 3.31 (Petterson Elektronik AB, Uppsala Sweden) and stored as wav?? files.

In total, 733 vocalizations were visually inspected using spectrograms of the software Batsound Pro 3.31 (FFT: 512, Hanning window) and visually classified into three call types: (1) the avahee call, (2) the whistle call, and (3) the growling call (Figure 1). Based on this sample, 405 calls of high quality (i.e. calls not disturbed by background noise and minimum amplitude difference of 7% between background noise and maximum amplitude of the call) were selected to conduct a multi-parametric sound analysis using call-type specific self-written macros for the software Signal 4.1 (Kim Beeman, Engineering design). The avahee call consists of two harmonic syllables (Figure 1), for which four temporal and nine spectral parameters were measured (Table 2). The first syllable is characterized by a clear fundamental frequency, often not visible in the second syllable. Thus, in the latter we characterized the dominant frequency band and the most prominent side-band. We measured for the first syllable acoustic parameters related to the fundamental frequency (F0) and for the second syllable acoustic parameters related to the dominant frequency (Fd) band and the first side
band above or below the dominant frequency. The whistle call is harmonic and characterized
by an almost constant narrow frequency band (Figure 1). For the whistle calls one temporal
and four spectral parameters were measured (Table 2). The growling call consists of a high
number of broadband pulses (Figure 1). Measurements of pulse duration and distance
between pulses were related to the first five pulses of a call. For the growling call, 11
temporal and one spectral parameter were measured (Table 2).

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Figure 1

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Table 2

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For further statistical analysis only individuals that contributed at least five calls were
considered. To characterize the acoustic structure of the calls, we calculated the mean and
standard deviation of each parameter per call type based on the individual means. To
investigate individual differences in Avahi vocalizations, we conducted analyses for each call
type as follows: first, to investigate whether acoustic parameters differ statistically between
individuals, we performed a One-way ANOVA and calculated the effect size using eta². To
control for multiple testing we applied the Fishers Omnibus test combining multiple p-values
(Haccou and Melis, 1994). Second, to investigate whether calls can correctly be classified to
the respective individual, we performed an independent DFA and a nested permuted
discriminant function analysis (pDFA; Mundry and Sommer, 2007). To rule out that acoustic
parameters used for both DFAs did not correlate with each other, prior to the DFA we
performed a correlation analysis using a Pearson correlation. We selected acoustic parameters with a correlation coefficient < 0.500. For the independent DFA, we calculated the degree of agreement between predicted and observed classification using the Kappa test (Scheumann et al., 2007c). According to the literature (Landis and Koch, 1977, Stemler, 2001) the level of agreement is defined as follows: Cohen’s kappa <0.00 poor agreement, 0.00–0.20 slight agreement, 0.21–0.40 fair agreement, 0.41–0.60 moderate agreement, 0.61–0.80 substantial agreement and 0.81–1.00 almost perfect agreement. For the pDFA we used subject as test factor and sex as control factor. The number of permutation was set at 1000 and the number of random selections was set at 100.

Analysis of calling activity, location, context and counter-calling

For analysing the calling activity, we calculated the call rate of the respective call type for each individual by dividing the number of calls of the respective call type by the total contact time per hour per individual. Due to the fact that data were not normally distributed, non-parametric statistics were performed. To investigate sex differences in the calling activity, we compared the call rate of the respective call types between sexes using Mann-Whitney U tests.

To investigate the effect of resource site, we compared the call rate between the resource and the non-resource site. Since the duration an individual spent at the resource or non-resource site was not equally distributed (82% of the time individuals spent at the resource site and 18% at the non-resource site), we first calculated the call rate per minute per individual at the resource site. Thus, we counted the number of calls and divided them by the duration in minutes the individual was observed at the respective location. Then, we compared the call rate between resource and non-resource site using the Wilcoxon signed-rank test.
To investigate the effect of context, we compared the call rate between the three main contexts which were observed shortly before the focal individual vocalized: locomotion, resting and feeding. Since the duration an individual spent in the respective contexts varied strongly between contexts (% of total contact time a context was observed: locomotion: 1%, resting: 58%; feeding: 37%), we first calculated the call rate per minute per context. Thus, we counted the number of calls and divided them by the time the individual was observed in the respective context per minute. Then, we compared the call rate between the three contexts using the Friedman test. When the Friedman test was significant, pairwise comparisons were conducted using the Wilcoxon signed-rank test. To control for multiple testing the Bonferroni correction was used ($p_{corr} =$ No. of comparisons*$p$-value).

To analyze counter-calling, we analyzed, at first, for each call type separately whether the calls of the focal animal were given spontaneously or as response to calls. Calls were counted as response call when they occurred immediately after a call of another individual. Thus, we calculated for each focal animal and call type the percentage of spontaneous calls and calls in response to avahee, whistle or growling calls from other individuals. To investigate whether other individuals responded to the call types of the focal animals, we calculated for each focal animal and call type the percentage of calls which elicited no response, avahee, whistle or growling calls.

**Spatial distribution of the calling positions**

The calling position for the three call types of each pair was overlaid with the home range by using ArcView GIS 3.3 to explore if calls were emitted at the core or non-core area of the home ranges. To define the non-core and core area of the home ranges, the 95% (non-core) and 50% (core) Fixed Kernel method was used (Horner and Powell, 1990; Harris, 2006).
The recorded geographical position of the pair’s calling position (place where a focal individual of the respective pair emitted a call) was mapped on the Kernel home range area. From the geoprocessing extension in ArcView 3.3 we quantified the number of caller positions for each pair within the core (Kernel 50%) and the non-core area (Kernel 95%). To investigate whether significant more calling positions were observed within the core compared to the non-core area, we calculated an observed versus expected Chi square test based on the number of calling positions in the respective areas. Since the 50% Kernel contour represents the 50% probability that the pair was found in the core area (Horner and Powell, 1990), the expected frequency for an equal distribution was set at 50% for the core area.

**Ethical considerations**

All procedures adhered to the legal requirements of Madagascar, and were approved by the Ministere de l’Environnement et des Forêts and Madagascar National Parks. This research was conducted in accordance with the American Society of Primatologists and German Society of Primatologists (GfP) ethical principles for the treatment of primates.

**RESULTS**

**Home range size and overlapping areas**

Using the MCP method, the median home range size of pairs was 1.96 ha (min-max: 1.2-3.1 ha). Home ranges of pairs overlapped about 25% (Median, min-max: 10%-51%) with those of neighboring pairs. One pair showed no overlapping area with the other focal pairs (Figure 2).

For males, median home range size was 1.79 ha (min-max: 1.08-2.72 ha), for females 1.57 ha (min-max: 1.1-3.01 ha). Home range size did not differ significantly between sexes
The home ranges of pair partners overlapped strongly with each other. Thus, home range of males overlapped with the respective female pair partner about 85% (Median, min-max: 65%-97%) of their total home range and home range of females overlapped with the respective male pair partner about 93% (Median, min-max: range 88%-100%, Figure 2). There was no significant difference in the percentage of home range overlap between pair partners (Mann-Whitney U=8, N_m=6, N_f=6, p=0.109). In contrast to the strong overlap between the home range of pair partners, neighboring animals showed a low percentage of home range overlap for both sexes. Home range of males overlapped with neighboring males about 13% (Median, min-max: 7%-44%) of their total home range, whereas home range of females overlapped with neighboring females about 6% (Median, min-max: 3%-12%). Thus, the overlap between male home ranges was significantly larger than between female home ranges (Mann-Whitney U=2, N_m=5 N_f=5, p=0.028). Home ranges of males overlapped with neighboring females about 12% (Median, min-max: 6%-20%) of their total home range and home ranges of females overlapped with neighboring males about 9% (Median, min-max: range 8%-38%).

Acoustic characteristics of call types

The avahee call (N=6, n=113; Figure 1, Table 3) represents a loud, harmonic call consisting of two syllables. The first syllable showed a fundamental frequency with several harmonics, whereas the second syllable showed a dominant frequency band with side bands, the fundamental frequency not being visible in the spectrogram. The mean duration of the first syllable was 107 ± 9 ms and for the second syllable 255 ± 53 ms, respectively. The total
duration of the whole avahee call was 393 ± 57 ms. The first syllable was characterized by a
harmonic structure with a fundamental frequency of 707 ± 84 Hz. The second syllable was
characterized by a dominant frequency of 4118 ± 348 Hz. Side-bands could be observed
around this dominant frequency. The mean frequency distance between the dominant
frequency and the first side band above or below was 937 ± 89 Hz.

The whistle call (N=11, n=195; Figure 1, Table 3) is a loud call of almost constant
frequency. It was given as a single call or in a call series. The mean call duration was 1268 ±
304 ms and the peak frequency was 4353 ± 220 Hz, respectively.

The growling call (N=7, n=97; Figure 1, Table 3) is a soft and broadband call
consisting of rapidly repeated short broadband pulses. The call duration was 941 ± 228 ms
and a call contained 17 ± 4 pulses of almost constant duration 23-26 ms ± 2-3. The peak
frequency was about 4538 ± 1020 Hz.

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Table 3

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**Acoustic discrimination of individuals**

For the avahee call all acoustic parameters differed significantly between individuals
(F≥2.73, df=5, N=113, p≤0.023; 0.113≤η²≤0.419; Fisher Omnibus test: χ²=300.47, df=26,
p<0.001; Table 3). Based on the correlation analysis, six parameters were selected to calculate
the DFAs: duration of the first syllable (Dur1), gap of two successive calls (Gap), total
duration of the call (Dur), fundamental frequency of the first syllable (F0), maximum
dominant frequency (Fdmax) and difference between dominant frequency and sideband
(DiffFd-s). An independent DFA correctly assigned 70.8 % of the calls to the correct
individual (cross-validation: 66.4%), with moderate agreement of classification (k=0.544,
Results of the pDFA controlling for sex support that calls were classified significantly correctly to the respective individual (selected cases: 75.27% correctly classified, chance level=47.81%, p=0.001; cross-validation: 57.48% correctly classified, chance level=21.67%, p= 0.001, Table 4).

For the whistle call, all acoustic parameters differed significantly between individuals (F≥2.89, df=10, N=195, p≤0.002; 0.136≤η²≤0.269; Fisher Omnibus test: χ²=146.81, df=10, p<0.001; Table 3). Based on the correlation analysis, three parameters were selected to calculate the DFAs: total duration of the call (Dur), minimum fundamental frequency (Fpmin) and bandwidth (Fpbandwith). An independent DFA correctly assigned 31.3 % of the calls to the correct individual (cross-validation: 24.6%), with fair agreement of classification (k=0.211, p<0.001). The results of the pDFA controlling for sex revealed that calls were classified significantly correctly to the respective individual (selected cases: 33.10% correctly classified, chance level=19.45%, p=0.001; cross-validation: 16.48% correctly classified, chance level=8.86%, p= 0.011, Table 4).

For the growling calls almost all acoustic parameters differed significantly between individuals (F≥2.31, df=6, N=97, p≤0.040, 0.134≤η²≤0.383 except for the duration of pulse 1 and 5 F≤2.18, df=6, N=97, p≥0.053, η²≤0.127; Fisher Omnibus test: χ²=154.56, df=24, p<0.001; Table 3). Based on the correlation analysis six parameters were selected to calculate the DFA: total duration of the call (Dur), peak frequency (Peak) and duration of the second and fourth pulse (DurPulse2 and DurPulse4) and distance between first and second (DisPulse1-2) and between third and fourth pulse (DisPulse3-4). An independent DFA correctly assigned 56.7 % of the calls to the correct individual (cross-validation: 50.5%), with moderate agreement of classification (k=0.420, p<0.001). The results of the pDFA controlling for sex revealed that calls were classified significantly correctly to the respective individual (selected cases: 65.86% correctly classified, chance level=50.52%, p=0.015; cross-validated cases: 29.15% correctly classified, chance level=17.55%, p=0.024, Table 4).
Calling activity and call types

The highest calling rate could be observed for growling calls with a median of 0.833 calls/h, followed by whistle calls with a median of 0.406 calls/h. The lowest calling rate was observed for avahee calls with a median of 0.070 calls/h. The avahee call was produced with the median rate of 0.071/h (25%-75% quartile: 0.020-0.140/h) by males and 0.087/h (25%-75% quartile: 0.038-0.385/h) by females. The median rate of the whistle call was 0.516/h (25%-75% quartile: 0.325-0.568/h) by males and 0.318/h (25%-75% quartile: 0.124-0.434/h) by females. The growling call was emitted with the median rate of 0.794/h (25% -75% quartile: 0.514-2.014/h) by males and 0.922/h (25%-75% quartile: 0.470-3.324/h) by females. Sexes showed no significant difference in calling rate for each call type (avahee: U=14, N_m=6, N_f=6, p=0.522; whistle: U=7, N_m=6, N_f=6, p=0.078; growling: U=15, N_m=6, N_f=6, p=0.631).

Calling rate, location, call associated context and antiphonal-calling

The calling rate of the three call types was significantly higher at the non-resource than the resource site (T=1, N=12, p≤0.004, p_corr≤0.012 for all call types) and differed significantly between contexts (χ²≥9.556, N=12, df=2, p≤0.008, p_corr≤0.024 for all call types, Figure 3).

For all three call types the calling rate during locomotion was significantly higher than during resting and feeding (T≤1.50, N=12, p≤0.013, p_corr≤0.039 for all call types; Figure 3), whereas no significant difference between the feeding and the resting contexts could be found.
for the avahee and growling call (T≥3.50, N=12, p≥0.107) nor after applying the Bonferroni correction for the whistle call (T=5.75, N=12, p≥0.030, p_{corr}=0.09).

A total of 89 avahee calls were recorded from 11 focal animals, both when pair partners were both visible to observer and when one partner was out of sight. 68 of 89 avahee calls (76.40%) were spontaneously given and 17 avahee calls (19.1%) were given in response to an avahee call from another individual. 79 of the 89 avahee calls (88.76%) of the focal animals evoked no vocal response. In 8 cases another individual answered an avahee call. In the majority of cases where the focal animal responded to an avahee call or other individuals responded to an avahee call of the focal animal, an avahee call was recorded as response call.

For the whistle call, 271 calls from all 12 focal animals could be recorded when pair partners travel without visual contact to each other (one pair partner out of sight of observer). 254 whistle calls (93.73%) were produced spontaneously. 14 whistle calls (5.17%) were given in response to a whistle call from another individual. 248 of the 271 whistle calls (91.51%) of the focal animals evoked no vocal response. Interestingly, in 12 cases (4.43%) another individual answered by producing a growling call and in 9 cases by also producing a whistle call (3.32%). Thus, in the majority of cases where the focal animal responded to a whistle call or other individuals responded to a whistle call of the focal animal, the growling call was recorded in response to a whistle call.

A total of 855 growling calls were recorded from all focal individuals, when pair partners were close to each other (pair partners could be seen by observer). The majority of growling calls (769, 89.94%) were given spontaneously, but 69 (8.07%) were produced in
response to a whistle call of another individual. 850 of the 855 whistle calls (99.42%) of the focal animals evoked no vocal response. To sum up, in the majority of cases focal animals responded to whistle calls using a growling call, whereas growling calls themselves evoked less vocal responses.

**Spatial distribution of calling positions**

Using the 95% Kernel, the median home range size of pairs was 1.61 ha (min-max: 1.1 – 2.94 ha; Figure 4–6) which is comparable to the MCP method. The median core area of pairs was 0.14 ha (min-max: 0.06 – 0.38 ha) using the 50% Kernel. No overlapping of the core area existed between neighboring pairs or neighboring individuals. Plotting the caller’s positions per pair in the respective pair home range, 87% of avahee (Median, 25-75% quartile: 50-100%, Figure 4), 86% of whistle (Median, 25-75% quartile: 60-97%, Figure 5) and 84% of growling call positions (Median, 25-75% quartile: 73-94%, Figure 6) occurred outside the core area of the respective pairs. These findings were statistically supported: for each call type, significantly more calling positions than expected by chance were found outside of the core area (avahee: $\chi^2=26.98$, df=1, N=99, p<0.001; whistle: $\chi^2=84.14$, df=1, N=271, p<0.001; growling: $\chi^2=342.32$, df=1, N=855, p<0.001).
DISCUSSION

Our study suggests for the first time that in a nocturnal, cohesive pair-living primate, the woolly lemur, acoustic signaling acts as an important mechanism to govern pair coordination and inter-group spacing. Call structure and usage of the reported three call types provided the first empirical evidence for their functions. Findings will be discussed regarding the suggested functions reported in the literature. Furthermore, we will compare findings with other lemur species to explore two commonly discussed hypotheses for the evolution of communicative complexity in primates, the phylogenetic and the social complexity hypothesis.

Vocalizations of woolly lemurs and their potential functions

In our study we investigated the three most common vocalizations of the western woolly lemur, the avahee call, the whistle call and the growling call, with regard to their acoustic structure and usage to deduce their function. All three call types were used primarily in the locomotion context in the non-core-area of home ranges. Additionally, specific differences between call types were observed in relation to loudness, calling rate and antiphonal calling behavior suggesting specific functions for spacing and social cohesion. The least common avahee call was mainly responded by the avahee call. The more common whistle call, given when partners become visually isolated, and the growling call, emitted at close distances, was answered by the whistle or the growling call. Avahee and growling calls displayed statistically moderate individual discrimination, while discrimination in the whistle call was only fair.

As outlined in the following, our findings support some speculations (Table 1) in the literature on the function of these call types for intra- and inter-group coordination.
empirically. The avahee call was given with high amplitude and displayed energy concentrated at lower frequencies. These acoustic characteristics are known to minimize degradation and attenuation by high ambient noise and maximize sound transmission and localization over longer distances (Marten and Marler, 1977; Waser and Waser, 1977; Waser and Brown, 1984). Thus, this call type can be considered a loud call. The avahee call rarely occurred compared to the second loud call type, the whistle. Both call types were given in the same context, namely locomotion in the non-core area. In contrast to the whistle call, the avahee call was answered by the avahee call from individuals, which seemed to be farther away and thus most probably not pair partners. Direct agonistic conflicts between neighboring pairs were never observed in the study period. These findings support that the avahee call is used for territory demarcation as suggested by Warren and Crompton (1997) and has an inter-group spacing function.

In contrast to the avahee call, the whistle call and the growling call were given with much higher rates. Whistle calls were responded by growling calls, suggesting that both call types function for the coordination of pair movement and thus contribute to pair cohesion and decision making. Both call types differ in sound amplitude. The growling call has fairly low amplitude with call attributes that are recognizable only at short distances and that also evoked less vocal responses than the two other call types. In the majority of cases, both pair partners were together when producing growling calls, suggesting that the growling call is given as a close-distance location marker to keep in contact as is also described for diurnal lemurs (e.g. sifakas: Trillmich et al., 2004; for redfronted lemurs: Pflüger and Fichtel, 2012). In contrast, the whistle call can be considered as a loud call due to its high amplitude and the fact that it is emitted at farther distance when visual contact between pair partners is broken by vegetation, comparable to diurnal lemurs living in cohesive social systems, e.g. the lost call in sifakas (Trillmich et al., 2004) or the meows in ringtailed lemurs (Oda, 1996) and redfronted lemurs (Pflüger and Fichtel, 2012). Findings support that the whistle call functions
as a means of communication between pair partners travelling and foraging together at farther distances (Warren and Crompton, 1997).

Acoustic analysis revealed individual distinctiveness across all call types, with variation in agreement between observed versus predicted classification. Whereas the discriminant analysis revealed a moderate agreement for the avahee and growling call, characterized by a multiple set of acoustic parameters, a fair agreement was yielded for the whistle call. The tonal whistle call displays a much simpler acoustic structure than the other two call types, resulting in the fact that only two non-correlating parameters could be included in the discriminant analysis. These lower numbers of parameters may also affect the result of statistical discrimination. A further explanation for the statistically yielded low classification may be sample size. We expect that correct classification may increase when more calls per individual become available, enabling a better balanced data set. Furthermore, future studies should pay special attention to playback experiments to explore whether the statistically revealed differences are biologically meaningful and sufficient to identify and discriminate pair partners and neighbors.

No sex difference in calling rate for all call types was found. This suggests that sexes play an equal role in keeping contact and/or leading travelling movements and that males do not play a more prominent role in resource defense than females, e.g. in helping females save energy for other beneficial purposes such as investment in offspring (e.g. Jolly, 1984; Warren and Crompton, 1997; Méndez-Cárdenas and Zimmermann, 2009) by overtaking the expenses for loud calling. However, vocal displays are only one means of defending a territory, olfactory demarcation is another. Western woolly lemurs exhibit chin glands (Hill, 1953) and in our study chin marking was observed at resource sites. These findings coincide with olfactory behavior of sifakas (Pochron et al., 2005; Lewis, 2006; Lewis and van Schaik, 2007) and suggest that multimodal signaling using olfactory and acoustic signaling serves as a mechanism for inter-group spacing. Thus, it would be interesting in further studies to explore
under which conditions multimodal signaling is involved in territorial advertisement and defense.

The effect of social complexity and phylogeny on vocal communication

The social complexity hypothesis (e.g. McComb and Semple, 2005; Freeberg et al., 2012; Pollard and Blumstein, 2012) predicts that increases in social complexity may have driven the evolution of communication. The phylogenetic hypothesis (e.g. Harvey and Pagel, 1991; Ord and Garcia-Porta, 2012) argues that signal complexity between closely related members of a taxonomic group is more similar than between more distantly related taxonomic groups, based on the shared evolutionary history. Comparable bioacoustic data to our study in nocturnal woolly lemurs are available from the nocturnal sportive lemurs of the taxonomic family Lepilemuridae, which are considered as pair-living (Rasoloharijaona et al., 2003; Rasoloharijaona et al., 2006; Méndez-Cárdenas and Zimmermann, 2009) and from the diurnal indris and sifaks (Pollock, 1975; Petter and Charles-Dominique, 1979; Macedonia and Stanger, 1994; Maretti et al., 2010; Patel and Owren, 2012) of the same taxonomic family (Indriidae), the former with the same (pair-living), the latter with a more complex social system (living in cohesive groups). This comparable data set allows us to explore these two hypotheses.

According to the social complexity hypothesis (see above), taxa that share a similar social system should match in vocal complexity, even if they belong to different taxonomic families within the Lemuriformes. Both, the Milne Edwards’ sportive lemur and the western woolly lemur, are clingers and leapers of comparable body size. Both species share the same activity pattern and habitat and thereby the same predation risks as well as the same ambient noise and habitat transmission conditions for acoustic signaling. Both species are described as pair-living, form long-term pair bonds, but species differ in pair cohesion during foraging (Ramanankirahina et al., 2011, 2012; Rasoloharijaona et al., 2003; Rasoloharijaona et al.,
When we compare the vocal behavior of the western woolly lemur (Indriidae) to the Edwards’ sportive lemur (Lepilemuridae), the vocal behavior is quite distinct. In the context of intra-group cohesion and inter-group spacing woolly lemurs of both sexes use three different call types, two of them loud, the third soft, each in a slightly different context. In contrast, sportive lemurs govern social cohesion and inter-group spacing by using a large set of nine different loud call types, which are in part sex-specific. Furthermore, pair partners in the latter species often engage in duetting at resource sites (Rasoloharijaona et al., 2006; Méndez-Cárdenas and Zimmermann, 2009). In contrast to Edwards’ sportive lemurs, our study revealed that western woolly lemurs do not engage in loud calling, duetting or chorusing behavior at resource sites. Thus, all in all these findings suggest that the pattern of pair cohesiveness and/or phylogeny may influence divergence in signaling behavior.

If phylogeny shapes vocal complexity then we would expect similarities in the size of the vocal repertoire as well as in vocal behavior within the same taxonomic family despite differences in activity pattern or social complexity. Indeed, acoustic signaling systems for governing intra-group cohesion are functionally and structurally similar across members of the same taxonomic family, the Indriidae. Thus, as our study reveals, the woolly lemurs use the soft growling call and the loud whistle call for governing intra-group cohesion, dependent on pair dispersion during foraging. The growling call is functionally and structurally similar to the soft hums reported for indris and the soft grumbles for sifakas, which function to hold contact with other group members at short distance (Pollock, 1975; Petter and Charles-Dominique, 1979; Macedonia and Stanger, 1994; Maretti et al., 2010; Patel and Owren, 2012). Loud howl calls, comparable in their function to the loud whistle call in woolly lemurs, are described for sifakas and indris and are given when an individual loses visual contact to the group (Maretti et al., 2010; Patel and Owren, 2012; Torti et al., 2013). Loud calling in the context of spacing between pairs is present across all studied taxa of the Indriidae. However,
as revealed in our study, only one pair partner emitted loud two-component avahee calls across the non-core areas of the home range, and these were answered with the same type of call, most likely by a neighbor. In contrast, in the diurnal pair-living indris and group living sifakas, both pair partners/all adult and subadult group members engage in loud duetting or chorusing (simultaneous calling of all group members), in particular at resource sites at the beginning of their activity (Pollock, 1986; Thalmann et al., 1993; Maretti et al., 2010; Fichtel and Kappeler, 2011; Patel and Owren, 2012). Songs in indris consist of nine different call types/notes/syllables, which can also be used context-specifically (Maretti et al., 2010; Torti et al., 2013). Certain syllables of the choruses of sifakas and the duets/choruses of indris such as the roars may also function as loud alarm calls for advertising the detection of disturbances or predators (e.g. Patel and Owren, 2012; Torti et al., 2013). Consequently, our results in combination with the literature does not support either the phylogenetic hypothesis or the social complexity hypothesis since indri and woolly lemurs, which exhibit the same social pattern, but differ in activity mode, also differ in the complexity of vocal signaling systems for intergroup spacing. All in all, these findings suggest that primate vocal complexity is driven by a mixture of at least three different factors, the degree of social cohesiveness, activity and phylogenetic history.

**Conclusion and areas for future research**

This study provides first quantitative information on the role of acoustic communication for spacing and cohesiveness in the nocturnal western woolly lemur. As in diurnal socially cohesive strepsirrhine as well as haplorrhine primates, acoustic signaling represents an important mechanism to regulate distances within pair partners and between pairs in space.

Future bioacoustic studies with satellite and audiocollars allowing for simultaneous recordings of pair partners are now needed to better understand the role of multimodal
signaling for governing inter-group spacing as well as coordination and decision making within woolly lemur groups. Such studies should be complemented by playback experiments, in order to explore to which extent woolly lemurs use and perceive messages acoustically conveyed in their calls. Furthermore, as loud calls are often used successfully to assess taxonomic and phylogenetic relations or migrations patterns in primates (e.g. Zimmermann, 1990; Davila Ross and Geissmann, 2007; Méndez-Cárdenas et al., 2008; Meyer et al., 2012), the role of loud calls in examining endangered woolly lemur species diversity and evolution as well as the conservation potential of loud calls (e.g. in studying species presence and abundance) would merit specific attention.

Altogether, our study provided the first empirical research on the vocal signaling system of the western woolly lemur as model for the hitherto neglected nocturnal, cohesive pair-living primates. Our results emphasize the effect of the degree of social cohesiveness, activity and phylogenetic history on the evolution of signaling systems and contribute to a better understanding of the evolutionary roots of primate vocal communication.

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