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**VOCAL COMMUNICATION AND ITS RELATION TO
SOCIALITY IN SPORTIVE LEMURS OF MADAGASCAR:
AN ECOETHOLOGICAL AND BIOACOUSTICAL APPROACH**

THESIS

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LIST OF ABBREVIATIONS

CHAPTER 2

ANOVA	Analysis of variance
BW	Bandwidth
DDE	Dynamic data exchange parameter file
DFA	Discriminant function analysis
Dur	Call duration
FFT	Fast Fourier Transform
HP	High-pitched call
IRSs	Inter-River-Systems
No of syll	Number of syllables per call
OC	Ouah call
PAUP	Phylogenetic Analysis using Parsimony
PF	Peak frequency
PFIs	Peak Frequency of last syllable
PFm	Peak Frequency mean
PFms	Peak Frequency of middle syllable
PFss	Peak Frequency of start syllable
Syll dur	Syllable duration
Syll rate	Syllable rate

CHAPTER 3

DBA	Different activities before calling
DBSA	Different activities before calling and the same activity after
HP	High-pitched call
HR	Home ranges
IS	Isolate shrill
JBA	Jardin botanique A

MWU- Test	Mann-Whitney U Test
RS	Related shrill
SBDA	Same activity before calling and different activities after

CHAPTER 4

DC	Duet call
DC-L	Duet call during lactation period
DR	Duration of a response
FSLC	Female solo loud call
FSLC-nm	Female solo loud call-during non-mating period
FSLC-m	Female solo loud call-during mating period
F	Female
IS	Isolate shrill
L	Lactation period
LR	Latency of response
LS	Loudspeaker
nm	Non-mating period
m	Mating period
M	Male
MSLC	Male solo loud calls
MSLC-nm	Male solo loud calls- during non-mating period
MSLC-m	Male solo loud calls- during mating period
MWU- Test	Mann-Whitney U Test
NOR	Non-orientation responses
OR	Orientation responses
RS	Related shrill
SPL	Sound pressure level
TR	Type of response

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GENERAL INTRODUCTION

Biodiversity in Madagascar

Madagascar broke off from the Gondwanaland super-continent around 80-60 million years ago (Reeves and de Wit, 2000; Ali and Aitchison, 2005) and nowadays represents a living example of species evolution in isolation. Despite close proximity to Africa, the island does not share any of the typical animal groups of nearby Africa (Sparks and Smith, 2004, 2005). Instead, it has evolved a unique assemblage of species, with high levels of genus and family level endemism (Yoder and Nowak, 2006). The natural vegetation of this hotspot is quite diverse. The tropical rainforests along the eastern escarpment and in the eastern lowlands give way to western dry deciduous forests along the western coast. A unique spiny desert covers the extreme south. The island is also composed of several high mountain ecosystems in which transition zones show many of the island's endemic species (www.eoearth.org).

The hallmark of the flora and fauna of Madagascar Island is not necessarily their diversity, which is also high, but their remarkable high occurrence of endemism. The high level of species unique to Madagascar resulted from tens of millions of years of isolation from the African mainland. Endemism is marked not only at the species level, but also at higher taxonomic levels: such is the case of the five primate families that live nowhere else on Earth. Currently it is generally acknowledged that these five primate families belong to the superfamily Lemuroidea. According to Mittermeier et al., (2006), Madagascar contains 11% of primate species and subspecies and fully 21% of all primate genera. Malagasy lemurs constitute one of six major radiations of extant primates (Martin, 1990). Lemurs show a remarkable species diversity, making them an excellent mammalian radiation to explore mechanisms and processes underlying speciation and evolution (Craul et al., 2007).

Sportive lemurs – an endangered lemur group

Sportive lemurs are nocturnal clingers and leapers belonging to the smallest folivorous primates (Smith and Jungers, 1997). They generally weigh less than one kilogram and are approximately half a meter tall including the tail. Originally, two species of sportive lemurs with a distribution in either the eastern rain forest or the western dry deciduous forests of Madagascar were recognized based on morphological traits (Schwartz, 1931). Since the early 1970s, 7 species of sportive lemurs have been characterized karyotypically and phenotypically and all of them are, as far as it is known, distributed discretely within inter-river-systems (Craul et al., 2007).

In the last decades, new biosystematic techniques have allowed the use of an extensive range of characters to delimit taxa using molecular genetics, morphometrics and bioacoustics. Molecular phylogenetics has been widely applied to determine species identities and boundaries. Particularly in sportive lemurs, 25 cryptic species have been recognized based on differences in mt-DNA sequences (Andriaholinirina et al., 2006; Craul et al., 2007; Louis et al., 2006; Rabarivola et al., 2006). In the first chapter we focus on three north-northwestern species *L. edwardsi*, *L. dorsalis*, and *L. ankaranensis*, and in the two last chapters we focus on the *L. edwardsi*.

The Milne Edwards' sportive lemur *Lepilemur edwardsi* (Fig. 1-1a) is a dispersed pair living nocturnal primate and it lives in dry deciduous forests in northwestern Madagascar found in Ampijoroa, Mariarano and Tananvaovao (Rasoloharijaona et al., 2000, 2003 2006, Craul et al., 2007; Thalmann and Ganzhorn, 2003; Zinner et al., 2003). The grey-backed sportive lemur *L. dorsalis* (Fig. 1-1b) is one of the smallest species belonging to the *Lepilemur* genus with a total length of 480-620mm and weight of 660-1100g. It is found only in humid and subhumid rain forests e.g. the Sambirano region of northwest Madagascar, including the Ampasindava Peninsula and the islands of Nosy Be and Nosy Komba (Garbut, 1999). The Ankarana sportive lemur *L. ankaranensis* (Fig. 1-1c) is a sportive lemur with a total length of about 530 mm, including 250 mm of tail, its average body weight is approximately 750 grams (Mittermeier, 2006) and it is found in northern

Madagascar, living in dry lowland forests in Ankarana, Andrafiarana and Analamerana, and in the moist montane forest of Montagne d'Ambre (Mittermeier, 2006).

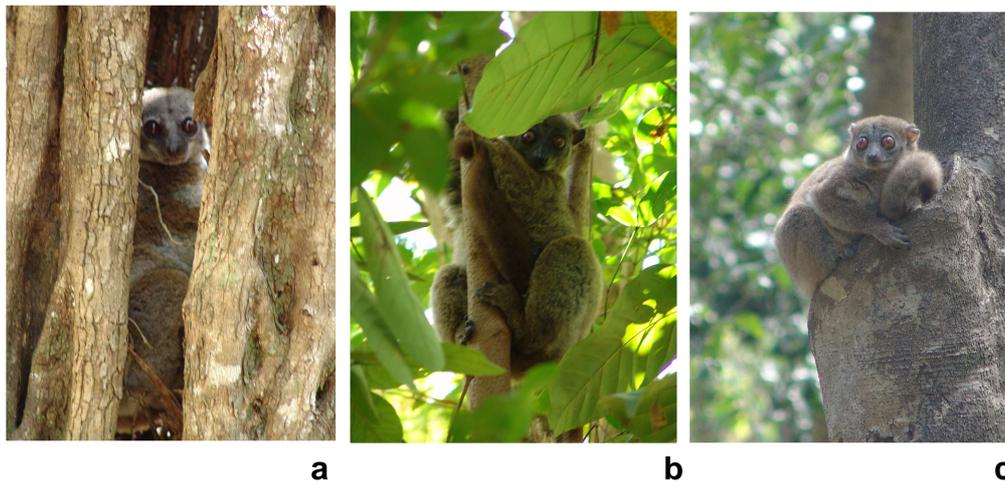


Fig. 1-1 Photographs of a) *Lepilemur edwardsi* in Ampijoroa b) *Lepilemur dorsalis* at Nosy Be and c) *Lepilemur ankaranensis* at Ankarana Park, taken by MG Méndez Cárdenas

Some of these species have limited distributions (Andriaholinirina et al., 2006; Craul et al., 2007; Louis et al., 2006; Rabarivola et al., 2006) and low reproduction rates (Randrianambinina et al., 2007), making them highly threatened for disturbance of their forest habitats. Within the *Lepilemur* genus *L. dorsalis* and *L. mustelinus* have been classified as data deficient, *L. ankaranensis* as endangered and *L. edwardsi* as vulnerable by the IUCN red list (2009) (<http://www.iucnredlist.org/search>).

Sportive lemurs, as most of the lemurs in Madagascar, are under high risk of extinction, due to the high rate of deforestation. From 1950 to 1985, half of Madagascar's forests disappeared. In 1985, only 34% of the original forests existing in Madagascar remained (Sussmann et al., 1994). The most important criteria for determining protected areas are the number of known species, their conservation state and their distribution. In other words, only what is known can be protected. Consequently, recently explored areas, in which a high number of cryptic species of sportive lemurs are being discovered, must be protected. In order to establish conservation strategies, we need to know their precise distribution, abundance and

life histories. One way to develop non-invasive tools to identify and monitor cryptic species in the nature is to use their vocal variations as cues for species diagnosis and abundance assessment. Therefore, in the second chapter we evaluate the extent to which vocal variation is informative for identifying cryptic species in the field.

Bioacoustics as a tool for systematics and phylogeny

The first question that comes when researchers want to use vocal characters as taxonomic units for species identification is which of those characters are relevant for reconstructing the evolutionary history (homologous characters) and informative for species diagnosis. And alternatively which of them may be influenced by social and/or environmental factors giving place to convergent evolution of the characters (homoplastic characters). Variation in vocal communication can be explained by several factors such as the environmental acoustics, (Piercy et al., 1977; Wiley and Richards, 1978) the physical properties of sound transmission, (Morton, 1975; Wiley, 1991; Tubaro et al., 1993) and the morphological variation of the phonatory organ (e.g. trachea, Fitch, 1999; laryngeal air sacs, Hewitt et al., 2002).

In contrast, numerous studies on mammals suggested that genetic factors play a predominant role in determining the acoustic structure of calls (Jürgens, 1979; Gautier and Gautier, 1977) and that vocal production is under strong genetic control (Snowdon and Hausberger, 1997). Call structure in squirrel monkeys, rhesus macaques and gibbons is totally inborn or almost not modified by the environment (Hauser, 1996). Thus in the Chapter 2 we explore if the variation of those characters can be informative for reconstructing the evolutionary group history due to having been less influenced by social and/or environmental factors and thus, can be used for cryptic species diagnosis.

During the last decades, acoustical characters have been treated as morphological entities and used to diagnose species and to reconstruct hypotheses of evolutionary relationships within a group (Geissmann, 1993; Davila Ross and Geissmann, 2007) and to discriminate cryptic nocturnal primates as it has been already reported (e.g., mouse lemurs: Braune et al., (2005, 2008) Zimmermann et al.,

(2000), galagos: Zimmermann (1990), Masters (1991), Anderson et al., (2000) and Ambrose (2003); tarsiers: Nietsch and Kopp (1998) and Merker and Groves (2006)). Phylogenetic diversity and taxa position in lemurs is still controversial and conservation priorities should be re-evaluated (Lehman, 2006).

Chapter 2 will contribute to enhancing our knowledge on the *Lepilemur* genus by providing an extensive analysis of the geographical variation on loud calls of genetically defined sportive lemur species within four geographically separated regions from northwestern to northern Madagascar. We analyzed the loud calls as a potential and non-invasive tool for identifying cryptic nocturnal sportive lemurs in the field and for testing evolutionary hypothesis of phylogenetic relationships of taxa.

Furthermore, we evaluated at which taxonomic level each character is informative. We assume that vocal characters with a highest retention index are the most informative ones to reconstruct the evolutive history of the group and therefore, more reliable as homologous characters, shaped by the evolutionary mechanisms of speciation. Since large rivers function as geographical barriers for gene flow in sportive lemurs in this geographical range, (Craul et al., 2007) we are interested in exploring the extent to which genetic variation coincides with the variation of vocal traits, and also if this variation can be explained by the same process that gave place to genetic diversification.

One of the main aspects on the evolution of communication is its relation to sociality. Thus in chapter 3 and 4 we explore vocal displays as a mechanism governing social interactions between pair partners in a dispersed pair-living primate society by two different approaches. The first uses descriptive information based on radiotelemetry and behavioral focal observations and the second uses experimental data based on the individual and pair responses to loud call playbacks broadcasted in the field.

Sociality and communication: the case of vocal coordination in sportive lemurs.

Sociality is the tendency to associate with others and to form social groups. Sociality formed an integral part of the adaptive nature of primate origins (Müller and Soligo, 2005). However, the causes and mechanisms by which species form or do not form social networks are still controversial. Understanding the rules that link communication and social behavior is an essential prerequisite for discerning how a communication system might have evolved in primates (McComb and Semple, 2005). A social system includes three interrelated factors: social organization, social structure and mating system (Sterling, 1993; Kappeler and van Schaik, 2002). Social organization describes group size, sexual composition and cohesion of a society (Müller and Thalmann, 2000; Kappeler and van Schaik, 2002). Social structure describes the relationships and the pattern of social interactions among individuals. Mating systems describe mating behavior (Emlen and Oring, 1977) and a genetic component, which describes the reproductive output (Clutton-Brock, 1989; Kappeler and van Schaik, 2002).

Especially in primates, the focus on pair-living has been a central aspect in studies of the evolution of social organization (Lovejoy, 1981; van Schaik and Dunbar, 1990; Jolly, 1998; Palombit, 1999; van Schaik and Kappeler, 2003). Theoretical considerations have shown that pair-living evolved independently several times in primates from a most likely solitary ancestor with a promiscuous mating system (van Schaik and Kappeler, 2003). This contributed to the search for similar evolutionary origins and selection pressures that drive males to mate with only one female. Consequently researchers proposed several hypotheses that try to explain the occurrence of this puzzling social organization (reviewed in Fuentes, 2002).

Social factors and fundamental life-history traits, in addition to ecological factors, contribute importantly to the variation in social systems among lemurs (Kappeler, 1997). Within these factors, vocal communication plays an important role to regulate social interactions. Nocturnal lemurs with their broad diversity in social patterns provide unique models to understand the evolution of communication signals for inter-group spacing, group coordination and its relation with sociality in

primates. Patterns of home range overlap between individuals are key parameters used in the study of sociality in nocturnal prosimians (Sterling et al., 2000).

Studies of nocturnal primates in their natural environment showed that acoustic signals control intra-group cohesion while olfactory signals represent an important mechanism for inter-group spacing (Braune et al., 2005). In territorial cohesive pair-living primates, duets, an interactive male-female vocal display, are suggested to act as a mechanism to deter territorial intrusions (Haimoff and Gittins, 1985), to be present during inter-group conflicts (Tilson and Tenaza, 1976; Robinson, 1979; Raemaekers et al., 1984; Haimmof, 1986; Leighton, 1987) and to function as a territorial declaration (Pollock, 1975). The Milne Edwards' sportive lemur (*L. edwardsi*), is a pair-living primate (Rasoloharijaona et al., 2003) in which male-female home ranges highly overlap while pairs are spatially dispersed during foraging. Thus *L. edwardsi* is a suitable model to explore the original mechanisms favoring cooperation and sociality in more cohesive pair-living primates.

In Chapter 3, I present our findings on duetting as a mechanism to strengthen pair bonds in a primate model with a dispersed social system, the Milne Edwards' sportive lemur. Radio-telemetric studies were necessary to determine the use of home ranges and sleeping sites by males and females in order to recognize mated-pairs through home-range overlaps and focal behavioral observations during one year. In order to assess how sex, home range, seasonality and social context affect calling activity and call structures during social encounters, senders and receivers have been characterized morphologically, genetically and socially.

We investigated the origin and function of an interesting vocal display, called "duetting" in territorial birds and territorial cohesive pair-living primates in which mated- pairs coordinate vocal interactions that occur predictably and repeatedly in time (Langmore, 2002). First we analyzed if duetting reported in territorial and cohesive pair-living species (Pollock, 1975; Tilson and Teneza, 1976; Robinson, 1979; Raemaekers et al., 1984; Haimoff and Gittins, 1985; Haimmof, 1986;) also occurs in a territorial nocturnal primate but with a dispersed pair-living social system, named the Milne Edwards' Sportive Lemur. Afterwards we examined the temporal

and spatial occurrence of duets along one year by exploring with which environmental sites and/or specific times of the activity cycle the duets are linked.

Furthermore we investigated the extent to which duets are associated with a specific behavioral context, thus exploring the potential function of duetting between mated- pairs during their nocturnal activities. We analyzed the duetting structure, who within the pair initiated the duetting display, and who lead activities after duetting. Finally we tested to what extent the functions of duetting in a dispersed pair- living primate correspond to hypotheses of mate reunion (e.g. birds: Stokes and Williams, 1968) pair reunion (e.g. primates: Nietsch, 2003) and joint territory defense (e.g. primates: Mitani, 1987) as tested in tropical birds and in primates living as cohesive pairs.

The results of this study provided more information about how vocal interactions influence sociality in pair-living primates and how pair-bond systems might lead the evolution of vocal duetting. However, at the same time, these findings also opened new questions about other factors which may have driven the evolution of duetting such as nutritional stress on lactating females (Ganzhorn, 2002) and infanticide prevention (van Schaik and Dunbar, 1990). The confounding effects of these new hypotheses are experimentally analyzed in Chapter 4. Furthermore, in that chapter, we will describe the results of playback experiments of solo and duet calls conducted during the non-mating period (which coincides with lactation) and we will compare it with experiments during the mating period with the aim of investigating the joint territory defense hypothesis independently from the confounding effects of sexual conflict over mating.

Playback experiments - a tool to determine the function of social calls

Calls in primates may have important social functions, such as bringing companions together, enhancing cooperation, keeping rivals apart and reducing conflicts (Marler, 1968). Calls also contain information on species, population, individual, status and external referents (Cheney and Seyfarth, 1990; Zimmermann et al., 1995; Hauser, 1996). However, it is often not easy to distinguish which part of the call contains these specific messages. One way to investigate this is to study the

physical structure of the call along with the behavior of the sender and the effects that this call has on a conspecific. Studies of call perception indicate how acoustic variations (explored through specific interactions between call and context, investigated in the Chapter 3), are classified into functionally meaningful categories by the receiver and thereby provide insights into the signal's informational content (Hauser, 1998).

Since 1980, there have been several playback experiments with non-human primates, in laboratories and in the field testing the hypothesis of Seyfarth and Cheney on the specific information content on predator calls. This heterospecific alarm calls have been found in primates (Hauser and Wrangham, 1990; Oda and Matasaka, 1996; Ramakrishan and Coss, 2000; Zuberbühler, 2000; Fichtel, 2004). In lemurs playback experiments studying the responses toward ground and aerial predators were carried out in several species: (e.g. red-fronted brown lemurs (*Eulemur fulvus rufus*) Fichtel and Kappeler, 2002; verreaux's sifakas (*Propithecus v. verreauxi*) Richard, 1985; Fichtel, 2004; Fichtel and Kappeler, 2002; the black-and-white ruffed lemurs (*Varecia variegata v.*) Macedonia, 1990, 1993; the ring-tailed lemurs (*Lemur catta*) Macedonia, 1990, 1993; Oda and Masataka, 1996; the red tailed sportive lemurs (*Lepilemur ruficaudatus*) Fichtel, 2007; tarsiers, Gursky, 2002). In mouse lemurs playback experiments showed species-specific recognition (Braune et al. 2008).

However less attention has been paid at the phenomena of kin and individual vocal recognition, which have important implications for the evolution of social behavior in primates (Hamilton, 1963, 1964; Trivers, 1971), thus, the capacity to signal and perceive identity has become a major focus of research (Rendall et al., 1996). Information on how lemurs perceive solo loud calls and/or duet calls of neighbors and react to them is lacking and our study contributes to filling this gap. Furthermore, the sex-specific vocal repertoire and the call-individuality reported in *L. edwardsi* (Rasoloharijaona et al., 2006) allowed us to experimentally test the function of duetting in a dispersed pair living primate.

Thus, in Chapter 4 and as a complementary evidence for our descriptive study on duet function, we test, for the first time in a nocturnal prosimian, two of the major

hypotheses of duet functioning: the territory joint defense and the mate attraction/mate defense by the use of playback experiments. Both hypotheses have been proposed to explain the origin and evolution of duetting in territorial birds and cohesive pair-living primates. For the mate attraction/mate defense hypothesis Darwin explained that sexual selection gave rise to a kind of communication, in which one sex produced signals (like songs) to attract the other (Darwin, 1871) and went as far as to suggest that human language originated in this kind of sexual advertisement signaling: *“the early progenitor of man, probably used his voice largely, as does one of the gibbon apes of the present day, in producing true musical cadences, that is in singing; we may conclude. . . that this power would have been especially exerted during the courtship of the sexes. . . The imitation by articulate sounds of musical cries might have given rise to words expressive of various complex emotions”*.

Sir Julian Huxley (1914, 1923) described the elaborate display of the great crested grebe *Podiceps cristatus* and the red-throated diver *Gavia stellata*. Both species perform amazing displays, particularly during the period before the eggs are laid. Because displays were not related with courtships, Darwin's theory of sexual selection was difficult to apply. A number of explanations have been suggested for courtship after pair formation, such as pair bonding (Huxley, 1923) and continuing partner evaluation (de Lope and Møller, 1993; Moreno et al., 1994). Finding a new mate in these species can be very time consuming and a change of mate appears to have little, and sometimes even a negative, effect on reproductive success (Ens et al., 1996).

Therefore, some authors seem to suggest that the function of duetting is to produce a stronger bond seen by more cooperation and less likelihood of separation (see e.g. Armstrong, 1965; Wickler, 1980; Bradbury and Vehrencamp, 1998). In contrast, the manipulation theory requires that partners are in a constant arms race conflict to evolve conspicuous displays (Arak and Enquist, 1995; Weichmeister, 2001). It has been suggested that the function of duetting in primates may be the same as for bird songs in general, serving both territorial and pair formation functions (Farabaugh, 1982; Catchpole and Slater, 1995). Currently, the function of duetting after pair formation is still not fully understood.

In Chapter 4, we aim to investigate the extent to which duetting functions as a cooperative display to defend shared resources by signaling coalition strength, thus duetting should be extended after the mating period. If duetting functions as a competitive display between sexes to avoid being usurped and to repel extra-pair mates, then duetting should be restricted to the mating period. Information on how nocturnal dispersed pair-living primates perceive solo loud calls or duets is lacking.

We carried out playback experiments of male and female solo loud calls and duets of neighboring mated- pairs during the mating and non-mating period to sportive lemur individuals of six mated- pairs. We performed comparisons of the latency, duration and type of response to these playbacks. We expected that if duetting functions as a display for “joint territory defense” of a mated- pair’s partner then duet playbacks would elicit a stronger response and more duet responses than solo playbacks during the non-mating period (period of female lactation). If duetting functions as a mechanism for “mate defense and mate ownership signaling” then more duets would be elicited in response to solo calls than in response to duets during the mating period. Furthermore we aimed to investigate if infanticide prevention may have driven the evolution of duetting. We analyzed vocal and behavioral reactions of females with infants and of females without infants towards solo loud calls and duet calls playbacks. Chapter 4 of my thesis will contribute to enhancing our knowledge about the function of duetting in pair-living primates based on the individual and pair reactions to single and duet calls and it will give us more insights about the origin of cooperative and/or competitive mechanisms explaining the evolution of duets.

CHAPTER 2

GEOGRAPHIC VARIATION IN LOUD CALLS OF SPORTIVE LEMURS (*LEPILEMUR SSP.*) AND THEIR IMPLICATIONS FOR CONSERVATION

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Abstract

Bioacoustical studies in nonhuman primates have shown that loud calls can be reliably used as a non-invasive diagnostic tool for discriminating cryptic taxa, for their monitoring in the field as well as for the reconstruction of their phylogeny. To date, it is unknown, whether loud calls can be used for these purposes in sportive lemurs, for which current genetic studies suggest the existence of at least 24 cryptic species.

The aim of this study was to compare the structure of loud calls of populations of sportive lemurs to characterize informative acoustic traits for taxa discrimination and to establish a phylogenetic tree based on acoustic structure. We have based our study on Inter-River-Systems (IRSs) as operational taxonomic units. Samples were collected from 9 different localities of four IRSs along a transect from northwestern to northern Madagascar. Two call types, the ouah and the high-pitched call, were present in almost all IRS. Six temporal and eight spectral parameters were measured in 196 calls of the best quality given by 21 different males. Variation within and between IRSs was assessed by multivariate statistics. Loud calls differed significantly among the different IRSs. The IRSs varied most in spectral parameters whereas temporal parameters were less variable. Phylogenetic Analysis using Parsimony (PAUP) yielded 11 out of 17 acoustic characters as phylogenetically informative. The acoustic tree had an average branch support of 78%. Its topology coincided less with geographic distances than with genetic tree topology. Altogether our findings revealed that loud calls separated geographically isolated populations of sportive lemurs specifically. Based on these results, non-invasive tools for diagnosis and monitoring of cryptic species in nature can be developed for conservation management.

Introduction

In recent decades the implementation of new techniques in biosystematics has allowed the use of an extensive range of characters to delimit taxa by means of molecular genetics, biometry and bioacoustics. As a result, species diversity in Malagasy lemurs has increased manifold from 32 in 1994 to currently 92 in 2007 (Andriaholinirina et al., 2006; Craul et al., 2007; Louis et al., 2006; Mittermeier et al., 2006; Olivieri et al., 2006; Rabarivola et al., 2006). In particular, new approaches in molecular systematic have led to an enormous and sometimes disputed diversification of lemurs on a macro- and micro-taxonomic scale (Tattersall, 2007). An example of this recent diversification is provided by sportive lemurs. Whereas in 1931 two species of sportive lemurs with a distribution in either the eastern rain forest or the western dry deciduous forests of Madagascar were recognized as being based on morphological traits (Schwartz, 1931), nowadays 25 cryptic species are diagnosed based on differences in mt-DNA sequences (Andriaholinirina et al., 2006; Louis et al., 2006; Rabarivola et al., 2006; Craul et al., 2007). These species are difficult to distinguish using size, pelage coloration and other external body characteristics. As a consequence, non-invasive species diagnosis in the field is extremely difficult. Furthermore, some of these species have fairly limited distributions (Andriaholinirina et al., 2006; Louis et al., 2006; Rabarivola et al., 2006; Craul et al., 2007) and low reproduction rates (Randrianambinina et al., 2007), making them highly vulnerable to human caused disturbances of their forest habitats. From an analysis of satellite images of the Malagasy forest it is known that the deforestation rate is enormous with a rate of 100.000 ha lost per year (Sussman et al., 1994 <http://american.edu/TED/MADAGAS.HTM>) endangering all forest-dwelling organisms. To establish effective management strategies, rapid species assessment methodologies are urgently needed. Here, we explore therefore the extent to which comparative bioacoustics can be used as a non-invasive tool to identify taxa in the field.

Sportive lemurs are nocturnal, cat-sized clingers and leapers belonging to the smallest folivorous primates (Smith and Jungers, 1997). They live either as dispersed pairs or solitary in the forests of Madagascar (Rasoloharijaona et al., 2000, 2003, 2006, Thalmann and Ganzhorn 2003; Zinner et al., 2003). Recent radiotelemetric studies revealed that sportive lemurs are territorial and may form stable and long-term sleeping associations (Warren and Crompton, 1997; Rasoloharijaona et al., 2003, 2006, 2007; Zinner et al., 2003). Furthermore, sexes in sportive lemurs are monomorphic with regard to size and pelage coloration. However, they are highly vocal and use an elaborate set of loud calls with partly sex-specific notes or syllables for long distance communication (Rasoloharijaona et al., 2006). Loud calling in this case seems to function as a device for intra-group cohesion and inter-group spacing (Rasoloharijaona et al., 2006).

Loud or long-distance calls are among to the most distinctive primate vocalizations. They travel over long distances, show a highly stereotypic and species-specific acoustic structure across a broad range of primate taxa and have therefore been used for species monitoring in the field as well as for the reconstruction of phylogeny (e.g., prosimians: Zimmermann (1990) for galagos; Macedonia and Stanger (1994) for lemurs Stanger (1995) for cheirogaleids, new world monkeys: e.g., Ploog (1974) for squirrel monkeys; Whitehead (1995) for howler monkeys; old world monkeys: Oates and Trocco (1983); Oates et al. (2000), Struhsaker (1981) for colobus, Gautier (1988) for guenons; Davila Ross et al. (2007) for orang-utans). Loud calls have also been used successfully as a non-invasive diagnostic tool for discriminating cryptic nocturnal primate species (e.g., mouse lemurs: Zimmermann et al., 2000, galagos: Anderson et al., 2000, Ambrose, 2003; Masters, 1991; Zimmermann et al., 1988, tarsiers: Nietsch and Kopp, 1998 and Merker and Groves, 2006).

Since loud call data can be easily and non-invasively collected in wild populations and spectrographic analysis of calls is relatively cheap due to affordable and commercially available software tools installed on a laptop, species diagnosis based on a specific loud call signature can be a more suitable and economical field method than bar coding (Species “identification” based on DNA sequences or

“barcodes”, DeSalle, 2006). Furthermore, local people can be easily involved in the survey of the animals in the forest. Likewise, didactic material for education in communities and ecotourism can be developed. Thus, locals can be trained for continuous monitoring of diversity and density of cryptic primates. Many researchers have demonstrated the importance of such an approach for conservation (e.g. Driscoll, 1998; Shirose et al., 1997; Bridges and Dorcas, 2000).

The aim of this study was to compare the structure of loud calls of genetically specified sportive lemurs in northwestern and northern Madagascar. As large rivers function as geographical barriers for gene flow in sportive lemurs in this geographical range (Craul et al., 2007), we have based our study on Inter-River-Systems (IRSs) as operational taxonomic units. Specifically, we were interested in exploring potential informative acoustic traits for taxa discrimination among IRS and the effect of geographic distance on acoustic variation. Furthermore, we established an acoustic tree based on vocal characteristics to explore the extent to which its topology coincides with genetic topology. We discussed results with regard to their relevance for systematics and taxon management in conservation.

Methods

Collection of sound samples

From June until November 2002-2004, data were collected from sportive lemurs populations at nine different localities along a 560 km transect from northwestern to northern Madagascar and one additional location in eastern Madagascar (Fig. 2-1, Table 2-1). Localities of northwestern and northern Madagascar were grouped into four different Inter-River-Systems (Craul et al., 2007): IRS I, IRS V, IRS VI and IRS VII in accordance with large rivers forming geographic barriers. Furthermore, studied individuals belonged to populations that were already characterized genetically (Craul et al., 2007).

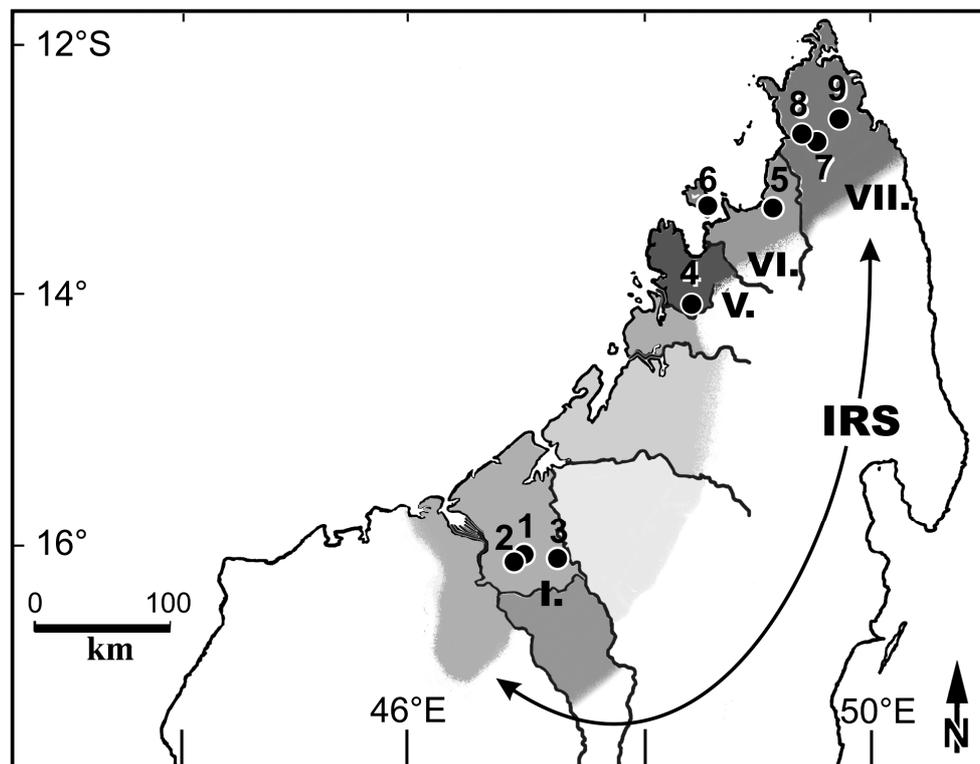


Fig 2-1. Studied sportive lemur localities within the four IRSs from northwestern to northern Madagascar.

Table 2-1. List of samples with information on Inter-River-Systems (IRSs), localities, geographical coordinates, number of sampled calls per call type, recorder and recording year.

Areas	IRS	localities	coordinates	individuals	High-pitched call	Ouah call	Recorder, recording year
Northwest	I	Ampijora (1)	S 16°35' E 46°82'	M4403	5		Méndez-Cárdenas M. 2004 (1)
Northwest	I	Ampijora (1)	S 16°35' E 46°82'	M4803	5		(1)
Northwest	I	Ampijora (1)	S 16°35' E 46°82'	M5303	5		(1)
Northwest	I	Ampijora (1)	S 16°35' E 46°82'	M0502		10	(1)
Northwest	I	Ampijora (1)	S 16°35' E 46°82'	M6777		10	(1)
Northwest	I	Ambodimanga (2)	S 16°18' E 46°49'	M4604	5	14	Rabesandratana A. 2004 (2)
Northwest	I	Ambodimanga (2)	S 16°18' E 46°49'	M4704	5	15	(2)
Northwest	I	Ambodimanga (2)	S 16°18' E 46°49'	M5204	4		(2)
Northwest	I	Ampatika (3)	S16°03' E47°07'	M2803	5	7	(2)
Northwest	I	Ampatika (3)	S16°03' E47°07'	M3003	5	9	(2)
North	V	Mahilaka (4)	S 14°17' E 48°13'	3M	5		Rasoloharijaona S. (3) Randrianambinina B. 2002
North	V	Mahilaka (4)	S 14°17' E 48°13'	4M	5		(3)
North	VI	Lokobe (5)	S 13°23' E 48°20'	M0402	10	9	(3)
North	VI	Lokobe (5)	S 13°23' E 48°20'	M0502		8	(3)
North	VI	Lokobe (5)	S 13°23' E 48°20'	M0602	5	6	(3)
North	VI	Manehoka (6)	S 13°25' E 48°47'	M0902	1	1	(3)
North	VII	Ankarana (7)	S 12°57' E 49°07'	M1603	7	11	(3)
North	VII	Ankarana (7)	S 12°57' E 49°07'	M1203	7	6	(3)
North	VII	Ankarana (7)	S 12°57' E 49°07'	M1303	3		(3)
North	VII	Andrafiabe (8)	S 12°55' E 49°03'	M0104	3		(3)
North	VII	Andrafiarena (9)	S 12°46' E 49°22'	M1603	5		(3)
East	OG	Mantadia	S18°47' E48°25'	M0199	2	2	(3)
Total	4	9		21	90	106	

Loud calls were collected from 21 radio-collared adult males during foraging in the first 2 hours of the activity period (for further details on capturing and on morphological and bioacoustic data collection see Rasoloharijaona et al., 2006). Calls were recorded at all localities with comparable sound recording equipment, consisting of a Sennheisser ME65/K6 directional microphone with windshield and a Sony WMD6C Professional walkman.

Research conducted in this paper adhered to the legal requirements of the country in which the research was conducted and permission was obtained from the appropriate agencies. Capturing procedures, animal handling and radiotracking techniques followed standard protocols and were authorized by the appropriate institutional and governmental bodies.

Acoustic analysis

Sounds were digitalized with a sample rate of 22.05 kHz and a sample size of 16 bit. Sonograms (time vs. frequency displays) of the sound material were generated using the software AviSoft SAS Lab Pro 4.39 (Specht, 2003), with the following parameter adjustments (FFT length: 512 points, frame size: 100; Hamming window, filter bandwidth: 56 Hz, temporal resolution: 1.45 ms). Sonograms were inspected visually for sound quality. Sounds -20 dB above background noise were selected for measurement. In the spectral dimension we used the automatic function (automatic parameter measurements) which transferred frequency parameters as a dynamic data exchange (DDE parameter file) to an Excel table. Temporal-related parameters were measured manually.

In the following, a call is defined as either a monosyllabic or multisyllabic sound separated from others by a gap of silence of at least the double of its call duration. Call types were defined visually by their specific structure in the sonogram. Two call types were present at almost all localities and IRSs, the high-pitched call (HPC) and the ouah call (OC) (for call use and definition see Rasoloharijaona et al., 2006). For the HPC we determined 5 temporal and 3 spectral parameters (Table 2-2). For the OC we determined one temporal and 5 spectral parameters, (Table 2-2).

We calculated the mean peak frequency (PF mean) of the HPC by adding up the values of the first, the middle and the last syllable and dividing this by three. In total, we included in our analysis 90 HPCs from 18 individuals of 9 localities from four IRSs and 106 OCs from 12 individuals of 6 localities from three IRSs (Table 2-1).

Table 2-2. Acoustic parameters measured from sonograms

Acoustic parameter	Description
High-pitched call (HP)	
<i>Temporal</i>	
Call duration (Dur, sec)	Time between onset of first syllable and offset of last syllable
	Time between onset and offset of a syllable
Syllable duration (Syll dur, sec)	Time between onset of a syllable and onset of the successive
Interval duration (Interval, sec)	syllable
	Number of syllables per second
Syllable rate (Syll rate)	Number of syllables within a call
Number of syllables per call (No of syll)	
<i>Spectral</i>	
Peak frequency (PF, kHz)*	Median frequency at maximum amplitude: PF = $\frac{PF_{ss} + PF_{ms} + PF_{ls}}{3}$
PF of start syllable (PF _{ss} , kHz)	Frequency at maximum amplitude of start syllable
PF of middle syllable (PF _{ms} , kHz)	Frequency at maximum amplitude of middle syllable
PF of last syllable (PF _{ls} , kHz)	Frequency at maximum amplitude of last syllable
Bandwidth 1 (BW1, kHz)	PF _{ms} - PF _{ss}
Bandwidth 2 (BW2, kHz)	PF _{ms} - PF _{ls} .
Ouah call (OC)	
<i>Temporal</i>	
Call duration (Dur, sec)	Time between the onset and offset of a syllable
<i>Spectral</i>	
PF start (kHz)	Frequency at the start of a syllable
PF maximum (PF _{max} , kHz)	Frequency at maximum amplitude of a syllable
PF end (kHz)	Frequency at the end of a syllable
Bandwidth 1 (BW1, kHz)	PF _{max} - PF start
Bandwidth 2 (BW2, kHz)	PF _{max} - PF end.

Statistical Analysis

We calculated medians for all measured acoustic parameters per individual and per IRS. To analyze the extent to which variation can be contributed either to individuals or IRS we applied nested analysis of variance (ANOVA) with hierarchically nested design (MS type I Satterthwaite's method; calls were nested in individuals and individuals were nested in IRS, with IRS as fixed factor and calls and individuals as random factors). Results were treated as significant with $p \leq 0.05$. All statistics was carried out using Statistica 6.0 (StatSoft Inc.).

To explore to which extent IRS can be discriminated based on acoustic parameters sets of the respective loud call, we conducted a forward stepwise discriminate function analysis (DFA). For model calculations we used the stepwise forward method (statistic, Wilk's L) with the criteria $F_{\text{to enter}} = 3.84$ and $F_{\text{to remove}} = 2.71$, and a tolerance level of ≤ 0.01 (SPSS 11.0, SPSS, Inc). Correct classification is indicated by % of correct assignment to each IRS. Results were cross-validated and the "leave-one-out" method where each case of the analysis was classified by the functions derived from all cases other than that case.

In order to establish an acoustic tree based on the measured acoustic parameter sets of both loud calls we used the gap-weighting method for coding characters (Thiele, 1993), MacClade for matrix construction (Maddison and Maddison, 2000) and PAUP for the phylogenetic analysis (Swofford, 1999). For the gap-weighting method, discrete character states were defined.

For each character (acoustic parameter), the value was standardized according to $\log(\text{value} + 1)$. Character states were calculated for each IRS according to the following formula: $X_s = ((X - \text{min}) / (\text{max} - \text{min})) * 4$ where X is the standardized value for IRS, 4 is the number of ordered states, min and max the lowest and highest standardized value across IRS (Thiele, 1993). Altogether, 17 characters of the high-pitched call and the ouah call and its character states were transferred into a Matrix using the software MacClade 4.0 (Maddison and Maddison, 2000). A list of all characters and their states is provided in Appendix 2-1. Cladograms were calculated using PAUP 4.0 software (Swofford, 1999). Maximum parsimony was used, which

minimizes the number of character states that are interpreted as synapomorphies (Swofford and Olsen, 1990). In order to ascertain the shortest tree, we used the exhaustive search procedure. The accuracy of the obtained tree was determined by Bootstrap analysis. Bootstrap values were calculated based on 1000 replicates (Kitching et al., 1998). To root the tree we used loud calls of sportive lemurs from eastern Madagascar (locality Mantadia, near Andasibe, (Rasoloharijaona, 2001)).

Results

1. Comparison of the HPC and the OC among the different IRSs

The HPC

The HPC is a harmonically structured call overlapped by noise. It can be discriminated between IRSs by ear (Fig. 2-2a, 2-3a). It consists of multiple syllables with an inversed v- shaped frequency contour in all IRS. The longest median call duration, the longest median inter-syllable interval and the lowest median syllable rate of all IRSs is found in IRS I, whereas the median PF is relatively high. The shortest median, call duration, the shortest syllable duration, the shortest median inter-syllable interval, the highest median syllable rate and the lowest median PF among all IRSs is found in IRS V. Finally, the highest median PF occurs in IRS VII (see also Table 2-3).

Hierarchically nested ANOVA showed that acoustic variation of loud calls was affected specifically by IRS and individuals (Table 2-4). Thus, individuals explained acoustic variation between IRS for seven out of 11 acoustic parameters (four temporal parameters: Dur, Interval, Syll rate, No of syll and three spectral parameters: PFss, PFms and PFm). Four spectral parameters (PFss, PFms, PFIs and PFm) also explained separation between IRS. PFIs explained the variation among IRS exclusively.

Forward stepwise discriminant function analysis has excluded four out of eight measured parameters. Thus, the model was based on four non-correlated parameters: PF m, No of syll, Syll rate and Syll dur. The most discriminant variable to assign calls to the respective IRSs was PF m (Fig. 2-3a). 72.2 % of acoustically defined individuals were correctly assigned to their respective IRS after cross-validation (Table 2-5).

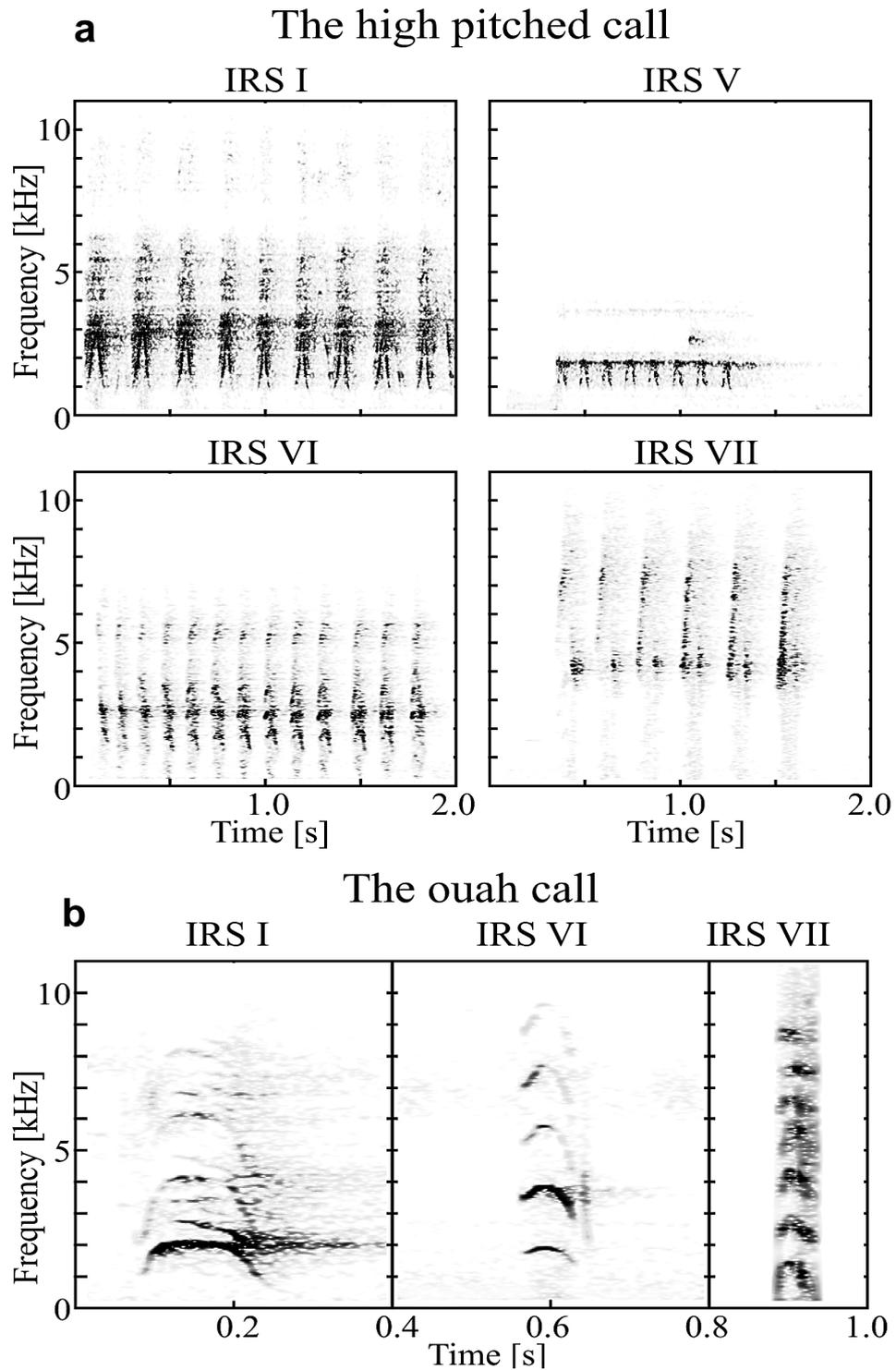


Fig. 2-2. Sonogram of the high-pitched call of four IRSs (a) and sonogram of the ouah call of three IRSs (b).

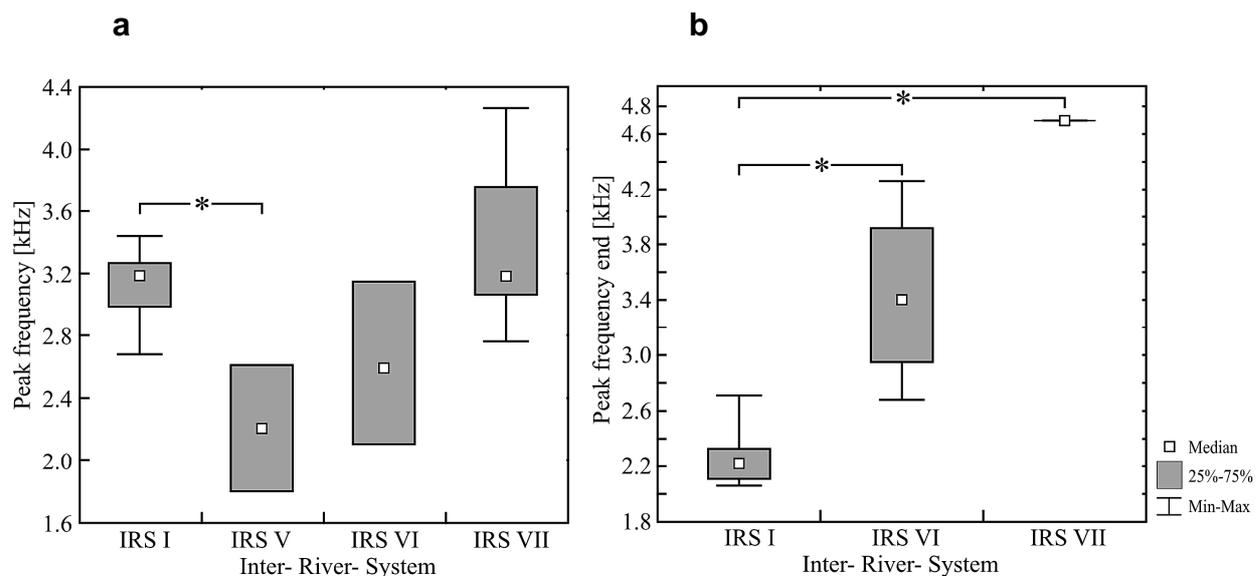


Fig. 2-3. The most discriminant variable of the high-pitched call separating four IRSs (a) and the most discriminant variable of the ouah call separating three IRSs (b).

The OC

The OC is a monosyllabic and harmonically structured call and can be easily discriminated between IRSs by ear (Fig. 2-2b, 2-3b). It consists of one syllable with an inverse u-shaped or downward modulated frequency contour. The ouah call of IRS I is characterized by the longest median call duration and the lowest PF, whereas that of the IRS VII is characterized by the shortest median call duration and the highest PF (see also Table 2-3).

Hierarchically nested ANOVA (Table 2-4) showed that both acoustic variation between individuals and between IRSs contributed to IRS separation. Thus, individuals explained acoustic variation in five out of six acoustic parameters (four spectral: PF start, PF max, BW1 and BW2 and one temporal: Dur). Four out of six acoustic parameters (three spectral: PF start, PF max and PF end and one temporal: Dur) also explained differences between IRSs. PF start and PF end explain differences among IRS reliably.

Forward stepwise discriminant function analysis, has excluded four out of six parameters. Thus, the model was based on two non-correlated parameters: PF end and BW1. The most important acoustic variable to assign calls to the respective IRSs

is PF end. 91.7% of acoustically defined individuals were correctly assigned to the respective IRS after cross-validation (Table 2-5, Fig. 2-4).

Table 2-3. Comparisons of non-correlated acoustic parameters of the high-pitched call and the ouah call of four / three Inter-River-Systems (IRS); N=number of individuals, M= medians, Q= quartiles; further abbreviations are explained in Table 2-2.

The high-pitched call									
		PF med		No of syll		Syll rate		Syll dur	
IRS	N	M	Q	M	Q	M	Q	M	Q
I	8	3.08	2.98 3.17	9.80	7.50 11.50	5.72	5.00 6.34	0.09	0.06 0.11
V	2	2.27	1.94 2.59	7.20	4.20 10.20	8.67	8.22 9.13	0.08	0.06 0.09
VI	3	2.60	2.10 3.19	9.70	5.40 18.00	7.31	4.59 9.33	0.09	0.07 0.12
VII	5	3.45	3.07 3.76	11.00	6.40 11.00	7.55	7.41 8.37	0.09	0.08 0.09
Total	18	3.04	2.71 3.20	9.85	6.40 11.00	6.83	5.12 8.22	0.09	0.07 0.11
The ouah call									
		PF end			BW 1				
IRS	N	M	Q	Q	M	Q	Q		
I	6	2.27	2.12	2.35	0.45	0.34	0.49		
VI	4	3.39	2.93	3.96	0.15	*0.56	0.28		
VII	2	5.19	5.02	5.37	*0.20	*0.42	0.01		
Total	12	2.66	2.27	3.96	0.26	0.07	0.45		

Table 2-4. Results of nested ANOVA. Acoustic parameters were dependent variables; individuals, populations and IRS were categorical variables. Abbreviations are explained in Table 2-2.

High-pitched call		
Acoustic parameters	Effect of individual	Effect of IRS
Dur	F=2.254590 p=0.013172	F=2.081047 p=0.157839
Syll dur	F=1.596755 p=0.100958	F=0.247375 p=0.861480
Interval	F=8.962510 p=0.000000	F=1.407952 p=0.283757
Syll rate	F=2.763700 p=0.002513	F=3.165728 p=0.063717
No of syll	F=5.140564 p=0.000001	F=0.209777 p=0.887815
PFss	F=3.955695 p=0.000053	F=4.949392 p= 0.017175
PFms	F= 2.592848 p=0.004393	F=4.899394 p=0.019059
PFIs	F=0.884774 p=0.577958	F=6.842715 p=0.012251
PFm	F=2.752327 p=0.002609	F=6.097254 p=0.009133
BW1	F=1.617562 p=0.095015	F=1.599620 p=0.246683
BW2	F=0.610867 p=0.847534	F=1.415412 p=0.323768
Ouah call		
Acoustic parameters	Effect of individuals	Effect of IRS
Duration	F=13.37645 p=0.002258	F=2.33266 p=0.020296
PF start	F=3.21504 p=0.001941	F=77.22408 p=0.000003*
PF max	F=8.50726 p=0.000000	F=33.75744 p=0.000070
PF end	F=1.74414 p=0.089712	F=76.36747 p=0.000004*
BW1	F=4.887568 p=0.000023	F=3.084009 p=0.096434
BW2	F= 2.456409 p=0.014681	F=2.058546 p=0.185591

Table 2-5. Classification table. Assignment of individuals to IRS (after cross-validation) based on four acoustic variables for the high-pitched call and two acoustic variables for the ouah call.

	High-pitched					Ouah					
	% correct	IRS I	IRS V	IRS VI	IRS VII	Total	% correct	IRS I	IRS VI	IRS VII	Total
IRS I	62.5	5	0	3	0	8	100.0	6	0	0	6
IRS V	100.0	0	2	0	0	2					
IRS VI	33.3	2	0	1	0	3	75.0	0	3	1	4
IRS VII	100.0	0	0	0	5	5	100.0	0	0	2	2
Total	72.2%					18	91.7%				12

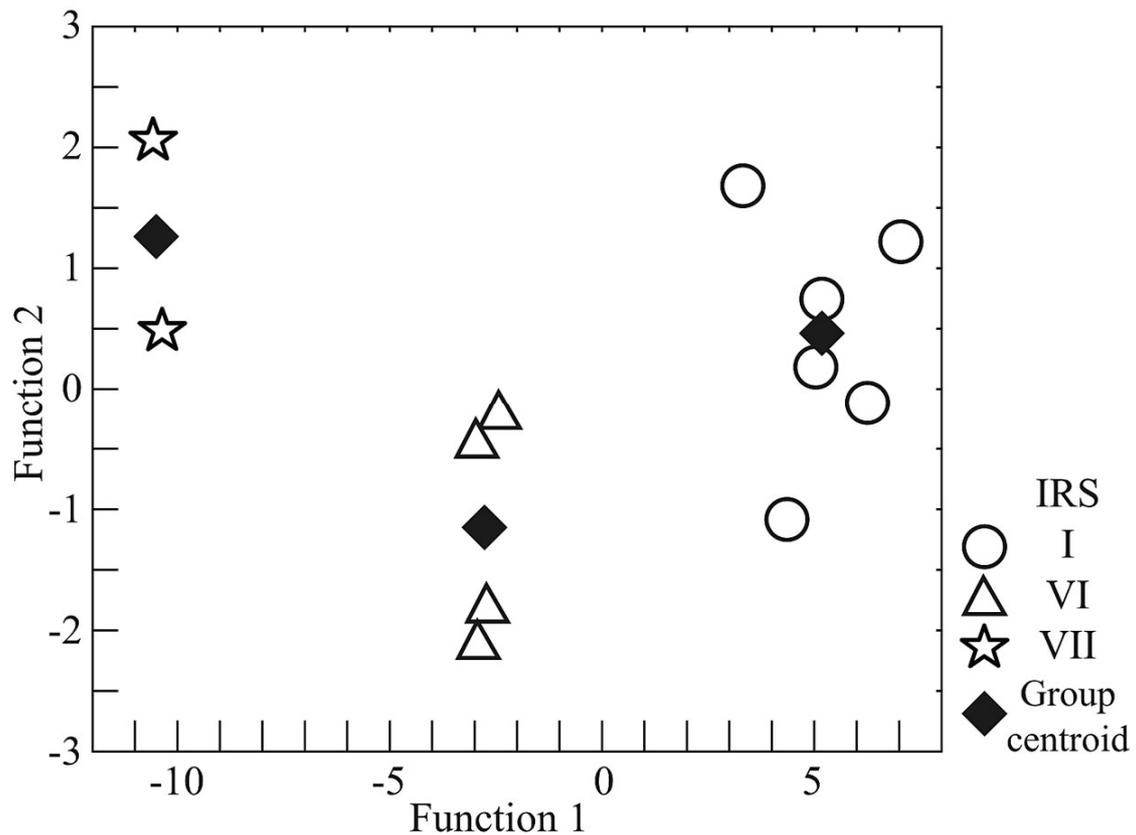


Fig. 2-4. Discriminant functions of the ouah call separating three IRSs.

2. Geographic distance and loud call variation

Based on a Mantel test we found that acoustical distances among localities based on the HPC had no relationship to geographic distance ($r=-0.117$, $p=0.79$). In contrast, acoustical distances among localities based on the OC were significantly correlated with geographic distances ($r= 0.717$, $p=0.006$).

3. Phylogeny of loud calls

14 out of 17 acoustic parameters were phylogenetically informative. For the HPC, four spectral parameters (PFm, PFms, PFIs, and BW2) were the most informative, whereas two spectral (PFss, BW1) and two temporal (Interval and Syll rate) parameters were informative, but showed less support for the reconstruction. For the OC, all acoustic parameters were informative and only duration showed less support (Appendix 2-1).

Using the eastern sportive lemurs from Mantadia as an outgroup, exhaustive searches for the most parsimonious tree using all characters for both call types yielded one shortest tree with a length of 75 steps, a consistency index of 0.90 and a retention index of 0.68 (Fig. 2-5). In the topology of this tree, IRS I separated first from the northern clade with a branch support of 86%; IRS V and IRS VI are close together and separated from IRS VII with a branch support of 70 %.

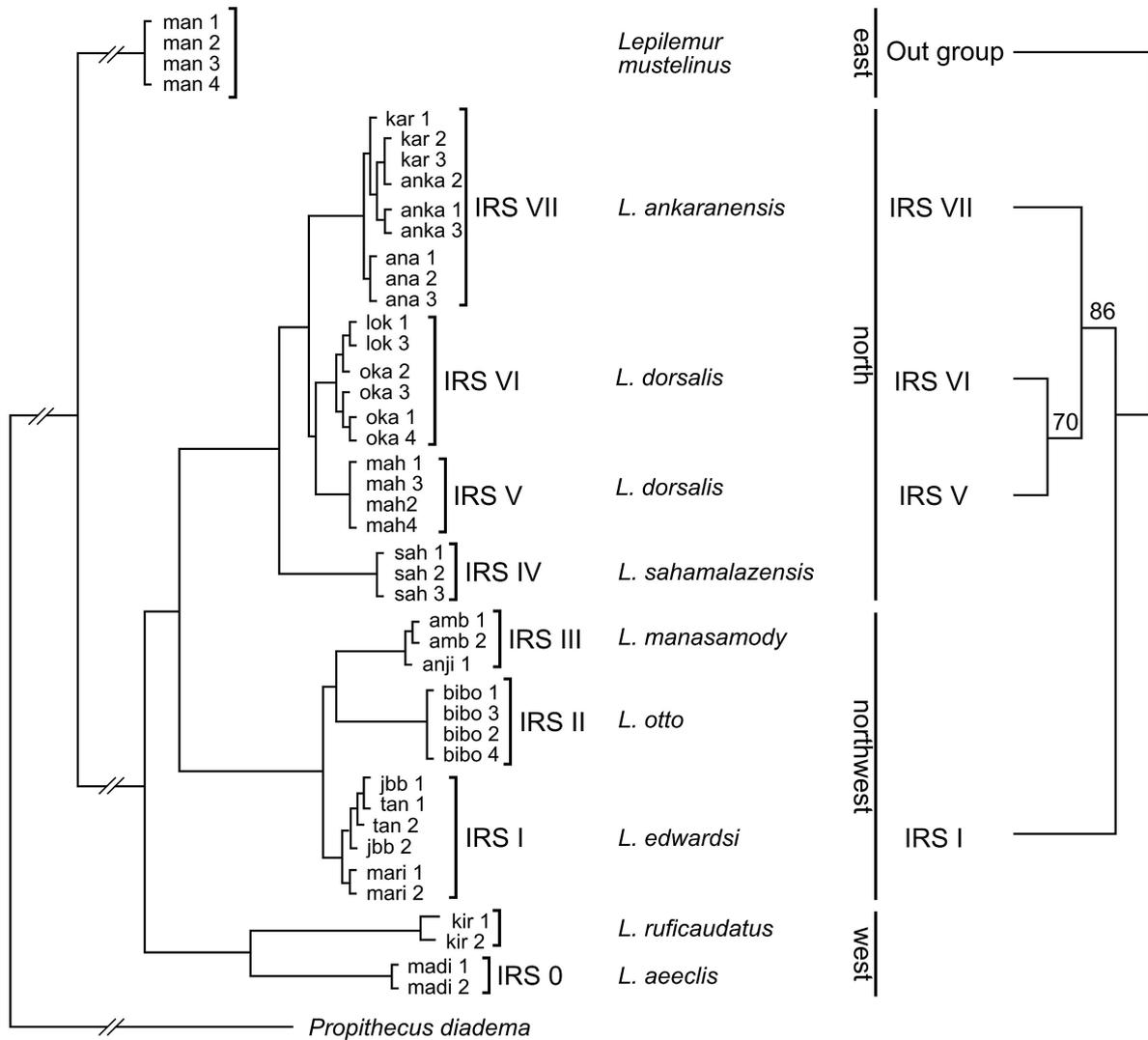


Fig. 2-5. Comparisons between the acoustic tree based on loud calls and the genetic tree obtained with mtDNA (Craul et al., 2007).

Discussion

Loud calls of sportive lemurs showed hearable and specific acoustic differences among IRSs, but call types contributed differently to IRS separation. Although the OC (with a clear harmonic structure) separated IRS with a probability of 91.7 %, the HPC (with a less harmonic spectrum) yielded a probability of only 72.2 %. The acoustic topology obtained by PAUP was robust and coincided well with the genetic topology proposed by Craul et al. (2007).

1. Loud call variation among IRS and geographic distance.

In our study we revealed that acoustic variation of two loud call types of sportive lemurs was related differently to geographic distance. Thus, acoustic distances between sample localities and geographic distances did not show a significant correlation for the HPC, a possible explanation may be that the HPC with its more noisy sound quality is difficult to quantify with common sound analysis techniques. In contrast to that, the harmonic contour of the OC is easy to characterize. Indeed, this call type showed a clear clinal acoustic variation in four out of six acoustic parameters (Dur, PF start, PF max, PF end and BW2). Values increased from northwestern to northern Madagascar. Similar clinal variations in loud calls were described from a variety of different species (e.g. in primates: Mitani et al., 1999, in passerines: Isler et al., 2005, in anurans: Pröhl et al., 2006).

A potential factor explaining clinal variation in long-range communication calls of mammals is body size. Thus, for example Kinsler and Frey (1982) and Fitch and Hauser (2002) hypothesized that body size is linked to frequency in so far as larger species produce lower frequencies. Our results on sportive lemurs may render additional support for this hypothesis. Thus, sportive lemurs of IRS I showed the highest-body mass (Craul et al., 2007) and the lowest peak frequency and those of IRS VII showed the lowest-body mass (Craul et al., 2007) and the highest peak frequency.

2. Acoustic topology of loud calls and its relation to phylogeny

The acoustic topology of loud calls in sportive lemurs, as determined in this study, showed a clear first dichotomy between sportive lemurs of northwestern (IRS I) and northern Madagascar (IRS V, VI, VII), and a further dichotomy between the three northern IRSs, in which IRS VII branched first. This grouping pattern is in accordance with the large river model put forth by Olivieri et al. (2007) and Craul et al. (2007) which argued that large rivers acted as insurmountable barriers for gene flow in lemurs of northwestern and northern Madagascar, leading to cryptic speciation events. When comparing the genetic topology of sportive lemurs based on mtDNA sequences (Craul et al., 2007) with the acoustic topology based on loud call variation (see Fig 2-5), both match very closely. Localities in IRS I correspond to the species *L. edwardsi*, those in IRS V to *L. dorsalis* (Mahilaka), those in IRS VI to *L. dorsalis* (Lokobe and Manehoko) and those in IRS VII to *L. ankaranensis* (Ankarana, Andrafiabe and Andrafiamena).

Individuals of IRSV and VI were considered by Craul et al. (2007) as sub-species of *L. dorsalis*. Both IRSs were cluster separately based on acoustics with a bootstrap support of 70%. This finding suggests that both IRS are not only separated genetically, but also bioacoustically. As the sample size was small, further studies with a larger sample size are necessary to decide whether on a sub-species or species level. Altogether our findings revealed that despite of the small sample size, loud calls separated geographically isolated populations of sportive lemurs specifically. Harmonic loud calls were more informative than noisy ones. Based on these results, non-invasive tools for diagnosis and monitoring of cryptic species in nature can be developed for conservation management.

3. Bioacoustics and conservation

Conservation decisions require a thorough knowledge of a particular species, its distribution and abundance. Although widely distributed and generally not hard to find, sportive lemurs are among the least-known of the lemurs (Tattersall, 2007). *L. edwardsi* and *L. mustelinus* have been classified as low risk (not endangered) and *L. dorsalis* as vulnerable (not critically endangered) (IUCN red list, 2006). However, all sportive lemur species are highly threatened by forest destruction and hunting, with a tendency of high risk future extinction. Based on the information about the phylogenetic diversity and taxa position (Lehman, 2006), low reproductive rate (Randrianambinina et al, 2007) and new species with limited distribution range (Craul et al., 2007) conservation priorities should be re-evaluated. Thus, species like *L. edwardsi* which is classified as low risk and *L. dorsalis* that has been classified as vulnerable should be treated as endangered.

The cryptic nature of sportive lemurs and the difficulties in locating them during the night or at the sleeping sites during the day make their detection, species identification and population density estimation difficult. The highly vocal activity of sportive lemurs, (Rasoloharijaona et al., 2006; Rabesandratana, 2006), along with the call species-specificity described in this study, provides an important basis to apply bioacoustics as a tool for surveying and monitoring populations as successfully carried out in gibbons (e.g. Geissmann and Nijman, 2006) and in anurans (e.g. Bridges and Dorcas, 2000) using observational fixed points and transects.

Vocal-monitoring represents an important additional tool in walk census and focal observations for assessing species diversity and abundance. Vocal monitoring by loud calls is a cheap and affordable method in regions where scientific resources are limited. Immediate measures to prevent further destruction and fragmentation within the IRSs are essential, with the help of vocal monitoring: 1) locals communities can be encouraged to participate in conservation projects, under the supervision of volunteer biologists, 2) information can be used for environmental educational and sensitization purposes and 3) new materials for ecotourism may be created at the different national parks in Madagascar.

Appendix

Appendix 2-1. Acoustic parameters, names and character states, with IRS-specificity. The * denotes uninformative characters.

CI	RI		
Character state and codification for the high-pitched call			
<i>Temporal related</i>			
1	1.00*	0/0	Call duration (Dur sec) 0 = < 0.55 (1) = 0.56-0.59 (2) = 0.60-0.66 (3) = 0.67-0.77 (4) = 0.78- 0.91
2	1.00*	0/0	Syllable duration (Syll dur sec) 0 = < 0.05 (1) = 0.06-0.60 (2) = 0.61-1.19 (3) = 1.20-1.86 (4) = 1.87- 2.63
3	0.80	0.00	Interval duration (Interval sec) 0 = < 0.04 (1) = 0.05-0.07 (2) = 0.08-0.11 (3) = 0.12-0.14 (4) = 0.15- 0.17
4	0.80	0.00	Syllable rate (Syll rate syll/sec) 0 = < 6.00 (1) = 7.00-8.00 (2) = 9.00-10.0 (3) = 11.0-12.0 (4) = 13.0- 14.0
5	1.00*	0/0	Number of syllables per call (No of syll) 0 = < 0.055 (1) = 0.56-0.59 (2) = 0.60-0.66 (3) = 0.67-0.77 (4) = 0.78- 0.91
<i>Source related</i>			
6	0.80	0.50	PF at start syllable (PF ss kHz) 0 = < 2.15 (1) = 2.16- 2.69 (2) = 2.70-3.23 (3) = 3.24-3.78 (4) 3.79- 4.32
7	1.00	1.00	PF at middle syllable (PF ms kHz) 0 = < 2.32 (1) = 2.33- 2.83 (2) = 2.84-3.33 (3) = 3.34-3.84 (4) 3.85- 4.35
8	1.00	1.00	PF at last syllable (PF ls kHz) 0 = < 2.33 (1) = 2.34- 2.91 (2) = 2.92-3.49 (3) = 3.50-4.07 (4) 4.08- 4.65
9	0.80	1.00	PF median (PFm. kHz) 0 = < 2.27 (1) = 2.28- 2.81 (2) = 2.82-3.35 (3) = 3.36-3.89 (4) 3.90- 4.43
10	0.67	0.33	Bandwidth 1 (BW1. kHz) 0 = < *0.06 (1) = *0.05-0.05 (2) = 0.06-0.15 (3) = 0.16-0.26 (4) = 0.27-0.37
11	1.00	1.00	Bandwidth 2 (BW2. kHz) 0 = < *0.30 (1) = *0.29-*0.17 (2) = *0.16-*0.05 (3) = *0.06-0.08 (4) = 0.09-0.21
Character state and codification for the ouah call			
<i>Temporal related</i>			
12	0.66	0.00	Call duration (Dur sec) 0 = < 0.04 (1) = 0.05-0.07. (2) = 0.08-0.09. (3) = 0.10-0.12. (4) = 0.13-0.15
<i>Source related</i>			
13	1.00	1.00	PF start (kHz) 0 = < 1.98 (1) = 1.99-3.04 (2) = 3.05-4.10 (3) = 4.11-5.16 (4) = 5.17-6.22
14	1.00	1.00	PF maximum (DFmax kHz) 0 = < 2.39 (1) = 2.40-3.30 (2) = 3.31-4.20 (3) = 4.21-5.11 (4) = 5.12-6.02
15	1.00	1.00	PF end (kHz) 0 = < 2.26 (1) = 2.27-3.00 (2) = 3.01-3.73 (3) = 3.74-4.46 (4) = 4.47-5.19
16	1.00	1.00	Bandwidth 1 (BW1 kHz) 0 = < *0.20 (1) = *0.19-*0.05 (2) = *0.04-0.11 (3) = 0.12-0.26 (4) = 0.27-0.42
17	1.00	1.00	Bandwidth 2 (BW2 kHz) 0 = < 0.13 (1) = 0.14-0.30 (2) = 0.31-0.48 (3) = 0.49-0.65 (4) = 0.66-0.82

CHAPTER 3

DUETTING - A MECHANISM TO STRENGTHEN PAIR BONDS IN A DISPERSED PAIR-LIVING PRIMATE (*LEPILEMUR EDWARDSI*)?

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Abstract

Duetting is defined as an interactively organized pair display in which one pair partner coordinates its vocalizations in time with those of the other. It is widespread among tropical birds and cohesive pair-living primates, in which it is suggested to strengthen pair bonds. We know very little about the presence and function of duetting in dispersed pair-living mammals. We studied duetting behavior in a solitary foraging, but pair-sleeping primate, the Milne Edwards' sportive lemur, in a dry deciduous forest of northwestern Madagascar. We radio-tracked six pairs throughout one year and recorded their sleeping sites and associations, home-range use and vocal and behavioral interactions. Three different periods were covered (mating, pregnancy and offspring care). Sleeping partners form long-term pair bonds, indicated by an almost exclusive pair-specific usage of sleeping sites and home-ranges across periods. We explored three functional hypothesis of duetting: mate reunion, pair reunion and joint-territorial defense. Pairs regularly engaged in duet calling. Duetting increased significantly during the offspring care period. Duetting occurred significantly more often at feeding sites than at sleeping sites. Pair partners synchronized behavioral activities after duetting. The activity most often synchronized was locomotion. Pair partners played an equal role in duetting with no difference between sexes in starting or terminating duetting. Altogether, our results provide support for the hypothesis that in dispersed pair-living primates, duetting evolved as a mechanism to coordinate activities between pair partners dispersed in space, to strengthen pair bonds, and, perhaps, to limit infanticide and nutritional stress in lactating females.

Introduction

Duetting represents a form of interactive acoustic communication in which a mate coordinates vocalizations in time with those of another (Wickler, 1980; Wickler and Seibt, 1982). Interactive calling of mating partners occurs repeatedly and predictably in time (Langmore, 2002). Duetting is found in a wide variety of animal taxa, from insects to mammals (e.g. insects: Bailey, 2003; e.g. anurans: Tobias et al., 1998; e.g. birds: Thorpe, 1972; Farabaugh, 1982; Langmore, 1998 and mammals: Robinson, 1979; Haimoff, 1986; Geissmann, 1999; Nietsch, 1999; Müller and Anzerberger, 2002). The independent evolution of duetting in phylogenetically distinct groups suggests that selection for duetting is strong (von Helversen, 1980). Several functional hypotheses have been put forth to explain the evolution of duetting.

The mate reunion model predicts the occurrence of duetting in insects such as bushcrickets (von Helversen and von Helversen, 1983), grasshoppers (Bailey, 2003; von Helversen et al., 2004), cicadas (Nuhardiyati and Bailey, 2004) as well as in frogs (Tobias et al., 1998), toads (Bush and Bell, 1997) and some birds (Stokes and Williams, 1968). In these animal groups duetting helps mating partners dispersed in space to detect and localize mating partners over large distances as well as to coordinate and synchronize mating activities (Bradbury and Vehrencamp, 1998). Duetting is thus restricted to the breeding season.

The pair reunion model represents an explanation for the evolution of duetting in nocturnal cohesive pair-living anthropoid primates, such as tarsiers. Here, pair partners spend more time duetting in the mornings when families congregate at sleeping sites than in the evenings when they start foraging (MacKinnon and MacKinnon, 1980; Nietsch, 2003). According to this model, there is no link between duetting and the breeding season.

The joint territory defense model was proposed to explain duetting in pair-living territorial birds and primates (Thorpe, 1972; Mitani, 1987). In birds, duetting is a joint aggressive display of both pair partners during territorial or spatial disputes (Wickler, 1976; Arrowood, 1988), often linked to prominent sites of the environment

(Wickler, 1976) and/or specific times of the activity cycle (Sonnenschein and Reyer, 1983). Duets are suggested to be too loud to have solely an intrapair function (Seibt and Wickler, 1977). For example, studies on duet and solo song activity of pair partners in rufous and white wrens (Topp and Mennil, 2007) showed that during the pre-breeding stage, pair partners maintain an equal level of responsiveness to each other's songs, supporting the hypothesis that duetting is a cooperative display allowing pairs to defend resources from conspecific rivals.

In territorial cohesive pair-living primates, duetting is believed to act as a mechanism to deter neighbors or strangers from intruding upon an occupied territory (Haimoff and Gittins, 1985). Duetting is present during inter-group conflicts in titi monkeys, indris, Mentawi langurs and different gibbon species (Tilson and Tenaza, 1976; Robinson, 1979; Raemaekers et al 1984; Haimmof, 1986; Leighton, 1987). In indris, a rapid exchange of duet songs between groups occurred when a group approached or crossed the border of another group's territory, indicating that duetting may function as a territorial declaration (Pollock, 1975). Playback experiments in some territorial birds and cohesive pair-living primates supported the notion that duetting helps to coordinate territorial defense among pair partners (Seibt and Wickler, 1977; Robinson, 1979; Mitani, 1987, 1990; Hall, 2000; Mulder et al., 2003; Rogers et al., 2006).

To date, very little is known about the presence and function of duetting in dispersed pair-living primates who forage solitarily during the night but form sleeping groups during the day. Primates with a dispersed social system are suitable models to explore mechanisms favoring cooperation and sociality in more complex social groups. The Milne Edwards' sportive lemur (*Lepilemur edwardsi*) is a dispersed pair-living nocturnal primate that lives in dry deciduous forests in northwestern Madagascar. Sportive lemurs are predominantly folivorous (Smith and Jungers, 1997). Toward the end of the dry season when trees become nearly bare of leaves, food may be expected to exert a selective pressure (Hladik, 1980) and competition for food resources increases (Warren and Crompton, 1997). Indeed, a study of the closely related, folivorous *Lepilemur ruficaudatus*, (Ganzhorn, 2002) revealed that differences in body mass variation and food requirements of both sexes during the

year indicate severe nutritional constraints for females during the period of lactation. Energetic constraints of females during the breeding season are also discussed to be linked to female dominance or female feeding priority, a common trait among lemurs (Jolly, 1984; Radespiel and Zimmermann, 2001; White et al., 2007).

The Milne Edwards' sportive lemur lives in dispersed male-female pairs as the smallest social unit (Rasoloharijaona et al., 2000, 2003, 2006; Thalmann and Ganzhorn, 2003; Thalmann, 2006). Pair partners share a home-range (HR) with suitable sleeping and feeding sites and use sleeping sites and HRs exclusively throughout several months of the year (Rasoloharijaona et al., 2003, 2006). This lemur species reproduces seasonally. Females are in estrous for a short period in May/June, most likely for only a few hours in a night, as is typical for lemurs (Randrianambinina et al., 2007). Babies are born in October. We documented one case of infanticide at the onset of the offspring care period beginning in November (Rasoloharijaona et al., 2000). In the latter period a male stranger attacked and injured the baby of a female whose male partner had disappeared.

Pairs are highly vocal. Pair partners display an elaborated vocal repertoire consisting of a total of nine structurally different call types (Rasoloharijaona, 2001; Rasoloharijaona et al., 2006) of which most are sex-specific: one call type, the high-pitched call (HP), is shared between the sexes, three call types (bark 1, bark 2, oooai), are used only by females and four (ouah; isolate shrill (IS); related shrill (RS); tchen-tchen) only by males.

Thalmann (2006) suggested that the occurrence of complex and coordinated vocalizations in pairs should be present during the mating season if enhancing and advertising the pair-bond and territorial advertising are the only functions.

Within this study we aim to investigate to what extent duetting in a mammal with a dispersed social system, such as the Milne Edwards' sportive lemur, corresponds to models established for duetting in tropical birds and in primates living as cohesive pairs. First we explore the extent to which adult sportive lemurs captured at the same sleeping site, are bonded pairs. If they are bonded, we expect pair partners to share HRs and sleeping sites over the whole year and to use them exclusively.

Second, we explore the extent to which the evolution of duetting in this sportive lemur species can be explained by the three previously described duetting models, established for birds and socially cohesive primates: the mate reunion model, the pair reunion model and the joint territory defense model. According to the mate reunion model, duetting should be restricted to the mating season. The pair reunion model predicts differences in duetting activity between dusk and dawn. The joint territory defense model proposes that vocal activity is higher during the offspring care period (lactation of females) when females are under severe nutritional constraints and their babies threatened by infanticidal males, than outside of this period (mating or pregnancy period). The latter model furthermore suggests that pair partners show significantly more duetting events at feeding sites than at sleeping sites. Likewise, it predicts that pair partners that exhibit different behavioral activities before duetting show significantly more synchronized behaviors afterwards. Third, we tested to which extent females are dominant over males by starting and terminating a duet call sequence and in leading locomotion by determining the direction of travel after calling.

Methods

Study site and animals

We performed the study in the western Malagasy dry deciduous forest in the National Park of Ankarafantsika (16°19'S, 46°49'E), located about 110km southeast of Mahajanga. For a detailed description of the forest and climate conditions see Rendigs et al. (2003). The study took place from July 2004 until July 2005 at the study site "Jardin Botanique A (JBA)". Sportive lemurs were captured at the onset of their activity period with a mist net fastened around the sleeping hole. We used Ketasetel 50 (50mg Ketasetel/ml) in the dose recommended by the manufacturers as anesthetic. The lemurs were briefly anaesthetized for measuring, weighing, marking and radio-collaring (collars were fixed around the neck) and released at their capture site the same night after recovery. Radio-collars were removed at the end of the study period. All procedures adhered to the legal requirements of the country in which the research was conducted and permission was obtained from the appropriate agencies. Capturing procedures, animal handling and radio-tracking techniques followed standard protocols and were authorized by the appropriate institutional and governmental bodies.

We radio-collared 6 males and 6 females via TW-3 button-cell tags (Biotrack, Dorset, UK). We marked each lemur individually by cutting patterns into the hair of its tail. We sexed each lemur, aged it as adult or non-adult according to body length and weight, (see Rasoloharijaona et al., 2003) and its reproductive status (Randrianambinina et al., 2007). We divided the year into three biologically relevant periods (see Randrianambinina et al., 2007): mating period (May to June), pregnancy (July to October), and offspring care (November to April).

1. Determination and analysis of sleeping sites, sleeping group composition and home-ranges

On a total of 180 days, we located the sleeping site of each animal by radio-tracking during the day and observing the sleeping sites at dawn and dusk. Thus sleeping associations were determined. The sleeping sites were numbered and marked on a map. We scored all different sleeping sites used by individuals throughout the year and defined a sleeping group as adult individuals that repeatedly slept together. According to this criterion, six females and six males were defined as sleeping pairs (Table 3-1). In order to determine if there are sex differences in the number of sleeping sites used by males and females, we summarized sleeping sites per individual and per sex and applied a Mann-Whitney U Test (MWU- Test).

Table 3-1. Percentage of days when the six pairs shared sleeping holes during almost 180 localization days per animal.

Pair code	Days together/ Total days	Percentage(%)
F31 M55	172/179	96.09
F72 M67	125/133	93.98
F71 M69	78/102	76.47
F79 M90	80/174	45.98
F88 M51	63/171	36.84
F78 M64	52/178	29.21
6 pairs		63.09%

HR sizes, locations and overlaps of all radio-collared lemurs were determined telemetrically according to Rasoloharijaona et al. (2006). A portable TR-4 receiver and a RA-14K antenna (Telonics, Inc., Impala, AZ) were used. For determining HRs, triangulation data points per individual were collected at intervals of at least 30 min for 3-8 hours per night during 8-11 nights per period (from 17:00 pm – 5:00am). A total of 60 data points per period were collected for each animal. HRs were analyzed

using Trackasc (software A. Ganzhorn, 1996, unpub.) and Ranges 6 v1.8 software (Institute of Terrestrial Ecology, Wareham, UK; (Kenward et al., 2002). Minimum convex polygons were applied to estimate HRs (White and Garrott, 1990). Extreme outliers (two points from two individuals) were eliminated manually maintaining more than 50 data points per individual/period. Mean HR overlaps were calculated intra- and inter-sexually for all possible dyads of radio-collared animals, considering overlap in both directions. Mean HRs per year were calculated and mapped for each individual using Arc View GIS 3.3 (ESRI). To determine if there were sex differences in HR size between males and females we calculated the mean value for each individual and each sex and applied a MWU- Test.

2. Recording and analysis of social behavior and vocal activity.

Direct focal observations were carried out on all radio-collared individuals, using focal animal sampling with continuous recording (Altmann, 1974; Martin and Bateson, 1993). One full night per period was covered for each radio-collared individual (e.g. one night from 5:00 p.m. -12:00 a.m., the successive night from 12:00 a.m. -05:00 a.m.). Lemurs were observed by dimmed light using headlamps. Individual behaviors, affiliative encounters or agonistic encounters (Table 3-2) were recorded and analyzed 1 min before and 1 min after the onset of vocalizations. Vocalizations were recorded on a stereo cassette-recorder (Sony Professional WM-D6C and a Sennheiser ME67/K6 directional microphone with a windshield). All sound recordings were made with Sony Super Chrome Class UX-S IECII/Type II tapes. Since the animals were habituated to researchers and to our equipment, high-quality recordings were made at relatively close range (3-5 m). Additional information relating to spatial and ecological factors (e.g. location within the HR, climate conditions) was recorded. All information was subsequently transferred to data sheets (Excel tables). To analyze call contexts, the noted behavioral activity associated with a call was linked to one of eight major behavioral categories (Table 3-2).

Table 3-2. Ethogram used for describing call context

Behaviors	Definition
<i>Resting</i>	Lies horizontally or vertically on a branch or sits there, sometimes closes the eyes.
<i>Locomotion</i>	Climbs up or down a tree or leaps to another tree
<i>Feeding</i>	Eats leaves branches, barks, fruits, licks gum, bites trunks
<i>Scanning/focused attention</i>	Moves the head horizontally from one side to the other in a fast manner or looks straightforward in a particular direction, looks in a sleeping hole, looks to sound source
<i>Self-grooming</i>	Grooms itself using toothcomb or grooming claw
<i>Affiliative</i>	Grooms another animal (partner or young) by using its toothcomb
<i>Aggression vs strangers</i>	Pair chases together, when strangers or neighbors approach, both members of a pair shake branches.
<i>Offspring care</i>	Mother or potential father carries baby in mouth or grooms it

We calculated the loud calling activity per individual and per period as follows: we summarized all vocal events per full night for each individual and divided it by the contact time. Out of that, we calculated the vocal rate/hour for each individual for each period. To explore potential sex differences in vocal activity we calculated the mean vocal rate per sex per period and we applied MWU- Tests. We also compared mean vocal rates between the three periods by performing a Friedman ANOVA, followed by a Wilcoxon matched pair test. Furthermore, we constructed a table with all vocal events given by an individual within each hour of a full night and summarized all the loud calling events per individual per hour per period.

3. Analysis of the behavior before and after duetting and pair's activity synchronization.

To explore to what extent duetting may function as a means for synchronizing behavioral activities of pair partners, we scored the behavioral activity (see Table 3-2) of pair partners 1 min before and 1 min after calling. We established three different sound-associated categories: 1) Pair partners showed different activities before

calling and different activities after (DBA); 2) Pair partners showed the same activity before calling and different activities after (SBDA); 3) Pair partners showed different activities before calling and the same activity after (DBSA).

All loud calling events were linked to one of the three sound-associated categories (DBA, SBDA, DBSA). We summarized the number of sound-associated categories for each pair and calculated the mean for each sound-associated category. To examine potential differences between categories, we applied a Friedman ANOVA and if significant differences exist, we applied Wilcoxon matched pairs test.

To explore which behavior category was related to the sound-associated category DBSA, we scored the occurrence of the respective behavior categories after calling for each pair. To examine whether there are differences between the eight established behavioral categories we applied a Friedman ANOVA, followed by a Wilcoxon matched pairs test with a Bonferroni correction. To determine whether there are sex differences between who determines direction of locomotion after a duet sequence, we scored which sex moves first immediately after calling and applied a MWU- Test.

4. Vocal analysis, location of calling and determination of call types

Loud calls were digitized with a sample rate of 44 kHz and a sample size of 16 bit using the software Bat Sound Pro Sound analysis 3.31 (Elektronik, 2001). Duetting was visualized as sonograms with the software Avisoft-SAS lab Pro V 4.39 (Specht, 1990). Call types were determined according to the vocal repertoire published by Rasoloharijaona et al. (2006). Duetting events were scored in the period of the year with the highest vocal rate, for two nights per pair. A duetting event started when one pair partner produced a call type and the other responded within an interval of less than 1 second. In most cases, vocal sequences developed in which different call types were exchanged between pair partners. Pair partners produced call types either alternatively or simultaneously.

To examine whether duet calling activity differed between feeding and sleeping sites, we recorded where (i.e. sleeping site or feeding site) duetting events occurred and summarized the frequency of duetting events per individual at each site during the period of the highest calling rate and applied a MWU-Test. In order to examine whether there were sex differences we performed a MWU-Test.

5. Composition of duet calling and sex dominance at the start and end of call sequences

To explore whether call types at the beginning of a duet calling sequence differed from those at the end of the sequence and whether there are sex-specific differences, we noted which sex produced the first and the last call type within a sequence, as well as the type of call emitted. We summarized the frequency of each call type occurrence for each individual and each sex during one full night and applied a MWU-Test.

Results

1. Sleeping sites, sleeping group composition and home-ranges

Individuals used sleeping sites in tree holes with one or several entrances and from 1 to 10 m above the ground. The focal animals slept together as male-female pairs, on average, 63.09% of their localization days (N=179), with high variation among pairs (Table 3-1). Three pairs shared sleeping sites for more than 70% whereas the other three pairs shared it for less than 46%. Most of the time, an adult lemur of one sex shared sleeping tree holes with an adult lemur of the other sex, with infants and other immature animals. Groups either slept together in the same hole, in different holes in the same tree, or in holes of an adjacent tree (distance about 5m). A total of 36 sleeping sites were recorded. Sleeping sites were used exclusively by the respective pairs (just in one case a young female used one sleeping site of a pair, for 2 days when the pair was not at that site). The number of sleeping sites used by adult males was 4.5 ± 2.16 with a maximum of seven and a minimum of two sites. The number of sleeping sites used by adult females was 4 ± 1.41 with a maximum of six and a minimum of two sites. There was no significant sex difference in the number of sleeping sites used (MWU- Test, $N_f=6$, $N_m=6$; $p=0.81$). Both sexes showed a high constancy of sleeping site usage.

Across all seasons, the mean HR size for females was 2.07 ± 1.00 ha (N=6), with a maximum of 4.10 ha and a minimum of 1.49 ha. HR size for males was 2.13 ± 0.77 ha (N=6), with a maximum of 3.57 ha and a minimum of 1.57 ha. No difference was found between the sexes (MWU- Test, $N_f=6$, $N_m=6$; $p=0.48$). The overall mean HRs of sleeping partners overlapped extensively in form and size (male overlap with female on average 71.92%, female overlap with male on average 87.47%; see Fig. 3-1). Sleeping pairs used their HR almost exclusively during the whole year. No differences were found between the sexes in HR overlaps (MWU- Test, $N_f=6$, $N_m=6$; $p=0.13$).

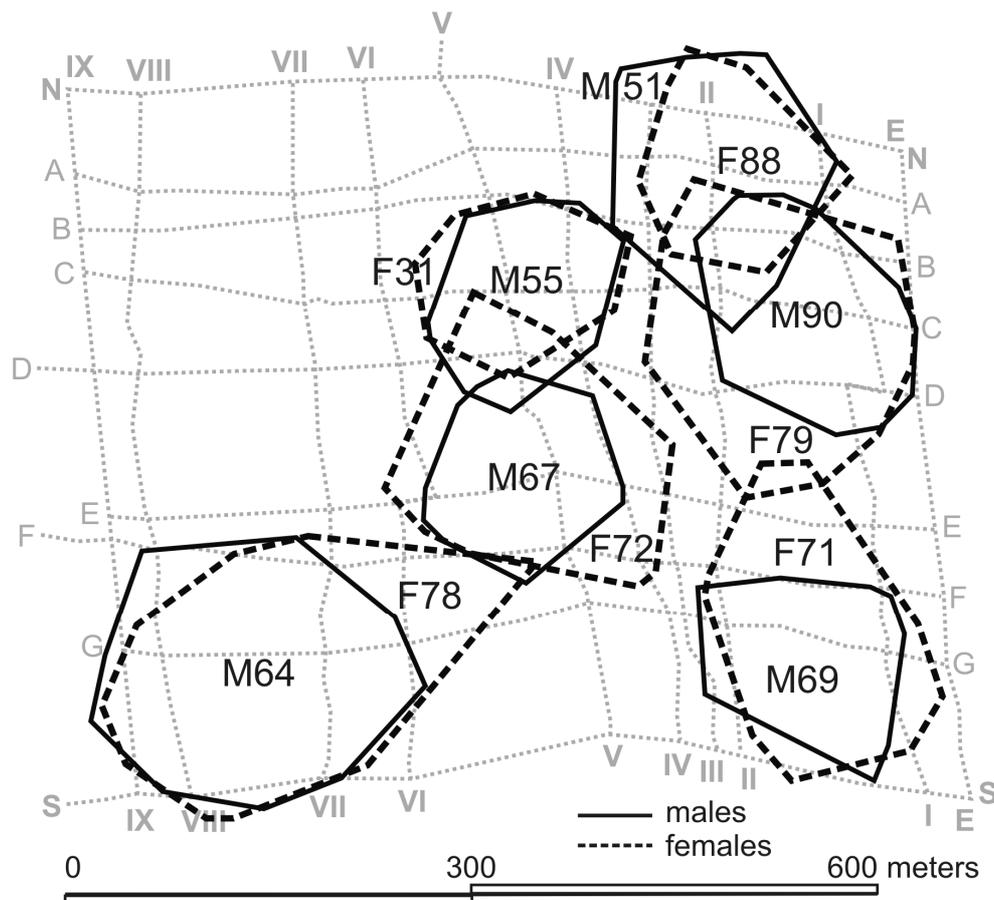


Fig. 3-1. Home-ranges of the six pairs of sportive lemurs, throughout the year, calculated by the minimum convex polygons method. Females are indicated by dotted lines, males by solid lines.

2. Vocal activity during the year

Mean vocal rate for females for the year was 3.42 ± 2.64 with a minimum of 1.78 ± 0.48 and a maximum of 5.39 ± 1.37 , males showing a mean of 2.91 ± 2.57 with a minimum of 0.72 ± 0.75 and a maximum of 4.86 ± 4.33 . We found no significant difference between sexes in the vocalization rates for each of the three periods, ((MWU- Test: pregnancy ($N_f=6$, $N_m=4$; $p=0.61$); offspring care ($N_f=6$, $N_m=6$; $p=0.81$); mating ($N_f=6$, $N_m=6$; $p=0.81$)). Therefore, we analyzed males and females together and tested the effect of the period on the vocalization rate. Friedman ANOVA analysis showed significant differences among periods, ($N=10$, $df=2$; $p=0.007$) the

mean vocalization rate was significantly higher during the offspring care period than during the pregnancy (Wilcoxon matched pair test $N=10$, $p=0.009$) and the mating periods (Wilcoxon matched pair test $N=12$, $p=0.004$), respectively. No difference was found between the pregnancy and the mating period (Wilcoxon matched pair test $N=10$, $p=0.57$; Fig. 3-2). No significant sex differences were revealed for any hour of the night, in any period. For the period with the highest vocalization rate (offspring care period), we tested the effect of hours on the mean vocalization rate for both sexes together. Vocal activity started around 6:00 pm and lasted until around 4:00-5:00 am. The mean vocalization rate varied between 4.03/h and 6.54/h with no significant differences between hours (Friedman ANOVA $N_{hr}=11$, $df=10$; $p=0.52$).

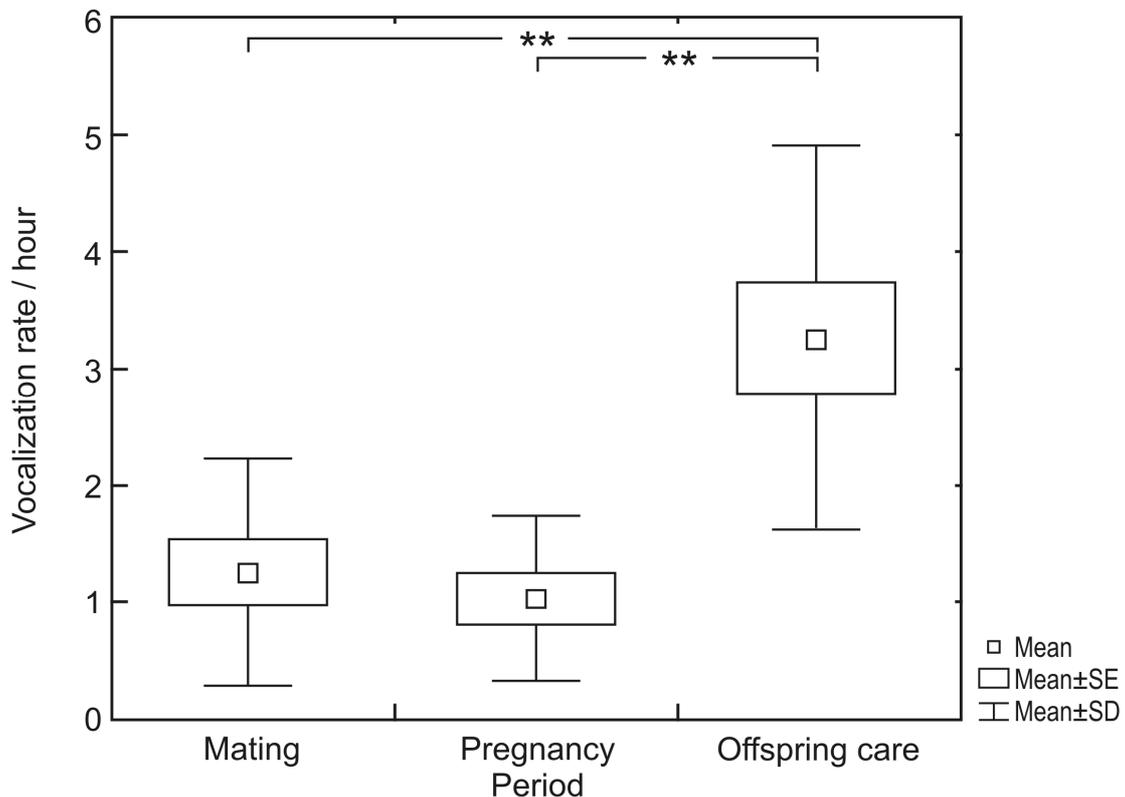


Fig. 3-2. Vocalization rates during different periods of the year. ** $P = 0.005$.

3. Duetting and pair synchronization

We explored the hypothesis that duetting acts as a means to synchronize pair activities after calling. We compared whether a difference exists in the frequency of the three sound associated categories: DBA, SBDA and DBSA. Friedman ANOVA analysis showed significant differences among periods, ($N_p=6$, $df=2$; $p=0.007$). We found that after calling, pairs showed a significantly higher probability of DBSA than DBA (Wilcoxon matched pair test $N_p=6$, $p=0.027$) or SBDA (Wilcoxon matched pair test $N_p=6$, $p=0.027$). We concluded that calling leads to the synchronization of pair activities (Fig. 3-3).

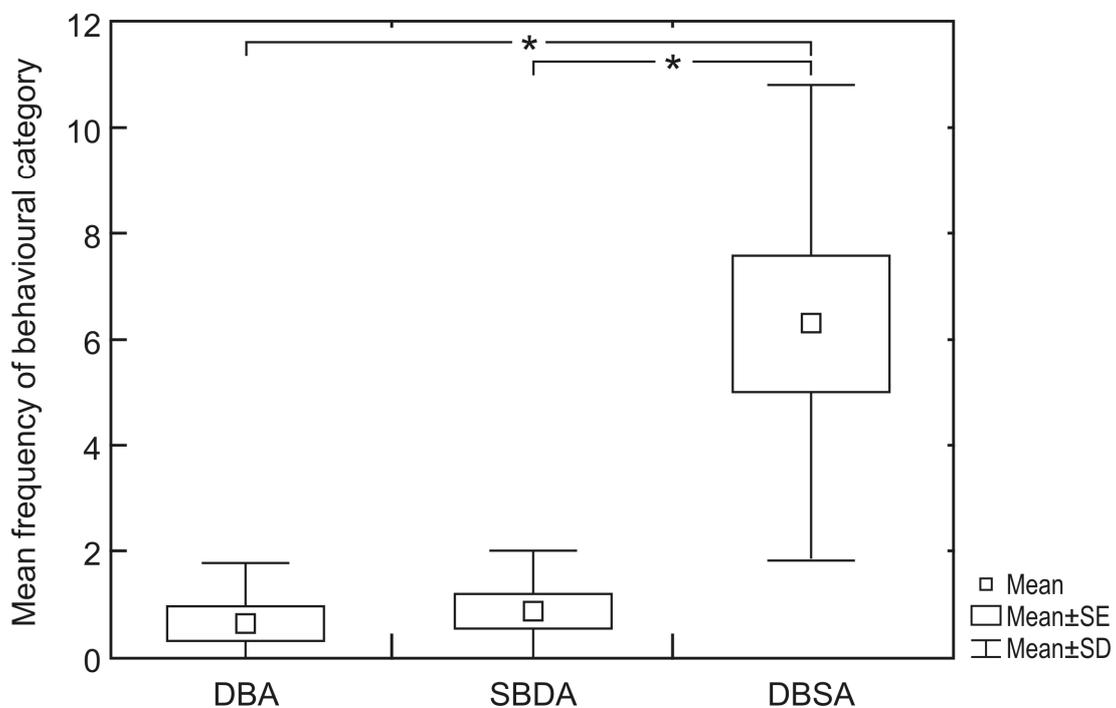


Fig. 3-3. Mean frequency of three behavioral categories associated with duet calling: DBSA, SBDA, and DBA. *P = 0.05.

Additionally, we examined what type of behavior was synchronized after calling. Figure 2-4 describes the type of behavior and its frequency. The frequency of synchronized activities differed significantly (Friedman ANOVA, $N_p=6$, $df=5$; $p=0.009$). Pairs synchronized locomotion more often than other activities. Our findings

showed that females moved first and males followed them in 63.63 % of all duetting events (N=33). However, there was no significant difference between the sexes in who leads the direction of locomotion or who follows (MWU- Test, $N_f=6$, $N_m=6$; $p=0.24$).

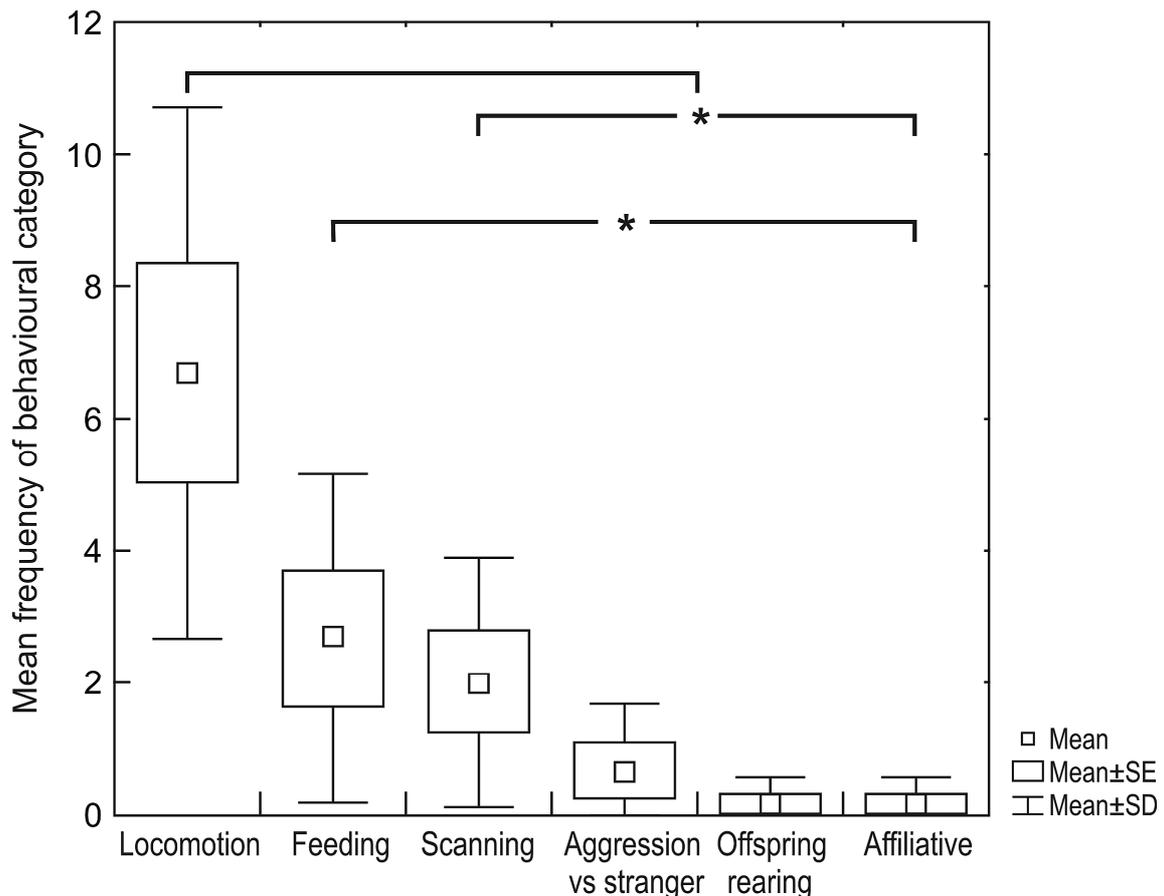


Fig. 3-4. Frequency of behaviors of category DBSA performed after duetting. *P = 0.05.

4. Duet calling location and occurrence of call types in duetting sequences

Duets occurred primarily in two contexts: when the sportive lemurs were travelling from one tree to another and when they were feeding/sitting at feeding/sleeping sites. From 68 duets for which we have recorded the location (during 103 contact hours), 72% were related to feeding sites.

The frequency of duetting was significantly higher at feeding sites than at sleeping sites (Wilcoxon matched pair test, $N=11$, $p=0.019$). The mean number of vocalization events at the feeding sites was 4.45 ± 3.2 with a maximum of 11 and a minimum of 1. At the sleeping sites it was 1.72 ± 1.84 with a maximum of 5 and a minimum of 0.

Lemurs gave different call types to start or terminate a duet sequence. In 67 duetting sequences analyzed, pairs used eight call types. Females of all pairs initiated the duet sequence using the high-pitched call, whereas four out of six males used the related shrill. Females of all pairs terminated a duet sequence using the bark, whereas four out of six males used the isolated shrill. The distribution of call types is displayed in Figure 3-5. A comparison among the frequency of call types between the start (Fig. 3-5a) and the end (Fig. 3-5b) of a duet sequence showed that the male related shrill call was significantly more used at the start than at the end of a sequence, in contrast the male isolated shrill call was significantly more used at the end (Wilcoxon matched pair test $N=11$, $p=0.034$ and $p=0.029$ respectively).

5. Sex dominance during starting and terminating of duetting sequences

To explore potential sex differences in starting or terminating duet sequences, we analyzed 51 call sequences available for the first call and 61 available for the last call. No significant differences between the sexes were revealed in the frequency that a sex took a position in the sequence (start: MWU- Test, $N_f=6$, $N_m=5$; $p=0.94$), (end: MWU- Test, $N_f=6$, $N_m=5$; $p=0.56$). Females called first in 47.06 % of the calling events and last in 55.74 % of the events.

Duet sequences consist of different call types (Fig. 3-6). Three call types were shared between the sexes (the high-pitched call, (HP), tchetchen, and the ouah call) and five were sex-specific (male-specific: the isolate shrill, and the related shrill and female-specific: the bark 1, bark 2 and the oaii). A male and a female may start duetting either alternatively or simultaneously. In the latter case, both used either the high-pitched or the tchetchen call. The sexes used different call types to start a duet sequence. In 69 duetting sequences analysed, the pairs used seven call types.

Females of all pairs used the HP, whereas four out of six males used the related shrill.

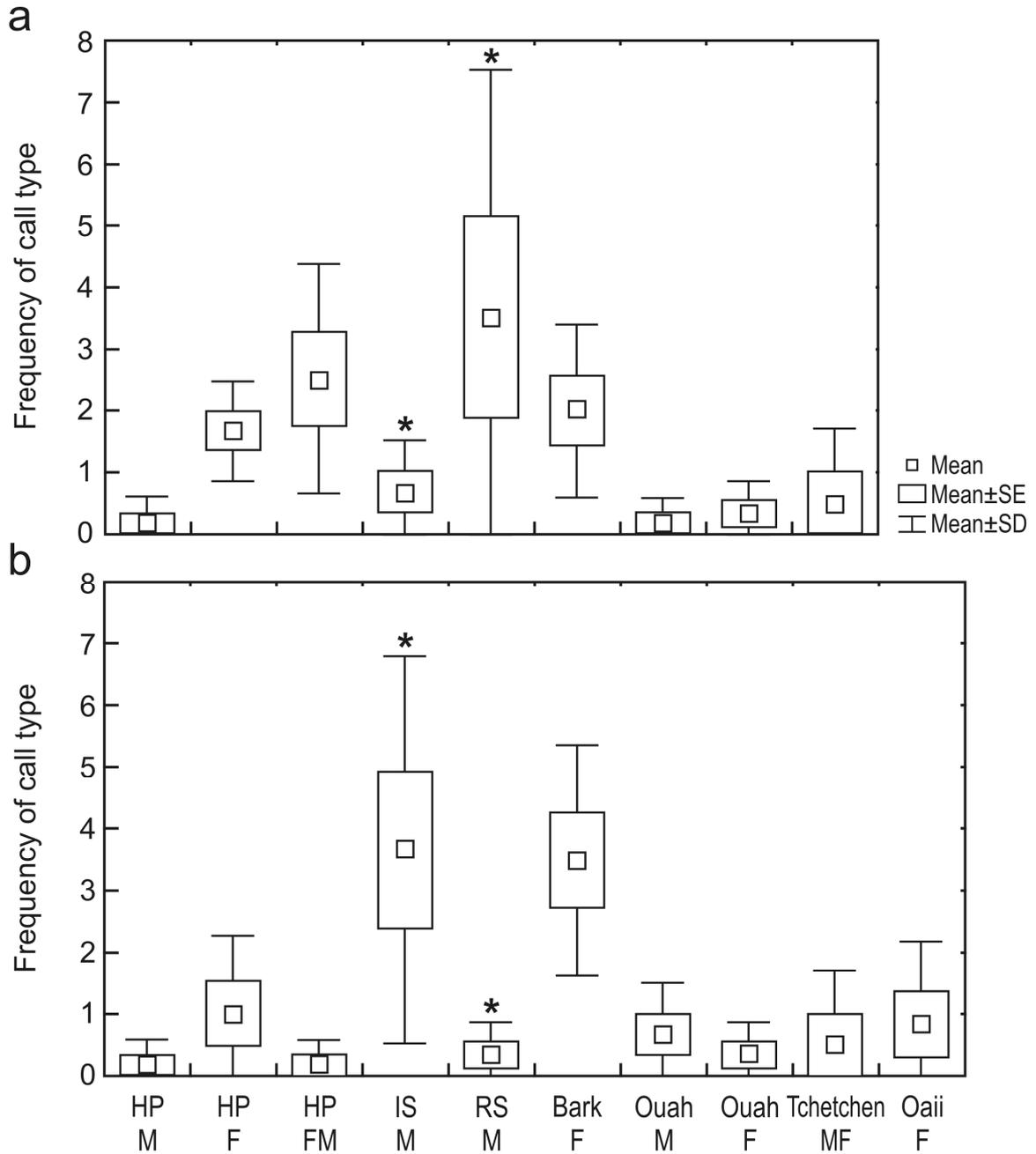


Fig. 3-5. Distribution of call types: (a) at the beginning of a duet and (b) at the end of a duet. *P= 0.05.

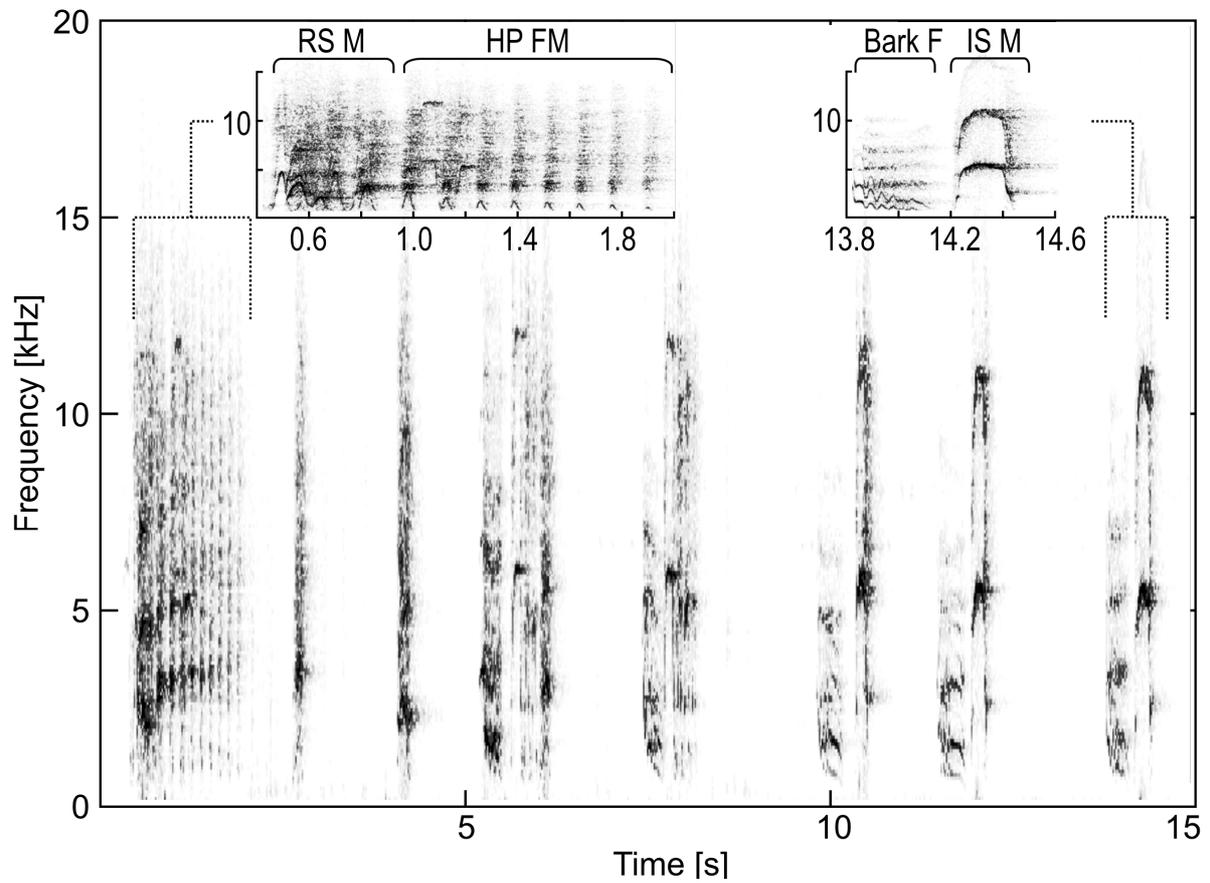


Fig. 3-6. Representative duetting sequence of a pair of sportive lemurs. First bracket represents the introductory sequence with the male call RS and HPMF calls, and second bracket represents the duet unit with a female call (bark) and male answer (IS= isolate shrill) that is repeated along the duetting sequence.

Discussion

Adult male-female pairs of Milne Edwards' sportive lemur, captured at the same sleeping sites, used shared HRs and sleeping sites almost exclusively throughout the whole year. These findings provide support for the hypothesis that the pairs are indeed pair-bonded and territorial. Duetting acted as a synchronized vocal display of pair-partners, enhanced during the period of food abundance which coincides with offspring care and female lactation. Duetting activity decreased during food scarcity which coincides with mating and pregnancy. Duet displays occurred more frequently at feeding sites than at sleeping sites during the offspring care period. Pairs used duetting to synchronize activities such as locomotion. Pair partners participated equally in duetting. There was no difference in who starts or terminates a duet sequence or who determines the direction of locomotion following duetting. Our findings provide the first evidence for the hypothesis that duetting functions as a mechanism to coordinate pair activities for inter-group spacing to signal the joint ownership of a territory. Additionally, it may also function as a mechanism to prevent infanticide and/or to limit nutritional stress of a lactating female.

Pair bonding, exclusive space use and monogamy in mammals

The pair bond is considered a basal unit in evolutionary models of many primates' societies (Fuentes, 2002). In the primatological literature, bonds between adults can be described as predictable relationships assessed by rates of affiliative interaction, proximity scores and a measure of reciprocity between two individuals (Hinde, 1983; Krebs and Davies, 1997). Studies have searched for the evolutionary and functional origin of pair bonds, the ecological factors that have selected for them, and how bonding influences individual fitness (Rasmussen, 1981).

Our results provide support for the hypothesis that sportive lemurs, considered as dispersed and pair-living, (Rasohalarijaona et al., 2003) are pair bonded. Pair

partners showed not only a massive overlap of HRs and sleeping sites throughout the year, as found in a previous study of shorter duration (Rasohalarijaona et al., 2006), but partners also used these sites and ranges almost exclusively and spent time synchronizing their activities during the night. The high variation in the percentages of pairs sharing the same site may indicate a variation in the strength of the pair bond. Across the three observation periods, pair partners seemed to remain closer together during the offspring care and mating period than during the pregnancy period (personal observation). HR overlaps of pair-partners, as found in our study, suggest spatial monogamy (Müller and Thalaman, 2000). Spatial monogamy is also reported for other dispersed pair-living prosimian taxa (e.g. *Cheirogaleus medius*: Fietz, 1999; e.g. *Phaner furcifer*: Schülke et al., 2004; e.g. *Galago zanzibaricus*: Harcourt and Nash, 1986). However, spatial monogamy does not always coincide with genetic monogamy. Thus, extra pair copulations and extra pair paternity may occur (e.g. birds: Benedict, 2008, e.g. prosimians: Fietz et al., 2000). Our ongoing genetic study will explore to what extent the male-partner of a female is not only socially bonded, but also genetically related to the offspring of a sleeping group.

Duetting among pair partners and its evolution in primates

Many monogamous bird and primate species are known to perform joint vocal displays, described as duetting (Thorpe, 1972; Farabaugh, 1982; Haimoff, 1986; Malacarne et al., 1991; Hall, 2004). Duetting has been reported in more than 200 species of birds (Thorpe, 1972; Kunkel, 1974; Farabaugh, 1982). Furthermore, the majority of monogamous primates, such as indris, tarsiers, gibbons, siamangs, titi monkeys and Mentawi langurs perform duets (Marshall and Marshall, 1976; Tilson and Tenaza, 1976; Haimoff, 1986; Pollock, 1986; Robinson et al., 1987; Geissmann, 1999; Nietsch, 1999; Müller and Anzenberger, 2002). Several models have been put forth to explain the evolution of duetting (see Introduction).

The mate reunion model (von Helversen and von Helversen, 1983; Tobias et al., 1998; Stokes and Williams, 1968; Bradbury and Vehrencamp, 1998) proposes

that duetting activity in some insects, frogs and birds is restricted to the breeding season. Our results on duetting activity in the dispersed pair-living sportive lemurs, showed that duetting is not restricted to the short mating season, defined by the presence of oestrous females (Randrianambinina et al., 2007) and copulations (personal observation), but instead duetting occurs throughout the whole year. Thus, the mate reunion model can not fully predict the occurrence of duetting in sportive lemurs.

The pair reunion model (Nietsch, 2003) predicts that the occurrence of antiphonal calling in nocturnal dispersed living primates, is higher during reunions in the mornings than during dispersals in the evenings. Comparing duetting activity in sportive lemurs between dawn and dusk, we did not find any differences between these two time periods. Thus, the occurrence of duetting does not coincide with the predictions of the social pair reunion model.

The joint territory defense model predicts that duets are used during territorial conflicts with neighbors (Sonnenschein and Reyer, 1983). Duetting is linked to prominent sites of the environment (Wickler, 1976) and increases during food scarcity (Fedy and Stutchbury, 2005). Our results indicated that in sportive lemurs, duetting occurs in territorial conflicts (personal observation) and is significantly higher at feeding than at sleeping sites. Furthermore pair partners showed higher duetting activity when food resources were abundant than when they were scarce. Despite this obvious contradiction to the model, we argue that joint territory defense may explain the evolution of a synchronized vocal display between pair partners in sportive lemurs.

A previous study on the distribution of the folivorous sportive lemur, *Lepilemur ruficaudatus*, in relation to seasonal variation in food resources in the highly seasonal dry deciduous forest of Kirindy in western Madagascar (Ganzhorn, 2002) revealed that the lean dry season is not the most stressful time of the year for these lemurs in energetic or nutritive terms. For males, the lean time seems to be the mating season, whereas for females, it seems to be the offspring care period, coinciding with lactation. Males are heavier than females in the offspring care season, but not in the mating season. In *Lepilemur edwardsi* a similar tendency was found during the onset

of the offspring care season (Randriananbinina et al., 2007) suggesting that food constraints for females are more severe during the time of lactation. Under these environmental conditions, pair partners may optimize reproductive success by joint defense of a feeding territory that provides sufficient young leaves rich in proteins for a lactating female.

The defense of food resources is more efficient when two individuals cooperate than when they defend on their own (Noe, 2006). Cooperation in the defense of food resources by duetting is a widespread phenomenon in pairs of tropical birds (Stutchbury and Morton, 2001; Grafe and Bitz, 2004; Fedy and Stutchbury, 2005). Our findings indicated that this may also explain the evolution of duetting in the Milne Edwards' sportive lemur. The frequency of duetting at feeding sites rises significantly after the birth of infants (during the offspring care period) when the risk of infanticide is higher (Rasohalarijaona, et al., 2000). The highest duetting activity occurred in October and November (63%) and decreased toward December (22%) and January (14%); (these percentages were calculated based on the total duetting events during the offspring care period). Since the period of female lactation and the period of infanticide overlaps in time, duetting may act not only as a mechanism to signal the joint ownership of a territory, but it may also simultaneously function as a mechanism to prevent infanticide. Altogether, these findings render support for the hypothesis that cooperative defense of a feeding territory induced by nutritional stress of a lactating female as well as infanticide prevention may have driven the evolution of the pair bond and duetting in primates.

Sex dominance and duetting in primates

High reproductive costs drive female dominance and feeding priority (Jolly, 1984; Radespiel and Zimmermann, 2001) in many Malagasy lemurs (White et al., 2007). Lemurs are usually not sexually dimorphic with regard to body size and mass. Since females are usually dominant across different social systems in Malagasy lemurs, (White et al., 2007) we expected that females in sportive lemurs are also dominant and will lead the duets. Leading a duet is variable across duetting taxa.

Many duetting bird species are sexually monomorphic and it has been assumed that males initiate the duet and females reply (Thorpe, 1972). In contrast, Levin (1996) found that it was females leading the duets, a fact which has also been described in four bird species (Todt, 1970; Kroodsma et al., 1987; Coates, 1990). In primates, such as gibbons, females lead the duet (Mitani, 1987). However our results showed that in *L. edwardsi*, there was no sex difference in duetting behavior or in who determines the direction of locomotion after duetting. This unexpected finding suggests that in the Milne Edwards' sportive lemur, both pair partners participate equally in the defense of a territory. Play back studies described in the next Chapter are going to test some of these hypotheses.

CHAPTER 4**FUNCTION OF DUETTING IN A DISPERSED PAIR-LIVING PRIMATE
(*LEPILEMUR EDWARDSI*)**

Méndez-Cárdenas M.G., and Zimmermann E. In preparation for publication.

Abstract

Many territorial birds and pair-living primates display duets. Most of the studies on the function of duetting have focused on birds, thus less is known about primates. One controversial issue is the extent to which duetting functions as a cooperative display to defend shared resources by signaling coalition strength, or if duetting functions as a competitive display between sexes to avoid being usurped and to repel extra-pair mates. In order to investigate the extent to which duetting in a dispersed pair-living mammal, the Milne Edwards' sportive lemur (*Lepilemur edwardsi*), functions as a cooperative "joint territory defense" or as a mechanism for "mate defense", we simulated territorial intrusions by presenting three categories of playback stimuli to six radio-collared, bonded pairs: duets, solo loud calls of males and solo loud calls of females. Responses to playback experiments were videotaped. All responses of the 12 individuals belonging to six mated- pairs were scored using a frame-by-frame video analysis. The response strength of the pairs was measured by the duration of orientation responses. The mean duration of responses of the six pairs to duet playbacks was significantly longer than the mean duration of responses to solo loud call playbacks. More duets were provoked in response to duet playbacks than to solo loud call playbacks, suggesting that duets are more threatening than solo loud calls. These findings coincide with those in territorial cohesive pair-living primates and birds. Additionally, data suggests that female solo loud calls function to attract mates and that duetting does not signal a conflict between pair partners or a sexual conflict over mating, but instead, functions to coordinate territory defense of valuable shared resources. Thus, our study presents the first evidence in a mammal living in a dispersed social system that duetting acts as an important mechanism to maintain pair bonds and govern social cohesion.

Introduction

Cooperation in the defense of feeding resources by duetting is a widespread phenomenon in pairs of tropical birds (Stutchbury and Morton, 2001; Grafe and Bitz, 2004; Fedy and Stutchbury, 2005). In duetting species, simulated intrusion elicits coordinated songs in the form of duets, and partners are often reported to approach the speaker together (Hall, 2000; Grafe and Bitz, 2004; Rogers et al., 2004; Mennill, 2006; but see Rogers et al., 2007). Thereby, it is suggested that duetting in birds represents a more threatening territorial display than solo songs (Hall, 2000; Marshall et al., 2006). In territorial cohesive pair-living primates, duetting is suggested to act as a mechanism to exclude intruders from an occupied territory (Haimoff and Gittings, 1985), to maintain inter-group spacing (Mitani, 1985), and may act as a reinforcement of the pair bond (Geissmann, 1999). Playback experiments in several territorial birds and cohesive pair-living primates supported the hypothesis that duetting helps to coordinate territorial defense among pair partners (Seibt and Wickler, 1977; Mulder et al., 2003; Rogers et al., 2006, 2007; Hall, 2000; Mitani, 1987, 1990; Robinson, 1979).

The joint territory defense model

The joint territory defense model in birds (Farabaugh, 1982; Langmore, 1998; Hall, 2000; Logue and Gammon, 2004; Hall and Peters, 2008) proposes that males and females should approach intruders of either sex together (Hall, 2000; Rogers et al., 2004). Furthermore, playback experiments presented to pairs of birds at the territory boundaries showed that more duets and more vigorous and aggressive responses to duets were given in response to duet playbacks than in response to solo song playbacks; duetting intruders represent a greater territorial threat than solo calling intruders, suggesting that the function of duetting is joint territory defense (Hall, 2000; Grafe et al., 2004). In cohesive pair-living primates such as gibbons, joint territory defense involves aggression (e.g., approaches, chases, vocalizations) between two or more mate-pairs during encounters along shared range boundaries

(Brockelman and Srikosamatara, 1984). While this aggression is primarily intrasexual, individuals involved in agonistic behaviors were intersexually supported and aided by their mates (Mitani, 1984). In *Hylobates mulleri*, singing females were joined vocally by their mates after a playback of a female song and after a playback of a duet. Most females lead duets and approaches in response to duets and to playbacks of female songs, whereas males lead approaches to playbacks of male songs, but no duet was elicited by a male song playback (Mitani, 1984). Playback experiments conducted in the field on *H. agilis*, and *H. muelleri* showed that individuals responded less frequently to playbacks of solo loud calls of males than to those of duet calls (Mitani, 1984, 1987).

The mate defense vs the joint territory defense model

The mate defense model in birds predicts that males guard females in the way of being more acoustically responsive to female songs by producing duets when a male intruder is present (Sonnenschein and Reyer, 1983; Seddon et al., 2002; Grafe and Bitz, 2004). Females sing to attract mates and males respond to deter other males (Stock and Williams, 1968; Levin, 1996). If males are not attracted to the female song, then acoustic mate guarding is not necessary. Paired males should answer the songs of their mate more frequently when a male intruder is present (Hall, 2000). In cohesive pair-living primates, aggression maintaining the pair bond takes place between pair members and solitary intruders within ranges occupied by these mated- pairs (Mitani, 1984). Agonism in this context is almost always intrasexual.

Playback experiments showed that responses to female solo calls were at least as intense (Mitani, 1984, 1987) or even more intense (Raemaekers and Raemaekers, 1985) than responses to duet songs. Duets are suggested to maintain and/or strengthen the pair bond (Geissmann, 1999). Thalmann (2006) suggested that if enhancing and advertising the pair-bond and territorial advertising are the only functions of complex coordinated vocalizations in pairs of sportive lemurs then their occurrence should be present during the mating season. In previous Chapter we found that these complex vocalizations increase, not during the mating but during the offspring care period (Méndez-Cárdenas and Zimmermann, 2009).

Mated male agile gibbons approached simulated solitary males with the intention of evicting them, whereas mated females did not approach or sing in response to solitary males because these intruders were not sexual competitors (Mitani, 1987). Mated females approached and sang when confronted by simulated solitary females (Mitani 1984; Raemaekers and Raemaekers, 1985). Females defend their territories from solitary female intruders, preventing the intruder from stealing her food supply and/or her mate (Mitani, 1984). This intra-sexual aggression is suggested to be a mechanism to regulate monogamy (Mitani, 1987). Responses of males towards females have been interpreted in two different ways; males may have approached simulated solitary females calls and responded vocally for mating purposes. Alternatively, males may have assisted their mates to enforce monogamy by approaching solitary females aggressively (Mitani, 1987) and joined its mate-female in a duet. This means that if intersexually-supported, female intrasexual aggression maintains monogamy in gibbons (Mitani, 1984).

Information on how nocturnal dispersed pair-living primates perceive solo loud calls or duets is lacking and further studies are needed to determine the function of duetting. In the Milne Edwards' sportive lemur, (*Lepilemur edwardsi*) a folivorous (Smith and Jungers, 1997), nocturnal primate that lives in dry deciduous forests in northwestern Madagascar, male-female pairs are the smallest social unit (Rasoloharijaona et al., 2000, 2003, 2006; Thalmann and Ganzhorn, 2003; Thalmann, 2006). Pair partners share a home-range with suitable sleeping and feeding sites and they use those sleeping sites and home-ranges exclusively for several months of the year. Pair partners have a dispersed pair-living system (Rasoloharijaona et al., 2003, 2006). Sportive lemurs are highly vocal and they have sex-specific loud calls (Rasoloharijaona et al., 2006). The study of the Chapter 3 on Milne Edwards' sportive lemur provided support for the hypothesis that duets are present in this species and may function as a mechanism to coordinate activities between pair partners dispersed in space.

A study of *Lepilemur ruficaudatus*, (Ganzhorn, 2002) a closely related folivorous species, revealed differences in body mass variation and food requirements of both sexes during the year. For males, the lean time seems to be the

mating season, whereas for females it seems to be the lactation period. A similar tendency was found in *Lepilemur edwardsi* (Randriananbinina et al., 2007). In Chapter 3 we proposed that pair partners may optimize reproductive success by joint defense of a feeding territory.

In the present Chapter we explored to what extent duetting in these primates coincides with two of the models put forth to explain the evolution of duetting in birds and in cohesive pair-living primates; the joint territory defense and the mate defense model. To allow us to investigate the joint defense behavior of a territory independently of confounding effects, we minimized the potential for sexual conflict over mating by conducting the experiments during the non-mating period (which coincides with lactation) and we compared it with experiments during the mating period.

The following hypotheses and predictions were investigated: 1) if coordination of male and female calls has evolved for cooperative territorial defense, then duets should be more threatening territorial signals than solo loud calls. We predicted that simulated pair intrusion by duet playbacks would increase the likelihood of duetting more than simulated individual intrusion by solo loud call playbacks during the non-mating period. Additionally duet playbacks would elicit stronger non-vocal responses in both sexes than solo loud call playbacks.

Alternatively, 2) if duets function to maintain the mate status and to deter mate competitors then we expected that individuals would be more likely to produce duets with their partners and would react stronger to solo loud call playbacks of the same sex during the mating period. Solitary intruders would be more of a threat to the partnership in this period than outside. Thus, if male participation in duets functions as acoustic mate defense, then males should answer the calls of their mates joining them in a duet more frequently when a male intruder is present.

The results of this study will give us more insights into the function of duetting in a dispersed pair-living primate, the *L. edwardsi*, and will establish a firmer basis for the discussion of how social interactions among individuals are regulated vocally and may be influenced by the reproductive state of the females.

Methods

Study site and Animals

Play back experiments were conducted on six radio-collared pairs of the Milne Edwards' sportive lemur (*Lepilemur edwardsi*) at the study site known as "Jardin Botanique A (JBA)" in the western Malagasy dry deciduous forest in the Réserve Forestière d'Ampijoroa (16°19'S, 46°49'E), located about 110km southeast of Mahajanga. The study took place from January 2004 to July 2005. For a detailed description of the radiotelemetric techniques used, see Méndez-Cárdenas and Zimmermann, (2009). Playback experiments cover mating (May-June) and non-mating periods (see Randrianambinina et al., 2007), particularly lactation phase (January-April).

Stimuli preparation

We simulated the presence of intruders by playing back conspecific communication calls of the Milne Edwards' sportive lemur recorded by Mendez-Cardenas and Zimmermann (2009). Three different categories of playback stimuli were selected: females solo loud calls (**FSLC**) referred as bark-barkoaii calls. Males solo loud calls (**MSLC**) referred as isolated shrill and related shrill calls. Duet call (**DC**) referred as related-shrill-high-pitched, bark-shrill. Seven sequences of female solo loud calls containing five to six barkoaiis, six sequences of male solo loud calls containing six isolate shrill calls, six sequences of the male solo loud call containing six related shrill calls and one sequence of duet call were selected as stimuli, duration of the sequences were standardized between 8 to 10 s in order to make them comparable (Fig. 4-1).

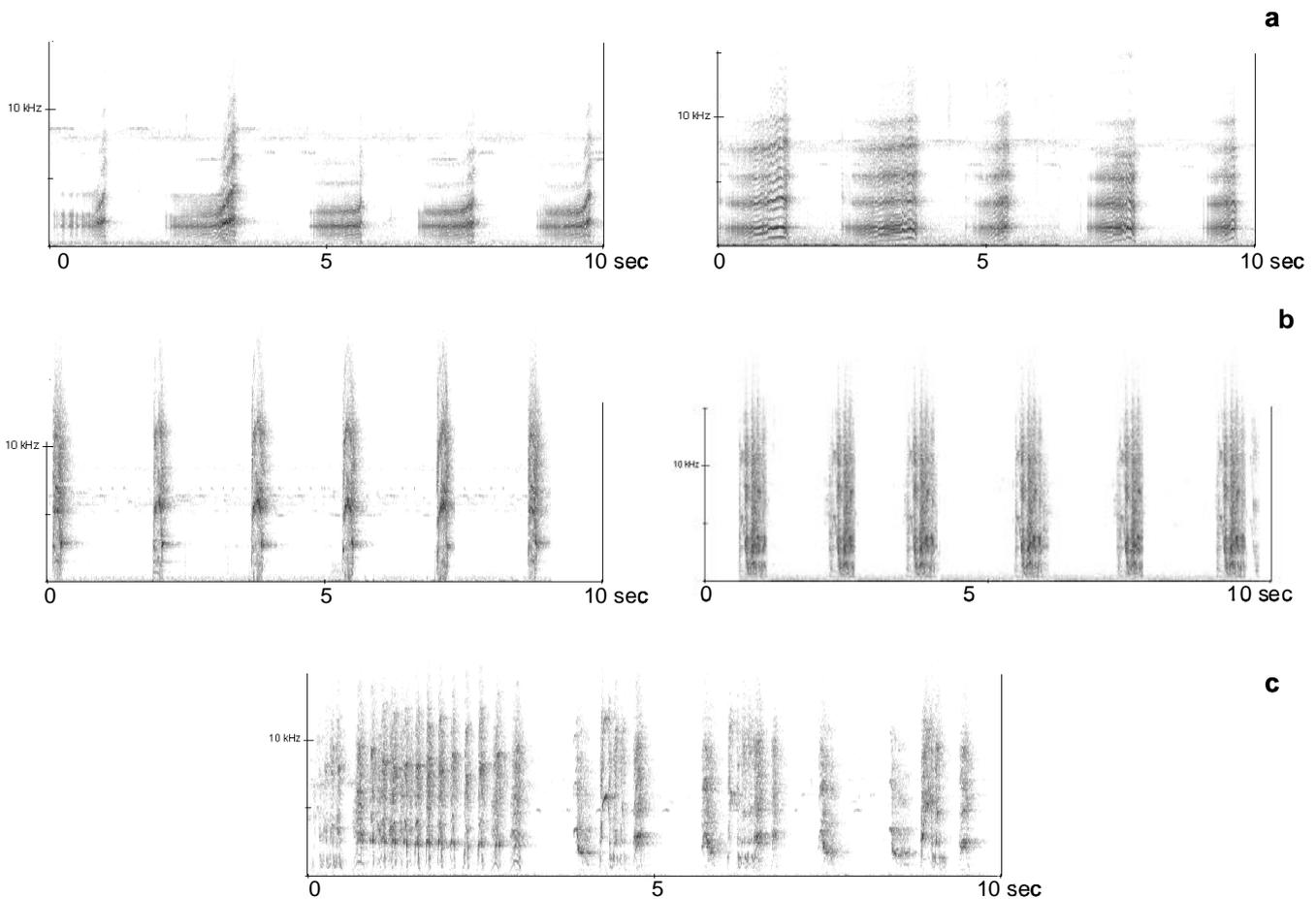


Fig. 4-1 Representative sonograms of the three categories of playback stimuli: a) Female solo loud calls (FSLC), b) Male solo loud calls (MSLC), and c) Duet calls (DC).

The selection was based on the quality of the recordings. Calls were stored as wave files. To ensure that the volume of the playback would be the same for all animals, we controlled the SPL of the playback stimuli with a sound level meter (RMS, Brüel and Kjaer Measuring Amplifier Type 2233). Stimuli ranged between 75.2 and 83.0 dB SPL measured at a distance of 1 m from the loud speaker (see Table 4-2). Recordings from 12 neighbor individuals were used for each of the three stimuli categories: 6 males for the two types of solo loud calls broadcasted during mating, 6 females for one type of loud calls broadcasted during mating and non-mating periods and one pair for the duet playbacks during non-mating period.

Play back experiments

All playback experiments were performed at a known sleeping site of a pair, either at the onset of the activity at dusk before the pair partners left their sleeping sites or at the end of the activity at dawn when pair partners returned to their sleeping sites. In each playback session the three different stimuli were played back in a random order on the same night.

Sessions started when the animal emerged from the sleeping hole or when it arrived at the mornings and sat on the sleeping tree. Each individual was tested a maximum of three times, with different playback stimuli in each session. In order to minimize potential habituation to the experimental conditions, the experiments were conducted with a minimum interval of five nights, i.e. individuals were not tested again until at least five days after the last playback stimuli. In some cases when individuals moved away or were not visible, they heard one stimulus a maximum of twice per day; we needed two days separated by a minimum interval to test the following playback stimuli. An experiment was complete for each individual when the different stimuli playbacks for each of the three categories had been performed

Playbacks were broadcasted with a Sony portable CD player D-EJ250 situated at 2-5 m from the sleeping site and connected to a loud speaker amplifier (Easy Port FP1pro/CD). The loudspeaker was placed 10-15 m from the sleeping hole and was hidden by vegetation or placed behind a bush or a tree, in an area that the animal couldn't see. It was fixed at an elevated site 1.5 m to 2 m above the ground (Fig.4-2).

Behavior before the onset of a playback and behavioral responses during and after the playback stimulus were videotaped at a distance of about 5m using a head lamp and a night shot camera (Sony digital CM 16K, 120x digital zoom). We started recording the behavior 1 min before the onset of each playback stimulus and for at least 1 minute after its offset. If reactions took longer or the animal stayed, we continued recording.



Fig. 4-2. Set-up of playback experiments, the animal is indicated with an arrow.

Vocalizations produced as a response to playback experiments were recorded with a Sony WMD6C Professional walkman and a Sennheiser ME65/K6 directional microphone with a windshield, and they were digitalized with a sample rate of 44 kHz and a sample size of 16 bit, using the software Bat Sound Pro Sound analysis 3.31 (Elektronik, 2001).

Video analysis of the playback responses

All the video-tapes recorded during the playback experiments were digitalized with the software 8 Pinnacle studio quick start (Pinnacle Systems Inc. Version 8.9.8; 2003). For the analysis of the behavioral responses we edited 54 playback stimuli (see Table 4-2). Using the Interact software version 8.0, (Mangold Int, GmbH, 2006) we scored the responses for each experiment with regard to the type, latency, and duration of the response for each individual.

The latency of a response (LR) was the time interval from the onset of the playback until a subject interrupted its ongoing behavior and reacted to the playback stimuli. It was measured during the broadcasted playback and one minute afterwards. The type of response was the behavioral reaction immediately after the interruption of the ongoing activity and it was coded as shown in Table 4-1. The duration of response was the time interval from the interrupted ongoing behavior until a subject stops reacting and returns to its previous behavior, measuring during a maximum span of one minute after the onset of the playback. Videotapes were analyzed frame-by-frame with a resolution of 25 frames per second. For each subject and for each stimulus, we calculated the number of orientation and no-orientation responses. For each type of response we gave a rank code from less to more intense reactions (Table 4-1).

Table 4-1. Behavioral responses scored.

<i>Parameters</i>	<i>Categories</i>	<i>Type of response</i>	<i>Rank</i>
Latency of response	No Interruption	No reaction	0
	Interruption	Reaction	1
Duration of response	No orientation	Head uncertain	0
		Ear movements	0
		Vocalise: duet, single	4
	Orientation	Looks to the loudspeaker	1
		Moves: up, down, in, out looking to LS	2
		Moves towards loudspeaker	3

Statistical methods

To obtain the mean duration of orientation responses we summarized the responses of each individual to the same playback stimuli during mating and non-mating periods. To analyze if males and females responded significantly differently towards the playback stimuli, we applied a Mann-Whitney U test for the two measured parameters, the latency of response (LR) and the duration of response (DR). The Friedman ANOVA has been used to analyze the effect of playback stimuli on the latency and the duration of response of individuals (Conover, 1999). We tested whether the Milne Edwards' sportive lemurs discriminate between solo loud calls of males (isolate-shrill and related-shrill), solo loud calls of females (bark-barkoaii) and pair duets (shrill-high-pitched-bark-shrill) during mating and non-mating periods. If differences were revealed, we applied a Wilcoxon matched pairs test in order to know the extent to which individuals discriminate calls by responding differently to these playback stimuli. All statistics were performed in Statistica 6.0 (StatSoft Inc.).

Results

Description of the type of response to solo loud call and duet call playbacks

Data on the type of response revealed that duets elicited stronger responses than solo loud calls did; the highest level of orientation responses was caused by duet playbacks, and the lowest level was caused by male shrill solo loud calls (Appendix 4-2). Duet playback stimuli elicited orientation responses in all individuals, while only 88.09% of the solo loud call (male and female) playback experiments elicited orientation responses. From the six pairs that showed orientation responses towards duet playbacks, three responded by duetting. All females initiated the vocal responses to duet playback stimuli and the males joined them in a duet and followed them by approaching the loudspeaker (LS).

During the non-mating period, the intensity of the type of responses caused by the female solo loud call playbacks ranked the second highest, while the highest level of intensity was caused by duet playbacks. In one pair the female went towards the LS and in another pair, the female who was seated half inside the sleeping hole, went out of the hole; in both cases, the respective males looked to the direction of the LS. Only one pair displayed a duet when a female solo loud call playback stimulus was broadcasted; the male approached the loudspeaker and began with a high-pitched call and the female joined him in a duet (Fig. 4-3).

During the mating period, the types of responses to the female solo loud calls (FSLC) stimuli were less intense than the responses during the non-mating period. However, in one pair, the female moved out of the hole and the male looked to the loudspeaker and in another pair the female went towards the LS and responded with a solo loud call that started during the playback and lasted for 65.17s, while the male followed her without vocalizing (Fig. 4-3).

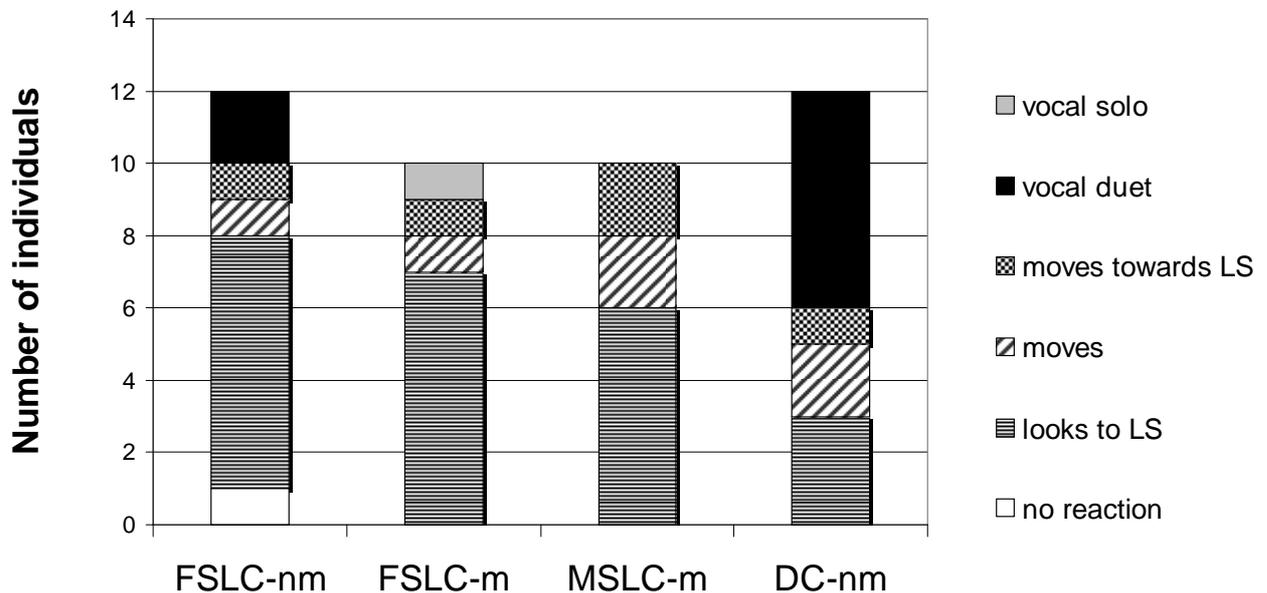


Fig. 4-3. Responses of individuals to the three playback stimuli: female solo loud calls (FSLC), male solo loud calls (MSLC) and duet calls (DC) during mating (m) and non-mating period (nm) linked to five scored behaviors. LS=loudspeaker

During duet playback stimuli, three pairs responded vocally to duets; duetting responses of these pairs showed a mean duration of 35.43 ± 13.76 s. After duetting, pairs moved toward the border of their home-range and continued duetting for an average of 76.55 ± 17.83 s; this second part of the duet included agonistic vocal interactions with neighboring pairs heard from around 10 m distance.

In two pairs with infants the females led the approach to the LS and initiated the duets, and the males followed the females and joined them in a duet after the females had begun to call. A third pair, a female without infants initiated the duet but stayed at the sleeping hole and the male approached the loudspeaker and shook branches while the female kept vocalizing biphonally. In the three remaining pairs the non-vocal responses were: male and female looked to the LS, male and female moved outside the hole and in the last pair (with an infant), the male moved towards the LS and the female stayed.

Comparisons between responses to solo loud calls and to duet playbacks

Latency of orientation responses

Playback stimuli played in mating and non-mating periods did not have significant effects on the latency of response (LR) of all individuals (F-ANOVA $N=10$, $df=3$; $p=0.92$). The mean latency of the responses of all individuals to solo loud calls of male and female playbacks ($N=12$, 1.86 ± 1.88) and the mean latency of the response to duet playbacks ($N=12$, 2.31 ± 3.75) did not differ (Wilcoxon matched pair Test, $N=12$, $p=0.43$). Within the responses to solo loud call playbacks, no sex differences were found in the mean duration of responses (MWU- Test, $N_f=6$, $N_m=6$; $p=0.81$); and in the mean duration of responses to duet playbacks (MWU- Test, $N_f=6$, $N_m=6$; $p=0.59$). Mann-Whitney U Tests in mating and non-mating periods (nm = non-mating and m = mating) showed that there were no sex differences in the latency of response for all types of solo loud calls and duets (MWU- Test for **FSLC-nm**, ($N_f=5$, $N_m=5$; $p=0.55$); **FSLC-m** ($N_f=5$, $N_m=5$; $p=0.69$); **MSLC-m** ($N_f=5$, $N_m=5$; $p=0.42$); **DC-nm** ($N_f=6$, $N_m=6$; $p=0.59$)). Uneven data is due to individuals absent or not found at the end of the study. Since non significant differences were found in the mean latency of response of individuals and sexes to all playback stimuli, we will focus just on the duration of orientation responses.

Duration of orientation responses to solo loud call and duet call playbacks

Playback stimuli had significant effects on the duration of orientation responses (DR) of all individuals (F-ANOVA $N=10$ $df=3$ $p=0.0087$). During the mating period, one pair was not longer located in its original area; therefore we have only 10 individuals in these comparisons. The mean duration of orientation responses of all individuals during mating and non-mating periods to all types of solo loud call (male and female) playbacks, was significantly shorter than the duration of responses to duet playbacks (Wilcoxon matched pairs test $N=12$, $p=0.05$ see Fig. 4-4).

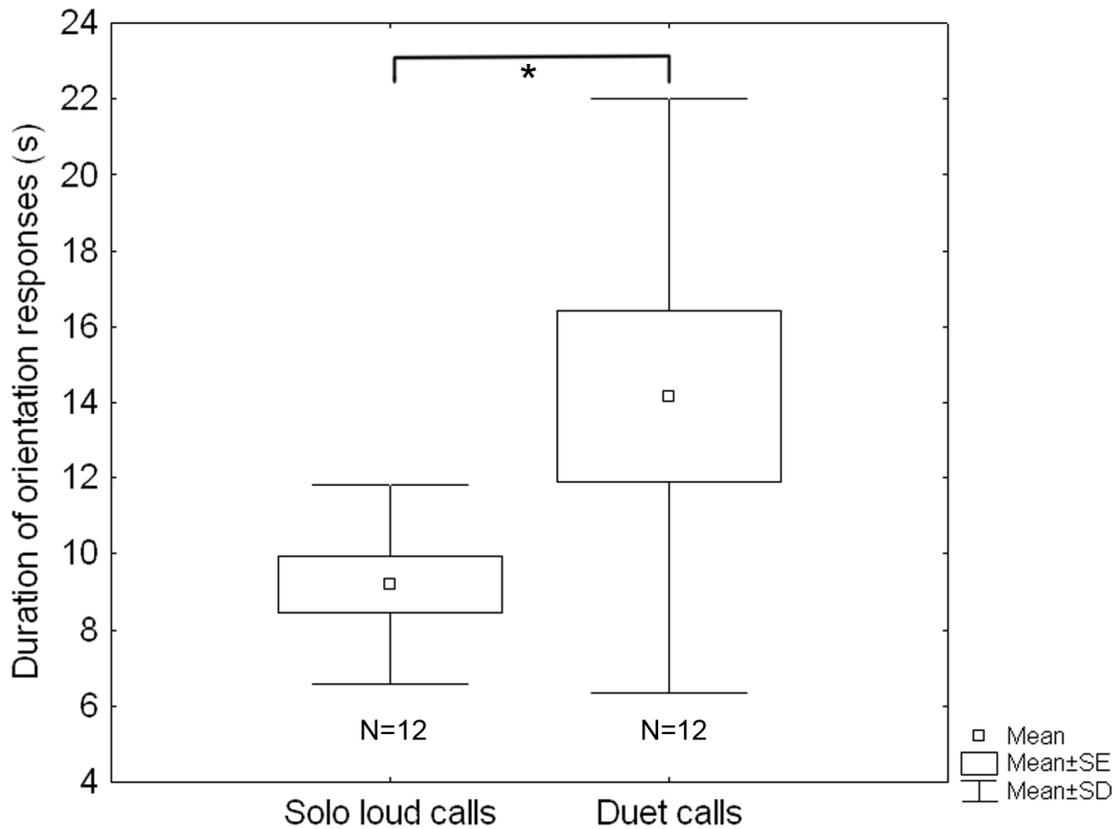


Fig. 4-4. Comparison between the mean duration of orientation responses of all individuals to solo loud call playbacks and to duet playbacks.

Duration of orientation responses to solo loud call and duet call playbacks between mating and non-mating periods

Differences were significant between the mean duration of orientation responses to female solo loud calls and to the duet playback stimuli during the non-mating period (Wilcoxon matched pairs test $N=12$, $p=0.0022$; see Fig. 4-5). In contrast, no differences were found between the duration of responses elicited by the female solo loud call during the mating period and those elicited by the duet playback during the non-mating period (Wilcoxon matched pairs Test, $N=10$, $p=0.64$). The duration of orientation responses to both types of male solo loud calls were not significantly different (Wilcoxon matched pairs test $N=10$, $p=0.95$). Thus, we calculated the mean duration of both calls and we used it in further analyses. The

mean duration of responses to male solo loud calls during the mating period and the mean duration of responses to duet playbacks during non-mating period were not significantly different (Wilcoxon matched pairs test $N=10$, $p=0.13$ see Fig. 4-5). Significant differences between the mating and non-mating period were found in the mean duration of orientation responses to female solo loud calls (Wilcoxon matched pairs test $N=10$, $p=0.02$).

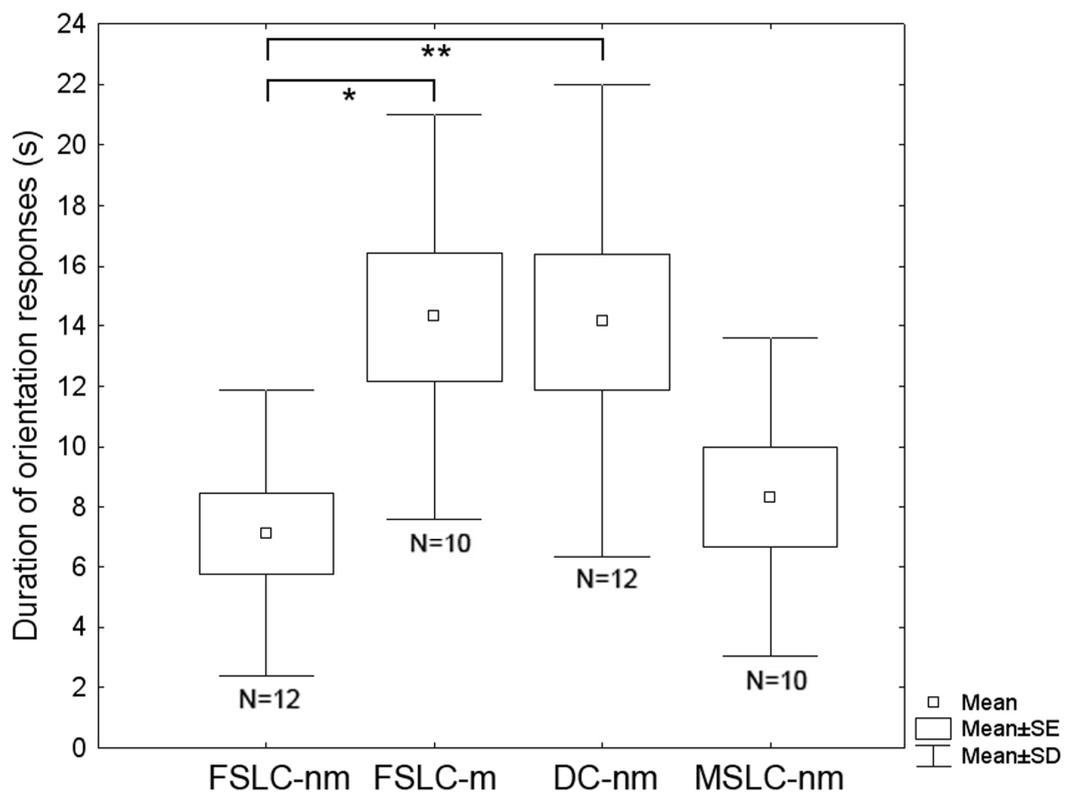


Fig. 4-5. Comparison between the mean duration of orientation response, to the three playback stimuli: female solo loud call (FSLC), male solo loud call (MSLC) and duet call (DC) during mating (m) and non-mating period (nm).

Sex differences between the mean duration of orientation responses to solo loud calls and to duet playback stimuli

Within the responses to solo loud call playbacks during the non-mating period, no sex differences were found in the mean duration of responses due to the female

solo loud calls (MWU- Test, $N_f=6$ $N_m=6$; $p=0.82$), nor in the mean duration of orientation responses to both types of male solo loud calls (IS, MWU- Test, $N_f=5$ $N_m=5$; $p=0.10$ and RS, MWU- Test $N_f=5$, $N_m=5$; $p=0.69$), nor in the mean duration of responses to duet playbacks (MWU- Test, $N_f=6$, $N_m=6$; $p=0.18$). However, sex differences were found in the mean duration of the response to female solo loud call during the mating period (MWU- Test, $N_f=5$, $N_m=5$; $p=0.05$). During this period, males showed larger non-vocal orientation responses ($N=5$, $18.75\pm 5.12s$) than females ($N=5$, $9.8\pm 5.07s$) (see Appendix 4-3).

Intersexual comparisons between the mean duration of orientation responses to solo loud calls and to duet playback stimuli

When we analyzed separately the responses of males and females during the non-mating period, differences between the mean duration of orientation responses to female solo loud call playbacks and the mean duration of orientation responses to duet playbacks remained significant in both sexes (Wilcoxon matched pairs Test in males ($N_m=6$, $p=0.028$) and in females ($N_f=6$, $p=0.028$) see Fig. 4-6).

In contrast the duration of orientation responses to female solo loud call playbacks during the mating and non-mating period showed that males and females responded differently. Males showed significantly shorter orientation responses to the female solo loud call during the non-mating period than during the mating period (Wilcoxon matched pairs Test in males ($N_m=5$, $p=0.04$), while females did not differ in orientation responses to female solo loud call playbacks in both periods ($N_f=5$, $p=0.22$) (see Fig. 4-6).

Furthermore, the mean duration of responses of males and females also revealed that during the mating period only the males showed significantly shorter orientation responses to the male solo loud calls than to the female solo loud calls (Wilcoxon matched pair Test, ($N_m=5$, $p=0.043$), while females did not differ in orientation responses to male or female solo loud calls ($N_f=5$, $p=0.68$) (see Fig. 4-6).

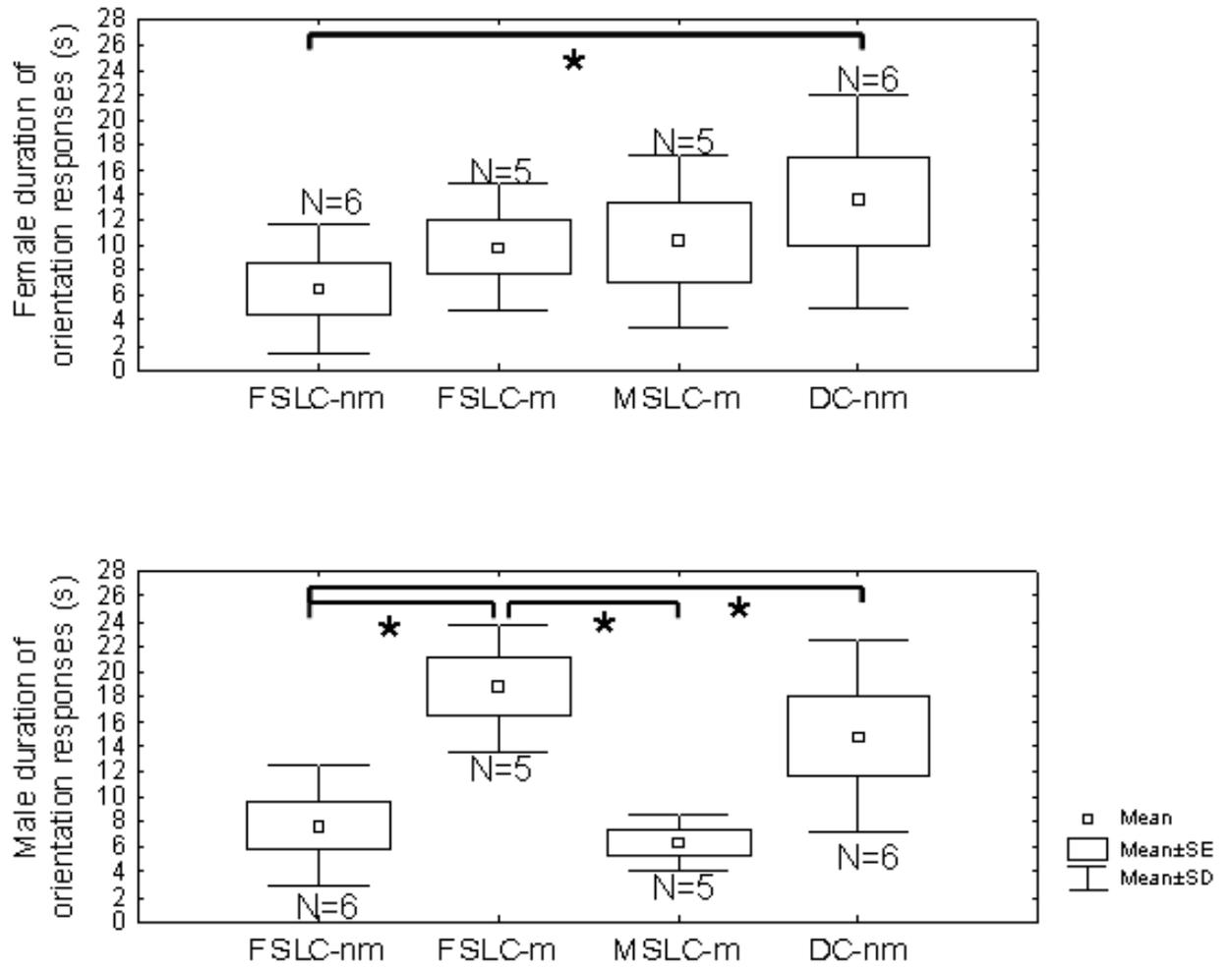


Fig. 4-6. Comparison of the mean duration of orientation response to the three playback stimuli: female solo loud call (FSLC), male solo loud call (MSLC) and duet call (DC) during mating (m) and non-mating period (nm). Females (top) and males (below)

Discussion

Mated- pairs of Milne Edwards' sportive lemur display more duets in response to duet calls than in response to solo loud calls. Pairs reacted significantly stronger to playbacks of duets than of solo loud calls. During the non-mating period males and females reacted stronger and jointly to duets than to female solo loud calls. Males were more attracted to female solo loud calls during the mating period than during the non-mating period, whereas females showed no differences in the mean duration of responses to female solo loud calls for the two periods. However, in the mating period solo loud female call playbacks elicited a stronger reaction in a paired female. Data also revealed that male intruder solo loud call playbacks elicited no vocal responses or strong reactions in males (agonistic) or in females (affiliative). We conclude that duets are a more threatening display than solo loud calls, at least during the non-mating period or the period of female nutritional stress, the lactation period, and that duets function as a cooperative mechanism for territory defense. Duets do not seem to be directly related to mate defense nor to signal conflict within the pair. Instead, female solo loud calls during the mating period may represent a competitive intrusion for females and might be attractive to males.

Duets as a more threatening display than solo loud calls for joint territory defense.

Duetting as a joint defense of feeding resources is a widespread phenomenon in tropical birds (Stutchbury and Morton, 2001; Grafe and Bitz, 2004; Fedy and Stutchbury, 2005). It helps to coordinate territorial defense among pair partners in some territorial birds and cohesive pair-living primates (Seibt and Wickler, 1977; Robinson, 1979; Mitani, 1987, 1990; Hall, 2000; Mulder et al., 2003; Rogers et al., 2006) and it is expected to be a more threatening territorial display than solo calls (Hall, 2000; Marshall et al., 2006). Our results showed that sportive lemur pairs

reacted significantly stronger to duet playbacks than to solo loud call playbacks. This difference is larger between female solo loud calls and duet playbacks during the non-mating period (period of female lactation). The lactation period is the most stressful energetically and nutritionally for females (Ganzhorn, 2002), thus pair partners may optimize reproductive success by joint defense of a feeding territory which provides sufficient young leaves rich in proteins for a lactating female. Our previous study showed that males and females display more duets during the lactation period than during the mating or pregnancy periods (see Méndez-Cárdenas and Zimmermann, 2009).

Playback experiments in birds showed that duets elicited more duetting responses and more vigorous responses than solo loud calls. This effect has been interpreted as a joint territory defense display (Grafe and Bitz, 2004). In cohesive pair-living primates, intrasexual conflicts for maintaining the pair bond are normally not intersexually supported, but a cooperative joint defense of territory should be intersexually supported (Brockelman et al., 1974; Chivers 1977). Furthermore, in our study, pairs displayed more duets in response to duet playbacks than in response to solo loud female or male calls. All females initiated the vocal responses to duet playback stimuli and the males joined them in a duet and followed them by approaching the LS, supporting the hypothesis of joint territory defense enhanced during the nutritional stress period of lactating females.

Duetting as a signal of conflict between sexes in mate defense

Duetting as a mate guarding defense in birds, showed stronger responses to solo songs during playback of the same sex than during duet playbacks (Grafe and Bitz, 2004). In the Milne Edwards' sportive lemurs, separate analyses on the duration of orientation responses of males and females found that individuals did not react stronger to the same sex than to the opposite sex, or that more duets were given in response to solo loud calls than in response to duets. Thus the mate defense model, as it is explained in birds, was not supported. Moreover, playback experiments in

territorial cohesive pair-living primates (e.g. gibbons Mitani, 1984, 1987) showed that male and female responses towards female solo loud calls and duets are equally strong, which has been interpreted as intra-sexual female range competition to maintain monogamy (Mitani, 1984, 1987).

Our results showed that the duration of orientation responses to female solo loud calls during the mating period and to duet call playbacks during the non-mating period were equally long in all pairs. However, the longer durations of orientation responses to female solo loud call playbacks during this period were only sustained by males, which we interpreted it as males being more attracted to females during the mating period than outside of it. Thus, even if males were attracted to solo loud calls of female intruders as proposed by Hall (2000) in the model of duetting as mate defense, in our study, males were not more vocally responsive to their female mates when we played back solo loud calls of male intruders and did not join their females in a duet, which could be also due to the absence of female vocal responses to solo loud calls of male intruders.

Male gibbons are suggested to be more interested than females in defending territory and mates, thus male intruders should elicit a stronger reaction in males than in females (Reichard and Sommer, 1997). In contrast to this, our data revealed that male intruders did not elicit stronger reactions in males than females. Furthermore, males elicited no vocal responses from males or females. Thus, the mate defense hypothesis was not supported by our data.

A female's response to intruders is expected to vary in relation to her reproductive state (van Schaik and Dunbar, 1990). This was partially supported by our results, in that females showed different behavioral reactions to female solo loud calls during non-mating and mating periods but no differences were found in the duration of orientation responses. However, evidence of a stronger, intrasexual conflict during the mating period was revealed by a female who approached the loudspeaker and began a solo loud call, in response to a playback of a solo loud call female intruder; later the male approached her, but did not join her in a duet, which indicates that intra-sexual conflict was not inter-sexually supported as suggested by Mitani (1984, 1987).

In contrast, during the non-mating period we found some evidence in which a male defended his lactating female by approaching a playback of a female intruder and responding with a high-pitched call used in confrontations (pers.obs). The female stayed with the infant and later she joined him in a duet. Thus, our data does not support the hypothesis that female range competition is inter-sexually supported to maintain monogamy as found in gibbons (Mitani, 1984, 1987) but instead female range competition was inter-sexually supported to defend territory resources jointly.

Duet and the infanticide hypothesis

Since biparental care and the evolution of monogamy are exceptional in mammals and seem to contradict sexual selection theory (Darwin, 1871; Trivers, 1972; Kleiman, 1977; Wittenberger and Tilson, 1980; Clutton-Brock, 1991), one explanation has been that monogamy evolved in species with sexually-selected infanticide, (birds e.g. Freed, 1986; gibbons e.g. Mitani and Rodman, 1979). In *L. edwardsi*, infanticide has been reported (Rasoloharaijaona, 2000) and our previous study found that during the lactation period, responses to intruders were collaborative and stronger than outside this period. In gibbons (Mitani, 1987; van Schaik and Dunbar, 1990) females carrying infants were less willing than non-lactating females to participate in territorial interactions and stopped calling, which was attributed to the attendant risks of male infanticide (van Schaik and Dunbar, 1990).

Our data showed that, out of four pairs with infants, three pairs approached the loudspeaker and the females led the duets in response to duets, which is opposite to Mitani's findings. This can be explained by differences in where and when experiments were carried; in our study playbacks were broadcast close to the sleeping hole, giving the chance to reduce the risk by leaving infants inside; in comparison to female gibbons which were carrying their infants. Males joined their females in defending the territory and kept the infant inside the hole. Thus if reducing the risk of infanticide is the primary motivation of females' responses to playbacks (van Schaik and Dunbar, 1990), then mated females might represent less risk than

mated- pairs of intruders; however male risk needs to be tested during the offspring-care period. Neighboring pairs of intruders with offspring might compete for resources since lactating females need more specific nutrients (Ganzhorn, 2002). Thus mated- pairs could represent a major risk for unprotected infants. Additional experiments on a larger sample size testing responses to solitary and mate male solo loud call playbacks are needed to evaluate the attendant risks of infanticide by males.

Appendix

Appendix 4-1 Orientation responses (OR), and non-orientation responses (NOR) of males (M) and females (F) tested toward three different playback stimuli during the mating (m) and the non-mating periods (nm). Rank is the intensity code give to each type of response, (TR). 12 individuals tested during non-mating period (Lactation period of females) and 10 individuals during the mating period. *db* is the sound pressure level at which each playback stimuli were broadcast. T1 and T2 are the two types of male solo loud calls (isolate-shrill and related-shrill).

Stimuli	OR	OR	OR	NOR	NOR	NOR	Tot exp	TR	TR	TR
	F	M	Tot	F	M	Tot		Rank F	Rank M	Rank Tot
Female solo loud calls (nm) 75.2 <i>dB</i>	6	5	11	0	1	1	12	12	8	20
Female solo loud calls (m) 80.8 <i>dB</i>	5	5	10	0	0	0	10	9	7	16
Male solo loud calls T1 (m) 83.0 <i>dB</i>	5	2	7	0	3	3	10	6	6	12
Male solo loud calls T2 (m) 78.98 <i>dB</i>	4	5	9	1	0	1	10	5	6	11
Duet calls (nm) 82.2 <i>dB</i>	6	6	12	0	0	0	12	16	18	34
Total number of playback stimuli	26	18	44	1	3	4	54	48	45	93

Appendix 4-2. Comparison of the duration of orientation responses between sexes, based on a Mann-Whitney U test (MWU- Test). N= number of individuals tested, nm=non-mating period, m=mating period, T1 and T2 are the two types of male solo loud calls (isolate-shrill and related-shrill) and SD= standard deviation.

Stimuli	Females		Males		Total		MWU- Test
	Means	SD	Means	SD	Means	SD	p value
Female solo loud calls (nm)	6,53	5,11	7,71	4,75	7,12	4,74	0,82
N	6		6		12		
Female solo loud calls (m)	9,86	5,07	16,92	8,08	13,39	7,37	0,05
N	5		5		10		
Male solo loud Calls T1 (m)	12,82	9,44	3,85	4,87	8,34	8,51	0,10
N	5		5		10		
Male solo loud calls T2 (m)	7,75	6,10	8,84	0,76	8,30	4,14	0,69
N	5		5		10		
Duet (nm)	13,55	8,60	14,77	7,76	14,16	7,83	0,18
N	6		6		12		

GENERAL DISCUSSION

A major aspect of understanding the evolution of vocal communication is to study the variation of the acoustic characters based upon the phylogenetic history of the group by evaluating which characters diversified in relation to the speciation events. This requires determining which characters are homologous and which characters are homoplastic and influenced by environmental or social factors. The first aim of this study was to examine whether vocal characters of loud calls vary geographically and coincide with the speciation events of *Lepilemur* species genetically determined. The second aim was to examine vocal individual variation, and specific call context interactions exploring the potential functions of solo loud calls, its relation to sociality and how these calls are organized within interactive male-female sequences as a cooperative/competitive display in the Milne Edwards sportive lemur. I will summarize the most relevant results of this study and discuss them with regard to the geographic and species-specific variation and to the specific contexts observed during vocal interactions and during the different playback stimuli in which vocal behavioral responses took place. All this is based on the causes, mechanisms, and selective forces that would favor the evolution of sociality and the behavioral rules of vocal communication underlying and governing social cohesion and explaining the origin of synchronized vocal displays.

Variation in loud calls and its implications for conservation.

In sportive lemurs the revealed species-specific acoustic differences are probably caused by genetic divergence between species. This genetic divergence can be due to the specific phylogeographic scenarios which gave place to allopatric speciation events. The phylogenetic topology based on acoustic characters matched very closely with the genetic topology proposed by Craul et al. (2007). This divergent pattern is in accordance with the large river model put forth by Olivieri et al. (2007) and Craul et al. (2007) who argued that large rivers acted as reproductive barriers for

gene flow in lemurs of northwestern and northern Madagascar, leading to cryptic speciation events. Therefore vocal characters of loud calls used as an indicator of common evolutionary history in diverse taxa such as birds and primates among others (Kroodsma, 1982; Zimmermann et al., 1995) were also informative for the phylogenetic reconstruction of sportive lemurs. These results supported the hypothesis that vocal production is under strong genetic control (Gautier and Gautier 1977; Snowdon and Hausberger, 1997) and vocal characters with a high consistency index are largely unmodified by the environment and therefore useful for reconstructing phylogenies (Hauser, 1996).

Although loud calls of sportive lemurs are species-specific and the likelihood of being inherited is high, call types did not contribute in the same way to the species-specific classification. The clear harmonic structure of the ouah call assigned loud calls to its corresponding species with a higher probability than the less harmonic spectrum of the high pitched call. These differences could be explained by the different selective pressures during sound transmission and its relationship with the forest habitat (Wiley, 1991; Naguib and Wiley, 2001). The effect of open and closed habitats (Morton, 1975) and further studies on the physical transmission of the sound comparing different habitats will be necessary to explain those differences. Only the ouah call showed a clear clinal acoustic variation in four out of six acoustic parameters which can be explained by its harmonic structure and the ability of the auditory system to detect pure tone signals in noise, as has been suggested by Scharf (1970).

Acoustic values increased from northwestern to northern Madagascar. Similar clinal variations in loud calls were described from a variety of different species (e.g. in primates (Mitani et al., 1999); in passerines: (Isler et al., 2005), in anurans: (Pröhl et al., 2006)). However, body size can be a potential factor explaining this clinal variation in loud calls in mammals, as was reported by Kinsler and Frey (1982) and Fitch and Hauser (2002). They hypothesized that larger species produce lower frequencies. Our results render additional support for this hypothesis. Sportive lemurs of northwestern Madagascar showed the highest-body mass (Craul et al.,

2007) and the lowest peak frequency and the northern ones showed the lowest-body mass (Craul et al., 2007) and the highest peak frequency.

An interesting result was that individuals in IRS V (Mahilaka) and IRS VI (Lokobe and Manehoko), which correspond to *L. dorsalis* and are considered as sub-species of *L. dorsalis* (Craul et al., 2006), were also clustered separately in our study. This finding suggests that acoustic characters also have the potential to differentiate at the level of the subspecies. Vocal characters should be considered as evolutionarily informative depending on their level of contribution to explaining the variation at different taxonomic categories and separating the conservative properties of the call from those that vary with the environment acoustics. Since species concepts are still controversial, further studies with a larger sample size and combined data from genetic, morphologic and acoustic characters, will be necessary to decide whether a local sample is a sub-species or a species. Information is also needed to determine phylogenetic diversity and taxa positions (Lehman, 2006), to estimate the reproductive rates (Randrianambinina et al, 2006), and to investigate species with limited distribution range (Craul et al., 2007). Based on all this information, conservation priorities should be re-evaluated. Loud calls should be considered to be a potential non-invasive tool to identify cryptic nocturnal sportive lemurs in the field and should be included in new conservation strategies.

Duetting and its evolution in pair bonded primates

As I described in Chapter 2, species-specific vocal variation is determined by the same allopatric process that gave place to genetic speciation. However, individual-specificity in vocal variation is normally conveyed in calls by temporal features and acoustic parameters related to spectral energy (e.g. humans: Bachorowski and Owren 1999; bats: Bastian and Schmidt 2008; tree shrews: Schehka and Zimmermann, 2009; deer: Reby et al., 1998; wolves: Tooze et al., 1990; elephants: McComb et al., 2003; rhesus macaques: Rendall et al., 1998). In duetting species, particularly in *L. edwardsi*, calls conveying individuality (Rasoloharijaona et al., 2006) are integrated in a male-female vocal display which is

most probably determined by social factors. Social factors and ecological factors contribute notably to the variation in social systems among lemurs (Kappeler, 1997). At the same time vocal communication plays an important role in regulating social interactions within these systems. The pair-living system has been a central aspect in studies on the evolution of social organization; such a system has evolved several times independently (van Schaik and Kappeler 2003). Duetting also shows the same independent evolution in phylogenetically distinct groups, suggesting that selection for duetting is strong (von Helversen, 1980) and seems to be associated with monogamy (Thorpe 1972; Farabaugh 1982; Haimoff 1986; Malacarne et al., 1991, Hall, 2004). Our results on the sociality of *L. edwardsi* support these hypotheses. In our study, sportive lemurs, considered as dispersed and pair-living, (Rasohalarijaona et al., 2003) were found to be pair bonded with pair-partners having overlapping home-ranges, suggesting spatial monogamy (Müller and Thalaman, 2000; Schülke et al., 2004). Duetting, as reported in territorial birds and cohesive pair-living primates, is also present in the dispersed pair-living sportive lemurs.

Studies in nocturnal primates showed that acoustic signals control inter-group spacing (Braune et al., 2005) and cohesiveness (Rasohalarijaona et al., 2006). Our results also render support to these findings. The dispersed pair-living sportive lemurs synchronized significantly more their activities after the males and females performed a coordinated vocal display. Thus, interactive loud calls in the *L. edwardsi* described in the third chapter acted as a mechanism between partners in a close proximity to coordinate social activities such as locomotion, feeding and scanning, and as a mean for maintaining and strengthening the pair bond, as previously reported in cohesive pair-living gibbons (Geissmann, 1999).

These findings led us to conclude that the dispersed pair-living system of the Milne Edwards sportive lemur seems to have more cohesive bonds than previously thought and may illustrate the previous step towards a more cohesive pair-living system. Freeberg, (2006) suggested that the level of sociality may determine the complexity of the type of vocal exchanges. Thus, more studies and comparative methods are needed to test the extent to which dispersed pair-living primates would

show a less complex vocal repertoire than cohesive pair-living primates or cohesive group-living primates.

Synchronization of social activities in the sportive lemurs after a duet display described in this thesis should be beneficial for both sexes because they shared territorial resources and both may be able to protect their offspring through a joint territorial display. The presence of a duet, together with the long-term pair bonding observed in the Milne Edwards' sportive lemur, can be explained by the honesty and deception model proposed in animal communication (Gouzoules and Gouzoules, 2002). In this model inexpensive signaling can evolve when both sender and receiver benefit from coordinated interactions in which individuals interact repeatedly and can use past interactions to assess the honesty of signals (Di Paolo, 1997, Gouzoules and Gouzoules, 2002); individuals do not assess quality as proposed by Zahavi (1975), but confidence in long-term honest signals probably based on commitment.

Learning of honesty signals arises through social interactions in which individually distinctive calls produced specific effects experienced by the receiver (Janik and Slater, 2000), which learns to produce adaptive responses. Learning would be specific to the history of interactions between the individuals involved, as showed in highly synchronized songs in birds and primates (Wickler, 1980; Geissmann, 1999). Thus vocalizations must carry acoustic features for individual identity. In sportive lemurs, parameters related with the emphasized frequency and temporal properties are found to be highly individual specific (Rasoloharijaona et al., 2006) in the isolated and related shrill (solo loud call of males) and the barkoaii call (solo loud calls of the females). We found that *L. edwardsi* pairs use these individually specific loud calls during duetting, producing pair specificity in the calls.

Models to explain the function of duetting

Joint territory defense model

From the three models of the evolution of duetting that were tested and described in Chapter 3, only the joint territory defense was supported by our data. Duetting as a joint defense of food resources helps to coordinate territorial defense

among pair partners in some territorial birds and cohesive pair-living primates (Seibt and Wickler, 1977; Robinson, 1979; Mitani, 1987, 1990; Hall, 2000; Mulder et al., 2003; Rogers et al., 2006) and it is expected to be a more threatening territorial display than solo calls (Hall, 2000; Marshall et al., 2006). The joint territory defense model predicts that duets are used during territorial conflicts with neighbors (Sonnenschein and Reyer, 1983). It is predicted to be linked to prominent sites of the environment (Wickler, 1976) and to increase during food scarcity (Fedy and Stutchbury, 2005).

Our results indicated that duetting occurs in territorial conflicts (personal observation) and it was significantly higher at feeding than at sleeping sites. Duetting activity was higher when food resources were abundant as opposed to when they were scarce. Despite this obvious contradiction to the model, we argue that joint territory defense may still explain the evolution of duetting in pair partners of sportive lemurs. In *L. edwardsi* (Randriananbinina et al., 2007) as in *L. ruficaudatus* (Ganzhorn, 2002) males are heavier than females in the offspring care season suggesting that food constraints for females are more severe during the time of lactation. Thus, pair partners may optimize reproductive success by jointly defending a feeding territory that provides sufficient young leaves rich in proteins for the lactating female.

Across the three observation periods, pair partners seemed to remain close together during the offspring care and mating period more than during the pregnancy period (personal observation) and the frequency of duetting was higher during the offspring care period (lactation) and at the feeding sites. These results lead us to the conclusion that duets were used as a cooperative display to defend shared resources during the period of female nutritional stress, but at the same time, duets may limit the risk of infanticide.

Mate defense model

Thalmann (2006) suggested that the occurrence of complex and coordinated vocalizations in mated- pairs should be present during the mating season if

enhancing and advertising the pair-bond are the only functions. Because playbacks are usually conducted on duetting species during the breeding season when intrusion elicits both territorial and mate guarding responses, this makes it challenging to distinguish between the two functions (Hall, 2004). Therefore, in the last chapter and in order to allow us to investigate territorial joint defense behavior independently of confounding effects of sexual conflicts over the mates, we conducted duet and solo loud call playback experiments during the non-mating period. To test if duets function as a mate guarding strategy, we performed solo loud call playback experiments during the mating period.

Our results revealed that, during the non-mating period, mated- pairs of Milne Edwards' sportive lemurs displayed more duets and reacted more strongly to duet calls than to solo loud calls. Males and females reacted more strongly and jointly to duets than to solo loud calls of females. In contrast, during the mating period males were more attracted to female solo loud calls than during the non-mating period, but they didn't answer vocally. Females showed no differences in the mean duration of responses to female solo loud call playbacks across the two periods.

Evidence of a stronger, intrasexual conflict during the mating period was revealed by a female who approached the loudspeaker and began a solo loud call, in response to a playback of a solo loud call female intruder; in this case intra-sexual conflict (Mitani, 1984, 1987) was not inter-sexually supported. In contrast, during the non-mating period a male defended his lactating female by approaching a playback of a female intruder and responding agonistically while his female joined him in a duet. Thus, our data does not support the hypothesis that female range competition is inter-sexually supported to maintain monogamy as found in gibbons (Mitani, 1984).

Data also revealed that solo loud calls of male intruders elicited neither vocal responses nor strong reactions in males (agonistic) or in females (affiliative). Instead, female solo loud calls during the mating period may represent a competitive intrusion for females and might be attractive to males. Female loud calls did not elicit duets as a mechanism of mate defense during the mating period, but they did during the non-mating period. These results, along with findings of Chapter 3 in which duetting is not restricted to the short mating season (defined by the presence of

oestrous females (Randrianambinina et al., 2007)) but occurs throughout the whole year, suggested that the mate defense model cannot fully predict the occurrence of duetting in sportive lemurs. Duets seem not to be directly related to mate guarding or to conflict signaling within the pair. Instead, duets appear to be related to joint territory defense. Further studies on pair-living mammals with different social organization systems are needed to examine whether duets' functions coincide and to explore the extent to which the cooperative rather than competitive origin of coordinated signals, as is supported in this study, can be also extended to other pair-living mammals.

Infanticide prevention model

Since bi-parental care and the evolution of monogamy are exceptional in mammals and seem to contradict sexual selection theory (Darwin, 1871; Trivers, 1972; Kleiman, 1977; Wittenberger and Tilson, 1980; Clutton-Brock, 1991), one explanation has been that monogamy evolved in species with sexually-selected infanticide, (birds e.g. Freed, 1986; gibbons e.g. Mitani and Rodman, 1979). In *L. edwardsi*, infanticide by a stranger male has been reported (Rasoloharijaona et al., 2000) and our previous study found that during the onset of lactation period, responses to intruders were collaborative and stronger than outside this period.

Data in Chapter 3 revealed that the frequency of duetting at feeding sites rises significantly after the birth of infants (Méndez and Zimmermann, 2009) when the risk of infanticide is higher (Rasohalarijaona, et al., 2000). In gibbons (Mitani, 1987; van Schaik and Dunbar, 1990) females carrying infants were less willing than non-lactating females to participate in territorial interactions and stopped calling, which was attributed to the attendant risks of male infanticide (van Schaik and Dunbar, 1990). Our data showed that, out of four pairs with infants, three pairs approached the loudspeaker and the females led the duets and the approaches to duets, which is opposite to Mitani's findings. We explained these contrasting results as methodological differences on how the playback experiments were conducted and by the differences on the nature of the sleeping sites. Males joined their females in defending the territories and both mates approached towards the loudspeaker but

kept the infant inside the hole as a more effective strategy to avoid aggression to the offspring. Thus, if reducing the risk of infanticide is the primary motivation of females' responses to playbacks (van Schaik and Dunbar, 1990), then mated females might represent less risk than mated- pairs of intruders. Neighboring mated- pairs of intruders with offspring might compete for resources since lactating females needed more specific nutrients (Ganzhorn, 2002). Thus, mated- pairs could represent a major risk for unprotected infants. Additional experiments on a larger sample size comparing responses of females with and without infants need to be tested. The attendant risk of infanticide by males needs to be tested during the offspring-care period by comparing responses to mated male and solitary male solo loud call playbacks.

Duetting as a more threatening display for territory defense might also limit the risk of infanticide; however it needs more extensive testing to be conclusive. Females benefit by defending the food resources with the aid of their mate (intersexually-supported) and both sexes profit by simultaneously protecting their offspring. Thus, nutritional constraints on lactating females may have driven the evolution of the pair bond governing social cohesion and duetting in primates.

SUMMARY

A central issue for understanding the evolution of primate vocal communication is to explore how vocal variation is related to function and to the evolution of the taxa. While there is much information about this aspect in anthropoid primates living in cohesive social systems, almost nothing is known about prosimian primates living in dispersed social systems. The goal of this thesis was to explore the inter-specific acoustic variation for informative characters for species diagnosis. In addition it was to examine the link between intra-specific acoustic variation in loud calls and its function in sportive lemurs as a model for primates living in a dispersed social system. Because of its high vocal activity and sex-specific vocalizations, sportive lemurs represent an interesting study model in which to investigate vocal function and its relationship to sociality. The thesis is based on three major chapters.

The aim of the first study described in Chapter 2 was to compare the structure of loud calls of populations of sportive lemurs to characterize informative acoustic traits for taxa discrimination and to establish a phylogenetic tree based on acoustic structures. We have based our study on Inter-River-Systems (IRSs) as operational taxonomic units. Samples were collected from nine different localities of four IRSs along a transect from northwestern to northern Madagascar. Two call types, the ouah and the high-pitched call, were present in almost all IRSs. Six temporal and eight spectral parameters were measured in 196 calls of the best quality given by 21 different males. Variation within and between IRSs was assessed by multivariate statistics. Loud calls differed significantly among the different IRSs. The IRSs varied most in spectral parameters, whereas temporal parameters were less variable. Phylogenetic analysis using parsimony yielded 11 out of 17 acoustic characters as phylogenetically informative. The acoustic tree had an average branch support of 78%. Its topology coincided less with geographic distances than with genetic tree topology. According to that, genetically confirmed species can be reliably identified by their specific loud calls. Thus, loud calls are important for conservation since they

provide non-invasive tools for species diagnosis and monitoring of cryptic species in nature.

The aim of the second study described in Chapter 3 was to explore duetting behavior in a solitary foraging, but pair-sleeping primate, the Milne Edwards' sportive lemur, in a dry deciduous forest of north western Madagascar. We radio-tracked six pairs throughout 1 year and recorded their sleeping sites and associations, home range use, and vocal and behavioral interactions. Three different periods were covered (mating, pregnancy, and offspring care). Sleeping partners form long-term pair bonds, indicated by an almost exclusive pair-specific usage of sleeping sites and home-ranges across periods. We explored three functional hypothesis of duetting: mate reunion, pair reunion, and joint-territorial defense. Pairs regularly engaged in duet calling. Duetting increased significantly during the offspring care period. Duetting occurred significantly more often at feeding sites than at sleeping sites. Pair partners synchronized behavioral activities after duetting. The activity most frequently synchronized was locomotion. Pair partners played an equal role in duetting with no difference between sexes in starting or terminating duetting. Based on these results we concluded that duetting evolved as a mechanism to coordinate activities between pair partners dispersed in space, to strengthen pair bonds, and, perhaps, to limit infanticide and nutritional stress in lactating females.

In the third study described in Chapter 4 we aimed to explore to which extent duetting in a dispersed pair-living mammal, the Milne Edwards' sportive lemur, functions as a cooperative joint territory defense display of pair partners or as a competitive mechanism for "mate defense". We simulated territorial intrusions by presenting three categories of playback stimuli to six radio-collared, bonded pairs: duets, solo loud calls of males and solo loud calls of females. Responses to playback experiments were videotaped. All responses of the 12 individuals belonging to the six mated- pairs were scored using a frame-by-frame video analysis. The response strength was measured by the duration of the pairs' orientation responses. The mean duration of responses of the six pairs to duet playbacks was significantly longer than the mean duration of responses to solo loud call playbacks. More duets were provoked in response to duets than to solo loud call playbacks, suggesting that duets

are more threatening than solo loud calls. These findings coincide with those in territorial cohesive pair living primates and birds. Data suggests that female solo loud calls but not the duets function to attract mates. Duets do not signal a conflict between pair partners or sexual competition over mating, but function to coordinate territory defense of valuable shared resources. Thus, our study presents the first evidence in a mammal with a dispersed social system that duetting act as an important mechanism to maintain pair bonds and govern social cohesion.

To summarize, the results presented in this thesis show that vocalizations of sportive lemurs encode information at different levels. Sex-specific loud calls were informative at the individual level and were used to signal the territorial ownership of the pair. This was conveyed within a coordinated sequence of vocal displays “duets”. The discussion illustrates factors underlying vocal variation and the origin and functions of coordinated displays, as a mechanism of cooperative communication in the defense of territory, rather than a mechanism for competition for mates. A cooperative vocal display for territory defense, in which, the male cooperates with his female to defend a feeding territory and to simultaneously protect their offspring; may be a beneficial mechanism for the reproductive success of both sexes. Altogether this thesis provides first information on how vocal variation is linked to function in sportive lemurs. Furthermore, it illuminates the importance of vocalizations for governing social cohesion and spacing in a primate living in a dispersed social system.

ZUSAMMENFASSUNG

Ein zentrales Thema, um die Evolution der akustischen Kommunikation von Primaten zu verstehen, ist es, zu untersuchen in wie weit vokale Variationen einer Art in Zusammenhang mit ihrer Funktion und Evolution stehen. Während viele Informationen über diesen Aspekt bei kohäsiv-lebenden anthropoiden Primaten bekannt sind, gibt es fast keine Informationen über Halbaffen, die in dispersen Sozialsystemen leben. Ein Ziel dieser Doktorarbeit ist daher die interspezifische akustische Variation hinsichtlich ihres Informationsgehaltes zur Artunterscheidung bei Wieselmakis (*Lepilemur sp.*), die als Modell für Primaten mit einem dispersen Sozialsystem gelten, zu untersuchen. Zusätzlich wurde der Zusammenhang zwischen der intraspezifischen akustischen Variation und ihrer Funktion am Beispiel von *loud calls* erkundet. Aufgrund ihrer hohen vokalen Aktivität und ihrer geschlechtsspezifischen Rufe, stellen die Wieselmakis ein interessantes Studienmodell dar, um den Zusammenhang zwischen vokaler Funktion und Sozialsystem zu untersuchen. Diese Doktorarbeit besteht aus drei Hauptkapiteln.

In Kapitel 2 haben wir die Struktur der *loud calls* von verschiedenen Wieselmakipopulationen verglichen, um taxaspezifische akustische Merkmale zu charakterisieren und einen auf diesen akustischen Merkmalen basierenden phylogenetischen Baum zu erstellen. Die Untersuchung basierte auf Zwischen-Fluss-Systemen (=IRSs; Inter-River-Systems), die als funktionelle taxonomische Einheiten dienten. Die Lautproben wurden, entlang eines Transekts vom Nordwesten zum Norden Madagaskars, an neun verschiedenen Orten aus vier IRSs gesammelt. Zwei Rufotypen, die *ouah* und *high-pitched* Rufe, waren in fast allen IRSs präsent. Es wurden sechs temporale und acht spektrale Parameter für 196 Rufe von 21 Männchen vermessen. Die Variationen innerhalb und zwischen den IRSs wurden mit Hilfe von multivariater Statistik untersucht. Die *loud calls* unterschieden sich signifikant zwischen den verschiedenen IRSs. Die Rufe der einzelnen IRSs unterschieden sich am meisten in ihren spektralen Parametern, wohingegen die temporalen Parameter weniger variabel waren. Die phylogenetische Analyse, die auf der Methode der sparsamsten Erklärung beruhte, zeigte, dass 11 von 17 akustischen

Parametern phylogenetisch informativ waren. Der akustische Stammbaum hatte eine durchschnittliche Zweigunterstützung von 78 %. Seine Topologie stimmte weniger mit der geographischen Entfernung als mit der genetischer Stammbaumtopologie überein. Demnach konnten auch Arten, die genetisch bestimmt wurden, verlässlich an ihren spezifischen *loud calls* unterschieden werden. Somit sind *loud calls* ein wichtiges nichtinvasives Werkzeug für den Umweltschutz, da sie Informationen zur Artunterscheidung und zur Überwachung von kryptischen Arten in der Natur liefern.

In Kapitel 3 meiner These untersuchten wir das Duett-Verhalten des Edwards-Wieselmakis (*Lepilemur edwardsi*), einer Primatenart, die solitär auf Nahrungssuche geht, aber als Paar schläft. In einem trockenen Laubwald im Nordwesten Madagaskars folgte ich ein Jahr lang 12 Wieselmakis von sechs Paaren, die einen Sender trugen, und nahm an ihren Schlafplätzen oder während ihrer Zusammenkünfte ihr Verhalten sowie ihre vokalen Äußerungen auf. Die Tiere wurden in drei verschiedenen Zeiträumen beobachtet (Paarungszeit, Schwangerschaft und Jungtieraufzucht). Aufgrund einer fast ausschließlichen paarspezifischen Nutzung der Schlafplätze und des Verbreitungsgebiets über alle Zeiträume hinweg, kann geschlussfolgert werden, dass Schlafpartner eine langfristige Paarbeziehung ausbilden. Paare tauschen regelmäßig Duettgesänge aus. Das Auftreten von Duettgesängen nahm in der Jungtieraufzuchszeit signifikant zu und trat signifikant häufiger bei Futter- als bei Schlafplätzen auf. Nach den Duettgesängen synchronisierten die Paarpartner ihre Verhaltensaktivitäten. Die am meisten synchronisierte Aktivität war die Fortbewegung. Beide Partner trugen gleichwertig zu den Duettgesängen bei. Es gab keine Unterschiede zwischen den Geschlechtern hinsichtlich des Beginnens oder Beendens eines Duettgesangs. Auf der Grundlage dieser Ergebnisse kann festgestellt werden, dass sich Duettgesänge als Mechanismus zur Koordination von Verhaltensaktivitäten zwischen zwei Partnern, die sich getrennt im Raum bewegen, entwickelt haben. Dies führt zur Verstärkung der Paarbeziehung und begrenzt vielleicht auch das Risiko eines Infantizids oder Ernährungsstress der säugenden Weibchen.

In Kapitel 4 meiner These untersuchten wir am Edwards Wieselmaki, inwieweit die Duettgesänge zur kooperativen Verteidigung des Territoriums oder zur

Verteidigung eines „Paarungspartners“ dienen. Ich simulierte territoriale Eindringlinge, in dem ich sechs Paaren Playbackstimuli aus drei verschiedenen Kategorien vorspielte: Duettgesänge, einzelne *loud calls* von einem Männchen sowie einzelne *loud calls* von einem Weibchen.

Die Antwortreaktionen während der Playbackexperimente wurden videographisch aufgezeichnet. Alle Reaktionen der zu den sechs Paaren gehörenden 12 Tiere wurden mit einer "frame-by-frame" Videoanalyse ausgewertet. Die Antwortstärke wurde mittels der Dauer der Orientierungsreaktion der Paare gemessen. Die mittlere Antwortstärke der sechs Paare auf Playbacks von Duettgesängen war signifikant länger als auf Playbacks von einzelnen *loud calls*. Playbacks von Duettgesängen provozierten mehr Antwortduettgesänge als Playbacks von einzelnen *loud calls*. Dies deutet darauf hin, dass Duettgesänge als bedrohlicher wahrgenommen werden als einzelne *loud calls*. Diese Ergebnisse stimmen mit Befunden bei territorialen, kohäsiv lebenden Paaren von Primaten und Vögeln überein. Weiterhin deuten sie an, dass einzelne weibliche *loud calls*, nicht aber gesamte Duettgesänge, zur Anziehung von Paarungspartnern dienen. Demnach signalisieren Duettgesänge keinen Konflikt zwischen Paarpartnern oder sexuelle Konkurrenz während der Paarung, sondern sie dienen zur koordinierten Verteidigung des Territoriums und der darin enthaltenen, wertvollen Ressourcen. Diese Studie präsentiert somit den ersten Hinweis darauf, dass Duettgesänge bei einem Säugetier mit dispersem Sozialsystem ein wichtiger Mechanismus zur Erhaltung der Paarbeziehung und zur Steuerung des sozialen Zusammenhalts bilden.

Zusammenfassend präsentieren die Ergebnisse dieser Doktorarbeit, dass die Vokalisationen von Wieselmakis Informationen auf verschiedenen Ebenen codieren. Artspezifische Signaturen wurden in den *loud calls* gefunden, die keine individual- und geschlechtsspezifischen Variabilität zeigten. Im Gegensatz dazu werden individualspezifische Informationen genutzt um den Territoriumsbesitz eines Paares anzuzeigen. Diese Information wurde mit Hilfe einer koordinierten Sequenz von vokalen Äußerungen übermittelt, dem Duettgesang. Die Diskussion zeigt Faktoren auf, die der vokalen Variation und dem Ursprung und der Funktion von koordinierten

Signalen bei Paarpartner zu Grunde liegen. Zusammenfassend enthält diese Doktorarbeit erste Informationen über den Zusammenhang zwischen vokaler Variation und deren Ruffunktion bei Wieselmakis. Darüber weist sie auf die Bedeutung von Rufen für den sozialen Zusammenhalt und die Distanzregulierung von Primaten hin

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EIDESSTATTLICHE ERKLÄRUNG

Hiermit erkläre ich, dass ich die Dissertation "Vocal communication and its relation to sociality in Sportive lemurs of Madagascar: an ecoethological and bioacoustical approach" selbstständig verfasst habe. Ich habe keine entgeltliche Hilfe von Vermittlungs- bzw. Beratungsdiensten (Promotionsberater oder andere Personen) in Anspruch genommen. Niemand hat von mir unmittelbar oder mittelbar entgeltliche Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.

Ich habe die Dissertation an folgender Institution angefertigt:
Institut für Zoologie, Stiftung Tierärztliche Hochschule Hannover

Die Dissertation wurde bisher nicht für eine Prüfung oder Promotion oder für einen ähnlichen Zweck zur Beurteilung eingereicht.

Ich versichere, dass ich die vorstehenden Angaben nach bestem Wissen vollständig und der Wahrheit entsprechend gemacht habe.

August-2009

Datum,

A handwritten signature in black ink, reading "Malte G. Mensch". The signature is written in a cursive style with a period at the end.

Unterschrift

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University Background

2009-2004 PhD in Primatology, at the Institute of Zoology, Hannover.
2001-1998 Masters degree in "Systematics" at the Ecology Institute; Xalapa Veracruz México.
1996-1992 Diploma degree in Biology, Faculty of Sciences at the "National Autonomous University of Mexico" (UNAM).

Thesis

2008 PhD topic: "Vocal communication and its relation to sociality in sportive lemurs of Madagascar: an ecological and bioacustical approach" supervised by Prof. Elke Zimmermann. Institute of Zoology Hanover.
2003 Masters topic: "Analysis of the vocal variation in sympatric and allopatric populations of howler monkeys genus *Alouatta*. A phylogenetic approach" supervised by Dr. Francisco. Ornelas, Institute of Ecology A.C. Xalapa Ver. México.
1998 Diplom topic: "Phylogeny of Mexican pines base on the internal transcribed spacers (ITS) of nrDNA and its application to biogeographical and adaptive hypotheses" supervised by Dr. Elena Alvarez Buyllá-R and Dr. Daniel Piñero Dalmau. Institute of Ecology, México city UNAM.

Awards

- 2008 Travel grant from the Frauenforderung at Veterinary University to attend the International Primatology Congress St Andrews.
- 2008 Travel grant from the PhD on Veterinary Science at Hanover to attend the Birds and Mammals Communication Conferences in Edinburgh.
- 2007 Travel grant from the PhD on Veterinary Science at Hanover to attend the Prosimians Congress in South Africa.
- 2006-2005 Complementary scholarship from México through the SRE (Secretaría de Relaciones Exteriores) for PhD research in Madagascar.
- 2005 Travel and research grant from the Society of Experimental Biology in England for Madagascar research.
- 2007-2003 PhD Scholarship from the German Academic Exchange Service DAAD. Veterinary and Animal Biology program at the Veterinary University.
- 1999 Research grant from the Institute of Ecology at Xalapa, for taxonomic research in Natural Museums of USA. Institute of Ecology. Veracruz, México.
- 2000-1998 Masters scholarship from the National Council of Science and Technology in Mexico, CONACYT. Institute of Ecology at Xalapa, Veracruz, México.
- 1997 Travel grant from the DGPA-UNAM to attend and participate in the Annual Meeting organized by the American Society of Naturalists, Society of Systematic Biologists and Society of Evolution in Boulder Colorado.
- 1997-1996 Diploma scholarship from the DGPA-UNAM for my research on molecular phylogenetics in Pines. Laboratory of Genetics and molecular evolution at the Ecology Center, UNAM.

1993-1992 Social Service Scholarship, from the DGPA-UNAM for assisting behavioral research at Isabel Island in México. Laboratory of Ecology Behavior at the Ecology Center UNAM.

Publications

- In prep Méndez-Cárdenas M.G. & Zimmermann E. Function of duetting in a dispersed pair-living primate (*Lepilemur edwardsi*).
- 2009 Méndez-Cárdenas M.G. & Zimmermann E. 2009. Duetting a mechanism to strengthen pair bonds in a dispersed pair-living mammal: (*Lepilemur edwardsi*)? *Am J of Phys Anthropology*. 139: 523-532.
- 2008 Méndez-Cárdenas M, Randrianambinina B, Rabesandratana A, Rasoloharijaona S and E Zimmermann. 2008. Geographic variation in loud calls of sportive lemurs (*Lepilemur ssp*) and their implications for conservation. *Am J Prim*. 70:1-11.
- 2000 Méndez-Cárdenas M.G. 2000. Vocal variation on allopatric and sympatric populations of Howlers monkey *Alouatta* and its use for phylogenetic analysis. *Laboratory Primate Newsletter* 39: (2)15-16.
- 1997 Quijada A, Méndez-Cárdenas G. & Ortiz S. 1997. "The region of the ITS of the ribosomal DNA of the nucleus nr DNA, source of molecular characters in Systematic of Gymnosperms ", *Bulletin of the Botanical Society of Mexico*.60:159-164.

Participation in research projects

- 2005-2004 "Geographical variation in socioecology, communication, morphology and genetics of nocturnal lemurs" at the National Park of Ankarafantsika". Supported by VW I/776 828 + VW I/76 828-1 + VW I/81906, DAAD, private organizations, DFG beantragt. Supervised by Prof. Elke Zimmermann and Dr. Ute

- Radespiel. Institute of Zoology, Hannover Germany.
- 2004-2003 “Phylogeny, phylogeography and conservation genetics of nocturnal lemurs”. Supported by DFG (Ra 502/7), VW (I/81906), DAAD, Conservation International, Bundesamt für Naturschutz. Supervised by Dr. Ute Radespiel and Prof. Elke Zimmermann. Institute of Zoology, Hannover Germany.
- 1998-1995 “Molecular phylogeny and specificity of fungi endophytes of the genus *Pinus* L.” supervised by Dr. Elena Alvarez-Buylla Rocés and Dr. Daniel Piñero Dalmau. Laboratory of Genetic and Molecular Evolution: Ecology Center at the UNAM.
- 1994 Study of the “Dominance effects, between young sisters of *Sula nebouxi*” (blue foot booby bird) and “Reproductive success on adults of the same species” supervised by Dr. Hugh Drummond. Ecology Center at the UNAM.
- 1992-1991 “Obtention of auxotrophic mutants in yeast”, supervised by Dra. Aurora Brunner Liesbhard. Institute of Cellular Physiology, UNAM.
- 1991-1990 “Young to Research Program” promoted by CONACYT, with the work entitled “Eclipsed effect on the NADPH production during the photosynthesis on de algae *Dictyota* sp” UNAM Mexico D.F.

Teaching

- 2003-2002 “Biochemistry for Physical Anthropologists” Teacher at the National School of Anthropology and History ENAH.
- 1998-1996 “Biology and New Techniques in Biology” Teacher at the National Preparatory School, UNAM.

Congresses and Seminars

- 2008 Vocal communication in birds and mammals Conference at St. Andrews University, Scotland with the poster presentation

- “Duetting in a nocturnal mammal (*Lepilemur edwardsi*) a mechanism for joint territorial defense and prevention of infanticide?.
- 2008 International Primatology Society XXII Congress, Edinburgh, Scotland, with the oral presentation “Duetting in a Nocturnal, Dispersed Pair-living Lemur (*Lepilemur edwardsi*)?”
- 2007 1st International Prosimians Congress, Ithala, South Africa, with the oral presentation “Biogeographical variation in loud calls of sportive lemurs of Madagascar”.
- 2005 Primate Diversity-Past, Present and Future Congress. Deutsches Primatenzentrum (DPZ) Göttingen, Germany with the oral poster presentation “Species-specificity in loud calls of sportive lemurs from northwestern and northern Madagascar”.
- 2005 Scientific Seminars at the Bachelor School of Xochimilco México; with the conference “Vocal communication between primates one way to avoid aggressive conflicts: a study in Madagascar’s Lemurs”.
- 2004 Veterinary and Life Sciences PhD Meeting Hanover, Germany. With the Poster “Screening microsatellites for the analysis of genetic relationships within a population of the Milne-Edwards’ Sportive Lemur in Madagascar”.
- 2004 97 Jahresversammlung der Deutschen Zoologischen Gesellschaft in Rostock Germany with the poster “Phylogenetic reconstruction of howler monkeys (*Alouatta*): A co-evolutive relationship between vocal and hyoid bone characters.
- 2001 First Mexican Congress of Primatology with the talk, “Sympatric effect on vocal variation in two species of howler monkeys of México and the use of morphological characters to phylogenetic reconstruction” Mérida, Yucatán .
- 2000 Annual Meeting of the American Society of Primatologists Boulder, Colorado; with the poster “Vocal variation in howler

- monkeys on sympatric and allopatric population".
- 1998 Meeting of genetic engineer and bio-devastation organized by the green parties of the United States and Europe, conference on "Mexican policy about genetic modify organism GMO`s" in St. Louis, Missouri.
- 1997 Annual Meeting organized by the American Society of Naturalists, Society of Systematic biologists and Society of Evolution, with the poster: "Morphological evolution and biogeography in pines using phylogenetic evidence from ITS sequences and restriction site variation". Boulder, Colorado.

Courses and workshops offered

- 2002 First meeting about "Alternative Tourism on Rural areas and Federal District" organized by the department of Rural Development Direction from Commission of Natural Resources (CORENA). México D.F.
- 2002 "Environmental Culture and Sustainable Tourism" seminar workshop organized by the Minister of Environment and Natural Resources through the Education and Training center for Sustainable Development. México, D.F.
- 2002-2001 "Social organization and planning for sustainable development in the wetland area on San Gregorio Atlapulco Advisor at UAM-X (Universidad Autonoma Metropolitana, Xochimilco).
- 1997 "Indigenous Communitarian Education" with the topic "Health and Ecology" operates by the special Delegation of Public Education, SEP (Secretaria de Educación Pública) in Chiapas. NGO, Grupo cinco.

1996 Theoretical and practical course of “Molecular Systematic”, supported by the Scientific Research Coordination at the UNAM. Laboratory of Genetic and Molecular Evolution. Institute of Ecology, UNAM.

Working experience

2008-2007 Student Research Assistant at the Institute of Zoology, Hannover, Germany.

2005-2004 Consultant for the Ankarafantsika National Park in Madagascar financed by the KFW, in order to create a scientific council for this Park and an Interpretation Centre.

2003-2002 Specialized Advisor to elaborate productive and ecotourism projects at rural areas in Mexico City, working as part of Advisors in Professional Services PESPROS Program at the Commission of Natural Resources (CORENA); and Secretary of Agriculture, Cattle raising, Natural resources and Fishing (SAGARPA).

2002-2001 Assessor to propose an Ecotourism project and design an ecological diagnosis of Wetland Systems, at San Gregorio Atlapulco, Xochimilco, Delegation.

1998-1997 Advisor in Ecology for the President of the green party in Mexico PVEM, during the journal interviews, political campaigns and attending public demands about environmental problems.