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Vocal Complexity in *Indri indri*

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Contents

Curiosity, that insolent, stubborn curiosity to know how the story ends.
Q - Luther Blissett

Abstract

The acoustic channel is fundamental in the communication system of a species, in particular for those species inhabiting low-visibility environments, as dense tropical forests, where the vocal communication seems to be preferred over other signaling strategies. Among primates, whilst the use of extremely diversified vocal signals is widespread among taxa, few species exhibit the so-called singing behavior. This peculiar communicative trait, that relies on the use of sequences of vocal emissions, commonly defined songs, is indeed rare and is found in only four families within the order. The songs are complex vocal displays that seem to be related to the evolution of territorial and monogamous social systems. Indeed, they are involved in territorial occupancy and defense, in mate attraction, and they can also have a cohesion function. Primate species are humans' closest relatives, therefore singing primates, which possess a language-relevant behavior, represent a captivating example for investigating the evolution of communicative complexity and flexibility. Hence, the relevance of investigating what evolutionary pressures, and to what extent, may have shaped such peculiar vocal behavior. The aim of this research was thus, to deepen the knowledge on primate species vocal behavior, by considering different measures of communicative complexity, to better understand which factors may have contributed to shaping vocal communication in basal primates. I tackled this fascinating topic by focusing on the vocal behavior of the only singing lemur: *Indri indri.* The species is a pair-living, monogamous, forest-dwelling primate living in small family units consisting of a reproductive pair and their offspring. Each social group occupies and actively defends an exclusive and stable territory, mainly using songs in signaling its occupancy. Indris' songs, complex sequences of vocal units organized in phrases of two to six notes, are choruses in which all the individuals within a group from two years of age may utter their contribution in a coordinated manner. This research includes data collected

between the years 2005 and 2018 on 21 wild groups inhabiting four different forest patches in North-Eastern Madagascar: seven groups in the Analamazaotra Reserve (Andasibe-Mantadia National Park, 18° 56′ S, 48° 25′ E), two groups in Mantadia (Andasibe-Mantadia National Park (18° 28′ S, 48° 28′ E), three groups in the Mitsinjo Station Forestière (18° 56′ S, 48◦ 24′ E), and nine groups in the Maromizaha Forest (18° 56′ S, 48° 27′ E). Primate songs have canonically been considered inherited, I therefore first examined the relationship between genetic distance and acoustic similarity, by analyzing whether the acoustic structure of phrases would be related to the kinship degree. The results indicated how the similarity of phrases, both at spectral and temporal level, correlates with kin mainly for males. A crucial feature of songs is their rhythmicity, I thus investigated whether the rhythmic pattern of different individuals' contributions could vary according to chorus size, to the total duration of the song or that of the individual contribution, considering both the rhythmic structure of the phrases given within the song and that of the whole song. I found a variation pattern significantly higher in females, whose contribution change in relation to the number of singing individuals, namely the chorus size. Hence, the findings suggested that females lead the rhythmic structure of songs. Indris choruses are powerful loud calls that usually travel over multiple neighboring territories. Pair-living species tend to occupy stable and exclusive areas, whose occupancy and defense are noticeably mediated through those vocal displays. I, therefore, investigated the inter-group dynamic of the species, and the song's role in negotiating the relationships among neighboring groups, finding a strike territory stability in both location and size, with singing locations evenly spread throughout the territory. The results confirmed both the pattern of territorial stability, typical of pair-living primate species, and the role of the songs in regulating intergroup dynamics. Moreover, being involved in the inter-group dynamics, it seems likely that songs are used for exchanging information about group size or the chorus size. I, thus, investigated whether an increase in the number of singers would lead to a lengthening in the song duration, finding that the song duration predicted both the actual numbers of singing individuals within a chorus and the group size. Lastly, the vocal repertoire size of a species is an indicator commonly implied in the evaluation of its vocal capacities. Indris’ songs are just a part of a larger set of vocalizations. I, therefore, quantified indris’ vocal repertoire, which resulted to include nine vocal types, besides the song units. Overall, the sex-dimorphic structure of indri’s songs, both in terms of its spectral and temporal features, together with the higher genetic similarity encoded in male phrases and the higher female flexibility, suggest that different evolutionary pressures acted on the two sexes. In the meantime, the presence of remarkable individual-identity cues is consistent with the necessity of enhancing individual recognition, related to the evolution of social life. Moreover, the territory stability over time, where the songs play a pivotal role in the spacing between neighboring groups, and where eventual shifts necessarily involve the neighbors’ spacing, underlines the existence of coordination between neighbors. In this picture, indris’ groups, rather than a single unit, should be considered part of a network where individual-related information could be more important than the group-specificity of signals. In conclusion, this investigation shed light on different aspects underlying indris’ communicative complexity, where different factors could have played a role in an evolutionary framework leading to such a particular vocal behavior.

General introduction

Vocal signals are fundamental mediators of communication systems; they are indeed involved, for instance, in species recognition, resources defense, mate attraction, in a broad range of taxa (Kroodsma and Byers 1991; Wich and Nunn 2002). Unlike other signals, they do not require proximity between senders and receivers and, being informative about signalers' identity, gender, and location, they decrease the risks connected with direct encounters. Proven to mediate discrimination within and between species, divergence in acoustic traits has therefore been proposed to play a role in speciation and evolution (Wilkins et al. 2013; Zimmermann 2017; Smentili-Cardoso and Donatelli 2019), although the processes underlying the divergence of acoustic signals and the consequences in terms of speciation have not been unraveled yet (Zimmerman 2017). Identifying the key determinants guiding the diversification of communication systems and vocal flexibility is, therefore, fundamental to infer about evolutionary diversification and versatility of animal signals (Schuster et al. 2012). The diversity of sensory systems, signals, and signaling behavior is astonishing: considering how these traits influence each other's evolution may be helpful to explain some of this variety (Endler 1992). Different frameworks have been assumed to affect acoustic signal evolution (Wilkins et al. 2013). One of the engines evoked to explain the coevolution of sensory systems, signals, and signaling behavior, is the *Sensory drive hypothesis*. Natural selection favors signals, receptors, and signaling strategies for maximizing the signals vehiculation and reception relative to the background noise while minimizing their degradation (Endler 1992). Environmental features are known to impact signal perception (Marler et al. 1967; Waser and Brown 1986): canopy, humidity, and temperature may affect the sound transmission, attenuation, degradation, or modulation (Wiley and Richards 1982). Habitat conditions, therefore, may induce vocal selection and, being temporally and spatially heterogeneous, may also affect the evolutionary direction of behavior, and by consequence, microhabitat preferences and time and place of signaling. The variation in these conditions, over space and time, will generate signal diversity (Seddon 2005). Recently, the relationship between vocal repertoire and brain architecture (Dunn and Smaers 2018) and neural control (Fitch 2010) has also been proposed. Still, although the role of environment in shaping acoustic characteristics of signals have long been hypothesized (Marler et al. 1967), not all the evidence converges towards the same direction, and data corroborating that the structure of calls is highly conserved among closely related species has been found (Fischer et al. 2017). Rather than as adaptation to the habitat only, the structure of calls seems indeed currently to be better explained by the phylogenetic inheritance (Fischer et al. 2017); in agreement with the *Phylogenetic Hypothesis* (Ord and Garcia-Porta 2012), the vocal behavior of a species is largely determined by its phylogeny, where the closer the relatedness between members of a taxonomic group, the higher the similarity in their signal complexity (Zimmerman 2017). A further driver of diversification might lie in the social system of a species. Following the *Social Complexity Hypothesis*, indeed, the evolution of vocal communication appears to be related to the evolution of social life (McComb & Semple 2005; Freeberg et al. 2012; Pollard and Blumstein 2012; Bouchet et al. 2013). According to this idea, whose origin can be led back to Darwin (1872), more complex societies are suggested to generate complex communication, where the number of distinct signals or units (namely the vocal repertoire of a species) is commonly used as a proxy for communicative complexity (Zimmerman 2017; Peckre et al. 2019). A further source of vocal diversification is the ability to combine single units into complex sequences (Kershenbaum et al. 2016) that may, or may not, have an organized structure over time (Ravignani and Norton 2017). Therefore, a single measure of complexity is unlikely to comprehend all the sources of variation (Kershenbaum et al. 2016). Thus, the substantial vocal diversification (Wilkins et al. 2013) existing among species is the result of an intricate tangle of selective pressures (Fischer et al. 2017) that have been found to underline vocal abilities.

The order Primates includes more than 300 extant species ranging from nocturnal to diurnal habits, living in dense or dry forests, where they may live in small groups up to thousands of individuals and may show arboreal to terrestrial adaptation (Ramsier and Quam 2017). This variety is reflected in the diversity of auditory sensitivity and perceptual abilities underlying the production and perception of a wide range of vocalizations. Indeed, although primate communication may rely on chemical, tactile, visual, and auditory signals (Snowdon 1982; Napier and Napier 1996), the acoustic channel seems to be crucial for the communication of diurnal, arboreal social species dwelling in low-visibility environments like that of dense tropical forests. In this kind of environment, the co-evolution of circadian activities, habitat structure, ecological and social needs, seems to have favored vocal communication over other signaling strategies (Altmann 1967; Waser and Brown 1986). The massive diversity of species within the order, showing an extensive variation in their vocal production and usage (Cheney and Seyfarth 2018), makes them an excellent model for investigating the evolution of vocal communication. Given their unique evolutionary history (Charles-Dominique and Martin 1972) and adaptive peculiarities (Fleagle 2013), lemurs represent the optimal phyletic lineage to investigate proximate and ultimate causes that may have contributed to shaping the vocal capacities of basal primates. Primate vocal output reaches one of its most complex and intricate displays with the generation of synchronized sounds leading to chorusing and singing behaviour (Ravignani et al. 2014). The latter is a rare capacity mainly associated with a territorial and socially monogamous lifestyle, only found in indris (*Indri indri*), tarsiers (*Tarsius* spp.), gibbons (*Hylobates* spp., *Nomascus* spp., *Hoolock* spp., *Symphalangus syndactylus*), and titi monkeys (*Plecturocebus* spp., *Cheracebus* spp., and *Callicebus* spp.) (the so-called *singing primates*, Haimoff 1986). The monophyletic *Indri indri* is the only lemur species able to emit coordinated vocal displays, or songs. Usually introduced by a series of harsh sounds (roars), these long sequences of vocal emissions include three main components: long notes, single notes, and descending phrases, series of two to six units with a descending frequency pattern (Thalmann et al. 1993; Sorrentino et al. 2013; Torti et al. 2013). These organized behavioral displays, where each signaler has a precise pattern, involve the chorusing of two or more interacting individuals (usually the reproductive pair, although all individuals from two years of age may join the chorus; Pollock 1986). Still, the terrific song is just a part (although essential in mediating several aspects of the species behaviour, as the territorial announcement and defense; Torti et al. 2013) of a broader set of vocalizations (Maretti et al. 2010). The temporal organization of acoustic signals represents one of the most complex and crucial traits of human and animal communication (Bowling et al. 2013; Ravignani et al. 2014). Investigating the individual timing during collective displays may be critical to understanding processes underlying the chorusing dynamics and the adaptive role of rhythm (Ravignani et al. 2014). Moreover, it also may help in identifying the selective pressures that may have driven the evolution of this trait only in four primate families (Haimoff 1986). Indris’ songs include calls that can be given with different degrees of overlap (absent to complete) and in an alternated or simultaneous way. These characteristics make the species an excellent model to investigate vocal complexity in terms of singing coordination and rhythmic abilities. Moreover, because of its peculiar vocal behaviour duality, *Indri indri* represents an ideal model for deepening strepsirrhine vocal complexity, to whose investigation the following sections are devoted.

Several studies explored the process leading to song diversification in birds, that seems to be the result of an interplay between genetics, social experience, and learning (Marler 1997; Marler 2004; Warren et al. 2010). Current evidence on primate singing behavior is less clear: vocal development has canonically been thought to follow a fixed trajectory with little or nothing learning or modification in structure or usage of calls (Hammerschmidt and Fischer 2018). To explore processes shaping indris' vocal capacities, in the first chapter, I, therefore, investigated the relationship between genetic distance and acoustic similarity. I found a relation between acoustic similarity (considering both time and frequency parameters) and genetic distance for males only. I also examined the potential of the song structure in vehiculate cues about individuality and group membership of the callers, finding support for the distinctive signature only. Chapter I. Torti V., Bonadonna G., De Gregorio, C., Valente D., Randrianarison R.M., Friard O., Pozzi, L., Gamba M., Giacoma C. An intrapopulation analysis of the indris' song dissimilarity in the light of genetic distance. 2017. *Scientific Report 7: 10140*.

Most of the previous research on singing primates has concentrated on duet function (Geissmann 2002; Clarke et al. 2006), but few studies have explored coordination abilities and rhythmic capacities in non-human primates. Indris vocal behaviour represents a remarkable model to investigate factors underlying the evolution of complex rhythmic signals that involve input from multiple individuals (Gamba et al. 2016). I, therefore, deepened the results of Gamba and colleagues (2016), by exploring which factors might have influenced the rhythmic structure and its variation in indris. I considered two different proxies of the rhythmic organization: the inter-onset interval of units within a phrase (wpIOI) and the inter-onset interval between the beginning of the last unit of and the beginning of the next phrase (bpIOI). I then deepened the knowledge on the rhythmic structure of indri's song, investigating the rhythm variation according to the sex of the emitter, the duration of both its phonation and its contribution to the song, the song duration, and the number of individuals participating to the song. I also aimed to outline a potential scenario to investigate how the synchronization of emissions is achieved. Gamba and colleagues (2016) found highly sexually dimorphic rhythmic features and an effect of the adult male emission on the adult female singing, for most of the songs. I hypothesized that the indris achieved the coordination of their songs through the matching of an individual rhythm to the singing pattern of others, and predicted that would be the female to match the male’s singing. Moreover, given that the number of singing individuals might affect individual singing (Gamba et al. 2016), I also predicted that female contribution would change with the number of singers. In fact, female indris seem to be more flexible when compared to males and lead the rhythmic gaiting of the song, where the higher the number of singing individuals, the higher the cost they sustain.

Chapter II. De Gregorio C., Zanoli A., Valente D., Torti V., Bonadonna G., Randrianarison R.M, Giacoma C., Gamba M. Female indris determine the rhythmic structure of the song and sustain a higher cost when the chorus size increases. 2019. *Current Zoology 65(1),89–97*.

Singing behavior has also been reported to be mainly related (although not exclusively) to two particular traits: a socially monogamous system and a territorial lifestyle (Adret et al. 2018). *Indri indri* is a family-living, territorial (Bonadonna et al. 2017) and monogamous primate (Pollock 1975; where only one case of Extra Pair Copulation has been described (Bonadonna et al. 2014). In monogamous systems, sexual selection should evenly affect the two sexes (Snowdon 2017) that might be expected to have similar repertoires, and similar spectral characteristics of calls. Given that the findings of a recent work supported a genetically monogamous mating system in this species (Bonadonna et al. 2019), and that territoriality is the one of the traits mainly associated with the emergence of singing behavior (Adret et al. 2018), in the third chapter I investigated the pattern of territorial stability and the role of songs in mediating the relationships among neighboring groups. Indris live in small family groups composed of two to six individuals (Pollock 1975), that occupy exclusive territories (Bonadonna et al. 2017). The results confirmed both the pattern of territorial exclusivity and stability over time and the use of songs in regulating the spatial dynamics of the species.

Chapter III. Bonadonna G., Zaccagno M., Torti V., Valente D., De Gregorio C., Randrianarison R.M., Tan C.L., Gamba M., Giacoma, C. Intra- and intergroup spatial dynamics of a pair-living singing primate, *Indri Indri*. A multiannual study of three indri groups in the Maromizaha Forest, Madagascar. 2020. *International Journal of Primatology*.

Many primate species living in social groups often rely on the use of loud vocal signals to transmit information at long distances (Haimoff 1981, Mitani and Nishida 1993). When these signals involve the simultaneous participation of several individuals, a chorus is generated. This may include or not all the animals within a group (Harrington and Mech 1979). According to previous findings, the number of singers may influence individual singing (Gamba et al. 2016), and female contribution changes according to the number of singers (Chapter II). In chapter four, focusing on the chorus size estimation, I investigated whether in indris the duration of the song may vehiculate information about the number of singing individuals or even the group size, hypothesizing that an increase in the number of singers would correspond an increase in chorus duration. I found support for the song duration predicting both the effective numbers of singers within a particular chorus and the group size.

Chapter IV. Torti V., Valente D., De Gregorio C., Comazzi C., Longondraza M., Ratsimbazafy J., Giacoma C., Gamba M. Call and be counted! Can we reliably estimate the number of callers in the indri's (*Indri indri*) song? 2018. *PlosOne 13(8): e0201664*.

Although likely the most impressive, the singing behavior is only one trait of indri vocal production. The species indeed emits at least eight vocal types that, together with the song units, represents the whole repertoire of the species (Maretti et al. 2010). The number of distinct call types a species emit has primarily been used as a proxy for communicative complexity (Peckre et al. 2019). The identification of units, however, has mainly relied on the spectrograms audio-visual inspections, a relatively subjective procedure (Fischer et al. 2017; Peckre et al. 2019). Despite quantitative approaches for repertoire estimation are lately being applied. Furthermore, an additional source of subjectivity is represented by the diversity of methods employed (Fischer et al. 2017). Hence, the panorama is currently dominated by scarce homogeneity among studies, and no agreement on the methods to be used for the objective identification of vocal types and, consequently, for the vocal repertoire size and complexity assessment (Peckre et al. 2019). The missing of shared methodical techniques, and the diversity of approaches employed when investigating vocal abilities, indeed impend the feasibility to perform comparative evaluations across species (Zimmermann 2017). In chapter five, I highlighted the results of a novel computational technique I conceived and tested by using the indri's vocal repertoire as a model. Chapter V. Valente D., De Gregorio C., Torti V., Miaretsoa L., Friard O., Randrianarison R.M., Giacoma C., Gamba, M. Finding Meanings in Low Dimensional Structures: Stochastic Neighbor Embedding Applied to the Analysis of *Indri indri* Vocal Repertoire. 2019. *Animals, 9(5), 243*.

In the latter chapter**,** I briefly summarized the main findings emerging from the thesis, discussing them in light of the mechanisms that may have driven the evolution of vocal capacities and diversification in basal primates.

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Chapter I

An intra-population analysis of the indris' song dissimilarity in the light of genetic distance

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An intra-population analysis of the indris' song dissimilarity in the light of genetic distance

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Abstract

The increasing interest in the evolution of human language has led several fields of research to focus on primate vocal communication. The ‘singing primates’, which produce elaborated and complex sequences of vocalizations, are of particular interest for this topic. Indris (*Indri indri*) are the only singing lemurs and emit songs whose most distinctive portions are “descending phrases” consisting of 2-5 units. We examined how the structure of the indris’ phrases varied with genetic relatedness among individuals. We tested whether the acoustic structure could provide conspecifics with information about individual identity and group membership. When analyzing phrase dissimilarity and genetic distance of both sexes, we found significant results for males but not for females. We found that similarity of male song-phrases correlates with kin in both time and frequency parameters, while, for females, this information is encoded only in the frequency of a single type. Song phrases have consistent individual-specific features, but we did not find any potential for advertising group membership. We emphasize the fact that genetic and social factors may play a role in the acoustic plasticity of female indris. Altogether, these findings open a new perspective for future research on the possibility of vocal production learning in these primates.

Introduction

Vocal signals often play a critical role in animal communication[1]. While many species make a conspicuous use of vocalizations, a limited number of taxa communicate using a sequence of vocal emissions, usually termed songs[2]. A song is a combination of different components that can be described hierarchically. Individual sounds are referred to as ‘units’, ‘elements’ or ‘notes’. One or more units that occur together can be called song ‘syllables’, and a sequence of one or more syllables is described as either a ‘phrase’ or ‘motif’. Since Darwin, scholars have been interested in understanding whether the complexity of singing is genetically determined, impacted by social experience, or the result of a learning process[3, 4]. The processes leading to song diversity have been widely investigated in birds. Endogenous and exogenous factors modulate the interplay between genetic characteristics, social experience, and learning[5]. For instance, early findings on song development in the zebra finches (*Taeniogypta guttata*) suggested that, while male birds develop their song during a sensitive period for vocal production learning[6], song culture appears as a multi-generational phenotype, partially encoded in the genes of an isolated founding population. In this species, juvenile birds, that imitate isolated tutors, changed particular characteristics of the songs. These alterations can be accumulated over learning generations until a new natural song emerges[7].

Although birds are the only animals in which vocal production learning has been rigorously associated with the modification of the cerebral connections, previous research showed that learning in vocal communication is not unique to them. Some terrestrial and marine mammals may possess the ability to learn the production of particular emissions[8], and individuals of other species can learn the context in which to produce a particular call or how to modify their response to others’ vocalizations[9]. Communication using songs is widespread in different groups of birds and marine mammals, but is rare in primates[10]. A key question is to what extent primate vocalizations can be shaped by vocal production learning processes (hereafter, vocal learning) and social factors, and whether they may possess information useful for kin recognition. The current evidence is contradictory, with data suggesting both vocal learning and genetic relatedness as forces in shaping primate vocal signals. For instance, Marshall and colleagues[11] suggested that genetic relatedness had a limited effect on the acoustic similarity in chimpanzee's vocalizations (*Pan troglodytes*), since unrelated males showed a similar acoustic structure in their pant hoots. Further studies on chimpanzees consolidated the idea that vocal learning and convergence may play a role in the acoustic structure of food grunts shared within two groups[12]. The influence of social factors in shaping vocal signals has also been suggested for monkeys. Lemasson and colleagues found that social bonding better explained the acoustic similarity in the vocalizations of Campbell's monkeys (*Cercopithecus campbelli*) when compared to genetic relatedness[13]. Although evidence seems to suggest that primate calls are not completely genetically determined, the question whether information about genetic relatedness, crucial for kin recognition, is retained in vocalization is still open. A study of mandrill's vocalizations (*Mandrillus sphinx*)[14] showed that the acoustic structure of contact calls was more similar between relatives than among unrelated individuals, suggesting that mandrill acoustic signals contain kin-specific information.

Because vocal learning has been typically associated with singing, studying the effects of genetics and social factors on the vocal output of primates that communicate using songs (i.e., tarsiers, gibbons, indris, titi monkeys[15]) is of great interest. Unfortunately, limited knowledge about the ontogenesis of primate singing behavior prevents a proper comparison with studies on birdsongs. However, previous research on gibbons reported that juveniles and young females produce immature vocalizations (*Hylobates lar* and *H. agilis*[16]) and that the structure of the songs may reach a mature form at about six years old (*Nomascus gabriellae*[17]). Moreover, the co-singing of mothers and daughters in *Hylobates agilis* has been interpreted as a possible form of tutoring to switch from an immature to a mature female great call[18]. On the other hand, concordance between song and genetic diversity across the crested gibbons[19] suggests that genes may play a major role in shaping song structure. Additionally, both hybrid males and females showed intermediate song structure compared to the songs of the parental species (*Hylobates lar* x *H. muelleri*[20]; *Hylobates lar* x *H. pileatus*[21]). Interestingly, female songs tended to diverge more from their parents' songs, while males' appeared to resemble those of their father.

To further explore the processes shaping primate songs, we investigated the relationship between genetic distance and acoustic similarity in the indris. We also aimed to understand whether the acoustic structure of phrases differed when analyzed within closely (e.g.; father-offspring, mother-offspring) and distantly related indris in the population of Maromizaha.

The indri (*Indri indri*[22]) is the only lemur that communicates through songs. The indris' songs are long sequences of vocal units that are organized in phrases[23, 24]. They have the form of a chorus in which all the adults and the subadults of a group utter their contribution in a precise and coordinated manner[25]. Songs have various functions depending on the context in which are emitted and they are used for both inter and intra-group communication[26, 27]. Furthermore, songs are likely to provide information about the group composition and mediate the formation of new groups[25, 28, 29]. Because this species lives in familiar groups (Bonadonna, *unpublished data*) and the song has a rich repertoire of units[25, 30, 31], the indris can be an excellent model to investigate the relation- ship between genetic relatedness and song similarity. Recent studies by Gamba and colleagues[25] showed that the acoustic structure of phrases did not significantly change between age classes, suggesting that a limited variation may occur during ontogeny.

The inheritance of song characteristics has been inferred from the studies of hybrid gibbons. Geissmann[21] studied the song of a male and a female hybrid (*Hylobates pileatus* x *H. lar*) finding that their songs differed markedly from the song characteristics of the parent species. However, studies on ground squirrels (*Spermophilus suslicus*) found a weak correlation between acoustic similarity and kinship, showing that other factors, such as the need for an individually distinctive acoustic structure, may play a critical role in vocal communication[32–34].

In this study, we hypothesize that, if genetics strongly determines song characteristics, vocal learning may not play an important role in shaping the indris' songs. We predicted that if song traits are mainly inherited, a high genetic distance will correspond to a reduced song similarity and that this reduction would be consistent within and between sexes. But it is also possible that emitters possess the potential for modifying their utterances and use songs to advertise their individuality and their belonging to a group[35–37]. In this second scenario, measures of genetic relatedness are not associated with song traits and we predicted that genetic distance and song similarity should not covary, but individuality and group membership would instead explain most of the acoustic variation.

Methods

Observations and recordings.

We studied seven groups living in the Maromizaha Forest (18°56′49″S, 48°27′53″E). We collected data in the field from 2011 to 2016, for a total of 24 months. We observed a social group per day, approximately from 6 AM to 1 PM. We identified the indris individually thanks to natural marks. Group composition ranged from 2 to 4 indris (Fig. 2; details are provided in Table 4). Recordings were made using solid-state recorders (SoundDevices 702, Olympus S100 and LS05, and Tascam DR-100, DR-40, and DR-05) equipped with Sennheiser (ME 66 and ME 67) or AKG (CK 98) shotgun microphones. The microphone signal was recorded at a sampling rate of 44.1 kHz, 16 bit. We recorded all the songs at a distance comprised between 2 and 20 m, keeping visual contact with the vocalizing animals (Fig. 3). We made all efforts to orientate the microphone toward the focal uttering individuals. All recordings were carried out without the use of playback stimuli, and nothing was done to modify the behavior of the indris. When in the field, we had one observer per individual in a group. Using the focal animal sampling technique[65], we were able to attribute each vocalization to a signaler. From the individual song contributions, we extracted 1066 descending phrases consisting of two units (hereafter, DP2), and 1259 descending phrases consisting of three units (DP3; Fig. 4). We focused on DP2s and DP3s because they are the most common phrase types in the indris' song[25]. The sampling included phrases emitted by seven males (of which six sired at least one offspring), seven siring females, 9 offspring (five males and four females, Table 4). We included in our analyses only those individuals contributing at least four DPs. Our final sample included 23 individuals for the DP2s and 22 individuals for the DP3s.

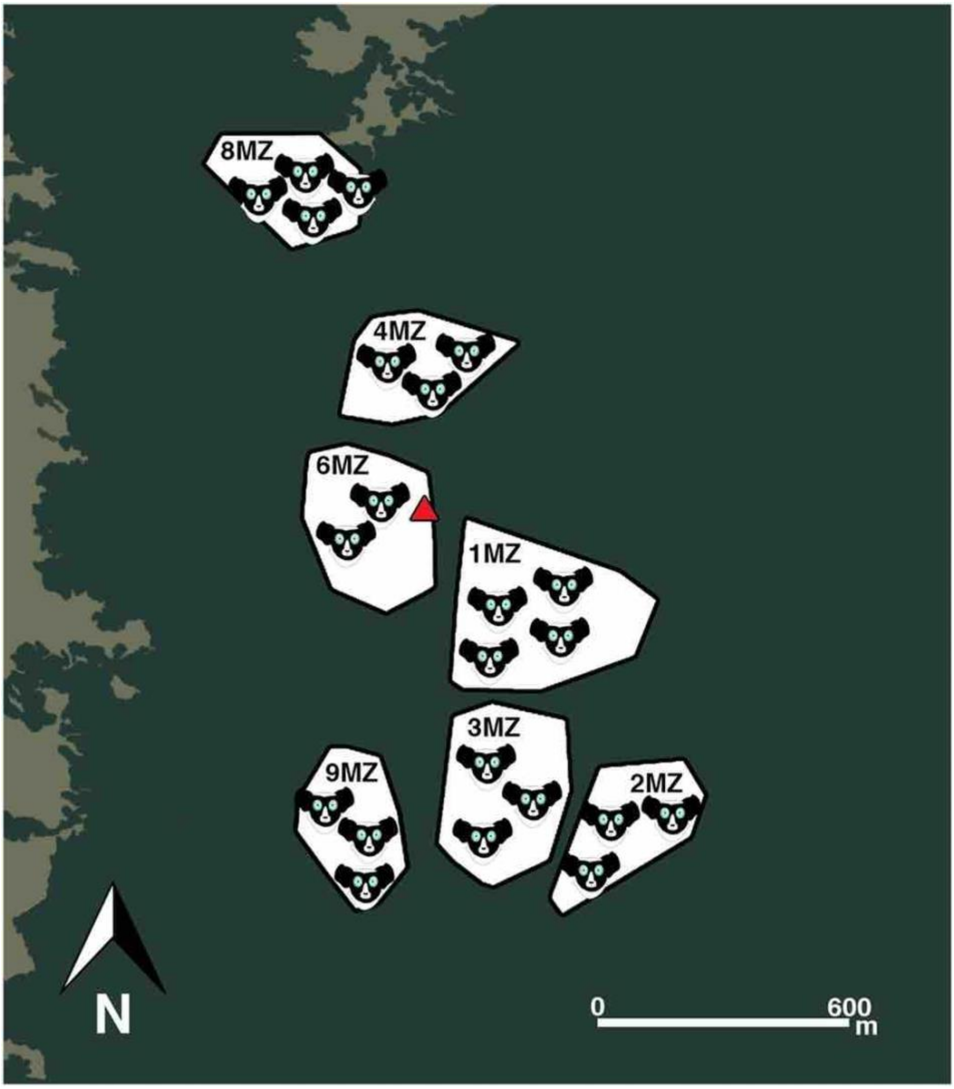

Figure 2. MapofthestudyareaintheMaromizahaForest.MinimumConvexPolygons(MCP)generated with ArcGIS 9.1 (ESRI) correspond to the 2016 home range of the study groups. Group ID is reported onto each MCP, and the indris' face icons indicate the number of animals per group. The red shape indicates the geographical location of the Maromizaha Research Center (18°58′34.06″S 48°27′53.88″E). Drawings by Dr. Valeria Torti.

Group	**ID**	**Sex/YOB**
1MZ	Jery	♂
	Bevolo	♀
	Maintso	♀ 2010
	Berthe	♀ 2012
2MZ	Max	♂
	Soa	♀
	Fanihy	♀ 2012
3MZ	Ratsytarehy	♂
	Mena	♀
	Tsaratarehy	♂ before 2009
	Zandry	♀ 2010
4MZ	Koto	♂
	Eva	♀
	Hendry	♂ before 2009
6MZ	Zokibe	♂
	Befotsy	♀
8MZ	Jona	♂
	Bemasoandro	♀
	Cesar	♂ before 2009
	Zafy	♂ 2012
9MZ	Emilio	♂
	Sissie	♀
	Ovy	♂ 2013

Table 4. Summary of group, ID, sex and year of birth of offspring. For each group, the reproductive pair is listed first. The year of birth is not reported for the reproductive pair.

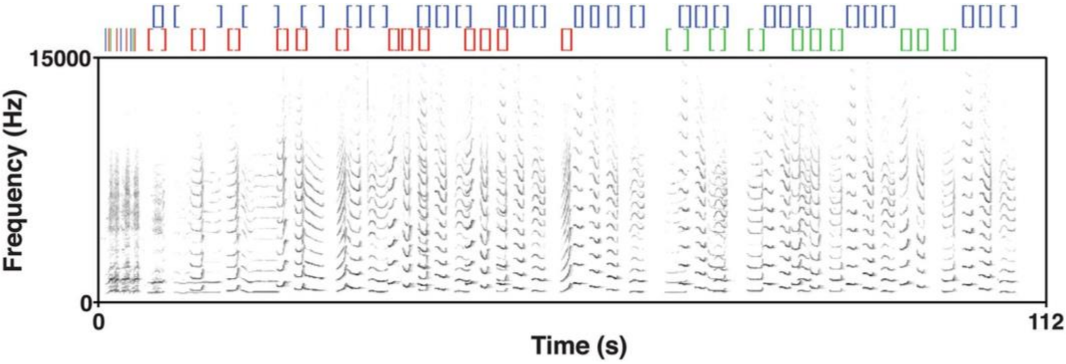

Figure 3. Spectrogram of the indris'song generated using PRAAT. In this song recorded in the Maromizaha Forest, a reproductive pair is singing with a female offspring (Group 2MZ). At the top of the spectrogram, the color brackets indicate the start ("[") and the end ("]") of each male's units (in blue), reproductive female's (in red), and female offspring's (in green).

Genetic analyses

The genetic analysis confirmed the identification of the reproductive pairs we independently derived from our behavioral observations[25, 26]. We collected fecal samples from 23 individuals and we stored all the samples in RNAlater$_{®}$ Ambion[66] at room temperature in the field and at 4 °C in the laboratory.

DNA extraction

We extracted genomic DNA from feces using the QIAamp DNA$_{®}$ Stool Mini Kit (Qiagen$_{®}$, Hilden, Germany) with the following changes from the manufacturer's protocol (QIAamp DNA Stoll Handbook 04/2010). We used 300 mg stool; we added 35 µl of proteinase K and incubated at 70° Celsius for 30 minutes during the purification phase. We applied 75 µl Buffer AE on the QIAamp membrane for the first DNA elution and incubated the spin column with Buffer AE at room temperature for 15 minutes.

For the samples collected in 2014, we used the automated robotic workstation QIAcube HT supported by the software QIAxtractor 4.17.1 (Qiagen$_{®}$) to conduct DNA purification. We set the protocol for QXT Liquid DNA V1. First, we bathed the 2.0 mL tubes containing 300 mg of smashed feces and 1.6 mL of Buffer ASL at 70 °C for at least 5 minutes. After that, tubes were centrifuged at 13000 RPM for 10 minutes. We transferred 200 μl of supernatant to separate wells of the QIAextractor lysis plate and we started the run. At the end of the process, we obtained 70 μl of DNA elution for each sample. We stored the extracted DNA at 4 °C for immediate use.

DNA genotyping

We selected a set of 6 microsatellite marker loci identified as potentially variable in indri that provided good quality amplification products for multiplex PCRs[67] (Table 5). A fluorescent dye (FAM, HEX) labeled the 5′ end forward primer of each locus to analyze simultaneously loci of similar allelic size. PCR amplification was carried out in 10 μL reaction volume containing: 5 μL Multiplex PCR Master Mix (Qiagen$_{®}$), 0.1 μm of each primer, 2 μL DNA template, 2 μL RNase-free water. We set the cycle conditions as follows: a pre-incubation step at 95 °C for 15 min; 50 cycles with denaturation at 94 °C for 30 s, annealing at 54 °C or 60 °C (depending on the locus, see Table 5) for 90 s. The first extension phase was at 72 °C for 60 s; the final extension phase at 60 °C for 30 min.

We estimated the allele size by electrophoresis using a 48-capillary ABI 3730 DNA Analyzer (Applied Biosystems). We mixed 1 μL of PCR product with 6.85 μL HiDi formamide (Applied Biosystems) and 0.15 μL Genescan 500-ROX size standard (Applied Biosystems). We conducted automated allele calling using the soft- ware GENEMAPPER 3.7 (Applied Biosystems). We then confirmed by eye all the allele sizes and checked for consistency across replicate PCRs of the same sample or from the same individual for a certain locus (minimum three replicates for heterozygotes and five replicates for homozygotes).

Locus	Forward primer	Reverse Primer	Repeat motif	Annealing temp. (°C)	Size range (bp)
67HDZ25	GGACCCTAATTCAAATATCACCTC	GGCATTTCTACTCCAGGTTGG	$(CA)_{16}$	54	218-253
67HDZ62	AGCCCTTTCTCTCAATGCC	CCTTCTTTGTTATCTTTCTGCATC	$(GT)_{21}$	54	203-217
67HDZ18	GGACTGGTAGATTTCTGGGTTTAG	CAGCCACTCCAATGCAAAG	$(CA)_7C(CA)_{15}$	60	164-190
67HDZ55	TCAGGAGTTGGGACCAGGG	ATGAAGGGATGGAGGTGGG	$(GT)_{18}$	60	312-334
67HDZ180	TCCCCTCCTCAGTCATTTCTC	CGTGAAGCTCGTGTGTATGG	$(CA)_{17}$	60	113-136
67HDZ39	CAGAGCCAGGGTTCAAATTC	TTGTCTTTTCTGCCACTGTAGG	$(CA)_{11}$	60	148-162

Table 5. Microsatellite loci used in this study, with respective primers, and annealing temperatures. The number of PCR cycles is 50.

Relatedness analysis

We estimated relatedness among individuals using the R package *related*[68]. First, we compared seven different relatedness estimators commonly used in the literature, five moment estimators[69–73] and two likelihood-based estimators, the dyadic likelihood estimator – *dyadml*[74] and the triadic likelihood estimator – *trioml*[75]. Using the allele frequencies observed in our dataset, we simulated datasets of 100 pairs for four known relatedness categories (parent–offspring, full-sibling, half-sibling, and unrelated). We chose the *trioml*[75] estimator to calculate relatedness for all possible dyadic combinations because it showed the highest consistency and obtained a matrix in which the more positive the index, the more two individuals are genetically related. Since indri lives in family group[28, 47], the historical record of group composition since 2009 allowed us to infer parental relationships among individuals based on behavioral observations, especially between mother and offspring. We were able to assign a social father to each of the offspring included in the study. To define parental information for the comparison of acoustic distances, we run paternity analyses including as potential fathers all the adult males sampled (Bonadonna, unpublished data) and could assign paternity for nine offspring (out of 10).

Acoustic and statistical analyses

Because the singers' phrases could overlap each in the temporal and frequency domain, we first extracted the fundamental frequency using a manual procedure and then obtained the pitch contour using a semi-automatic process in Praat[76]. We then added 0.5 s of silence at the beginning and the end of each phrase. Because each unit within a phrase went through the same set of measurements, we collected a minimum of 10 measurements in the temporal domain and a minimum of 20 measurements of pitch variability for each DP. The complete list of variables we measured is in Supplementary Table S6, while some parameters are presented in Fig. 4. Further details about the methodology used can be found in Gamba and colleagues[25].

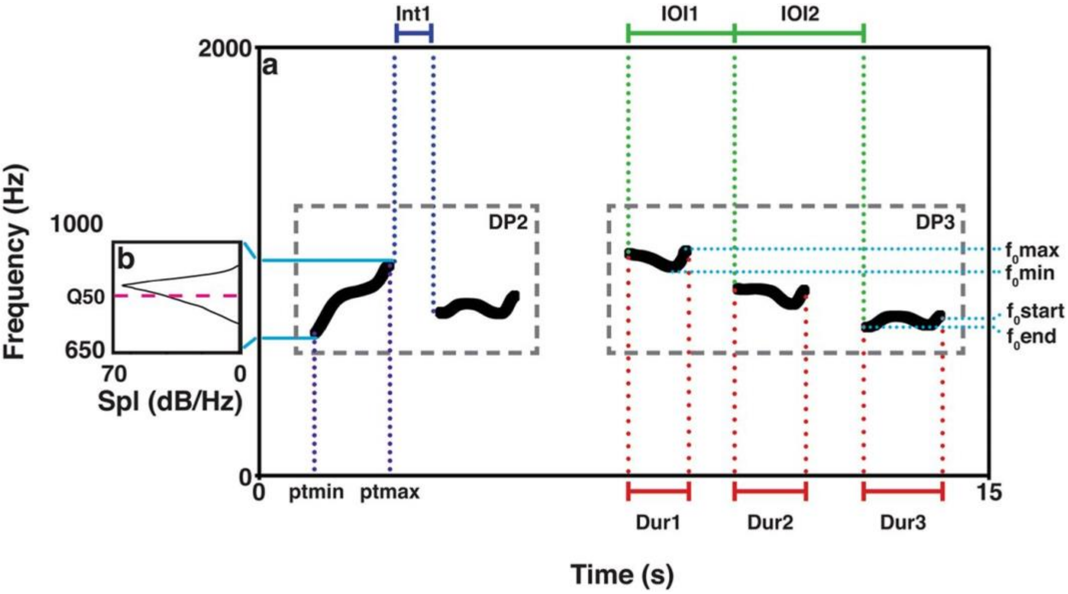

Figure 4. Schematicrepresentationofthespectrogramoftheisolatedfundamentalfrequencyoftwo descending phrases, a DP2 and a DP3 (a). The sound spectrogram displays time (seconds) on the x-axis, frequency (Hz) on the vertical axis. We describe acoustic parameter collection of unit duration (in red); intervals (in blue); inter-onset intervals (in green); percentage of time to the minimum (ptmin) and maximum of pitch (ptmax, in purple); maximum and minimum pitch (f0max and f0min), fundamental frequency at the beginning and at the end of a unit (f0start, f0end, in light blue). In the spectrum (b), the fuchsia dotted line marks the frequency corresponding to the upper limit of the second quartile of energy in the spectrum (Q50). The sound spectrum displays sound pressure level (Spl/dB) on the x-axis, frequency on the vertical axis.

We used principal components analysis (Factor analysis in IBM SPSS 24.0.0.1) to reduce the data to uncorrelated principal components (PCs) using separately temporal and frequency measurements of DP2s and DP3s. We obtained four PCs exceeding eigenvalue 1 for the temporal measurements and five for frequency variables of DP2s. We obtained six PCs exceeding eigenvalue 1 for each of the two sets of variables of DP3s. To understand whether genetic relatedness could explain acoustic similarities, we transformed the PCs obtained for each of these sets in a Euclidean distance matrix (function *dist* in R 3.2.3) and then calculated the average individual means. We then run the Mantel tests (9999 randomizations[77, 78]) on the average individual means against the matrix of genetic indices (package *vegan* in R[79]). All matrix indices were normalized to have a value between 0 and 1 before entering the analyses.

We used the Mantel test to evaluate whether the acoustic distance differed among individuals paired by categories (‘father-daughter’; ‘mother-daughter’, ‘father-son’, ‘mother-son’). We assessed the correlation between the acoustic similarity matrix and a binary matrix indicating the category (e.g.; ‘father-daughter’; refs 77 and 80) for each pair of song phrases. A significant correlation would indicate a difference in the similarity of phrases given by one of the pairs mentioned above (e.g.; father-daughter) when compared with phrases emitted by unrelated reproductive adults (e.g.; other ‘fathers’ in the sample) and offspring (e.g.; ‘daughters’ of other pairs).

To understand whether we could identify a potential for individual recognition or group membership we submitted the component scores to permutated linear discriminant function analysis[81] in R (using a custom script by Roger Mundry). When testing for individual differences, we used the individual as test factor and the song from which the DPs were extracted as a control factor. We also ran the analyses split by sex. When testing for group membership, we used the group as test factor and the individual identity as control factor. We split all the analyses into two phases, a training phase and a testing phase (R. Mundry, personal communication) for which we collected the correct classification rate and the p-value.

Results

Extraction of the principal components.

Four principal components accounted for 83.2% of the total variance of the temporal variables of the descending phrases of two units (DP2, see Supplementary Table S1), and five components accounted for 92.5% of the variance of frequency parameters (see Supplementary Table S2). We found six components for the temporal variables of the descending phrases consisting of three elements (DP3s) explaining 83% of the observed variance. We then found six components for the frequency variables of DP3s explaining 89.5% of the observed variance (see Supplementary Tables S3 and S4). The acoustic parameters showing the highest loadings on the principal components PC1 of each PCA were the first inter-onset interval (IOI1) and the total duration of unit 1 (Dur_unit1) for the temporal parameters of DP2. For frequency parameters of DP2, they were the average fundamental frequency of unit 2 (f0mean_unit2) and unit 1 (f0mean_unit1). For the temporal parameters of DP3, they were the second inter-onset interval (IOI2) and the duration of the second interval (Dur_int2). For the frequency parameters of DP3, they were the average fundamental frequency of unit 2 (f0mean_unit2), the frequency at the upper limit of the second quartiles of energy (Q50_unit2). A complete list of PCs and the loadings of the acoustic parameters are listed in the Supplementary Information (see Supplementary Tables S1–S4).

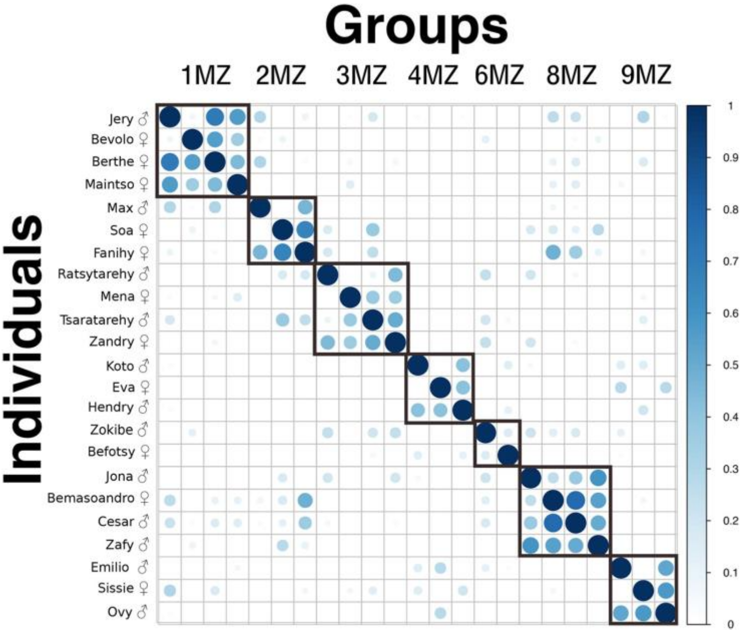

Figure 1. Plot of the trioml estimator[75] showing the genetic relatedness among the individuals in the study groups of the indris of Maromizaha (see also Supplementary Table 3). Individual names and sexes are shown on the vertical axis; group composition is shown on the horizontal axis. Dot size and color refer to genetic relatedness: the darker and bigger the dot, the more genetically related are the individuals. The correlation plot was generated using the R package corrplot[82].

		MALES			FEMALES			OVERALL		
		N	**R**	**P-VALUE**	**N**	**R**	**P-VALUE**	**N**	**R**	**P-VALUE**
DP2	Temp.	12	0.306	0.004	11	0.119	0.201	23	0.119	0.034
	Freq.		0.058	0.309		0.077	0.309		0.072	0.136
DP3	Temp.	12	0.164	0.080	10	0.006	0.473	23	0.062	0.171
	Freq.		0.172	0.055		0.015	0.234		0.094	0.074

Table 1. Results of the Mantel tests analyzing the correlation between acoustic similarity for the temporal (temp.) and frequency (freq.) parameters of descending phrases DP2 and DP3 and genetic relatedness.

Acoustic similarity and genetic relatedness.

We found a variable degree of genetic variation within the groups of indris we sampled, confirming that each social group consisted of a male, an unrelated female, and their offspring (Fig. 1, Supplementary Table S5). When analyzing whether genetic distances had an effect on the temporal and frequency characteristics of DP2 at the population level, Mantel tests revealed a significant positive effect for temporal but not for frequency parameters (Table 1). For DP3, we did not find a significant effect either for temporal or frequency parameters (Table 1). Since male and female phrases are sexually dimorphic[25], we replicated the Mantel tests separately for each sex. We found that combining the sexes together was underestimating the effect of genetic distance on male song dissimilarity, which showed a highly significant value for the temporal characteristics of DP2 (Table 1). We did not find any significant p-values for DP3 temporal and frequency features (Table 1). While there was a tendency for frequency parameters to be related to genetic relatedness in males, Mantel tests revealed a non-significant correlation between acoustics and genetics among females for DP2 and DP3 (Table 1).

The genetic similarity of adults ranged between 0-0.30, 0.09-0.77 for parent-offspring, and 0.11-0.50 for siblings. When we compared the pairwise acoustic indices of the categories father-daughter, father-son, mother-daughter, and mother-son to those of unrelated adults-offspring pairs, we found significant correlations of fathers and sons for the temporal and frequency parameters of DP2 and DP3 (Table 2), showing that similarity was higher within phrases emitted by related than unrelated male indris. We have also found that similarity was higher within phrases emitted by mothers and daughters than unrelated females, but only for the frequency parameters of DP2.

			SONS			**DAUGHTERS**		
			N	**R**	**p**	**N**	**R**	**p**
DP2	Temp.	FATHERS	7	0.397	0.010	7	0.033	0.410
		MOTHERS		0.126	0.295		0.227	0.114
	Freq.	FATHERS	7	0.281	0.019	7	0.026	0.533
		MOTHERS		0.152	0.191		0.311	0.038
DP3	Temp.	FATHERS	7	0.509	0.010	5	0.221	0.200
		MOTHERS		−0.001	0.486		0.115	0.200
	Freq.	FATHERS	7	0.342	0.010	5	0.059	0.500
		MOTHERS		0.183	0.171		0.001	0.500

Table 2. Results of the Mantel tests analyzing the correlation between acoustic similarity for the temporal (temp.) and frequency (freq.) parameters of descending phrases DP2 and DP3 and kin information.

Individual signature and group membership.

We investigated whether the variation of the DPs was consistent with the individual ID and the group membership. Despite an overall within-sex structural similarity between the DPs recorded, we found a remarkable similarity at the individual level, which reflected in the high rates of correct classification of the permuted Discriminant Function Analysis (pDFA) (Table 3). DP2s and DP3s could be assigned to the individual with accuracy greater than chance using both temporal and frequency parameters (Table 3). These results were confirmed when we repeated the analyses separating sexes (Table 3).

In contrast, indris produced phrases that did not signal group membership accurately. In fact, pDFAs using the temporal and frequency parameters of DP2 and DP3 showed statistically significant p-values neither during the training, nor during the testing (Table 3).

				TRAINING	**P-VALUE**	**TESTING**	**P-VALUE**
DP2	INDIVIDUALITY	Temporal	Overall	44.03	0.001	24.07	0.001
			Males	51.64	0.024	29.23	0.001
			Females	50.50	0.003	37.30	0.001
		Frequency	Overall	46.62	0.001	19.59	0.001
			Males	53.56	0.046	18.15	0.002
			Females	56.39	0.001	32.13	0.001
	GROUP MEMB.	Temporal		41.47	0.475	31.09	0.258
		Frequency		45.13	0.380	29.98	0.225
DP3	INDIVIDUALITY	Temporal	Overall	81.02	0.001	28.73	0.001
			Males	91.83	0.009	35.92	0.001
			Females	71.40	0.001	45.91	0.001
		Frequency	Overall	73.50	0.001	27.79	0.001
			Males	83.00	0.009	23.04	0.001
			Females	70.47	0.001	44.60	0.001
	GROUP MEMB.	Temporal		56.04	0.443	35.56	0.125
		Frequency		56.89	0.325	33.72	0.269

Table 3. Percentage of correctly assigned descending phrases DP2 and DP3 to the individuals, overall and by sex (N=23 for DP2, of which N=12 for males and N=11 for females, N=22 for DP3, of which N=12 for males and N = 10 for females), and groups (group memb., N = 7) for the indris of Maromizaha.

Discussion

The results presented here come from the first intra-population analysis of indris comparing the acoustic characteristics of song phrases and genetic relatedness. Using this approach, we demonstrated that the structure of males' phrases transmits information about relatedness more consistently than females' ones, and that song phrases possess the potential to provide conspecifics with a cue to individual identity of the emitter in both sexes. Male song-phrases DP2 transmit information about relatedness in the form of time parameters, but this information is not encoded in female calls. We found a tendency for encoding of relatedness information in the frequency structure of male song-phrases DP3. We also found that similarity of both temporal and frequency parameters in male phrases DP2 and DP3 correlate significantly correlate with genetic distance in the 'father-son' category. A result that for female phrases is limited only to the frequency parameters of DP2. We did not find significant correlations across sexes. Overall, we can confirm our prediction that the indris' song phrases contain information about genetic relatedness as it has been found in other primate species (e.g.: *Mandrillus sphinx*[14]). It is interesting to notice that the encoding of this information is strong in the temporal characteristics of the descending phrases when analyzed using genetic similarity indices, but significantly stronger in both temporal and frequency parameters of DP2 and DP3 when compared between related and unrelated males. The correlation between temporal patterns and genetic relatedness is especially interesting in the light of those studies that investigated how hybrids differed from their parental species in the acoustic characteristics of their utterances[38, 39]. In gibbons and lemurs, pulse structure and rhythmic characteristics have a particular relevance in the discrimination between hybrids and parental species[21, 38]. Our results are in agreement with analyses of intra-population variation on ground squirrels (*Urocitellus beldingi*) showing that individuals produce calls more similar to their relatives than to unrelated individuals[40]. We extended these findings showing that, in indri, relatedness is also encoded by frequency parameters of both DP2 and DP3 in males, and DP2 in females.

While the vast amount of data about primate kin recognition is devoted to the highly complex social groups of African monkeys[41, 42], Kessler and colleagues[43] demonstrated that the advertisement calls for the grey mouse lemur (*Microcebus murinus*) possess patrilineal signatures that mediate paternal kin recognition. Male vocal signatures have been indicated as an important mechanism for inbreeding avoidance[43]. This mechanism may have sense in the light of the long-term pair bonding of indris[44], but may also play a role in a scenario in which extra-pair copulation can potentially contribute to increasing levels of genetic diversity within a population[45]. Moreover, even though physical fights between individuals of neighboring groups are rare in indris[46], they involve primarily males and always include choral vocal displays[47]. Thus, song similarity between related males may mediate kin recognition and de-escalate aggressions. From a different perspective, the descending phrases are paradigmatic examples of vocal emissions with remarkable frequency modulation[23]. It is therefore possible that those phrases are acoustically more flexible and less genetically-determined. This interpretation can explain only part of our results, but it is interesting to notice that in the study by Lemasson and colleagues[48] the genetic similarity between Campbell's monkey females (*Cercopithecus campbelli campbelli*) did not explain the acoustic similarities of their contact calls. A higher degree of acoustic plasticity within females is supported by previous research on baboons (*Papio cynocephalus ursinus*[49]), Campbell's monkeys (*Cercopithecus campbelli campbelli*[50]), and Japanese macaques (*Macaca fuscata*[51]). Whether social factors may play a role in the acoustic plasticity of female indris need further investigation, but there is evidence that the contribution of the temporal structure to the song is less genetically determined in females than in males. Previous studies have found that females change the duration of their song to partially overlap with males' singing[29]. Females may also adjust duration according to the number of males in their social group, while males tend to avoid overlapping each other[25, 26].

The lack of knowledge about dispersal patterns in the indris prevents further speculation regarding the relatively higher genetic signature in male calls. In socially monogamous species there is a tendency towards female-biased dispersal[52] that data from the field do not support for the indris. Data collected over 14 years and in different forests suggest that both male and female indris disperse (Giacoma, *unpublished data*), although dispersal frequency and distance are currently not available.

Individual variation in the vocal signals is a precondition for individual recognition, which can result in both affiliative and aggressive situations[53]. Previous research has demonstrated that sex differences may override individual differences[25], but the results of the present study complement those findings showing that strong individuality is nevertheless encoded in the indris' phrases. The presence of an individual signature is confirmed by both the training and testing phase of the permuted Discriminant analysis and it is valid for both temporal and frequency parameters. Our findings are in agreement with previous studies that have reported a strong individual signature in the acoustic signals of social mammals (e.g.; yellow-bellied marmots[54]; Speckled ground squirrels[32, 33]). The individuality encoding was also found in the Cao Vit gibbon male phrases[55] and in the Bornean gibbon female great calls[56], two species that emit songs like the indris. Our analyses suggest that individuality is encoded in both males and females, tracing an interesting path for future research in other singing primates. While most of the previous research on the indris' song suggests that the temporal parameters play a major role in the sex-specific encoding of the vocal emissions[25,

[29], we found that also frequency parameters have potential for individual recognition. This evidence expands the recent findings of Gamba and colleagues[25] about a sex-specific difference in pitch patterns during the song. In general, both time and frequency variables appeared to play a role in encoding individuality as they probably do for sex-specificity.

Behavioral observations support our results that singing in indris may facilitate the exchange of identity information in the context of distant communication[26, 46]. This idea is in agreement with previous studies on other primates, where long distance vocalizations were found to be useful for identifying individuals[55] and estimating male fighting ability[57]. Although we do not have data in support of the hypothesis that song may be useful to estimate individuals' fighting ability, our behavioral observations suggest that they indris can vocally discriminate individuals. Two lines of evidence support this idea. Torti and colleagues[26] showed that songs elicited regrouping of particular individuals within a group. Bonadonna and colleagues[45] observed that a female that has just been involved in extra-pair copulation did not join the song of her group mates, which were singing at a distance. We found support for the hypothesis that the use of song phrases to broadcast individuality may be essential during pair formation at distance[26] and for the regulation of territorial spacing, where other communicative signals may be ineffective[58].

When we analyzed the dissimilarity of phrases emitted by members of a social group, we found that pDFA could assign neither DP2 nor DP3 to the group with a classification rate higher that those predicted by chance. This result is in contrast to the findings of Knörnschild and colleagues on greater sac-winged bats (*Saccopteryx bilineata*[59]) and Vester and colleagues on pilot whales (*Globicephala melas*[60]), where differences of vocalizations within social groups were significantly lower than intergroup differences. The work on the greater sac-winged bats is particularly interesting because the authors found that pups modified their emissions during ontogeny and learned their songs through vocal imitation of their harem males, independently of their genetic relatedness[59]. Studies on apes and monkeys suggested a consistent degree of acoustic plasticity in nonhuman primate calls, which was observed in particular after social changes[61, 62] or during vocal interactions[63]. A previous investigation on the rhythmic structure of the indris' song showed that the structure of the descending phrases did not change significantly during ontogeny[25]. Combined with our finding that group membership cues do not appear to be encoded in the phrase structure, these two elements seem to demonstrate that the indris' song has limited flexibility when compared to other animals' utterances and that learning may play a secondary role in song acquisition. Our results disagree with previous findings by Baker-Médard and colleagues[27] that found significant differences in chorus songs between three groups in the Analamazaotra Reserve. There can be multiple reasons for these different findings. First, the number of songs and the number of groups considered by Baker-Médard and colleagues[27] is smaller than those we used in the present study, possibly reducing the variation of the acoustic measurements and leading to an intergroup dissimilarity that we were not able to find. Second, it is possible that the fact that Baker-Médard and colleagues[27] considered all the units in the songs for their analysis contributed some essential trait for group discrimination that is lacking when only the DPs are considered. However, we think that considering the most common DP types occurring in the songs should allow a proper evaluation of the acoustic variability exhibited by this species. As for the individual discrimination, we lack any evidence that the indris make similar discriminations regarding groups. However, according to our data, the potential for group recognition appears weaker than that for individual recognition. We can hypothesize that the song may play a role in the numerical assessment of group size, as McComb and colleagues[64] have demonstrated on the

African lions (*Panthera leo*). Future playbacks of the indris' songs may improve our knowledge about the amount of information encoded by the songs.

Overall, our results confirm that vocal signals can be shaped by both genetic factors and social experience in the indris. Even in primates that emit songs with complex temporal and frequency patterns, phrases contain information about genetic relatedness and subtle variation in the acoustic structure may play a role in providing conspecifics with cues for kin identification and individual discrimination.

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Author Contributions

V.T., G.B., and C.G. contributed to study design, analyzed the data, performed literature review, helped to interpret the results, and helped to draft the manuscript. C.D.G., D.V., L.P., and O.F. analyzed the data, performed literature review, helped to interpret the results and to draft the manuscript. R.M.R. oversaw field studies and data collection, performed data collection in 2013 and helped collate the data. M.G. contributed to study design, collated and analyzed the data, interpreted results, and drafted the manuscript. All authors contributed to writing the final manuscript.

Data availability

Data and programs used for the analyses presented in the paper are available to the Editorial board members and the referees upon request or already included in the Supporting Information.

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Additional Information

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Supplementary information

Supplementary Table S1. Factor loadings for temporal parameters of DP2 on the first four principal components.

	Parameter	Component 1	Component 2	Component 3	Component 4
DP2 Temporal Parameters	IOI1	0.957	-0.132	0.088	0.107
	Dur_Unit1	0.833	0.288	0.435	0.146
	Dur_Int1	0.757	-0.498	-0.281	0.03
	Dur_Unit2	0.688	0.547	0.151	-0.392
	PtMin_Unit1	0.478	0.454	-0.447	0.217
	PtMax_Unit2	0.337	-0.674	0.179	-0.185
	PercDur_Unit1	0.021	0.352	0.731	0.505
	PercDur_Unit2	-0.179	0.786	0.109	-0.55
	PtMin_Unit2	-0.334	0.395	-0.246	0.501
	PtMin_Unit1	-0.407	-0.4	0.646	-0.093

Supplementary Table S2. Factor loadings for frequency parameters of DP2 on the first five principal components.

	Parameter	Component 1	Component 2	Component 3	Component 4	Component 5
DP2 Frequency Parameters	f0mean_Unit2	0.889	0.276	-0.339	0.007	0.041
	f0mean_Unit1	0.874	-0.395	0.19	-0.154	0.031
	Q75_Unit1	0.864	-0.362	0.107	-0.259	0.086
	Q50_Unit1	0.857	-0.386	0.102	-0.249	0.083
	Q25_Unit1	0.849	-0.409	0.098	-0.328	0.08
	Q50_Unit2	0.849	0.211	-0.436	-0.1	0.07
	Q75_Unit2	0.849	0.192	-0.436	-0.103	0.078
	Q25_Unit2	0.848	0.228	-0.434	-0.096	0.06
	f0min_Unit2	0.813	0.273	-0.413	0.005	0.008
	f0max_Unit1	0.812	0.011	0.478	-0.043	-0.058
	f0max_Unit2	0.76	0.389	0.041	0.355	0.122
	f0start_Unit1	0.664	0.573	0.066	0.338	-0.236
	f0start_Unit2	0.664	0.573	0.066	0.338	-0.236
	f0min_Unit1	0.656	-0.683	0.084	0.189	-0.087
	f0end_Unit1	0.51	-0.735	0.145	0.286	-0.07
	f0end_Unit2	0.51	-0.735	0.145	0.286	-0.07
	f0abs_slope_Unit1	0.444	0.518	0.673	-0.1	-0.094
	f0abs_slope_Unit2	0.444	0.518	0.673	-0.1	-0.094
	f0rangeratio_Unit2	0.021	0.058	0.215	0.446	0.845
	f0rangeratio_Unit1	0.009	0.464	0.296	-0.401	0.345

Supplementary Table S3. Factor loadings for temporal parameters of DP3 on the first six principal components.

	Parameter	Component 1	Component 2	Component 3	Component 4	Component 5	Component 6
DP3 Temporal Parameters	IOI2	0.896	0.257	-0.232	-0.118	-0.178	-0.015
	Dur_Int2	0.849	-0.186	-0.331	-0.138	-0.256	-0.176
	IOI1	0.835	0.24	0.35	0.218	0.127	-0.144
	Dur_Int1	0.801	-0.149	0.399	-0.105	0.322	0.028
	Dur_Unit2	0.58	0.753	0.001	-0.041	0.003	0.212
	PercDur_Int2	0.557	-0.591	-0.41	-0.126	-0.351	-0.128
	Dur_Unit1	0.556	0.566	0.166	0.482	-0.13	-0.273
	PtMax_Unit3	0.512	-0.141	-0.341	0.133	0.101	0.404
	PtMax_Unit2	0.36	-0.275	0.225	0.466	0.429	0.025
	PercDur_Int1	0.297	-0.626	0.533	-0.088	0.375	0.178
	PtMin_Unit1	0.207	0.115	0.608	-0.338	-0.312	-0.075
	Dur_Unit3	0.204	0.808	-0.115	-0.288	0.299	-0.231
	PercDur_Unit2	-0.098	0.724	0.048	0.027	-0.072	0.537
	PtMin_Unit2	-0.138	0.073	0.413	0.066	-0.453	0.403
	PtMax_Unit1	-0.139	-0.067	-0.555	0.566	0.217	0.069
	PercDur_Unit1	-0.315	0.202	0.249	0.789	-0.288	-0.209
	PtMin_Unit3	-0.391	-0.168	0.429	-0.07	-0.077	-0.235
	PercDur_Unit3	-0.593	0.568	-0.13	-0.305	0.329	-0.196

Supplementary Table S4. Factor loadings for frequency parameters of DP3 on the first six principal components.

	Parameter	Component 1	Component 2	Component 3	Component 4	Component 5	Component 6
DP3 Frequency Parameters	f0min_Unit2	0.897	-0.029	-0.11	-0.355	-0.172	0.068
	Q50_Unit2	0.878	-0.062	-0.22	-0.358	-0.099	0.03
	Q75_Unit2	0.878	-0.078	-0.224	-0.35	-0.088	0.025
	Q25_Unit2	0.876	-0.047	-0.213	-0.365	-0.11	0.036
	f0mean_Unit2	0.818	-0.463	0.239	-0.036	0.074	-0.077
	Q75_Unit1	0.812	-0.455	0.172	-0.164	0.049	-0.092
	Q50_Unit1	0.81	-0.461	0.166	-0.154	0.043	-0.097
	Q25_Unit1	0.808	-0.466	0.163	-0.142	0.039	-0.099
	f0min_Unit2	0.796	-0.046	-0.23	-0.314	-0.288	0.051
	f0max_Unit1	0.784	-0.174	0.426	-0.025	0.195	-0-064
	f0mean_Unit3	0.768	0.449	-0.371	0.194	0.116	-0.001
	f0max_Unit2	0.739	0.045	0.144	-0.316	-0.312	0.241
	f0min_Unit3	0.721	0.377	-0.426	0.099	0.064	0.04
	Q25_Unit3	0.716	0.348	-0.476	0.179	0.262	-0.035
	Q50_Unit3	0.713	0.348	-0.476	0.184	0.272	-0.042
	Q75_Unit3	0.709	0.333	-0.475	0.188	0.279	-0.048
	f0min_Unit1	0.597	-0.635	0.162	0.397	-0.059	0.039
	f0max_Unit3	0.533	0.604	0.131	0.276	-0.33	-0.14
	f0end_Unit1	0.513	-0.613	0.159	0.541	0.04	0.023
	f0end_Unit2	0.513	-0.613	0.159	0.541	0.04	0.023
	f0end_Unit3	0.513	-0.613	0.159	0.541	0.04	0.023
	f0start_Unit1	0.475	0.708	0.178	0.354	-0.116	0.068
	f0start_Unit2	0.475	0.708	0.178	0.354	-0.116	0.068
	f0start_Unit3	0.475	0.708	0.178	0.354	-0.116	0.068
	f0abs_slope_Unit1	0.343	0.462	0.741	-0.182	0.188	0.014
	f0abs_slope_Unit2	0.343	0.462	0.741	-0.182	0.188	0.014
	f0abs_slope_Unit3	0.343	0.462	0.741	-0.182	0.188	0.014
	f0rangeratio_Unit3	0.043	0.243	0.234	0.191	-0.511	-0.473
	f0rangeratio_Unit1	-0.031	0.246	0.039	-0.348	0.381	-0.4
	f0rangeratio_Unit2	-0.056	0.068	0.157	-0.011	0.072	0.73

Supplementary Table S5. Matrix of genetic relatedness (*trioml* estimator) among the individuals of the study groups of the indris of Maromizaha.

	befotsy	bema	berthe	bevolo	cesare	emilio	eva	fanihy	hendry	jery	jonah	koto	maintso	max	mena	ovy	ratsy	sissie	soa	tsara	zafy	zandry	zokibe
befotsy	1	0	0	0	0	0.038	0	0	0.114	0	0	0.142	0	0	0	0	0	0	0	0.051	0	0	0.155
bema	0	1	0.101	0	0.77	0	0	0.483	0	0.268	0.252	0	0.111	0.076	0	0	0	0.06	0.169	0	0.54	0	0.139
berthe	0	0.101	1	0.55	0.153	0	0	0.043	0	0.079	0	0	0449	0.302	0.053	0	0	0.167	0	0	0	0.063	0
bevolo	0	0	0.55	1	0.04	0	0	0	0	0.07	0	0	0359	0.032	0	0	0	0.0007	0.088	0	0.089	0	0.118
cesare	0	0.77	0.153	0.04	1	0	0	0.361	0	0.222	0.375	0	0.147	0.04	0	0	0.053	0	0.111	0	0.5	0.04	0.173
emilio	0.038	0	0	0	0	1	0.274	0	0.003	0	0	0.148	0.068	0	0	0.525	0	0	0	0	0	0	0.118
eva	0	0	0	0	0	0.274	1	0	0.417	0	0	0	0	0		0.277	0	0	0	0	0	0	0
fanihy	0	0.483	0.043	0	0.361	0	0	1	0	0.088	0.027	0	0	0.465	0	0	0.18	0.061	0.655	0.25	0.105	0	0
hendry	0.114	0	0	0	0	0.003	0.417	0	1	0.037	0	0.417	0	0	0.005	0	0	0.203	0	0	0	0	0
jery	0	0.268	0.709	0.07	0.222	0	0	0.088	0.037	1	0	0.037	0.566	0.293	0.037	0.037	0	0.304	0	0.188	0	0.026	0
jonah	0	0.252	0	0	0.375	0	0	0.027	0	0	1	0.057	0	0	0	0	0.204	0	0.172	0	0.595	0.204	0.212
koto	0.142	0	0	0	0	0.148	0	0	0.417	0.037	0.057	1	0	0	0	0	0	0.149	0	0	0	0	0
maintso	0	0.111	0.449	0.359	0.147	0.068	0	0	0	0.566	0	0	1	0	0.148	0	0	0	0	0	0	0	0
max	0	0.076	0.302	0.032	0.04	0	0	0.465	0	0.293	0	0	0	1	0	0	0	0	0	0	0	0	0
mena	0	0	0.053	0	0	0	0	0	0.005	0.037	0	0	0.148	0	1	0	0	00.063	0	0.375	0	0.36	0
ovy	0	0	0	0	0	0.525	0.277	0	0	0.037	0	0	0	0	0	1	0	0.578	0	0	0	0	0
ratsy	0	0	0	0	0.053	0	0	0.18	0	0	0.204	0	0	0	0	0	1	0	0.164	0.08	0	0.455	0.248
sissie	0	0.06	0.167	0.007	0	0	0	0.061	0.203	0.304	0	0.149	0	0	0.063	0.578	0	1	0	0.134	0	0	0
soa	0	0.169	0	0.088	0.111	0	0	0.665	0	0	0.172	0	0	0	0	0	0.164	0	1	0.375	0.276	0	0
tsara	0.051	0	0	0	0	0	0	0.25	0	0.188	0	0	0	0	0.375	0	0.08	0.134	0.375	1	0	0.5	0.206
zafy	0	0.54	0	0	0.089	0.5	0	0	0.105	0	0	0.595	0	0	0	0	0	0	0.276	0	1	0	0
zandry	0	0	0.063	0	0.04	0	0	0	0	0.026	0.204	0	0	0	0.36	0	0.445	0	0	0.5	0	1	0.248
zokibe	0.155	0.1390	0	0.118	0.173	0.118	0	0	0	0	0.212	0	0	0	0	0	0.248	0	0	0.206	0	0.248	1

Supplementary Table S6. List and abbreviations of the acoustic parameters.

Parameter	Description
Dur_Unit(x)	Total duration of the unit, measured in seconds
PercDur_Unit(x)	Unit's duration percentage related to the total phrase length
$f_0$mean_Unit(x)	Mean fundamental frequency value across the unit (Hz)
$f_0$max_Unit(x)	Maximum fundamental frequency value across the unit (Hz)
$f_0$min_Unit(x)	Minimum fundamental frequency value across the unit (Hz)
$f_0$start_Unit(x)	Frequency value of f_0 at the start of the unit
$f_0$end_Unit(x)	Frequency value of f_0 at the end of the unit
ptmax_Unit(x)	Time % occurring from the unit beginning up to the maximum f_0, assuming that the unit duration is 100
ptmin_Unit(x)	Time % occurring from the unit beginning up to the minimum f_0, assuming that the unit duration is 100
$f_0$abs_slope_Unit(x)	Decline of the unit f_0 over time (Hz/s)
FM_rate	Number of complete cycles of f_0 modulation per second (s^{-1})
$f_0$rangeratio_Unit(x)	Range between maximum and minimum f_0
Q25_Unit(x)	Frequency values at the upper limit of the first quartiles (Q25%) of energy
Q50_Unit(x)	Frequency values at the upper limit of the second quartiles (Q50%) of energy
Q75_Unit (x)	Frequency values at the upper limit of the third quartiles (Q75%) of energy
IOI1	Inter-onset interval for the first and the second unit
IOI2	Inter-onset interval for the second and the third unit
Dur_int(x)	Duration of the interval between units
PercDur_Int(x)	Interval's percentage duration related to the phrase total length

Chapter II

Female indris determine the rhythmic structure of the song and sustain a higher cost when the chorus size increases

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Female indris determine the rhythmic structure of the song and sustain a higher cost when the chorus size increases

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Abstract

Among the behavioral traits shared by some nonhuman primate species and humans there is singing. Unfortunately, our understanding of animals' rhythmic abilities is still in its infancy. Indris are the only lemurs who sing and live in monogamous pairs, usually forming a group with their off- spring. All adult members of a group usually participate in choruses that are emitted regularly and play a role in advertising territorial occupancy and intergroup spacing. Males and females emit phrases that have similar frequency ranges but may differ in their temporal structure. We examined whether the individuals' contribution to the song may change according to chorus size, the total duration of the song or the duration of the individual contribution using the inter-onset intervals within a phrase and between phrases. We found that the rhythmic structure of indri's songs depends on factors that are different for males and females. We showed that females have significantly higher variation in the rhythm of their contribution to the song and that, changes according to chorus size. Our findings indicate that female indris sustain a higher cost of singing than males when the number of singers increases. These results suggest that cross-species investigations will be crucial to understanding the evolutionary frame in which such sexually dimorphic traits occurred.

Keywords: chorus· coordination· duets· lemurs· singing· synchrony

Introduction

The ability to coordinate vocal emissions is universal in humans (Stivers et al. 2009; Henry et al. 2015). Usage of strikingly diverse languages commonly present turn-taking, which also sets early in ontogeny (Stivers et al. 2009; Casillas et al. 2016). This evidence raises the question whether the ability to coordinate our utterances has evolved under particular pressures or it was already present in the last common ancestor we had with other species (Levinson 2016). Studies on birds, insects, amphibians, and mammals showed that also animals produce vocal signals in which different individuals emit in a coordinated manner. The common feature in animal's choruses is the temporal organization of participants' contribution to favor or avoid overlap (Ravignani et al. 2014). This mechanism has evolved independently in species in which the mutual influence in the timing of signals involves an interaction among emitters driven by a complex short-scale timing behavior (Geissmann 2002; Takahashi et al. 2013). For instance, duetting between the sexes in the broad-winged bush katydid *Scudderia pistillata* showed rhythmic and synchronization abilities. In this species, the number of ticks female produce, as well as the timing of her response, depends on the number of pulses generated by the male (Villarreal and Gilbert 2013). Studies on tropical songbirds suggested that the evolution of coordinated resource-defense signals may be driven by ecological conditions that favored sedentary lifestyles and social stability (Logue and Hall 2014). In fact, males and females of duetting species usually establish long-lasting bonds, live in forested habitats, and show a territorial behavior (Thorpe 1963).

The characteristics of duetting bird species may partially overlap those showed by primates that produce songs. There is evidence of vocal turn-taking or alternating duetting in nocturnal and diurnal lemurs (Méndez-Cárdenas and Zimmermann 2009), marmosets (Takahashi et al. 2013), and Campbell monkeys *Cercopithecus campbelli* (Lemasson et al. 2011). A critical example of coordination during vocal displays is singing, which is infrequent in primates, and it was observed only in tarsiers, gibbons, indris, and titi monkeys (the so-called “singing primates”; Haimoff 1983). In these species, males and females forming a mating pair or extended family group, engage in coordinated duets or choruses where temporal features play a central role to convey information to conspecifics (Brockelman and Schilling 1984; Merker 1999). Researchers suggested that singing has evolved independently in these different taxa, driven by similar selective ecological pressures. Cowlishaw (1992) indicated that song is associated with group encounters and aggression at the territorial boundary, supporting the claim that duets and choruses advertise the presence of pair-bonding mates to threaten intruders and reduce the cost of territorial confrontation.

Studies on gibbons suggested that duet behavior is achieved by vocal interactions between mates of the reproductive couple (Maples et al. 1989; Geissmann 1999, 2000). A crucial point for the understanding of the mechanisms involved in determining song structure is to understand which factors influence male and female contribution to the song (Geissmann 2002) and to what extent the singing of a partner influences the other. In particular, a study by Traeholt et al. (2006) showed that song structure of female pileated gibbons *Hylobates pileatus*, significantly affects male utterances. On the contrary, the male song of white-cheeked gibbon *Nomascus leucogenys*, influences the duration of female song bouts (Deputte 1982). Müller and Anzenberger (2002) demonstrated that duets of the titi monkeys *Callicebus cupreus* are composed of partially overlapping songs, in which synchrony lies in transitions between phrases. Interestingly, the authors pointed out that the length of these sections is determined by the female, which seems to induce the transitions, accordingly to what previously found by Robinson (1979).

The indris, which are the only lemur species that produce songs, were indicated as good candidates for further investigations of the evolution of rhythmic abilities. Their songs exhibit turn-taking between individuals of different sexes and a variable degree of overlap between group members (Gamba et al. 2016). They produce songs that differ in their acoustic structure between contexts. The songs may serve to inform the neighboring groups about the occupation of the territory, to resolve territorial fights during a confrontation between different groups, and have a cohesion function (Pollock 1986; Torti et al. 2013). The indris' songs are composed by a long sequence of vocalizations that usually starts with a harsh emission ("roar"), followed by a series of slightly frequency modulated units ("long notes"). There is then a series of units organized in phrases with a descending frequency pattern (descending phrases [DPs], Thalmann et al. 1993; Sorrentino et al. 2013) composed of 2–6 units. The indri's vocal behavior provides a model system for studying the evolution and production of complex rhythmic signals that involve input from multiple individuals (Gamba et al. 2016). Most of the previous research has concentrated on the function of duets (Geissmann 2002; Clarke et al. 2006), but few studies have investigated rhythmic and coordination abilities in non-human primates. Our first aim was to examine rhythmic features in paired adult indris to understand which factors, and to what extent, could influence the rhythmic song's structure and its variation. We hypothesized that the indris' song output would show variability related to different variables, depending on the duration of the song, the number of singers, and the individual contribution per singer. Our second aim was to define a possible scenario to understand how the synchronization of utterances is achieved. The work by Gamba et al. (2016) showed that the indris' rhythmic characteristics are highly sexually dimorphic. They also found an effect of the adult male singing on the adult female output on most songs. A recent paper by Torti et al. (2017) showed that the females' song features are less genetically constrained than those of males, in fact they found that similarity of both temporal and frequency parameters of DPs significantly correlate with genetic distance only for males, whereas for females this result was limited only for frequency parameters of the DPs composed by two units. We hypothesized that the coordination in the indris' song is achieved by the matching of an individual rhythm to the other’s singing. We predicted that the female could match male’s singing, showing a higher degree of rhythmic variation if compared with males. According to the previous findings, which showed that the number of singers in a chorus might affect individual singing (Gamba et al. 2016), we also predicted that female’s contribution would change according to the number of singers.

Materials and Methods

Observations and recordings

We studied 8 groups living in the Maromizaha Forest (18° 56’ 49’’ S, 48° 27’ 53’’E; see Figure 1A). We collected data in the field from 2011 to 2017, for a total of 27 months. We observed one group per day, approximately from 6 AM to 1 PM. We used natural marks to identify the indris individually (see Figure 1C). The groups consisted of 2–5 indris. We used solid state recorders (Sound Devices 702, Olympus S100 and LS05, and Tascam DR-100, DR-40, and DR-05) equipped with Sennheiser (ME 66 and ME 67) or AKG Acoustics (CK 98) shotgun microphones to record the songs. We set the recorders at a sampling rate of 44.1 kHz, 16 bit during all the recording sessions. When recording the songs, we were always at a distance comprised between 2 and 20m from the indris, with the microphone orientated toward the focal singing individuals. The data were recorded without the use of playback stimuli, and nothing was done to modify the behavior of the indris. When in the field, a researcher observed a particular individual in a group, and we attributed each vocalization to a signaler using the focal animal sampling technique (Altmann 1974). We recorded 119 songs uttered by 35 individuals (see Table 1) but focused our analyses on the emissions of the reproductive pairs, consisting in 8 dominant females and 9 males. We excluded subadults and nonreproductive individuals because their contribution could provide the analyses with a potential confounding factor in understanding the indri’s rhythm characteristics as we know that offspring may sometimes try to match parents vocal output during ontogeny (Merker and Cox 1999). We took into account the individual identity of the singer (“individualID”), its social group (“groupID”) and the song (“songID”).

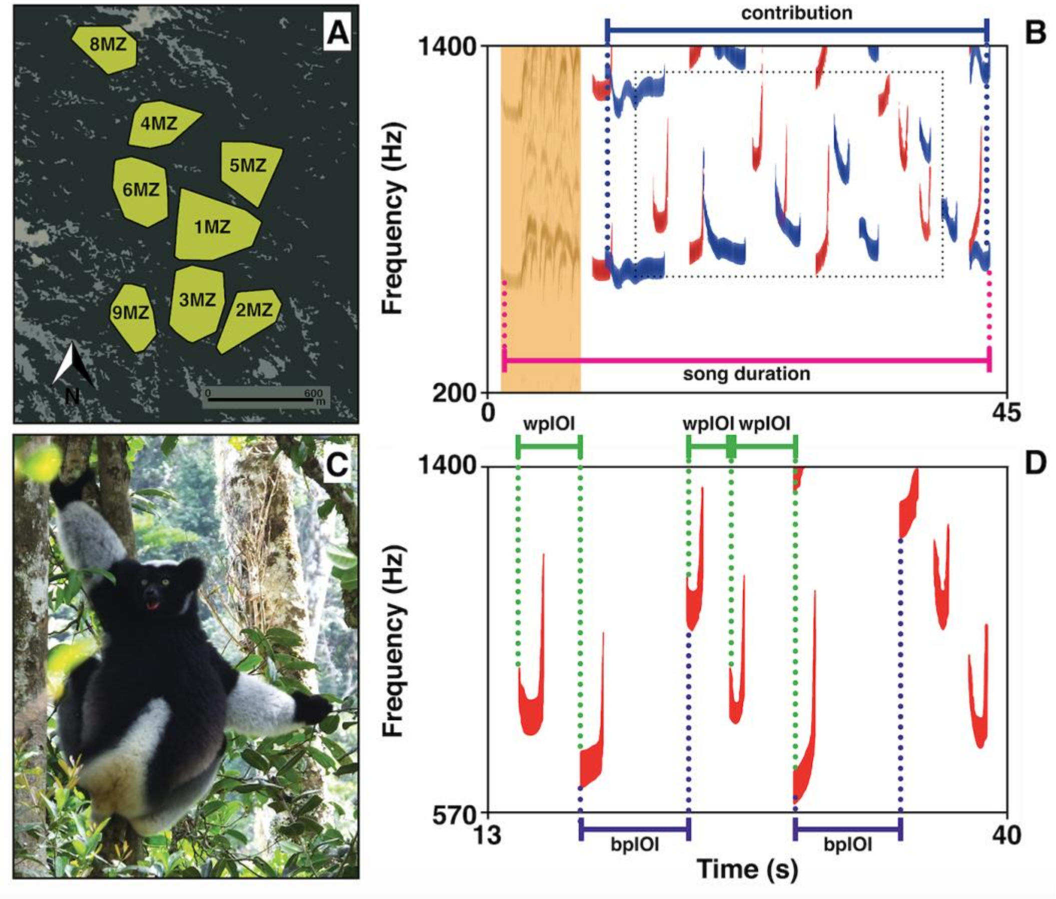

Figure 1. (A) Map of the study area in the Maromizaha Forest. Minimum Convex Polygons (MCP) generated with ArcGIS 9.1 (Environmental System Research Inc.) correspond to 2016 home range of the study groups. Group ID is reported onto each MCP. **(B)** Spectrogram of the indris' song generated using Praat. A reproductive pair is singing in the song. The initial portion is characterized by the emission of roars (shaded in orange), then the contribution of the male is high- lighted in blue and female's one in red. Song duration and contribution (for the male) are exemplified. Phonation, which is the cumulative duration of each note, is not shown. The small black dots indicate the area magnified in 1D. **(C)** A female indri from the Maromizaha Forest while singing. The natural marks (e.g., fur color pattern) are crucial for the identification of different individuals. **(D)** Schematic representation of the spectrogram of the isolated fundamental frequency of three DPs. The sound spectrogram displays time (s) on the x-axis, frequency (Hz) on the vertical axis. We describe acoustic parameter collection of bpIOI and wpIOI.

Acoustic analyses

Using Praat 6.0.14 (Boersma and Weenink 2016), we edited portions containing the indris' songs. We saved each song in a single audio file (in WAV format). Using field notes and video recording, we selected and saved the individual contribution for each singer in a Praat TextGrid. We identified units and silences through visual inspection of the spectrograms. We then generated textgrids of all the singers in a song and labeled the units according to their position in a DP (Gamba et al. 2016). A DP usually begins with a high-frequency note, followed from 1 to 6 units that start at a progressively lower frequency (Thalmann et al. 1993). For each of the above units and intervals, we extracted the timing of the starting point and duration using Praat and saved them to a Microsoft© Excel spreadsheet (Gamba and Giacoma 2007; Gamba et al. 2012). We extracted the inter-onset intervals of two following notes within a phrase (wpIOI) and between two subsequent phrases (bpIOI, see Figure 1D) and used them as a proxy for the rhythmic structure of phrases and songs (Sasahara et al. 2015). We also calculated the total song duration and the duration of individual contribution. We rescaled all variables to a logarithmic scale. To understand whether the singing behavior was influenced by the number of singers ("NoS" or "chorus size") in a song, we calculated the total song duration ("song duration"), the duration of the individual contribution ("contribution," see Figure 1B), the cumulative duration of the units uttered by an individual ("phonation") and the total number of DPs ("nDPs") in the song. Finally, we calculated the coefficient of variation (CV) for the wpIOI and the bpIOI, both for males and females.

Statistical analyses

We used a first Generalized Linear Mixed Model (GLMM, lme4 package, Bates et al. 2015) in R (R Core Team 2017; version 3.4.3), to understand how song rhythm was influenced by contribution and phonation, the number of singers, the total song duration, the number of DPs uttered, the mean inter-onset interval between notes (wpIOI), the mean inter-onset interval between phrases (bpIOI), and the sex of a singer. To analyze the rhythm variation, we ran six models using wpIOI or bpIOI as the response variable. Two models were run using the total dataset and contribution, phonation, nDPs as fixed factors. We also considered the interaction between sex and number of singers (Sex x NoS), and we used bpIOI or wpIOI as a covariate (depending on which was the response variable). We used the individualID, the groupID, and songID as random factors. We also ran 4 models in which the dataset was consisting of only males or females, in which we included the same predictors as above, plus song duration.

We ran 3 more models using the duration of individual contribution as the response variable, one on the total dataset, one for females and one for males. We used phonation, nDPs, bpIOI, wpIOI, and NoS as fixed factors. The random factors were individualID, groupID, and songID. When running the model on the total dataset, we included sex as a predictor, when the sexes were separated we used song duration as a predictor. We ran the same models using phonation as the response variable and contribution as a predictor.

For each model, we calculated the P-values for the individual predictors based on likelihood ratio tests between the full and the respective null model by using the R-function “drop1” (Barr et al. 2013). We used a multiple contrast package (multcomp in R) to per- form pairwise comparisons for each level of the factors with the Tukey test (Bretz et al. 2010), for which we then adjusted the *P*-values using the Bonferroni correction. We reported estimate, standard error (SE), z- and P-values for each test. We examined the variance inflation factors (vif package; Fox and Weisberg 2011) and then tested the significance of the full model against a null model comprising the random factor exclusively, by using a likelihood ratio test (see Gamba et al. 2016 for details).

We ran another GLMM to confirm whether the wpIOI were sexually dimorphic and to understand whether the CV of the wpIOI and bpIOI differed between males and females which were chorusing together. We used the paired t-test to understand whether the coefficients of variation of wpIOI and bpIOI differed between the sexes. In the paired t-test only, we considered Groups 3A and 3B as two different entries (Table 1).

Group ID	N songs	Individual	Sex
1	23	Jery[a]	M
		Bevolo[a]	F
		Berthe	F
		Fotsy	M
2	19	Max[a]	M
		Soa[a]	F
		Fanihy	F
		Afo	F
		Tovo	M
3a	11	Mahagaga[a]	M
		Mena[a]	F
		Tonga	F
		Faly	M
		Laro	M
3b	3	Ratsy[a]	M
		Mena[a]	F
		Faly	M
		Zandry	F
4	16	Koto[a]	M
		Eva[a]	F
		Hendry	M
		Gibet	M
5	9	Graham[a]	M
		Fern[a]	F
		Voary	M
6	18	Zokibe[a]	M
		Befotsy[a]	F
		Hira	M
8	9	Jonah[a]	M
		Bemasoandro[a]	F
		Cesare	M
		Mika	F
		Zafy	M
9	11	Emilio[a]	M
		Sissie[a]	F
		Dosy	F

Table 1. Summary of group ID, N of recorded songs per group, individual ID, and sex of the individuals of the social groups considered. All groups were sampled from 2011 to 2017, except Groups 3a (2015–2017) and 3b (2011–2014), because the reproductive male changed in 2015. The members of reproductive pairs are listed first for each group. [a] Denotes individuals aged 6 years or more at the time of recordings.

Results

Rhythmic features

The average wpIOI was 2.698 ± 0.710 s. Phonation positively influenced the average individual wpIOI, whereas it had a negative effect on individual bpIOI, showing that for an increase of the wpIOI there was a decrease of bpIOI. The full model significantly differed from the null model ($\chi^2 = 278.151$, df = 7, $P < 0.001$; see Table 2). Moreover, the nDPs negatively influenced the individual wpIOI. This result showed that the higher the number of the DPs in the song, the shorter were the IOIs within a phrase. The model (Table 2) revealed the tendency of the males to show longer wpIOI than females. In fact, average female wpIOI was 2.228 ± 0.408 s and 3.169 ± 0.630 s for males. When we ran models for separated sexes, they confirmed that phonation had a significant positive effect on wpIOI and that nDPs had negative effect on the wpIOI (null vs. full, females: $\chi^2 =139.538$, df = 7, $P < 0.001$; males: $\chi^2 =140.301$, df = 7, P < 0.001). These models also showed a positive correlation between song duration and wpIOI, but only for females (see Table 3). A GLMM considering both sexes showed that wpIOI had a positive effect on bpIOI, whereas phonation showed a negative correlation with bpIOI. The average bpIOI was 2.219 ± 0.330 s and the full model significantly differed from the null model ($\chi^2 =157, 912$, df = 7, P < 0.001; Table 2). We also found that the duration of the bpIOI was positively affected by nDPs. We found no influence of the interaction between the sexes and the number of singers. The females' average bpIOI was 2.037 ± 0.204 s, whereas the males' one lasted 2.340 ± 0.332 s. We found that bpIOI was positively correlated with song duration and nDPs for both sexes, but for males there was also a positive effect of the wpIOI and a negative effect of phonation (null vs. full, females: $\chi^2 = 91.550$, df = 7, P < 0.001; males: $\chi^2 =81.607$, df = 7, P < 0.001, see Table 3). We summarized the variation of wpIOI, bpIOI, and phonation and wpIOI, bpIOI, and contribution, in males and females, according to chorus size in Figure 2.

We have also found that the CVs of both wpIOI (paired t-test, t = 5.786, df = 8, $P < 0.001$) and bpIOI (paired t-test, t = 5.9627, df = 8, $P < 0.001$) were significantly higher in the females compared with males (Figure 3).

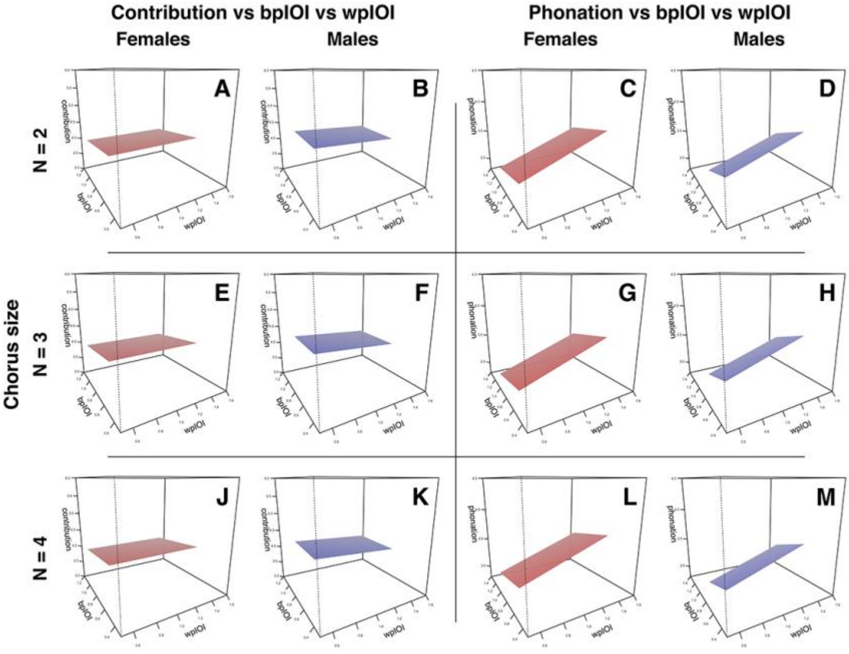

Figure 2. Interaction between contribution and phonation with bpIOI and wpIOI during the indris' song (N=119). The individual mean durations are 2.698 ± 0.705s for the wpIOI, 2.219 ± 0.330s for the bpIOI, 69.768 ± 32.452s for contribution and 28.416 ± 11.096s for phonation. The interaction is presented using the 3-dimensional surface (visreg package in R; Breheny and Burchett 2017; females in red A, E, J, C, G, and L; males in blue, B, F, K, D, H, and M).

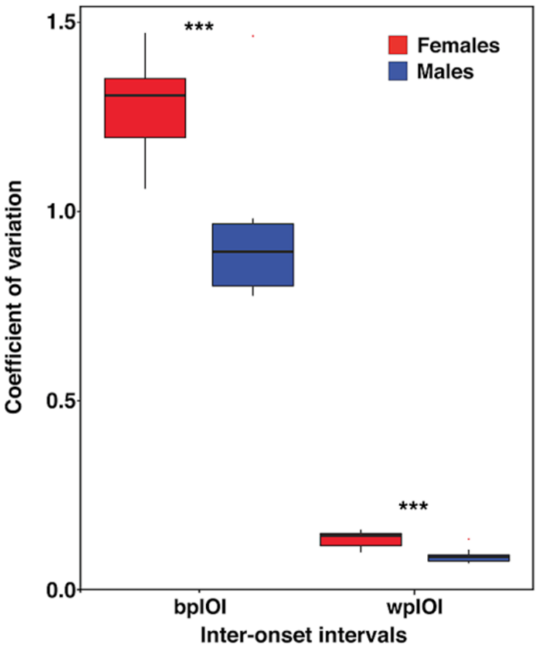

Figure 3. Boxplot of the CV of bpIOI and wpIOI in the sexes (males in blue and females in red). The values shown are calculated from the average individual means. Paired *t-test* significance at $P < 0.001$ is denoted by ***.

Contribution

We found that phonation and nDPs affected the contribution of an individual to the song when considering both the sexes together. The average song duration was 88.610 ± 39.512 s, whereas the duration of an individual's contribution was 69.768 ± 32.452 s. The full model significantly differed from the null model (χ^2 = 431.492, df = 7, $P < 0.001$, see Table 2). We also found a significant effect of the interaction between sex and number of singers on the duration of individual contribution. The Tukey test (see Table 4) revealed that there was a significant difference between sexes in the duration of the individual contribution, with female indris showing a more extended contribution than males when the number of singers in a chorus arose from 2 to 3 singers. We found that song duration and phonation had a positive effect on the average contribution for both the sexes when they were considered separately, but the models showed that only for male indris the contribution was positively correlated also with nDPs (null vs. full; females: $\chi^2 = 282.490$, df = 7, $P < 0.001$; males: $\chi^2 = 304.266$, df = 7, $P < 0.001$, see Table 5).

Phonation

Contribution, the wpIOI, and nDPs had a positive effect on phonation. We also found that phonation was negatively affected by the bpIOI. The average phonation of an indri was 28.416 ± 11.096 s. The full model significantly differed from the null model ($\chi^2 = 529.037$, df = 7, $P < 0.001$, see Table 2). The Tukey test (Table 4) showed that the females' phonation decreased when chorus size increased from 2 to 3 singers. We also found that phonation that the male's phonation was significantly longer than those of females in the songs with 3 singers. Running the models for each sex, we found that the phonation of both sexes was positively influenced by the contribution, the wpIOI, and the nDPs. We found that song duration had a negative effect on the females' phonation, and that the bpIOI had a negative effect on the males' phonation (null vs. full, females: $\chi^2 = 324.649$, df = 7, $P < 0.001$; males: $\chi^2 = 301.978$, df = 7, $P < 0.001$; see Table 5).

Contribution						Phonation					
Factors	Estimate	SE	df	t	P	Factors	Estimate	SE	df	t	P
(Intercept)	1.197	0.135	[a]	[a]	[a]	(Intercept)	0.280	0.120	[a]	[a]	[a]
SexM[b,c]	-0.121	0.039	30.611	-3.161	**0.004**	SexM[b,c]	0.133	0.037	17.385	3.610	**0.002**
3 singers[b,c]	0.080	0.025	121.147	3.263	**0.001**	3 singers[b,c]	-0.033	0.017	231.892	-1.923	0.056
4 singers[b,c]	0.093	0.058	117.284	-1.595	0.113	4 singers[b,c]	-0.023	0.039	228.877	-0.593	0.554
Phonation	0.835	0.062	190.165	13.406	**<0.001**	Contribution	0.474	0.035	230.439	13.408	**<0.001**
bpIOI	-0.089	0.159	155.135	-0.557	0.578	bpIOI	-0.518	0.124	222.756	-4.182	**<0.001**
wpIOI	0.065	0.119	228.283	0.547	0.585	wpIOI	0.605	0.079	223.753	7.624	**<0.001**
nDps	0.118	0.058	227.794	2.040	**0.042**	nDps	0.419	0.034	229.963	12.407	**<0.001**
wpIOI						**bpIOI**					
(Intercept)	-0.316	0.082	[a]	[a]	[a]	(Intercept)	0.565	0.051	[a]	[a]	[a]
SexM[b,c]	0.061	0.017	20.270	3.608	**0.002**	SexM[b,c]	0.040	0.026	8.904	1.501	0.168
3 singers[b,c]	0.022	0.013	113.004	1.693	0.093	3 singers[b,c]	-0.004	0.008	225.495	-0.510	0.610
4 singers[b,c]	0.035	0.030	107.552	1.154	0.251	4 singers[b,c]	-0.004	0.019	222.023	-0.214	0.831
Contribution	0.010	0.033	174.785	0.306	0.760	Contribution	-0.009	0.023	224.506	-0.401	0.689
bpIOI	1.003	0.059	70.350	16.997	**<0.001**	wpIOI	0.453	0.032	237.055	14.230	**<0.001**
Phonation	0.299	0.040	94.548	7.496	**<0.001**	Phonation	-0.128	0.031	231.333	-4.139	**<0.001**
nDps	-0.304	0.024	207.741	-12.741	**<0.001**	nDps	0.119	0.020	228.190	6.013	**<0.001**

Table 2. Influence of the fixed factors on contribution, phonation, wpIOI, and bpIOI. Statistically significant values are indicated in bold.

[a] Not shown as not having a meaningful interpretation. [b] Estimate ± SE refer to the difference of the response between the reported level of this categorical predictor and the reference category of the same predictor. [c] These predictors were dummy coded, with the “SexF,” “2 singers” being the reference categories.

	Females					Males				
	Estimate	SE	df	t	P	Estimate	SE	df	t	P
wpIOI										
(Intercept)	-0.493	0.113	[a]	[a]	[a]	-0.195	0.139	[a]	[a]	[a]
3 singers[b,c]	-0.004	0.017	115.460	-0.247	0.805	0.018	0.022	118.615	0.820	0.414
4 singers[b,c]	-0.043	0.036	113.106	-1.172	0.244	0.087	0.051	116.410	1.695	0.093
Song duration	0.127	0.038	113.533	3.362	**0.001**	-0.020	0.036	116.866	-0.569	0.570
Contribution	-0.039	0.047	115.127	-0.835	0.406	-0.133	0.082	118.975	-1.617	0.109
Phonation	0.234	0.058	116.536	4.052	**<0.001**	0.492	0.079	118.197	6.223	**<0.001**
bpIOI	0.977	0.092	116.307	10.608	**<0.001**	1.029	0.082	64.541	12.482	**<0.001**
nDPs	-0.271	0.035	118.642	-7.789	**<0.001**	-0.325	0.038	118.782	-8.617	**<0.001**
bpIOI										
(Intercept)	-0.586	0.069	[a]	[a]	[a]	0.541	0.087	[a]	[a]	[a]
3 singers[b,c]	0.001	0.012	114.010	0.072	0.943	-0.007	0.014	112.982	-0.529	0.598
4 singers[b,c]	0.025	0.026	112.085	0.980	0.329	-0.057	0.032	110.738	-1.804	0.074
Song duration	0.494	0.047	118.742	10.565	**<0.001**	0.054	0.023	112.993	2.397	**0.018**
Contribution	-0.044	0.028	112.440	-1.581	0.117	0.000	0.053	112.170	-0.003	0.998
Phonation	-0.011	0.033	114.443	-0.341	0.734	-0.196	0.054	112.254	-3.601	**<0.001**
wpIOI	-0.060	0.044	118.794	-1.373	0.172	0.427	0.043	117.899	9.896	**<0.001**
nDPs	0.083	0.030	118.732	2.804	**<0.001**	0.145	0.027	111.786	5.295	**<0.001**

Table 3. Influence of the fixed factors on male's and female's inter-onset intervals. Statistically significant values are indicated in bold. [a] Not shown as not having a meaningful interpretation. [b] Estimate ± SE refer to the difference of the response between the reported level of this categorical predictor and the reference category of the same predictor. [c] These predictors were dummy coded, with "2 singers" being the reference category.

Sex x Chorus size	Contribution				Phonation				wpIOI			
	Estimate	SE	z	P	Estimate	SE	z	P	Estimate	SE	z	P
F(3)-F(2)	0.162	0.031	5.262	**<0.001**	-0.091	0.023	-3.293	**<0.001**	0.027	0.017	1.559	0.592
F(4)-F(2)	0.193	0.075	2.567	0.091	-0-006	0.054	-0.101	1000	-0.014	0.041	-0.341	0.999
F(4)-F(3)	0.031	0.074	0.416	0.998	0.086	0.052	1.652	0.518	-0.041	0.040	-1.045	0.888
M(2)-F(2)	-0.040	0.038	-1.061	0.880	0.081	0.040	2.087	0.256	0.061	0.019	3.232	**0.013**
M(3)-F(3)	-0.202	0.040	-5.105	**<0.001**	0.198	0.040	5.004	**<0.001**	0.050	0.021	2.348	0.154
M(4)-F(4)	-0.237	0.092	-2.582	0.087	0.045	0.078	0.568	0.991	0.155	0.052	2.979	**0.029**
M(3)-M(2)	0.001	0.031	0.014	1.000	0.025	0.022	1.102	0.858	0.015	0.017	0.921	0.932
M(4)-M(2)	-0.003	0.073	-0.046	1.000	-0.042	0.053	-0.802	0.959	0.080	0.040	2.037	0.291
M(4)-M(3)	-0.004	0.073	-0.053	1.000	-0.067	0.051	-1.314	0.743	0.064	0.039	1.659	0.524

Table 4. Results of the Tukey test for the interaction including sex and chorus size (bpIOI not shown as not significant for the interaction). Statistically significant values are indicated in bold. M, males; F, females; (2), 2 singers; (3), 3 singers; (4), 4 singers.

	Females					Males				
	Estimate	SE	df	t	P	Estimate	SE	df	t	P
Phonation										
(Intercept)	0.632	0.173	[a]	[a]	[a]	-0.160	0.136	[a]	[a]	[a]
3 singers[b,c]	-0.038	0.025	113.389	-1.517	0.132	0.000	0.022	118.946	0.008	0.994
4 singers[b,c]	0.048	0.053	112.552	0.902	0.369	-0.074	0.052	117.132	-1.416	0.160
Contribution	0.453	0.054	112.175	8.327	**<0.001**	0.689	0.054	111.911	12.769	**<0.001**
Song duration	-0.147	0.056	113.328	-2.632	**0.010**	0.045	0.035	101.780	1.282	0.203
woIOI	0.523	0.126	116.454	4.157	**<0.001**	0.498	0.079	111.659	6.281	**<0.001**
bpIOI	-0.269	0.188	118.421	-1.429	0.156	-0.452	0.112	56.421	-4.040	**<0.001**
nDPs	0.550	0.038	112.533	14.357	**<0.001**	0.153	0.046	116.192	3.312	**0.001**
Contribution										
(Intercept)	-0.821	0.291	[a]	[a]	[a]	0.748	0.139	[a]	[a]	[a]
3 singers[b,c]	-0.016	0.034	107.015	-0.472	0.638	-0.036	0.024	117.760	-1.527	0.129
4 singers[b,c]	0.013	0.073	117.788	0.179	0.858	-0.070	0.057	114.501	1.233	0.220
Song duration	2.253	0.256	118.988	8.795	**<0.001**	0.115	0.038	118.902	3.025	**0.003**
Phonation	0.653	0.083	15.228	7.899	**<0.001**	0.827	0.065	118.456	12.695	**<0.001**
wpIOI	-0.034	0.168	34.494	-0.200	0.842	-0.162	0.100	118.895	-1.623	0.107
bpIOI	-0.302	0.228	35.668	-1.326	0.193	0.049	0.139	91.725	0.353	0.725
nDPs	-0.081	0.075	33.630	-1.083	0.286	0.161	0.051	118.046	3.165	**0.002**

Table 5. Influence of the fixed factors on male’s and female’s phonation and contribution. Statistically significant values are indicated in bold. [a] Not shown as not having a meaningful interpretation. [b] Estimate ± SE refer to the difference of the response between the reported level of this categorical predictor and the reference category of the same predictor. [c] These predictors were dummy coded, with “2 singers” being the reference category.

Discussion

We have analyzed how the rhythm of an indri's song may change depending on the sex of the emitter, the duration of its contribution, the cumulative duration of its phonation, the duration of the song, and the number of singers. We took into account 2 proxies of the rhythmic structure: the inter-onset interval of units within a phrase and the inter-onset interval between the beginning of the last unit of and the beginning of the next phrase. For the first time, we provide a more in-depth analysis of the structural variation of the song considering both phrase timing and unit timing. We demonstrated that these descriptors of the rhythmic variation show different trajectories in males and females.

Similarly to birds, the song of the indris comprises hierarchical sets of vocal gestures that reflect in a hierarchical organization of the indris' song in phrases and units (Yu and Margoliash 1996; Gamba et al. 2011). This organization suggests that indris are producing and potentially perceiving their songs at the level of both phrases and units, in agreement with evidence on gibbons (Terleph et al. 2018a). In agreement with previous studies, we found that, overall, the average duration of the wpIOI is positively correlated with the average duration of pauses between phrases (bpIOI). The number of DPs in the song was also positively correlated with bpIOI, suggesting that when the song has a high number of DPs the silent parts between the DPs are longer. In agreement with the work of Gamba et al. (2016), our findings showed that the average duration of wpIOI is strongly influenced by the sex of the emitter, with males showing significantly longer intervals between the onset of units than females. We have also found that the rhythmic structure of phrases is independent of the number of singers but wpIOI increased with the total song duration in females. Thus, we asked whether a higher number of singers would lead to other changes in song structure. We found that those changes correspond to females emitting longer contribution and diminishing phonation, leaving male singing invariant. In agreement with studies on the chimpanzees' pant hoots (Fedurek et al. 2017), the decrease of phonation for females could be due to the need of facing the higher cost of uttering a more extended contribution.

The singing rhythm, as defined by bpIOI, appeared influenced by different parameters between the sexes. In fact, in both sexes bpIOI is influenced by the number of DPs and song duration, but for males it also depends on phonation and wpIOI. We should expect that adult males should show a less variable input to the song than those of females, which instead should react more clearly to the increase in the number of singers. In fact, we found that the females' phonation is influenced by the increase in the number of singers from 2 to 3 and their phonation has a significant effect on song duration. This event is not happening in males whose phonation is not influenced by song duration and the number of singers. This evidence stresses the fact that changes in group size, which may, of course, result in changes in chorus size, would impact more on the females' singing than on males' singing as we know that song duration increases with the number of singers (Gamba et al. 2016). Given the fact that female contribution is a major determinant of the total song duration (Giacoma et al. 2010) and they overlapped with several group members (Gamba et al. 2016), we can hypothesize that female singing has a role in regulating the duration of males' contribution. Thus, we could ask whether females may have evolved higher flexibility to accommodate more singers in the song. In agreement with Gamba et al. (2016), we found that wpIOI duration differed significantly be- tween males and females. We found that the CV of both wpIOI and bpIOI was significantly higher in females, suggesting that they possess more flexible rhythmic abilities than males.

Since we know that indris can synchronize their utterance showing nonrandom overlap between singers (Gamba et al. 2016), an open question is whether the male more than the female or vice versa is changing the rhythm of its song to synchronize with the other singer. The results presented in this paper indicated that female contribution is critical in determining the temporal span of the song and that their contribution indeed changes more remarkably according to chorus size. The fact that females are more flexible than males in their contributions is in agreement with findings on the white-cheeked gibbons *N. leucogenys* (Deputte 1982) and indris (Torti et al. 2017). Deputte (1982) also argues that male singing may stimulate a longer duration of the female song, but lead to a re- duction in the number of bouts. Our findings are also in agreement with the results of recent studies on white-handed gibbons Hylobates lar, where female contribution varied in length. However, in the white-handed gibbon male contributions were more flexible than females' ones (Terleph et al. 2018b). On the contrary, female indris appeared to change the structure of their contribution to the song more consistently than males showing more flexibility than males in song expression. We observed a mechanism in the song of the indris that may resemble a turn-taking system with short turns and rapid responses in which most of the variation lie not in the internal structure of the phrases but the timing of phrases. This fact is in agreement with the experimental studies on cotton-top tamarins (Miller et al. 2003; Egnor and Hauser 2006) and common marmoset (Roy et al. 2011), which demonstrated that New World monkeys are capable of adjusting the timing of their contribution, exhibiting a certain degree of vocal control. These studies and the data presented in this paper support the hypothesis that vocal turn-taking has an ancestral origin in the primate order (Levinson 2016).

Different pieces of evidence support our first prediction that indri male's song has a more fixed pattern, whereas females could adjust their contribution. Whereas wpIOI can be considered as a proxy for the rhythmic structure of a phrase (Sasahara et al. 2015), bpIOI can inform about the rhythmic structure of the song. We found that bpIOI is correlated with the number of phrases and the duration of the wpIOI in both the sexes, but it was negatively correlated with phonation only in males. This result confirmed our second prediction, suggesting that males have a more predictable rhythmic song pattern than females, who are more flexible and their contribution may change with the number of singers. In fact, our works confirmed that female indris show higher coefficients of wpIOI and bpIOI variation than males. This result is in line of what previously found by Torti et al. (2017), where males showed acoustic and temporal characteristics that were more genetically determined than female’s ones, which showed a higher degree of plasticity. Thus, indris showed a pattern in line with the plasticity shown by female *Hylobates agilis* (Koda et al. 2013a, 2013b), but in contrast with what previously found by Traeholt et al. (2006) on *H. pileatus*, where the male calling structure seems to be affected by the female one. Our findings suggest that female indris sustain a higher cost of singing than males when the number of singers in chorus arises from 2 to 3. When we considered songs emitted by 4 singers, our results were inconsistent possibly because of the small sample (N = 5). Songs with a chorus size exceeding 3 indris are rare (Torti et al. 2018) because groups are usually smaller (Bonadonna et al. 2017). Large group size may indicate a difficulty to disperse of the offspring (Reichard and Barelli 2014), but further studies are needed to clarify this evidence.

In conclusion, we can assume that the rhythmic structure of indri’s songs depends on factors that are different for males and females. These differences have sense in the light of the different roles that sexes may have in achieving coordination in their vocal output and how do they adjust their singing. Our study demonstrates that, as suggested by Hall (2009), it is fundamental to distinguish the pair level aspects of duets and underlying individual behavior to understand how duets occur.

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Author Contributions

C.D.G. and M.G. designed the computational framework and analyzed the data. C.D.G., A.Z., D.V., V.T., G.B., and R.M.R. collected the data. C.D.G., A.Z., D.V., V.T., and M.G. performed the measurements. C.D.G., D.V., V.T., and M.G. wrote the manuscript with support from A.Z., G.B., R.M.R., and C.G.

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Chapter III

Intra- and intergroup spatial dynamics of a pair-living singing primate, *Indri Indri*: a multiannual study of three indri groups in the Maromizaha Forest, Madagascar

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Intra and intergroup spatial dynamics of a pair-living singing primate, *Indri Indri*. A multiannual study on three indri groups in the forest of Maromizaha, Madagascar.

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Abstract

Territorial pair-living species tend to occupy and defend stable areas, assumed to contain all the resources needed for the lifetime of the group. Furthermore, groups have to mediate spatial relationships with neighboring groups. We investigated the relationship between social and spatial dynamics at the intra- and intergroup level in a pair-living territorial singing primate: the indri (Indri indri). We collected spatial data on three neighboring groups during 396 sampling days between 2009 and 2014 in Maromizaha forest, Madagascar. We evaluated the stability of territories in terms of size and location using minimum convex polygons, defined the presence and stability of core areas, and investigated if singing locations and intergroup encounters were concentrated in the core areas. Territories were generally stable in location and size, although some degree of territorial shift occurred, leading to readjustment of intergroup spacing. Groups had core areas that were not stable across years but were concentrated in the area of the territories that groups occupy consistently over time (stable areas). Singing locations were equally distributed inside and outside core areas, suggesting an even distribution through the territories; meanwhile 9 of 12 intergroup encounters took place in the core areas at the edge of territories. Together, our results support the pattern of territorial stability predicted for a pair-living species, where groups regulate territory exclusivity and spacing with neighbors. Singing behavior also plays an important role in mediating intergroup spatial dynamics. The spatial pattern we found in indris is comparable with that found in other territorial and pair-living primates with different ecological needs, suggesting that in addition to ecological factors, social dynamics influence intergroup spatial dynamics.

Keywords: Core area· Indri· Intergroup spacing· Song spatial distribution· Territorial stability

Introduction

Animals adjust their spatial distribution to balance competing demands such as feeding, defending resources, reproducing, dealing with the presence of neighboring individuals or groups, and avoiding predators (Beyer et al. 2010; Lazaro-Perea 2001; Waser and Wiley 1979). The area where animals range to meet their survival needs is referred to as a home range (Burt 1943). One way to regulate the spacing between neighboring individuals or social units is to defend the exclusive use of an area against the intrusion of conspecifics, which we define as a territory and implies limited or no overlap between areas occupied by neighboring groups (Burt 1943; Clutton-Brock 1974; Maher and Lott 1995). When groups defend and exclusively occupy their entire home range, territory and home range coincide (Burt 1943). In territorial species, animals exhibit aggression toward conspecifics attempting to enter a territory (Grant et al. 1992). Strategies of territorial advertisement and defense may influence space use to maintain exclusive access to resources (Brown and Orians 1970; López-Sepulcre and Kokko 2005).

The degree of overlap between territories is the result of changing space use over time and is regulated by the stable occupation of an area, visiting certain areas more or less frequently and signaling territory occupancy to conspecifics (Van Belle and Estrada 2020). The degree of overlap between territories, although generally limited, may indicate the quality of the relationship between adjacent groups, with lower overlap indicating less tolerance against intrusions of conspecifics (Wrangham et al. 2007). Hence, territorial control leads to spatial dynamics that have a critical role in intergroup social dynamics at the population level (Furuichi 2020), requiring a consideration of social systems in territorial species.

In several species, and across taxa as diverse as Crustacea, Insects, Annelids, Fishes, Amphibians, and Mammals, pair-living co-occurs with a territorial model where the pattern of space use reflects mate guarding strategies and reproductive success (Clutton-Brock 1989; Emlen and Oring 1977; Lang and Jaeger 2000; Mathews 2002; Park and Choe 2003; Roberts and Ormond 1992). Territorial pair-living species tend to occupy a stable defended area that is assumed to include all the resources needed for survival and reproduction in the long term (Börger et al. 2008). Studies suggest that a pattern of stable use of an area over time, defined as site fidelity, is due to the predictability of food resources distribution (Asensio et al. 2012; Ramos-Fernandez et al. 2013). Furthermore, the presence of neighboring exclusive territories can limit the shift of territories over time, imposing a system of site fidelity. Consequently, site fidelity has implications for territoriality, because it can ensure limited investment in interactions with neighboring conspecifics (Bartlett et al. 2016). Studies of nonhuman primates have shown that when the habitat is not homogeneous, or resources are not evenly distributed in space and time, knowledge of food resource availability and distribution can make site fidelity advantageous (Janmaat et al. 2009; Ramos- Fernandez et al. 2013; Wartmann et al. 2014). Site fidelity appears weaker when food resources are abundant and evenly distributed, which is more common for folivorous species, such as gorillas (*Gorilla gorilla beringei*) (Watts 1998a), although gorillas tend to limit foraging costs by balancing the intensity of use of an area with the regeneration of food resources (Watts 1998b).

The stable occupation of an area does not imply an even use of the space therein. On the contrary, the intensity of use of certain areas depends on resource distribution and interactions with conspecifics or neighboring groups. Group-living primates may use some areas of their range more intensively for resting, feeding, and social behaviors (Bates 1970). Such regions are defined as core areas and are considered to have important biological functions for survival (Asensio et al. 2014; Burt 1943; Samuel and Green 1988). Core areas do not necessarily coincide with the geometrical center of the range; indeed, intensively used areas can be located at the periphery of a territory or home range (Asensio et al. 2014), and can be more or less stable over time depending on the distribution of preferred food resources and the need for territorial defense (Asensio et al. 2014; Vander Wal and Rodgers 2012). The concepts of core area and territory are based on different assumptions: a core area is defined in terms of intensity of use, while a territory is defined as the area exclusively occupied and defended by a group (Asensio et al. 2014). Core areas can be identified within territories or in nondefended home ranges; however, a core area can be considered a territory if it represents the area of the range that is exclusively occupied and defended against intrusions (Bates 1970; Wartmann et al. 2014).

In addition to preferring core areas, groups may also tend to avoid other areas if there is a risk of potentially dangerous intergroup encounters (Wrangham et al. 2007). For example, in Javan gibbons (*Hylobates moloch*), individuals —especially males— select sleeping sites away from the location of encounters (Yi et al. 2020), and capuchin monkeys (*Cebus capucinus*) tend to reduce the risk of encounters by avoiding shared or peripheral areas of territories or home ranges (Tórrez-Herrera et al. 2020). A strategy to reduce the costs associated with territorial defense is the use of signals that allow long- distance communication, reducing the occurrence of physical encounters or fights. Loud calls play an essential role in signaling territory occupancy or defense and can trigger responses affecting the spacing patterns of neighboring groups (Cowlishaw 1992; Pollock 1986). The spatial responses to neighboring loud calls can also be influenced by groups' relative dominance and resource availability, as in howler monkeys (*Alouatta palliata*) (Hopkins 2013). The pattern of emission of such signals within a territory depends on the broadcast distance, the cost of emission, and the behavioral response of the receivers (da Cunha and Byrne 2006; Van Belle et al. 2013). In wild gray-cheeked mangabeys (*Lophocebus albigena*), for example, long-distance calls can influence the movements of resident individuals with respect to feeding resources (Brown, special issue).

Because loud calls can travel long distances, the broadcasting location in a relatively small territory does not limit communication with neighboring groups. Groups can advertise the occupancy of a territory and regulate intergroup spacing without needing to concentrate loud calls at the boundaries. Indeed, when the function of the call is to advertise territory occupancy and defensive potential, loud calls tend to be spread out within a territory, to advertise occupation (da Cunha and Byrne 2006).

The indri (*Indri indri*) is a pair-living primate that lives in groups of two to six individuals, consisting of a reproductive pair and their offspring (Pollock 1986). Genetic monogamy is the norm in this species (Bonadonna et al. 2019), and only one case of extra-pair copulation has been reported between two reproductive individuals of neighboring groups (Bonadonna et al. 2014). Individuals pair for years. Most reproductive pairs in our study population in Maromizaha, Madagascar, have been together since they were habituated in 2009, although rare cases of takeover or new pairing following the death of the partner have been reported (Bonadonna et al. 2019).

Both sexes disperse in indris, and in our study population, offspring remain with the family group for 4 years, on average (*unpubl. data*). Each group's range is an exclusive and defended area; the home range coincides with the territory and there is little or no overlap between neighboring territories. The ranging pattern does not indicate a constant patrolling of the boundaries and groups take *ca.* 2 weeks to range in the whole territory, requiring at least 16 days of observation to reach an accurate estimate of territory size (Bonadonna et al. 2017; Pollock 1986).

Indris emit songs in which the number of singers ranges from two—usually the reproductive pair—to five (Torti et al. 2018). The song is a cost-efficient way to communicate over long distances and one of the main functions is to regulate territorial occupancy, in addition to broadcasting individual cues (Gamba et al. 2016; Torti et al. 2017). Advertisement and territorial songs have different characteristics and can be recognized from their acoustic structure and the context of emission. Advertisement songs are emitted in the absence of visual contact between groups, they are shorter in duration, and the overlap between notes is limited. Territorial songs are emitted only when groups are in visual contact on a territorial boundary, they can last five times longer than the average advertisement song and individuals' contributions overlap highly (Torti et al. 2013). Intergroup encounters are infrequent (on average one encounter every 20 days) and restricted to the peripheral areas of the territory (on average within 22 m of the boundaries). In the majority of cases (86%), the encounters are solved with the emission of territorial songs and encounters rarely (13%) involve chases and physical fights (Bonadonna et al. 2017).

We investigated the relationships between social and spatial dynamics in indris and tested whether differential intensity of space use within a territory is related to inter- group dynamics. Because of the fine regulation of territory exclusivity between neigh- boring groups and the rare use of songs among primates, the indri is an interesting model to study the implications of space use for intergroup dynamics over time. We aim to extend previous findings concerning the spatial behavior of this species (Bonadonna et al. 2017), investigating 1) the stability of territories across time, 2) the presence and stability of core areas over time; and 3) the spatial distribution of singing locations and intergroup encounters within the territories, comparing core areas and noncore areas.

According to the hypothesis that high territorial stability reduces the costs associated with spatial mediation between neighboring groups, indris should show a pattern of territorial stability similar to that found in other pair-living territorial primates, and that over time the pattern should reflect the history of spatial dynamics between groups (Bartlett et al. 2016; Fernandez-Duque 2016; Van Belle et al. 2018; Wartmann et al. 2014). Therefore, we predict that indri groups will occupy the same area across successive periods with a limited territorial shift.

Core areas are assumed to contain important resources for survival and can shift over time according to spatial needs such as the distribution of preferred food resources. However, these ecological needs might not be the only drivers of the pattern of space use and groups may also spend more time in particular areas in response to the need for exclusive territories (Asensio et al. 2014). We hypothesize that indri groups regulate space use according to the distribution of preferred food resources and their need for exclusive territories, resulting in changes in the intensity of space use over time. Therefore, we predict that core areas will show low stability over time.

In the indri, the overlap between territories is extremely low, and intergroup encounters have a medium risk of aggression (Koch et al. 2016; Wrangham 2007) and are restricted to the peripheries of territories (Bonadonna et al. 2017). We hypothesize that indri adopt a strategy to reduce the costs associated with territorial defense concentrating core areas in the stable and exclusive area of the territory while limiting the encounters in areas intensely used on the periphery of the territory. Hence, we predict higher intensity of use within overall stable areas—areas constantly occupied by groups across years—and a concentration of intergroup encounters in the core areas rather than in noncore areas.

Indris' calling has an effective distance that extends up to 2 km, beyond the range of a single territory (Pollock 1986; Torti et al. 2017), so the advertisement can reach receivers independently from the sender's location within the territory. We test the hypothesis that loud calls function to signal territory occupancy and that call distribution is spread out in the territory (da Cunha and Byrne 2006). Thus, we predict that calling locations are equally distributed in the core and noncore areas.

Methods

Study Site and Subjects

The New Protected Area (Nouvelle Aire Protégée [NAP]) of Maromizaha (18°56′S, 48°27′E) is part of the forest corridor Ankeniheny–Zahamena (CAZ). It is located in the Alaotra–Mangoro region, in the district of Moramanga, in eastern central Madagascar. Maromizaha extends for 1880 ha covered with tropical mid-altitude (800–1200) evergreen rainforest, comprising both primary and anthropologically disturbed secondary succession, with annual rainfall of 1779 mm and an endemism of 77% (Randrianarison et al. 2015). This kind of forest is characterized by a single stratum 20–25 m high, above an undergrowth of plentiful shrubs and herbaceous plants (Koechlin 1972). Maromizaha includes an ecotourism area, but the indri groups included in this study are located in the off-limit research area, avoiding exposure to tourists that might affect the behavior of the focal groups.

We collected spatial data on three habituated indri groups (1MZ, 2MZ, 3MZ; Table 1).

Data Collection

Researchers and trained research guides that contributed to data collection collected data on three focal groups during four study periods between 2009 and 2014 (Table I). We did not have a data set robust enough for all the three groups to analyze 2009 and 2013 separately (Bonadonna et al. 2017). Because we wanted to investigate spatial dynamics across time, we prioritized continuity and pooled data collected during November and December 2009 with data for 2010, and data collected in February and March 2013 with data for 2012.

Given the indris' diurnal habits (Pollock 1975), we started observations early in the morning, at ca. 06:00 h, when individuals begin to be active, and we followed groups until they became inactive at ca. 13:30 h. We identified individuals based on their pattern of fur color.

We recorded the location of the center of group members using a hand-held global positioning system (GPS Garmin MAP 76CSX), with an accuracy of ≤5 m. A previous study of indris’ spatial behavior showed that their ranging pattern is characterized by progressive directional displacements, and a group takes ca. 2 weeks to visit the entire territory (Bonadonna et al. 2017). We followed the same methodology as in the previous study, recording a new GPS point each time the animals reached a new location after having interrupted their previous activities and had moved ≥20 m from the previous location; we defined each recorded location as a stationary area (see Lair 1987; Bonadonna et al. 2017). Consequently, each waypoint has a different time value, and we weighted each location based on the time indris spent in each of them. Groups visited a mean of three stationary areas per day (Table 1). This method, based on biological relevance rather than arbitrary time intervals between recorded locations, allowed us to avoid autocorrelation between points while maintaining biological information. We included all waypoints recorded during focal observations in analyses.

Every time the focal group emitted a spontaneous advertisement song or was involved in intergroup encounters during focal observations, we noted the geographical coordinates. We did not use playback (which could bias singing locations).

Group code	**Study periods**	**Group size**	**Group composition**	**Total locations recorded**	**Total days of sampling**	**Sampling months**
1MZ	2009-2010	3	af, am, sam	107	29	Dec 2009 Apr; Oct-Dec 2010
	2011	4	af, am, sam, jf	122	30	Sept-Dec
	2012-2013	4	af, am, saf, jf	141	45	Jan-Feb; Apr-Oct; Dec 2012
	2014	3	af, am, j	86	32	Feb-Apr; Jun-Sept
2MZ	2009-2010	3	am, af, j	109	33	Nov-Dec 2009 Mar; Oct-Dec2010
	2011	2	am, af	90	23	Aug-Dec
	2012-2013	2	am, af, jf	150	45	Feb; Apr-Dec 2012 Feb-Mar 2013
	2014	3	am, af, saf	42	17	Feb-Sep
3MZ	2009-2010	4	am, af, sam, jf	128	40	Nov-Dec 2009 Feb-Mar; Oct-Nov2010
	2011	4	am, af, sam, jf	149	38	Aug-Dec
	2012-2013	3	am, af, saf	83	30	Apr-Jul; Sept-Dec 2012 Feb-Mar 2013
	2014	4	am, af, saf, jm	84	34	Feb-Sept
Total				1291	396	

Table 1. Data set description. For each group, we provided group size and composition (af/am: adult female and adult male, sam/saf: sub-adult male and female, jf/jm: juvenile female and male; j: juvenile sex unknown) total number of locations recorded for that study period, the number of sampling days and months of data collection.

Spatial and Statistical Analysis

We analyzed all spatial data in ArcGIS 9.3 or ArcGIS 10.1 (ESRI 2012). We calculated the linear extension of the territories using minimum convex polygon (MCP) 100% because the data set is robust enough to obtain estimates of the territories with an accuracy higher than 90% (see Bonadonna et al. 2017 for methodological details). We performed all statistical tests in IBM SPSS 22. We report means with their standard deviation (SD).

Territory Stability

We investigated the degree of stability of territories across four study periods using three different parameters: the variability in size of a territory between two consecutive study periods, the extent of territory persistently occupied by a group across the years (overall stable area), and the difference in the geometric centroids of a territory between consecutive study periods. We report the size of territories in hectares (ha) for each study period and calculate the size variability as the absolute change in percentage of territory size between two consecutive study periods. To describe the extent of variability of territory size across years, we calculated the coefficient of variation (CV) for each group.

To obtain the persistent area occupied by a group over time, we followed the methodology described in previous studies of territories and home range stability in primates (Asensio et al. 2012; Bartlett et al. 2016; Janmaat et al. 2009). We overlapped all the annual MCPs of a group first, and then calculated the Minta Index (1992):

$$\frac{\bigcap_{i=1}^{n} a^{i}}{\sqrt[n]{\prod_{i=1}^{n} a^{i}}}$$

Where $\cap_{i=1}^{n} a^{i}$ the overall intersection of *n* areas (*n* representing the number of annual territories), and $\Pi_{i=1}^{n}$ is the product of those areas, so that $\sqrt[n]{\Pi_{i=1}^{n} a^{i}}$ is the geometric mean of all the territory extensions obtained for each group. The Index can range between 0% (no overlap) and 100% (complete overlap) among areas. Values between 0 and 33% are classified as low overlap, 34–66% as moderate overlap, and 67–100% as high overlap (Kernohan et al. 2001). We also report the percentage of overlap of annual MCP with the overall stable area for each group (and respective CV) and calculated the overlap of territories between two study periods, in terms of percentage of territories maintained in the following year.

Finally, to quantify the centroid shifts, we computed X and Y coordinates of the geometric centers of annual MCPs (100%) using the Spatial Analyst tool in ArcGis 9.3 (Hooge and Eichenlaub 1997). We then calculated the linear distance (m) between centroids observed in two consecutive periods.

Core Area Designation and Stability

In our data set, the time spent by a group at each recorded GPS waypoint may vary, depending on how long a group remained stationary. Hence, the number of waypoints does not reflect the intensity of use of an area. To measure the differential intensity of use within each territory, we created a grid with hexagonal cells of 0.5 ha each, using the ArcGis extension Patch Analyst (Asensio et al. 2012; Rempel et al. 2008; Rempel and Kaufmann 2003). By summing the minutes spent at each waypoint included in a cell, considering only cell grids containing stationary area centroids, we obtained the cumulative time spent at each hexagon by a group.

To evaluate differential intensity of use throughout the territory, we used ArcGis to identify four classes of intensity of cell use based on their time value. By setting 25% thresholds in the distribution of time values, we obtained a map showing four categories of intensity of use throughout the territory, with the lowest 25% representing the least most intensely used cells and the highest 25% the most intensely used cells. We report the territory size calculated using the hexagonal grid for comparison with the MCP method in Electronic Supplementary Material (ESM) Table S1.

Next, we ordered the cells according to their time values and identified the smallest number of cells that made up to 50% of the observation time. This allowed us to identify the smallest area of the territory in which a group spent at least 50% of the time, which we defined as the core area. There was no significant correlation between the percentage of territory representing the core area and absolute territory size (Pearson's correlation: $r = 0.1$, $P = 0.656$, $N = 12$). In addition, there was no correlation between sampling effort (number of months per study period) and the size of the core area (Spearman's rho: $r_s = 0.4$, $P = 0.227$, $N = 12$).

To estimate the stability of the core areas across study periods, we quantified size variability between study periods and the Minta Index, both calculated as described for territory stability. We defined the stable core area as the hexagons maintained consistently as core areas across study periods. We report the extent of core area shared between two consecutive periods for each group, calculated as the percentage of the core area maintained from the previous period. We obtained the proportion of core area included in the territory in two consecutive study periods by calculating the percentage of core area for one study period that was included in the MCP of the next study period. Finally, we reported the percentage of the core area included in the overall stable area of territories.

Spatial Distribution of Intergroup Encounters and Singing Locations

We recorded 12 intergroup encounters during the study, 4 for each group, and noted if encounters were resolved through the emission of territorial songs or if they involved physical fights. We plotted the encounters in the territories and reported the percentage located in the core areas. To account for movements of the groups during intergroup encounters, we applied a 20 m buffer (10 m radius) to each encounter point.

We recorded 191 singing locations for the three groups during the study: 77 for 1MZ, 64 for 2MZ, and 50 for 3MZ. We plotted the singing locations on the annual core area for each of the three indri groups, then used the ArcGis tool point count to obtain the number of singing locations inside and outside the core area for each territory. We then compared the frequency of the total number of singing locations inside and outside the core areas for each group. We used a chi-squared test of goodness-of-fit ($\alpha \leq 0.05$) to compare the number of singing locations between core and noncore areas. We defined expected values based on the null hypothesis of an even spatial distribution of singing locations, given that the groups spent half of the observation time inside or outside the core areas.

Results

Territory Stability

The three groups tended to have stable territories across the study period (2009–2014) (Fig. 1a). The results are consistent across the three parameters considered: size variability, intragroup territory overlap, and centroid shift, although we observed a degree of flexibility in the territories (Tables 2 and 3).

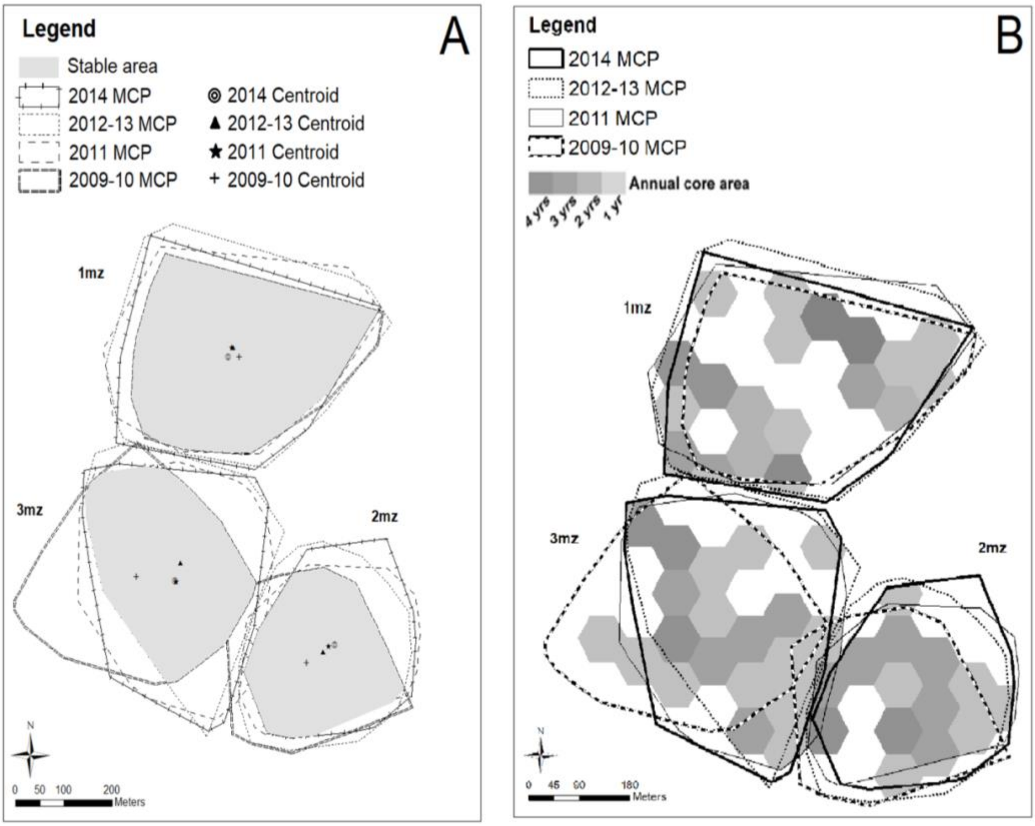

Figure 1. Territory and core area stability for the indri groups 1MZ, 2MZ, 3MZ over four study periods (2009-2014) in the Maromizaha forest, Madagascar. Dashed lines indicate the annual MCP for each group. **A.** Intragroup territories overlap wit and centroids for each study period. The gray area represents the overall stable area for each group. **B.** Overlap of the core areas obtained in each study period. Progressive darker shades represent core area shared in multiple study periods (one to four). White areas never resulted classified as core area during the study periods.

Size Variability

We found an overall mean territory size of 12.7 ± SD 2.8 ha (N = 12), ranging 9.2–17.5 ha [with the grid method we obtained an overall mean territory size of 16.0 ± SD 2.5 ha (N = 12), ESM Table S1]. The overall mean difference in territory size between two consecutive study periods was 10.6 ± SD 5.5 % (N = 9), ranging 13–22% for group 1MZ, 4–7% for group 2MZ, and 8–12% for group 3MZ. Group 1MZ showed the greatest increase in territory size over time, group 2MZ had the smallest and most stable territory size across years, and group 3MZ was intermediate in terms of territory size (Table 2). The CVs show comparable variability in territory size across years among groups (Table 2).

Study period	**Territory size (ha)**			**CA size (ha)**		
	1MZ	**2MZ**	**3MZ**	**1MZ**	**2MZ**	**3MZ**
2009-2010	12.7	9.2	13.4	3.4	2.5	4
2011	15.3	9.6	14.7	3.8	2.2	4.2
2012-2013	17.5	10.2	12.9	4.8	3.5	3.8
2014	15	9.6	13.9	2.7	3.1	2.9
Mean ± SD	15.1±2.0	9.7±0.4	13.7±0.9	3.6±0.9	2.8±0.6	3.7±0.5
CV (%)	13	4.3	6.8	34	21	14,7
Overall Stable Area (ha)	12.2	6.88	8.7	0.9	0	0

Table 2. Territory and core area size and stable area. Territory and core area size for each indri group in each sampling period and the respective mean, standard deviation (SD), and coefficient of variation (CV). The table includes the area constantly occupied by a group across sampling periods (overall stable area) for territories and core areas, respectively.

Intragroup Territory Overlap

Groups 1MZ and 2MZ show high territorial overlap across time, and group 3MZ is at the upper limit of medium overlap (Table 3). The overall mean overlap between the MCP of a given study period and the overall stable area was 63.9 ± SD 14.0% (N = 12), ranging between 59.2% (group 3MZ) and 96.3% (group 1MZ). Although group 1MZ presents the highest value of overall overlap of its territory across time, it is also the group with the highest CV (Table 2), indicating higher flexibility in annual territory overlap with the overall stable area. The degree of territory overlap between consecutive study periods increased over time for groups 1MZ and 2MZ, but not for group 3MZ (Fig. 2a).

Study period	**Overlap MCP-overall stable area (%)**			**Overlap CA-overall stable CA (%)**		
	1MZ	**2MZ**	**3MZ**	**1MZ**	**2MZ**	**3MZ**
2009-2010	96.3	73.7	65.2	29.1	0	0
2011	79.6	71.1	59.2	26.2	0	0
2012-2013	69.9	66.7	67.4	20.6	0	0
2014	81.2	70.9	62.7	37.1	0	0
Mean ± SD	81.7±11.0	70.6±2.9	63.6±3.5	28.2±6.7	0	0
CV (%)	13.4	4.1	5.5	24	0	0
Minta Index (%)	81.2	70.5	63.5	27.6	0	0

Table 3. Territory and Core Area Overlap with Respective Overall Stable Area and Minta Indices. Percentage of overlap between the Minimum Convex Polygon (MCP) of a sampling period and overall stable territory; percentage of overlap between the core area of a sampling period and the overall stable core area. We reported mean, standard deviation (SD), and coefficient of variation (CV) for each indri group. Minta Indices represent the degree of overlap for each group (high 67-100%, moderate 34-66%, low 0-33%, Kernohan et al. 2001).

Centroid Shift

The centroids showed little shift over time with an overall mean of 32 m ± SD 24 (N = 12), ranging between 2 m (group 1MZ) and 82 m (group 3MZ). The mean centroid shift between two consecutive periods was 14 ± SD 11 m (range: 2–22 m) for group 1MZ, 32 ± SD 19 m (range: 15–53 m) for group 2MZ, and 50 ± SD 28 m (range: 34–82 m) for group 3MZ (N = 3 for each group). We found the greatest centroid shift for group 3MZ between 2010 and 2011, which reflects the lowest values of interannual territory overlap found for this group (Fig. 2a). During the same time interval, we observed a 53 m centroid shift, the second largest, for the adjacent territory occupied by group 2MZ (Fig. 1a).

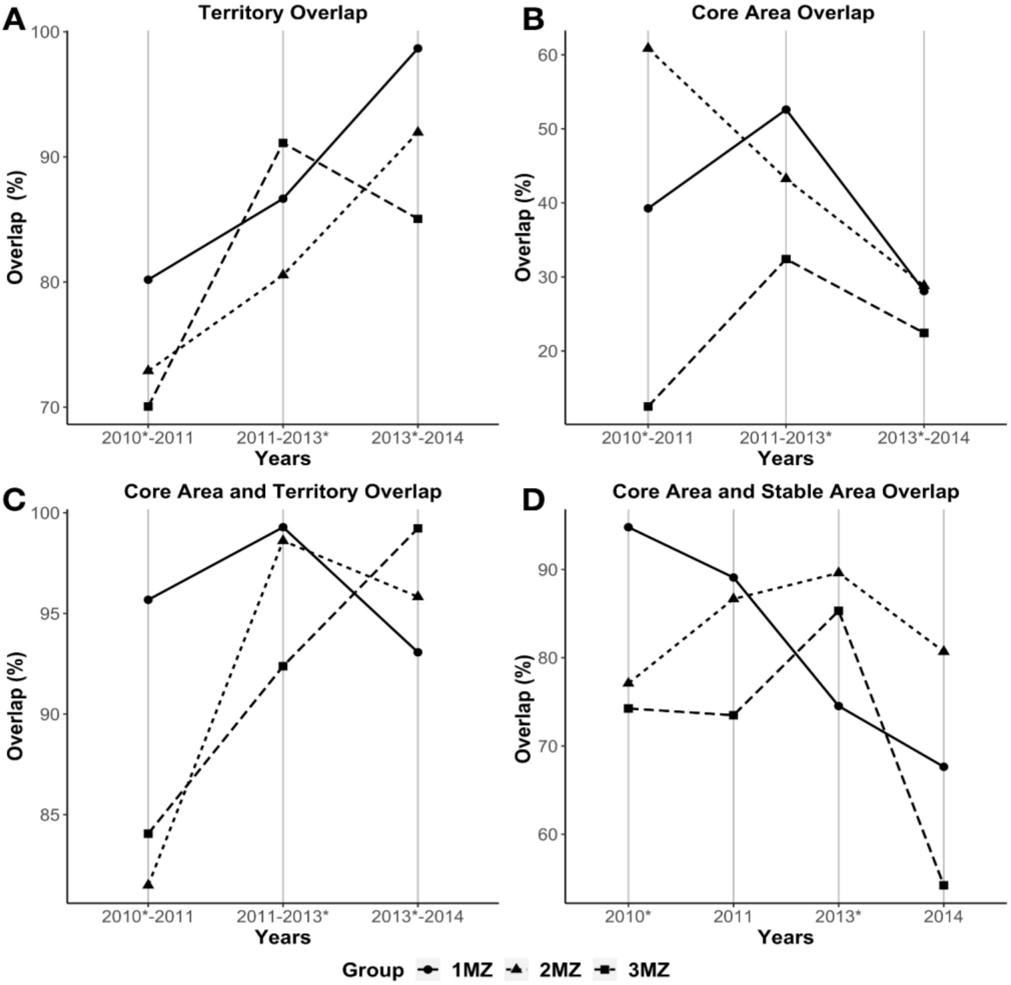

Figure 2. Territory and core area overlap for the indri groups 1MZ, 2MZ, 3MZ over four study periods (2009-2014) in the Maromizaha forest, Madagascar. Lines indicate the inter-annual variation for each group. The star (*) indicates a study period comprising two different years (e.g. 2010* comprises both 2009 and 2010). A. Intragroup territories overlap between study periods (%). B. Intragroup core area overlap between study periods (%). C. Extent of core area included in the territory of the consecutive study period (%). D. Extent of core area included in the overall stable area (%). In A, B, and C overlaps are expressed as percentage in relation to the total extension of the consecutive study period. In D. overlap is expressed as percentage in relation to the core area total extension, within the same study period.

Intensity of Use and Core Area

We found that areas with a higher intensity of use are scattered through a group's range and can be located in the center as well as in the peripheral areas of a territory (Fig. 3). The same is true for the areas used less intensely by a group in a given study period (Fig. 3). Groups spent 50% of their time in a mean area of 3.4 ± SD 0.8 ha (N = 12), with the core area representing 26.7 ± SD 4.7% (N = 12) of the territories (ESM Table S2). Considering each group separately across the four study periods, the core area represented 24.0 ± SD 4.4% (N = 4) of the territory for group 1MZ, 29.0 ± SD 5.1% (N = 4) for group 2MZ, and 27.1 ± SD 4.1% (N = 4) for group 3MZ. Core areas of neighboring groups never overlapped during a study period but could be adjacent to each other (Fig. 4).

We found considerable variation in core area size and location across time (Table 2). We found a greater change of core area size between study periods than for territory size, with an overall mean change of 22.2 ± SD 18.7% in size between consecutive study periods (N = 9). The high CVs indicate that the core area size of a group can greatly vary from one study period to another (Table 2).

All three indri groups exhibited low overlap of core areas across years (Table 3). Only group 1MZ showed core area overlap throughout the four study periods. Neither group 2MZ nor group 3MZ had an overall stable core area, although they occupied smaller territories than group 1MZ (Tables 2 and 3). We found a degree of intersection in the overlap of core areas between two consecutive study periods for all groups (ESM Fig. S1). Group 2MZ showed the highest percentage of core area overlap between two consecutive periods (61%), although the overlap decreased over time. Groups 1MZ and 3MZ showed a similar change in overlap size between 2 years over time, with group 3MZ presenting the lowest values, ranging 13–32% (Fig. 2b). Overall, 93.3 ± SD 6.5% (N = 9) of core areas were still included as part of the territory in the following year. Group 1MZ showed an overlap higher than 90% in all years; the other two groups showed a general increasing trend, with the last period showing overlap values >95% (Fig. 2c). Across the study period, 78.9 ± SD 11.2 % (N = 12) of core areas were located in the overall stable area, ranging between 54.2% (group 3MZ) and 95.8% (group 1MZ) (Fig. 2d).

Spatial Distribution of Intergroup Encounters and Singing Locations

Nine of 12 intergroup encounters were located in the core areas. All but one of the encounters were resolved through the emission of territorial songs. Groups 2MZ and 3MZ had a physical fight in 2011. Three of the four encounters recorded in 2011 involved the groups 2MZ and 3MZ, following a shift of the group 3MZ's territory toward the east that resulted in an overlap between the two groups (Fig. 4). In the following study periods, the vocal activity and intergroup encounters decreased com- pared to 2011, and the territories of the group 2MZ and 3MZ did not overlap (Fig. 4). Group 3MZ was larger than the other group: in 2011 group 3MZ was composed of four individuals including three singers and a 2-year-old nonsinging juvenile, meanwhile group 2MZ was composed of the reproductive pair only, after losing their infant in 2010 (Table 1).

Of the 191 singing locations recorded, 98 were inside the core areas (Fig. 4). A mean of 55.9 ± SD 5.2% (N = 3 groups) of songs were emitted from the core areas. We did not find a significant difference in the frequency of singing locations in core and noncore areas for any of the three groups: for group 1MZ, 37 locations (48%) were in the core area ($\chi_2 = 0.117$, $df = 1$, $P = 0.732$, $N = 77$); for group 2MZ, 32 locations (50%) were in the core area($\chi 2=0.000$, $df=1$, $P=1$, $N=64$);and for group 3MZ 29 locations (58%) were in the core area ($\chi_2 = 1.280$, $df = 1$, $P = 0.258$, N = 50).

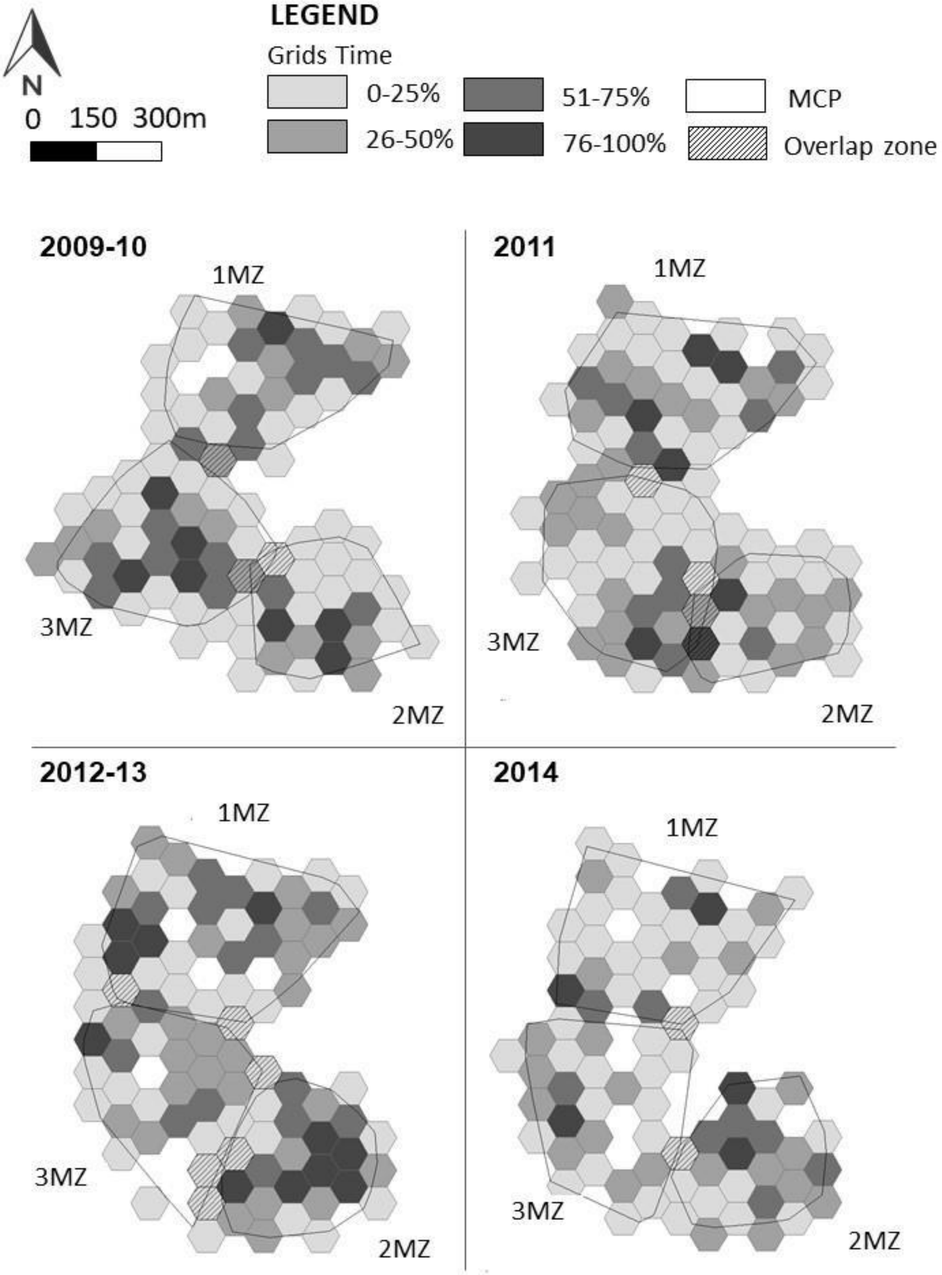

Figure 3. Intensity of use. The territories of three indri neighboring groups in four study periods between 2009 and 2014 are shown. The outlines represent the Minimum Convex Polygons (MCP). Different shades of gray represent the intensity of use in terms of time spent in a cell by a group as percentage of the total time of observation. The lined pattern indicates overlapping cells between groups.

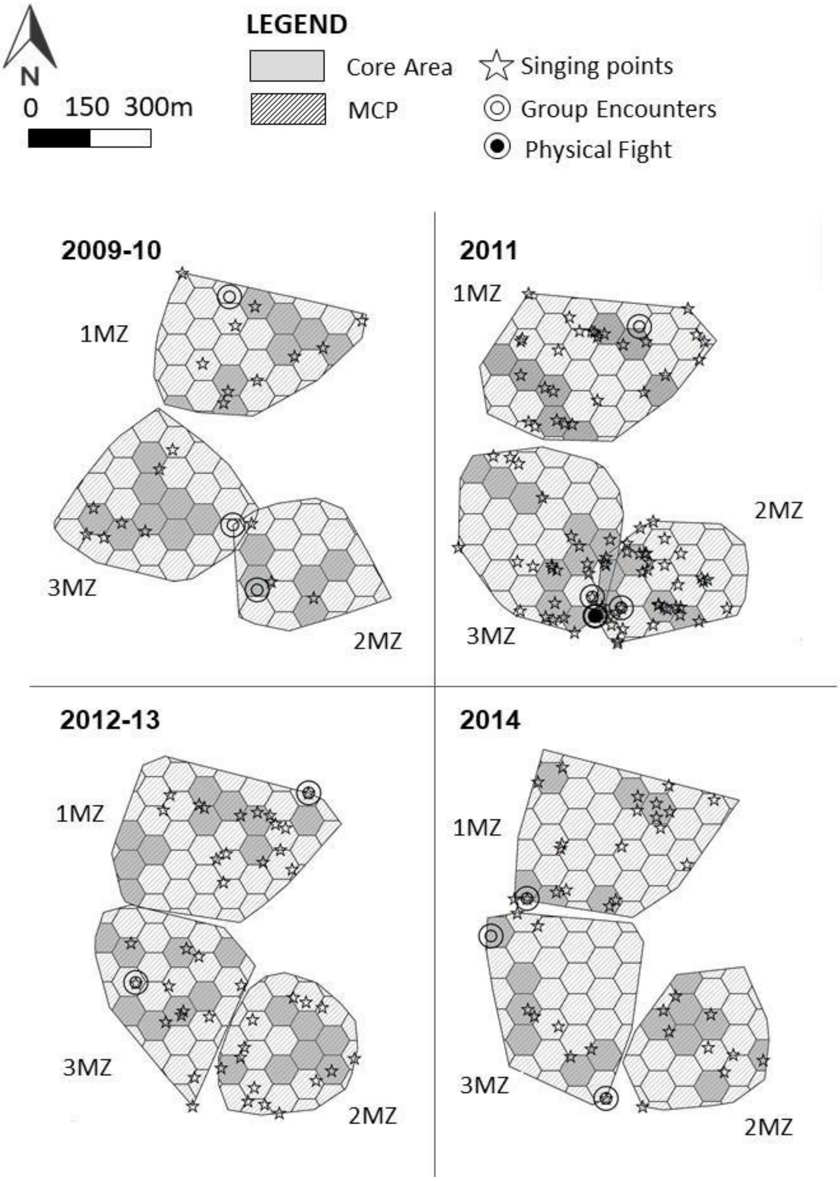

Figure 4. Core Area, spatial distribution of singing locations and intergroup encounters of three indri neighboring groups in Maromizaha, Madagascar, during four study periods.

Spatial distribution of core area (gray cells), singing location (stars), and intergroup encounters (circles) in each indri territory (MCP) during four study periods between 2009 and 2014 (each box represents a different period). The encounters include a 10 m radius buffering area. The black dot marks the only intergroup encounter that involved a physical fight besides the emission of territorial songs (2011).

Discussion

We found that indri territories were stable in terms of both size and location over four study periods. Core areas shifted over time but remained part of the territory in successive years and tended to be concentrated in the stable area of a territory. In particular, we found indris used some core areas over several years, while other areas were never included in a core area during the study. None of the three indri groups called more frequently from core areas than the rest of the territory, and intergroup encounters—although rare—were more frequent in peripheral core areas

Territory Stability

The indri groups showed a degree of site fidelity comparable with that of other pair-living primates: white-handed gibbons (*Hylobates lar*: Bartlett et al. 2016), Kloss' gibbons (*Hylobates klossii*: Tenaza 1975), titi monkeys (*Callicebus* spp.: Robinson et al. 1987), fat-tailed dwarf lemurs (*Cheirogaleus medius*: Fietz and Dausmann 2003), and owl monkeys (*Aotus azarae*: Wartmann et al. 2014). This pattern of high stability implies that a territory contains all the resources needed to support a group over the long term (Bartlett 2015; Vander Wal and Rodgers 2012). In a mainly folivorous species that occupies small territories, such as the indri, the pattern of food distribution and its availability in space and time does not seem to represent the principal variable in determining the pattern of space use.

Our results suggest that the high stability is a strategy to limit the costs of spatial competition between groups, keep exclusive use of the territory, and ensure exclusive access to the partner in a pair-living system. Once territorial boundaries are set, opportunities to shift territories without risking conflicts with neighboring groups are rare. The territorial dynamics and sequential shift observed between groups 2MZ and 3MZ (including the only case of physical fight reported in our study) suggest that territorial advertisement and defense are related to the territory exclusivity, which is considered a prerequisite in maintaining a monogamous mating system (Reichard and Boesch 2003). The group with weaker stability (3MZ) was involved in the extra-pair copulation reported in 2011 (Bonadonna et al. 2014), suggesting that territorial stability may coincide with social and reproductive intergroup dynamics.

Core Area

We found evidence that all the groups invested half of their time spent in stationary activities in less than a third of their territory. The intensive use of a core area within a territory is a common pattern among primates, e.g., chimpanzee (Pan troglodytes: Herbinger et al. 2001), howler monkeys (*Alouatta guariba clamitans*, *Alouatta caraya*: Agostini et al. 2010), owl monkeys (*Aotus azarae*: Wartmann et al. 2014), and gibbons (*Hylobates lar*: Asensio et al. 2014).

We found that core areas shift but tend to be included in the next year's territory, and they cover the whole territory over the years. Similar results have been found in multiannual studies of white-handed gibbons (Bartlett et al. 2016) and spider monkeys (*Ateles geoffroyi*: Asensio 2011), suggesting that a territory needs to include future core areas. Unlike the indri, white-handed gibbons and spider monkeys are highly frugivorous, and the shift of core areas has been explained by changes in the availability preferred food over time (Asensio et al. 2014). In contrast, a long-term study of a different population of spider monkeys found that core areas were more stable than the home range, probably owing to high fidelity to high-quality habitat (Ramos-Fernandez et al. 2013). This suggests that intraspecific variation can be due to the spatial distribution of food resources (Ramos-Fernandez et al. 2013). Finding a similar pattern of spatial stability among species with different diets, and a different pattern between populations of the same species, suggests that resource location is important in defining space use dynamics over time, but the role of territorial defense and control of mate access in pair-living species is also a contributing factor, as mate location is less predictable than the location of food resources.

Factors such as the regeneration of young leaves (>70% of the diet, Powzyk and Mowry 2003), the distribution of preferred resources in space and time, and geographic features of territories (i.e., presence of rivers and waterfalls, topography) may also influence the differential intensity of space use in indris. Our current data do not allow us to test this hypothesis.

Group Dynamics and Intergroup Encounters

Although limited in size and occurrence compared to home range overlap in nonterritorial species, overlapping zones between territories tend to be under-used, especially when encounters with neighboring groups involve the risk of dangerous fights (Tórrez-Herrera et al. 2020; Wrangham et al. 2007). In agreement with previous findings, we found that indri territories barely overlap (Bonadonna et al. 2017); thus, it is not surprising that intergroup encounters are rare in this species, although they can occur. Most of the encounters were located in heavily used areas located at the periphery of territories, which suggests that spending time at the territorial border increases the probability of an intergroup encounter, despite the limited or no overlap between territories. Groups may also spend more time in areas where an intergroup encounter took place to defend a disputed area of territory. Furthermore, we found that even if core areas were located in the peripheral area of a territory, they were concentrated in the overall stable area of a territory, suggesting that indri groups concentrate their activities in areas less affected by territorial changes, which can limit the risk of encounters in contentious areas.

Through the emission of songs, indris maintain an exclusive use of the territory, limiting the necessity of physical confrontation. This strategy of territorial defense and exclusivity can also reinforce an active mate guarding strategy: having exclusive territories and minimizing the risk of physical encounters can be a strategy to monopolize access to females (Reichard and Boesch 2003). During intergroup encounters, males of territorial species can discourage neighboring males attempting to mate with the resident female (Koch et al. 2016), while at the same time displaying their ability to defend a territory (Kempenaers and Dhondt 1993).

A good example of the role of territorial defense on maintaining exclusive access to the partner is given by comparing intergroup encounters and extra-pair copulations rates between indris and gibbons. Intergroup encounters in indris are rare. Only one observation of extra-pair copulation has been reported (Bonadonna et al. 2014) and genetic monogamy seems to be the norm in this species (Bonadonna et al. 2019). In contrast, gibbons have a high rate of intergroup encounters, and pair-living females show higher rates of extrapair copulation compared to the indris (Barelli et al. 2013; Reichard and Barelli 2008).

Singing Locations

We found that the indri's advertisement songs are equally distributed inside and outside the core areas, relative to the time a group spent in those areas. The fact that core areas shift over time may explain the strategy of advertising group presence throughout their territories rather than concentrating the advertisement in areas more intensely used in the relatively short term. Indri groups emit on average 2.2 advertisement songs per day (Torti et al. 2013) and the signal can reach far beyond the territory, eliminating the need to broadcast the signal from certain locations so that it would reach receivers located outside the territory.

Our results are in line with the proposed role of advertisement songs for indris: maintaining territory occupancy and reducing the need to engage in costly intergroup confrontations (Geismann and Mutschler 2006; Pollock 1986). This description fits with the model of regular advertisement of occupation, which predicts that loud calls are spread all over the range when they function to announce territory occupancy (da Cunhna and Byrne 2006). The same model has been suggested for howler monkeys (Alouatta pigra: Van Belle et al. 2013), while in Kloss's gibbons most post-dawn singing locations are in what the authors call the "most used area" (Whitten 1982). In contrast, in indris the spatial distribution of territorial songs is limited to the boundaries, fitting with a model of territorial boundary marking and defense (da Cunhna and Byrne 2006) and in accordance with the exclusive emission of territorial song in the context of intergroup encounters (Torti et al. 2013). In conclusion, we consider that given the pronounced territoriality of the indri, and the efficient spacing between neighboring groups, the pattern of space use in indris is influenced by intergroup dynamics and vocal communication.

The maintenance of stable and exclusive territories, as we found for indri, is a prerequisite for the evolution and maintenance of a pair-living social system and sexually or genetic monogamous mating system (Reichard 2003). However, not all pair-living primates are territorial and vice versa. A system in which pair-living and territoriality are linked may require the evolution of strategies to regulate communication and relationships between units, because units compete and are not independent of each other (Bartlett 2003; Fuentes 2000; Furuichi 2020; Tsai 2002).

From their studies on white handed gibbons, Bartlett et al. (2016) suggested that social factors can also drive space use, in addition to ecological factors, and that to better understand the behavioral ecology of a species, social units should not be considered as independent but as part of a network. We found a similar pattern in the indri, a species with a different feeding ecology from that of gibbons, but with many similarities in their social organization and vocal communication. Both social organization and ecological needs play a role in intergroup spatial and territorial dynamics, the link between social organization and external and ecological factors was suggested decades ago (Bartlett 2003;

Emlen and Oring 1977; Tsai 2002). Studies of the role of ecological variables, such as the spatial and temporal distribution of preferred food resources, are needed to better understand the role of ecological factors alongside social factors, and reach a more complete understanding of the drivers behind the intergroup dynamics in a pair-living territorial singing primate.

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Author Contributions

GB, MZ, and VT developed methodology and performed the analyses. GB, MZ, VT, DV, CDG, and RMR conducted the fieldwork. CT, MG, and CG contributed in developing the methods, interpreting of the results, and provided editorial advice. GB, MZ, VT, DV, CDG, and MG wrote the manuscript.

Data Availability

The data sets analyzed during the current study are available from the corresponding author on reasonable request.

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Ethical Note

We conducted this study on a wild population of the Critically Endangered (IUCN 2014) species *Indri indri* in the New Protected Area (NAP) of Maromizaha (Madagascar), managed by the GERP (Group d'Étude et de Recherche sur les Primates du Madagascar). During observations, we followed the groups at 10–50 m, avoiding any unnecessary disturbance. All the groups studied have been habituated to human presence since early 2009. The groups inhabit an area of primary forest accessible only when a research permit is granted; thus none of the groups has been subjected to ecotourism. None of the field workers were in physical contact with the animals. The study followed the legal requirements of Madagascar: the Ministère de l'Environnement et des Forêts (MEF) of Madagascar reviewed and approved the research methods and issued research permits for fieldwork and data collection in Maromizaha (N° 243/09/MEF/SG/DGF/DCB.SAP/SLRSE, N° 118/10/MEF/SG/DGF/DCB.SAP/SCBSE; N° 293/10/MEF/SG/DGF/DCB.SAP/SCB, N° 274/11/MEF/SG/DGF/DCB.SAP/SCB, N°245/12/MEF/SG/DGF/DCB.SAP/SCB, N°066/14/MEF/SG/DGF/DCB.SAP/SCB; N°066/14/MEF/SG/DGF/DCB.SAP/SCB).
The contents of this document are the sole responsibility of the authors and can under no circumstances be regarded as reflecting the position of the European Union. The authors declare that they have no conflict of interest.

Supporting Information

Estimates of territory size obtained with the method of the grids and minimum convex polygons (ESM1 - Table S1), the complete data set used to conduct the analysis on the core extensions and stability (ESM2 - Table S2), and the map showing core areas overlap between two following sampling periods for each group (ESM3 - Fig. S1) are available online.

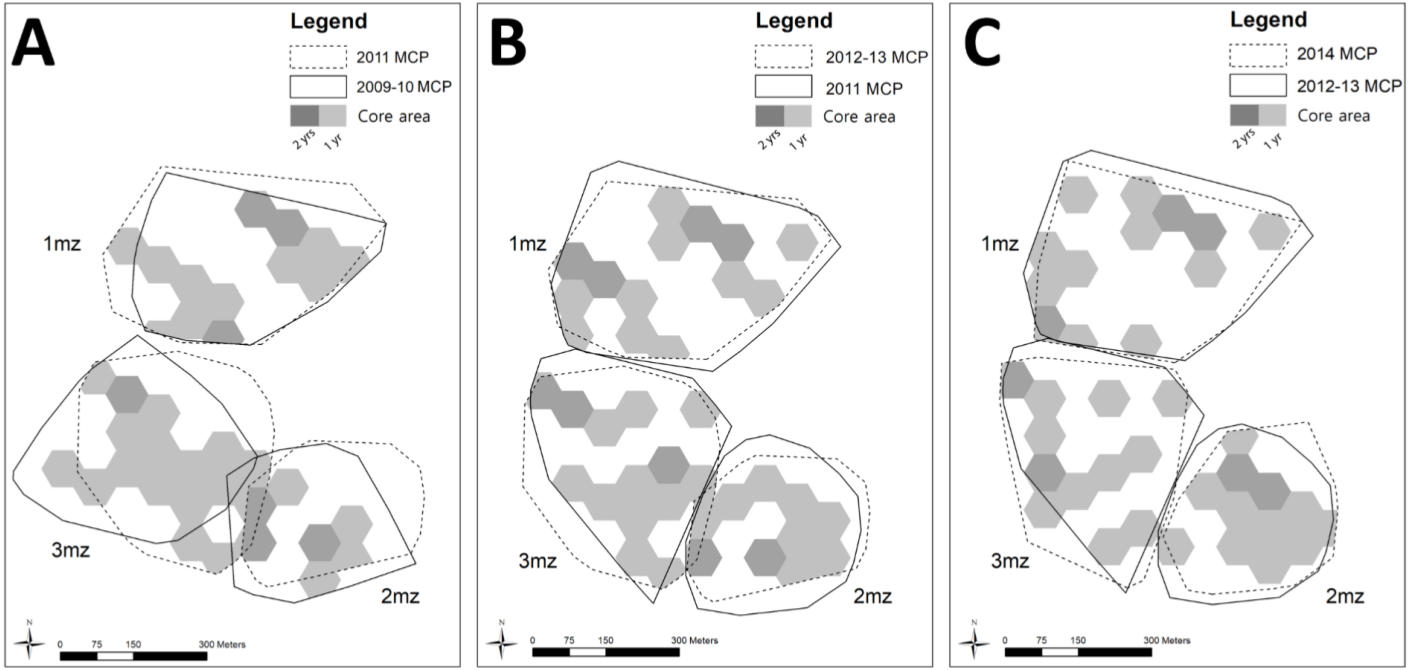
ESM1. Estimates of territory size in ha with the method of the hexagonal grids and the MCP 100%. We reported mean and standard deviation (SD) for each method.

Group	Sampling period	Territory size in ha (grids)	Territory size in ha (MCP 100%)
1mz	2009-10	15.5	12.7
	2011	19	15.5
	2012-13	19	17.5
	2014	19	15.0
2mz	2009-10	12.5	9.2
	2011	12.5	9.6
	2012-13	14.5	10.2
	2014	14.5	9.6
3mz	2009-10	18	13.4
	2011	18	14.7
	2012-13	15	12.9
	2014	15	13.9
Mean ± SD		16± 2.5	12.9 ± 2.7

ESM2. Core area (CA). Number and percentage of waypoints included in the core area, extension of the core area and proportion on the total size of the territory obtained with MCP for each sampling period and each group. Mean and standard deviation (SD) are reported for the percentage of waypoints included in the core area, the core area extension and as percentage of the territory.

Group	**Sampling Period**	**Total number of points in the CA**	**% points in the CA**	**CA extension (ha)**	**% CA on the territory**
1mz	2009-2010	47	43.9	3.4	26.6
	2011	52	42.6	3.8	24.4
	2012-2013	67	47.5	4.8	27.3
	2014	38	44.2	2.7	17.6
2mz	2009-2010	54	49.5	2.5	26.8
	2011	39	43.3	2.2	22.8
	2012-2013	74	49.3	3.5	34.1
	2014	20	47.6	3.1	32.2
3mz	2009-2010	58	45.3	4.0	29.6
	2011	68	45.6	4.2	28.3
	2012-2013	39	47.0	3.9	29.4
	2014	35	42.2	3.0	21.1
Mean ± SD			45.7 ± 2.5	3.39 ± 0.76	26.7 ± 4.8

ESM3. Core area overlap between two following sampling periods for three indri groups between 2009 and 2014. The Minimum Convex Polygons represent the territories of the three focal groups for each sampling period. The gray area represents the core area and the darker gray represents the overlap between sampling periods. A) Overlap between 2009-2010 and 2011; B) overlap between 2011 and 2012-2013; C) overlap between 2012-2013 and 2014.



Chapter IV

Call and be counted!

Can we reliably estimate the number of callers in the indri's (*Indri indri*) song?

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Call and be counted! Can we reliably estimate the number of callers in the indri's (*Indri indri*) song?

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Abstract

Estimating the number of animals participating in a choral display may contribute reliable information on animal population estimates, particularly when environmental or behavioral factors restrict the possibility of visual surveys. Difficulties in providing a reliable estimate of the number of singers in a chorus are many (e.g., background noise masking, overlap). In this work, we contributed data on the vocal chorusing of the indri lemurs (*Indri indri*), which emit howling cries, known as songs, uttered by two to five individuals. We examined whether we could estimate the number of emitters in a chorus by screening the fundamental frequency in the spectrograms and the total duration of the songs, and the reliability of those methods when compared to the real chorus size. The spectrographic investigation appears to provide reliable information on the number of animals participating in the chorusing only when this number is limited to two or three singers. We also found that the Acoustic Complexity Index positively correlated with the real chorus size, showing that an automated analysis of the chorus may provide information about the number of singers. We can state that song duration shows a correlation with the number of emitters but also shows a remarkable variation that remains unexplained. The accuracy of the estimates can reflect the high variability in chorus size, which could be affected by group composition, season and context. In future research, a greater focus on analyzing frequency change occurring during these collective vocal displays should improve our ability to detect individuals and allow a finer tuning of the acoustic methods that may serve for monitoring chorusing mammals.

Introduction

Species living in social groups may communicate at long distance transmitting information through the use of loud vocal signals (e.g., carnivores [1]; primates [2,3]). Sometimes, the pro- duction of these signals is not limited to a single emitter but can involve the participation of several callers, resulting in a chorus display, that may or may not include all the animals in a group [4]. Although we do have limited knowledge of the ability of conspecifics in extracting information about the size of the group and its spatial position, behavioral observations suggested that these emissions play a significant role in spacing neighboring groups within an area and in regulating their social interactions [5,6].

Understanding the dynamics of choral singing is crucial in bioacoustics studies for many reasons [7,8], but possibly the most critical information is related to the potential of group chorusing in providing human listeners with information on the number of emitters. In fact, estimating the number of animals participating in a choral display is becoming essential in different research fields, from conservation biology to management censuses [9,10,11]. Under- standing the number of animals in a chorus may also play a crucial role in the definition of reliable population estimates, particularly when the possibility of visual surveys is restricted by environmental or behavioral factors [12,13,14].

Investigating the vocal activity of a species instead of planning visual surveys is a highly cost-efficient technique [15] and is very popular in marine mammal studies [16,17,18]. Passive acoustic monitoring (PAM) is increasingly used in terrestrial and marine habitats [19,20,21]. One of its main benefits is that of minimizing the potential effect of the researcher on the behavior of the target animals. Of course, monitoring the presence of a species often represents only the first step before developing censuses that may provide researchers with estimates of the density and abundance of a particular species [22]. The potential advantage of using PAM recordings to count subjects is evident, but previous research has raised several methodological issues that led to use PAM as a complement to traditional recording techniques [21], especially in the case of terrestrial animals. Since acoustic signals do not propagate efficiently in air as they do in water, PAM only partially resolved the problems encountered with active monitoring for those species for which visual detection is largely limited [23]. However, the new wave of studies of ecoacoustics and the renovated interest in the study of soundscapes will surely contribute data for species and individual identification [24,25,26].

Although the spreading of new algorithms for automatic segmentation of the recordings and species identification are impressive [27,28], there is still a lack of information on the actual dynamics of group vocal displays and the potential for counting individuals. Studies on those terrestrial mammals which engage in complex choral displays represent a challenging natural example for individual recognition and may play a major role in estimating the relative or even absolute abundance of a species across an area [29,30]. In the case of several species displaying choruses (e.g. wolves [31,32]; jackals [33,34]; gibbons [35,36]), however, it has been impossible to validate the minimum number of emitters recognizable in a group because recordings could not be supplemented by traditional survey methods. The difficulties in pro- viding a reliable estimate of the number of singers in a chorus are many, from background noise masking to individual behaviors that may affect acoustic propagation characteristics [37,38,39,40].

Canids were usually targeted as one of the most interesting cases of chorusing animals because their monitoring is one of the central issues in conservation biology [41,42]. The study of Passilongo and colleagues [43] showed that a spectral examination of the chorus howling allowed to estimate real versus bioacoustically predicted chorus size in a way far more precise and objective that field estimations by ear. The seminal work of Filibeck and colleagues [44] indicated that methods based on spectral sound decomposition could be effective in censuses of wolves via howls simulated by the howling technique [1]. In later studies, Root-Gutteridge [7,45], Passilongo [43,46], and colleagues found that fundamental frequency and amplitude variations could help identifying wolves with high accuracy. Passilongo and colleagues [43] showed that estimates generated by the screening of the spectrograms were closer to the real chorus size than the aural estimations of an expert operator. They also found that the reliability of chorus size estimates decreased with the increase of the real chorus size, especially when the latter exceeded four animals [43].

Thus, it is clear that direct observations of chorusing animals can vastly improve our under- standing of the development, the dynamics and the structure of animal chorusing [47,7]. For this work, we contribute data on the vocal chorusing of a primate species, *Indri indri*. This species lives in socially monogamous family groups (2–6 individuals; [48]), usually consisting of the adult breeding couple and the offspring of up to four different generations. This lemur is mainly folivorous [49] and occupies and actively defends territories, whose extension varies according to the forest site [50]. Indris have a rich vocal repertoire (with eight different vocal types besides the song [51]) but they mainly rely on the emission of the so-called “songs” for regulating inter- and intra-group relationships [48]. indris are, in fact, among the so-called “singing primates” [52] (like gibbons, tarsiers and titi-monkeys) and are the only lemur species producing songs, that can be heard up to 2 kilometers [53].

The indris’ song consists of a long sequence of vocalizations that usually starts with a harsh emission ("roar"), followed by a series of slightly frequency modulated units ("long notes"). There is then a series of units organized in phrases with a descending frequency pattern (descending phrases) composed of 2–5 units [54]. Male and female indris within a group, including juveniles (aged up to one year, [55]), take part in a chorusing song, which lasts 40– 250 s [51]. Previous studies have shown that male and female contributions to the song differ, both quantitatively and qualitatively, in the overall temporal structure of calling, repertoire size, and acoustic structure of the note types [56, 57]. Sex dimorphism is also present in the modulation of the frequency of vocal emissions, in the duration of note types and the rhythmic structure of a contribution [47]. The indris produce songs that differ in their acoustic structure between contexts [53, 54]. The songs may serve to inform the neighboring groups about the occupation of the territory ("advertisement songs"), to resolve territorial fights during a confrontation between neighbors ("territorial songs"), and have a cohesion function between group members ("cohesion songs").

By visually detecting and observing every individual during the emission of their songs, we were able to apply the spectrographic methodology used in previous studies. We focused on estimating chorus size, and then we compared those estimates with the behavioral observations we did in the field. Direct observations not only allowed to assign each emission to an individual caller, but also improved our understanding of how many animals participated in the song, and how do they behave during singing. Because of these particular conditions, we were able, from time to time, to record videos that were used to describe how the indris emit their howling cries (e.g. S1 Movie).

Previous studies have shown that a weaker, but still useful information of group size could be derived from the total length of the choruses. Durbin [58] and Comazzi and colleagues [33] found that solo howls of the Asiatic wild dog (*Cuon alpinus*) and golden jackals (*Canis aureus*) were significantly shorter than chorus howls. Servin [59] showed that the average duration of chorus howling of the Mexican wolf (*Canis lupus baileyi*) varied significantly throughout the year, thus possibly related to a change in the number of vocalizers. Those evidences could explain why Harrington [60], who concentrated his sampling on a single season, found that the average duration of chorus howls by wolves did not vary with pack size or composition, while works on captive and wild animals showed that more wolves in a group might stimulate each other to longer howling [4,61,62]. The duration of an elicited chorus howl of free-ranging wolves (*Canis lupus*) significantly increased with group size [32]. Nowak and colleagues [32] reported that howls of single wolves or pairs lasted less than a minute (average: 34–40 s), whereas those of 5–7 wolves could reach nearly 4 minutes (average 67–95 s).

Palacios [63] examined the group howls of the Iberian wolf (*Canis lupus*) finding a correlation between chorus size and group size and between howl duration and group size. They showed that the number of wolves simultaneously vocalizing coincided for 73% of the howls with group size. The same authors found that the number of wolves vocalizing simultaneously and the length of the chorus increased with the number of wolves in a group. The correlation between group size and total duration of a chorus is far less investigated in nonhuman primates. Geissmann and Nijman [10] found a significant difference in the duration of female song bouts with one versus two participants.

At the light of the previous research, we examined whether we could estimate the number of indris emitting in a chorus using screening of the spectrograms and the reliability of this methods when compared to the real chorus size. We were expecting to find a correlation between chorus size and group size and between song duration and the number of vocalizing indris.

We could thus hypothesize that the spectrographic analyses of the indri’s choruses, based on the visual inspection of the fundamental frequency of each overlapping singer, could be predictive of the number of callers. We could also attend that the estimate we could generate with the spectrographic count would become less reliable [43] at the increase in the number of vocalizers. Moreover, we asked whether the duration of the song may be informative about the number of vocalizing animals or even of the size of the group. We could thus hypothesize that at the increase in the number of indri vocalizers we would observe an increase in chorus’ duration. Finally, we wanted to focus on whether song duration could provide cues about groups size, but we could not formalize a precise prediction for this last hypothesis because of the controversial data currently available from other species. We also tested whether the most com- mon acoustic indices could provide information about the number of singers in a chorus.

Methods

Study areas, subjects and recordings

We studied 21 groups of indris living in three different areas of dense tropical forest in Madagascar (S1 Table): 9 groups in the Analamazaotra Reserve (Andasibe-Mantadia National Park, 18˚56' S, 48˚25' E), 3 groups in the Mitsinjo Station Forestière (18˚56' S, 48˚24' E), and 9 groups in the Maromizaha Forest (18˚56' 49" S, 48˚27' 53" E). We collected data in the field every year, from 2005 to 2016, for a total of 45 months. We observed one group per day from 06:00 am to 1:00 pm. We used natural marks to identify the individuals, and we had one observer per single indri during the observations to ensure we could correctly track each singer during the song.

Recordings were made using Sennheiser ME 66 and ME 67 and AKG CK 98 microphones. The microphone output signal was recorded using a solid-state digital audio recorder (Marantz PMD671, SoundDevices 702, Olympus S100 or Tascam DR-100MKII 24/96) at a sampling rate of 44.1 kHz. All utterances were recorded at a distance of maximum 10 m since all the study groups were habituated. We made all efforts to orient the microphone towards the vocalizing animal.

For this work, we selected songs emitted by two (duets) to five vocalizers (we use the term choruses for songs uttered by more than two indris) in 194 days of sampling (S1 Table). All the selected signals were advertisement songs [54].

Acoustic and statistical analyses

We analyzed a total of 258 songs (duets and choruses), which we edited using Praat 5.3.46 [64] and Boris 4.0.3 [65]. We used field notes and video recordings to assign every utterance to the correct emitter, as an individual profile (Fig 1). To extract information from the songs, we focused on the fundamental frequency (e.g.

the lowest frequency produced by the vibration of the vocal folds). We reported this information in a Praat textgrid [47].

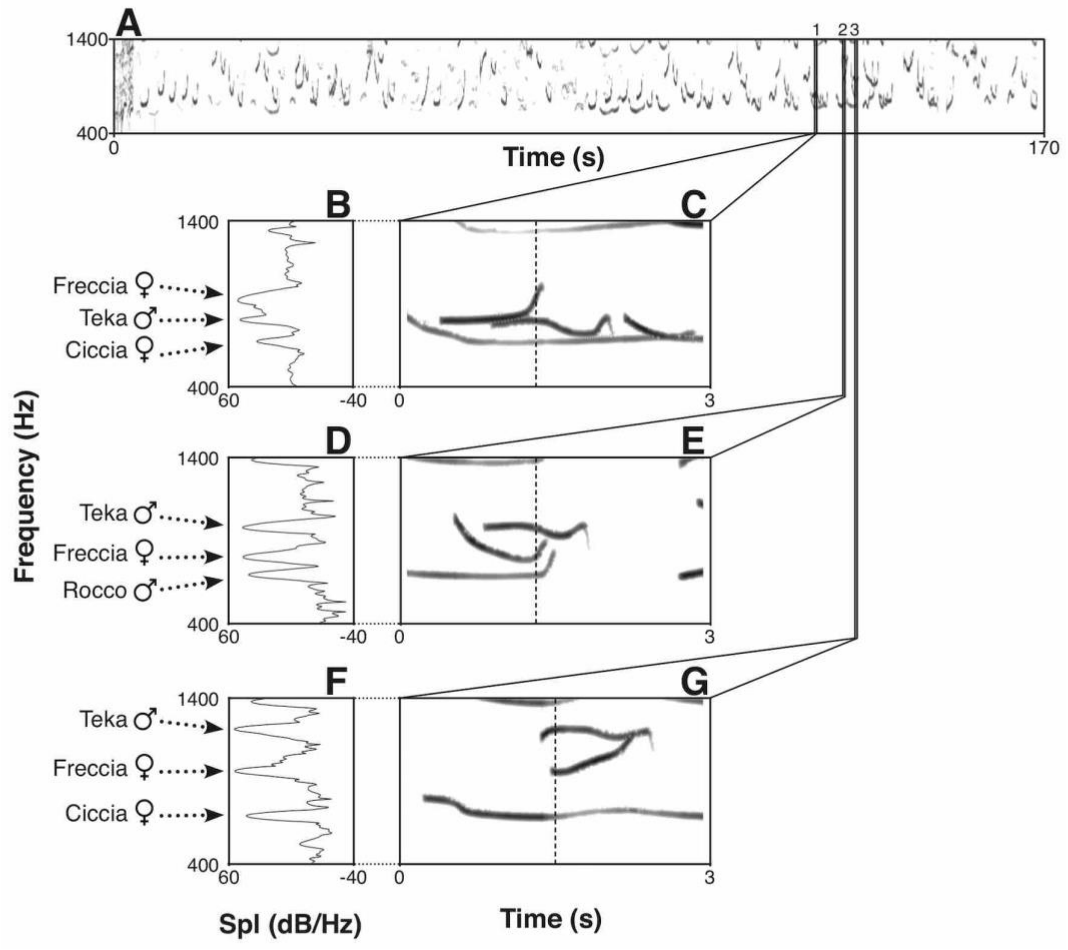

Figure 1. Spectrograms of the indris' song. A spectrogram (A) of a complete song generated using Praat (time step: 20 ms). We enlarged three overlap occurrences between the singers (C, E, and G) and presented the spectra (B, D, and F) calculated at the point indicated by the dashed line in each spectrogram. The occurrence of different fundamental frequencies (f0s) and harmonic structures at the same time allows indicating the emission of different animals. The arrows indicate the name and sex of the vocalizing individual and the respective spectral peak. Notice the variation of the Sound Pressure Level (SPL) of an individual across the different spectra. Other peaks may correspond background noise and harmonics.

To understand whether we could estimate the minimum number of callers in a song, we carefully screened the spectrogram (e.g. the visual representation of the spectrum of frequencies of a sound in relation to time) of each song in Praat, focusing on a frequency range of 0 to 3000 Hz and using a window length of 0.025 s. Following a methodology similar to those utilized in the works of Filibeck et al. [44], Passilongo et al. [43], and Comazzi et al. [33], we visually identified the portions of the chorus in which we could recognize multiple vocalizers, by identifying overlapping notes (each with its fundamental frequency profile) and counting the number of overlapping fundamental frequencies. Given the fact that we attributed each vocalization in the song to its respective vocalizer, the maximum value of simultaneous emitters was taken as the estimate of the minimum number of participants for that chorus (Predicted Chorus Size, CSp, following Passilongo et al. [43]). We used our field notes and video recordings to track the real number of animals singing in a particular chorus ($\mathrm{CS_r}$) and the group size of the group in that season (GS). We used a Praat script to extract and save the total duration of the chorus in a text file, which could be then exported to a comma-separated file [66,67] or used in R (The R Foundation for Statistical Computing 2017; version 3.3.3).

We investigated to what extent we could spectrographically predict the exact number of callers in a song by calculating the percentage of songs in which CSp and CSr coincided. We did this calculation by grouping data according to CSr. We then calculated four acoustic diversity indices for each indri’s song (seewave [68] and soundecology packages [69] in R): 1) the acoustic complexity index, ACI [70]; 2) the acoustic richness index, AR [71]; 3) the acoustic diversity index, ADI [72] and the Acoustic Entropy Index, H [73]. The four indexes (see for details [74, 75]) are applied here to understand whether they could inform about the numerosity of singers in a chorus. We considered the frequency range between 150 Hz and 10000 Hz (comprising the fundamental frequency and the visible harmonics) and used a Fast Fourier Transform window of 512 samples.

We ran the Spearman correlation test in R (The R Foundation for Statistical Computing 2017; version 3.3.3) to understand whether there was a correlation between the duration of the choral songs and CSp, CSr, and GS. We also run the Spearman correlation test to reveal whether there was a significant correlation between each index (ACI, AR, ADI, H) and the real number of singers (CSr) in the choruses. We also calculated the confidence interval for each set of data by using the spearman.ci function (package RVAideMemoire 0.9–68; [76]). We visualized all our analyses and calculations using the package ggplot2 2.2.1 [77].

We ran four separate linear mixed-effects models (LMM, lme4 package in R) for song duration and CSp, with the real number of animals singing in a particular chorus (CSr) and the group size of the group in that season (GS), entered as the response variables. We tested for the distribution of both the responses and the fixed variables. We log-transformed the data when the data were not normally distributed.

The two LMM models we used to investigate the variation in the real number of singers (CSr) included the predicted chorus size (CSp) and the song duration alternatively, as fixed factors. The two models in which the group size of the group in that season (GS) was the response variable included the predicted chorus size (CSp) and the song duration alternatively, as fixed factors. We entered group identity as a random factor in all the four models. We finally ran a chi-square test in R to define which variables turned out to be predictive.

We then run four separate linear mixed-effects models (LMM) to investigate the variation of the four different acoustic indexes (ACI, AR, ADI, H), adding the real number of singers (CSr) in the choruses as a fixed factor. We entered group identity as a random factor in all the four models.

For every LMM model, we verified the assumptions that the residuals were normally distributed and homogeneous by looking at a quantile-quantile plot and the distribution of the residuals plotted against the fitted values with a specific function written by R. Mundry (Max Planck Institute, Germany). To test the significance of each full model [78] we compared it against a null model comprising the random factor (group identity) exclusively, by using a likelihood ratio test (Anova with argument test “Chisq” [79]). Then, we calculated the P values for the predictors based on likelihood ratio tests between the full and the respective null model. We, finally, calculated conditional R-squared (R^2_C) measures for each LMM model, providing an absolute value for the goodness-of-fit of each model [80].

Results

Predicted chorus size vs real chorus size

We compared CS_p with CS_r to understand to what extent the spectrographic investigation could provide accurate estimates of the number of chorusing animals (see S2 Table for differences between groups). It has been interesting to notice that only in 165 cases all the group members actively sung in the chorus. In 74 recordings, more than 50% took part to the song, and in 19 songs we observed 50% or less of the group participating to the chorus. The estimated number of emitters ranged from two (N = 175) to four (N = 2), while the real chorus size ranged from two (N = 124) to five (N = 4; Fig 2).

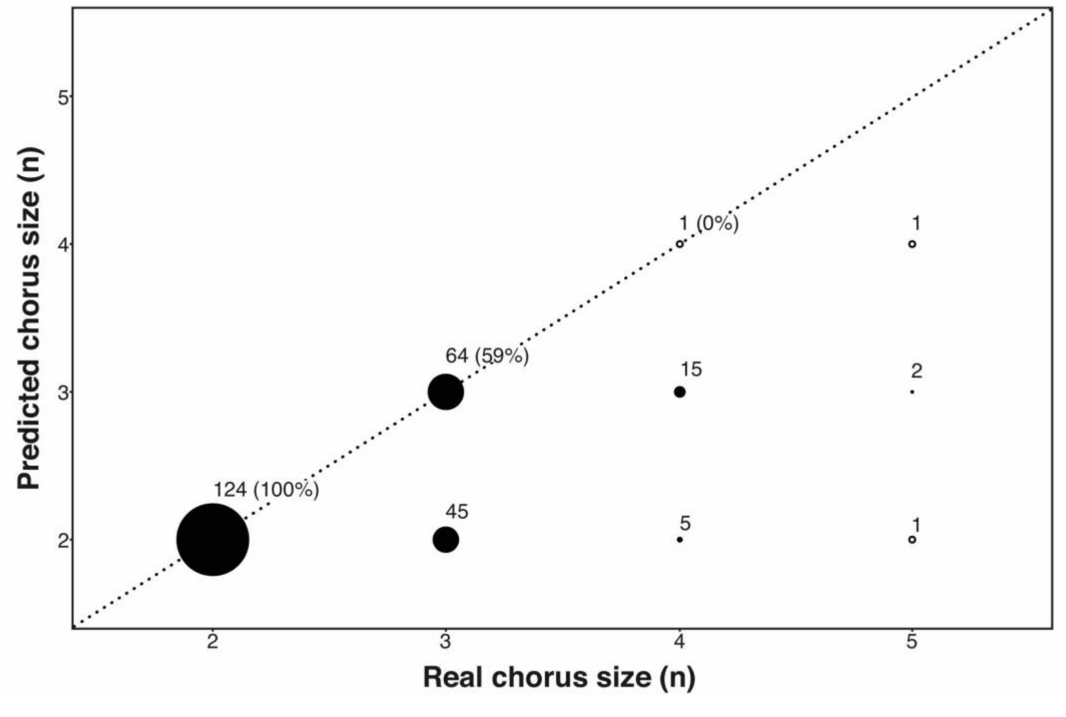

Figure 2. Spectrographically predicted versus real chorus size in the indris. Scatter plot of Spectrographically predicted (CSp) versus Real chorus size (CSr) in the indris. The radius of each point is proportional to the number of cases, which is reported with the percentage of correct estimates. A circled white dot denotes N = 1. The diagonal indicates the accurate predictions.

In all the songs emitted by pairs (N = 124), we could indicate two callers from the spectrogram screening. Thus, for the groups where we had a pair only, real group size and estimated group size coincided. Songs emitted by three indris (N = 109) showed the potential to indicate all the emitters only in 64 cases (59%). The remaining 45 songs allowed indicating only two emitters. We had 21 songs in which four singers coordinated their emissions. In five of them (24%) we could indicate only two emitters from the spectrograms (Fig 2). In 15 of the remaining songs (71%), we could indicate three singers, and only one song allowed indicating from the spectrogram the real chorus size of four emitters (Fig 2). Four songs, in which we observed five singers participating, revealed an estimate of 2, 3 and four emitters (Fig 2).

Duration vs real chorus size

The duration of indri choruses ranged between 25.32 s and 533.79 s. We found a positive correlation between the real number of singers and the overall duration of the song (Spearman correlation test; N = 258, P < 0.001; rho = 0.601, Fig 3A) as it has already been reported by Gamba and colleagues [47]. The correlation between the estimated number of singers and the total duration was also positive and significant (Spearman correlation test; N = 258, P < 0.001; rho = 0.313, Fig 3). When we considered duration in the light of real chorus size, we found that songs emitted by pairs ranged between 25.320 s and 191.960 s (76.533 ± 29.649, N = 124). Songs emitted by three indris lasted from 42.300 s to 533.790 s (119.576 ± 59.067, N = 109), while those given 4 or 5 lemurs showed a minimum duration of 91.510 s and a maximum of 404.900 s (183.531 ± 73.980, N = 25). It may be of interest to notice that also group size (calculated on the potential singers of the group, e.g., adults and subadults according to Pollock 1986) positively correlated with song duration (Spearman correlation test; N = 258, P < 0.001; rho = 0.418, Fig 3).

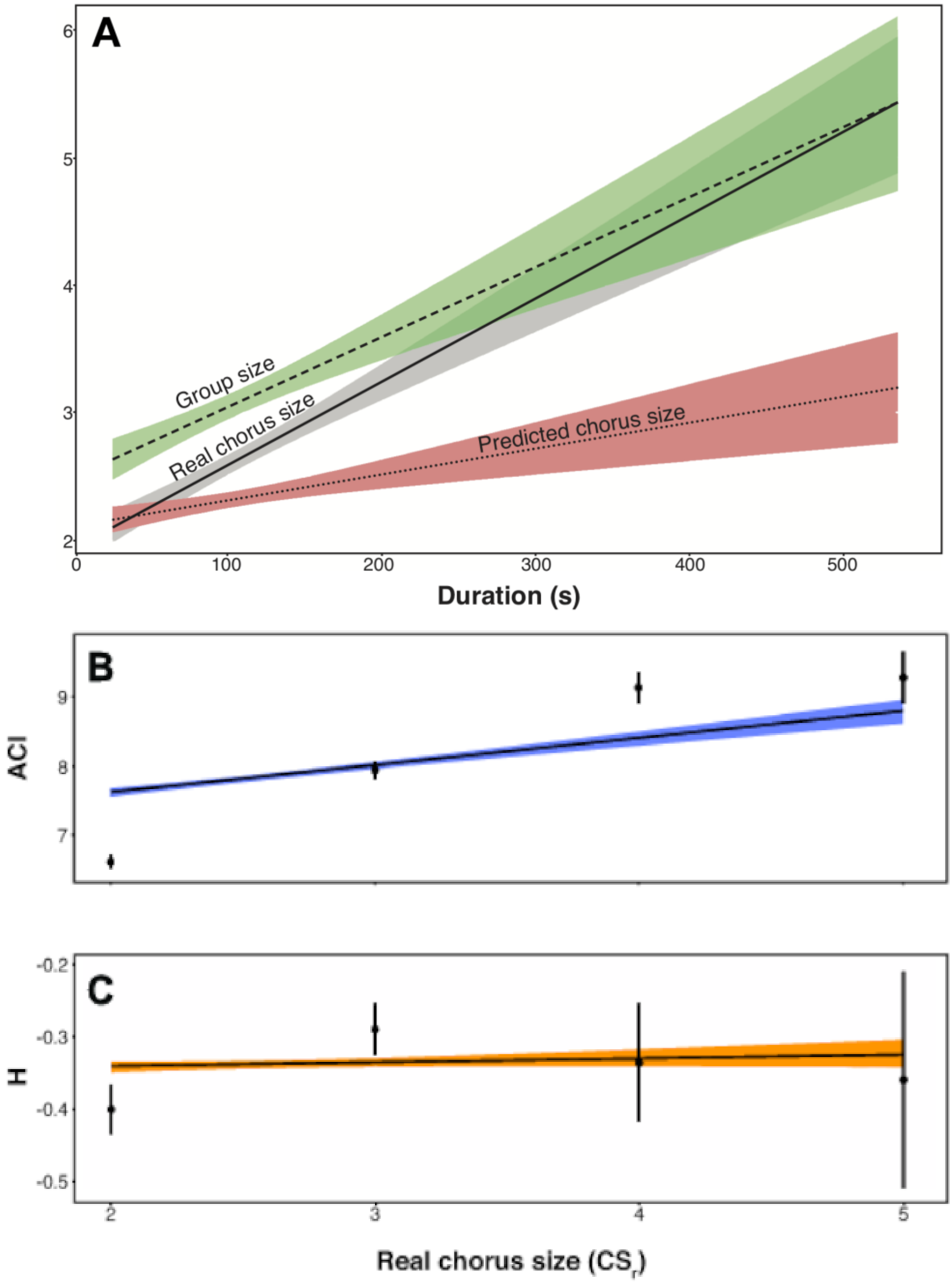

Figure 3. Indri's song duration. The indris' song duration is (A) presented as a function of Group size (dashed line), Real chorus size (solid line), and Predicted chorus size (dotted line). The Real chorus size is presented as a function of (B) the Acoustic complexity index (ACI) and (C) the Acoustic entropy index (H). Each regression line is represented with the associated 95% confidence interval range (shades). Error bars indicate standard errors.

Comparison between models

We first estimated to what extent the real number of animals singing in a particular chorus (CS_r) could be predicted by CS_p and by song duration. CS_p and song duration significantly explained the CS_r variability (Table 1). CS_p values (R^2_C = 0.519) and song duration (R^2_C = 0.522) explained the same percentage of variation in CS_r (52%). The two models did not differ (Chi square = -148.05; df = 4; P = 1.000). We then tested whether group size (GS) could also be predicted by CS_p and by song duration. The GLM full models (Table 1) returned a conditional R-squared of R^2_C = 0.788 and R^2_C = 0.795 respectively. Both predictors significantly explained GS variability (Table 1). When compared, the two models were significantly different (Chi square = -268.95; df = 4; P < 0.001). We also observed that song duration better predicts group size (GS) if compared to CS_p.

	Real number of animals singing in a particular chorus (CS_r)				**Group size of the group in that season (GS)**			
	Estimate	SE	t	P	Estimate	SE	t	P
(Intercept)	0.362	0.057	6.381	a	0.972	0.067	14.574	a
CSp	0.684	0.060	11.313	< 0.001	0.160	0.048	3.337	< 0.001
(Intercept)	-0.271	0.119	-2.280	a	0.718	0.102	7.032	a
Song duration	0.265	0.026	10.377	< 0.001	0.085	0.019	4.428	< 0.001

Table 1. Results of the LMM full models for CS_r and GS. Influence of the fixed factors on the real number of animals singing in a particular chorus (CS_r) and on group size (GS). [a]Not shown as not having a meaningful interpretation. **Real number of animals singing in a particular chorus (CS_r): CS_p model**: full vs. null; chisq = 101.0795, df = 1, P < 0.001; **Song duration model**: full vs. null; chisq = 88.877, df = 1, P < 0.001. **Group size of the group in that season (GS): CS_p model**: full vs. null; chisq = 10.860, df = 1, P = 0.001; **Song duration model:** full vs. null; chisq = 18.888, df = 1, P < 0.001.

Bioacoustics diversity vs real chorus size

We found a positive correlation between the acoustic complexity index (ACI) and the real number of singers in a chorus (Spearman correlation test; N = 258, P < 0.001; rho = 0.578). We did not find a correlation between the real number of singers in a chorus and the ADI (Spearman correlation test; N = 258, P = 0.454; rho = -0.047), the AR (Spearman correlation test; N = 258, P = 0.955; rho = -0.004), and the H (Spearman correlation test; N = 258, P = 0.113; rho = 0.098) indices. The real chorus size had a significant effect on the acoustic complexity index (ACI), and the acoustic entropy index (H, Table 2). The LMM models did not show this significant relationship for the other two indexes (ADI and AR).

By direct comparison of the R-squared values for the acoustic diversity indexes, we observed that the real chorus size better predicts the acoustic complexity index variation ($R^2_C = 0.386$) than the acoustic entropy index variation ($R^2_C = 0.219$) (Fig 3B and 3C).

		Estimate	SE	t	P
Acoustic Diversity Index	(Intercept)	0.736	0.015	47.646	[a]
	CSr	-0.004	0.015	-0.279	0.780
Acoustic Complexity Index	(Intercept)	6.829	0.105	64.900	[a]
	CSr	1.109	0.106	10.473	<0.001
Acoustic Entropy Index	(Intercept)	0.692	0.008	82.219	[a]
	CSr	0.018	0.008	2.253	0.025
Acoustic Richness Index	(Intercept)	0.302	0.076	3.958	[a]
	CSr	-0.302	0.076	-0.426	0.671

Table2. Results of the LMM full models for the acoustic indices. **Acoustic Diversity Index (ADI):** full vs. null; chisq = 0.078, df = 1, p = 0.780. **Acoustic Complexity Index (ACI):** full vs. null; chisq = 87.559, df = 1, p < 0.001. **Acoustic Entropy Index (H):** full vs. null; chisq = 5.024, df = 1, p < 0.001. **Acoustic Richness Index (AR):** full vs. null; chisq = 0.180, df = 1, p = 0.671

Discussion

In this paper, we evaluated whether chorusing displays may provide reliable insights into the number of individuals participating in the chorus, contributing new data from direct observation of singing lemurs. The spectrographic investigation provided reliable information on the number of animals engaging in the chorusing only when this number is limited to two or three singers. This outcome supports a systematic underestimation of the number of callers, which has been already indicated in a previous study [43]. Our findings are congruent with those presented by Passilongo and colleagues [43] and Comazzi and colleagues [33] on wolves and jackals. Even if the spectrographic investigation is useful for predicting the real number of singers, the method allowed estimating the actual chorus size correctly only for small chorus sizes. At the increase in the number of emitters, the reliability of the estimate decreased. Thus, whether minimum counts of the individuals in a group are useful, it must be considered that they may not reflect the actual chorus size.

In *Indri indri* solo singing is rare [47] and is mainly observed in dispersing individuals (VT personal observations), playing a significant role in mate attraction and new pairs' settling [53,54,47]. Songs are frequently emitted by all the singing individuals in a family group, even if we observed that all group members participate to the song more frequently during territorial fights than in advertisement songs [54], in agreement with data of Bonadonna and colleagues [48]. Besides, despite high levels of overlapping between singers', indris show significant non- overlapping rates in the song emission. We previously found that an indri dominance status (e.g. reproductive vs non-reproductive members) plays a crucial role in the amount of overlap in the song [47]. Animals showed overlapping avoidance in between dominants and non-dominants (which are often sub-adults and offspring in our sampling) and more frequent overlapping between the adult reproductive couple. Thus, the underestimation of the number of callers could be directly influenced by the low levels of overlapping between the breeding couple and more than one non-dominant individual. The underestimation for more than three singers can be partially explained by the fact that, when more than three animals are singing in a chorus, they may avoid or show small overlap [47]. We observed, in fact, that at the increase in the number of singers in a chorus the amount of co-singing between two individuals significantly decreased [47].

We tested whether the most common acoustic indices could inform about the number of indris vocalizing in a song. We found the acoustic complexity index and acoustic entropy index correlated with the observed chorus size. These results are particularly interesting because acoustic indexes are more often used in the field of ecology, but their application to investigate animal behavior is rare. The current result confirms that ACI and H would be suit- able for further evaluation of animal chorusing. The number of indris participating to the song had no significant influence on the other indices we tested.

The extent to which these estimates can reflect the actual group size in the indris is still difficult to understand, as the participation of all members of the group to the chorus appears variable. The relation between chorus size and group size may be affected by group composition (e.g.; age and sex of the group members), season and context of emission.

A different conclusion can be taken when considering whether the number of singers may affect song duration and to what extent song duration can inform about group size. Overall, we found evidence of the higher predictive power of total duration if compared to the spectro- graphic investigation. Song duration appeared to better explain the variability of both the real chorus size and the group size if compared to spectrographically predicted chorus size. Describing the temporal properties of the indris' singing, we found that the individuals tend to overlap more in duets than in choruses. While adults tend to overlap, in fact, sub-adults are more likely to avoid co-signing. Consequently, preventing overlap between individual contributions could result in increasing the total duration of the songs. Song duration could better predict the number of singers, even if this effect could be masked by several factors (e.g. season, sex of the co-emitter, age, etc.). Both song duration and CSp, in fact, show the same power in predicting the real number of singers. The reliability of both methods tends to decrease at the increase in the number of singers in a chorus. For those reasons, the song duration model shows a remarkable variation that remains unexplained.

Finally, we could confirm that song duration better predicts both the real number of animals singing in a particular chorus and group size (GS) if compared to the analysis of the fundamental frequency profiles. At least for the advertisement songs, total duration appears to a reliable of real chorus size.

In future research, a greater focus on analyzing frequency variation occurring during these collective vocal displays should improve our ability to detect individuals [81] and allow finer tuning of the acoustic methods that may serve for monitoring chorusing mammals.

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Supporting information

S1 Table. Summary of the dataset

Summary of the dataset with group ID, site, year of the recording, individual, sex, number of singers (mean±sd), group size (mean±sd) and number of days in which the songs were recorded. * symbol denotes that the individual is an adult (aged more than 6 years) at the time of the recording (column Year); R symbol indicates that the individual is member of the reproductive couple; R2 symbol denotes that the individual has been involved in a takeover and is the new reproductive member of the couple. Numbers in round brackets represent the year in which an individual moved away from the group. When we have evidences of the death of an animal we inserted the year with the †.

Group ID	*Site*	*Year*	*N songs*	*Individual*	*Sex*	*N° singers (mean±SD)*	*Group size (mean±SD)*	*N° days of recordings*
1MZ	Maromizaha	2011 2012 2016	46	Jery*,R	M	2,70±0,55	3,00±1,00	29
				Bevolo*,R	F			
				Fotsy* (2013)	M			
				Maintso (2014)	F			
				Berthe (2014)	F			
2MZ	Maromizaha	2011 2012 2016	21	Max*,R	M	2,00±0,00	2,00+0,00	13
				Soa*,R	F			
3MZ	Maromizaha	2011 2012 2015 2016	42	Ratsy*,R (†2015)	M	2,64±0,62	3,30±1,00	31
				Mahagaga*,R2	M			
				Mena*,R	F			
				Zandry (2015)	F			

				Tonga	F			
				Faly	M			
4MZ	Maromizaha	2015 2016	5	Koto*,R	M	3,60±0,55	4,00±0,00	5
				Eva*,R	F			
				Hendry*	M			
				Gibet	M			
5MZ	Maromizaha	2016	2	Graham*,R	M	2,00+0,00	4,00+0,00	2
				Fern*,R	F			
				Sonny Bill	F			
				All Black	M			
6MZ	Maromizaha	2016	5	Zokibe*,R	M	2,00+0,00	3,00±0,00	4
				Befotsy*,R	F			
				Hira	M			
8MZ	Maromizaha	2016 2017	6	Jonah*,R	M	3,50±1,38	4,00±1,00	5
				Bemasoandro*,R	F			
				Cesare*	M			
				Mika	F			
				Zafy	M			
9MZ	Maromizaha	2016	4	Emilio*,R	M	2,50±0,58	3,00+0,00	4
				Sissie*,R	F			
				Dosy	F			
10MZ	Maromizaha	2016	2	Tia*,R	M	2,00+0,00	3,00+0,00	2
				Joeline*,R	F			
				Voandalana	?			
1R	Analamazaotra		17	Curvo*,R	M	2,76±0,44	3,70±0,60	13
				Tozza*,R	F			

		2005 2007 2008		Cleo	M			
				Norma (2008)	F			
				Panda	?			
2R	Analamazaotra	2005 2007 2008	11	Rocco*$_{,R}$	M	3,64±0,92	4,70±0,60	10
				Freccia*$_{,R}$	F			
				Ciccia	F			
				Teboka*$_{,R2}$	M			
				Stringo (†2006)	M			
				Forchette (†2007)	F			
				Teka	M			
3R	Analamazaotra	2005 2006 2007 2008	24	Fano*$_{,R}$ (2007)	M	3,04±0,69	4,30±0,50	20
				Mamatin*$_{,R}$	F			
				Tete Dure*$_{,R2}$	M			
				Chef*	M			
				Zaza	M			
				Lanto	M			
5R	Analamazaotra	2005 2006 2007 2008	15	Ranga*$_{,R}$	M	2,00±0,00	2,00±0,00	13
				Sola*$_{,R}$ (2007)	F			
				Clara*$_{,R2}$	F			
6R	Analamazaotra	2005 2007 2008	12	Plateau*$_{,R}$	M	2,67±0,49	3,70+0,60	6
				Nbola*$_{,R}$	F			
				Ninja	M			
				Cammela	F			
XR	Analamazaotra	2005 2007 2008	10	Fumo*$_{,R}$	M	2,00±0,00	2,00+0,00	8
				Vola Mena*$_{,R}$	F			

YR	Analamazaotra	2007	2	Bekibo*,R	M	3,00±0,00	3,00+0,00	2
				Celeste*,R	F			
				Armando	M			
1M	Mantadia	2006 2007	4	Maquillage*,R	M	2,75±0,50	3,50+0,70	3
				Cristina*,R	F			
				Faralahy	M			
				Mère Noel	F			
4M	Mantadia	2007	3	Jobby*,R	M	2,00+0,00	2,00+0,00	3
				Chiara*,R	F			
ASF	Mitsinjo SR	2005 2008	10	Rambo*,R	M	3,10±0,57	3,50+0,70	9
				Maya*,R	F			
				Lion* (2008)	M			
				Jule (2006)	M			
				Sabotsy	M			
				Talata	M			
WSF	Mitsinjo SR	2005 2008	6	Rasta*,R	M	2,00+0,00	2,00+0,00	3
				Tina*,R	F			
				Mauro	M			
YSF	Mitsinjo SR	2005 2007 2008	11	Panza*,R	M	2,45±0,52	3,7+0,60	9
				Lisa*,R	F			
				Diadème	F			
				Miss Scarlet	F			
				Christo	F			

S2 Table. Results of the Tukey's HSD (honestly significant difference) applied to the group size during our study. The groups are listed in order of ascending harmonic means (mean±se). Subset 1: p = 0.122; Subset 2: p = 0.060.

Group	Subset 1	Subset 2
10MZ	2.00±0.00	
2MZ	2.00±0.00	
4M	2.00±0.00	
5MZ	2.00±0.00	
5R	2.00±0.00	
6MZ	2.00±0.00	
WSF	2.00±0.00	
XR	2.00±0.00	
YSF	2.45±0.16	2.45±0.16
9MZ	2.50±0.29	2.50±0.29
3MZ	2.64±0.09	2.64±0.09
6R	2.67±0.14	2.67±0.14
1MZ	2.70±0.08	2.70±0.08
1M	2.75±0.25	2.75±0.25
1R	2.76±0.11	2.76±0.11
XR	3.00±0.00	3.00±0.00
3R	3.04±0.14	3.04±0.14
ASF	3.10±0.18	3.10±0.18
8MZ		3.50±0.56
4MZ		3.60±0.24
2R		3.64±0.28

Chapter V

Finding Meanings in Low Dimensional Structures: Stochastic Neighbor Embedding Applied to the Analysis of *Indri indri* Vocal Repertoire

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Finding Meanings in Low Dimensional Structures: Stochastic Neighbor Embedding Applied to the Analysis of *Indri indri* Vocal Repertoire

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Simple Summary

The description of the vocal repertoire represents a critical step before deepening other aspects of animal behaviour. Repertoires may contain both discrete vocalizations—acoustically distinct and distinguishable from each other—or graded ones, with a less rigid acoustic structure. The gradation level is one of the causes that make repertoires challenging to be objectively quantified. Indeed, the higher the level of gradation in a system, the higher the complexity in grouping its components. A large sample of *Indri indri* calls was divided into ten putative categories from the acoustic similarity among them. We extracted frequency and duration parameters and then performed two different analyses that were able to group the calls accordingly to the a priori categories, indicating the presence of ten robust vocal classes. The analyses also showed a neat grouping of discrete vocalizations and a weaker classification of graded ones.

Abstract

Although there is a growing number of researches focusing on acoustic communication, the lack of shared analytic approaches leads to inconsistency among studies. Here, we introduced a computational method used to examine 3360 calls recorded from wild indris (*Indri indri*) from 2005–2018. We split each sound into ten portions of equal length and, from each portion we extracted spectral coefficients, considering frequency values up to 15,000 Hz. We submitted the set of acoustic features first to a t-distributed stochastic neighbor embedding algorithm, then to a hard-clustering procedure using a k-means algorithm. The t-distributed stochastic neighbor embedding (t-SNE) mapping indicated the presence of eight different groups, consistent with the acoustic structure of the a priori identification of calls, while the cluster analysis revealed that an overlay between distinct call types might exist. Our results indicated that the t-distributed stochastic neighbor embedding (t-SNE), successfully been employed in several studies, showed a good performance also in the analysis of indris' repertoire and may open new perspectives towards the achievement of shared methodical techniques for the comparison of animal vocal repertoires.

Keywords: lemurs; vocal communication; unsupervised analyses

Introduction

Recent technological innovations in many areas of animal behavioral research, allow the collection of huge, complex, and often high-dimensional data sets. These can be daunting to be analyzed and may fail to satisfy the assumptions required by common statistical models [1]. Still, despite the high-dimensionality, because of the redundancy and multicollinearity of variables, data can be reduced and represented by fewer features [1]. The data reduction, indeed, allows the decrease of the storage amount and that of computational time, an easier understanding of data distribution, the improvement of visualization, classification and clusterization of high dimensional data [1,2]. Moreover, the dropping of uninformative attributes may help to highlight the best predictors and to improve the model's accuracy [1,3]. Dimensionality reduction can be performed with different kinds of procedures [1,2]: Classical methods like the metric multi-dimensional scaling (MDS) [4] and the principal components analysis [5] are fast and efficient but they may fail to identify the real structure of datasets when they contain a nonlinear configuration [6]. Both techniques also embed a cost function more reliable with the modeling of large dissimilarities rather than the small ones. Therefore, they may not provide a good visualization of data [6,7]. More recent methods, such as the stochastic neighbor embedding (SNE) [8] or the local linear embedding (LLE) [9], aim to represent the similarity structure of objects by involving a two-dimensional visualization, where the higher the similarity between pairs, the less the distance between them [7]. The SNE foundation is the modeling of pairwise similarities by transforming Euclidean distances into likelihoods of selecting neighbors [2] and, being centered on a probabilistic model, it uses different bi-dimensional spaces and combines them into a single model of similarity, therefore leading to a good visualization of data [7]. Still, albeit the latter, massive use of the SNE is prevented because of its "crowding problem" (the tendency to pack points together in the center of the plan) and because it uses a cost function difficult to be optimized [10]. We used a variation of stochastic neighbor embedding [8] the t-distributed stochastic neighbor embedding (t-SNE) [10] that differs from the first one by using a symmetrized variant of the SNE cost function with simpler gradients as introduced by Cook, J.A. et al [7]. It also uses a Student's t-distribution to compute the pairwise dissimilarities in low-dimensional space, instead of a Gaussian distribution [10]. The t-SNE heavy-tailed distribution allows confining both the optimization and the crowding problem of SNE, producing notably improved visualization [10]. Since its introduction, due to its flexibility, efficiency, and accuracy, various studies successfully applied the t-SNE and its extensions to the visualization and the classification of different kinds of objects: Paintings [11], single nucleotide polymorphisms (SNPs) [12], data collected by computer-aided diagnosis systems (CADx) [13], and high-dimensional cytometry data in mouse tumors [14]. t-SNE has also been employed in several studies investigating a wide range of acoustic aspects: To solve problems in the estimation and characterization of pitch content in musical audio [15], to examine similarities among words and phrases in natural language processing [16], to visualize relevant selected features of audio data [17], to characterize singing styles and to discriminate vocal and non-vocal contours [18], and to perform a dimensionality reduction in the building of an efficient technique of speaker recognition [19]. Still, this promising technique has hitherto rarely been applied to the study of animal behavior in general (stereotyped behavior of freely moving fruit flies, *Drosophila melanogaster*) [20], and never to investigate animals' vocal behavior. However, vocal repertoires may represent an ideal model for this kind of analysis. Indeed, the sounds investigation often implies the analyses of huge, high-dimensional datasets [21]. We used t-SNE to analyze the vocal repertoire of *Indri indri*, the largest living lemur and the only one producing coordinated vocal displays. Nonetheless, the particular song is not the only noteworthy trait of the species, which also possesses an interesting vocal repertoire. Non-human primates' vocal repertoires have been usually classified either as discrete (e.g., *Macaca fuscata* [22]; *Macaca Sylvanus* [23], with acoustically distinct call types clearly distinguishable from each other, or graded (e.g., *Cercopithecus diana* [24]; *Cercopithecus nictitans* [25]), when the acoustic structure of the vocalizations does not show neat boundaries between call types [26–28]. Yet, the dividing line between these two categories is not always clear and the classification of a whole repertoire as either continuous or discontinuous, may constitute an oversimplification [27,29], as repertoires may show both graded and discrete features (e.g., *Papio ursinus* [29]; *Cercopithecus neglectus, Cercopithecus campbelli, Cercocebus torquatus*, [30]), and the differentiation within vocal types may occur to varying degrees [31,32]. Traditionally, a large number of studies relied on the comparison of sounds similarity using clustering methods [33] based on acoustic features extracted from spectrograms. Still, although these algorithms showed good results in the classification of sounds, they could fail to describe the graded transition of call types that may occur in vocal repertoires [29]. Moreover, the gradation level is precisely one of the main reasons for the lack of consistency in vocal repertoire sizes assessments. Indeed, the higher the level of gradation, the higher the potential for information diffusion but also the higher the complexity in grouping the components of a system [28]. We expected to find a repertoire containing both graded and conspicuous signals [29,30] and, according to the call social function hypothesis, an acoustic variation of calls associated with their function [27,28,30,34]. Calls related to social contexts show the highest variation level when associated with affiliative value, while the highest level of stereotypy is associated with agonistic contexts (*Cercopithecus campbelli* [35]); alarm calls show an intermediate gradation level. Hence, we expected to find great flexibility in those calls having an affiliative social function, a rigid structure of signals associated with negative contexts, and an intermediate variation in the alarm calls. Accordingly, in agreement with Peckre and colleagues [28], we expected to find a clearer clusterization of discrete calls and a weaker grouping accuracy of graded ones. Finally, in agreement with the “social complexity–vocal complexity hypothesis” [30] and the social complexity hypothesis for communicative complexity [28], we expected indris to possess a small repertoire size if compared to that of other lemurs [21] or other primates [36] living in larger social groups.

Materials and Methods

Data Collection

We recorded spontaneous vocalizations of 18 groups of indris at four different forest sites: Six groups (1R, 2R, 3R, 5R, 6R, and XR) were recorded in Analamazaotra Special Reserve (18°56’ S, 48°25’ E), one group (1M) in Mantadia National Park (18°28’ S, 48°28’ E), three groups (ASF, YSF, and WSF) in Mitsinjo Forest Station (18°56’S, 48°24’ E), eight groups (1MZ, 2MZ, 3MZ, 4MZ, 5MZ, 6MZ, 8MZ, and 10MZ) in Maromizaha Forest New Protected Area (18°56’ S, 48°27’ E). Data from all forest sites, apart from Maromizaha, were collected from 2005–2008. Indris inhabiting the Maromizaha forest were sampled from 2008–2018. Recordings were collected using a Sennheiser shotgun ME 66 and ME 67 (Sennheiser electronic GmbH & Co. KG, Wedemark, Hanover, Germany) and AKG CK 98 microphones (AKG Acoustics, Harman International Industries, Vienna, Austria). The signals were recorded at a sampling frequency rate of 44.1 kHz using a solid-state digital audio recorder: Marantz PMD671 (Marantz, Kew Gardens, NY, USA), SoundDevices 702 (Sound Devices, LLC, Reedsburg, WI, USA), Olympus S100 (Olympus Corporation, Shinjuku, Tokyo, Japan), or Tascam DR-100MKII 24 bit/96 kHz (TEAC Corporation, Montebello, CA, USA), with a 16-bit amplitude resolution. Vocalizations were recorded at a distance from 2–10 m since all the study groups were habituated, and all efforts were made to ensure that the microphone was oriented toward the vocalizing animal. Focal animal sampling [37] and the presence of individual-specific natural marks, allowed the attribution of each vocalization to a signaler. Only spontaneous utterances were recorded, avoiding the use of playback stimuli.

Acoustical Analysis

We visually inspected all recordings using spectrograms (Praat 6.0.28) (Phonetic Sciences, University of Amsterdam, Amsterdam, The Netherlands) [38] and then cut high-quality vocal emissions, normalized, saved into single files (*n* = 3360), and assigned to nine putative categories on the basis of their acoustic and spectrographic evaluation, according to the vocal types identified in a previous study [39]: Clacsons (*n* = 622), grunts (*n* = 1145), hums (*n* = 418), kisses (*n* = 296), long tonal calls (*n* = 31), roars (*n* = 62), short tonal calls (*n* = 44), wheezes (*n* = 150), and wheezing grunts (*n* = 297). Moreover, all indris within a familiar group participate in a chorusing song, mainly consisting of harmonic frequency modulated notes [40]. We also isolated units from the songs and grouped them in a tenth category (songbits, *n* = 295). Eight vocal types and 1275 vocalizations out of 3360 were included in a previous analysis [39]; wheezing grunts were previously identified [41] but not detected by Maretti and colleagues [39], and song units were not considered in that former repertoire description. For each call, we extracted spectral coefficients using a custom-made script in Praat [38]. The script first calculated the overall duration of a sound and then split it into ten portions of equal length. For each portion, the frequency range between 50 Hz and 15,000 Hz was divided into sets of frequencies called bins or bands (e.g., 50–500 Hz, 501–1000 Hz, 1001–1500 Hz, and 2001–2500 Hz). For each bin, we extracted the energy value using the function ‘Get band energy’ in Praat. The resulting dataset contained 3360 samples with 151 attributes for each; one hundred and fifty parameters were frequency parameters, the last was the duration of sounds.

Acoustic Embedding and Classification Procedure

We embedded the spectral features vectors into a bi-dimensional space using t-distributed stochastic neighbor embedding [10] with a Barnes-Hut implementation, using the Rtsne package [42] in R (R Core Team 2018; version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria) [43]. We then used the t-SNE model (perplexity = 40, theta = 0.5, dims = 2) to group the cases, using k-means clustering [44]. t-SNE was also used for data visualization. We then used the WEKA 3.8 (Waikato Environment for Knowledge Analysis) [45] machine learning tool for the implementation of two classification algorithms. We applied multi-layer perceptron (MLP) [46,47], for the quantitative categorization of both the cluster assignment and the vocal type prediction, using the 67% of the dataset to train the neural network. We then computed two mean confusion matrices, one from the vocal types assigned a priori and the classes predicted by the MLP, the other one from the cluster assigned with the t-SNE procedure and the classes predicted by the network. Finally, to compare the results of the t-SNE cluster assignment to that of a k-means clustering (with $k = 7$, calculated through an average silhouette width) performed on a dataset reduced with a principal components analysis (and indicating six principal components), we applied a third network for the quantitative categorization of the cluster assignment.

Results

t-SNE Mapping

The t-SNE algorithm identified eight clouds (Figure 1a), we, therefore, performed a k-means clustering with $k = 8$. As highlighted in Figure 1a,b, the analysis recognized eight different clusters; all groups but three were consistent with the acoustic structure of the a priori identification. Cluster one, two, and three exclusively contain a vocal type each: Wheezing grunts (Figure 2f, Figure S1e), songbits (Figure 2i, Figure S2c), and clacsons (Figure 2j, Figure S2b), respectively (Table 1). Kisses and wheezes (Figure 2d,e, Figure S1c,d) were grouped in cluster five (66.37% and 33.63%, respectively), while grunts and hums (Figure 2b,c, Figure S1a,b) were both included in clusters four, seven, and eight. Specifically, cluster four contained mainly grunts (85.04%) and a small percentage of hums (14.96%); cluster seven, just as cluster four, comprised mostly grunts (99.00%). Conversely, cluster eight included a great portion of hums (82.06%) and a smaller part of grunts (17.94%). Short tonal (Figure 2g, Figure S1f), long tonal calls (Figure 2h, Figure S2d), and roars (Figure 2k, Figure S2a), although emerging as single clouds in the map, were grouped together in cluster six (respectively, 22.63%, 45.36%, and 32.12%, Table 1).

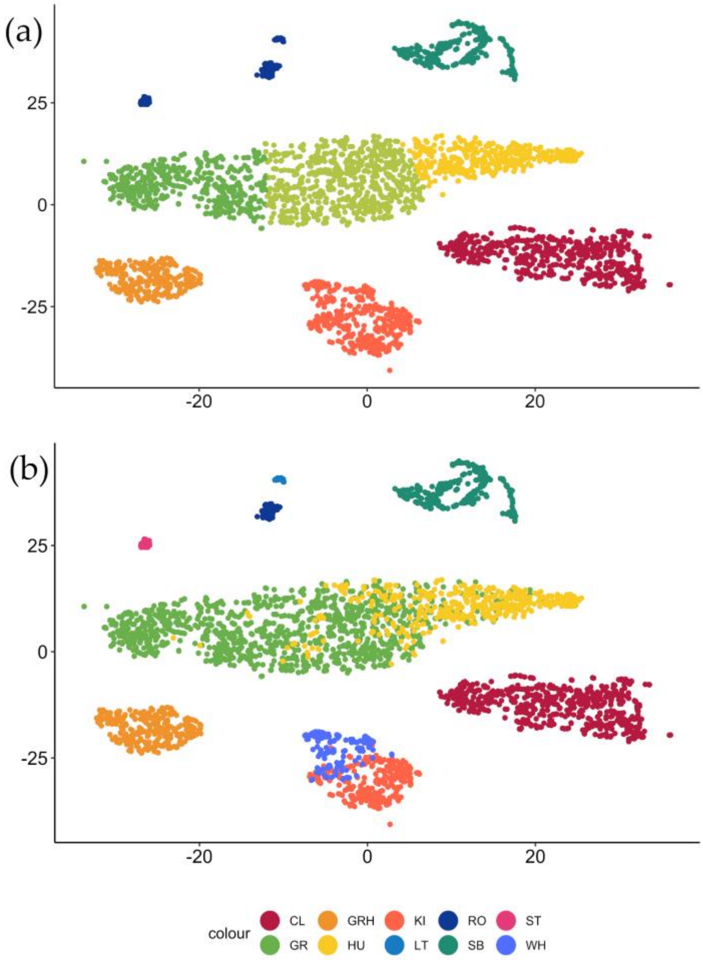

Figure 1. Bi-dimensional representation of the whole sample of sounds obtained initializing the t-SNE algorithm with perplexity = 40 and theta=0.5. (**a**) Output of the t-SNE mapping combined with the k-means clustering results. (**b**) Remapping the t-SNE output with the a priori classification and distribution of the vocal types in the clouds identified by the algorithm (cl=clacsons, gr=grunts, grh=wheezing grunts, hu=hums, ki=kisses, lt=long tonal calls, ro=roars, sb=songbits, st=short tonal calls, wh=wheezes).

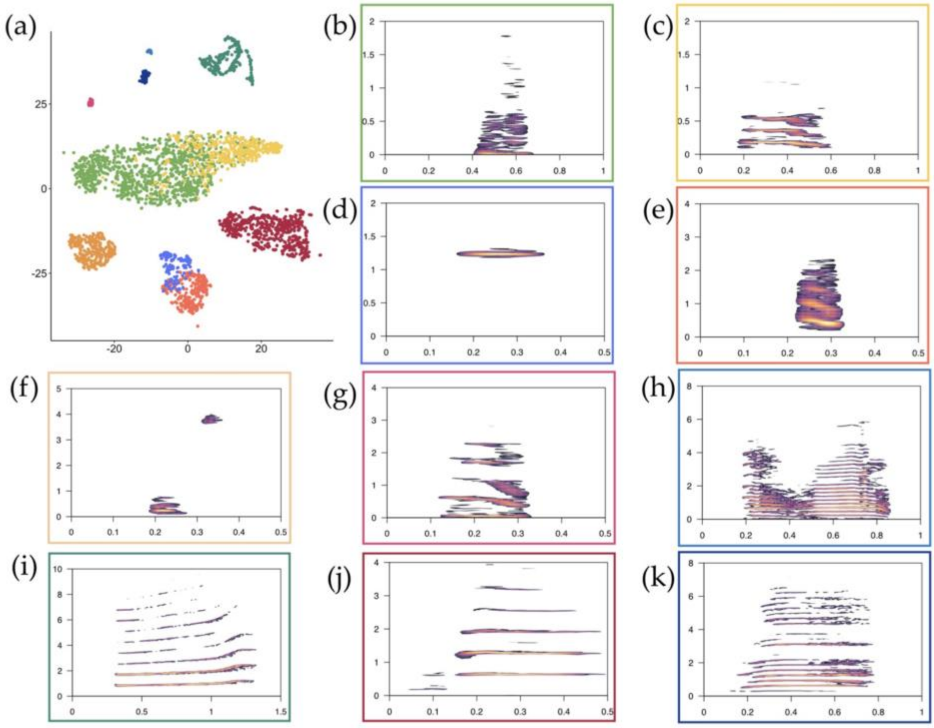

Figure 2. (**a**) Distribution of the vocal types in the clouds identified by the t-SNE map and their spectrographic representation: (**b**) Grunt (**c**) Hum; (**d**) Wheeze; (**e**) Kiss; (**f**) Wheezing grunt; (**g**) Short tonal call; (**h**) Long tonal call; (**i**) Songbit; (**j**) Clacson; (**l**) Roar. Almost all classes (except kisses and wheezes and hums and grunts), are well separated. Spectrograms -Frequency (kHz) on the y axis and Time (s) on the x-axis- were obtained with a Hanning window, 512 samples, 0% overlap, no zero-padding, using the Seewave package [48,49].

Cluster	CL	GR	WG	HU	KI	LT	RO	SB	ST	WH
1st	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2nd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
3rd	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4th	0.00	85.05	0.00	14.96	0.00	0.00	0.00	0.00	0.00	0.00
5th	0.00	0.00	0.00	0.00	66.37	0.00	0.00	0.00	0.00	33.63
6th	0.00	0.00	0.00	0.00	0.00	22.63	45.26	0.00	32.12	0.00
7th	0.00	99.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
8th	0.00	17.94	0.00	82.06	0.00	0.00	0.00	0.00	0.00	0.00

Table 1. Distribution of the vocal types in the eight clusters (expressed in %). Cl: clacsons; GR: grunts; WG: wheezing grunts; HU: hums; KI: kisses; LT: long tonal calls; RO: roars; SB: songbits; ST: short tonal calls; WH: wheezes.

Call Recognition

For the quantitative categorization of both the cluster assignment and the vocal type prediction, the network we selected, trained for 500 iterations yielded the best performance by using a learning rate =0.2 and momentum 0.2. The correct attribution for the vocal type prediction achieved the 85.57% (n = 949, kappa statistic: 0.820; mean absolute error: 0.034; root mean squared error: 0.157; Table 2). The network recognized all vocal categories with percentages of correct classification ranging from 58.76% for the wheezing grunts to 100.00% for the long tonal calls and roars. Clacsons and songbits were almost totally correctly classified (99.03% and 98%, respectively). The classification of grunts achieved lower performances (84.25%), as that of hums (84.56%), kisses (77.89%), short tonal calls (75.00%), and wheezes (78.57%, Table 3).

Vocal Type	TP Rate	FP Rate	Precision	Recall	F-Measure	MCC	ROC Area	PRC Area
CL	0.99	0.00	0.99	0.99	0.99	0.99	1.00	1.00
GR	0.82	0.08	0.84	0.84	0.83	0.74	0.94	0.88
WG	0.71	0.04	0.59	0.71	0.64	0.61	0.96	0.65
HU	0.83	0.02	0.85	0.83	0.84	0.81	0.98	0.90
KI	0.79	0.02	0.78	0.79	0.78	0.76	0.98	0.87
LT	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00
RO	0.81	0.00	1.00	0.81	0.90	0.90	1.00	0.98
SB	1.00	0.02	0.98	1.00	0.99	0.99	1.00	1.00
ST	0.69	0.00	0.75	0.70	0.72	0.72	0.98	0.76
WH	0.75	0.01	0.79	0.75	0.77	0.76	0.95	0.84
Weighted Average	0.86	0.04	0.86	0.86	0.86	0.82	0.97	0.90

Table 2. Vocal type assignment detailed accuracy by class. TP rate: Rate of true positives; FP rate: Rate of false positives; precision: Proportion of instances that are truly of a class divided by the total instances classified as that class; F-measure: Combined measure for precision and recall; ROC area: Receiver operating characteristics measurement area; PRC area: Precision recall area.

Classified as	A	B	C	D	E	F	G	H	I	J
CL	99.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.76
GR	0.00	84.25	38.14	14.09	10.53	0.00	0.00	0.00	0.00	4.76
WG	0.00	4.99	58.76	0.00	4.21	0.00	0.00	0.00	0.00	0.00
HU	0.00	6.30	1.03	84.56	0.00	0.00	0.00	0.00	0.00	2.38
KI	0.00	3.15	1.03	0.67	77.89	0.00	0.00	0.00	16.67	9.52
LT	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
RO	0.97	0.00	0.00	0.00	0.00	0.00	100.00	2.00	0.00	0.00
SB	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
ST	0.00	0.79	0.00	0.67	0.00	0.00	0.00	0.00	75.00	0.00
WH	0.00	0.52	1.03	0.00	7.37	0.00	0.00	0.00	8.33	78.57

Table 3. Confusion Matrix on vocal type prediction. Cl: clacsons; GR: grunts; WG: wheezing grunts; HU: hums; KI: kisses; LT: long tonal calls; RO: roars; SB: songbits; ST: short tonal calls; WH: wheezes.

The model built for the cluster assignment showed better results. A total of 1109 instances were correctly classified in 1059 cases (95.49%, kappa statistic: 0.947; mean absolute error: 0.016; root mean squared error: 0.088; Table 4). The network recognized all clusters with high percentages of correct classification (Table 5). Five groups (clusters 1, 3, 5, and 6) were entirely correctly classified, with a rate of correct assignment of 100%. The last three groups' classification showed almost as good results. The lowest performance was achieved by cluster 4 that was correctly classified in 85.35% of cases. Cluster 7 and cluster 8 showed the highest results: The first was correctly classified in 96.92%, while the second reached 95% of correct assignation. These groups, containing almost the totality of cases misclassified with respect to the clustering assignment, corresponded to the clusters showing a less homogeneous composition (Table 1): Cluster 4 and 7, contained mainly grunts (85.04% and 99.00%, respectively) and smaller percentages of hums (14.96% and 1%, respectively). On the other side, cluster 8 included a great portion of hums (82.06%) and a smaller part of grunts (17.94%). The third model, built using the PCA-based clustering as class, showed slightly weaker results when compared to the t-SNE model (93.05% vs. 95.49%; kappa statistic: 0.897; mean absolute error: 0.02; root mean squared error: 0.13).

Vocal Type	TP Rate	FP Rate	Precision	Recall	F-Measure	MCC	ROC Area	PRC Area
3rd	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00
1st	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00
4th	0.96	0.05	0.85	0.96	0.90	0.88	0.99	0.97
7th	0.83	0.00	0.97	0.83	0.90	0.88	1.00	0.98
8th	0.83	0.01	0.95	0.88	0.92	0.91	1.00	0.98
5th	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00
6th	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00
2nd	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00
Weighted Average	0.95	0.01	0.96	0.95	0.95	0.95	1.00	0.99

Table 4. Cluster assignment detailed accuracy by class. TP rate: Rate of true positives; FP rate: Rate of false positives; precision: Proportion of instances that are truly of a class divided by the total instances classified as that class; F-measure: Combined measure for precision and recall; ROC area: Receiver operating characteristics measurement area; PRC area: Precision recall area.

Classified as	A	B	C	D	E	F	G	H
3rd	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1st	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
4th	0.00	0.00	85.35	3.08	5.00	0.00	0.00	0.00
7th	0.00	0.00	9.16	96.92	0.00	0.00	0.00	0.00
8th	0.00	0.00	5.49	0.00	95.00	0.00	0.00	0.00
5th	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
6th	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00
2nd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

Table 5. Confusion Matrix on cluster assignment.

Discussion

We described the use of a computationally simple but powerful method applied in the automatic recognition of acoustic signals. The t-SNE embedding and the use of MLP allowed an efficient analytical performance: Our results indicate that it was possible to automatically identify vocal types by using a dataset consisting of high-dimensional vector representations of objects, assigning similarities between those objects as conditional probabilities [10]. Still, although both t-SNE [15–19] and neural networks [50,51] are widely used to analyze acoustic characteristics in a wide range of research fields, ours represents the first attempt to combine these kinds of computational tools and apply them to the identification of vocal repertoire in nonhuman primates. Our findings support what was found in a previous analysis on indris' vocal repertoire [39]. Indeed, our analysis confirmed the presence of the eight call types emerged in the study, but we also identified two further categories: The songbits, consisting of all units given by an indri during the choral song of the group, were not considered to the purposes of the qualitative assessment of *Indri indri* vocal repertoire; and the wheezing grunts [41], particular vocalizations given after agonistic physical interactions (pers. obs.), were not detected by Maretti and colleagues [39]. Albeit our analysis allowed us to easily distinguish the different vocal types, the algorithm's map contained some points clustered within the wrong class. Most of these points correspond to sounds belonging to vocal classes showing a certain degree of gradation one another and therefore may be difficult to be identified [29]. In particular, we found an overlay between hums and grunts and kisses and wheezes. Hums (also known as weak grunts) [52] and grunts are both low-frequency and low-intensity calls; hums show a more defined harmonic structure when compared to grunts that, in contrast, show a clearer and low-pitched pulsed structure [39]. Furthermore, hums serve as group-cohesion calls [39] and their gradation level is following what was found in Campbell's monkeys (*Cercopithecus campbelli*), where calls associated with high affiliative social values show an elevated gradation level [35]. The great gradation in these calls may allow for flexible usage and the encoding of multiple elements of information, in agreement with the findings of Keenan and colleagues on *Cercopithecus campbelli* [27]. Overall, our results are in line with findings on red-capped mangabeys (*Cercocebus torquatus*), whose contact calls show more acoustic dissimilarity than long-distance and alarm signals [53], in contrast with findings on chacma (*Papio ursinus*), olive (*P. anubis*), and Guinea (*P. papio*) baboons, whose loud calls are more differentiated than grunts [54]. Kisses and wheezes, on the other hand, are both brief medium-intensity vocalizations, often uttered together (85% of cases) [39]. They are stress-related vocalizations that can be emitted as contact-rejection call, before a song, or in response to anxiety-causing stimuli [39,41,55]. In our analysis, the categories identification relied on a human visual assessment, and the vocal classes grouping, although supported by our findings, may imply dissimilarities perceived by humans but not necessarily by the species [56,57]. Moreover, in agreement with what was hypothesized, our results indicated the presence of signals showing features of both conspicuousness and gradedness, as found in other primate species [27,29,30] and the analysis showed a stronger accuracy in the classification of discrete calls, than that of graded ones [28]. We expected the variation of calls to be associated with their social function [35], with calls having affiliative value showing the highest variation level, calls associated with agonistic contexts showing the highest stereotypy, and alarm calls showing an intermediate gradedness. This prediction was not entirely supported by our results, as we found the two alarm calls (roars and clacsons), well separated from one another. The result seems instead to be in line with studies on calls referentiality [58–60]. Additionally, the roars were grouped together with long tonal and short tonal calls; these three vocal types are the only with a chaotic component [39] and the result may depend by their spectral features, known to affect the vocalization recognition [21,61].

Finally, in agreement with the social complexity–vocal complexity hypothesis [30] and the social complexity hypothesis for communicative complexity [28], the vocal repertoire size is directly proportional to the group size. We expected indris to possess a small repertoire size compared to that of other lemurs [21] and other primates [36] living in larger social groups. A ten-categories vocal repertoire and an average group size of four to six individuals, seemed not to be in line with this theory, in accordance with findings on *Eulemur rubriventer*, owning a vocal repertoire of 14 vocal types and a group size of about three individuals [21]. Notably, both species also show a stable social monogamous organization [62,63], in agreement with the hypothesis stating that the diversity in communication signals may be favored by an egalitarian social structure or a stable social group [64]. These findings are also in agreement with the studies on Asian colobines *Pygathrix nemaeus* [65] and *Nasalis larvatus* [66,67], showing a repertoire size smaller or similar to that of indris, compared to an average group size sometimes even significantly higher.

Conclusions

As earlier hypothesized, the vocal repertoire structure may be determined by both the species' environment and social structure [68]. This could also be for the indris' case, where the presence of loud and discrete calls, like alarm calls [27,68] and even the song, may have evolved to cope with a noisy environment and poor visual ranges, like that of dense rainforests, to reduce the misinterpretation of signals in the long-distance and even in inter-group communication. On the other side, contact calls and in general vocalizations that may serve the intra-group and short-range communication, do not have to face such kinds of obstacles and may show a more graded structure.

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Conceptualization, D.V. and M.G.; data curation, D.V., M.G., O.F.; formal analysis, D.V. and M.G.; investigation, D.V., M.G., V.T., C.D.G.; methodology, D.V. and M.G.; resources, D.V., M.G., V.T., C.D.G, C.G., L.M.; supervision, M.G.; validation, D.V., M.G., V.T., C.D.G.; visualization, D.V., M.G., V.T., C.D.G.; writing—original draft preparation, D.V.; writing—review and editing, D.V., M.G., V.T., C.D.G., L.M.; R.M.R; O.F.

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217/16/MEEMF/SG/DGF/DSAP/SCB.Re], 2017 (73/17/MEEF/SG/DGF/DSAP/SCB.RE), 2018 (91/18/MEEF/SG/DGF/DSAP/SCB.Re).

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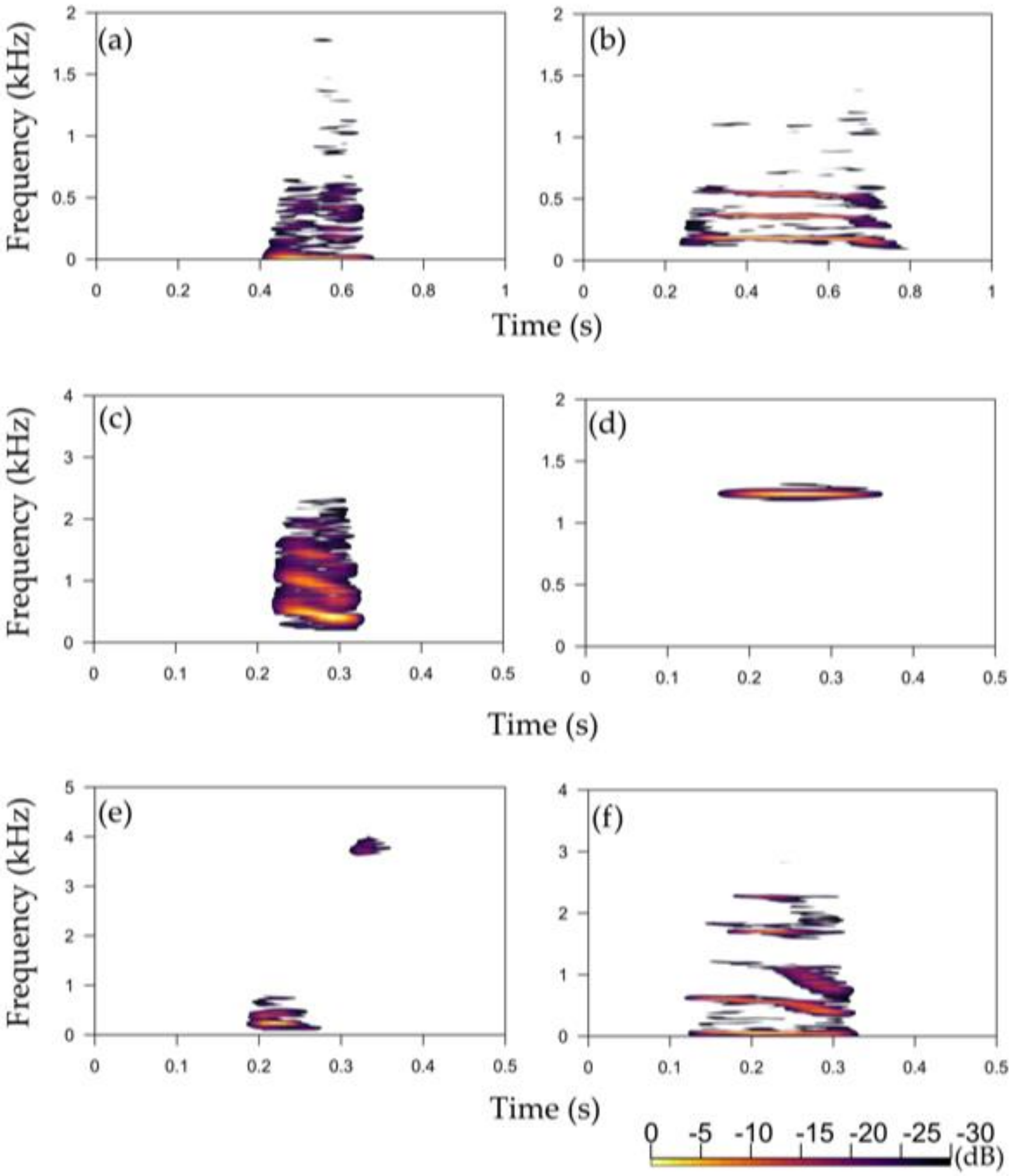

Figure S1: Sound spectrograms of *Indri indri* vocal types. **(a)** Grunt; **(b)** Hum; **(c)** Kiss; **(d)** Wheeze; **(e)** Wheezing grunt; **(f)** Short tonal call. All spectrograms were generated in R [43] through the Seewave package [48,49], using a Hanning window, 512 samples, 0% overlap, no zero-padding. Spectrograms of Grunt **(a)** and Hum **(b)** show a different time scale (1s) compared to the other (0.5s).

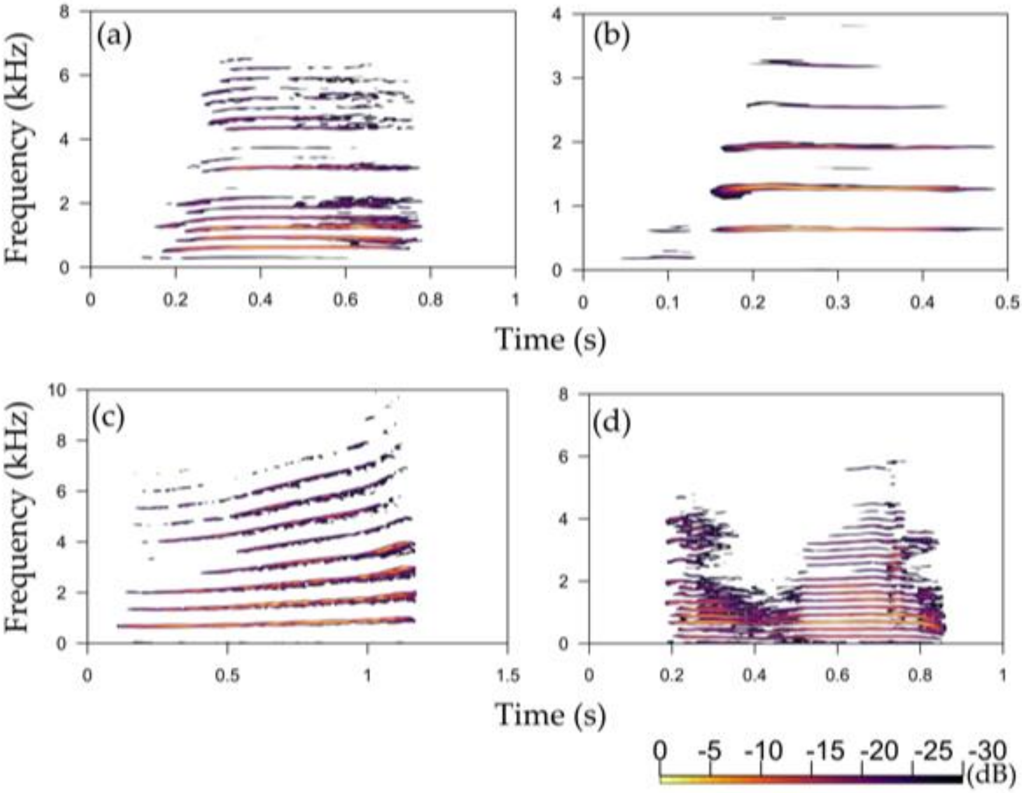

Figure S2: Sound spectrograms of *Indri indri* vocal types. **(a)** Roar; **(b)** Clacson; **(c)** Songbit; **(d)** Long Tonal Call. All spectrograms were generated in R [43] through the Seewave package [48,49], using a Hanning window, 512 samples, 0% overlap, no zero-padding. Spectrograms of roar **(a)** and long tonal call **(d)** are produced with a different time scale (1s) compared to both of songbit **(c,** 1.5s**)** and clacson **(b,** 0.5s**)**.

Discussion and concluding remarks

This thesis aimed to investigate the relationships between factors that may have shaped primate communicative traits, and that could have played a role in the evolution and diversification of vocal abilities in basal primates. Indeed, because of their particular evolutionary history, strepsirrhine primates are particularly well suited for investigating the evolution of vocal capacities in our lineage (Charles-Dominique and Martin 1972). Because of their peculiar vocal behaviour and social organization, indri is a particularly interesting species for exploring the potential factors responsible for the diversification of vocal communication (Wilkins et al. 2013), namely the degree of repertoire complexity. Several drivers have been considered to explain the variation in vocal production and complexity across species, including primates: genetics, habitat characteristics, sexual selection, and social organization (Freeberg et al. 2012).

Primate vocal production is regarded as mostly innate, although current evidence indicates more flexibility than previously thought (Hammerschmidt and Fischer 2018). Among singing primates, nonetheless, the evidence is conflicting; for instance, a certain degree of vocal ontogeny has been indicated in *Hylobates lar* (Koda et al. 2013) and *Nomascus gabriellae* (Merker and Cox 1999; Hradec et al. 2017). Still, other studies indicated a concordance between genetic and acoustic diversity in the *Nomascus* genus (Thin et al. 2011). Previous work on the indris' rhythmic abilities showed no differences across age cohorts (Gamba et al. 2016). I, therefore, investigated the relationship between vocal and genetic similarity by examining how the temporal pattern varied together with kin within a population (Chapter I), finding a relation between kin and vocal production, more marked in males than females. These results, combined with the lacking of membership cues encoded within the song structure (Chapter I), suggest that genetics may play a major role in shaping individual temporal features, at least for male indris.

The social system is considered another primary driver of mammalian communication (Blumstein and Armitage 1997; Freeberg et al. 2012; Pollard and Blumstein 2012), including primates (McComb and Semple 2005; Bouchet et al. 2013). The evolution of such a peculiar vocal output as singing seems to be related to a monogamous and territorial system (Adret et al. 2018), therefore expanding the knowledge of singing primate social organization may help in deciphering the evolutionary pressures that led to such an uncommon system among mammals, where monogamy and singing behaviour are rare. A first aspect related to the evolution of social life is the necessity of encoding individuality in vocal signals (Pollard and Blumstein 2011). Findings on indris indicate remarkable evidence of individuality, kin, (Chapter I), and sex of the emitters (Chapters I, II); phrases structure may be, therefore, used for conveying conspecifics cues for kin, gender, and individual recognition. Despite findings did not support the potential for group recognition (Chapter I), they suggest that songs may have a role in regulating the inter-group dynamics of the species (Chapter III), and in vehiculating information about group and chorus size (Chapter IV), although information on the actual dynamics of group chorusing and the potential for the evaluation of vocalizing individuals is lacking.

The temporal organization in the vocal interaction among individuals is a crucial trait of communication in pair-living duetting species (Bowling et al. 2013; Ravignani et al. 2014). I found indris capable to actively adjust the timing of their contributions (Chapter II), in agreement with other primate species, as cotton-top tamarins (*Saguinus oedipus*, Miller et al. 2003; Egnor and Hauser 2006) and common marmosets (*Callithrix jacchus*, Roy et al. 2011). Indris non-random pattern over time influences the achievement of a coordinated singing displays, where male rhythmic contributions to the songs are more fixed when compared to females' (Chapter II). The sexual dimorphism emerged in Chapters I and II confirms the result of a recent study indicating that in family-living, monogamous societies such as the indris (Bonadonna et al. 2019), a gender-related dissimilarity in temporal parameters and fundamental frequency does exist, although sexual selection should comparably affect the two sexes (Snowdon 2017). Moreover, vocal competition (Dunn et al. 2015) may explain the indris' dimorphic vocal structure. In indris, indeed, a case of extra-pair copulation has been reported (Bonadonna et al. 2014), and sexual monogamy co-occurs with the presence of unrelated individuals within a group (Torti et al. 2013). Therefore, inter-sexual selection may have played a role in the diversification of vocal signals (Singleton et al. 2009). Furthermore, despite the sex dimorphic structure of song (Chapters I, II), the individual contribution to the song is comparable between the sexes (Giacoma et al. 2010); this could also indicate that both sexes participate in territorial and resources defence, as suggested by Which and Nunn (2002).

Various studies investigating the role of social complexity as a driver of communication considered group size as a measure for social complexity, where the larger the group, the higher its social complexity (e.g., McComb and Semple 2005). Still, a more accurate layer of complexity lies in the evolution of bonded social systems. Indeed, the requirements for the maintenance of pair-bonded relationships over time, seem to be much more demanding than those needed to regulate less intense and more casual social interactions in larger societies (Freeberg et al. 2012, Bonadonna et al. 2019). An additional measure of social complexity concerned the home range: when groups or individuals occupy relatively small home ranges that are stable over both space and time, as found in indris (Bonadonna et al. 2017; Chapter III), individuals will interact with the same neighbors at home-range boundaries over the years (Freeberg et al. 2012). Consistently with findings on other territorial and pair-living monogamous primates (Adret et al. 2018), I found the singing behaviour crucial in regulating the spatial dynamics of the species, and its territory exclusivity and stability (Chapters III; IV). The peculiarity of the indris' vocal behaviour may be partially explained by considering the social organization of the species as a complex social network of family groups whose equilibrium is maintained through a complex communication system, rather than a single social unit, as indicated by indris inter-group dynamics (Chapter III).

I also faced the daunting task of investigating vocal behaviour in its diversity, examining vocal repertoire size. This measurement is particularly critical because diverse approaches may lead to strikingly different results (Gamba et al. 2015), but it provides one of the most robust proxies for vocal complexity (Oller and Griebel 2008; Zimmermann 2017; Peckre et al. 2019). Singing is indeed only one trait of the species vocal behaviour, that include at least eight additional vocal types, besides song units (Maretti et al. 2010). The analysis of the indris' vocal repertoire (Chapter V) will hopefully contribute data and techniques for future comparative analyses (Hammerschmidt and Fischer 2018).

Indeed, given the uniqueness of vocal behavior divergence across primate species, cross-taxa investigations and data sharing (both in terms of collection and analyses procedures) are fundamental to infer about the forces leading to communicative complexity in primates (Zimmermann 2017; Hammerschmidt and Fischer 2018). Currently, the scarce homogeneity among data processing and analyses mines the feasibility to perform comprehensive comparative investigations (Fischer et al. 2017; Peckre et al. 2019). Therefore, the methodology I tuned could constitute a step towards to achievement of common evaluation procedures for cross-species comparisons.

In sum, acoustic communication is a distinctive crucial trait of both humans and non-human primates. Therefore, the investigation of vocal abilities in non-human primates is fundamental to infer about processes that potentially may have played a role in the evolution of human language (Fedurek and Slocombe 2011; Hammerschmidt and Fischer 2018). The vocal tract and larynx basic layouts are highly conservative among terrestrial mammals, humans included, in both forms and functions (Fitch 2000; 2006; 2010; Fitch et al. 2016); the vast vocal diversity among primates, therefore, does not only depend on the morphological differences of the vocal tract (Fitch et al. 2016; Boë et al. 2017) but possibly on the volitional control over vocal production. Consequently, investigating the evolutionary basis for such an impressively diverse vocal production across primates may help in understanding the trajectories that shaped their communicative abilities and provide valuable insight into the evolution of human communicative complexity as well (Dunn and Smaers 2018).

In conclusion, this investigation highlighted different aspects of indris' vocal abilities, more flexible than previously thought. The song's coordinated pattern seems to be mediated by a series of rules also underlying other primate species communication, including humans (for instance, the sexually dimorphic structure, both in terms of spectral and temporal pattern, a variable degree of overlap among singers, and a flexible individual timing). Therefore, indris are excellent candidates to further investigate the extent of vocal capacities in singing species, and the forces guiding the evolution of such particular vocal traits and social systems, extremely rare among primates.

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