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It's all about rhythm:

Social, sexual, and developmental features

of the indris' songs

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"…to live a life, is not to cross a field"

Boris Pasternak – Doctor Zhivago

In loving memory of my grandmother, Angela

Takona, carrying her infant. Maromizaha 2019
(Photo by G. Binucci)

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Abstract

The study of primate vocal communication can offer useful insights into the selective pressures that may have shaped the evolution of language. In particular, among non-human primates, few taxa use complex sequences of vocal emissions, usually termed a song. Songs consist of notes that are combined into sequences and grouped at different levels hierarchically. It's intriguing how the use of songs and melodic sounds is shared between humans and non-human animals. Still, it is unclear which factors favored the emergence of this peculiar behavior that is likely to have evolved convergently. In primates, as in birds, singing behavior has been associated with territorial behavior and social stability. Among singing primates, *Indri indri* is the only lemur species that emits powerful and modulated songs, resonating in the montane rainforest of eastern Madagascar. Indris live in small family groups consisting of a reproductive pair and their offspring, and each social group occupies and actively defends an exclusive and stable territory. The fact that a strepsirrhine species characterized by long-term social bonds possess the ability to utter complex, coordinated signals that require turn-taking behavior represents a fascinating case among primates, and its songs well-deserve a fine-scale investigation. It may be interesting to understand which factors may have contributed to shaping vocal communication in basal primates and recognizing which of their traits are still shared with humans. The data used in this work were collected in Madagascar from 2005 to 2020 on 21 wild indris' 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). All these indris' groups may emit songs in the form of duet or choruses, depending on how many individuals perform the utterances simultaneously, taking turns. It is unclear how indris achieve the coordination of utterances; thus, I investigated the temporal domain of indris' songs, and I found that their rhythmic structure depends on factors that are different for males and females. Females, in particular, have

significantly higher variation in inter-onset interval duration and that, it 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, and, thus, we hypothesized that females lead the rhythmic structure of songs. Another way to investigate rhythmic patterns is to consider the ratio between two inter-onset intervals, calculated by dividing an interval by itself plus its adjacent one. This approach successfully highlighted similarities between human music and the song of thrush nightingales, which exhibited isochrony (ratio 1:1) but only a tendency for the 1:2 category. I adopted the same approach to investigate indris' songs. I found that these lemurs showed a link with human music even stronger than birdsongs since I successfully found both 1:1 and 1:2 rhythmic categories. This finding means that two consecutive intervals have more probably either the same duration or the second is twice the first one than having other durations. Moreover, while males and females have the same isochronous 1:1 ratio, males have a higher 1:2 ratio than females. This evidence enhances the sexual differences already found in inter-onset interval duration, which is dimorphic. Thus, we hypothesized that sexual selection may influence the interval duration and the higher 1:2 ratio in males. On the other hand, isochrony can effectively improve synchronization and coordination of utterances and, potentially, song learning. However, even if, to date, primate vocalizations are considered mostly inherited and already-in-place at birth, there is a growing corpus of evidence suggesting that social factors may indeed shape, at least in part, the ontogeny of primates' vocal behavior. For this reason, I investigate the developmental changes occurring in indris' songs, and I found that they underwent essential changes during growth. In particular, I found that frequency parameters showed consistent changes across the sexes, but the temporal features showed different developmental trajectories for males and females. Since the morpho-physiological modifications related to indris' development could only partially explain the difference I found, I hypothesized that factors like social influences and auditory feedback might affect songs' features, resulting in high vocal flexibility in juvenile indris. This has a sense as indris start participating in the complex turn-taking behavior of duets several years before reaching maturity. Finally, giving the peculiarity of singing behavior among primates, I was interested in understanding which socio-ecological traits might be the ones driving the emergence of these complex utterances. I looked at the link between pair-living, territoriality, and singing behavior in all the primates' species known to sing. I found that in all the cases investigated, territorial behavior co-occurred with singing behaviour, but that pair-living is not the rule in these primate taxa. In conclusion, this work sheds light on how rhythm is an essential feature of the indris' song. A strong sexual dimorphism is present in rhythmic features, from inter-onset interval durations to their ratios and their developmental trajectories. This aspect might be relevant to selective hypotheses for rhythm and musicality, speculating the role of sexual selection of an adaptive advantage on rhythm origins.

General introduction

The auditory channel is one of the most used signal modalities in the animal kingdom. Animals use vocalizations in several contexts, such as species recognition, resource defence, and mate attraction (Kroodsma & Byers 1991; Wich & Nunn 2002). Vocal signals can contain identity, sex, maturity, health cues (Bradbury & Vehrencamp 1998, Knörnschild 2013; Gamba et al. 2012). For instance, vocalizations of freely interacting mixed-sex pairs of mice showed marked sexual differences in the number of calls given and their acoustic structure (Warren et al. 2018). On the contrary, a study on the Australasian gannet calls indicated no evident sexual differences in their calls, but they had the potential to signal individual identity to conspecifics (Krull et al. 2012). Looking at what parameters encode individual differences, Favaro et al. (2017) found that fundamental frequency's features can discriminate among different penguin individuals (*Spheniscus* gen.). Interestingly, the human voice contains identity cues that can be used by animals of different species (Carlson et al. 2020). Humans also seem to discriminate animals individually based on their vocalizations (Friendly et al. 2014; Shaltz & Dickins 2021). This evidence shows how vocal expression and perception are fundamental aspects of vertebrate vocal communication.

Besides spectral features, temporal patterning plays a critical role in animal communication (Filippi et al. 2019). Early findings on ruffed lemurs and studies on gibbons showed that sequences of vocalizations might inform about phyletic differences. For example, red ruffed lemurs (*Varecia rubra*) showed a higher pulse rate than black and white ruffed lemurs (*Varecia variegata*), showing that temporal differences may be critical for species recognition (Macedonia & Taylor 1985, Brockelman & Schilling 1984).

Continuing to focus our attention on the temporal domain, since the discovery that rhythmic abilities are universal in humans (Stivers et al. 2009), rhythmic features of vocal communication have attracted a great deal of interest from scholars investigating animal behaviour and communication (Ravignani 2019). While the classical temporal parameter involved in acoustic analyses is the duration of a specific call or unit, one needs to consider the pattern of these individual temporal events to investigate rhythmic abilities. Rhythm has been proven a valuable tool in species discrimination (David et al. 2003); it can influence mate choice (Norton & Scharff 2016) and individual recognition (Mathevon et al. 2017). Another essential feature of a rhythmic output is that the single, temporally organized elements can be grouped at a different level, constituting a hierarchical metrical structure (Kotz et al. 2018). To date, the presence of categorical rhythms has been found only in humans and two species of songbirds (Savage et al. 2015; Roeske et al. 2020).

Moreover, adding another level of complexity, rhythm can be interactive, as different individuals emit vocal signals in a coordinated manner, such as duets or choruses. These peculiar behaviours are fascinating, as it has been suggested that group chorusing behaviour can shape individual timing abilities (Ravignani et al. 2014). Therefore, they can help understand the adaptive function of rhythm.

While scholars researching in the field of animal communication agree that we should investigate non-human communication for its own sake (Bräuer et al. 2020), many resources have been devoted to building a framework suitable to compare the human model. In this framework, besides experiments focused on humans, a comparative cross-species approach is fundamental to understanding our ancestors' communication (Ravignani & De Boer 2021). We do not have fossil evidence linked to the vocal modification that led to the rise of human language since they are of a behavioural nature. Therefore, it is interesting to investigate non-human primates' vocal behaviour to understand which mechanisms were already in place when human language evolved. Indeed, the great diversity of species in the Primate order and the variety of vocal signal characteristics and usage (Cheney & Seyfarth 2018) make non-human primates an excellent model to investigate the selective pressures involved in evolution vocal communication. Primates include more than 300 extant species ranging from nocturnal to daily habits, living in different forested habitats. They may live in small groups or up to thousands of individuals and show arboreal and terrestrial adaptation (Ramsier & Quam 2017). In many primate species, the chemical, tactile, and visual are essential communication channels (Snowdon 1982; Napier & Napier 1985), acoustic emissions are critical for diurnal primates inhabiting densely forested habitats. In fact, in the savanna habitat, the availability of a non-obstructed visual channel may permit greater elaboration of bi-modal visual/acoustic displays. The environmental characteristics may favour the acoustic channel (Waser & Brown 1984, 1986; Brown 1989).

While many species make conspicuous vocalizations, a limited number of species communicate using a sequence of vocal emissions, usually termed *songs*. Even if there is no unique definition of a song, most authors agree to distinguish a *song* from a *call* based on the duration of the sequence, the apparent structural hierarchy, and the frequency modulation (Spector, 1994). In mammals, song production is limited (Haimoff 1983) and about 16% of primate taxa can be included among the singing primates (Haimoff 1986, Gamba et al. 2014). To this limited circle belong the Hylobatidae family, *Indri indri*, some species of the genus *Tarsius* and the genus *Callicebus* (Robinson, 1979; MacKinnon & MacKinnon, 1980; Haimoff, 1986; Niemitz et al., 1991; Geissman, 1993; Thalmann et al. 1993; Nietsch & Kopp, 1998). However, in the last decades, the phylogeny of some of these taxa has dramatically changed and, currently, it is not clear to which extent singing behavior is currently present in these groups: for example, *Tarsius* and *Callicebus* have been split in different genera.

Indeed, as Ravignani et al. (2014) pointed out, one of the most complex and intricate displays in primate communication is the generation of synchronized sounds leading to chorusing and singing behaviour. In primates, singing behaviour has been associated with a territorial and socially monogamous lifestyle (Geissmann 2000). In line with the idea that the evolution of communal signals is related to the defence of ecological resources by individuals of both sexes forming stable coalitions, duetting species usually show territorial defence, long-lasting bond, and life in forested habitat (Thorpe, 1963; Tobias et al. 2016). It has been hypothesized that the evolution of coordinated signals has been guided by ecological conditions favouring sedentary lifestyles and social stability, as emerged from studies on tropical songbirds (Logue & Hall, 2014). Geissmann (2000) suggested that the evolution of primates' singing behaviour and duet singing behaviour could be related to the development of monogamy. However, these two grouping factors (territoriality and monogamy) for singing primates were defined decades ago, and a series of phylogenetic revision have been made in this taxon. Moreover, these groups of primates are not closely related; thus, it is likely that singing behaviour has evolved independently within the order (Geissmann 2000).

Since Darwin, singing behaviour has been the target of researchers interested in investigating the evolution of human language. Indeed, most of the works have focused on the *learning* aspect of communication, as this trait has been universally considered the basis for modern human speech. On one side, scholars urge to point out that the comparative approach is essential to truly understand the biological basis behind the evolution of this trait. On the other hand, as pointed out by Lattenkamp & Vernes (2018), considering only vocal learning, the vast majority of investigations have been conducted on birds (84 %) while few on them (4%) on non-human primate's vocal abilities. If we focus on singing primates, the values drop dramatically, given that they represent 16% of all primate species, from lemurs to apes. It is not difficult to understand why: singing primates are among the most challenging animals to study. Many of them are classified as Critically Endangered or Endangered by the IUCN (King et al. 2020; Rawson et al. 2020; Shekelle and Salim 2020). They live in lush tropical forests, generally with low population density. Also, only a few individuals are available for ex-situ studies (Gibbons – Melfi 2012), giving the breeding difficulty (Tarsiers – Řeháková-Petrů 2019) or impossibility (Indris – Petter et al. 1977). For these reasons, manipulation experiments are complicated to be carried out and impossible for some species. Thus, if we want to investigate endangered primate populations, we still have to rely on data collected in the field. It can take decades to study essential communication features, such as developmental ones or the effect of social aspects in vocal communication. This might be why there are currently some crucial lacks in the knowledge on primates' species communication and ecology, species that would be critical for unravelling the biological basis of modern human communication. For example, gibbons' songs can offer significant insights to the study of primate vocal communication since Geissmann (2000) suggested that "*loud calls in modern apes and music in modern humans are derived from a common ancestral form of loud call*." On the other hand, basal primates as indris and tarsier may provide promising new avenues to trace the evolutionary origins of primate communication: they exhibit unique diversity, complexity, and flexibility of vocalizations for social communication (Zimmermann 2017).

Moreover, given their unique evolutionary history (Charles-Dominique & Martin 1972) and adaptive peculiarities (Fleagle 2013), lemurs, in particular, represent the optimal phyletic lineage to investigate proximate and ultimate causes that may have contributed to shaping the vocal capacities of basal primates.

However, since songs are not used to convey complex referential meanings, there is not a direct correspondence between songs' notes and human language' words. Even if songs are considered "complex for the sake of advertising complexity" (Ravignani & De Boer 2021), their complexity is the condition *sine qua non* most of the information carried by this peculiar vocal display could not have been transmitted. A complex signal composed of several modulated units organized in phrase holds the potential for a greater degree of variability than a simpler call. Thus, information can be encoded in different songs' aspects such as notes' combination (Clarke et al. 2006), the rhythmic structure (Norton & Schraff 2016), degree of overlap (Hall & Magrath 2007), and different songs characteristics correspond to different contexts (Torti et al. 2013). Moreover, primates' songs can be uttered as solos, duet, or choruses, being an excellent model for studies focused on the interactive aspects of vocal communication and the biological function of rhythmic behaviours.

Indri indri is the only lemur species that emits song, as coordinated vocal displays, involving two or more individuals of a family group. These displays are usually introduced by harsh sounds (*roars*), followed by three main components: *long notes*, *single notes*, and *descending phrases*. The phrases are two to six units with a descending frequency pattern (Thalmann et al. 1993; Sorrentino et al. 2013; Torti et al. 2013). These notes can be given with different degrees of overlap between callers (absent to complete) and in an alternated or simultaneous way. These characteristics make the species an excellent model to investigate vocal complexity in singing coordination and rhythmic abilities. Currently, it is unclear how the coordination of utterances is achieved. Examining the individual timing during collective displays may be critical to our understanding of the processes underlying the chorusing dynamics and the adaptive role of rhythm (Ravignani et al. 2014). Since the first studies on rhythmic aspects of indris' song in 2016 (Gamba et al. 2016), it was clear that rhythm has been an overlooked aspect of singing primates' communication and that indeed we need more investigation to come close to our understanding of vocal rhythms in our closest living relatives, the primates (Ravignani et al. 2019). Most of the previous research on singing primates was concentrated on duet function (Geissmann 2002; Clarke et al. 2006), but few studies have explored coordination abilities and rhythmic capacities in non-human primates.

For this reason, the first chapter of this thesis intends to deepen 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 first is the inter-onset interval of units within a phrase (wpIOI), and, for the first time, I introduced the inter-onset gap between the beginning of the last note in a phrase and the beginning of the next one (bpIOI). This categorization was done because indris' utterances possess a hierarchical structure in which notes are organized in phrases, and a series of phrases constitutes a song. I investigated how the rhythmic structure varied according to the sex of the emitter, the duration of its phonation and contribution to the song, the song duration, and the number of individuals participating in 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 the adult male emission effect on the adult female singing for most of the songs. I hypothesized that the indris achieved the coordination of their songs by matching an individual rhythm to the singing pattern of others and predicted that the female would 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. Female indris seem to be more flexible when compared to males (Torti et al. 2017) and lead the rhythmic gaiting of the song, where the higher the number of singing individuals, the higher the cost they sustain.

Chapter I. 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*.

It is becoming increasingly evident that vocal expression and perception are fundamental aspects of vertebrate vocal communication, with shared capacities between humans and animals. This sharing regards not only the spectral domain (Filippi et al. 2017), but also the temporal one (Savage et al. 2015; Roeske et al. 2020).

In fact, in 2020 Roeske and colleagues published a research showing that, besides humans, also songbirds have, and may have convergently evolved, categorical rhythms. These categories consist in the relative and clustered timing of intervals and are considered "rhythmic universals". This is intriguing, as rhythm is a fundamental feature of another trait shared between humans and animals: melodic songs. Music is a common, widespread behaviour in humans, but it is not clear what are the biological origins of musical rhythm in humans and other primates. Recent work by Haiduck and colleagues (2020) showed that humans perform better in remembering song-like vocal phrases than speech-like ones, suggesting an evolutionary advantage of memorization of songs over speech. The authors also pointed out that we, as humans, have a long tradition of using songs to transmit cultural information over the generations, from the works of Homer to the Vikings saga and the Aboriginal Songlines (or *Dreaming tracks*). It is quite clear that only comparative studies involving non-human primates, and in particular the singing ones, can enlighten the evolutionary path that made music such an important feature of human culture. Both music and language consist of elements that are combined into sequences and group at different levels hierarchically. For this reason, given its peculiar hierarchical organization in singing behaviour, *Indri indri* represents an ideal model for deepening to which degree complex rhythmic features are shared between human and non-human primates. Chapter II aims to answer an interesting question raised by Burchardt and Knörnschild (2020): "Can we learn something about rhythm in animals that will help us understand their communication better and also find underpinnings of the abundance of rhythm in human biology and culture?". The same authors also suggested that results coming from these investigations should be comparable between species and contexts. I used the methodological approach developed by Roeske and colleagues (2020), which successfully highlighted rhythmic similarities between birdsong and human music. I, therefore, hypothesized that rhythmic categories should appear in species showing coordinated group singing and that the song of *Indri*

indri contains temporal intervals related by small integer ratios. I first aimed at statistically substantiating the intuition that indris' songs possess two types of inter-onset, namely bpIOI and wpIOI. I then extracted rhythmic ratios and evaluated if they belonged to different rhythmic categories (e.g., 1:1, 1:2, 2:1).

Chapter II. De Gregorio C., Valente D., Raimondi T., Torti V., Miaretsoa L., Friard O., Giacoma C., Ravignani A., Gamba M. Categorical rhythms in a singing primate. *Submitted to Current Biology*

An issue arising from the scarcity of long-term studies devoted to a fine-scaled analysis of vocal communication in all the species belonging to the families and genera of primates in which there is singing behaviour is that likely we still do not know the degree of vocal flexibility of these fascinating species. In particular, the ontogeny of vocal rhythms is the least studied aspect of rhythmic behaviours from a comparative perspective (Ravignani 2019).

Although it is generally agreed that non-human primates' calls are largely innate (Hammerschmidt & Fischer 2008), recent evidence indicated that not always inheritance and physiological modification could explain the developmental changes in vocal communication observed during growth (Takahashi et al. 2015). Moreover, in some cases, practising seems to be more important than exposure to an adult model to achieve the adult-like call form (Hammerschmidt et al. 2000). We only have a few indications concerning singing primates, coming from some works on gibbons, that indeed showed mixed evidence. While it seems that inheritance is a substantial factor in shaping gibbon song features (Tenaza 1985; Geissmann 1984), Koda et al. (2013) also indicated that practice during vocal interaction might be essential for the song developmental process. Also, the protracted nature of song development in gibbons (Merker & Cox 1999; Hradec et al. 2017) suggested that other primate species could follow a similar developmental trajectory.

For this reason, in chapter III, I explored the developmental processes shaping indris' vocal capacities, focusing on spectral and temporal parameters, including rhythm. I investigate juvenile indris' vocal behaviour, and I hypothesized that morpho-physiological modifications, such as elongation of vocal folds and lung capacity, will affect both spectral and temporal parameters. Therefore, I expected a decrease in fundamental frequency values of notes and an increase in their duration and/or number. I also predicted that, since the rhythmic structure of phrases did not change between adults and non-adults (Gamba et al. 2016), the song structure (the between-phrase IOI, introduced in the first chapter) would not change during ontogeny. Another prediction would be the emergence of sexually dimorphic traits in song ontogeny, given that in adults, there is a marked sexual dimorphism in song features. Finally, I also expected to find higher song variability in juveniles' songs than adult ones, in line with growing evidence showing that juvenile primates are more flexible than conspecific adults (Takahashi et al. 2015).

Chapter III. De Gregorio C., Carugati F., Estienne V., Valente D., Raimondi T., Torti V., Miaretsoa L., Ratsimbazafy J., Gamba M. and Giacoma C. Born to Sing! Song development in a singing primate. 2021. *Current Zoology,* 10.1093/cz/zoab018

An open question in the field of primates' communication is which factors have driven the emergence of the peculiarity of singing behaviour in primates. Among these factors, a link between territoriality, pair living and song emission has been suggested (Geismann 2000). Moreover, although the complexity of animals' songs has gathered much interest from scholars of different fields, it is not clear how much different songs organizations (solos, duets and choruses) are shared among singing primates' taxa. A further issue is the lack of a shared and unambiguous terminology for different songs' types. For this reason, the last chapter of this thesis represents the first review about primate singing displays and re-analyses the traits considered as potential factors in driving the evolution of singing behavior. I also reviewed the most used song definitions in this field of research and proposed new ones with the aim of removing possible ambiguity.

Chapter IV. De Gregorio C., Carugati F.., Valente D., Raimondi T., Torti V., Miaretsoa L., Gamba M. and Giacoma C. "Notes on a tree: reframing the relevance of primate choruses, duets, and solo songs." *Submitted to Ethology, Ecology and Evolution.*

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Chapter I

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 the 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 & 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 & 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 & 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 pro- vides 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”).

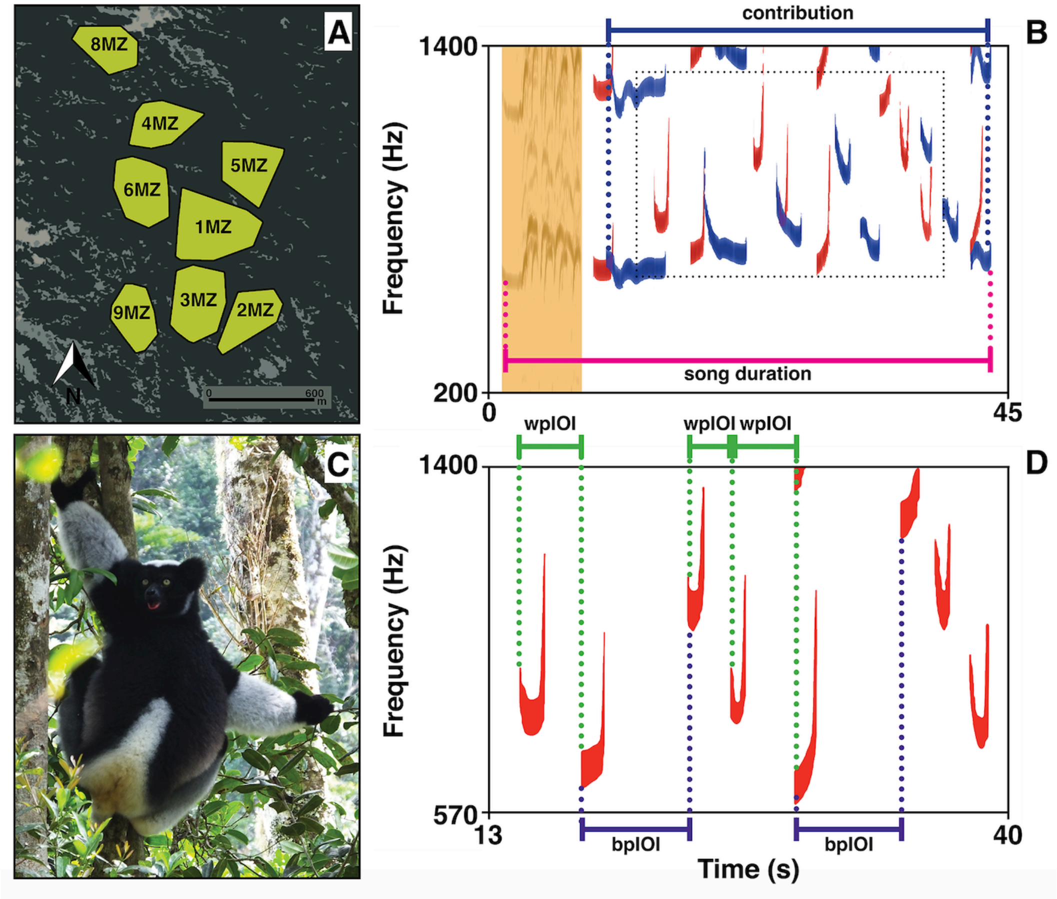

Figure 1. (A) Map of the study area in the Maromizaha Forest. Minimum Convex Polygons (MCP) generated with ArcGIS 9.1 (Environmental System Research Inc.) correspond to 2016 home range of the study groups. Group ID is reported onto each MCP. **(B)** Spectrogram of the indris' song generated using Praat. 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 perform 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).

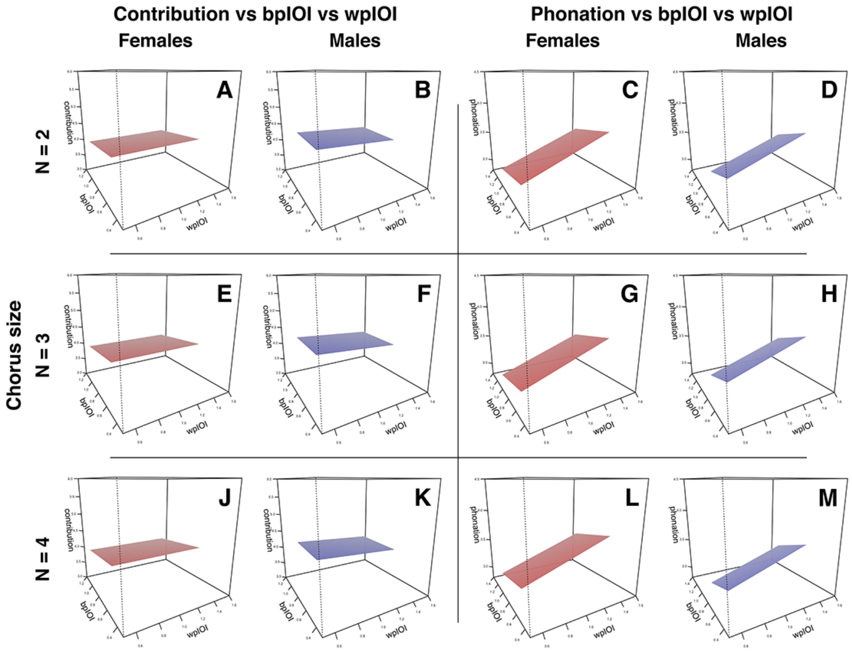

Figure 2. Interaction between contribution and phonation with bpIOI and wpIOI during the indris' song (N=119). The individual mean durations are 2.698 ± 0.705s for the wpIOI, 2.219 ± 0.330s for the bpIOI, 69.768 ± 32.452s for contribution and 28.416 ± 11.096s for phonation. The interaction is presented 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).

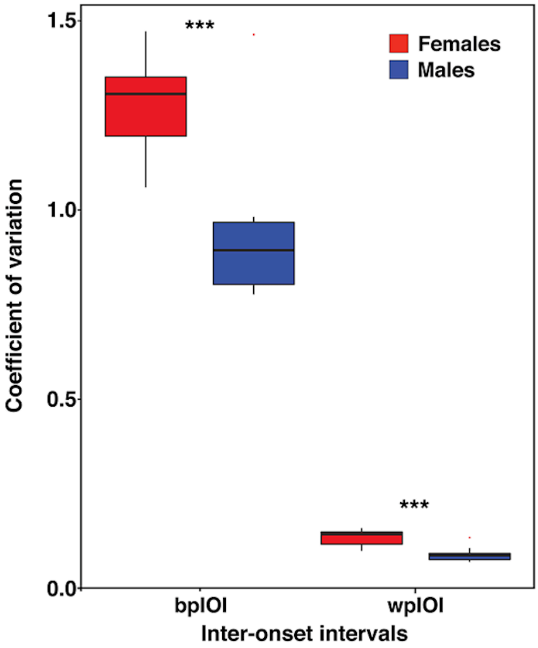

Figure 3. Boxplot of the CV of bpIOI and wpIOI in the sexes (males in blue and females in red). The values shown are calculated from the average individual means. Paired *t-test* significance at $P < 0.001$ is denoted by ***.

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 ($\chi^2 = 431.492$, 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

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 & 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 & 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 & 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 II

Categorical rhythm in a singing primate

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Categorical rhythms in a singing primate

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What are the origins of musical rhythm? One approach to the biology and evolution of musical rhythm consists in finding common musical traits across species. These similarities allow biomusicologists to infer when and how musical traits appeared in our species (Kotz et al. 2018). A parallel approach to the biology and evolution of music focuses on finding statistical universals (Savage et al. 2016). These include rhythmic features that appear above chance across human musical cultures. One such universal is the production of categorical rhythms (Roeske et al. 2020), defined as those where temporal intervals between note onsets are distributed categorically rather than uniformly (Savage et al. 2016; Jacoby & McDermott 2017; Ravignani et al. 2016). Because of categorical rhythms, any song sung (reasonably) faster or slower is still recognizable. Prominent rhythm categories include those with intervals related by small integer ratios, such as 1:1 (isochrony) and 1:2, which translates as some notes being twice as long as their adjacent ones. In humans, universals are often defined in relation to the beat, a top-down cognitive process of inferring a temporal regularity from a complex musical scene (Kotz et al. 2018). Without assuming the presence of the beat in other animals, one can still investigate its downstream products, namely rhythmic categories with small integer ratios detected in recorded signals. Here we combine the comparative and statistical universals approaches, testing the hypothesis that rhythmic categories and small integer ratios should appear in species showing coordinated group singing (Roeske et al. 2020). We find that a lemur species displays, in its coordinated songs, the isochronous and 1:2 rhythm categories seen in human music, showing that such categories are not, among mammals, unique to humans (Roeske et al. 2020).

Beyond melodic features (Figure 1A), individual animal vocalizations have onsets (blue lines in Figure 1C). Two onsets delimit an inter-onset interval (tk), i.e. the time between the onset of a note and the next one (Ravignani et al. 2016; Ravignani *in press*). Ratios between these intervals are calculated by dividing an interval by itself plus its adjacent one (Roeske et al. 2020). Patterns in these ratios may emerge: for instance, two identical intervals generate a 1:1 ratio, and an interval followed by another twice its duration generates a 1:2 ratio. This approach successfully highlighted rhythmic similarities between birdsong and human music (Roeske et al. 2020). In particular, for the first time in a non-human species, songs of thrush nightingales showed not only a significant isochronous rhythmic category (1:1 ratio), but also a potential bias towards 1:2 categories (Roeske et al. 2020). Apart from songbirds, singing mammals are promising species to look for musical universals, offering the advantage of phylogenetic proximity to humans (Roeske et al. 2020).

We focused on one of the few singing primates, the lemur Indri indri, sampling approximately 1% of all living individuals from this critically endangered species (see Supplement for details). All members of a family group sing in temporally-coordinated duets and choruses [De Gregorio et al. 2021; Gamba et al. 2016). Over 12 years, we recorded songs from 20 indri groups (39 individuals) living in their natural habitat, the rainforest of Madagascar (Figure 1B). We extracted temporal features of indris' songs, analyzing inter-onset intervals (Figure 1E) and their ratios (Figure 1D).

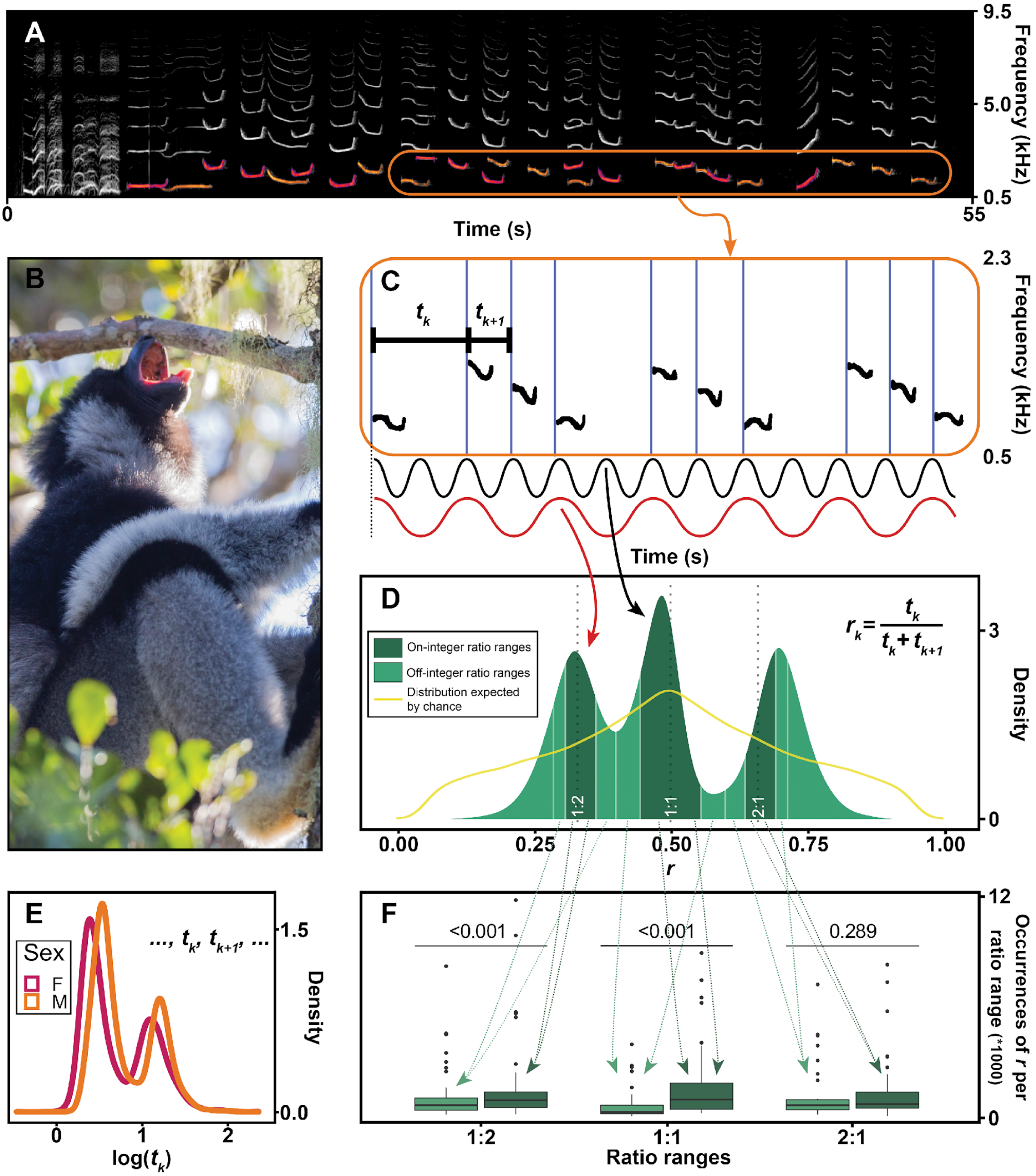

Figure 1. Indris' songs: Extracting individual rhythms from group choruses.
(A) Spectrogram of the indris' song, highlighting the fundamental frequencies of male (orange) and female (pink) notes (iZotope RX). The orange inset highlights the male song portion detailed in C. (B) Adult male indri singing in the Maromizaha New Protected Area, Madagascar (Credit: Filippo Carugati). (C) Schematic representation of onsets (solid blue lines) in a male

indri song. Pairs of onsets define inter-onset intervals tk, marked with solid black lines. Sine waves (bottom) exemplify which purported oscillatory processes might generate the rhythmic categories found in the data: 1:1 ratio in black, 1:2 ratio in red. (D) Probability density function of rhythm ratios (rk), which we calculated across 39 adult indris and 636 individual contributions to songs. On-integer (dark green) and off-integer (light green) ratio ranges are highlighted. A null distribution (yellow line), showing how the ratios would be distributed if the underlying intervals were uniformly distributed (hence in the absence of rhythmic categories, see Supplement), differs from the empirical distribution (2-samples Kolmogorov-Smirnov test: $0.109 < D < 0.127$, $p < 0 .001$). (E) Probability density function of inter-onset intervals (tk), which significantly differs from a uniform distribution (2-samples Kolmogorov-Smirnov test: $D = 0.656$, $p<.001$) and shows dimorphism between sexes (i.e., a statistically significant sex difference, see Supplement). (F) Boxplots of adjusted r occurrence for on-integer (dark green) and off-integer (light green) ratio ranges. The ratio counts for individual indris constitute the data points for the analysis, and counts are normalized by bin size (see Supplement). Indris significantly produce more on-integer than off-integer ratios for the 1:2 (Wilcoxon signed-rank Test, $V = 81$, p-values in figure) and the isochronous 1:1 categories ($V = 0$) but not for the 2:1 category ($V = 313$).

First, we find that intervals between note onsets are not uniformly distributed (Figure 1E), and that their ratios appear to form three clusters (green density function in Figure 1D). In other words, the intervals between notes are not sampled with the same probability among all possible values (yellow line in Figure 1D), similarly to the discretely-sampled note durations found in human music (Roeske et al. 2020; Jacoby & McDermott 2017). Note that finding these clusters does not, in principle, imply that they match specific values nor that these values are integer ratios (Ravignani et al. 2016).

Second, we find that ratios match two rhythmic categories: 1:1, isochronous, similar to the pace of a metronome, and 1:2, a fundamentally small integer ratio. We ask whether ratios produced by each individual fall more frequently on-integer, i.e. in the vicinity of a small integer ratio, than off-integer, i.e. in the vicinity of its adjacent non-integer ratio (Figure 1D, Supplement, and Roeske et al. 2020). By pairing the number of on-integer to off-integer ratios produced by each indri, we find that the empirical rhythmic ratios from songs fall statistically more often on small integer ratios than off integer ratios (Figure 1F). In particular, and similarly to songbirds (Roeske et al. 2020), indri songs have a strong, above chance, isochronous component with some tempo flexibility; indris sing at a slightly decreasing isochronous tempo, similar to musical ‘ritardando’ (Roeske et al. 2020). While songbirds have not been shown to produce 1:2 categories above chance (Roeske et al. 2020), indris' empirical ratios do statistically match the theoretical 1:2 category (Figure 1F). This provides direct evidence for one musical universal, categorical rhythms (Savage et al. 2016; Ravignani et al. 2016). The small integer ratios, falling at 1:1 and 1:2, are exactly those expected for beat production and binary, metrical subdivisions (Ravignani et al. 2016); both of these are aspects of human musicality rare in other species (Kotz et al. 2018). The 1:1 and 1:2 integer ratios we witness are not direct evidence of beat or meter, though they may hint at similar nested periodic processes (bottom of Figure 1C).

Third, our data show that males and females use different inter-onset intervals (Figure 1E and S1F) but the same 1:1 ratio (see Supplement). This may be relevant to selective hypotheses for music, hypothesizing a role of sexual selection, social bonding, etc. on rhythm origins (Savage et al. *in press*). Productive inference on how rhythm evolved requires multi-component thinking (Haimoff 1986, Kotz et al. 2018; Ravignani *in press*) rhythm as a whole is a mystery but some of its defining features may be present in other species, as we show here. Male and female indris may produce different singing tempi and interval durations, but 1:1 ratios are the same between sexes. This suggests that sexual selection should not affect the evolution of isochrony in indris; it might however play a role in single interval timing and in non-isochronous rhythmic categories (Kotz et al. 2018).

Why should another primate, apart from humans, produce categorical rhythms? As the last common ancestor between humans and indris lived 77.5 MYA, common ancestry of categorical rhythms is unlikely; instead, this ability may have convergently evolved among singing species, such as songbirds, indris, and humans (Savage et al. *in press*). As in songbirds, isochrony and rhythmic categories in indris may facilitate song coordination, processing, and potentially learning (Kotz et al. 2018; Roeske et al. 2020; De Gregorio et al. 2021, Ravignani *in press*). We encourage comparative work on indri and other endangered species to gain more data before it is too late to witness their breath-taking singing displays.

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Supplement

Methods

1. Observations and recordings

Indris (*Indri indri*) are lemurs whose ancestor diverged from ours approximately 77.5 MYA[S1]. Indris are classified as 'critically endangered' by the IUCN Red List of Threatened Species[S2]. While their population size is currently unknown, it has been estimated that only around 1000 - 10000 individuals remain in the wild[S3]. Indris have never been successfully bred in captivity[S4].

We recorded songs produced by 20 indri groups (39 individuals) living in four different rainforest patches in Madagascar (Figure S1A): six groups in the Analamazaotra Reserve (Andasibe-Mantadia National Park, 18°56' S, 48° 25' E), two groups in Mantadia (Andasibe-Mantadia National Park), three groups in the Mitsinjo Station Forestière (18°56'S, 48°4' E), and nine groups in the Maromizaha Forest (18°56'49" S, 48°27'53"E). Genetic analyses on seven groups inhabiting Maromizaha Forest confirmed that indris live in groups composed of an unrelated mating pair plus one to three individuals, usually their offspring[S5].We collected data in the field from 2005 to 2016 for a total of 41 months. We observed one group per day from 06:00 a.m. to 1:00 p.m., using natural marks to identify each individual. Our analyses focused on adult reproductive individuals because temporal features in the vocalizations of adult reproductive indris may differ from those found in young or non-reproductive individuals[S6,S7] .

We recorded songs 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. We set the recorders at a sampling rate of 44.1 kHz and an amplitude resolution of 16 bits during all the recording sessions. We recorded the animals at a distance ranging from 2 to 20 meters, with the microphone aimed at the focal singing animals. The recordist pointed the microphone towards a particular individual and attributed each vocalization to the signaler via the focal animal sampling technique[S8]. Songs can take the form of duets when two indris (usually the reproductive pair) sing together, or choruses, when one or more non-reproductive individuals join the pair in singing. In the indris' songs, notes can be either organized in phrases or produced as isolated units. Phrases are characterized by adjacent units with a descending frequency pattern, while isolated units are single notes between two phrases.

2. Acoustic analyses

We recorded a total of 636 individual contributions uttered by 39 reproductive indris, 20 females and 19 males. Using Praat 5.3.46[S9], we edited and saved the recorded portion containing the indris' song as a single mono audio file (WAV format). Using field notes and video recordings, we then identified, annotated, and saved the onsets and offsets of each note for each individual as a Praat textgrid. We identified units and silences via visual inspection of the spectrograms. We labeled the notes, differentiating if they were organized in a sequence or produced as isolated units. Our analysis does not include 'roars', which are harsh and chaotic emissions that usually introduce the song[S10].

We then labeled the silent portions of the individual contributions, differentiating silences depending if they occurred between two notes of the *same phrase,* or, conversely, if they occurred between two *different phrases* or two different *isolated notes* . 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[S11]. We imported them in R (R Core Team 2017; version 3.4.3) and calculated the inter-onset intervals (t_k, Figure 1C) to evaluate the rhythmic structure of contributions[S12]. Notice that a sequence of n notes will produce n-1 inter-onset intervals, i.e. $t_1,...,t_{n-1}$. Based on previous work[S3,S7], we used the information on notes and silences type (belonging to a phrase or an isolated note) to calculate two types of t_k: the within-phrase t_k (WP: Within-Phrase inter-onset intervals, that is, all and only the intervals between notes *within* a phrase) and the between-phrase t_k (BP: Between-Phrase inter-onset intervals, that is, all and only the intervals *between* two different phrases or between two isolated notes).

We then calculated rhythmic ratios r_k following the methodology of Roeske and colleagues[S14], namely by dividing each t_k for its duration plus the duration of the following interval: $r_k = t_k / t_k+t_{k+1}$. Since each t_k can either be a WP or a BP, we obtained four ratio types: WP/WP+WP, WP/WP+BP, BP/WP+BP and BP/BP+BP, depending on the type of two adjacent t_k. These four ratio types and their corresponding intervals in indris' songs are depicted in Figure S1B-E. Further analyses were performed both on the t_k values and their ratios r_k.

3. Statistical analysis

Four main analyses were performed. All statistical analyses in 3.1-3.3 below were performed in R (R Core Team 2017; version 3.4.3). Analyses in 3.1 aimed at statistically substantiating the intuition in Figure 1E, namely that the distributions of t_k durations have 4 distinct peaks, potentially different by sex and t_k type. Analyses in 3.2 aimed at testing this same hypothesis on the r_k data. Analyses in 3.3 correspond to, and provide details for, the main result reported in the manuscript and in Figure 1F. When compared to 3.3, analyses in 3.2 afford the possibility of zooming in on the overall pattern of ratios and analyzing them by sex and ratio type. In addition, comparison between results in 3.1 and 3.2 allow inference on potential sexual differences in durations and/or ratios. Finally, the simulation described in 3.4 aimed at providing a plausible baseline scenario of how empirical ratios would look if indris had no rhythmic categories. While density functions (Figure 1D and 1E) were calculated for illustrative purposes, all statistical analyses were based on the original data points, not the density functions.

3.1. t_k durations

We tested whether sex and the type of t_k statistically predicted the inter-onset interval duration, using a Linear Mixed Model (LMM, lmer function of lme4 package[S15]). Before fitting the models, t_k duration was log-transformed ($\log_e$) because it was not normally distributed. We entered the values of t_k as response variable, and sex of the emitter, t_k type (WP and BP) and their interaction as fixed factors. We included the individual's identity and the specific song contribution from which we extracted the t_k as nested random factors. We used the Tukey test[S16] to perform all the pairwise comparisons for all the interaction levels between sex and t_k type (multiple contrast package *multcomp* in R).

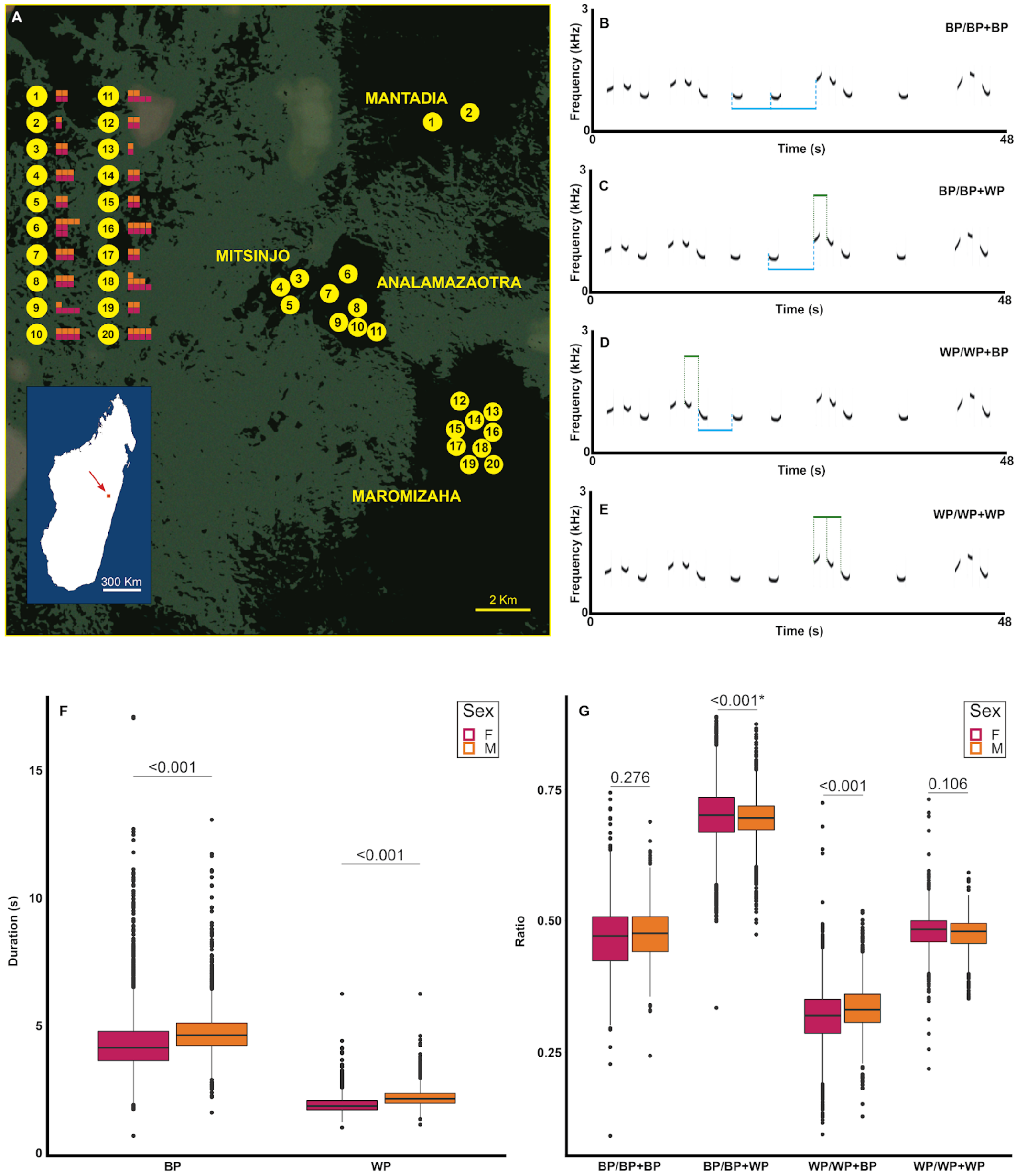

Figure S1. Song recording locations, with schematic representation of durations and ratios types and their values. (A) Map showing the four sites where indris were recorded. Yellow numbered circles represent the ID of the familiar group sampled. Colored lines next to the circles indicate that a reproductive male (orange) or female (pink) belonged to that group; the number of squares in each line denotes the number of years that individual was recorded. **(B)** Schematic representation of a "BP/BP+BP" r_k type; the light blue line indicates two BP intervals: a t_k between two isolated notes, and a t_k between an isolated note and the first note of a phrase. **(C)** Schematic representation of a "BP/BP+WP" r_k type; the light blue line indicates a BP interval, and the green line indicates a WP interval: a t_k between an isolated note and the first note of a phrase, and a t_k between the first two notes of the same phrase. **(D)** Schematic representation of a "WP/WP+BP" r_k type; the light blue line indicates the BP interval, the green line indicates the WP interval: a t_k between two notes of the same phrase, and a t_k between a note belonging to a phrase and an isolated note. **(E)** Schematic representation of a "WP/WP+WP" r_k type; the green line indicates twoWP intervals: in this case a t_k between the first and second note of the same phrase, and a t_k between the second and third note of the same phrase. **(F)** Boxplots of t_k duration by type. Notice that the WP boxplots here correspond to the first and second peaks in Figure 1E, and the BP boxplots here correspond to the third and fourth peaks in Figure 1E. **(G)** Boxplots of r_k values by type. The * denotes that, although the Tukey test is statistically significant, the difference is negligible because of the small effect size of the comparison.

3.2. t_k ratios (r_k)

To test whether the values of t_k ratios (r_k) were statistically influenced by the sex of the singer and the r_k type (WP/WP+WP, WP/WP+BP, BP/WP+BP and BP/BP+BP), we used a Generalised Linear Mixed Model (GLMM, *glmmTMB* package[S17]) fitting a beta distribution, continuous between 0 and 1. Beta was chosen via the package *fitdistrplus*[S18] as a suitable theoretical distribution. The values of r_k were entered in the model as response variable, and sex of the emitter, r_k type and their interaction as fixed factors. We included the individual identity and the specific song contribution from which we extracted the ratios r_k as nested random factors. We used the Tukey test (within the multiple contrast package *multcomp* in R) to perform all pairwise comparisons for all levels of the interaction between sex and r_k type and for all levels of r_k type[S16].

For both models, we verified the assumptions of normality and homogeneity of residuals by visually inspecting the *qqplot* and the residuals' distribution (a function provided by R. Mundry). We also excluded the presence of collinearity among predictors based on variance inflation factors (*vif* package[S19]). To test for significance of our full models[S20] we compared them against null models containing only the random factors, with a likelihood ratio test (Anova with argument test "Chisq"[S21]). We report estimates, standard error (S.E.), z- and p-values for the Tukey tests (Table S1C-E).

Finally, we used Cohen's d test to compute the magnitude of the effect size for the Tukey tests' comparisons between different types of durations and ratios between the two sexes.

3.3. Rhythmic categories: Ratios distribution and their peaks

To evaluate the empirical occurrence of small integer ratios, following the methodology of Roeske and colleagues [S14], we divided the ratio distribution into on-integer and off-integer ratio ranges, centering the on-integer ratio ranges around 1:2 (or 0.333; a fundamentally small integer ratio), 1:1 (or 0.500; corresponding to isochrony), and 2:1 (or 0.666; a fundamentally small integer ratio). While the 1:1 ratio corresponds to two intervals of equal duration, the 1:2 and 2:1 ratios correspond to the second interval being, respectively, double and half the duration of the first.

Conversely, again following the methodology of Roeske and colleaguesS14, off-integer ratio ranges were centered around 1:3.5 (or 0.285), 1:2.5 (or 0.400), 1-1:2.5 (or 0.600), and 1-1:3.5 (or 0.710); the boundaries of all on- and off integer ratio ranges were 1:3.25 (or 0.307), 1:2.75 (or 0.363), 1:2.25 (or 0.444), 1-1:2.25 (or 0.555), 1-1:2.75 (or 0.637), and 1-1:3.25 (or 0.693). All these ratios were those used in Ref[S14]. We then counted all occurrences of ratio values that fell in each on- and off-integer ratio range for each individual, and we normalized these counts according to the size of their range on the x-axis[S14]. Notice, for instance, how the on-integer ratio range of 1:2 in Figure 1D is narrower than the 1:1 range; normalization allowed to correct and account for this and other inequalities. The Shapiro-Wilk tests confirmed that our count data did not follow a normal distribution, so we compared on-integer and off-integer ratio ranges using three (paired) Wilcoxon signed-rank tests.

3.4. Simulated ratio distribution

For reference, we simulated the null ratio distribution N expected by chance. The yellow line in Figure 1D is based on 100,000 simulated ratios produced using a custom script in Python 2.7.10. Based on the upper (681 msec) and lower (17089 msec) bound of inter-onset intervals naturally produced by indri[S13], we simulated what would happen if no rhythmic categories existed by sampling inter-onset intervals from two uniform distributions U and V with bounds at 681 and 17089 msec and calculating each ratio as in the formula in Figure 1D. In other words, this sampling simulated the distribution N of the ratio between one random variable U and its sum with another random variable V, both random variables being uniform.

Finally, to understand if the simulated ratio distribution (yellow line in Figure 1D) significantly differed from the real one, we resampled 17100 observations (the number of observations in our dataset) from the 100,000 simulated ones. This was done 1000 times, and every resampled distribution was compared with the real one with a Kolmogrov-Smirnov in R.

Results

1. t_k durations

The average duration of t_k was 2.114 ± 0.298s for WP and 4.651 ± 1.008s for BP. This difference emerged also in Figure 1E, where the probability density function clearly indicated the presence of two clusters in the t_k duration. Males showed longer t_k than females (Figure S1F, Table S1A), both overall (M = 3.429 ± 1.372s, F = 2.919 ± 1.403s; $p < 0.001$) and by t_k type (WP: M = 2.339 ± 0.340s, F = 1.899 ± 0.258s; $p < 0.001$; BP: M = 5.002 ± 0.928s, F = 4.318 ± 1.083s; $p <0.001$; Table S1D; Figure S1F). WPs had a shorter duration than BPs ($p < 0.001$; Table S1A). For the comparison between the two sexes, Cohen's d was 1.477 for WP and 0.679 for BP, confirming that the significant sexual differences were non-negligible. In brief, there is both a sexual dimorphism in durations and a significant difference between the t_k types BP and WP, exemplified as the four peaks in Figure 1E.

2. t_k ratios (r_k)

The average of ratios r_k was 0.499 ± 0.161. Our model showed significant differences among r_k types (Table S1B), and the Tukey test confirmed that all four r_k types differed significantly ($p < 0.001$, for every comparison; Table S1C). The average WP/WP+BP was 0.324 ± 0.054, WP/WP+WP was 0.477 ± 0.033, BP/BP+WP was 0.698 ± 0.054, and BP/BP+BP was 0.470 ± 0.068. Notice how both values linked to isochrony (BP/BP+BP and WP/WP+WP) are slightly smaller than 0.5, suggesting increasing duration of adjacent intervals, i.e. ritardando.

Males presented overall higher ratio values than females ($p = 0.021$; Table S1B). However, the Tukey test indicated no significant sex differences for the ratio's types linked to isochrony (BP/BP+BP, $p = 0.276$; WP/WP+WP, $p = 0.106$; Table S1E) emerged. Instead, we did find sexual dimorphism in r_k type WP/WP+BP, where males showed lower values than females ($p < 0.001$), and in BP/BP+WP, where males were the ones showing higher values ($p < 0.001$). When comparing between sexes, Cohen's d was 0.152 for WP/WP+WP, 0.059 for BP/BP+BP, 0.278 for WP/WP+BP. Instead, it was 0.111 for BP/BP+WP, suggesting that this difference between males and females is negligible, even if it is statistically significant (Figure S1G). Therefore, the only actual (significant and non-negligible) difference in r_k types concerns the value of WP/WP+BP between males and females. Notice that this value is always greater than 0.5 because BP>WP. WP/WP+BP corresponds to the third peak in Figure 1D and the third pair of boxplots in Figure 1F, also corresponding to the one non-significant peak out of three. No significant peak in Figure 1D shows sexual dimorphism. In brief, there is little sexual dimorphism in ratios and categories and no dimorphism at all for significant rhythmic categories, speaking *against* sexual selection hypotheses for rhythm.

Summary and details of both models and Tukey tests appear in table S1.

3. Rhythmic categories: Ratios distribution and their peaks

Visual inspection of the occurrence of different r_k types indicated a possible presence of three clusters (green density function in Figure 1D). Statistical comparison between data points from this density function and from the distribution expected by chance (yellow line in Figure 1D) suggested that our empirical ratios differed from those expected by chance. The dependent 2-group Wilcoxon tests between on-integer and off-integer ratio ranges (Figure 1F) confirmed that indris produce songs characterized by at least two rhythmic categories: isochrony, corresponding to 1:1 ratio ($p < 0.001$,$V = 0$) and a 1:2 ratio ($p < 0.001$, $V = 81$). A V value of 0 for the isochronous 1:1 categories means that each of the 39 indris produced more on-integer than off-integer ratios, not only statistically but also numerically. However, we did not find significant differences between on-integer and off integer ratio ranges for 2:1 ($p = 0.289$, $V = 313$). In other words, the first and second (but not the third) peaks in Figure 1D result from indris producing ratios falling on small on-integer ratio neighborhoods rather than equally-sized off-integer neighborhoods.

A	**[LMM] Response Δ**	**Fixed Factor**	**Random Factors**	**Package**	
	t_k duration	Sex * t_k type	ID ind, ID contrib	lme4	
Predictors	*Estimate*	*SE*	*df*	*t*	*p*
(Intercept)	1.423	0.028	[a]	[a]	[a]
WP[b]	-0.080	0.038	13770	-207.956	**<0.001**
M[b,c]	0.163	0.033	40.130	5.443	**<0.001**
WP* M[b,c]	0.037	0.006	13760	5.875	**<0.001**

B	**[GLMM] Response Δ**	**Fixed Factor**	**Random Factors**	**Package**
	r_k	Sex * r_k type	ID ind, ID contrib	glmmTMB
Predictors	*Estimate*	*SE*	*z*	*p*
(Intercept)	-0.130	0.008	[a]	[a]
BP/BP+WP[b]	0.982	0.009	105.460	**<0.001**
WP/BP+WP[b]	-0.632	0.009	-68.020	**<0.001**
WP/WP+WP[b]	0.046	0.009	4.860	**<0.001**
M[b,c]	0.034	0.015	2.300	**0.021**
BP/BP+WP* M[b,c]	-0.069	0.016	-4.170	**<0.001**
WP/BP+WP* M[b,c]	0.040	0.016	2.430	**0.015**
WP/WP+WP* M[b,c]	-0.054	0.016	-3.270	**0.001**

C		**[Tukey Test] r_k type**		**Package:** multcomp
	Estimate	*SE*	*z*	*p*
BP/BP+WP - BP/BP+BP	0.982	0.009	105.457	**<0.001**
WP/WP+BP - BP/BP+BP	-0.632	0.009	-68.021	**<0.001**
WP/WP+WP - BP/BP+BP	0.045	0.009	4.858	**<0.001**
WP/WP+BP - BP/BP+WP	-1.614	0.006	-259.709	**<0.001**
WP/WP+WP - BP/BP+WP	-0.936	0.006	-144.786	**<0.001**
WP/WP+WP - WP/WP+BP	0.677	0.006	105.373	**<0.001**

D		**[Tukey Test] t_k type * Sex**		**Package:** multcomp
	Estimate	*SE*	*z*	*p*
(F:F) WP-BP	-0.796	-0.796	-207.956	**<0.001**
(M:F) BP-BP	0.163	0.038	5.443	**<0.001**
(M:F) WP-BP	-0.596	0.030	-19.994	**<0.001**
(M:F) BP-WP	0.958	0.030	32.090	**<0.001**
(M:F) WP-WP	0.199	0.030	6.697	**<0.001**
(M:M) WP-BP	-0.759	0.005	-153.539	**<0.001**

E		**[Tukey Test] r_k type * Sex**		**Package**: multcomp
	Estimate	*SE*	*z*	*p*
(M:F) BP/BP+BP-BP/BP+BP	-0.796	-0.796	-207.956	0.276
(M:F) BP/BP+WP-BP/BP+WP	0.163	0.038	5.443	**<0.001**
(M:F) WP/WP+BP-WP/WP+BP	-0.596	0.030	-19.994	**<0.001**
(M:F) WP/WP+WP-WP/WP+WP	0.958	0.030	32.090	0.106

Table S1. Summary and details of models and Tukey tests. (**A**) Influence of the fixed factors on *t_k duration*. Full model vs. Null model: $\chi2 = 24468.48$, df = 3, $p < 0.001$. (**B**) Influence of the fixed factors on *r_k type*. Full model vs. Null model: $\chi2 = 30155$, df = 11, $p < 0.001$. (**C**) Results of the Tukey test for *r_k type*. (**D**) Results of the Tukey test for the interaction between *t_k type* and *sex*. (**E**) Results of the Tukey test for the interaction between *r_k type* and *sex*. [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] This predictor was dummy coded, with “SexF,” being the reference category. Statistically significant values are indicated in bold; M, males; F, females.

4. Simulated ratio distribution

The results of the Kolmogorov-Smirnov two-sample test showed that the 1000 resampled ratio distributions were significantly different from the real ratio distribution: $0.109 < D < 0.127$, $p < 0.001$.

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Author Contributions

Conceptualization, C.D.G., D.V., T.R., A.R. and M.G.; Methodology, C.D.G., D.V., T.R., A.R., O.F. and M.G.; Investigation, C.D.G., D.V., V.T., T.R., and L.M..; Writing – Original Draft, A.R. and C.D.G.; Writing –Review & Editing, M.G., V.T., T.R., D.V., and L.M..; Visualization, C.D.G, D.V., T.R., A.R. and M.G.; Supervision, M.G. and C.G.

Data and Code Availability

An example video is available as supplementary information. Data, code, and custom-written scripts are available from the corresponding author upon request.

Declaration of Interests

The authors declare no competing interests.

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Chapter III

Born to sing! Song development in a singing primate

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Born to sing! Song development in a singing primate

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Abstract

In animal vocal communication, the development of adult-like vocalization is fundamental to inter- act appropriately with conspecifics. However, the factors that guide ontogenetic changes in the acoustic features remain poorly understood. In contrast with a historical view of nonhuman primate vocal production as substantially innate, recent research suggests that inheritance and physiological modification can only explain some of the developmental changes in call structure during growth. A particular case of acoustic communication is the indris' singing behavior, a pecu- liar case among Strepsirrhine primates. Thanks to a decade of intense data collection, this work provides the first long-term quantitative analysis on song development in a singing primate. To understand the ontogeny of such a complex vocal output, we investigated juvenile and sub-adult indris' vocal behavior, and we found that young individuals started participating in the chorus years earlier than previously reported. Our results indicated that spectro-temporal song parameters underwent essential changes during growth. In particular, the age and sex of the emitter influ- enced the indris' vocal activity. We found that frequency parameters showed consistent changes across the sexes, but the temporal features showed different developmental trajectories for males and females. Given the low level of morphological sexual dimorphism and the marked differences in vocal behavior, we hypothesize that factors like social influences and auditory feedback may af- fect songs' features, resulting in high vocal flexibility in juvenile indris. This trait may be pivotal in a species that engages in choruses with rapid vocal turn-taking.

***Key words**:* duet, flexibility, juveniles, lemurs, ontogeny, rhythm

Introduction

During ontogeny, juvenile individuals need to acquire crucial abilities to adult survival, like kin recognition or anti-predatory strategies. In many species, vocalizations undergo developmental changes that transform less structured utterances into fully functional adult calls (Margoliash & Tchernichovski 2015). An ongoing debate focuses on whether vocal developmental changes are determined by genetics and innateness (Mice—Kikusui et al. 2011), by variation in the social environment (Bats-Knörnschild et al. 2012) or in the internal environment, such as changes in hormone levels (Wetzel and Kelley 1983; Frogs-Kelley & Gorlick 1990); finally, they may be the result of a learning process (Bats-Knörnschild et al. 2010).

Studies focused on vocal development in birds showed that vocal production learning is essential to shape adult vocal signals (Ríos- Chelén et al. 2012). For instance, studies on parrots' vocal development, like the green-rumped parrotlet (*Forpus passerinus*—Berg et al. 2013), demonstrated that, in few weeks, the developmental pattern of their begging call underwent several changes in frequency and duration in order to reach the adult-like output.

Regarding non-human primates, over the past decades, there has been a general agreement that vocal production was largely innate and genetically determined (for review, see Snowdon 1989; Newman 1995; Seyfarth & Cheney 1997; Tomasello 2008). On the other hand, recent studies showed that inheritance and physiological modification could partially explain the developmental changes during growth. In marmosets *Callithrix jacchus*, the increase of call duration with growth is related to lungs' growth, which influences the respiration rate and expands the incidence and duration of calls (Zhang & Ghazanfar 2018). Still, parental feedback appeared to influence juvenile vocal ontogeny substantially, while the growth pattern could not explain precisely these changes across development (Takahashi et al. 2015).

Previous studies focused on the variation of infant and juvenile monkeys in acoustic communication (Hammerschmidt et al. 2001; Pistorio et al. 2006; Takahashi et al. 2015), have led researchers to suggest that the expansion of a flexible, juvenile period during individual development may be one of the fundamental steps in the evolution of language (Hage & Nieder 2016). Ontogenetic changes of vocal features were found in all call types of squirrel monkeys (*Saimiri sciureus*—Hammerschmidt et al. 2001), involving the frequency range and calls duration. In particular, the authors observed that both juvenile and adult form of calls was characterized by high variability, and pointed out that this may be a critical prerequisite for other structural changes during the life span (Hammerschmidt et al. 2001). Indeed, some primates do modify the structure of their vocal output during adulthood (*Cebuella pygmaea*—Elowson & Snowdon 1994; Snowdon & Elowson 1999*;* *Plecturocebus cupreus*—Clink et al. 2019). The work from Seyfarth & Cheney (1986) on vervet monkeys *Chlorocebus pygerythrus* indicated that, while most of the calls appeared "ready-made," in some cases animals have to learn "their correct pronunciation," a process involving, once again, changes in the fundamental frequency and duration of calls and intervals. Hammerschmidt et al. (2000) found a similar effect was found for rhesus macaques' coo calls *Macaca mulatta*, which showed changes in the spectro-temporal parameters during development. These authors suggested that practicing may be more important than exposure to an adult model to achieve the adult-like call form.

Although many primates show a certain degree of sex dimorphism in vocal behavior, we have scanty information on how these differences arise during ontogeny, and most studies focused on captive populations of macaques and marmosets. On the one hand, Hammerschmidt et al. (2000) did not find any significant difference in coo calls between male and female rhesus infants or in the development of coo call production. In this call type, the only sexual dimorphism was found in its usage, with infant females showing a higher emission rate than males (Tomaszycki et al. 2001). On the other hand, screams in the same species are sexually dimorphic in juveniles: in particular, screams of juvenile females were more similar to those of adults than were the screams of juvenile males (Tomaszycki et al. 2005). Similarly, in their first 6 months of life, male and female common marmosets *C. jacchus* are characterized by different developmental trajectories in terms of the spectral and temporal features of the calls they produce (Pistorio et al. 2006).

Primate vocal communication includes some very diverse acoustic outputs, ranging from low-frequency contact calls (e.g., spider monkeys—Ordóñez-Gómez et al. 2019) to elaborate vocal displays like songs (e.g. indris and gibbons—Geissmann 2000). Elaborate vocal outputs represent challenging cases to study primate vocal ontogeny. Liebal et al. (2013) underlined the difficulty of researching this topic due to mainly methodological constraints: large sample sizes are difficult to obtain from infant and juvenile individuals, especially in the wild, as in most cases mothers give birth to a single infant which has to be followed and studied over a long period. A particular case of vocal communication is the singing behavior of the so-called singing primates: members of the families Pitheciidae, Hylobatidae, Tarsiidae, and Indriidae, utter complex, coordinated vocalizations between 2 or more individuals, composed by a series of vocal elements—termed “units” or “notes”—forming a recognizable pattern in time, known as a song (Thorpe 1961; Dahlin & Benedict 2014). Recent research highlighted vocal plasticity and flexibility in primate song’s characteristics (gibbons—Terleph et al. 2018; tarsiers—Clink et al. 2020a; indris—De Gregorio et al. 2019a; titi monkeys—Clink et al. 2019), and it may be of interest to understand how the fully functional adult song develops from life’s early stages.

Almost all the limited information available on song development in singing primates comes from studies on gibbons, which, as all the primates that show singing behavior, are monogamous and characterized by low sexual dimorphism in body size (Leigh & Shea 1995). In general, those works attested that the developmental process leading to the full adult song could last several years (Merker & Cox 1999; Hradec et al. 2017), contrary to what happens with infants’ separation-induced calls, that appeared in early ontogeny with the same spectro-temporal parameters as those produced by older individuals (*Nomascus gabriellae*—Hradec et al. 2020). This evidence is interesting as it may indicate that the developmental process’s protracted nature does not involve the whole vocal repertoire of the species but is specific to the song. In particular, Merker & Cox (1999) found that song development in gibbons included an increase in song duration and the appearance of different song portions in different ontogenetic steps: the authors pointed out that the song, at 2.5 years old, was still not fully adult-like. The work of Koda et al. (2013), which proposed the presence of socially mediated vocal flexibility in the song ontogeny, may explain such an extended period of vocal development in gibbons, also suggesting that practice during vocal interaction may be an essential part of the process.

Furthermore, although gibbons' vocal repertoire is sex-specific, it has been reported that immature males can produce female-specific vocalization, called "great calls" (Koda et al. 2014), that showed different acoustic parameters and had a lower number of syllables than those produced by adult females (*N. gabriellae*— Hradec et al. 2017). Terleph et al. (2016) found that aging in the white-handed gibbon *Hylobates lar* led females to show lower fundamental frequency in their calls. Besides gibbons, the rate of emission of pulse elements in the titi monkeys' song decreased, while call duration increased (Clink et al. 2019). The authors mentioned that this could be an effect of aging, as quickly repeated elements may be challenging to perform.

Indri indri is the largest living lemur and the only Strepsirrhine primate that produces songs, emitted mostly during the morning (Pollock 1986) and consisting of multiple distinctive unit types. Members of a family group participate in the chorus simultaneously, usually showing duets between males and females in turn. Pollock (1986) reported that juveniles join the chorus after 3 years of age. Songs serve several functions, from inter- and intra-group communication to territory defence (Pollock 1986; Torti et al. 2013) and show a different acoustic structure depending on the context of emission (Torti et al. 2013). Songs may also mediate the formation of new groups (Giacoma et al. 2010; Bonadonna et al. 2014; Gamba et al. 2016) and possess the potential to inform conspecifics about individuals' genetic relatedness (Torti et al. 2017). Adult songs, which last 113.188 ± 39.682 s (mean ± standard deviation; Gamba et al. 2016), consist of units that are sexually dimorphic: females possess a higher number of unit types, which are also more frequency-modulated (Giacoma et al. 2010). Females' units also showed a higher fundamental frequency (De Gregorio et al. 2019). Overall, males emit longer units than females (Giacoma et al. 2010), but when considered in detail, only few unit types showed males' longer duration, likely because units organized in phrases are structurally constrained to phrase length (Gamba et al. 2016). Depending on the level of analysis, males may show a higher pitch (including descending phrase (DP) units only, Gamba et al. 2016) or a lower pitch (once taking into account the different types, e.g., long notes (LN), single notes (SN); De Gregorio et al. 2019). The rhythmic structure of indris' choruses also appears to be sexually dimorphic (Gamba et al. 2016), with males exhibiting longer intervals between the onset of units compared with females, which instead displayed more flexible intervals between phrases' units (De Gregorio et al. 2019a). Despite these marked differences, male and female indris are difficult to distinguish morphologically (Pollock 1986), and Dixson (1998) reported no sexual dimorphism in the air sac size. Air sacs have been suggested to play a role in shaping the acoustic and/ or temporal communication features (Fitch & Hauser 1995; Hewitt et al. 2002).

This work aims at examining the ontogeny of indris' song to understand the development of such vocalization. After an intense decade of data collection on wild, free-ranging indri groups, we pre- sent the first quantitative analysis on juvenile and sub-adults individuals (following Pollock 1986). Given the importance of practicing or learning in the development of calls in some primates' species (Seyfarth & Cheney 1986; Hammerschmidt et al. 2000; Koda et al. 2013), understanding if even a Strepsirrhine primate may show some degree of plasticity in the ontogeny of such complex vocal out- put may be indeed essential. Studies on family-living primates have indicated a more substantial effect of social and environmental factors on the development of vocal signals compared with other non- human primates (for review, see Snowdon 2017). Thus, it is likely that similar processes, together with maturational effects, may also affect the song production of juvenile indris, which join the family chorus for several years and remain in the family group until reaching adulthood.

Given that physical and physiological constraints can influence sound production, we expect that modification of the units' spectro- temporal parameters will mainly occur during the first years of life because growth rate toward maturity increases consistently from apes to prosimians (Kirkwood 1985). In particular, we predicted that 1) indris will decrease the fundamental frequency during growth since the elongation of vocal folds relates with a lower fundamental frequency (Titze et al. 2016). We also predicted that 2) some temporal features will increase in duration with age: unit duration and phonation amount will be positively affected by lung capacity (Zhang and Ghazanfar 2018). Consequently, we expect that the individual contribution within a song will increase with age. As a previous investigation found that the rhythmic structure of phrases did not change between adults and non-adults (Gamba et al. 2016), we predicted 3) the rhythmic structure of the whole song will be stable during ontogeny and that 4) juvenile indris would show dimorphic acoustic traits that will become more marked during growth. Finally, given the growing evidence showing that juvenile primates are more flexible than conspecific adults (Takahashi et al. 2015), we predicted that 5) juvenile indris would show higher variability in the songs' spectro-temporal features compared with adults.

Methods

Observations and recordings

The data were collected in the Maromizaha New Protected Area (18° 56'49'' S, 48° 27'53'' E), in Eastern Madagascar. We conducted field observations between 6:00 am and 1:00 pm, from 2011 to 2020, for a total of 59 months. We followed eight habituated groups of wild indris and we recorded their spontaneous songs from a close distance (between 2 and 10 meters), using different sound recorders (Sound Devices 702, Olympus S100 and LS05, and Tascam DR-100, DR-40, and DR-05) set at a sampling rate of 44.1 kHz, with a 16bit amplitude resolution. Semi-directional microphones (ME 67 and AKG CK 98) were oriented towards the singing individuals, and we recognized individuals using natural marks and annotated each emitter's identity for every unit in the song. We did not use playbacks or any particular action to avoid altering the natural behavior of the study subjects. For this study, we considered only the individuals we knew or could estimate the date of birth. We provided an accurate birthdate for those animals we observed from the day of birth. In contrast, an estimated birthdate (month of birth) refers to a newborn we found during its natal group's regular sampling. We set the estimated birthdate to the 15th of the actual month of birth, allowing an accuracy of 15days. We considered juveniles up to 4.5 years because all females dispersed from their natal group at that age. Our dataset comprised 128 choruses, resulting in 140 individual contributions and 2,151 units uttered by 20 individuals (10 males and 10 females). The indris' age ranged from 0.99 to 4.50years old for females, and 1.23 to 4.50years old for males (Table 1).

Individual	Sex	Group	Birthdate
Berthe	F	1MZ	15/06/2012
Cami	F	1MZ	15/05/2017
Fanihy	F	2MZ	15/06/2012
Afo	M	2MZ	07/07/2014
Tovo	F	2MZ	15/07/2016
Zandry	F	3MZ	15/05/2010
Faly	M	3MZ	31/05/2014
Laro	M	3MZ	31/05/2015
Ana	F	3MZ	15/05/2017
Gibet	M	4MZ	15/06/2012
Meva	F	4MZ	15/05/2017
Voary	M	5MZ	07/07/2014
Hira	M	6MZ	15/07/2014
Zafy	M	8MZ	15/05/2012
Mika	F	8MZ	07/07/2014
Eme	M	8MZ	15/05/2017
Ovy	M	9MZ	15/06/2013
Dosy	F	9MZ	31/05/2015
Beny	M	9MZ	15/06/2017
Maintso	F	10MZ	15/05/2010

Table 1. Summary of sex, age and familiar group of individuals considered in this study. When an accurate birthdate was not known, birthdate was estimated to the 15th day of the respective month

Acoustic Analyses.

In the indris' songs, units were mainly organized in phrases, including 2–6 units arranged in sequences of the progressively lower fundamental frequency (and so-called DPs; Figure 1A; Torti et al. 2013). After a series of roars, harsh emissions that introduce the song, indris uttered some long units (LN), usually longer and less modulated than the units emitted in the DPs. LN preceded a variable number of DPs and SN. We analyzed the songs using the software Praat 6.0.56 (Boersma and Weenink 2007) and identified each indri's contribution using annotations in Praat TextGrids.

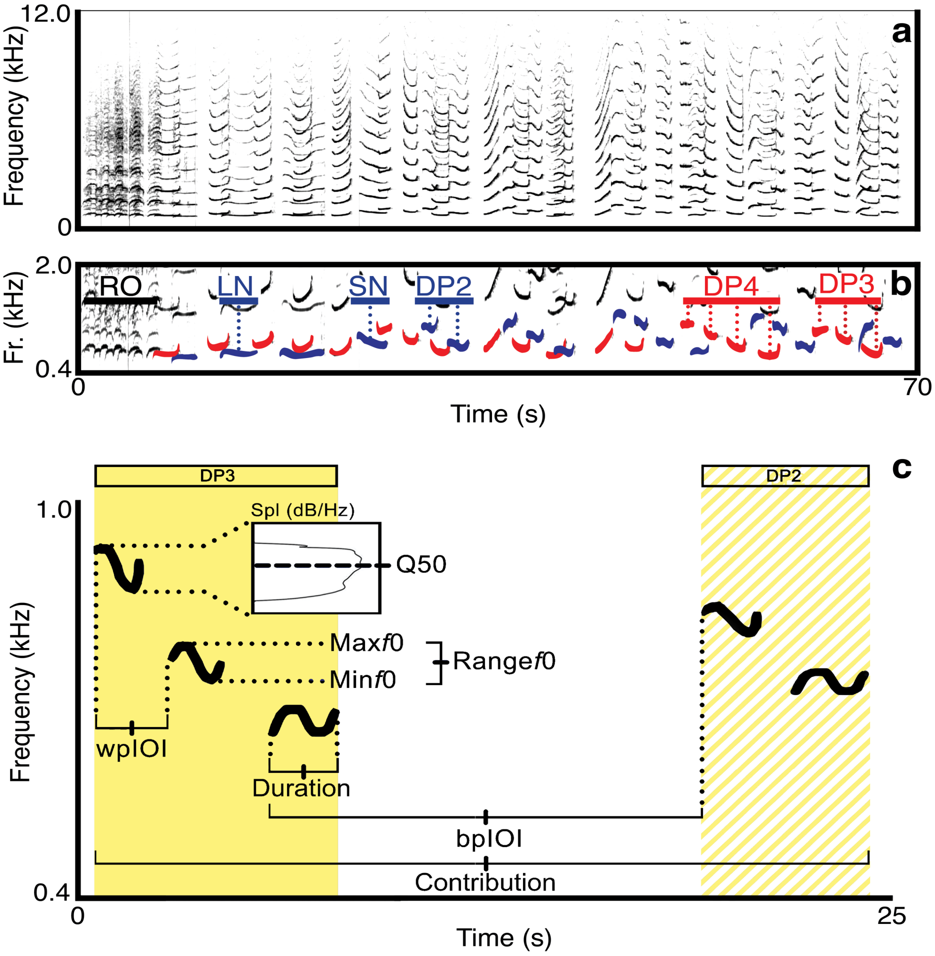

Figure 1. (a) A spectrogram of the indris' song. (b) A spectrogram of the song in which the singing of males (blue) and females (red) is highlighted. The different elements and phrases are also shown: roars (RO – *not included in the analysis*), a long note (LN), a single note (SN), descending phrases made of two (DP2), three (DP3), or four (DP4) units. (c) Schematic representation of a spectrogram describing the acoustic parameters we collected. Temporal features included the duration of a unit (Duration), the duration of the individual contribution to the song (Contribution), the Inter Onset Interval between two consequent units (wpIOI) and phrases (bpIOI). Phonation not represented as consisting in the cumulative duration of units in a contribution. Spectral features included the maximum, minimum and range value of each unit's fundamental frequency (respectively, maxf0, minf0, Range f0), the upper limit of the second quartile of energy in the spectrum (Q50). The sound spectrum displays sound pressure

level (Spl) on the x-axis, frequency on the vertical axis. We did not show the Mean absolute slope as it is the average absolute slope across 25 turning points in the pitch contour.

We labeled units according to their type and position (e.g., being part of a phrase or not) and indicated where intervals occurred between units within a phrase or between different phrases (Gamba et al. 2016; De Gregorio et al. 2019a). Each unit type had its code: LN, SN, or accordingly to the phrase type they belong (DP2, DP3, DP4, DP5, DP6 based on the number of units forming the phrase; Figure 1B); for the silences, the code identifies the position between (inter) or within (intra) DPs. A unit's fundamental frequency was then isolated and saved into a single audio file (WAV format). We used a custom Praat script to extract the duration and 5 spectral measurements from each unit (Figure 1C and Table 2): the frequency at the upper limit of the second quartile of energy (Q50), the maximum and minimum of the fundamental frequency (max*f*0 and min*f*0, respectively), the difference between the maximum and minimum fundamental frequency (range*f*0), and the fundamental frequency mean absolute slope, a measure of the frequency variation along with the unit (MA slope). We then calculated the total duration of the individual vocal output in a duet/chorus (Contribution), the cumulative duration of the uttered units (Phonation), and the number of units in each contribution (Number of units). We also calculated the inter-onset intervals (IOIs) to evaluate the contributions' rhythmic structure (Sasahara et al. 2015). Namely, we considered the within-phrase IOI (wpIOI) and the between-phrase IOI (bpIOI). For all parameters (Table 2), we calculated the mean and the standard deviation.

Abbreviation	**Parameter**
Max*f*0 (Hz)	Maximum fundamental frequency value across the unit
Min*f*0 (Hz)	Minimum fundamental frequency value across the unit
Range*f*0 (Hz)	Max*f*0 – Min*f*0
Q50 (Hz)	Frequency value at the upper limit of the second quartiles of energy
MA slope (Hz)	Mean *f*0 average absolute slope across 25 turning points in the pitch contour
Unit duration (s)	Time between the onset and offset of a unit
Contribution (s)	Total duration of an individual vocal output in a duet/chorus, from the first its first note to its last one
Phonation (s)	Cumulative duration of the units of each individual contribution
Number of units (N)	Number of units uttered in each individual contribution
bpIOI (s)	Inter-Onset-Interval between two subsequent phrases
wpIOI (s)	Inter-Onset-Interval of two following units within a phrase

Table 2. List and abbreviations of the parameters included in the analysis.

Statistical analyses.

To investigate developmental changes occurring in juvenile songs' spectro-temporal features, we used 11 linear mixed models (LMM, *lmer* function of *lme4* package, Bates et al. 2015) in R (R Core Team 2017; version 3.4.3). We used the subjects' age at the moment of recording (hereafter only "age") as a fixed factor in all models. Before fitting the models, bpIOI and wpIOI were log-transformed since they did not show a normal distribution and all continuous variables were z-transformed. We ran 5 models using Q50, max*f*0, min*f*0, range*f*0, and MA slope as response variables. Since we could not assume that duration was not affecting spectral features, we included duration as a predictor, and we then ran an additional model with unit duration as the response variable. Each model featured one of the above parameters as the response variable, and all the others as fixed factors altogether with an interaction between age and sex.

To understand how song temporal features are affected by growth, we ran five models using contribution, phonation, bpIOI, wpIOI, and unit number: when one of these parameters was used as the response variable, all the others were used as fixed factors. These models also included an interaction between sex and age. In the case of unit number, we used a Generalized Linear Mixed Model (GLMM, *glmer* function of *lme4* package, Bates et al. 2015) with a Poisson distribution. In the models concerning temporal features, we included "age" as a squared term. This is because we hypothesized that temporal variables would follow a non-linear trajectory, reaching an asymptote at some point during individual development, also considering that prosimians' growth curves show highest rates in the earliest years of development and decreases as individuals age (Kirkwood 1985), and that lung capacity correlates with body size (see Ey, Pfefferle & Fischer 2007). We ruled out correlation among the predictors by examining the variance inflation factors (*vif* package; Fox & Weisberg 2011) and tested the significance of the full model against a null model including only the random factors using a likelihood ratio test (see Gamba et al. 2016 for details).

To understand how song temporal features are affected by growth, we ran 5 models using contribution, phonation, bpIOI, wpIOI, and number of units. As for the spectral parameters, when we used a temporal parameter as the response variable in a particular model, the others were entered as fixed factors. These models also included an interaction between sex and age. We used a generalized LMM (GLMM, *glmer* function of lme4 package, Bates et al. 2015) with a Poisson distribution for the number of units. In the models concerning temporal features, we included "age" as a squared term because it should better fit with the expected growth rate of Strepsirrhine juveniles (Kirkwood 1985). Moreover, we know that fast growth rates may correlate with increased body size and lung capacity (see Ey et al. 2007). We included group ID, individual ID, contribution ID, and unit type as random factors, with a nested design. We ruled out correlation among the predictors by examining the variance inflation factors (*vif* package; Fox and Weisberg 2011) and tested the full model's significance against a null model including only the random factors using a likelihood ratio test (see Gamba et al. 2016). We adjusted all the p-values (*padj*) using the

Benjamini–Hochberg correction, controlling for false discovery rate.

To determine whether juveniles' song features were more variable than adults, we calculated the individual mean of the coefficient of variation (CV) for each parameter considered in our models (range*f*0, min*f*0, max*f*0, Q50, MA slope, number of units, contribution, phonation, wpIOI, bpIOI, and unit duration) and used independent 2-sample t-tests to compare CVs between adults and juveniles. Adult song parameters were extracted from the datasets of De Gregorio et al. (2019a; 2019).

Results

Occurrence of different unit types within the song

The occurrence of different unit types at different age and sex, summarized in Table 3, indicated that the mean number of SN emitted in the individual contribution to the song decreased with age, while the phrases composed by 2 units (DP2) showed a more variable trend, with a lower value at 1 year old and a higher one at 4 years old. Both phrases including 3 and 4 units (DP3, DP4) increased in number with age, while DP5 were more common at 1 year old and generally scarce compared with the other vocal types. The mean number of LN emitted per contribution remained more stable across years than phrases and SN, while the mean total number of units ($\mathrm{Unit}^{\mathrm{tot}}$) emitted per contribution increased with age.

Considering the 2 sexes, SNs were strongly predominant in young females between 1 and 2 years old (5.79 ± 2.53 per contribution), while males of the same age showed this vocal type only sporadically (0.13 ± 0.18 per contribution). Both males and females had lower values of DP2 per contribution at 1 year old, and while males reached their peak at the age of 2 (2.70 ± 2.05), females reached it at the age of 4 (2.17 ± 1.34). Phrases composed of 3 and 4 units (DP3, DP4) were more common in males of 1 year old (1.88 ± 0.18 for DP3; 0.38 ± 0.53 for DP4) than females of the same age (0.21 ± 0.29 for DP3; 0.17 ± 0.24 for DP4). For DP3, the trend showed an inversion at the age of 2 and 3 years old, with females emitting a higher number of this phrase type (1.45 ± 1.23 at 2 years old; 3.19 ± 1.89 at 3 years old) than males (1.30 ± 1.72 at 2 years old; 1.26 ± 0.79 at 3 years old). At 4 years old, males emitted again a higher number of phrases composed of 3 units (2.13 ± 0.61) with respect to females (1.26 ± 0.95). Concerning DP4s, males showed higher values than females at all developmental stages; in particular, while the mean number of DP4 emitted by males increased with age, for females increased until 3 years old and then decreased. The number of DP5, although generally low, had a peak in males at 1year old (0.25 ± 0.35). Moreover, this vocal type was absent in males aged 2 and 4 years old and females aged 3 years old. DP6s are not reported in the table since we recorded a single case emitted by a female at 4.5 years old.

The number of LN was always higher in males than females and, regarding the mean number of units emitted in an individual contribution (Unit^{tot}), males had their peak at 4years old (18.28 ± 8.78) while females at 3 years old (18.56 ± 5.03).

	Age											
	1			2			3			4		
Vocal Type	Tot	Males	Females	Tot	Males	Females	Tot	Males	Females	Tot	Males	Females
SN	2.47 ± 2.89	0.13 ± 0.18	5.79 ± 2.53	2.38 ± 2.33	2.20 ± 1.30	2.51 ± 2.97	1.73 ± 1.27	1.53 ± 1.17	2.02 ± 1.55	1.36 ± 0.67	1.48 ± 0.60	1.21 ± 0.80
DP2	0.61 ± 0.78	1.38 ± 0.53	0.71 ± 1.00	2.29 ± 1.87	2.70 ± 2.05	2.00 ± 1.83	1,68 ± 1.19	1.43 ± 1.07	2.06 ± 1.42	2.14 ± 1.21	2.12 ± 1.23	2.17 ± 1.34
DP3	0.53 ± 0.72	1.88 ± 0.18	0.21 ± 0.29	1.39 ± 1.38	1.30 ± 1.72	1.45 ± 1.23	2.03 ± 1.59	1.26 ± 0.79	3.19 ± 1.89	1.74 ± 0.87	2.13 ± 0.61	1.26 ± 0.95
DP4	0.18 ± 0.31	0.38 ± 0.53	0.17 ± 0.24	0.52 ± 0.53	0.70 ± 0.67	0.39 ± 0.40	0.63 ± 0.90	0.74 ± 1.15	0.46 ± 0.42	0.65 ± 0.91	0.89 ± 1.14	0.37 ± 0.51
DP5	0.10 ± 0.20	0.25 ± 0.35	0.04 ± 0.06	0.01 ± 0.04	-	0.02 ± 0.05	0.03 ± 0.08	0.04 ± 0.10	-	0.03 ± 0.12	-	0.08 ± 0.17
LN	1.68 ± 1.52	2.63 ± 0.53	0.92 ± 1.30	2.00 ± 1.30	2.80 ± 1.35	1.42 ± 0.98	2.11 ± 1.31	2.78 ± 1.03	1.10 ± 1.06	2.44 ± 1.73	2.60 ± 1.69	2.25 ± 1.95
UNIT^{tot}	8.17 ± 6.48	13.88 ± 4.07	9.63 ± 7.95	15.27 ± 6.05	17.10 ± 5.48	13.96 ± 6.50	15.93 ± 5.65	14.11 ± 5.68	18.65 ± 5.03	16.08 ± 7.28	18.28 ± 8.78	13.44 ± 4.47

Table 3. Mean number and Standard Deviation of different vocal types' occurrence within the song at different ages (Tot) and in the two sexes (Males, Females). SN = single notes; DP2= phrases composed by two units; DP3= phrases composed by 3 units; DP4= phrases composed by 4 units; DP5= phrases composed by 5 units; LN = long notes; Unit^{tot}= mean value of number of units emitted per individual contribution.

Spectral features

The average Q50 value was 808.00 ± 43.01 Hz, showing higher values for males, and it was positively influenced by age in both sexes: the older the indris, the higher the Q50 value (Figure 2A and Supplementary Table SM1). Unit duration influenced this response variable negatively.

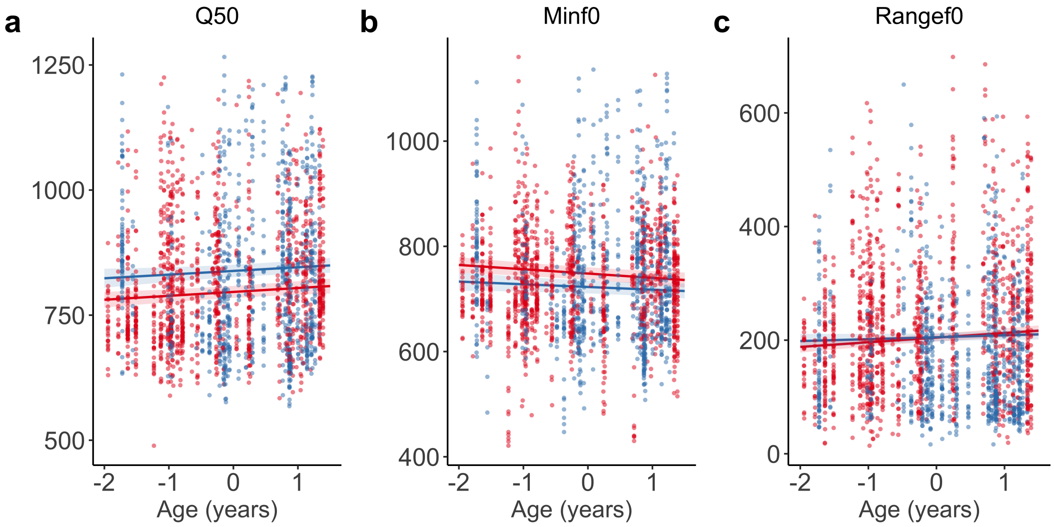

Figure 2. Effect of age on different spectral features (Hz) as response variable: a) Q50, b) min*f*0, c) range*f*0. Red line represents juvenile females, while blue line represents juvenile males. Dots represent the observed data; shaded areas indicate confidence intervals. Being a plot of the effects resulting from the model, the predictor age must be included as z.transformed.

The models showed a similar pattern for the minimum (min*f*0) and the maximum (max*f*0) fundamental frequency. While the overall average for max*f*0 was 930.75 ± 53.27 Hz, for min*f*0 was 727.29 ± 41.98 Hz. Moreover, while the individuals' age negatively influenced min*f*0 (Figure 2B and Supplementary Table SM1), no significant relationship emerged between age and max*f*0. Similarly, unit duration was negatively correlated with minf0, but not with maxf0. From the model also emerged an effect of sex on the average minimum value of fundamental frequency, with females showing higher values than males. Moreover, Q50 appeared to positively affect minf0, with higher frequencies corresponding to higher values of Q50, while the range of fundamental frequency was negatively correlated with it.

Considering the range of the fundamental frequency (range*f*0), its average was 203.02 ± 46.01Hz and were positively influenced by age (Figure 2C and Supplementary Table SM1) and by units' duration. Also, both max*f*0 and MA slope positively affected the fundamental frequency range. On the contrary, the model showed a negative correlation between the range*f*0 and Q50 value. No significant effect of sex emerged for this response variable.

Concerning the frequency variation along with the unit (MA slope), juvenile indris showed an average of 438.48 ± 131.08 Hz. From the model emerged that the Q50 value positively influenced this variable (Supplementary Table SM2). Max*f*0 and min*f*0 were both significantly correlated with MA slope, but where the first parameter had a positive influence, the second had a negative one. We did not find a significant correlation between age and MA slope. Unit duration negatively affected this response variable.

We did not find a significant effect of the interaction between age and sex on the response variables for all of the spectral features tested (Supplementary Tables SM1 and SM2).

Temporal features

The summary and detailed results of all models can be found in the Supplementary Materials (Supplementary Tables SM1–SM4). The average duration of Phonation was 23.107 ±7.463s, with higher values for males. The model showed that the amount of phonation was positively correlated with the duration of individual contribution and number of units. While age itself showed no significant correlation with phonation, the interaction between age and sex significantly affected phonation duration. In fact, males showed a decrease in phonation duration with age, contrary to females, which showed an increase instead (Figure 3A and Supplementary Table SM2). Concerning the rhythmic features, the model revealed that the wpIOI positively influenced the phonation, while no significant correlation with bpIOI emerged.

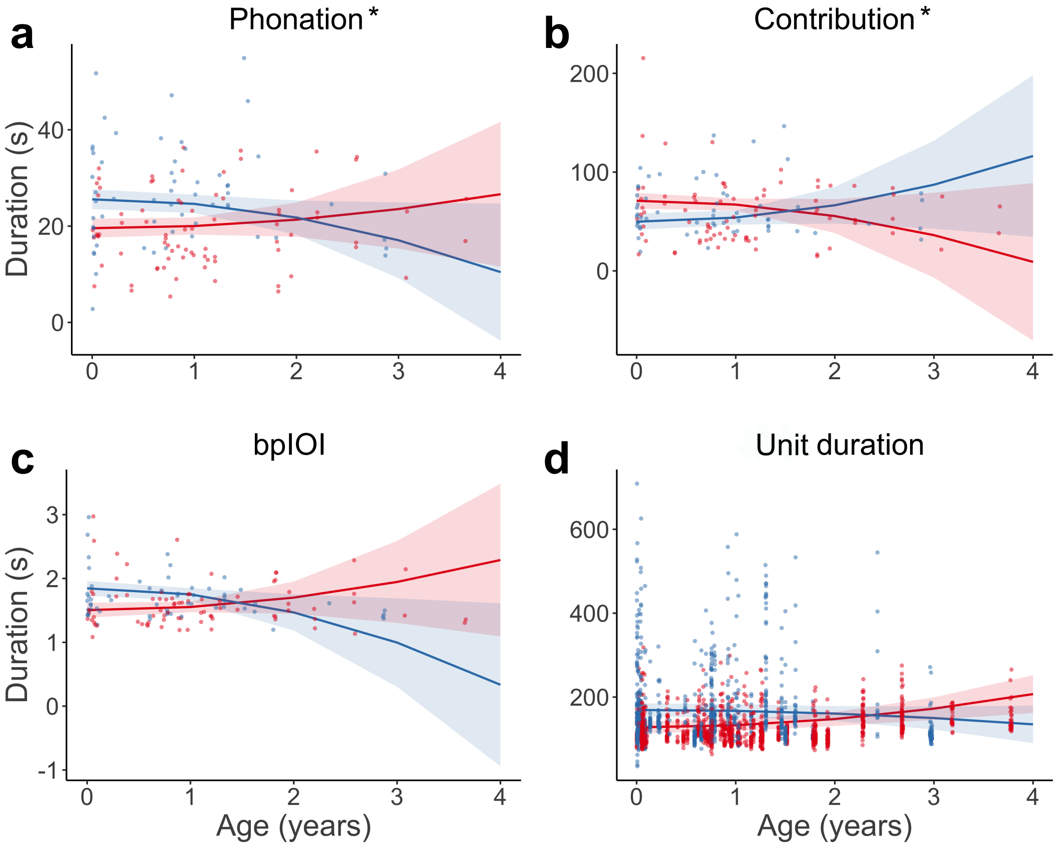

Figure 3. Effect of the interaction between age and sex on a) Mean phonation duration, b) Mean duration of individual contribution, c) Mean inter-onset-interval between phrases, D) Unit Duration. Red line represents juvenile females, while blue line represents juvenile males. Dots represent the observed data; shaded areas indicate confidence intervals. Being a plot of the effects resulting from the model, the predictor age must be included as z.transformed. *raw p-values of the interaction are 0.046 for Contribution and 0.038 for Phonation; adjusted p-values are respectively *0.057* and *0.064*.

The individual Contribution to the song lasted, on average, 63.883 ± 18.275s. The duration of the individual contribution was positively correlated with the amount of phonation. Moreover, the model showed that contribution was longer in females. The wpIOI had no significant influence on the response variable, differently from the bpIOI, which was positively correlated with the individual contribution duration. Again, while age was not correlated with the duration of individual contribution, the interaction between sex and age had a significant influence on the response variable, with an increase of the contribution duration for males and a decrease for females as they age (Figure 3B and Supplementary Table SM2).

The mean IOI between different phrases (bpIOI) was 6.097 ± 2.265s. From the model emerged that this parameter was higher in males, and it showed a significant correlation with the duration of individual contribution and phonation. In particular, while the increase of contribution duration corresponded to longer bpIOI, the phonation was negatively correlated with this parameter. The model did not show significant correlations with the wpIOI and with age. However, the interaction between sex and age negatively influenced the bpIOI: males showed a decrease in their intervals duration with age, while females increased it (Figure 3C and Supplementary Table SM3).

The IOI between different units of the same phrase (wpIOI) was, on average, 2.306 ± 0.304 s. Our results indicated that the duration of the wpIOI was positively influenced by the amount of phonation and was negatively influenced by the number of units emitted. No significant correlations emerged from the other tested variables: sex, age, the interaction between sex and age, the duration of the contribution, and the bpIOI (Supplementary Table SM3).

The songs uttered by juvenile indris were composed, on average, by 16.081 ±5.612 units, and the number of units was higher in females. Moreover, the number of units increased with longer contribution durations and phonation's values (Supplementary Table SM4). No significant influence of age and its interaction with sex emerged from the model. The wpIOI and the bpIOI both showed a significant and negative influence on the response variable: the longer the IOIs, the smaller the number of units.

Finally, our study subjects showed a mean value of unit duration of 1.078± 0.680s. The model indicated a general increase in the units' duration with age, with higher values for males. While Q50 did not significantly influence units' duration, the min*f*0 and the max*f*0, together with the MA slope, negatively influenced the units' duration: the higher the value of these parameters, the shorter the units' duration. Moreover, in this case too, males and female juvenile indris showed different developmental trajectories: while females increased the duration of their units with age, the males decreased it (Figure 3D and Supplementary Table SM2).

Juveniles versus adults' variability

When comparing the coefficients of variation, we found that juvenile and adult indris significantly differed for rangef0 ($t = -2.199$, $df = 16.795$, $P = 0.033$) and for bpIOI ($t = -5.321$, $df = 16.795$, $P < 0.001$), with juveniles showing higher CVs than adults (Figure 4). We did not find significant differences for min*f*0 ($t = -0.333$, $df = 29.450$, $p = 0.742$), max*f*0 ($t = -1.552$, $df = 40.212$, $p = 0.128$), Q50 ($t = 1.465$, $df = 38.420$, $p = 0.151$), MA slope ($t= 0.349$, $df = 41.994$, $p = 0.728$), number of units ($t = -0.930$, $df = 31.691$, 0.359), contribution ($t = -0.499$, $df = 30.447$, $p = 0.621$), phonation ($t = -0.244$, $df = 29.670$, p =

0.809), wpIOI (t = -1.517, df = 31.986, p = 0.139) and note duration (t = 0.764, df = 40.970, p = 0.449).

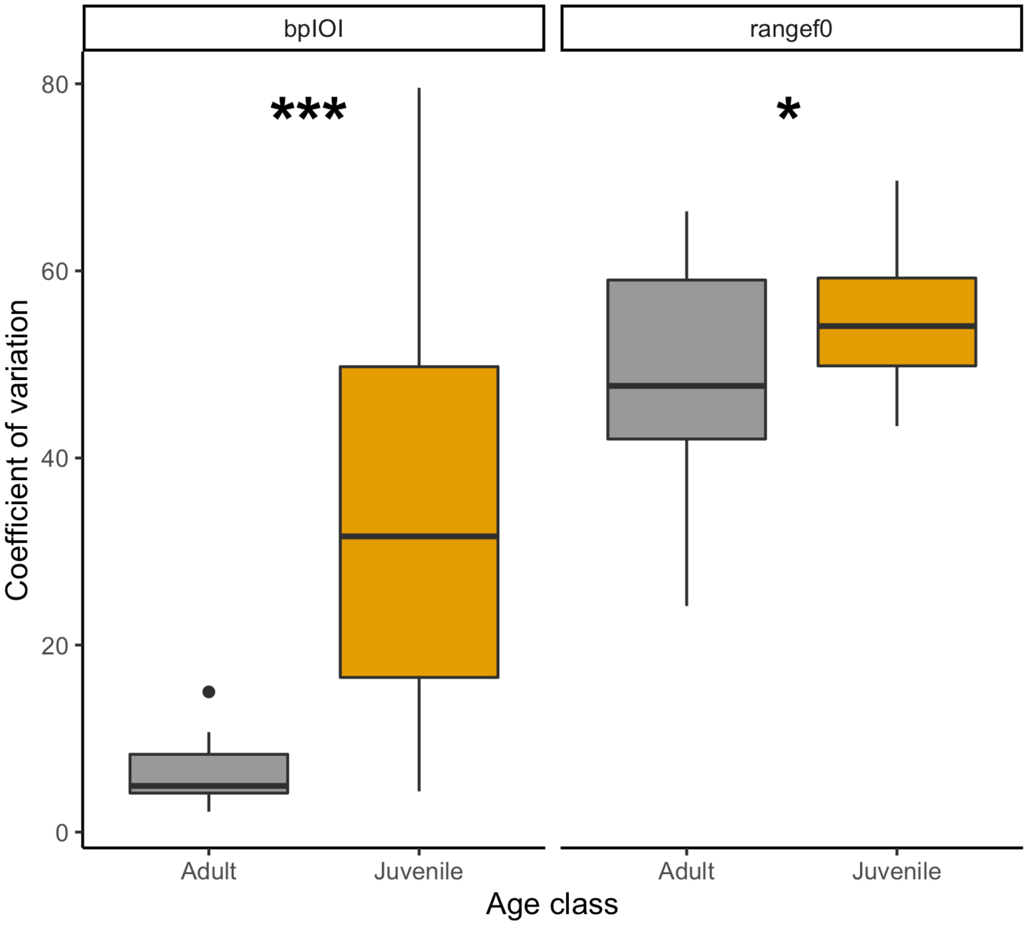

Figure 4. Boxplot of the CV of bpIOI and range*f*0 in the age classes (adults in grey and juveniles in orange). The values shown are calculated from the average individual means; *t*-test significance at $p < 0.001$ is denoted by ***, at $p = 0.033$ is denoted by *.

Discussion

We examined how song parameters of juvenile indris change during ontogeny, and we found that both spectral and temporal features were influenced by age. In particular, while in the tested frequency parameters developmental changes were consistent between sexes, the temporal features showed different developmental trajectories for males and females. Contrary to what was reported by Pollock (1986) on juveniles only emitting introductory roars until 3 years of age, female indris started to participate in choruses at 1 year of age, while males at 1.23. Moreover, our data indicated that females seem to disperse earlier than males from their natal group: this may suggest that, in this species, females reach maturity earlier than their male counterparts. This matches what previously reported for other primate species, where females enter puberty earlier than males (Behringer et al. 2014; Dixson & Altmann 2000).

Our results confirmed the presence of vocal sexual dimorphism in *Indri indri* also at early stages, with male emitting longer but fewer units (in agreement with Giacoma et al. 2010) with higher Q50 values than females (as reported for adult indris: Gamba et al. 2016), which instead have higher values of minF0. This is in line with what has been suggested by previous research (Giacoma et al. 2010, Gamba et al. 2016), about how differences emerging in the contribution of different sexes and age classes to the chorus may be informative about the characteristics of group composition. Our study confirms a crucial sexual influence on both temporal and frequency factors of vocal utterances in juvenile individuals. Interestingly, although a previous work conducted on adult individuals (Giacoma et al. 2010) found sex differences in the fundamental frequency range, with males showing a wider range than females, we did not detect any sexual dimorphism in this trait in our sample of juvenile indris. Moreover, De Gregorio and colleagues (2019b) found sex differences in adults' min*f*0 only for long notes, while our work considered the whole repertoire. This result supports the idea that at 4.5 years of age, indris do not perform the fully developed, adult-like song.

Our results regarding the developmental changes of units' frequency characteristics were only partially consistent with our first prediction: while the minimum value of *f*0 decreased with age, the Q50 and the range of *f*0 showed an increase. The fact that the range of the fundamental frequency increased with age may be directly linked with the decrease of the min*f*0, as max*f*0 was not affected by age. This effect may, indeed, be explained by the elongation of vocal folds' length with growth, which leads to the emission of vocalizations characterized by lower frequencies (Titze et al. 2016). The increase of the Q50 value suggests that, in indris, there is a modification of units' shape, where the minf0 of the units shift towards lower values, while the median frequency increase, thus resulting in a higher range of the fundamental frequency. This evidence is in line with what previously found on squirrel monkeys, whose mean range*f*0 increased with age (Hammerschmidt et al. 2001) and rhesus macaques, whose coo calls exhibited a sharp decrease of *f*0 during growth (Hammerschmidt et al. 2000). Similar results have been found in another singing primate, the white-handed gibbon, where older females showed lower call frequency (Terleph et al. 2016). Our work showed that in indris, juvenile females presented higher values of min*f*0 than males, and no differences emerged regarding the max*f*0: the fact that in adults the sexual difference in min*f*0 has been reported only for a particular type of notes (*long notes*), while differences in the max*f*0 were found in most units' type (De Gregorio et al. 2019b) can be a further indication that songs' vocal types undergo important changes during growth.

Our second prediction that temporal features will show an increase in duration with age was only partially confirmed, as developmental changes in unit duration differed for males and females. We found significantly different developmental trends between males and females in unit duration and IOIs between phrases (bpIOI). Moreover, the amount of phonation and individual contribution duration showed a tendency to differ in their developmental process between juvenile males and females. While females showed an increase of unit duration with age, male indris evidenced a decrease, overall, juvenile males emitted longer units than females. This is interesting since the analysis on adult indris' unit duration that considered the unit type (De Gregorio et al. 2019) as we did in present work evidenced differences only for a limited number of unit type. We can hypothesize that the developmental changes we observed may lead to a reduction in the sex dimorphism in unit duration, that may become more constrained to phrase length as individuals age (Gamba et al. 2016). An increase in units' duration with age has been reported in titi monkeys' broadband pulse (Clink et al. 2019), although this species showed no sex differences in the development pattern. This could be because, in titi monkeys, males and females sing the same units, while the indris' repertoire is strongly dimorphic (Giacoma et al. 2010; Zanoli et al. 2020). Similar results have been reported on marmosets, which increased their utterances' duration during the first 2 months of age (Takahashi et al. 2015). However, the authors did not consider a possible effect of sex in the development of vocalizations, and the temporal span they considered is shorter from the one we examined here. Moreover, Takahashi et al. (2015) focused on the transitions between different vocal types (cries and phee) and concluded that their timing was only partially due to maturation, but also affected by parental vocal feedback. This interpretation may be relevant to our findings on the development of temporal parameters. While the increase in units' duration may be in part due to an increase of lung capacity (Fitch & Hauser 1995), differences between sexes may indicate that vocal plasticity plays an essential role in the process lead- ing to adult vocal output. As reported by De Gregorio et al. (2019a) male and female indris seemed to play a different role in achieving the coordination of utterances, where females showed higher flexibility in the timing of their contribution and males, on the contrary, showed a more fixed pattern. Besides, adult females potentially suffered a higher cost when the number of singers in chorus increases: they had to diminish the phonation to emit a longer contribution, while male singing remained invariant (De Gregorio et al. 2019a). This aspect may explain the differences, even if limited, on unit duration that we found between juvenile and adult females, in agreement with De Gregorio et al. (2019). Our findings support the hypothesis that females' singing may reflect female dominance by regulating the extent of males' contribution (Pollock 1979). Therefore, the change in social status and the critical role that female singing has in coordinating male output may explain why we observed that juvenile females' unit duration increased with age, but adult female's units are usually shorter than the males'.

Other than unit duration, we also found that IOIs between different phrases changed with age, unlike IOIs between units of the same phrase. Contrary to our third prediction and to what previously found by Gamba et al. (2016) that only considered the IOIs between units, we found that songs' rhythm changed during development in a sex-specific way. However, as Gamba et al. (2016) reported, we also found that juvenile intervals between units did not differ from adults. Overall, our findings suggest that phrase rhythmic structure is constrained during ontogeny (see also Gamba et al. 2016). Because unit duration increased with age, juvenile indris must then modify the silent gaps between units. In contrast, the rhythmic structure of songs is more flexible (De Gregorio et al. 2019a). Future studies should aim to understand whether the extent to which young and adult indris can control their vocal output can differ and reflect in turn-taking between emitters during the song (*Lepilemur edwardsi*—Mendez-Cardenas & Zimmermann 2009; *Cercopithecus campbelli*—Lemasson & Hausberger 2011; *C. jacchus*—Takahashi et al. 2013). For marmosets, Chow et al. (2015) conclude that turn-taking is a learned vocal behavior developed under the parents' tutoring activity, similarly to what Koda et al. (2013) hypothesized for gibbons (*Hylobates agilis*). Whether or not this tutoring mechanism is present in the indris remains unclear. However, alongside practising, the auditory input may likely be involved in developing such a complex vocal output, which mostly occurs as a duet or a chorus and requires some degree of coordination among singers (Gamba et al. 2016).

Duration of an individual contribution (overall duration, including the silent gaps) and phonation (the cumulative vocal output) showed a tendency toward different developmental trajectories. We found that while females' overall duration decreased with age, it increased in males. In contrast, females' phonation increased with age, and males showed a decrease during ontogeny. These findings differ from adult reproductive indris, where males showed a higher phonation and a shorter individual contribution than females (De Gregorio et al. 2019a). This evidence may indicate that juvenile indris are still developing the fully adult song pattern despite joining the chorus at an early age, a process in which practice may be involved. Our finding also contrasts with the study of female's great call in gibbons (*N. gabriellae*—Merker & Cox 1999), which increased individual contribution during development.

Our study reveals more variability, at least in the range of the fundamental frequency and in the IOI between phrases, in juveniles than adults again suggesting that auditory experience may shape processing of the acoustic stimuli during growth. It is also possible that being dominant and reproductively mature can influence vocal characteristics, as previously reported for indris (Gamba et al. 2016) and other primates (e.g., male baboons, *Papio cynocephalus*— Fischer et al. 2004). At the same time, taking the role that song may have in the formation of new pairs (Bonadonna et al. 2014; Torti et al. 2017), juvenile females may exploit particular portion of the songs in which overlapping with the adults is less frequent, as previously suggested by Gamba et al. (2016). This result appears in agreement with the observation that overlapping rates decreased with juvenile females' development (*H. lar*—Reichard 2003; Koda et al. 2013). This strategy may allow broadcasting more efficiently their unpaired status, resulting in juvenile female songs characterized by lower total duration but higher phonation amount and longer intervals between phrases. On the other hand, we observed that juvenile males might remain in their natal group until 7 years of age: future studies may consider this mechanism to understand whether male singing may show more extended development.

Our study also revealed that the IOIs are sexually dimorphic in juveniles, unlike previous findings on adult indris (De Gregorio et al. 2019a). Duration of the between-phrases IOI in juveniles appeared to be more variable than adults, which instead showed sexually dimorphic wpIOI variability, unlike the juveniles we studied. These differences provide further support to the idea that some factors, other than physiological modification during growth or genetics, may play a role in the development of singing behavior in I. indri.

This species shows a little dimorphism in external morphology (Pollock 1986) and substantial differences in singing behavior (Giacoma et al. 2010). The sub-glottal air sac possessed by indris does not vary in size between males and females (Dixson 1998). Giacoma et al. (2010) results did not support an influence of body size on the f0 values, since both male and female indris utter units characterized by a wide range of f0 values.

Indeed, vocal plasticity may be an essential factor in shaping singing behavior, especially in the timing of phrases during vocal development, since animals, like indris, that participate in choruses uttered by several family members need to practice and acquire the ability to perform turn-taking (Gamba et al. 2016; De Gregorio et al. 2019b). Song production can be energetically costly (De Gregorio et al. 2019a; Clink et al. 2020), and an immature vocal apparatus may not be prepared to endure the full adult song, that can reach 110 dB (see Zanoli et al. 2020). In line with the above findings, there was a conspicuous use of SN and short phrases (DP2) in songs produced by indris around 1–2 years old, while reproductively mature animals tended to produce phrases consisting of more units (DP3, DP4). Energetic constraints and development of vocal control may likely drive the emission of different vocal types during growth, and, thus, we hypothesize that vocal plasticity, for example, in the articulation of vocal apparatus or the vocal tract tuning (Gamba et al. 2011), may play an essential role in the development of this complex vocal output. Our results are in line with recent evidence on the primate vocal plasticity and flexibility, which showed a certain degree of control on their vocal production (Terleph et al. 2018), even in juveniles (Koda et al. 2007). Parent tutoring activity, which requires further investigations, and auditory feedback may concur in driving some critical traits of such complex duetting behavior. As pointed out by Chow et al. (2015), some degree of learning may be indeed functional to the ontogeny of a signal that requires the ability to perform turn-taking between callers, as in the case of indris. Moreover, both internal and external factors, from maturation to motivation and social influences, may have a stronger effect on song characteristics than body size (Fitch 1997; Ey et al. 2007), and this may be in line with the fact that vocal development of primate family-living species is susceptible to social and environmental factors (Snowdon 2017).

Flexibility in juvenile primates is a pivotal condition in the evoluion of language (Hage & Nieder 2016): as difficult as it is defining the substrates that led to the rise of human language, our work indicates that indeed even in a basal primate as *I. indri* there is strong evidence for flexibility in the changes during the development of singing behavior.

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Authors contribution

C.D.G., F.C., V.E. and M.G. designed the computational framework and analysed the data. C.D.G., D.V., V.T., T.R., L.M. and R.M.R. collected the data. C.D.G., F.C., D.V., V.T., and M.G. performed the measurements, C.D.G., F.C. and M.G. wrote the manuscript with support from V.E., D.V., V.T., T.R., L.M, R.M.R., and C.G.

Q50

Predictors	Estimate	SE	df	t	P
(Intercept)	796.326	6.088	[a]	[a]	[a]
Age	6.470	2.625	130.616	2.465	**0.015**
SexM[b,c]	39.962	3.882	136.846	10.293	**<0.001**
Duration	-6.827	1.402	1194.221	-4.868	**<0.001**
Max*f0*	293.248	353.802	2026.048	0.829	0.407
Min*f0*	-101.187	244.539	2025.999	-0.414	0.679
Range*f0*	-151.101	265.116	2026.041	-0.570	0569
Age:Sex	-0.155	4.386	121.757	-0.035	0.972

Range*f0*

Predictors	Estimate	SE	df	t	P
(Intercept)	204.435	3.457	[a]	[a]	[a]
Age	8.159	2.010	132.632	4.060	**<0.001**
SexM[b,c]	0.716	3.188	184.480	0.224	0.823
Duration	5.301	1.391	1189.169	3.810	**<0.001**
Q50	-99.633	2.592	1582.899	-38.444	**<0.001**
MA Slope	60.851	1.564	1718.809	38.917	**<0.001**
Max*f0*	132.354	2.774	1806.005	47.716	**<0.001**
Age:Sex	-4.769	3.338	130.677	-1.428	0.155

Max*f0*

Predictors	Estimate	SE	df	t	P
(Intercept)	939.500	0.012	[a]	[a]	[a]
Age	-0.002	0.011	126.300	-0.226	0.822
SexM[b,c]	-0.024	0.020	153.100	-1.177	0.241
Duration	-0.010	0.010	1433.000	-0.963	0.336
Range*f0*	116.800	0.009	2021.000	12400.664	**<0.001**
Min*f0*	107.700	0.009	1424.000	11164.916	**<0.001**
Age:Sex	0.004	0.020	124.700	0.206	0.837

Min*f0*

Predictors	Estimate	SE	df	t	P
(Intercept)	748.275	6.083	[a]	[a]	[a]
Age	-8.169	2.385	132.959	-3.426	**<0.001**
SexM[b,c]	-25.506	3.574	146.610	-7.136	**<0.001**
Q50	111.577	1.216	2116.867	91.755	**<0.001**
Duration	-3.640	1.274	1137.736	-2.857	**0.007**
Range*f0*	-58.942	1.115	2119.059	-52.879	**<0.001**
Age:Sex	3.038	3.980	123.433	0.763	0.447

Table SM1. Influence of the fixed factors on Q50, Range*f0*, Max*f0*, and Min*f0*. Statistically significant values are indicated in bold. *Q50*: Full model VS null model: $\chi 2 = 4522.196$, df = 11, $p<0.001$*; Rangef0*: Full model VS null model: $\chi 2 = 3593.085$, df = 11, $p<0.001$; *Maxf0*: Full model VS null model: $\chi 2 = 3832.107$, df = 10, $p<0.001$; *Minf0*: Full model VS null model: $\chi 2 = 3830.951$, df = 10, $p<0.001$.[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] This predictor was dummy coded, with the “SexF,” being the reference category

Unit Duration

Predictors	Estimate	SE	df	t	P
(Intercept)	1.279	0.071	[a]	[a]	[a]
SexM[b,c]	0.414	0.107	97.351	3.853	**<0.001**
Age^2	0.049	0.014	2114.969	3.433	**<0.001**
Min*f0*	-0.051	0.015	2057.521	-3.444	**<0.001**
Q50	-0.012	0.022	2066.742	-0.539	0.590
MA slope	-0.037	0.013	2067.433	-2.726	**0.006**
Max*f0*	-0.040	0.019	2078.825	-2.075	**0.038**
Age^2*SexM[b,c]	-0.071	0.020	2103.358	-3.530	**<0.001**

MA Slope

Predictors	Estimate	SE	df	t	P
(Intercept)	441.474	18.078	[a]	[a]	[a]
Age	0.776	8.838	143919	0.088	0.930
SexM[b,c]	2.175	18.961	169.682	0.115	0.909
Min*f0*	-313.542	8.362	2141.535	-37.495	**<0.001**
Q50	300.948	14.322	2109.388	21.013	**<0.001**
Max*f0*	251.016	12.008	2095.244	20.905	**<0.001**
Duration	-19.345	6.393	2095.218	-3.026	**0.003**
Age^2*SexM[b,c]	-20.408	20.515	126.080	-0.995	0.322

Phonation

(Intercept)	19.572	0.068	[a]	[a]	[a]
SexM[b,c]	5.993	1.334	27.320	4.493	**<0.001**
Age^2	0.441	0.504	134.906	0.874	0.384
Contribution	1.143	0.458	129.879	2.497	**0.014**
bpIOI	-0.472	0.340	131.996	-1.391	0.167
wpIOI	1.331	0.336	136.008	3.956	**<0.001**
N of units	7.782	0.439	131.546	17.708	**<0.001**
Age^2*SexM[b,c]	-1.386	0.686	133.182	-2.019	**0.046**

Contribution

(Intercept)	70.995	4.086	[a]	[a]	[a]
SexM[b,c]	-21.191	6.003	43.040	-3.530	**<0.001**
Age^2	-3.870	2.699	106.216	-1.434	0.155
Phonation	24.098	1.574	137.994	15.305	**<0.001**
bpIOI	14.211	1.452	135.951	9.788	**<0.001**
wpIOI	-1.900	1.787	120.807	-1.063	0.290
Age^2*SexM[b,c]	8.027	3.823	133.078	2.100	**0.038**

Table SM2. Influence of the fixed factors on Unit Duration, MA Slope, Phonation and Contribution duration. Statistically significant values are indicated in bold. *Unit duration*: Full model VS Null model: $\chi2=3126.26$, df =7, $p<0.001$. *MA Slope*: Full model VS Null model: $\chi2= 2761.253$, df = 11, $p<0.001$. *Phonation*: Full model VS Null model: $\chi2= 691.29$, df = 7, $p<0.001$; *Contribution*: Full model VS Null model: $\chi2= 1167.69$, df = 6, $p<0.001$. [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] This predictor was dummy coded, with the "SexF," being the reference category.

bpIOI						wpIOI					
Factors	Estimate	SE	df	t	P	Factors	Estimate	SE	df	t	P
(Intercept)	1.504	0.059	[a]	[a]	[a]	(Intercept)	0.785	0.029	[a]	[a]	[a]
SexM[b,c]	0.341	0.086	40.706	3.959	**<0.001**	SexM[b,c]	0.004	0.045	43.260	0.087	0.931
Age^2	0.049	0.041	74.264	1.207	0.231	Age^2	0.031	0.018	113.423	1.737	0.085
Contribution	0.300	0.031	137.596	9.702	**<0.001**	Contribution	0.015	0.018	134.990	0.852	0.395
Phonation	-0.257	0.034	130.634	-7.635	**<0.001**	Phonation	0.145	0.027	103.617	5.300	**<0.001**
wpIOI	0.009	0.027	95.456	0.339	0.736	bpIOI	-0.020	0.013	132.915	-1.591	0.114
Age^2*SexM[b,c]	-0,143	0.059	121.165	-2.449	**0.016**	N. units	-0.158	0.026	109.854	-6.104	**<0.001**
						Age^2*SexM[b,c]	-0.003	0.026	134.952	-0.133	0.895

Table SM3. Influence of the fixed factors on bpIOI and wpIOI. Statistically significant values are indicated in bold. *BpIOI*: Full model VS Null model: $\chi2$= 19.90, df = 6, p<0.001. *WpIOI*: Null model VS full model: $\chi2$= -220.02, df = 7, p<0.001; [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] This predictor was dummy coded, with the "SexF," being the reference category

Number of units					
Factors	Estimate	SE	df	t	P
(Intercept)	17.434	0.698	[a]	[a]	[a]
SexM[b,c]	-3.294	1.039	31.988	-3.170	**<0.003**
Age	-0.351	0.373	137.146	-0,939	0.349
Contribution	1.048	0.333	132.381	3.142	**0.002**
Phonation	5.908	0.334	131.411	17.684	**<0.001**
wpIOI	-1.227	0.240	136.150	-5.122	**<0.001**
bpIOI	-0.608	0.247	134.718	-2.459	**0.015**
Age^2*SexM[b,c]	0.859	0.512	133.281	1.676	0.096

Table SM4. Influence of the fixed factors on Number of units emitted. Statistically significant values are indicated in bold. Number of Units: Full model VS Null model: $\chi 2= 606.56$, df = 8, $p<0.001$; [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] This predictor was dummy coded, with the "SexF," being the reference category

Chapter IV

Notes on a tree:

Reframing the relevance of primates' solos, duets and choruses

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Abstract

The complexity of primates' singing behavior has long gathered the attention of researchers interested in understanding the selective pressures underpinning the evolution of language. Among these pressures, a link between territoriality, pair-living, and singing displays has been suggested. Historically, singing primates have been found in a few taxa that are not closely related to each other, and, in the last years, their phylogeny has dramatically changed. Hence, we aimed at understanding if the dogmatic association between territorial behavior and a monogamous social structure still holds in the light of current research. Moreover, singing behavior has often been considered a whole, but, depending on how many individuals call simultaneously, animals can perform different types of singing. Currently, it is unclear to which extent these singing forms are widespread among these primate groups. Given that there is no unique definition for a *song*, *solo*, *duet*, and *chorus*, we envisioned some of the most used descriptions. We then formulated some new definitions that we followed in our review of the presence/absence of these different song organizations among singing primates' taxa. In particular, we suggested that tarsier species that are typically considered non-singers may indeed sing, and we pointed out that non-duetting gibbons may perform duet interactions. We found that, besides duets, chorusing behavior and solo songs are essential features of primates' communication, but their study is still in a descriptive phase. Moreover, while territorial behavior seems to be conserved in these singing taxa, we highlighted that the monogamous social structure is not the rule. Pair-living plus multi-female groups are common too. We suggest that ending to consider these taxa as uniform in their sociality and vocal behavior might be a significant turning point to unravel the different selective pressures that influenced the emergence and organization of such peculiar vocal behavior.

KEYWORDS: *singing, tarsiers, gibbons, titi monkeys, indris, territorial, pair-living, phylogeny.*

Introduction

Among the diversity of primates' vocal signals, the most complex and intricate displays are singing and chorusing (Ravignani et al. 2014). While many primate species make conspicuous use of vocalizations, a limited number of taxa communicates using combined or stereotyped sequences of long-range vocal emissions, usually termed songs. There is no unique definition of song, but most authors agree to distinguish it from a vocalization considering the duration, the typical frequency modulation, and the apparent temporal and structural hierarchy between song elements (e.g. Spector 1994). Mammals song production is limited (Haimoff 1983), and about 16% of primate taxa have grouped within the singing primates (Gamba et al. 2014, Haimoff 1986). Historically, singing primates have been found in the Hylobatidae family, in the genus *Indri*, some species of the genera *Tarsius* and *Callicebus* (Robinson 1979; MacKinnon & MacKinnon 1980; Haimoff 1986; Niemitz et al. 1991; Geissman 1993; Thalmann et al. 1993). These primates are not closely related (Fig.1A, 1B); thus, it is likely that singing behavior has independently evolved within the order (Geissmann 2000).

This aspect is intriguing since it is unclear which drivers led to this peculiar behavior in species that show such diverse traits. These primates can be nocturnal or diurnal, folivorous and faunivorous, and have very different morphology. In primates, singing behavior has been associated with a territorial and socially monogamous lifestyle (Geissmann 2000). It has been hypothesised that the evolution of long-range coordinated signals has been guided by stability of resources over the year, favouring resource acquisition via territorial defense by a collaborative stable group, as it has emerged from studies on birds (Thorpe 1963; Logue & Hall 2014; Tobias et al. 2016). Geissmann (2000) suggested that the evolution of primates' singing behavior and duet singing behavior could be related to the development of monogamy, which involves a pair-living social organization.

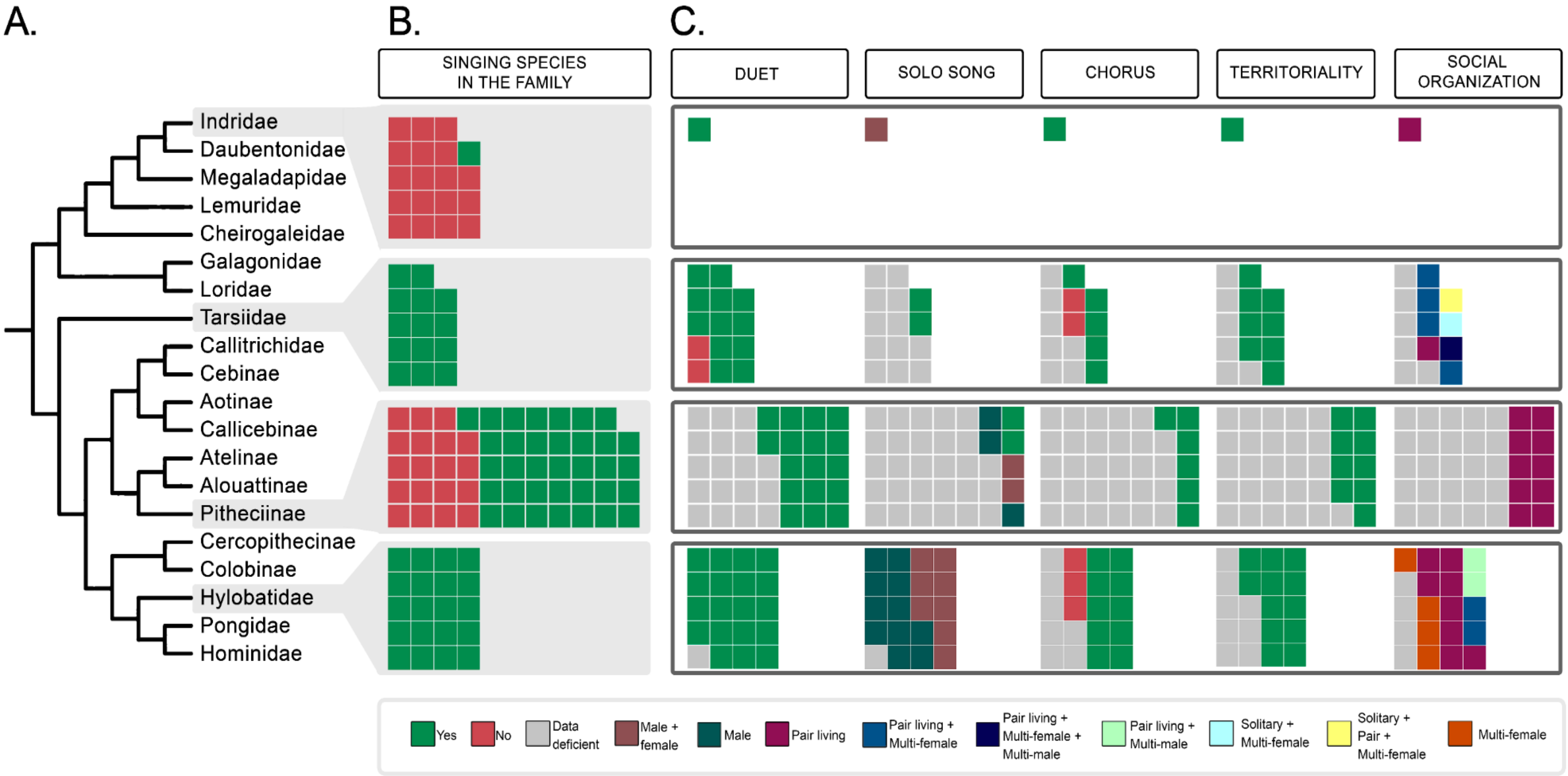

Fig.1. Schematic representation of the primates' singing behavior and characteristics. A. Phylogenetic relationship between primates' families historically considered as containing singing species. B. Occurrence of singing species in each primates' family. Each square represents a species. C. Overview of the presence/absence of different song types, territorial behavior and social organization in the singing species, that are the sum of green squares from panel B.

Currently, it is not clear to what extent territorial defence and pair-living social structure are shared among singing species. Moreover, “singing behavior” has been often considered as a whole. Still, different forms of singing can take place, depending on how many individuals perform the vocal display simultaneously (Fig. SM1), and there is not a clear picture of how much these forms are spread among these primate groups.

To what extent pair-living and territoriality are widespread among singing species of primates, according to recent studies? Can we map the occurrence of solo song, duets, or choruses across the singing taxa in association with their social and mating system and territoriality? Hence, we provide the first review about primate singing displays and re-analysing the traits indicated as potential factors that led to the evolution of singing. We propose a new definition for the terms *song* and *chorus*, and we point out that, following the most used definitions of duet song, species considered non-duetting might indeed be duetting. We reported the detailed information we found (see Supplementary Material), either at the mating system level (e.g. monogamy, polygyny) or social system level (e.g. pair living, multi-female). However, many data are missing as singing primates are extremely challenging to study. They live in dense tropical forests, generally with low population density. Also, only a few individuals and species are available for ex-situ studies (gibbons, Melfi 2012) given the difficulty, or impossibility, to breed them in captivity (tarsiers, Řeháková-Petrů 2019; indris, Petter et al. 1977).

Towards new systematics of singing

Song. Most authors agreed on differentiating songs from calls—the first showing remarkable duration, complexity, and the presence of notes (Spector 1994). In songs, emitter(s) utter units (also called notes or elements) following a precise temporal pattern. This temporal regularity is a crucial feature highlighted in most definitions. Thorpe (1961) described the birdsong as "a series of notes, generally of more than one type, uttered in succession and so related as to form a recognizable sequence of pattern in time". Geissmann (2002) and Haimoff (1986) adopted Thorpe's (1961) definition when rereferring to the gibbons’ song. This definition is particularly suitable, focusing on the utterance's spectral and temporal features rather than its function and its ontogeny (Logue & Krupp 2016;

Catchpole & Slater 1995). Thorpe's criteria would also easily separate songs from loud calls: despite being used for territorial advertisement and defense (Kitchen 2004), roaring bouts of Howler monkeys, for example, are composed of a series of harsh and chaotic emission, where melodic features are absent (Ybarra 1986). We hence propose that songs can be defined as a series of notes of different types, uttered following a hierarchical structure, and characterized by a frequency variation. Singing may occur with different degrees of interaction with conspecifics, we can then recognize solos where only a singer is involved (Seddon 2002), duets which is a dyadic interaction (de Reus et al. 2021; Fig. SM1A, B), and choruses that include multiple singers (e.g. birds, Seddon 2002; primates, Raemaerkers et al. 1984; Fig. SM1C).

Solos. Solo songs have generally been considered as a series of two or more syllables given by a single individual (Seddon 2002, adapted from Farabaugh 1982). A critical point when studying solos is the understanding of solo singing vs a duet contribution unanswered by the partner. For example, by answering each other's songs, paired birds prevent solo singing, advertising the mated status of their partner (Langmore 1998). Consequently, if a pair member does not answer, the result would be the other member emitting a solo song. This is quite different from what happens, for example, in Bornean gibbons. Males emit solos and duet at specific times of the day: solos before dawn, while duets with females occur later (Clink et al. 2020). For this reason, we point out that a solo derived from the absence of reply from a pair member (*unanswered duet)* should be considered different from a solo song who is usually emitted by a single individual (*solo singing*). However, in the present work we will not differentiate between these two categories due to the lack of information on the context in which these songs are emitted.

Duets, and choruses. A vocal duet is an acoustic interaction between two partners (Bailey 2003), whether they emit simple calls or more complex vocal displays. In avian studies, pair-mates are typically interacting in duets, which were regarded as joint vocal displays in which breeding pairs sing in combination with one another, either synchronously or alternately (Thorpe 1972; Farabaugh 1982). Male-male duets (Trainer et al. 2002) or juveniles performing with adults of the opposite sex were also observed (Seddon 2002). For this reason, as suggested by Langmore (2002), a more suitable definition of duet should consider spectro-temporal properties rather than the identity of the emitters which define the type of interaction. Hall (2009), for example, proposed that a duet is emitted by two individuals that coordinate their singing so that their phrase would alternate or overlap. To differentiate this particular behavior from a general two-individual vocalization (e.g. the calling of crickets, Zimmermann et al. 1989), we will refer to the coordinate song emission between two individuals as a duet song. Primates emit duets either with their partner or with an offspring of the opposite sex (e.g. indris, Gamba et al. 2016), or with an adult and an offspring of the same sex, often mother and daughter (e.g. gibbons, Raemaerkers et al. 1984). In these vocal interactions, daughters try to synchronise their vocal utterances to match the mother's, which has been supposed to be a practicing process (Koda et al. 2013), and they may also show this behavior while participating in the duet song of the parents (Merker and Cox 1999). It is intriguing that this particular kind of duet also occurs in species of gibbons that do not show duets (Kappeler 1984). Even if duets between members of the same sex in indris does not happen, juvenile females can sometimes match their mothers' notes in the duet emitted by the mated pair (De Gregorio, *pers. obs*.). While these cases would satisfy the criteria defined by Langmore (2002) and Hall (2009), we asked ourselves if would be the case of differentiating, at least for singing primates, same-sex duet as *song matching duets* and opposite-sex duets as duet songs. This may have also sense in the light of the dramatic differences in temporal structure between the two duet types: total synchronisation in the matching, from antiphonal to partial overlap in different sex duets.

In signing primates, offspring of the reproductive pair may join the duetting adults creating a family chorus. In the last decades, the term chorus has been widely used to indicate a range of different vocal displays, united by having more than two individuals emitting acoustic signals simultaneously (Gerhardt & Huber 2002). Different studies refer to the term chorus as the simultaneous emission of the same call type by more than two animals, either in a cacophonic or synchronized way (Yoshida & Okanoya 2005; Pika et al. 2018). In birds, the term chorus has been used either to refer to the simultaneous, cacophonic display of different species emitting song in the same time of the day ("bird dawn chorus", Gil & Llusia 2020) and the communal singing that takes place when two or more birds coordinate their emissions (Seddon 2002; Hale 2006). This can happen when a juvenile joins the parents' duet (Seddon 2002), similarly to what occurs in singing primates. While the "dawn chorus" and duetting behavior of birds have been extensively investigated, the chorusing of different individuals from the same group remains little studied. As for birds, the term chorus has been used to indicate both a display of a group or the event of more groups or individuals of different groups emitting songs. Haimoff (1986) described as chorus also the calls of one animal or the duet being sequentially followed by others, like howler monkeys, siamangs and gibbons; moreover, some studies on gibbons defined as a chorus the emissions at the dawn of male solo songs from different groups (Tenaza 1976). Since duet songs imply complex turn-taking processes between the two emitters, we suggest that a chorus song cannot be only defined by the temporal proximity of more than two song events. For this reason, in this review, we referred to "chorus song" as the coordinated and structured emission of songs displayed by more than two individuals within the same group members. In singing primates, a chorus song is uttered by the mated pair and their offspring. The participation of the juveniles in the adult song display has no clear function yet, but it may represent a form of practising towards a fully adult-like song (Koda et al. 2013; De Gregorio et al. 2021) and an occasion to broadcast their individuality (Gamba et al. 2016).

Social organization and mating systems. While the social organization of a species indicate who lives with whom, its mating system indicates who mates with who. Among the social organizations, pair living is the one that has been typically linked to a monogamous mating system, and it has been described as two adults of the opposite sex living together in their home-range with their non-reproducing offspring (Tecot et al. 2016; Huck et al. 2020). Following Huck and colleagues (2020), a pair living social organization can have a sexually monogamous mating system when an exclusive mating relationship between a single female and a single male take place, or a genetic monogamous mating system when a female and a male reproduce exclusively with one another over a set of multiple births. However, “monogamy” is an ambiguous concept that has been often used in referring to either a social or a mating system (Fernandez-Duque et al. 2020). In fact, when revising the literature, we found some inconsistencies in the usage of the word “monogamy”, and only few studies reported observations of copulations and genetic analysis to assess with certainty the presence of a particular mating system. Thus, if "monogamy" should be used when referring to an animal' mating behavior (Huck et al. 2020), when clear information about this trait lack one should only report the species social organization, and thus in our cross-taxa comparison we considered only this attribute (e.g. *noyeau*, pair-living, multi-male/female) to avoid confusing the social and the mating levels. However, we reported in Supplementary Material all the detailed information we found on both social and mating systems.

Overview

Tarsiers. The family Tarsiidae includes small-bodied, nocturnal, and faunivorous primates (Schwartz 2003), classified in three genera (Groves & Shekelle 2010): *Tarsius* is found in Sulawesi (12 species), *Cephalopachus* in Sumatra and Borneo (one species), *Carlito* on islands of the southern Philippines (one species). However, more species will likely be identified (Shekelle et al. 2017). Singing behavior is well known in the gen. *Tarsius*, where male and female emit a duet song (Gursky et al. 2017; Fig. SM1F, Tab. SM1), even if spectrograms of pygmy tarsier's duets (*T. pumilus*' - Grow et al. 2016) seem to lack a precise hierarchical structure and different note types resembling more a multi-individual vocalization than a duet song. However, further acoustic analysis would assess this duet structure. Besides, members of genera *Carlito* and *Cephalopachus* do not sing a duet (Groves & Shekelle 2010). Even so, since we did not find any mention of singing behaviour, do they sing at all? The acoustic repertoire of *C. Syrichta* comprises two vocal behaviors that may have the potential to be classified as solo songs: a) a series of different calls often occurring in a sequence, and b) trills, complex, harmonic calls consisting of several modulated notes (Řeháková-Petrů et al. 2012). Both these emissions might be involved in territorial advertising and mate attraction, functions linked to singing behavior. For the present study, we considered this species as non-duetting but as *data deficient* for solo songs. We suggest that further analyses may conclude that singing behavior is likely to be present. The calls of the Horsefield tarsier (*C. bancanus*) have been often described as bird-like (Crompton & Andau 1987), but more details are lacking. Thus, to our knowledge, 85.7 % of Tarsiidae species emit songs. Future investigations on phinippine Tarsier (*C. syrichta*) vocal behavior would clarify if this value will rise to 93.8%, possibly questioning the singularity of the non-singing behaviour in the Western tarsier. In contrast to duet, reports of solos are only anecdotal, and the occurrence of this particular case of singing behavior has not been extensively investigated. We found mention of solos in only two species (Tab. SM1), while duets in *T. pumilus* consist "of any call, where the male and female vocalise in synchrony," and the same calls can be used for social communication (Grow 2019). This may indicate something similar to duets in a solitary way even if there are indications in gen. *Tarsius*, all adults and some sub-adults can join the duet song of the pair, creating a family chorus (Groves & Shekelle 2010; Gursky 2015), we found reports of chorusing behavior only on 35.7% of the Tarsiidae.

Tarsiers show considerable variation in social structure. There are different social/mating systems in this family comprising genetic monogamy, pair-bonds with facultative polygyny and *noyau*/dispersed polygyny. The mating system of some species remains unclear, but their social system has been described as comprising multi-male and multi-female groups (Tab. SM1).

Finally, tarsiers are considered territorial, although most of the evidence seems to rely on qualitative rather than quantitative data, such as the idea that duets are emitted for territorial advertisement (MacKinnon & MacKinnon 1980) and scent marking behavior (Gursky-Doyen 2010). Thus, we found indications of territorial behavior for 57.1% of species.

Indris. Its distinctive black and white pelage and its loud song make indri one of Madagascar's best-known lemur species. Indris are diurnal, arboreal, and mostly folivorous (Powzyk & Mowry 2003). The indris' song is a long sequence of vocal units organized in phrases (Gamba et al. 2011). The song could be uttered by two (a duet) or more individuals (a chorus - Tab. SM2). Duets can take place between members of the pair, or between one pair member and an offspring of the opposite sex (Gamba et al. 2016). Floating solitary individuals rarely perform solo song bouts (Gamba et al. 2016). The family groups are composed of the adult pair and their offspring and contain two to six individuals (Torti et al. 2013). Despite the observation of an extra-pair copulation (Bonadonna et al. 2014), genetic monogamy is the norm (Bonadonna et al. 2019). Family units occupy relatively small territories that are stable in terms of size and location, with core areas that remain stable over the years (Bonadonna et al. 2020). Intergroup encounters are rare, and are frequently mediated by prolonged vocal interactions, also indicated as territorial songs (Torti et al. 2013).

Gibbons. The family Hylobatidae is the only member of the Catarrhine monkeys showing singing behavior. They are medium sized primates, arboreal and mostly frugivorous. According to the latest works on gibbons' phylogeny (Shi & Yang 2018), this family includes four genera: *Hylobates*, *Nomascus*, *Hoolock*, *Symphalangus*. Following the classification proposed by Roos (2016), we considered the family Hylobatidae as composed of 20 species. All gibbon species emit songs. Duet represents the most widespread form of song organization: in fact, nearly all species have been reported to perform singing vocal interaction, corresponding to the 95% of the Hylobatids (Tab. SM3). Duets have been reported to occur mainly between the mated pair, but duetting between mother and daughter (*song matching duets*) can take place (Koda et al. 2013), and, occasionally, vocal interactions between mother and son have also been described (Konrad & Geissmann 2006). Comparably to duets, direct evidence of solos is present for 95 % of the total (Tab. SM3). In 36.8% of cases, both sexes have been reported to emit solos, while for 57.9% only males. On the other hand, chorusing behavior has been reported in 47.4% of the species, and in most cases, authors roughly reported the participation of the juveniles in the adults' duet (Tab. SM3).

Although gibbons are largely considered territorial primates (Bartlett 2009), we found direct indications of territoriality only in 60% of species (Tab. SM3). For others, it is not clear to what extent the territory the familiar groups occupy is exclusive or not (Fei et al. 2012). Besides territoriality, monogamy has also been considered a common trait of this family. Nevertheless, we found a notable variability in social systems. Recent evidence suggests that the mating system in gibbons is not fixed and uniformly distributed as previously considered but comprises monogamy, polygyny, and polyandry (Tab. SM3).

Titi monkeys. Titi monkeys are primates of small/medium size living only in South America, mainly folivorous and found in various forest types. They belong to the subfamily Callicebinae, including three genera: *Callicebus*, *Cheracebus*, and *Plecturocebus* (Byrne et al. 2016), with 35 species in total (Gusmão 2019; Boubli 2019). Information about Callicebinae's vocal behavior is lacking (Adret et al. 2018). Authors often refer to song presence and features in the titi group as a whole, but when looking at the specific evidence, we found few studies actually describing song structure and organizations (e.g. Adret et al. 2018). 77.3% of titis have been reported to perform duets (Tab. SM4; Fig. SM1E). There is descriptive evidence for solos (31.8%), and the frequency of this behavior is poorly known. The pair and its offspring emitted choruses only in 27.3% of species (Tab. SM4). Titis are considered monogamous and pair-living (Fuentes 1998), but at the species level, we found indications on social systems only in the 25.7% of titis. Likewise, to our knowledge, territoriality has been proven for 25.7% of them, and in one case the evidence is mixed; Tab. SM4).

Discussion

This work aimed to provide an overview of current knowledge about the co-occurrence of song, territoriality, and a pair-living social organization in those primate taxa that historically have been considered showing singing behavior, as these traits have been often linked to one another. Singing primates groups have often been presented as being uniform in their vocal activity and social/mating system. Nevertheless, we found direct reports of singing behaviour only in 70% of species (Fig.1). For this reason, further considerations on the occurrence of different song organization in these taxa will refer to this percentage. Several studies often mention this vocal feature without providing explicit references or further details. We found the same for territorial behavior and social system: while many articles describe these animals as monogamous and territorial, in many cases, we found no work providing empirical evidence for the presence of these traits. As pointed out by Fernandez-Duque et al. (2020), this might be due to the habit of referring to studies of historical value, but that may have less importance in the light of current research, especially when considering the critical changes in primate phylogeny. In fact, it is not obvious that newly described species possess the same ecological features of the group they have been separated from.

Many singing primates' social and mating systems are still unclear, and we did not find direct information for 31.4% of them (Fig.1C). Thus, for our comparisons, we focused our attention on their social organization and avoided mixing different social traits often grouped under the concept of “monogamy” (Fernandez-Duque et al. 2020). In particular, although many species for which it was possible to find the information presented mixed social organizations, we found indications of pair-living in 71.4% of them. Still, despite being a strongly present trait, we found that pair-living is not the rule for this taxon. 42.9% of the species presented multi-female/multi-male groups, in addition to pair-living (Tab. SM1), or even as primary social systems (Tab. SM3).

This aspect is fascinating, not only because it suggests that the strong link between singing behavior and a pair-living social organization does not hold so firmly anymore, but also for it concerns song emission and duetting behavior. One might ask how singing behavior is regulated in polygynous or polyandrous species. To our knowledge, no studies have considered this aspect in singing primates. One speculation, for example, would be that, in polygynous species, duets would be emitted more frequently between the male and the higher-ranking female, if singing behavior advertises bonding (Geissmann 1999).

Concerning the territorial behavior, even if we found information only in 42.9% of species (Fig. 1C), it is worth noting that all cases suggested the presence of territoriality. Only for *C. personatus*, we found mixed evidence on both presence/absence of this trait (Price & Piedade 2001). Thus, in the light of the recent phylogenetic revisions of singing primates’ taxa, we can conclude that territoriality seems to be more conserved than the social system. While the association between a monogamous social lifestyle and territorial behavior still holds for titi monkeys and indris, we indeed found indications of multi-female and multi-male groups for gibbons and tarsiers. However, future research will elucidate the behavior of species that remain to be investigated.

Our work indicates that the duet is the most widespread among the different types of song organization (70% of species). In this category, we also considered the *song* *matching duets* given by gibbons' species typically considered non-duetting: while duets between paired individuals do not occur (Geissmann 2002), singing interaction between mothers and daughters can indeed take place. Besides duets, chorusing occurs in 60% of species, but we are confident that, given that we could not find evidence for chorus songs for some singing primates living in family groups, future studies will find more information regarding this behavior. Finally, we found mention of solo songs in 62.5% of the species, often only as anecdotes.

It is quite clear that the study of solos and choruses in singing primates is still in its descriptive phase. Future studies will elucidate if these are infrequent displays or if the scarcity of their reports may be due to the complexity of studying animals in the wild, which raises a wide range of difficulties concerning the intrinsic nature of their habitats and the need for long habituation periods (Pinto et al. 2013). Here, we reported the occurrence of different song organizations in different singing primates' group, the current scarcity of data limits our clear understanding on how these different behavioural traits have evolved in these fascinating animals. More information will be needed to understand which ecological conditions favoured the presence of a particular song organization over another in a given species. It is also possible that stepping away from the idea of pair-living as a rule, and starting considering the variety of social organization in these taxa, will be a helpful approach to disentangle the selective pressures that led to the emergence and organization of singing behavior.

AUTHOR CONTRIBUTION

C. De Gregorio: conception of the work idea. C. De Gregorio, F. Carugati, D. Valente, T. Raimondi. V. Torti and L. Miaretsoa: literature research and organization of information. T. Raimondi: visualization. M. Gamba and C. Giacoma: critical revision. All authors contributed to the final version of the manuscript.

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Supplement Materials

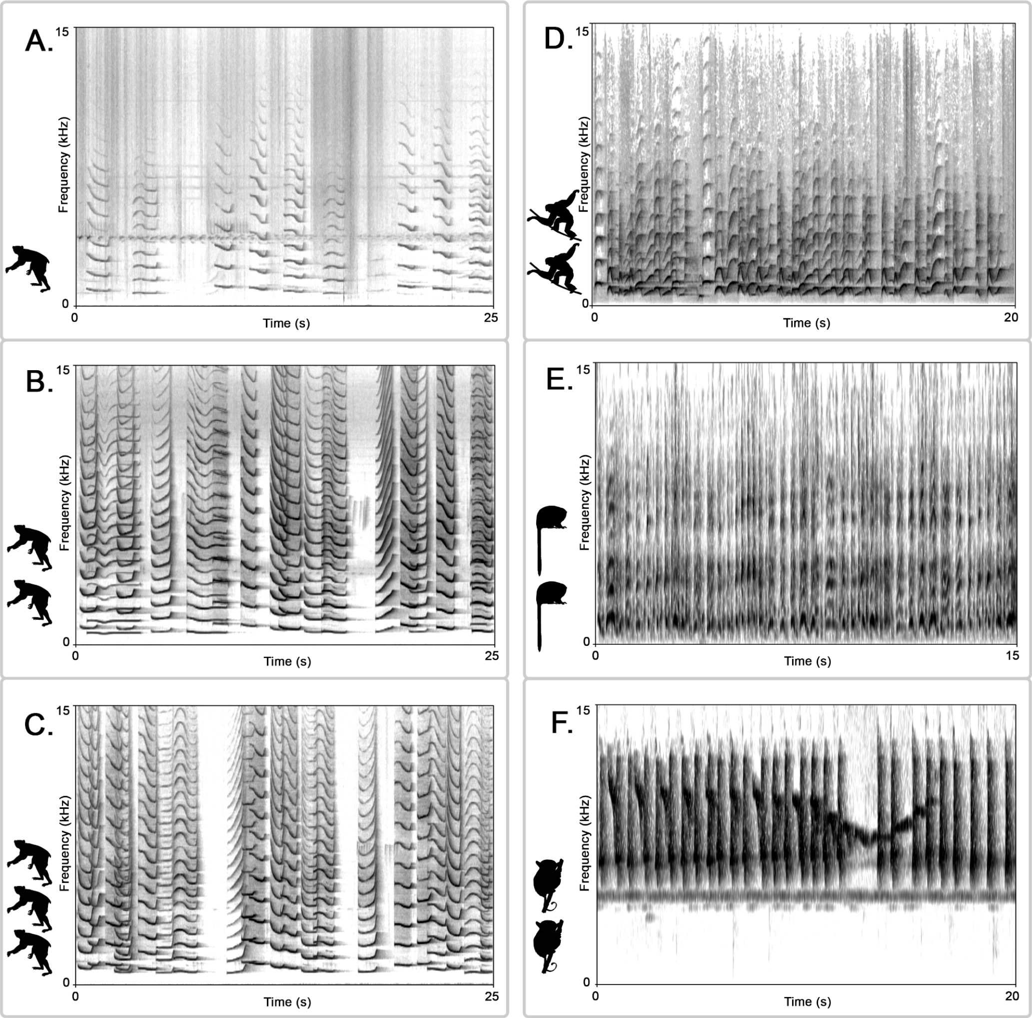

Fig. SM1. Spectrograms elucidating different song organizations in singing primates. A. Solo song of *I. indri*; B. Duet song in *I. indri*; C. Chorus song in *I. indri;* D. Duet song of *H. hoolock*; E. Duet song of *P. donacophilus*; F. Duet song of *T. spectrumgurskye.*

Genus	Species	Song Organization			Territoriality	Social system
		Duet	Solo	Chorus		
Carlito	*syrichta*	N[a]	-	N[a]	Y[b, c]	NO, PG[b, d]
Cephalopachus	*bancanus*	N[a, e]	-	N[a, e]	Y[c, e]	NO[f] PB[a] PG[e,]
Tarsius	*dentatus*	Y[h, i]	-	Y[j]	Y[k, l]	PL,PG[k]
	fuscus	Y[m, n]	-	Y[m]	Y[o]	-
	lariang	Y[i, p]	Y[i]	Y[p]	Y[k]	MO[q]
	niemitzi	Y[r, s]	-	-	-	-
	pelengensis	Y[m]	-	-	-	-
	pumilus	Y[t, u]	-	Y[u, v]	Y[t]	PL, MM, MF[w]
	sangirensis	Y[x]	-	-	-	PB, MF[y]
	spectrumgurskyae	Y[z]	Y[aa]	Y[ab]	Y[ac]	PL, PG[f, ad]
	supriatnai	Y[p, ae]	-	-	-	-
	*tarsier**	Y[m]	-	-	-	-
	tumpara	Y[h]	-	-	Y[x]	-
	wallacei	Y[x]	-	-	-	PL, MF[af]

Table SM1. (*) Selayar tarsier, *sensu* Groves and Shekelle 2010; minus symbol; (-) Indicates missing data; Y or N indicate the presence or absence of a character, respectively; M: males, F: females; Social system: NO: *noyeau*, PB: pair-bond; PL: pair-living, MO: monogamous; PG: polygynous; MF: multi-female; MM: multi-male. [a] Groves & Shekelle 2010; [b] Neri-Arboleda et al. 2002; [c] Gursky et al. 2017; [d] Dagosto et al. 2001; [e] Crompton & Andau 1987; [f] Gursky 1995; [g] Yustian 2007; [h] Niemitz et al. 1991; [i] Merker & Groves 2006; [j] Merker & Gursky-Doyen 2016; [k] Merker 2006; [l] Merker 2010; [m] Burton & Nietsch 2010; [n] Putri 2020; [o] Shekelle & Stilwell 2016; [p] MacKinnon & MacKinnon 1980; [q] Driller et al. 2009; [r] Nietsch & Niemitz 1993; [s] Nietsch 1999; [t] Grow & Gursky-Doyen 2010; [u] Grow 2019; [v] Grow et al. 2016; [w] Shekelle & Salim 2009; [x] Shekelle et al. 2008; [y] Shekelle 2013; [z] Shekelle et al. 2017 ; [aa] Kulander 2018; [ab] Gursky 2015; [ac] Gursky-Doyen 2010; [ad] Merker et al. 2005; [ae] Shekelle et al. 1997; [af] Merker 2016.

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Genus	Species	Song Organization			Territoriality	Social system
		Duet	Solo	Chorus		
Indri	*Indri*	Y[a]	M[a]F[b]	Y[a]	Y[c]	MO[d]

Table SM2. Y or N indicate the presence or absence of a character, respectively; M: males, F: females; MO: monogamous. [a]Gamba et al. 2016; [b]Torti pers obs; [c]Bonadonna et al. 2017; [d]Bonadonna et al. 2019

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Genus	Species	Song Organization			Territoriality	Social system
		Duet	Solo	Chorus		
Hoolock	*hoolock*	Y[a]	M[b]	Y*[c]	Y[d]	MO[d]
	leuconedys	Y[e]	M[a]	-	-	MO[f]
	tianxing	Y[g]	M[h]	-	-	MO[g]
Hylobates	*abbotti*	-	-	-	-	-
	agilis	Y[i] MD[j]	MF[k]	-	Y[l]	MO[k]
	albibarbis	Y[m]	M[m]	Y*[n]	Y[o]	MO[m]
	funereus	Y[p]	M[p]	-	-	-
	klossii	N[q] MD[r]	MF[a]	N[r]	Y[q]	MO[s]
	lar	Y[t] MD[t]	MF[l]	Y*[t]	Y[u]	MO[v], PA[w]
	moloch	N[x] MD[y]	MF[a]	N[y]	Y[z]	MO[aa]
	muelleri	Y[ab]	MF[ac]	N[ab]	Y[ad]	MO[ac]
	pileatus	Y[ae]	M[af]	Y*[ae]	Y[ag]	MO[ae] PG[ae]
Nomascus	*annamensis*	Y[ah]	M[ai]	-	-	-
	concolor	Y[aj]	M[ai] F[aj]	Y[aj]	-	PG[ak]
	gabriellae	Y[al] MD[am]	M[ai] F[an]	Y[am]	Y[ao]	MO[ao] PG[ap]
	hainanus	Y[aq]	M[ar]	Y[ar]	Y[as]	PG[at]
	leucogenys	Y[al]	M[ai] F[au]	Y*[al]	-	PG[av]
	nasutus	Y[aw]	M[ai]	Y[ax]	Y[aw]	PG[ay]
	siki	Y[az]	M[ai]	-	-	-
Symphalangus	*syndactylus*	Y[aaa]	M[l]	Y*[aab]	Y[aac]	MO[aad] PA[aad]

Table SM3 (*) Indicates that the chorus involves youngsters; minus symbol; (-) Indicates missing data; Y or N indicate the presence or absence of a character, respectively; M: males, F: females; MD: matching duet; Social system: MO: monogamous; PA: polyandrous; PG: polygynous social system; [a]Geissmann 2002; [b]Kumar et al. 2013; [c]gibbons.de; [d]Ahsan 2000; [e]Fan 2013; [f]Peng-Fei et al. 2011; [g]Chan et al. 2017; [h]Yang et al. 2020; [i]Haimoff 1984a; [j]koda 2013; [k]Mitani1987; [l]Gittins and Raemaekers 1980; [m]Wanelik 2013; [n]Cheyne 2008; [o]Cheyne et al. 2019; [p]Inoue 2021; [q]Tenaza 1976; [r]Whitten 1982; [s]Tenaza 1975; [t]Raemarkers et al. 1984; [u]Bartlett 2009; [v]Reichard 1995; [w]Reichard et al. 2012; [x]Geissmann & Nijman 2006; [y]Kappeler 1984; [z]Ham et al. 2016; [aa]Yi et al. 2020; [ab]Haimoff 1985; [ac]Mitani 1984; [ad]Mitani 1985; [ae]Srikosamatara & Brockelman 1987; [af]Brockelman and Srikosamatara 1993; [ag]Suwanvecho & Brockelman 2012; [ah]Thien et al. 2017; [ai]Geissmann et al. 2000; [aj]Fan et al. 2009; [ak]Fan et al. 2006; [al]Konrad and Geissmann 2006; [am]Merker and Cox1999; [an]Geissmann 1995; [ao]Kenyon et al. 2011; [ap]Barca et al. 2016; [aq]Haimoff 1984b; [ar]Deng et al. 2014; [as]Chan et al. 2005; [at]Zhou et al. 2005; [au]Dooley and Judge 2007; [av]Harding 2012; [aw]Ma et al. 2020; [ax] Chan et al. 2008; [ay]Fan et al. 2010; [az]Geissmann et al. 2007; [aaa]Geissmann 2000; [aab]Chivers 1976; [aac]Chivers 1974; [aad]Lappan 2008

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Genus	Species	Song Organization			Territoriality	Social
		Duet	Solo	Chorus		
Callicebus	*barbarabrownae**	-	-	-	-	-
	coimbrai	Y[a]	-	-	-	-
	melanochoir	-	-	-	Y[b, c]	PL[c]
	nigrifrons	Y[d]	M[d,]	Y[d]	Y[d]	PL[c]
	*personatus**	-	-	-	Y[b] N[f]	-
Cheracebus	*lucifer*	Y[g]	-	Y[g]	-	-
	lugens	-	-	-	-	-
	medemi	-	-	-	-	-
	purinus	-	-	-	-	-
	regulus	-	-	-	-	-
	torquatus	Y[h]	M[h]	-	Y[d, i]	PL[h]
Plecturocebus	*aureipalatii*	Y[c]	-	-	-	-
	*baptista**	-	-	-	-	-
	bernhardi	-	-	-	-	-
	brunneus	Y[j]	-	-	-	-
	caligatus	-	-	-	-	-
	caquetensis	-	-	-	-	-
	cinerascens	-	-	-	-	-
	cupreus	Y[b,]	M F	-	Y[n, o, q, r, s, t, u]	GM[v]
	discolor	Y[g,]	MFJ	Y[g]	Y[n, w, x]	PL[w, aa,]
	donacophilus	Y[g]	M[g]	Y[g]	-	-
	grovesi	-	-	-	-	-
	*hoffmannsi**	-	-	-	-	-
	miltoni	-	-	-	-	-
	modestus	Y[g,]	-	-	Y[ab, x]	SM[x]
	*moloch***	-	-	-	-	-
	oenanthe	Y[g]	Y[ad]	Y[g, x]	-	PL[ae, af]
	olallae	Y[g,]	-	-	Y[ab, x]	SM[x]
	ornatus	Y[n]	Y[n]	-	Y[n, x]	SM[x]
	pallescens	Y[g]	-	-	-	-
	parecis	Y[ag]	-	-	-	-
	stephennashi	-	-	-	-	-
	toppini	Y[g]	-	Y[g, ah]	-	-
	urubambenis	Y[g]	-	-	-	-
	vieirai	-	-	-	-	-

Table SM4. Evidence for reply to playback but no quantitative/qualitative description of the call or of the individuals participating. ** Studies referring to former *Callicebus moloch* are hard to attribute to the modern taxonomy. The individuals studied by Robinson have been attributed to *Plecturocebus cupreus*. Moynihan recognized three subspecies, now recognized as species, among his individuals with no certitude about their fine scale geographical origin (probably *P. cupreus, discolor* and *ornatus*). (-) Indicates missing data; Y or N indicate the presence or absence of a character, respectively; M: males, F: females; J: juveniles; Social system: PB: pair bonding, GM: genetic monogamy, SM: social monogamy; [a] Müller 1995; [b] Pearce et al. 2013; [c] Caselli et al. 2014; [d] Caselli et al. 2015; [e] Price & Piedade 2001; [f] Adret et al 2018; [g] Kinzey & Robinson 1983; [h] Easley & Kinzey 1986; [i] Robakis et al. 2019; [j] Clink et al. 2019; [k] Robinson 1979a; [l] Robinson 1981; [m] Moynihan 1966; [n] Robinson 1979b; [o] Müller & Azenberger 2002; [p] Dolotovskaya et al. 2020a; [q] Lau et al. 2020; [r] Fernandez-Duque 1997; [s] Mendoza-Mason 1986; [t] Cubicciotti & Mason 1978; [u] Dolotovskaya et al. 2020b; [v] Van [y] Belle et al. 2020; [w] Fernandez-Duque et al. 2020; [x] Ragen 2012; [ah] Dacier et al. 2011; [y] Fernandez-Duque et al. 2013; [z] Martinez & Wallace 2016; [aa] Martinez & Wallace 2007; [ab] Aldrich & Shanee 2017; [ac] DeLuycker 2006; [ad] DeLuycker 2012; [ae] Gusmão et al. 2019; [af] Wright 2013; [ag] Chagas & Ferrari 2010;

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C. baptista

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C. hoffmannsi

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Discussion and concluding remarks

This thesis aimed to deepen our understanding of *Indri indri*'s songs, a unique trait among lemurs and rare among primates, that can offer valuable hints for multidisciplinary, cross-species approaches to explore musicality and human language evolution.

Even if animal songs are not characterized by syntax, as singular units are meaningless (Zuberbühler 2019; Engesser et al. 2019), indris' songs, like music and language, consist of elements combined into sequences and grouped at different levels hierarchically (Fee & Long 2013). For this reason, indris represent an ideal model for deepening rhythmic aspects of communication, understanding how its peculiar songs develop during ontogeny and change during adult life. These studies helped broaden our knowledge about which crucial components of human language are shared with basal primates (Zimmermann 2018).

In particular, I have investigated how the rhythm of the indris' song may change depending on the sex of the emitter and the number of individuals participating in the chorus, and I found that these descriptors of the rhythmic variation show different trajectories in males and females (Chap. I). Gamba and colleagues (2016) have demonstrated that indris' duets possess a non-random overlap. Thus, songs effectively coordinate vocal displays between singers, implying that these animals may show turn-taking behaviour. I have hypothesized that female singing has a role in regulating the duration of males' contribution and may have evolved higher flexibility to accommodate more singers in the song, showing higher variability in rhythmic features than males.

Once deepened the relationship between sex, chorus size and songs' rhythmic features, I was interested in understanding if onsets' organization would reflect the presence of complex rhythm categories (Chap II). The analysis confirmed my early intuition that indris' songs contain two distinct inter-onset intervals (between phrases and between notes and the exact phrase). The study of two consecutive IOIs' ratio revealed that indris' songs possess two categories of rhythm: an isochronous (when the ratio is 1:1) and a non-isochronous one (when the ratio is 1:2). This is the first evidence that primates other than humans possess this music universal, suggesting convergent evolution of this ability, as the last common ancestor between humans and indris lived 74 MYA (Masters et al. 2013.).

It's intriguing how rhythm, in the form of inter-onset intervals' duration, is sexually dimorphic (Chap I, II, III) while isochronous rhythmic categories are the same in males and females (Chap. II). Why should males have evolved longer inter-onset interval between notes and phrase than females? Interestingly, even if it's only speculative for now, the rhythmic differences between the sexes can be affected by sexual selection via female choice. Darwin (1871) believed that music could be an outcome of sexual selection used by our ancestors to attract each other before the emergence of language. In birds, for example, the female choice may have driven the evolution of male vocal signals (Ligon 1999).

Indris' social system is characterized by female dominance, and an unsolicited mating attempt by the male of the reproductive pair are aggressively rejected by the female (Pollock 1979; Pollock, 1986). This may suggest that females can operate the mate choice towards males. Female mate choice seems to be more suitable as one of the forces driving language evolution since male-male contests can hardly explain the complexity of human language (Hurford 2007). In an environment characterized by dense vegetation and where encounters between family groups are rare due to territorial exclusivity (Bonadonna et al. 2017), songs are a fundamental vehicle of information about individuality and kinship (Torti et al. 2017), un-paired status (Gamba et al. 2016), sex and individual identity (Giacoma et al. 2010) and possibly age (Chap. III). It is unknown how long the indris' pair stay together, but there are indris in Maromizaha that are paired at least since the last 20 years (Fig. 1)

Fig. 1 Max and Soa grooming each other. They are together at least since 2001.

Given the low level of sexual dimorphism in external morphology and body size, is it likely that if a female needs to choose a male as a partner for decades, she will select a suitable candidate considering its on song features. So, what may be the benefits associated with longer intervals?

One is that they might be linked to more extended songs. In fact, given the cost of song production (Chap I.; Clink et al. 2020; Cowlishaw 1996), longer intervals may permit to extend the signalling time, and this may be a sign of quality, as has been hypothesized for unmated male gibbons, which call more frequently and longer than mated ones (Raemaekers et al. 1984; Tenaza 1976). Moreover, Cowlishaw (1996) suggested that male gibbons use high-energy assessment signals to show resource-holding potential to possible mates.

Another benefit associated with extended signalling, other than duration itself, might be the redundancy of the vocal output (Shannon & Weaver 1949). This may allow one side to better broadcast longer phrases in the dense vegetation through different territory that might present unpaired females. Even if it is still a mystery how indris choose their mates and what exactly they do when they go to dispersal, it is likely that an extended time of self-advertisement, as seen in gibbons, would be a critical feature of the process leading to the finding of a mate.

Other than longer intervals, male indris posses, in part, longer notes than females, especially the ones uttered at the beginning of the song (De Gregorio et. al 2019). Indris songs are incredibly loud, considering that the loudest terrestrial animal is the white bellbird (*Procnias albus*, Jacobsen et al. 2021), that reaches 125 dB, and indris reach 110 dB (Zanoli et al. 2020). This is a further indication that singing behaviour has high energetic costs, and thus males' longer notes could be a product of sexual selection on traits indicating high quality males.

This could also explain the sexual dimorphism we found in 1:2 ratio. This difference in males and females might be a byproduct of males generally emitting longer notes than females, so that the last inter-onset interval of a phrase given by male may end up being longer. In fact, if males utter a series notes that have a more extended duration than females's ones, may have sense that they have also a longer onset between the last note of a phrase and the first note of the subsequent one. This longer onset would give a higher ratio in males than females, as we have found.

Unlike 1:2 ratio, indris' isochronous rhythmic category is consistent between the sexes (Chap. II). Thus, I hypothesized that sexual selection had not been involved in the evolution of this trait. Then, one might ask what benefit may arise from preserving isochrony in males and females. The answer is that it may facilitate duet coordination, processing, and potentially learning (Kotz et al. 2018; Roeske et al. 2020). But rhythmic categories have also been linked to cultural transmission (Ravignani et al. 2016; Roeske et al. 2020). Despite the decades of effort to study this fascinating, critically endangered primate, there are still many things of its behaviour that we do not know—assessing whether wild indris show song learning or transmission is indeed a difficult task. At the moment, our results highlight either an alternative path to isochrony and rhythmic categories or the promise of finding cultural transmission in indri.

Aiming to shed light on this aspect, I looked at the ontogeny of indris' singing behaviour. I found that indris' songs are not fixed, and already-in-place at birth, but their spectral and temporal features evolve during development, with juveniles showing, in part, a higher degree of variability than adults (Chap. III). Interestingly, some aspects of song development seem to be mediated by other factors than morpho-physiological constraints. I speculate that auditory feedback or practice may be necessary to shape a complex display that requires input from different individuals (Chap. I, III). This aspect may leave some space for vocal production learning, a scenario in which categorical rhythms may have evolved to enhance the coordination of utterances and turn-taking behaviour.

Finally, the last chapter of this thesis (Chap. IV) has been devoted to understanding which traits are associated with the peculiarity of singing behaviour in primates. Songs can be emitted in different forms depending on the number of animals joining the vocal display. Historically, researchers have proposed a link between songs, territoriality and pair living social organization. However, I found that choruses and solo songs are still descriptive for most of the species examined. While singing behaviour has evolved independently in primates (Geismann 2000), current knowledge does not allow us to hypothesize which socio-ecological factor has favoured the presence of a song organization over another in the singing primates' taxa. Besides, even if precise indications on the social system and territorial behaviour lacks for many species, it seems that while territoriality is a relatively conserved trait, the pair-living social organization is not the rule for singing primates. Often, they show a mixed social system between pair-living and multi-female groups, while in many cases, the multi-female or multi-male group are the primary social organization. Thus, I suggest that territorial behaviour is a stronger driver for the evolution of singing behaviour than a social system based on pair-living individuals.

This work aimed to examine indris' vocal behaviour, a peculiar case among primates. In sum, this investigation highlighted different aspects of indris' vocal abilities that are flexible than previously thought. I found that rhythm is an essential aspect of indris' songs (Chap. I, II, III) and that a strong sex-dimorphism is present in rhythmic features (Chap. I, II), even during development (Chap. II). For the first time, I showed that a non-human primate possesses a musical universal, shared with humans and songbirds, that is, categorical rhythms (Chap. II). Complex rhythm categories are not, among primates, unique to humans. Moreover, territoriality seems to have been one of the major drivers for the emergence of singing behaviour in primates (Chap. IV)

My work is essential not only to interpret evolutionary precursors of crucial components of human language (Seyfarth et al. 1980; Zuberbühler 2005) but also to highlight the benefits of a cross-species and multidisciplinary approach to studying music and singing behaviour.

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My comic and beautiful family, Tonino & “la” Lilly, Mari and Jenny, Marco, which I consider a great new crew member, and Fil which has a cameo also in this section (Highly cited!), gramma and grampa that are playing *briscola* somewhere, in a dimension so close to ours that I can hear their laughs and the sounds of cards slammed on the table, and all the crazy cats and dogs that we had and we will have. Altogether we are a tornado of love and craziness that I would not exchange with anything else in the world.

All the precious friends on which I can always count, but in particular my ladies Laura, Sara, Francesca, Maddalena, Donatella, Caterina, Giorgia, Ambra. You have always been there for me, and so I’ll always do too.

And Maromizaha, where I spent almost a year of my life, and where so many magical things have happened that sometimes it feels like it was only a nice, weird, green dream.